Deadline for Proposals
for SOT 2015
Annual Meeting
Sessions: April 30, 2014

Session Types

**Continuing Education**—Emphasis on quality presentations of generally accepted, established knowledge in toxicology
*Note: CE courses will be held on Sunday.*

**Symposia**—Cutting-edge science; new areas, concepts, or data

**Workshops**—State-of-the-art knowledge in toxicology

**Roundtables**—Controversial subjects

**Continuing Medical Education**—Emphasis on state-of-the-art knowledge to assist medical doctors, health professionals, and researchers in life-long learning for providing high-quality health care
*Note: Any session type may be considered for CME.*

**Historical Highlights**—Review of a historical body of science that has impacted toxicology

**Informational Sessions**—Scientific planning or membership development

**Education-Career Development Sessions**—Sessions that provide the tools and resources to toxicologists that will enhance their professional and scientific development

**Regional Interest**—Central topics of relevance that describe public health and/or ecological problems of a particular region

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**Why Submit a Proposal?**

1. To present new developments in toxicology.
2. To provide attendees with an opportunity to learn about state-of-the-art technology and how it applies to toxicological research.
3. To provide attendees with an opportunity to learn about the emerging fields and how they apply to toxicology.

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Submit your proposal online at www.toxicology.org
Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 53rd Annual Meeting of the Society of Toxicology, held at the Phoenix Convention Center, March 23–27, 2014.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 627.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 655.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

NOTE: Abstract numbers including a lower-case letter were programmed during second submission phase.

Scientific Session Types:

| EC | Education-Career Development Sessions |
| HH | Historical Highlights Session         |
| IS | Informational Sessions                |
| PL | Platform Sessions                     |
| PS | Poster Sessions                       |
| RI | Regional Interest Session             |
| R  | Roundtable Sessions                   |
| S  | Symposium Sessions                    |
| W  | Workshop Sessions                     |

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Use the enhanced planning and networking tools to access the latest meeting information, connect with fellow attendees, build your own schedule, view presentation details, abstracts, and ePosters, request meetings with attendees and exhibitors, and navigate ToxExpo with an interactive floor plan. In addition to these networking and meeting planning tools, use the mobile event app and website to access a complete Phoenix city guide including hotels, restaurants, attractions, nightlife, and shopping. One-on-one technology training and support is available during the meeting; visit the @SOT Center—Internet Access and App Training located outside the ToxExpo on the Lower Level or the SOT Pavilion in ToxExpo.

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1 Combination Products: Toxicology and Regulatory Challenges

J. N. Cammack1, C. Ghosh1 and T. Nguyen2, 1AstraZeneca Biologics, Gaithersburg, MD and 2US FDA, Silver Spring, MD.

Therapeutic and diagnostic products that combine drugs, devices, and/or biological elements are termed and regulated by US FDA as combination products. Technological advances continue to merge product types and blur the historical lines of separation among traditional drugs, biologics, and medical devices. Concomitantly. FDA’s medical product centers, the Center for Biologics Evaluation and Research (CBER), the Center for Drug Evaluation and Research (CDER), and the Center for Devices and Radiological Health (CDRH), are employing ever-evolving collaborative efforts to address the regulatory complexities of combination products. Because combination products involve components that would normally be developed and regulated under different types of processes and policies (and frequently submitted to different FDA Centers), these products raise challenging development, regulatory, and review management questions. Differences in these pathways for each combination product type can impact the processes for all aspects of product development and management (especially preclinical testing), but also clinical investigation, marketing applications, manufacturing and quality control, adverse event reporting, promotion and advertising, and post-approval modifications. Trends and strategies for addressing the impact of overlapping technologies and evolving regulatory processes in developing a successful preclinical evaluation program will be highlighted. A regulatory overview of definitions and combination product examples, as well as a high-level review of FDA’s final rule (effective July 22, 2013), will be included. A primary focus of the course is discussion of approaches in optimizing a pre-clinical program for a hypothetical drug-device combination product (e.g., a monoclonal antibody packaged in a pre-filled syringe). Additionally, regulatory overview of the preclinical evaluation program will be provided. Future trends in combination product therapies will also be highlighted.

2 Computational and Experimental Aspects of microRNAs in Toxicology

S. C. Tilton1, K. Wang1, J. Popribia2, R. J. Brennan3 and K. Thompson3.

MicroRNAs (miRNAs) are small noncoding RNAs that function as post-transcriptional regulators of gene expression. miRNAs are increasingly recognized for their importance in regulating mechanisms of disease and exposure, including those associated with nervous system development, cardiac function, metabolism and cancer. miRNAs and their transcriptional targets are highly conserved across species. They are also stable in plasma and urine as biomarkers of tissue-specific damage or response. Furthermore, miRNAs are unique in that not only can they be experimentally measured along with their inhibitory effects on transcripts and protein levels, but their post-transcriptional regulation can also be computationally predicted based on sequence specificity and conservation across species. Given the overall importance of miRNAs in toxicology, it is necessary to understand both computational and experimental aspects of miRNAs for accurate miRNA quantification and discovery of the functional consequences of their disruption by chemical or drug exposure. The goal of the course is to provide toxicologists with a better understanding of miRNA biology (biogenesis, sequence, structure, function, and species similarities), the experimental and computational resources available for identification and target prediction and how these resources can be leveraged to identify mechanisms and biomarkers of toxicity.

3 Current Trends in Genetic Toxicology Testing

B. Gollapudi1, S. Dertinger2, R. H. Heflich3 and M. J. LeBaron4, 1Exponent, Midland, MI, 2Litron Laboratories, Rochester, NY, 3US FDA-NCTR, Jefferson, AR and 4The Dow Chemical Company, Midland, MI.

The scientific discipline of genetic toxicology has played an important role in the safety assessment of existing and new chemicals during the past four decades. This field has undergone significant changes during this time, not only in its regulatory applications, but also in the tools and technologies employed to identify adverse events. While the emphasis during the early years was on protecting germ cells and future generations from the deleterious effects of mutagenic agents, the focus shifted in later years towards identifying carcinogenic chemicals through the use of short-term assays. Furthermore, genetic toxicology tended to operate as a standalone discipline, generating qualitative data and placing little importance on dose-response analysis or integration with other toxicology measurements. The field is now in the midst of a sea change. Regulatory requirements across the globe are being harmonized, with emphasis on “3 Rs”. Recent changes to ICH and OECD testing guidelines promote the integration of genetic toxicology endpoints (e.g., Comet, micronucleus, and gene mutation) into repeat-dose general toxicology studies. This integrated approach benefits the interpretation of genotoxic findings by placing them in context with other toxicology data, including pharmacokinetics and pharmacodynamics. Regulatory initiatives such as REACH stress the importance of germ cell effects as part of a comprehensive assessment of genotoxicity. Guidelines for the study of mutations in germ cells of transgenic animals (OECD 488) have recently been finalized. Rapid advances in molecular biology are facilitating the integration of genomic biomarkers into standard toxicology studies to identify various classes of genotoxic agents (DNA reactive and DNA nonreactive). Genetic toxicology is moving from a qualitative science to the quantitative assessment of dose-responses including the identification of point-of-departure (PoD) metrics to extrapolate effects to realistic human exposure levels. The course is designed to provide a comprehensive overview of recent changes and newly established practices in the field with emphasis on their application in safety assessments.

4 Elucidating Adverse Outcome Pathways (AOPs) for Developmental Toxicity

T. B. Knudsen1, G. Daston2, D. L. Villenevuel3, N. Kleinstreuer4 and A. Terskhel.1, 1US EPA, Research Triangle Park, NC, 2Procter & Gamble Company, Mason, OH, 3US EPA, Duluth, MN, 4NHES, Research Triangle Park, NC and 5Sanford-Burnham Medical Research Institute, La Jolla, CA.

An Adverse Outcome Pathway (AOP) is a theoretical construct that integrates the biological plausibility and weight of evidence supporting a linkage between a Molecular Initiating Event (MIE) to adverse response at the individual or population level. Conceptually, an AOP spans multiple levels of biological organization and organizes the stepwise propagation of chemical disruption from MIE to toxicological outcome via a series of key events. Qualitatively, the concept of an AOP is basic to establishing plausible hypotheses and weight of evidence for chemical mode of action. This has practical use in the integration of high-dimensional data with knowledge of a complex biological system and focusing research planning on critical data needs identified as gaps in the AOP, thereby enhancing current risk assessment practices. Alternatively, development of more quantitative AOP constructs requires a framework to delineate causal relationships across a temporal series of events, and will support more realistic quantitative risk assessment. As AOPs are initially governed by signaling networks and metabolic processes, SNPs in key genes relevant to the AOP could point toward susceptible populations. The course will delve into the science of AOP elucidation from a system biology perspective, focusing on developmental processes and toxicities for early life stage susceptibilities. The presenters will each focus on making extensive use of current knowledge, informatics and data-mining tools to advance predictive toxicology.

5 Inhalation Studies: Challenges and Complexities


The successful execution of animal inhalation studies (e.g., acute, subchronic, and chronic) present many challenges and complexities not encountered with other routes of exposure. Five inhalation study challenges will be addressed: 1) Comparison of methods of exposure and potential impact on inhalation studies; 2) Using various test materials, generating simple atmospheres (e.g., exposures to gases, nanoparticles, bioaerosols, micron-sized aerosols) and mixtures (e.g., semi-volatile compounds and particles, tobacco smoke); 3) Selection of the appropriate animal species (e.g., species specific dosimetry); 4) Incorporating standardized and novel toxicological endpoints; 5) Deciding which regulatory guidance document or specifications (e.g., US EPA, US FDA, OECD and NTP) to follow. The diversity of presenters’ backgrounds (government, contract research organization, industry, and academic), and depth of experience, will provide a broad and rich resource for the participants.
6 Methodologies in Human Health Risk Assessment

J. T. Sullivan1, B. Mee2, H. A. Barton3 and J. C. Lipscomb1
1US EPA, Cincinnati, OH, 2University of Ottawa, Ottawa, ON, Canada and 3Pfizer Inc., Groton, CT.

This course provides an overview of more advanced aspects of chemical risk assessment, following up from a successful CE course on basic principles offered at the Annual Meeting in 2013. This new course will focus on methodologies, which incorporate increased use of biological and chemical specific data as a basis to provide more accurate risk assessment. In addition, it will address evolving areas such as problem formulation as a basis to better target toxicity testing and tailor assessments to the needs of risk management. The course will feature presentations and discussions focusing on the value of mode of action analysis for characterization of hazard, the fundamental tenets of physiologically based pharmacokinetic (PBPK) model development and implementation, use of benchmark dose (BMD) models to identify points of departure, and use of chemical specific adjustment factors to address inter- and intraspecies uncertainty and variability. The principles and key components of these methodologies will be illustrated with applied case examples from the regulatory risk assessment arena.

7 Nonclinical Animal Models Enabling Biopharmaceutical Advances in Translational Medicine

T. M. Monticello1, R. Dixit2, S. Morgan3, N. Ganner4, V. K. Kadambi2 and J. T. Sullivan1
1Amgen Inc., Thousand Oaks, CA, 2MedImmune, Gaithersburg, MD, 3Abbott, Abbott Park, IL, 4Ellegaard Göttingen Minipigs A/S, Dalmose, Denmark and 5Millennium, Cambridge, MA.

A fundamental theme in drug discovery and nonclinical development is the utilization of appropriate animal models that are predictive for efficacy or adverse events in humans administered a novel biopharmaceutical. The accurate prediction of human adverse effects using nonclinical animal toxicology studies remains a major goal in drug development and relies on appropriate animal models. Essential attributes for an appropriate animal model include similar target distribution, target pharmacology, systemic pharmacokinetics, metabolism, and distribution to those of humans. Utilization of the most appropriate animal model aligns with the 2011 US FDA Strategic Plan to advance regulatory science and modernize toxicology in order to enhance product safety and develop better models of human adverse responses. The Preclinical Safety Leadership Group (PSLG) of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) is creating a contemporary industry-wide database to determine accuracy with which the interpretation of nonclinical safety assessments in animal models correctly predicts human risk. The course will present considerations for the selection of an appropriate animal model for nonclinical safety, the use of animal models of disease in safety testing, emerging use of the minipig in safety testing, data from an industry-wide nonclinical to clinical translational database, and the use of animal safety data in the design and conduct of clinical trials. Output from the course will help identify advances and remaining gaps in the utilization of animal models in biopharmaceutical development.

8 Nanotoxicology: Past Achievements, Future Challenges, and Potential Solutions

S. M. Hussain1, D. B. Warheit2, L. K. Bravdo-Snolli3, C. Grubinski1, K. K. Comfort4 and C. Gera5
1US Air Force, Wright-Patterson AFB, OH, 2DrPept Hashold Laboratories, Newark, DE, 3University of Dayton, Dayton, OH and 4CDC-NIOSH, Cincinnati, OH.

Nanomaterials (NM) possess tremendous promise to advance consumer, military, and medical applications due to their unique physicochemical properties, such as enhanced surface area, tunable size, modifiable surface chemistry, and particulate reactivity. However, these same properties have made NMs a potential health hazard, thus giving rise to the field of nanotoxicology (NT), which has become a prominent player in toxicological advancement and research over the past decade. Initial NT studies were limited by a lack of both available materials and characterization tools. Through advances in material science, enhanced capabilities have been developed that allow for the synthesis of distinctive NMs and the ability to accurately evaluate their characteristics. Taking advantage of these developments, NT has made remarkable progress in evaluating the hazards of NMs and correlating specific properties, such as size, shape, coating, and composition, to observe cytotoxicity. However, even with these numerous advances, there are still a number of constraints plaguing the field of NT. One principal area of concern is the development of procedures that account for new NT facets; including NM behavior in a physiological environment, varied aggregate structure, role of ionic dissolution, and realistic modes of exposure. Another limitation is the need for new and more powerful characterization tools. Recently, the question of dosimetry has become a forefront topic and whether a universal, conceptual standard should be adopted, such as mass, surface area, or particle number. Arriving at a consensus on this issue is critical for the establishment of NM exposure limits and risk assessment metrics, which are significantly lacking. To accomplish accurate risk assessment and regulatory evaluations, NT will have to develop a means to improve the correlation of in vitro data to in vivo predictions, via enhanced cell models, relevant dosages (low vs. high), and realistic exposure scenarios. This CE course will evaluate where NT stands, by highlighting key research successes, identifying challenges facing the field today, and exploring solutions to overcome current limitations.

9 Epidemiology for Toxicologists: What the Numbers Really Mean

N. B. Beck1, M. Goodman2, S. Efron3, L. E. Goodman4 and J. Bus5
1American Chemistry Council, Washington, DC, 2Emory University, Atlanta, GA, 3George Washington University School of Public Health, Fairfax, VA, 4Harvard School of Public Health and GraduiMed, Cambridge, MA.

21st Century risk assessment relies on data from multiple lines of evidence. High quality human epidemiology data are generally preferred for regulatory decision-making, but the body of evidence often includes animal toxicity, in vitro, in silico, animal dosimetry, and human exposure data. The quality of individual epidemiology studies can be highly variable and dependent on study design as well as other critical factors that sometimes cannot be controlled for. For risk assessors to fully understand the implications of epidemiology evidence, they must understand how the overall integration of toxicity and mechanistic data with human epidemiology findings facilitates science-informed decision-making. A sufficient understanding of the epidemiology data is a necessary starting point at the regulatory decision-making process, to appropriately integrate all the available information. The course is geared towards the toxicologist who is trying to determine how to appropriately evaluate, use, and integrate epidemiology data in a weight-of-evidence evaluation or risk assessment. An overview of the field will be given, prior to providing a focus on different epidemiology study designs and their strengths and weaknesses. Attendees will also gain an understanding of exposure assessment and biomonitoring, and how this information is used and evaluated in epidemiology studies. Additional learning objectives of the course: How to determine when an association may be supportive of a causal relationship and what confidence intervals mean; how to use trend information; how to evaluate and understand adjustments that are made for potential confounding factors; and how to evaluate several epidemiology studies on the same topic, particularly in light of available toxicity and mechanistic data. Finally, attendees will learn to integrate all types of data streams with a real example. Attendees will leave the course with a stronger understanding of how to interpret and use epidemiology data in their weight-of-evidence analyses and risk assessments, and how epidemiology can help inform regulatory decision-making.

10 Innovations in Methodologies for Inhalation Exposure and Interpretations of In Vivo Toxicity

J. Pauluhn1, J. D. McDonald2, B. T. Chen3, T. E. Kleindienst4 and M. A. Higuchi5
1US EPA, Research Triangle Park, NC, 2Bayer HealthCare, Wuppertal, Germany, 3Love lace Respiratory Research Institute, Albuquerqu e, NM and 5NIOSH, Morgantown, WV.

The respiratory system presents most diverse structural and cellular heterogeneity suited to handle complicated aspects of air liquid interface such as the direct exposure of the delicate cellular and capillary surfaces to the atmosphere and the encounter of lung epithelial cells to complex mixtures of particles and gases. Not only the respiratory depositions of inhaled substances vary regionally but also the regional responses generated by the respiratory tract. Recently the field of inhalation technology and respiratory toxicology has seen revolutionary growth because of the emergence of the use of nanomaterials and renewable energy sources creating new environmental challenges. Moreover, the paradigm shift of toxicology testing to high throughput screening has led to the development of novel inhalational approaches for cells. Scientists will cover the recent advances in inhalation methodologies for various types of emerging inhalants and focus on generation of atmospheres for in vitro and in vivo toxicity assessment. These aerosols will include gas and particulate emissions from vehicles using old and new energy sources, forest fires, coal combustion, manufactured nanomaterials and mixtures formed from atmospheric aging. The dynamic of physicochemical composition of such mixed aerosols will be discussed to allow for identification of causative constituents and lung site-specific injuries. Structural differences in the respiratory tract of rodents and mammals, including humans, impacting dosimetry will be discussed. Respiratory system heterogeneity between humans and animals, and their differential neurohumoral mechanisms will be discussed to aid in interpretation of inhalational hazard for.
humans. This course will be useful for those involved in air pollution toxicology, nanotoxicology, novel drug delivery systems, pulmonary toxicology, and risk assessment.

11 Nonclinical Pediatric Drug Development: Considerations, Study Designs, and Strategies

Although nonclinical and clinical testing needs for drugs for pediatric populations have been discussed for more than 40 years, there is no default approach to evaluating safety in this age group. Over the last decade there has been a heightened awareness of the differences between the pediatric and adult patient, and these differences are being addressed by the pharmaceutical and healthcare industries, as well as the governmental and regulatory bodies that sanction the development and testing of drugs for children. As regulatory demands evolve for nonclinical safety assessments in juvenile animals, industry leaders are developing innovative ways to meet the regulatory expectations and to overcome the challenges associated with pediatric drug development. Many practical issues regarding nonclinical testing in immature animals have been surmounted, using novel and/or adapted approaches. There are considerations related to the differences in regional guidelines (US FDA, EU, and Japan), therefore development of appropriate information for submission to worldwide agencies is critical. History and experience provide the best scientific arguments as to why juvenile animals can be useful. There are numerous examples of drugs that have identified findings in various species, including information regarding kinetic and toxicity differences that highlight considerations regarding nonclinical testing models. Additionally, there are unique challenges associated with nonclinical juvenile toxicity testing for biopharmaceuticals, including selection of appropriate animal models, immunogenicity, dose selection (toxicity vs. pharmacology), and relevant endpoints. Developing a juvenile animal program requires an appreciation of the complexity of the nonclinical strategies to enable pediatric trials and an overview of the historical perspective and the current approaches to evaluating safety during this unique period of life.

12 Stem Cells in Toxicology

Stem cells are revolutionizing toxicological research and remain an area with tremendous potential. Recently, research on stem cells has generated tremendous public and professional interest. However, some areas of toxicological research have lagged behind in the integration of stem cells as a concept in toxicant-induced disease etiology. We will describe the utility and suitability of the assorted types of stem cell models (i.e. embryonic, fetal, progenitor, induced pluripotent, immortalized stem cell lines, etc.) for various research purposes, including disease modeling, drug discovery and toxicity testing in order to describe the potential applications of stem cells in toxicological research. This important overview of stem cells will highlight their nomenclature, properties, and their roles in the genesis of various diseases.

13 Translational Biomarkers in the Assessment of Health and Disease
V. S. Vaidya, M. Moses, D. L. Mendrick, J. Aubrecht and C. Leptak, Harvard Medical School, Boston, MA; US FDA-NCTR, Jefferson, AR; Pfizer Inc., Groton, CT; Children’s Hospital Boston, Boston, MA and US FDA, Silver Spring, MD.

Biomarkers serve as quantitative measures of chemical exposures and biologically effective doses, early warning signals of biologic effect, predict outcome in a patient with disease, and identify who will respond to an intervention and whether the intervention is working. The current era of scientific discovery has brought seemingly limitless opportunities for improvements in medical care. Translational biomarkers that can be measured in blood or urine in both experimental animals and man are of particular interest. Given the importance to the clinical, pharmaceutical, and regulatory communities motivated by more specific and timely diagnoses, early intervention and safer therapies, clinically useful biomarkers have evolved over time, reflecting the scientific and technologic progress made over the centuries. An increasing number of clinically relevant tests and procedures are available to estimate organ injury and guide treatment. The use of molecular signals in the assessment of health and disease is not new; however, the concept of what constitutes a useful biomarker has evolved considerably in the past two to three decades given the advanced enabling technologies, deeper molecular understanding of disease, and the advent of a regulatory framework for biomarker qualification. Our panel experts will highlight the potential of these biomarkers over a wide variety of applications spanning preclinical-clinical-safety in liver and kidney, to disease monitoring in cancer. The panel will also demonstrate the application of translational biomarkers in clinical trial design. Coordinated efforts at biomarker discovery and validation as well as technologies for biomarker measurement will help ensure that the ultimate goal of safer drugs, a cleaner environment, and improved patient outcomes are realized.

14 Air Pollution and Cardiovascular Effects: Mechanisms and Role of Lipid Peroxidation
D. J. Conklin, Cardiovascular Medicine, University of Louisville, Louisville, KY.

The mechanisms by which air pollution, including particulate matter (PM2.5) and volatile gases, affect human cardiovascular health are incompletely known. Yet many animal studies performed to probe these mechanisms have observed changes in the levels of lipids and/or lipid peroxidation products regardless of the specific form of air pollution, e.g., diesel engine exhaust, concentrated ambient particulate matter, or volatile gases, used in the exposure that associates with severity of injury. These studies suggest a potential common pathway of injury that is dependent on oxidative stress and an increased production of lipid mediators. The role of lipids and lipid peroxidation as final common mediators of air pollution-induced injury in the cardiovascular system is the focus of this symposium. Speakers will address how exposures to diverse types of air pollutants, including complex mixtures, traffic emissions, volatile acrolein, and concentrated ambient particulate matter, result in alterations in circulating lipid levels, lipids structure and function, and lipid peroxidation indicators in target organs including lungs, blood, and the vasculature. Collectively, these presentations will shed light on the specific role of lipid and lipid peroxidation in air pollution-induced cardiovascular injury.

15 Air Pollution, Lipid Oxidation, and Cardiovascular Effects in Humans and Animals Models
A. Bhattacharya, Cardiovascular Medicine, University of Louisville, Louisville, KY. Sponsor: D. Conklin.

Epidemiological studies indicate that ambient air pollution enhances the incidence and progression of cardiovascular disease and increases the risk of cardiovascular mortality primarily by promoting acute coronary syndromes and ischemic heart disease. Exposure to air pollutants associates with systemic lipid peroxidation, which has been shown to be an important marker and pathogenic mediator of atherosclerosis, acute plaque rupture and myocardial infarction. This symposium will address the relationship between air pollutants and lipid peroxidation that often accompanies exposures resulting in altered tissue and blood lipids. Moreover, this symposium will address the question whether and how these changes contribute to subsequent cardiovascular dysfunction and potentially increase the risk of cardiovascular disease.

16 Vascular Lipid Peroxidation Induced by Complex Emissions Indicates Gas-Particle Interactions in Driving Systemic Toxicity
M. Campana, Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.

Air pollution is associated with adverse cardiovascular events in epidemiological studies. Several recent studies have indicated a role for oxidative changes to phospholipids following inhalation of various pollutants, which leads to altered biological activity of those modified lipids. To better understand the important causal components of the ambient pollution mixture, we have investigated the relative lipid peroxidation induced in the murine aortic wall by diverse exposure conditions involving fresh emissions of gasoline and/or diesel engines, resuspended road dust, and secondary particulates of sulfate or nitrate composition. We noted that emissions containing mixtures of particulate and gas phase components typically induced greater vascular lipid peroxidation than did individual components. Moreover, our data suggest that gases may deposit on particulates, thereby driving toxicity in a surface area-dependent manner. Further studies highlight a role for modified lipids and metabolites in the circulation after exposures, in human and rodent studies, that may drive the accumulation of vascular lipid peroxides.
Effects of Acrolein or Concentrated Ambient Particulate Matter Exposure on Plasma Lipids and Vascular Targets

D. J. Conklin, Cardiovascular Medicine, University of Louisville, Louisville, KY.

Acute exposure to the inhaled carbonyl, acrolein, or to fine (concentrated) ambient particulate matter (CAP) leads to alterations in plasma lipids, oxidized lipids, circulating progenitor cells and vascular insulin resistance but how these divergent endpoints are related to each other is not clear. Because vascular dysfunction and vascular insulin resistance are two early events associated with metabolic syndrome and the progression to diabetes (and in order to interrogate mechanistic relationships between disparate endpoints), we studied whether changes induced in lipids and the vasculature were shared across different pollutant exposures (gas or CAP) and to similar degrees dependent on exposure level to reveal potential common mechanisms of action.

Vehicle Emissions-Exposure Results in Increased Cerebrovascular Lipid Peroxidation Associated with Altered Blood Brain Barrier Permeability

A. K. Lund, Biological Sciences, University of North Texas, Arlington, TX.

Traffic-generated air pollution-exposure has recently been associated with adverse effects in the central nervous system (CNS) including neuroinflammation and neurodegeneration. While alterations in the blood brain barrier (BBB) have been implicated as a potential mechanism of air pollution-induced CNS pathologies, there is currently little known about the pathways involved. Lipid peroxidation and oxidative stress are known to regulate air pollution-mediated effects in the systemic vasculature resulting in progression of vascular disease states; therefore, we investigated their role in altered signaling, transport, and permeability in the cerebral vasculature after inhalation exposure to vehicle emissions. We observed significant increases in lipid peroxidation in the cerebral vasculature of mice, resulting from exposure to vehicle emissions, which was associated with increased BBB permeability. Additionally, we measured increased matrix metalloproteinase-9 activity and decreased tight junction expression in the cerebral vessels from exposed animals. Further studies using plasma from exposed vs. control mice in an in vitro BBB co-culture model suggest that a circulating "reactive factor" may mediate the observed alterations in BBB permeability and transport. Collectively, these data suggest that lipid peroxidation may mediate alterations in signaling and structural proteins responsible for maintaining BBB integrity.

Air Pollution, Lipid Peroxidation, and Alterations in HDL Functionality

J. Araujo, Medicine, University of California Los Angeles, Los Angeles, CA. Sponsor: D. Conklin.

Exposure to ambient particulate matter (PM) lead to enhanced atherosclerosis in experimental animal models. Particle size and composition play a role in the extent of how atherosclerosis is promoted. We have reported that inhalation of concentrated fine and ultrafine ambient particles led to alteration of plasma HDL anti-inflammatory capacity in ApoE null mice that correlated with their ability to enhance atherosclerotic lesion formation. In addition, we have also shown that exposures to diesel exhausts result in alteration of HDL anti-oxidant capacity. This presentation will focus on our studies that have evaluated whether HDL functional changes, induced by air pollutants, are related to effects on lipid peroxidation in the lungs, circulating blood and systemic tissues.

Carbon Nanotube Exposure Assessment: An Evaluation of Workplace Exposures in the US

M. Dahm1, M. Schubauer-Berigan2 and A. Erdely2. 1CDC-NIOSH, Cincinnati, OH and 2CDC-NIOSH, Morgantown, WV.

Just as there has been much advancement in the field of toxicology over the past decade relative to health outcomes from carbon nanotube (CNT) exposures, similar strides are being made in the field of exposure assessment. Recent developments in sampling methodologies have led to more accurate estimates for worker exposure levels as compared to preliminary studies, which may have overestimated exposures. As part of an ongoing NIOSH exposure assessment and epidemiologic study, worker exposure to CNT has been examined by sampling 14 different workstations across the US over a three year period. Personal breathing zone exposure levels were measured using a chemical specific marker for the mass concentration of elemental carbon (EC) at both the inhalable and respirable size fractions. The sampling methodologies are in accordance with the NIOSH Current Intelligence Bulletin on CNTs and carbon nanofibers which set a mass based Recommended Exposure Limit at 1 μg/m^3 of EC at the respirable size fraction. Overall, personal workplace exposures at the respirable size fraction to EC ranged from 0.02 – 1.47 μg/m^3 with an 8 hr time weighted geometric mean of 0.16 μg/m^3. Inhalable personal breathing zone exposures ranged from 0.003 – 22.40 μg/m^3 with a geometric mean of 0.38 μg/m^3. Concurrent personal breathing zone samples were also collected and analyzed by electron microscopy using methods similar to the asbestos counting convention. CNT structure agglomerate sizes averaged > 4 μm while few single CNT fibers were found. This study also focused on how exposure level and type change within the various industries in which CNTs are being applied (composites, electronics, production), between types of materials (multi-walled vs. single-walled), and under varying work conditions. The detailed information from these exposure assessment findings bring awareness to industries with higher experimental studies to human relevance difficult. Initial findings from epidemiological studies of workers handling engineered nanomaterials, recent advancements in detailed facility exposure assessment, pertinent in vivo toxicology studies with dosimetry-based human health implications, regulatory aspects, and risk assessment based on results from animal inhalation studies will be included. The outcome of this session is to provide the most recent human exposure assessment and epidemiological findings and to gather perspective on in vivo toxicology studies involving risk estimates and potential carcinogenicity. This data should have direct influence on the course of newly designed studies and add perspective on previous studies of CNT-induced toxicity.
exposure potentials and provide valuable data on job specific tasks where exposures are likely to occur. In addition, the findings from this study can provide valuable insight for designing and interpreting in vivo exposures.

23 Relationship between In Vivo Carcinogenicity and Human Risk to Carbon Nanotubes

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In vitro genotoxicity investigations have shown that high aspect ratio carbon nanotubes induce cell cycle disruption and errors in chromosome number. A subsequent in vivo multiwalled carbon nanotube (MWCNT) whole body inhalation exposure study further demonstrated that MWCNTs promoted the growth of DNA damaged (initiated) lung cells to form lung adenomas and adenocarcinomas. Twenty-three percent of the filtered air controls, 27% of the MWCNT-exposed, and 52% of the methylethylbenzene (MCA) followed by air-exposed mice, had a mean of 1.3, 1.3 and 1.4 lung tumors per mouse, respectively. By contrast, 91% of the mice exposed to MCA followed by inhaled MWCNTs (MCA/MWCNT) had an average of 3.24 tumors per mouse. A total of 61 adenocarcinomas and 2 metastatic ad- enocarcinomas were observed in 55 MCA/MWCNT-treated mice. Furthermore, MWCNT inhalation increased the incidence of sexual tumors consistent with the diagnosis of sarcomatous mesothelioma from 2% in the MCA-exposed mice to 9% in the MCA/MWCNT, a 4.5 fold increase. These data demonstrate that inhaled MWCNTs are strong promoters of pulmonary adenomas and adenocarcinomas in B6C3F1 mice. The presence of metastatic disease in 15% of the MCA/MWCNT-exposed mice compared to 1.6% in the MCA-exposed group suggests that carbon nanotubes also induce cancer progression. The mouse MWCNT lung burden in this study approximated feasible human occupational exposures at the NIOSH Recommended Exposure Limit (REL). Given that recent measurements of carbon nanotubes in the workplace indicate levels of nanotubes that can be higher than the NIOSH REL, adverse outcomes in occupationally exposed humans is a possibility. The presentation will include a discussion of the mechanism of MWCNT-induced tumor promotion and progression as well as work in progress to examine the dose response of MWCNT-induced tumor promotion.

24 Review of CNT Health and Environmental Risks under TSCA, and Areas Where More Data Are Needed

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EPA, under the Toxic Substances Control Act, reviews numerous types of nano- materials prior to their entry into the market, including a substantial number of carbon-based materials. For each one a risk assessment and a risk management plan are developed that address human and environmental risks, including occupational risks. Approaches for addressing the risks of CNTs will be discussed, based on available hazard and exposure data available. More information is needed that would allow predictive determinations of hazards and exposures. Predictive frameworks could be based on hazard and exposure categories, streamlined testing strategies, and/or control banding. Additional data are needed to underpin such frameworks.

25 Evaluation of Human Risks on Animal Models

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Current inhalation testing approaches are designed to evaluate and assess systemic and portal-of-entry related toxicities of soluble and bioavailable chemical substances. Insoluble chemical substances may cause toxicity by two major mechanisms: The first depends on the site and acute dose of initial deposition and the cause of localized toxicity may depend mainly on physical factors, such as solubil- ity, particle surface activity including size, and shape. The second depends more on its durability and total dose accumulated with exposure time with time. It seems that the accumulated particle volume of inhaled low-density nanostructures delay clearance with associated faster manifestation of lung overload and toxicity. Each mechanism can dose-dependently destabilize the pulmonary barrier function, giving the structure access to the systemic circulation, depending on the degree of local inflammation. Toxicologists need to disentangle which of these etiopathologies is causing the critical toxic response under exposure conditions relevant to humans. Multiple chemical-, particle-, and experimental design-specific factors may affect the outcome of experimental studies with multi-walled Carbon Nanotubes (CNT). This adds another dimension of complexity as nanostructured particle morphologies change with their degree of agglomeration and purpose-driven modified surface characteristics. All may affect their compartmentalized retention within the lung and associated localized toxicity. Humans exposed chronically to CNTs may accumulate these structures over time and the mere exhaustion of the existing, physiological capacity of the lung may cause lung toxicity. Experimental models that most closely resemble these model-specific constraints may generate findings difficult to extrapolate to any real-life scenario of humans. This paper addresses how CNT-specific human risks can be evaluated and assessed in controlled experimental models and how kinetic modeling can be used to estimate safe Occupational Exposure Levels with a minimum number of animals.

26 Computational Approaches to Predict Repeat-Dose Toxicity: Lessons Learned from Cosmetic Ingredients

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Assessing the toxicity of cosmetic ingredients presents numerous challenges, the solution of which will contribute to the general understanding of chemically-in-duced toxicity. The goal is to predict the effects to humans of long-term repeated low-dose exposure to chemicals used in cosmetics. For risk assessment, the results of in vivo assays are often distilled in terms of NOAELs and LOAELs. However, alternatives are required for cases where animal test data are lacking or cannot be obtained due to cost, time, or legislation. The past decade has seen an im- mense growth in attempts to predict toxicity computationally, but even with these advances traditional approaches (e.g., quantitative structure-activity relationships (QSARs)) are limited due to the lack of understanding of xenobiotic targets and small molecular interactions associated with observed phenotypic effects. QSAR is also not appropriate for making reliable estimates of NOAELs; thus, a new paradigm is being sought. Despite these drawbacks, the key premise is that struc- ture-based computational analysis of a chemical of interest and close structural analogues for which experimental data are available will lead to improved predictivity due to greater association with mechanisms of toxicity. Exposure is often dermal, but inhalation or oral routes are also possible. Penetration and metabolism within the dermis, possibly followed by target organ toxicity, must all be considered and accounted for when necessary. The combined factors of relatively low-dose, dermal ADME properties and toxicity to specific organs are amongst the greatest chal- lenges facing computational toxicology for the prediction of the effects of exposure to cosmetics. This session will address these issues and review the current state of the art of computational modeling at the organ level to support risk assessment as it is being developed in a unique European Union Project called COSMOS. The funding of the COSMOS Project is from the European Commission and Cosmetics Europe.

27 Databases, Tools, and TTC Approach Applied to Chemicals in Cosmetics Products

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The COSMOS consortium developed new databases and tools central to its mission of assisting in the ultimate replacement of animal testing of cosmetic products. COSMOS Database includes a new Cosmetics Inventory of more than 19,000 substances reported by EU CosIng and the US Personal Care Products Council. The chemical in-use functions include antioxidants, hair dyes/colorants, emol- lients, emulsifiers/surfactants, perfumes, preservatives, skin/hair conditioners, and UV absorbers/filters. A new toxicity database enriched with oral repeat dose studies for cosmetics ingredients was developed by compiling data from public sources in- cluding US FDA, EPA, EU SCCS, ECHA, and literature. A new non-cancer TTC database for cosmetics-related chemicals has been compiled by augmenting the COSMOS DB with relevant Munro data. The resulting TTC database contains over 500 chemical structures with reported NOAEL. The inclusion and selection criteria of the NOAEL decisions were established. ILSI Europe experts reviewed toxicity data for the high impact portion of the database, the lowest 10th percentile plus compounds for which there is a large variation across different sources. The chemical space of the new TTC database was compared with other TTC databases to demonstrate the coverage suitable for the assessment of cosmetics. A decision tree workflow incorporating structural and usage exposure categories specific to cosmetics to address the oral-to-dermal extrapolation of repeat dose toxicity data was also devised. Thus this talk addresses overall COSMOS efforts on the TTC.
approach to chemicals used in cosmetics products, building from the databases to implementation of tools. Abstract does not reflect US FDA or EC JRC policies. Authors acknowledge ILSI Experts.

## 28 Role of Bioavailability in Risk Assessment of Cosmetic Ingredients: Kinetics, Permeation, and Metabolism


When assessing the impact of cosmetics ingredients on human health risk assessment, the chemical’s toxicity is influenced by the degree to which it is absorbed into the body (i.e. its bioavailability). When developing Threshold of Toxicological Concern (TTC) categories for chemicals used in cosmetics products the comparison of exposure from the oral and dermal routes is mandatory as existing TTC approaches for an assessment of repeated dose toxicity are based on the oral route of administration. This presentation demonstrates the role of the prediction of permeability from the dermal and the oral routes and their role in toxicity prediction. This includes the use of relevant QSARs (e.g. the Ports and Guy equation for skin permeability) and more recent models. These models have been evaluated using two new databases: a database of skin permeability values (over 2,000 entries for more than 350 compounds) and a database of PAMPA permeability values. Prediction of skin metabolism is also considered in the context of identifying chemotypes (i.e. the sets of enzymes or enzyme pathways) that may be activated or detoxified more significantly than from the oral route. This scheme is based on a “tiered approach” for chemicals’ bioavailability which identifies four general scenarios according to the degree of oral and dermal absorption with each being subsequently considered for differences in metabolism.

## 29 Adverse Outcome Pathways (AOPs) for Target Organ Effects: The Role of Structural Alerts and Chemotypes for Lipotoxicity to Group Compounds and Apply Read-Across


AOPs provide a framework to organise information at the mechanistic and pathway level, providing evidence that a molecular initiating event (MIE) is linked, through a pathway, to an (adverse) effect. This allows for a direct linkage of chemistry to adverse effects. Capturing the chemistry related to organ level mechanisms of action has provided the capability to profile, and hence group, compounds together. Over 100 structural alerts, which form the basis of chemotypes, have been developed for liver toxicity. These alerts are for effects such as reactive hepatotoxicity, phospholipidosis and other liver toxicities. They were developed from grouping of known liver toxicants and are supported by mechanistic information within the AOP framework. The alerts, in the form of chemotypes, have been developed to extend the traditional structural approach by incorporating not only structural information but also physico-chemical properties. They show great potential for group grouping chemicals together to perform read-across predictions for complex endpoints. The alerts are freely available as a stand-alone toxicity profiler or as part of KNIME Workflows. KNIME is a flexible computational workflow technology that allows the user and developer to build flexible, adaptable and transparent models for toxicity prediction. Together with AOP-based profilers for other organs and adverse effects they create computational approaches to predict systemic toxicity and assist in risk assessment.

## 30 Applying Databases and Tools from COSMOS to the Scientific Needs of US FDA’s CERES Project

K. Arvidson1, A. McCarthy2, D. Hristozov3, C. Yang4 and M. T. Cronin5.

The Chemical Evaluation and Risk Estimation System (CERES) project aims to establish a single, unified data repository that compiles available information on a food substance, including: chemical structures and properties, regulatory records, summarized toxicity data, and other biological screening assays for the Office of Food Additive Safety’s (OFAS) pre- and post-market safety evaluations. In cases where no information is available for a particular substance, CERES provides tools to identify potential safety concerns by applying mode of action-driven QSAR prediction models as well as to identify and analyze data on structural and biological analogs. Such, CERES was designed with a robust foundation for handling chemical and toxicity data as well as knowledge derived from these data. In order to expand the diversity of CERES’ chemical space and incorporate additional high-quality toxicity data from oral repeated dose studies, CERES is exchanging knowledge with the COSMOS DB project. Although OFAS does not regulate cosmetics ingredients, the chemical space within OFAS has significant overlap with many cosmetic ingredients, including colorants, flavors, antimicrobials, anti-oxidants, emulsifiers and surfactants. Thus, scientifically, the setting of study inclusion criteria for the COSMOS TTC DB and data and tools developed by COSMOS are relevant to the CERES project. The chemical space of the COSMOS DB and CERES has been profiled using appropriate chemotypes. Chemotypes representing both the COSMOS DB and CERES are correlated with various phenotypic effects in the oral repeated-dose toxicity studies available within COSMOS and CERES. Results from both the COSMOS DB and TTC databases are used in this analysis. This analysis is able to identify gaps in the chemical domain of the databases so that we may better understand the applicability domain of the systems as well as identify where additional biological data are needed. Abstract does not reflect US FDA policy.

## 31 US EPA’s ToxCast, Tox21, and COSMOS Projects: Cheminformatics Approaches to Creating Data Linkages and Synergies

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The ToxCast and Tox21 projects are generating high-throughput screening (HTS) data for thousands of structurally diverse chemicals, spanning multiple use categories and chemical functionalities, and probing a wide diversity of biological targets, pathways and mechanisms related to toxicity. The ToxCast and Tox21 chemical libraries include substances of environmental, agrochemical, and industrial relevance, including drugs, food contact substances and cosmetics. While there are a number of common chemicals within the ToxCast, Tox21 and the COSMOS databases, there are even greater overlaps in “chemotype” space in these libraries. Chemotypes are fundamental units of chemical structure (structural features and associated atom/bond properties) that have been distilled from existing knowledge of metabolic transformations and structure-based mechanisms of toxicity. Using the concept of chemotypes as a holistic guiding principle, cheminformatics approaches were applied to highlight overlapping areas of chemical space in the ToxCast, Tox21 and COSMOS inventories, to focus mechanistic investigations, and to establish data linkages and synergies across the different project data landscapes. In particular, we illustrate how ToxCast Phase II HTS assays that are significantly linked to chemotypes enriched within the chemical space of the COSMOS RepeatDose toxicity database provide a conduit for leveraging ToxCast data across Tox21 and COSMOS. Similarly, chemotype analysis of the COSMOS RepeatDose toxicity data has been projected back onto the ToxCast and COSMOS inventories to both focus (mechanistically) and broaden (chemically) investigations to cover a wider range of environmentally relevant chemicals beyond cosmetics. Abstract does not represent EPA policy.
32  **Induced Human Pluripotent Stem Cells and Their Differentiated Progeny Cells: Implementation in Toxicity Testing**


Over the past two decades stem cell technology has progressed from isolating murine and nonhuman primate embryonic stem cells (ESCs) to reprogramming fully differentiated human samples into induced pluripotent stem cells (iPSCs). Concurrent advances have been made in the differentiation of stem cells into progeny tissue cells and the ability of those cells to increasingly recapitulate native behavior. Together, these advances have driven the translation of this technology from small-scale basic biological investigations into large-scale use within the pharmaceutical industry and potential clinical applications. This symposium will focus on several examples of iPSC-derived tissue cell use in understanding and translating preclinical cardiototoxicity to the clinic; hepatocyte function, toxicity, and idiosyncratic drug-induced liver injury; and developmental neuronal toxicity. Particular emphasis will be placed on practical implementations in toxicity testing as well as the ever-expanding functional incorporation of samples from clinic populations.

33  **Engineering the Microscale Environment around iPSC-Derived Human Hepatocytes In Vitro**


Engineered in vitro models of the human liver have proven utility in assessing liver metabolism and toxicity in preclinical drug development. Isolated primary human hepatocytes (PHHs) are ideal for constructing such models, but there is a shortage of donor livers, and PHH quality can vary widely. Induced pluripotent stem cells (iPSCs) derived from somatic cells can address the limitations of PHHs and have future potential for personalized drug screening and therapy; however, current methods yield more fetal-like hepatocytes that display only a fraction (<10%) PHH functions. We utilized microfabrication to investigate the effects of controlled homotypic and heterotypic cell interactions and extracellular matrix (ECM) cues on long-term iPSC-HH liver functions in vitro. We found that 3T3-J2 murine embryonic fibroblasts, previously shown to positively affect PHH functions, also induced liver functions (albumin secretion, urea synthesis, CYP3A4 activity) in iPSC-HHs when micropatterned onto collagencoated domains. Further, the addition of a Matrigel overlay enhanced both albumin production and CYP3A4 activity. We also assessed the utility of the iMPCC model for assessing drug toxicity and showed that prototypical liver toxins (i.e. diclofenac, acetaminophen) caused dose-dependent down-regulation of liver functions, while non-toxins (i.e. aspirin, propranolol) had little to no effects. Our current focus is on expanding these studies to include growth factors as well as more systematic presentations of ECM mixtures towards designing differentiation protocols that will be useful for the field at large while exploring the role of liver-derived stromal cells on iPSC-HH differentiation in vitro.

34  **iPSC-Derived Liver Cultures to Study Mechanisms Underlying Idiosyncratic Hepatotoxicity**

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There are no accepted animal models of idiosyncratic drug-induced liver injury (IDILI); indeed, the vast majority of humans are not good models for IDILI since they are not susceptible to it. Because it is generally not possible to obtain liver biopsies for research purposes from patients who have experienced DILI, induced pluripotent stem cell (iPSC) technology is an alternative approach to develop liver culture systems capable of testing the components of the prevailing causal hypothesis. We have demonstrated that iPSC derived hepatocytes (iPSC-HHs) can be used to study mechanisms underlying IDILI due to other drugs implicated in the DILIN registry.
40 Inherited Effects of Low Levels of Methylmercury in Neural Stem Cells


Epidemiological studies have shown that chronic prenatal exposure to low levels of methylmercury (MeHg) can cause long-term neuropsychiatric alterations even in subjects who did not present signs of toxicity at birth. The mechanisms underlying the long-lasting consequences of MeHg exposure in early life are still unclear. The present study was designed to investigate short-term direct and long-term inherited effects of low levels of MeHg using primary cultures of rat embryonic neural stem cells (NSCs). We found that exposure to MeHg at nM concentrations decreased NSC proliferation and altered the expression of cell cycle regulators, such as p16 and p21, senescence-associated markers, including Bmi1 and Hmga1, and genes encoding mitochondrial respiratory chain proteins. MeHg induced a decrease in global DNA methylation, which pointed to the occurrence of epigenetic modifications. Interestingly, all changes occurring in NSCs directly exposed to MeHg (parent cells) were also present in their daughter cells kept in MeHg-free culture conditions. Data from adult mice exposed to low doses of MeHg in the perinatal period showed that the exposed mice, in addition to long-lasting behavioral alterations, exhibited a decreased proliferation of the neural stem/progenitor cells in the subgranular zone of the hippocampus, as well as long-lasting epigenetic modifications. The programming effects induced by MeHg in NSCs provide an explanation for the long-term consequences of developmental exposure to low levels of MeHg, which can predispose to neurodevelopmental disorders and/or neurodegeneration.
The Human Microbiome Project, a NIH initiative to understand the complexity, constitution, and diversity of microbes living on and in the human body, was recently completed in 2012. The term “Super-Organism” was coined to describe humans as a result of characterization of the breadth and diversity of microbes that live on the external surface as well as in the blood, tissues, and cells of the human body. What role do commensal organisms play in health and disease? What role do pathogenic microbes play in health and disease? For decades, a major emphasis in the field of immunotoxicology has been to understand the impact of environmental/occupational/therapeutic exposures on host defense against invading and opportunistic pathogens. Mounting evidence suggests that equal effort should be provided to understanding the relationship between the human microbiome and how alterations thereof can have profound implications for the development of complex immune and inflammatory diseases. Individuality of the microbiome contributes to immune-diversity, "metagenetic" diversity, and interindividual differences in susceptibility to many complex diseases, including allergic disease, autoimmune diseases, cancer, and others. Evidence suggests that development of an individual’s microbiome begins before birth, and the nature of this colonization can influence susceptibility to disease later in life. In addition, homeostasis of the microbiome is under continual attack due to exposures encountered in daily life. Recent research shows that exposure to toxic chemicals can shift the dominant characteristics of the microbiome, thereby providing a strong contribution to disease susceptibility. Therefore, it is important to consider this research in the context of human health risk assessment. The purpose of this symposium is to provide evidence of beneficial and detrimental contributions of the microbiome to the development of immune and inflammatory diseases and provide insight into how microbiome research integrates into human health risk assessment.

"The Completed Self" model for formation of the human-microbial superorganism in early life posits that: 1) symbiotic self completion is a critical step in the developmental programming of later-life health vs. disease, 2) the immune definition of self includes our internal microbial ecosystem, and 3) commensal-driven host metabolism and immune maturation are critical factors in subsequent tissue homeostasis. A consideration of humans as beyond-mammalian could shift our prevailing view of toxicity, health hazards, and preventive measures as well as the tools we employ for effective safety assessment. Prenatal, postnatal and even transgenerational epigenetic factors have the capacity to either support or interfere with the establishment of the microbiome and/or immune-microbiome interactions. Lack of self completion appears to be an important route to metabolic disorders, immune dysfunction, misregulated inflammation, tissue pathologies and chronic disease. Environmental risk factors (e.g., environmental chemicals, drugs, diet, physical and lifestyle factors) during development are considered in light of the goals of self completion in the child and a fully-developed immune system to support the newly-formed human-microbial symbiont.

It is recognized that the complex collection of microbes in our gut, the microbiome, plays a critical role in our health and well-being. It influences our responses, e.g., immunological, and reacts to environmental exposures, such as from food, drugs, and environmental contaminants. Regulatory agencies are putting a great deal of effort into understanding the sequence of mechanistic events that lead to disease. However, they haven’t generally incorporated the importance of the microbiome to a great extent and how it can provide insight into human health risk assessment. We will likely need to rethink some of the risk assessments to take into account the contribution of the microbiome. Research will greatly help assessments by addressing the lack of understanding of how xenobiotic exposures can affect the composition and function of the microbiome as well as how the microbiome can affect bioavailability of contaminants and susceptibility to pathogens. Also, the interplay between chemical exposures and pathogens with commensal gut flora and with each other need to be examined as potential risk factors. While the microbiome raises the complexity of the research in disease etiology, it will help human health risk assessors to rethink how chemicals and pathogens interact with exposed people.
emerging research from a number of laboratories demonstrates that maternal expo-
sure to environmental chemicals during the perinatal period alters the expression and function of metabolic and transport proteins in progeny later in life. Up- or down-regulation of key hepatic metabolic processes, including cytochrome (Cyp) P450 and carboxylesterase (Ces) enzymes, as well as secretory transporters, may have a significant impact on the pharmacological and toxicological responses to xenobiotics during puberty and adulthood and, additionally, impact systemic hemo-

nence levels. The purpose of this workshop is to bring together experts in the field of toxicoology to highlight the regulatory mechanisms underlying the developmental programming of hepatic metabolism and transport, and to discuss the potential impact of early exposure to xenobiotics on the ability of the liver to metabolize and excrete chemicals later in life. Experimental design and cutting-edge technologies will also be discussed. The workshop contains presentations and a roundtable dis-

cussion that will address four questions: What is the role of nuclear receptors and transcription factors in the ontogenic regulation of hepatic metabolism? What epigenetic mechanisms are involved in the hepatic programming of drug processes in newborns? Therefore, characterization of the gene expression profiles of drug processing genes and their regulatory mechanisms during liver maturation is needed for im-

proving drug therapy in pediatric patients. Mouse is frequently used as an experi-

mental model to determine how developmental expression of drug processing enzymes and epigenetic modifiers in mouse liver during development. Liver samples from male C57BL/6J mice were collected at 12 different ages from prenatal to adulthood (n=3 per age) for RNA-Seq. Several distinct ontogenic patterns were identified among the drug processing genes, which reflect a functional transition of liver from a hematopoietic organ in prenatal stage to a metabolic organ in postnatal stages during maturation. A correlation of the gene expression patterns among the Phase-I/II enzymes, transporters, and nuclear receptors was recognized, which provides a valuable foundation for mechanistic studies in the future. This study was supported by the National Institute for Environmental Health Science [RO1ES-019487] (to Xiao-bo Zhong, Curtis D. Klaassen, and Hong Lu); the National Institute of General Medical Sciences [R01GM-087376] (to Xiao-bo Zhong); the National Institute for Environmental Health Science [RO1ES-009669] (to Curtis D. Klaassen).

50 Xenobiotic Receptor CAR and Epigenetic Misprogramming

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Constitutive androstane receptor (CAR; NR1I3) is a central regulator of drug.


xenobiotic and endobiotic metabolism. CAR also regulates other metabolism, such as glucose and lipid metabolism. Interestingly, a transient activation of CAR during animal development induces epigenetic changes, which results in epigenetic mem-

ory and permanent changes of liver drug metabolism and detoxification. CAR activation by neonatal exposure to its ligand, 1,4-bis[(2-(3,5-dichloropyridylo)] benzene (TCPOBOP), persistently induces the expression of CAR target genes Cyp2B10 and Cyp2C37 throughout the life course. These mice also show a per-

manently reduced sensitivity to zoxazolamine treatment. The induced expression of CAR target genes in hepatocytes isolated from these mice is more sensitive to low concentrations of CAR agonist TCPOBOP in vitro. There are significant changes of histone modifications at the gene promoters of Cyp2B10 as analyzed by chromatin immunoprecipitation assays. These results demonstrate that epigenetic misprogramming mediated by CAR may result in a long-term epigenetic memory and permanent effect on the health of subjects.

51 Pharmacokinetic and Pharmacodynamic Consequences of Long-Term Increases in Carboxylesterase Expression following Developmental Pesticide Exposure

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Carboxylesterases (Ces) play important roles in mediating the metabolism of thera-

peutic drugs and environmental chemicals. As such, developmental exposures that cause changes in Ces expression and activity have the potential to exert significant influence on the metabolism of drugs and chemicals. We have identified that de-

velopmental pyrethroid exposure causes persistent increases in Ces mRNA expres-

sion, protein levels and activity that appear to be mediated through the constitut-

ive androstane receptor. These increases have functional consequences, decreased peak plasma and brain levels of methylphenidate, a drug commonly used to treat ADHD, are observed after oral dosing. These decreased levels of methylphenidate also result in decreased efficacy of methylphenidate in raising brain neurotransmitter levels. Data will also be presented regarding potential mechanisms responsible for persistent Ces up-regulation, including alterations in DNA methylation and histone modifications. Supported by NIEHS RO1ES015991, T32ES007149, and P30ES050520.

52 Developmental Bisphenol A Exposure Alters Hepatic Phase II Metabolism and Transport via Histone Deacetylase

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Developmental exposure to Bisphenol A (BPA), a component of plastics manufactur-

ing, is associated with adverse effects in rodents through epigenetic mechanisms. Multiple mechanisms and etiologies for BPA effects have been examined, with few examining BPA effects on hepatic metabolism and excretion, despite its importance in maintaining metabolic and endocrine balance for the body. We hypothesized that maternal BPA exposure could affect liver metabolism and disposition in re-

sulting progeny. Virgin females (C57BL/6, a/a) were fed one of the following diets containing corn oil, BPA, BPA in combination with genistein, or ethynyl estradiol (EE) through gestation and lactation. Exposure ceased at weaning and livers were collected from male progeny at 135 days of age or older. Developmental BPA and EE exposure decreased UDP-glucuronosyl transferase (Ugt) and Multidrug Resistance-Associated Protein 2 (MRP2/ABCC2) expression and function, which was associated with significant alteration in nuclear receptor expression and bind-

ing. Developmental BPA and EE exposure decreased BPA glucuronidation and hepatic dibromosulfotetraethane (DBSP) clearance. Lastly, down regulation of mouse MRP2/ABCC2 expression was associated with increased Histone Deacetylase 2 (HDAC2) and decreased acetylated Histone 3 recruitment to function oxidant response elements for the Nr2 transcription factor in the mouse MRP2/ 

ABCC2 promoter. These observations were not observed in progeny from dams that were fed diet containing BPA and genistein. Together, these data illustrate that BPA exposure early in life can reprogram liver function, which is associated with altered Nr2 receptor binding and histone recruitment.

53 New Concerns and New Science Addressing Environmental Asbestos Exposures

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Although extensive research has been conducted on asbestos health effects for spe-
cific fiber types, there are still many unresolved issues and controversies, particularly related to environmental asbestos exposures. Further, the health effects of mixtures of elongate mineral fibers have not previously been studied in detail. Approximately 120,000 asbestos-related deaths occur in the US and worldwide every year, and it has been well known for the past 30–40 years that occupational exposure to asbestos causes mesothelioma, asbestosis, and lung cancer. Moreover, environmen-
tal asbestos exposures have led to a declared public health emergency in Libby, Montana, an area known to have a higher incidence of asbestos-related diseases than the rest of the US. Other areas, such as El Dorado Hills, California, and Nooksack and Sumas, Washington, are also currently being evaluated for abes-
tos-contaminated soils and potential exposure to populations living in these areas. Although occupational asbestos exposures have been limited, there is increased concern related to exposures to environmental asbestos. For both environmental and occupational exposures, there are a number of critical issues, including: (1) How can complex mixtures of different forms of asbestos and nonasbestos contam-

inants be evaluated?; (2) What are the cellular and systemic mechanisms resulting
in fibrosis and/or tumor development; (3) What is the relative toxicity of different forms of asbestos?; (4) What is the proper dose-metric to consider (e.g., mass, fiber number, or surface area of fibers) when interpreting asbestos toxicity?; (5) What are the effects of asbestos exposure on susceptible populations (e.g., children and adolescents)?; and (6) How do we implement toxicological findings into risk assessment and clean-up efforts? This workshop has been designed to present the latest epidemiological and basic research findings in an attempt to address some of these questions, and to highlight the efforts of all stakeholders in determining the role of asbestos in various disease endpoints.

**54 Human Health and Environmental Exposure to Libby Amphibole Asbestos**

T. Larson, ATSDR, Atlanta, GA Sponsor: D. Carlin.

The public health situation in Libby, Montana has afforded a body of new research on the impacts of asbestos exposure on human health. While the focus of much of the preexisting asbestos literature has been on occupational cohorts comprising mostly men, the spectrum of exposures in Libby includes low levels among residents without occupational exposure. Consequently, ATSDR and EPA have funded a program of community-based epidemiologic research in Libby. Research topics include pulmonary disease progression using computed tomography, pulmonary health of child residents of Libby who subsequently moved away, and study of the relationship between residential exposure, system autoimmunity, and asbestos-related pulmonary disease. In addition to this in-process research program, several papers have been published examining health outcomes in sensitive subpopulations, health effects not classically associated with asbestos exposure, and health outcomes in residents. Self-reported pulmonary symptoms have been associated with frequency of handling vermiculite in Libby residents who were minors when the Libby vermiculite mine was operational and pleural plaque has been detected on the chest radiographs of Libby children. Residents of Libby who reported frequent contact with vermiculite also demonstrated elevated risk for systemic lupus erythematosus, scleroderma, or rheumatoid arthritis. Also among residents, radiographic pleural plaque was associated with restrictive spirometry.

**55 Autoimmune Responses following Asbestos Exposure**

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Immune dysfunction is well established as part of the response following asbestos exposures, ranging from the inflammatory component of fibrotic disease to increased autoantibody production. However, epidemiological data clearly linking asbestos exposure to autoimmune disease is lacking, with only one study showing an increased risk of systemic autoimmune diseases in an amphibole exposed population. Because that study population was Libby, MT, where both occupational and environmental exposures occurred, hypotheses regarding the autoimmune component focus on differences either in exposure levels or exposure type—environmental versus occupational and/or chrysotile versus amphibole. Recent work is beginning to tease apart these issues using both human cohort comparisons and mouse models, revealing that the immune dysfunction following fiber exposure is highly complex and fiber specific. While amphibole asbestos clearly affects autoantibody production in both mice and humans, the human health outcomes do not seem to fit into current diagnostic criteria for specific rheumatic disease, but may dramatically impact pulmonary outcomes for environmental amphibole exposures. A key objective for research, therefore, is to identify specific immune changes that may be predictive of severity or progression of disease.

**56 Determinants of Toxicity of Environmental Asbestos Fibers**

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Recent EPA-led studies have addressed the comparative toxicity and pathological mechanisms of environmental asbestos samples from Libby, Montana and other communities in the United States. Longer amphibole fibers induce a 4-10 fold greater induction of pro-inflammatory mediators COX-2 and HO-1 than Libby fibers in human airway epithelial cells, as well as a number of other genes involved in cellular stress and toxicity. Similarly, equal mass doses of longer amphibole fibers administered intratracheally to F344 rats cause greater pathological effects than Libby fibers, from 1 to 2 years post-exposure. However, both intratracheal and inhalation studies show comparable effects of Libby fibers and shorter UICC amphibole fibers. Dosimetry modeling and potency analysis studies are using these data to predict effects in humans. Libby fibers induce an acute phase response and systemic increases in selected markers of inflammation, and induce components of the NALP-3 inflammasome in the lung, while surface complexed iron inhibits these responses. Libby fibers alter genes involved in inflammation, immune regulation, and cell-cycle control, and also induce autoimmune responses in a rat model. Comparative toxicity studies showed that chrysotile fibers from Sumas Mountain, Washington caused greater lung interstitial fibrosis than Libby fibers, which were significantly smaller in diameter than tremolite fibers from El Dorado, California and act as “cleavage fragments” from Ontario, Canada. These data are improving the scientific basis for the risk assessment of asbestos-contaminated communities, defining key determinants of internal dose, and providing critical insight on additional key health or pathologic endpoints.

**57 Role of Inflammasomes in Malignant Mesotheliomas**

A. Shukla, University of Vermont, Burlington, VT Sponsor: D. Carlin.

Asbestos fibers cause a number of respiratory diseases including pleural fibrosis and malignant mesothelioma (MM), however, the mechanisms initiating these diseases are not well understood. We are the first to demonstrate that asbestos-induced MM development involves activation of the NOD like receptor protein 3 (NLRP3) inflammasome, caspase-1 activation and mature IL-1β release from human mesothelial cells (HMC). This priming and activation of NLRP3 in HMC by asbestos does not require exogenous TNFα or lipopolysaccharide to initiate the response. In addition, using an IL-1 receptor antagonist, Anakinra, we document that there exists an autocrine feedback loop for activation of the inflammasome and production of cytokines perpetuating inflammation in mesothelial cells. Other studies in human MM tumors and MM cells have shown significantly decreased levels of NLRP3 and caspase-1, in contrast to normal mesothelial cells, which may in part be responsible for increased drug resistance observed in these tumors. Based on our findings, the use of chemotherapeutic drugs including Bcl-2 inhibitors in combination with an IL-1 receptor antagonist could be projected as better treatment strategies for MMs. The data generated by our research will help in understanding the mechanistic(s) by which asbestos can cause MM and eventually may lead to development of beneficial therapeutic strategies for asbestos-associated diseases.

**58 Challenges and Recommendations for Future Asbestos Research**

A. Miller, NIEHS, Bethesda, MD.

In an effort to address a host of new challenges associated with environmental asbestos exposures the NIEHS, USEPA, ATSDR, NIOSH, and academic institutions have closely collaborated over the past several years. Multidisciplinary research teams comprised of epidemiologists, toxicologists, mineralogists, clinicians, and statisticians have worked through array of complex issues elucidated by the Libby, Montana site, as well as other sites around the United States containing hazardous mineral fibers. Such issues include exposures to non-work populations including children, development of more robust sampling and analytical approaches to better characterize environmental exposures, and new epidemiologic and medical studies to understand resultant health effects including autoimmune and inflammatory health endpoints. Utilizing collaborative research platforms, Interagency Working Groups, and workshops such as an NIEHS-sponsored Mechanisms of Asbestos Action Workshop that occurred in December 2009 (Chapel Hill, NC) experts from these organizations have identified data gaps and mechanistic, epidemiologic, and exposure assessment research needs. Recent collaborative research efforts also include investigations of exposures and risks among communities in North Dakota and Turkey exposed to erionite, an unregulated but very hazardous mineral fiber. Recently, the NIEHS National Toxicology Program has been designing projects (e.g. 2 year bioassays) to better assess the toxicity of Libby amphibole material in conjunction with a comprehensive program to study naturally occurring asbestos and related mineral fibers. These studies will consist of collecting toxicity data, complete detailed physical and chemical characterizations, dose-response characterization for the fibers, and the influence of mineralogy and morphology on toxicity. It is anticipated that these collective efforts will lead to an improved understanding of fiber induced illnesses and new risk assessment strategies to help protect impacted communities.
Predicting Children’s Internal Exposure to Pyrethroids Using a Physiologically-Based Pharmacokinetic Model with Age-Appropriate In Vitro Metabolism Data

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Due to species differences in physiological and biochemical maturation as well as differences in major metabolic pathways, predicting early age sensitivity to a chemical exposure based on animal data requires caution. In our previous modeling with deltamethrin (DLM), while lower metabolic clearance of DLM resulted in increased sensitivity in neonatal rats, no age related sensitivity was predicted for humans. In this study, the DLM model was tested as a general platform to predict age-dependent pharmacokinetic sensitivity to other pyrethroids using compound specific kinetic parameters for trans-permethrin (TPM). In addition to maturation physiology, developmental changes in pyrethroid hydrolysis by carboxylesterases (CESs), the major metabolic pathway for both DLM and TPM in humans, were incorporated in the model. Age-appropriate hydrolysis estimates were obtained based on adult data measured in hepatic microsomes and cytosol, and ontogeny of CESs. No substantial age-differences in brain exposure to pyrethroids were predicted for TPM at a constant daily exposure (0.1 mg/kg/d) as shown by less than a 2-fold difference in average daily brain AUCs across ages. This study demonstrates that taking into consideration species-specific enzyme ontogeny and the resulting age-dependent changes in metabolic clearance of a given pyrethroid is critical in estimating early age sensitivity. The difference between environmentally relevant exposure levels and dose levels used in animal toxicity studies also needs to be considered. This modeling platform can serve as a tool to properly evaluate children’s sensitivity to pyrethroids when combined with age-appropriate metabolism data and environmentally relevant exposure estimates for children (supported by the Council for Advancement of Pyrethroid Human Risk Assessment, LLC [CAPHRA]).

Using a Two-Dimension Monte Carlo Model to Develop Chemical Specific Adjustment Factors (CSAFs) for Chlorpyrifos and Chlorpyrifos Oxon

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The traditional values of 10 for inter- and intra-individual uncertainty factors have been recognized as resulting in overly conservative health-based standards for most chemicals (Baird et al. 1996; Gayler and Kedell, 2000). This paper presents chemical specific adjustment factors (CSAFs) for setting the acute oral Population Adjusted Doses (aPADs) for chlorpyrifos and chlorpyrifos oxon consistent with recent WHO and USEPA guidance. The CSAFs are developed using data generated by a Biologically Based Dose Response Model (BBDR). The BBDR is based on a PBPK/PD model (Smith et al. 2013) that simulates the inhibition of red blood cell acetylcholinesterase (RBC AChE). This model is placed in the framework of a two-dimensional Monte Carlo model of uncertainty and variation in the response. RBC AChE is the critical effect used by USEPA in setting the aPADs for chlorpyrifos. Using the predicted average response in humans and the 99th percentile of variation in sensitivity, the inter- and intra-individual CSAFs for chlorpyrifos were 1.3 and 2.7, respectively. The corresponding values for chlorpyrifos oxon are 0.9 and 2.4. The relatively small CSAFs (as compared to traditional values) for the compounds are due to the use of RBC AChE as the critical effect. RBC AChE is the main target for chlorpyrifos in the human body that occurs early in the compounds’ adverse outcome pathways (AOPs). The slightly larger values for chlorpyrifos CSAFs are due to the additional activation step (conversion of chlorpyrifos to oxon) in the chlorpyrifos AOP. Variation in activation increases the intra- and inter-species differences in response for chlorpyrifos and thus the CSAF values. The use of the BBDR and its use in setting CSAFs can be applied to other chemicals where chemical reactions are events, or markers of events, that occur early in a chemical’s AOP.

Predicting Inhalation Toxicity of Bifenthrin Using a Pharmacokinetic Rationale

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Numerous studies with the pyrethroid bifenthrin have demonstrated relatively low acute inhalation toxicity in rats. However, due to increased toxicity following inhalation exposure for some pyrethroids, such as flumethrin and cyfluthrin, there was hesitation to assign an inhalation BMDL for bifenthrin. The objective of this work was to use a pharmacokinetic (PK) basis for setting an inhalation BMDL for bifenthrin. To obtain internal exposure data and disposition characteristics, two PK studies were conducted in rats. These studies used far fewer rats than a Guideline 28-day or acute inhalation neurotoxicity study. In the first study, bifenthrin concentrations were measured in brain and plasma following inhalation exposure (3.1 mg/kg) or inhalation exposure (0.018 mg/L for 4 hours, equivalent to the same delivered oral dose), relative to time. The maximum concentrations (Cmax) and area under the concentration curves (AUCs) in both plasma and brain were slightly lower following inhalation than those after oral dosing. These new PK data for oral exposure were comparable to previous literature data. After intravenous administration to rats (1 mg/kg) in the second study, bifenthrin elimination was relatively rapid with a plasma half-life of 13.4 hours, i.e. limited tendency to bio-accumulate. Since bifenthrin peak neurotoxicity is proportional to Cmax in brain and/or plasma, an inhalation BMDL of 3.5 mg/kg/day was calculated based on the oral BMDL of 3.1 mg/kg/day and a ratio of 1.14 (brain oral Cmax/plasma concentration Cmax). These PK data demonstrate that there is an acceptable margin of safety even for the worst case worker exposure scenario, having an inhalation MDE=946 using the inhalation BMDL (3.5 mg/kg/day). Furthermore, the PK properties shown here for bifenthrin after multiple exposure routes, enable a complete picture of internal exposure levels to emerge for this pyrethroid, having predictive value for risk assessment without conducting additional animal toxicity studies.

Reframing of an Acute Inhalation Reference Concentration for 1,3-Dichloropropene

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The acute inhalation reference concentration (aRIC) is an important component of fugitive health risk assessment. For the soil fugitive 1,3-dichloropropane (1,3-D) there have been two aRIC values derived which differ significantly. We conducted a critical analysis of the basis for the differences between these values and provide an updated aRIC incorporating additional data. Key elements of the aRIC derivation process include point of departure (POD) selection, dosimetric adjustments, and uncertainty factor use. For POD selection, the acute studies have insufficient information to characterize the dose-response for the pertinent effects. Well conducted repeat-exposure studies are available, but comparison of dose and temporal concordance patterns indicated that these studies were inappropriate in terms of temporal relevance for the aRIC. For the updated assessment a LOAEL of 357 ppm, based on decreased body weight and clinical signs, was selected as the POD from a 4-hour inhalation study in albino Wistar rats. The two assessments also differ in terms of dosimetry methods used to estimate a human equivalent concentration (HEC). One based the HEC on partition coefficient ratios, while the other used respiratory rates for this conversion. The choice of methods depends on toxicokinetic behavior following acute exposure and on whether the critical effect is related to systemic or portal of entry toxicity. Additional analysis of the rate at which steady-state toxicokinetics would be reached for 1,3-D suggested that the approach based on partition coefficients ratios is most appropriate. Thus, the LOAEL of 357 ppm was adjusted for exposure duration (4 h/24 h) and a factor of 1 was applied to reflect species differences in steady-state blood concentrations, resulting in an HEC value of 60 ppm. An updated aRIC of 0.6 ppm was derived by applying a composite uncertainty factor of 100 to this POD. Overall, the analysis highlights the importance of a systematic analysis of each step in the aRIC derivation process.
Toxicokinetic (TK) data can provide valuable information for human health risk assessment. The following describes the advantages of integrated TK (no additional animal usage) to the process of risk assessment, using halauxifen-methyl, a new herbicide, as a case-study to show integrated TK allows:

Hazard Identification: Available TK data, including target tissue concentrations, provided insight as to why acid- and methyl-forms of halauxifen had different primary target organs, kidney and liver, respectively. Halauxifen-methyl is rapidly hydrolyzed in liver to the primary metabolite, halauxifen-acid, such that post-hepatic systemic exposure is exclusively to the acid. However, unlike the acid, its methyl ester was subsequently shown to selectively activate the hepatic AhR, and be responsible for its hepatotoxicity.

Dose-Response Assessment: Halauxifen-methyl has a rapid half-life of elimination (<6 hrs in rats); metabolism data in primary hepatocytes were used and AhR activation as measured by Cyp1a1 transcript levels was assessed. Subsequent primary hepatocyte studies in rats, mice and humans, using rat TK-based target tissue concentrations, provided data on AhR activation across species at relevant concentrations.

Exposure Assessment: PBPK models for rats and humans predicted similar systemic exposures, primarily to halauxifen-acid. Dietary exposures at the NOAEL of 10 mg/kg/day from the rat 90-day study were modeled. Similar results were obtained at relevant human exposure levels (i.e., proposed chronic RfD).

Risk Characterisation: In vitro, cross-species comparative hydrolysis data and integrated TK analyses supported quantitative non-human relevance of the halauxifen-methyl AhR-mediated hepatotoxicity in rodents by establishing: 1) a clear threshold for rat liver effects, 2) that systemic exposure is to acid, and 3) a chronic RfD significantly higher than any potential human exposure.

Mode of action (MOA) provides a central framework for assessing human relevance of adverse health outcomes observed in nonclinical safety studies. The goal of this study was to characterize MOA profiles for known rodent liver tumorogens identified from a database of pesticides assessed by the U.S. Environmental Protection Agency (EPA) Office of Pesticide Programs. Among 464 compounds, with mouse and rat carcinogenicity study data, liver tumor effects were observed for 23% of compounds (108/464; 95/464 for mouse and 32/464 for rat). Of these, 74% (80/108) were classified by the EPA as possible (43%) or probable/likely (31%) human carcinogens. Twenty-one compounds had a MOA proposed by the registrant for liver tumor outcomes used in quantitative risk assessment.

Of these, 16 MOAs were accepted by the EPA Cancer Assessment Review Committee. Molecular initiating events for these MOAs included constitutive androstane receptor (CAR) activation (10/16), peroxisome proliferator-activated receptor (PPARα) activation (4/16), and sustained hepatic cytotoxicity (2/16), followed by increased liver cell proliferation as measured by BrdU, Ki67, or PCNA labeling index (LI). A significant increase in proliferation was observed at ≥7 days for 13/14 compounds with accepted mitogenic (CAR- and PPARα-mediated) but not cytotoxic (0/2) MOAs, while short-term effects on proliferation LI were not provided (4/5) or were inconclusive (1/5). None of the compounds included decreased liver cell apoptosis as a key event. These findings highlight the central role of quantitative proliferation data in MOA evaluation and support current efforts to prioritize potent hepatotoxins based on early key biological effects.
To aid in completing the human health risk assessment (HHRA) for diesel exhaust (DE), inhalation dosimetry models (addressing deposition and clearance) were compared, with particular attention to applicability to DE particle (DEP) retention modeling. The literature was reviewed, and the six models of greatest relevance were identified. The model evaluation included: model compartments, clearance and deposition approach, species extrapolation approach, model assumptions and validation, usability and applicability to DEP. The model comparison highlighted model aspects that reflected state of the science/best practice, as well as issues that could decrease the reliability or validity of the results. For example, the Yu and Yoon (1992) model uses chemical-specific clearance information, including separate accounting for clearance of particles and of organics adsorbed to particles, but the human model has not been verified. Conversely, MPPD reflects the state of the science for deposition modeling, and the current version (2.2) includes alveolar clearance. We found that predicted or optimized deposition efficiencies were generally similar across the models; to a large degree, the observed differences reflected differences in the assumed ventilation rate, rather than inherent model differences. This observation is consistent with the finding by U.S. EPA (2002) that the alveolar deposition was very similar among the Yu and Yoon, ICRP, and MPPD (ver. 1.1) models. However, under conditions of continuous exposure, the net cumulative dose to the lung reflects the balance between delivery and clearance, and model differences in clearance appeared to be larger than for deposited dose. Based on these results, the MPPD model was deemed most appropriate for re-evaluation of the dosimetry and inclusion in a human health risk assessment of DE, to reflect the understanding of the science.

**68 Evaluation and Comparison of Inhalation Dosimetric Models for Applicability to Diesel Exhaust Particle (DEP)-Retention Modeling**

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Model aspects that reflected state of the science/best practice, as well as issues that could decrease the reliability or validity of the results. For example, the Yu and Yoon (1992) model uses chemical-specific clearance information, including separate accounting for clearance of particles and of organics adsorbed to particles, but the human model has not been verified. Conversely, MPPD reflects the state of the science for deposition modeling, and the current version (2.2) includes alveolar clearance. We found that predicted or optimized deposition efficiencies were generally similar across the models; to a large degree, the observed differences reflected differences in the assumed ventilation rate, rather than inherent model differences. This observation is consistent with the finding by U.S. EPA (2002) that the alveolar deposition was very similar among the Yu and Yoon, ICRP, and MPPD (ver. 1.1) models. However, under conditions of continuous exposure, the net cumulative dose to the lung reflects the balance between delivery and clearance, and model differences in clearance appeared to be larger than for deposited dose. Based on these results, the MPPD model was deemed most appropriate for re-evaluation of the dosimetry and inclusion in a human health risk assessment of DE, to reflect the understanding of the science.

**69 Cross-Species Dosimetry for Upper Respiratory Tract Naphthalene Exposure with a Hybrid CFD-PBPK Model**

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A hybrid CFD-PBPK model for naphthalene in the rat and human was developed to support cross-species dosimetry comparisons of naphthalene concentrations in the nasal respiratory and olfactory epithelium. The model reproduces time courses of naphthalene blood concentrations from intravenous and inhalation exposures in rats, and upper respiratory tract extraction data in both naïve rats and rats pretreated to inhibit nasal metabolism. Metabolic rates in the nasal tissue compartments were based on in vitro to in vivo extrapolation from published studies employing nasal tissue microsomes in rats and monkeys. The model was applied to estimate human equivalent inhalation concentrations (HECs) in dorsal olfactory and ventral respiratory tissues (i.e., nasal regions where tumors were observed in the NTP animal studies) corresponding to several endpoints for cancer and non-cancer effects of naphthalene in rats, as well as benchmark doses for genomic responses observed in rat studies conducted at The Hamner Institutes. Two approaches for cross-species extrapolation were compared: (1) equivalence based on tissue naphthalene concentration, and (2) equivalence based on amount metabolized per minute (normalized to tissue volume). At the NOAEL of 0.1 ppm (based on absence of hyperplasia in nasal tissue in a 90 day exposure study), the RGD based on naphthalene concentration was 0.2 for the dorsal olfactory; however, the ratio rises to 5.3 when based on the normalized amount metabolized due to the lower of expression of CYP2F2 isoforms in the nasal epithelium of primates and humans. HECs based on amount of naphthalene metabolized per minute could not be extrapolated to TWA concentrations above 0.54 ppm in the dorsal olfactory region or 0.018 ppm in the ventral respiratory region due to saturation of metabolic activity in the human nasal epithelium.

**70 A Regional Model of Lung Metabolism for Improving Species-Dependent Descriptions of 1,3-Butadiene and Its Metabolites**


1,3-Butadiene (BD), a volatile organic chemical used in synthetic rubber production and other industrial processes, is detectable at low levels in outdoor air and in tobacco smoke. Exposures to high concentrations of BD have been associated with cancer in humans and animals, although there are species differences in sensitivity. Following inhalation, respiratory tissues are a primary site of BD metabolism as well as targets for exsudate-induced damage and carcinogenicity. Pulmonary Type I epithelial cells, Type II epithelial cells and Clara Cells are metabolically active cells and thus potential targets of BD metabolite-induced toxicity. Metabolic capacities of these cells, their regional densities, and distributions vary throughout the respiratory tract as well as between species and cell types. Despite these differences in lung physiology/metabolic capacity, physiologically based pharmacokinetic (PBPK) models for BD to date have relied on metabolic parameters derived from whole-lung studies, and treat the lung as a homogenous organ. Here we present a novel regional model of lung metabolism for improving species dependent descriptions of BD metabolism for PBPK modeling. The overall structure of the model was also expanded by adding a kidney compartment and descriptions of higher order metabolism to allow consideration of available urinary and blood biomarker data in animals and humans to enable better model calibration between exposure and internal levels. Simulation results of inhalation exposures to BD indicate that incorporation of differential lung metabolism is important in describing species differences in pulmonary response and that these differences may have implications in cross-species extrapolation for risk assessment of BD.

**71 Development of an Inhalation PBPK Model for Benzo[a]pyrene in Rats and Humans**


Benzo[a]pyrene (BaP) is a by-product of incomplete combustion of fossil fuels and plant/wood products, including tobacco. Since human inhalation exposure to BaP is most commonly associated with particles, the bioavailability of inhaled BaP in humans is uncertain; however, PBPK models may assist in estimating internal dosimetry. The existing PBPK model of Crowell et al. (2011) for BaP in rats was extended to simulate inhalation exposures to BaP in rats and humans. An important step identified in modeling inhaled BaP was the impact of BaP-carrier particle dissociation on lung dosimetry. A kidney compartment was also added to allow preliminary reverse dosimetry using human urinary biomarker data to determine if carrier particle-specific BaP dissociation rates in the lung significantly impact long-term BaP dosimetry. Existing data for inhalation of BaP coated on various particle types suggested that lung clearance is bi--phasic. However, this behavior may be driven by rapid and slow particle desorption phases, the effect of mucociliary clearance, or a combination of these processes. Thus, parameter values for BaP-particle dissociation were estimated, tested in the extended inhalation model for rats, and optimized to the available rat data. Simulations of acute inhalation exposures suggest that diffusion-limited transfer of BaP from lung tissue to blood, ciliated clearance, swallowing of BaP particles, or all of these processes, may be critical for modeling lung dosimetry over time. Modeling of long-term human urinary biomarker data informed the relative significance of accounting for particle dissociation rates on chronic human dosimetry.

**72 Predicting Blood Lead Following Short-Term Exposures Using the All Ages Lead Model (AALM)**


Models currently used by EPA to predict blood lead concentration following exposure to lead (Pb) in environmental media are limited with respect to the exposure pathways and durations that can be simulated (e.g., the Integrated Exposure Uptake
The views expressed are those of the authors, and do not necessarily represent the choice of dose-metric has a significant impact on the evaluation of potential human for hexavalent chromium, and a variety of different internal dose-metrics may be between individuals. In addition, multiple PBPK models have been developed the GI lumen, and only total chromium can be analytically measured from a total of 109 based on completeness of information on cadmium levels in the current modeling approach to estimate the internal uptake of Cd and subsequent induction of metallothionein (MT) that retains Cd in the placenta. The simulated total Cd contents in the lung, blood, liver, placenta, uterus, and kidney in the dam on GD 17.5 were in good agreement with the observed values. The model predicted a rapid build-up of Cd in the placenta and uterus with daily inhalation exposure to CdO NPs compared to a relatively slower increase resulting from every other day exposure. Although the Cd levels were similar on GD 17.5, the AUC values in the placenta and uterus from the daily exposure were 1.4 and 1.8 fold higher, respectively, compared to those from every other day exposure indicating that the observed effects on fetal and neonatal development may be attributable to the difference in time profiles of Cd exposure in the placenta and uterus. The current modeling approach to estimate the internal exposure to CdO NPs in the placenta and the fetus can also be applied to other NPs (This work was supported by NIEHS Award #U19ES019525)

Hexavalent chromium (Cr(VI)) is an environmental and occupational contaminant, and is present in both soil and drinking water in the United States. In 2-year drinking water bioassays, the National Toxicology Program observed effects including carcinogenicity of the gastrointestinal (GI) tract in mice and oral cavity in rats. Physiologically-based pharmacokinetic (PBPK) models have been developed to estimate interspecies differences in toxicity and assess human health risk from oral exposure to Cr(VI). However, there are significant uncertainties and inter-individual variabilities to consider when modeling chromium in the gastrointestinal tract. Hexavalent chromium is rapidly reduced to trivalent chromium (Cr(III)) in the GI lumen, and only total chromium can be analytically measured in vivo. The reduction and absorption of hexavalent chromium will vary with intestinal pH, dietary intake, and gastric contents and physiology. These factors vary over time and between individuals. In addition, multiple PBPK models have been developed for hexavalent chromium, and a variety of different internal dose-metrics may be applied to link external exposure and toxic effects. Toxicity may be correlated to 1) concentration of Cr(VI) in the GI tract lumen; 2) absorption of Cr(VI) into specific GI tract tissue sites; 3) total absorption of Cr(VI) in the full GI tract. This work quantifies the impact of different modeling assumptions on the interpretation of toxicological data in rodents, and extrapolation to humans. It was found that the choice of dose-metric has a significant impact on the evaluation of potential human health effects from oral exposure to hexavalent chromium. The views expressed are those of the authors, and do not necessarily represent the views or policies of the U.S. EPA.

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Pharmacokinetic [IEUBK] model for Pb in children, and the Adult Lead Methodology (ALM)). In some cases, inputs into these models must necessarily approximate actual exposures, adding to uncertainty in model results and strategies to meet remediation goals. To improve estimates of Pb internal dosimetry following a wide range of exposure conditions and populations, EPA developed (and is testing) a PBPK model called the All Ages Lead Model (AALM). The AALM predicts blood and tissue Pb concentrations following Pb intake from contaminated air, drinking water, surface dust, food, or miscellaneous ingestion pathways. The AALM exposure module (an Excel™ interface) allows users to simulate multi-pathway exposures from 24 time constant or that vary in time increments as small as one day, and that occur at any age from birth to 90 years. Values for gastrointestinal absorption fractions for any age as well as values for relative bioavailability of Pb from all ingestion pathways are set by the user. The AALM model is implemented in acs[IEUBK] using either a Leggett et al. (AALM-LG) or O’Flaherty et al. (AALM-OF) based biokinetic submodel. The deposition and absorption parameter values (for either submodel) are those used in ICRP’s Human Respiratory Tract Model. Two case studies for short-term exposures (exposure 30 days) demonstrated the expanded capability of the AALM model: 1) exposures to construction workers, and 2) exposures to children following a home renovation. Additional AALM capabilities are listed, and next steps in the AALM model development and testing are discussed. [The views expressed are those of the authors, and do not necessarily reflect the views or policies of the U.S. EPA. Mention of any products does not constitute endorsement or recommendation for use.]


Hexavalent chromium is rapidly reduced to trivalent chromium (Cr(III)) in the GI lumen, and only total chromium can be analytically measured in vivo. The reduction and absorption of hexavalent chromium will vary with intestinal pH, dietary intake, and gastric contents and physiology. These factors vary over time and between individuals. In addition, multiple PBPK models have been developed for hexavalent chromium, and a variety of different internal dose-metrics may be applied to link external exposure and toxic effects. Toxicity may be correlated to 1) concentration of Cr(VI) in the GI tract lumen; 2) absorption of Cr(VI) into specific GI tract tissue sites; 3) total absorption of Cr(VI) in the full GI tract. This work quantifies the impact of different modeling assumptions on the interpretation of toxicological data in rodents, and extrapolation to humans. It was found that the choice of dose-metric has a significant impact on the evaluation of potential human health effects from oral exposure to hexavalent chromium. The views expressed are those of the authors, and do not necessarily represent the views or policies of the U.S. EPA.

Manganese to Include Rates of Cellular Uptake and Efflux

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Previously, we developed a series of physiologically based pharmacokinetic (PBPK) models for manganese (Mn) using the importance of Mn homeostasis and that this can be accounted for by a gender-specific uptake parameter.

Improved Toxicokinetic Model for Cadmium Using Urine, Blood, and Kidney Cortex Concentrations from Living Kidney Donors

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Several toxicokinetic models have been developed to describe the relation between external exposure, tissue levels and urinary excretion of cadmium. The aim of the present work was to analyse and validate one of the most common models (K&N, Kjellström & Nordberg, Environ Res, 1978) by using new data on cadmium concentrations in kidney cortex, blood and urine (Barregard et al., Environ Res, 2010). Measured data from 30 living kidney donors (14 males, 16 females) were selected from a total of 109 based on completeness of information on cadmium levels in urine, whole blood, plasma and kidney cortex, and donor status as never smokers. Markov Chain Monte Carlo simulations and flat priors were used to estimate posterior parameter distributions for the three most sensitive of the independent parameters in the model, including the systemic uptake (not to be confused with oral intake). Two additional parameters accounting for the covariate effects of gender and serum ferritin on uptake were also tested. For the gender effect, the uptake was 48% (posterior median) higher in women. For gender and serum ferritin combined, the uptake was 44% (posterior median) higher in women and 20% higher in individuals who had a serum ferritin level <30 µg/L. However, the uncertainty in the effect of serum ferritin included the null value, indicating that the parameter may not be significant. In conclusion, the K&N model described the new data well; however these new data suggest that the uptake is higher in females than in males and that this can be accounted for by a gender-specific uptake parameter.

Clinical and Pharmacological Studies for the Evaluation of Potential Human Health Effects of Cadmium Exposure in Pregnant Mice

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Fetal and neonatal periods may represent vulnerable life stages for exposure to nanoparticles (NP). In this study, we used a physiologically based pharmacokinetic (PBPK) model to simulate Cd disposition in pregnant mice from two different inhalation exposures to CdO NPs: every other day inhalation at 100 µg CdO/m3 and daily inhalation at 230 µg/m3 between gestation day (GD) 4.5 and 16.5. Although there was no difference in Cd levels in the maternal placenta or uterus between the two exposures, developmental effects were observed only with daily inhalation of 230 µg CdO NPs/m3. The respiratory tract deposition of inhaled CdO NPs was simulated using the Multiple-Path Particle Dosimetry model and was linked to the PBPK model. The saturable accumulation of CdO in the placenta was based on the reported involvement of active transporters such as DMT-1 and ZIP14 for uptake of Cd and subsequent induction of metallothionein (MT) that retains Cd in the placenta. The simulated total Cd contents in the lung, blood, liver, placenta, uterus, and kidney in the dam on GD 17.5 were in good agreement with the observed values. The model predicted a rapid build-up of Cd in the placenta and uterus with daily inhalation exposure to CdO NPs compared to a relatively slower increase resulting from every other day exposure. Although the Cd levels were similar on GD 17.5, the AUC values in the placenta and uterus from the daily exposure were 1.4 and 1.8 fold higher, respectively, compared to those from every other day exposure indicating that the observed effects on fetal and neonatal development and growth may be attributable to the difference in time profiles of Cd exposure in the placenta and uterus. The current modeling approach to estimate the internal exposure to CdO NPs in the placenta and the fetus can also be applied to other NPs (This work was supported by NIEHS Award #U19ES019525).
Cobalt (Co) supplements are available for sale, and concerns about metals released in patients with Co-containing hip implants have been raised. However, simultaneously collected whole blood, serum, urine, excretion and serum protein binding data have not been available to evaluate Co biokinetic models. Blood and urine data from ten healthy volunteers who ingested ~1.0 mg Co/day of a Co supplement for three months were used to refine previously published Co-biokinetic models. The model was modified to account for three key experimental observations: 1) an increase in serum protein bound Co from 9% during dosing to 99% after dosing; 2) a linear decrease in Co renal cell concentration post-dosing period, and 3) Co renal clearance consistent with estimated glomerular filtration rates. New model compartments included: 1) albumin bound Co in intravascular fluid (serum), 2) albumin bound Co in extravascular fluid exchanging with intravascular albumin, and 3) a series of compartments representing red blood cell ages between 1 and 120 days. Renal excretion was estimated by glomerular filtration rates and free Co2+ concentration. Agreement between the modeled and measured urine, serum, and whole blood concentrations were observed (r=0.8) with oral absorption rates between 10% and 65%, a RBC Co transfer coefficient equal to 10% of the rate suggested in vitro studies, and albumin binding rate constants adjusted to reflect the measured fraction of large molecular serum protein bound Co before and after dosing. Distribution of Co to systemic tissues over three months of dosing was approximately 1/10th of the amount suggested by historical single-dose radiological tracer studies, and the increase in albumin-bound Co after dosing is consistent with exchange of albumin-bound Co from extravascular fluid to intravascular fluid after source elimination. Our revised model provides useful insights on human Co kinetics, and should be helpful in the evaluation of elevated blood Co concentrations in MoM hip implant patients.

Predicting human pharmacokinetics for acid liver transporter substrates is needed due to the increasing frequency of novel acid substrates in drug discovery and development. Initially, a previously developed prediction method using sandwich cultured human hepatocytes (SCHH) and human liver microsomes (HLM) was applied to telmisartan. The human data show experimental and pharmacogenomic variability; the predictions were moderately successful using empirically determined in vitro to in vivo extrapolation scaling factors. Several improvements for the modeling were considered, Partition coefficients (Kp) were estimated from positron emission tomography (PET) data for tissues other than liver considered to have limited transporter activity. These Kp values were largely within the 3-fold error associated with Kp prediction methods, but improved the plasma profile predictions. Telmisartan is largely metabolized to a glucuronide conjugate, so a minimal model for the glucuronide was incorporated to predict PET data for the liver. Parameters were estimated from HLM, with cofactor added, and SCHH in which parent and glucuronide distribution in cells and media was measured. The longer times (>2.5 h) for the plasma telmisartan profiles were substantially underpredicted suggesting that enterohepatic recirculation played an important role. An empirical recirculation description was added incorporating meal induced gall bladder emptying, biliary efflux transport of the glucuronide, and instantaneous cleavage to parent in the gut. This provided reasonable simulation of the plasma profile. Overall, this modeling demonstrated that while prediction of liver transporter activity is necessary for transporter substrates, simulation of additional pharmacokinetic processes can be essential including better estimates of partition coefficients and enterohepatic recirculation.
Chlorpyrifos (CPF), an organophosphorus pesticide (OP), is metabolized by CYPs to the detoxified metabolite trichloro-2-pyridinol (TCPy), or to the active metabolite, chlorpyrifos-oxon (CPF-O), which is a potent esterase inhibitor. For CPF, urinary TCPy concentrations are a reliable biomarker of CPF exposure while RBC acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BuChE) are biomarkers of effect. A PBPK/PD model was developed to predict exposure to CPF, dermal absorption, distribution, metabolism, elimination and adverse health effects in Egyptian agricultural workers based on urinary TCPy, blood AChE and BuChE data collected in the field over a period of 33 consecutive days. Regression analysis of 24 hr urinary TCPy values was used to establish an array of 33 daily linear transfer coefficients of CPF to clothing and skin of 35 workers (14 backpack applicators, 9 technicians and 12 engineers). The array based model predicted skin exposure (dose) for the workers, ranging from 0.07 to 9.05 mg/kg/day, dermal absorption (1.88% of skin dose), daily wash-off (95.5% of dose), evaporation (2.14% of dose), and total overall recovery (99.6% of dermal dose). Model simulations estimate CPF and CPF-O blood levels as high as 124800 and 1275 pmol/L, respectively, with model Ki values for blood AChE and BuChE varying between 1.03 x 10−4 and 1.03 x 10−3 pmol-1hr-1 for applicators and between 1.03 x 10−4 and 6.0 x 10−3 for technicians and engineers. PBPK/PD modeling is generally consistent with human biomarker data, however, there is some variability between model estimates and observed ChE inhibition, suggesting that there may be genetic susceptibility components that can alter an individual's sensitivity to CPF. (US EPA STAR, grant R-83068301 and NIEHS grants ES016308, ES022163).

Chlorpyrifos (CPF) is an important pesticide used to control crop insects. Biological effects occur following metabolic activation to chlorpyrifos oxon, and these effects are monitored using RBC cholinesterase inhibition. Non-occupational exposures to CPF will occur primarily through residues on foods, with occupational exposure also from dermal contact or low-level inhalation exposures to the non-volatile CPF. A physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model has been developed that describes the relationship between oral, dermal, and inhalation doses of chlorpyrifos and key associated events in the pathway for cholinergic effects. Pharmacokinetic data is available for controlled human exposures via the oral and dermal routes and from multiple oral or inhalation studies in rats. The multi-route model was developed in these two species to validate all modeled scenarios in a parallel design. Since bioactivation is key to CPF effects, small amount of metabolism in tissues potentially have a great affect on PK and PD. Metabolism was therefore added in diaphragm, brain, lung, and skin, based on published data. For most tissues, metabolism that represented 2% of the measured hepatic metabolism resulted in fits to available cholinesterase inhibition and dosimetry. Due to the lipophilic nature of CPF most of the inhaled dose will either be delivered via superficial nasal deposition into the stomach or associated with the metabolically active pulmonary tissue, thus metabolism in the lung is comparable to liver. The resultant model fits to human and rat multi-route data, and shows that CPF Cmax will be higher through non-oral routes when cholinesterase inhibition in non-portal of entry tissues will be less following exposure via these non-oral routes. This resulting model will be highly useful in risk assessments for CPF from one or more routes of exposure.

A Physiologically-Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Model of Daily Chlorpyrifos Exposure in Egyptian Agricultural Workers

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A General PBPK Model for IV-Injected Nanoparticles

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In order to link NP exposure to toxicity, information about absorption, distribution, degradation and excretion (ADME) is essential. Experience from pharmaceuticals and industrial chemicals shows that the ADME can successfully be simulated, described and predicted by physiologically based pharmacokinetic (PBPK) modeling. The few PBPK models for NPs published to date have been developed and evaluated for a single type of NP. The aim of this study was to develop a general NP-PBPK model for intravenously injected NPs in rats. The model was based on a recently developed PBPK model for pegylated polyacrylamide (PAApeg) NPs (Dinghuang et al., submitted manuscript), which in turn was developed using detailed experimental data (time courses of NP mass in different tissues) after single intravenous administration (Wenger et al., Toxicol. Appl. Pharmacol. 2011). In addition to PAApeg, we retrieved intravenous rat data for nonpegylated PAA, gold and polyethylene maleate NPs (Lescure et al., Pharm. Res. 1994; Wang et al., Anal. Bioanal. Chem. 2010). NP-dependent parameters were adjusted by best fit to each data set, whereas NP-independent physiological and anatomical parameters were kept constant. The general model was able to adequately describe the ADME behavior of all four NP types. The fitted parameters that varied most were the clearance to urine and feces, the biodistribution permeability coefficients and the uptake rate and capacity of phagocytic cells. Additional data on NP properties such as corona formation and physiological parameters, such as number of phagocytic cells in different tissues and their capacity and turnover, are required to further improve the model. The study was supported by the Swedish Research Council for Health, Working Life and Welfare (Forte).

A PBPK Model of Hypobaric and Hyperbaric Toluene Exposure

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Modern high-performance jets achieve extreme altitudes and operate in hypobaric pressure environments. Conversely, divers operate at hyperbaric depths. Inhalation exposure to toluene in these extreme pressure environments may result in altered kinetics, and therefore, altered response and exposure safety guidelines. Such pressure-dependent differences in kinetics have been observed for some compounds, while absent for others. To test whether toluene kinetics are sensitive to pressure, we developed a physiologically-based pharmacokinetic (PBPK) model as a tool for examining potential physiological contributors to such differences. Our approach was to compare model predictions to hypobaric and hyperbaric toluene kinetics data in Sprague-Dawley (SD) rats, and then adjust model parameters and/or processes to fit the data, thereby identifying potential effectors of observed differences. The model was based on a typical Ramsey-Andersen model of isopropanol, with tissue compartments for the brain, liver, fat, skin, and lumped rapidly perfused and slowly perfused tissues. Toluene parameters were obtained from literature sources. The model was verified for normal pressure through comparison with previously published data and models in the literature, and produced good agreement with the data. For hyper/hypobaric simulations, the model was first adjusted with explicit implementation of the ideal gas law for conversion of ppm to mass at different pressures. Male and female SD rats were exposed to toluene via inhalation, and the data were compared with model predictions. Initial model predictions underestimated the experimental data. However, the model predicted decreased toluene in tissues with decreasing pressure, which was consistent with the experimental data. Predictions of published hyperbaric exposures slightly overestimated those data by about 30%. Future work will focus on comparison of predictions with additional hypobaric and hyperbaric, and examination of other potential physiological contributors to pressure-dependent kinetic differences.

Understanding the Differences in the Toxicity of Tolcapone and Entacapone: A Comparative Study Using the Virtual Liver Platform—HeptoX


The effect of two catechol-O-methyltransferase (COMT) inhibitors, entacapone and tolcapone are compared using our toxicity prediction platform, a combination of an in silico model of liver metabolism with a set of in vitro biochemical measure-ments. Although both tolcapone and entacapone show similar on-target inhibitory potencies (Ki for COMT~10 nM), their off-target effects in the liver and their pharmacokinetic and pharmacodynamic properties are different. We measured the dose- and time-dependent impact of both drugs on key liver metabolic pathways using a HepG2 cell system. HepG2 cells were treated for different time periods up to 72 hours, and we estimated both the direct action of the drug on enzyme activity as well as compensatory mechanisms that are invoked with time in response to the drug treatment. This information is used to simulate a virtual clinical trial for both the drugs using their respective pharmacokinetic data as input. Tolcapone with a three fold higher Cmax than entacapone as well as higher plasma half-life (2.9 vs 0.9 hrs) has a far greater impact on the off-targets leading to more toxicity compared to entacapone. In this study we show a relationship between drug-exposure and the toxic impact of these two drugs on different off-targets, and derive an explanation as to why tolcapone is more hepatotoxic than entacapone. Our simulations indicate the onset of tolcapone induced toxicity starts in after 6 weeks of treatment and predicts that over a period of 3 months, tolcapone induces mitochondrial damage, inhibits transport of ATP across the mitochondrial membrane and leads to a lowering of cytosolic ATP and eventual necrotic damage.

The Association between Prenatal Exposure to Perfluoroalkyl Substances (PFAS) and Reduced Birth Weight: Is Glomerular Filtration Rate the Underlying Cause?

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Prenatal exposure to PFAS has been associated with lower birth weight in epidemiologic studies. Because glomerular filtration rate (GFR) during pregnancy is associated with PFAS excretion and birth weight, the observed association between PFAS levels and reduced birth weight could be driven by GFR. We used an existing physiologically-based pharmacokinetic (PBPK) model of human pregnancy to simulate PFAS in maternal and cord blood and to subsequently assess how much of the PFAS-birth weight association observed in epidemiologic studies might be attributable to GFR. We modified the model to reflect the association of birth weight with GFR. Data from 100,000 Monte Carlo simulations was analyzed using linear regression to evaluate the association between simulated PFAS levels and birth weight. Based on the simulations, we estimated reductions of 2.61 g and 6.23 g in birth weight for each 1 ng/ml increase in cord PFOS and PFOA, respectively. Each 1 ng/ml increase in simulated maternal blood PFOS and PFOA levels at delivery was associated with reductions of 1.84 g and 6.99 g in birth weight. In comparison, a meta-analysis of published epidemiologic studies suggested that each 1 ng/ml increase in prenatal PFOS and PFOA levels is associated with 5.00 g and 14.72 g reductions in birth weight. Our results revealed that a substantial portion of the reported association between prenatal PFAS and birth weight might be attributable to GFR. This study shows that PBPK models can be used to assess how much of an epidemiologic association can be explained on the basis of pharmacokinetics.

PBPK Modeling of Human PFOA Exposure Predicts Measured Serum Concentrations from Consumption of Contaminated Drinking Water

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Human exposure to perfluorooctanoic acid (PFOA) is common and widespread - PFOA is regularly found in human blood samples as reported by the National Health and Nutrition Examination Survey (NHANES). A physiologically-based pharmacokinetic (PBPK) model was developed that predicts the behavior of PFOA in humans and computational research is underway to evaluate human biomonitoring data collected in a community with known exposures to PFOA as a result of contaminated drinking water. Exposure scenarios that may approximate those occurring in the community are simulated and the resulting PFOA-serum concentrations are compared to those measured in the field study. The water supply for this community was known to be contaminated with PFOA for at least 12 years and the contaminants were simulated and the resulting PFOA-serum concentrations are compared to those measured in the field study. The water supply for this community was known to be contaminated with PFOA for at least 12 years and the contaminants were simulated in humans and computational research is underway to evaluate human biomonitoring data. The few PBPK models for NPs published to date have been developed and evaluated for a single type of NP. The aim of this study was to develop a general NP-PBPK model for intravenously injected NPs in rats. The model was based on a recently developed PBPK model for pegylated polyacrylamide (PAApeg) NPs (Dinghuang et al., submitted manuscript), which in turn was developed using detailed experimental data (time courses of NP mass in different tissues) after single intravenous administration (Wenger et al., Toxicol. Appl. Pharmacol. 2011). In addition to PAApeg, we retrieved intravenous rat data for nonpegylated PAA, gold and polyethylene maleate NPs (Lescure et al., Pharm. Res. 1994; Wang et al., Anal. Bioanal. Chem. 2010). NP-dependent parameters were adjusted by best fit to each data set, whereas NP-independent physiological and anatomical parameters were kept constant. The general model was able to adequately describe the ADME behavior of all four NP types. Modern high-performance jets achieve extreme altitudes and operate in hypobaric pressure environments. Conversely, divers operate at hyperbaric depths. Inhalation exposure to toluene in these extreme pressure environments may result in altered kinetics, and therefore, altered response and exposure safety guidelines. Such pressure-dependent differences in kinetics have been observed for some compounds, while absent for others. To test whether toluene kinetics are sensitive to pressure, we developed a physiologically-based pharmacokinetic (PBPK) model as a tool for examining potential physiological contributors to such differences. Our approach was to compare model predictions to hypobaric and hyperbaric toluene kinetics data in Sprague-Dawley (SD) rats, and then adjust model parameters and/or processes to fit the data, thereby identifying potential effectors of observed differences. The model was based on a typical Ramsey-Andersen model of isopropanol, with tissue compartments for the brain, liver, fat, skin, and lumped rapidly perfused and slowly perfused tissues. Toluene parameters were obtained from literature sources. The model was verified for normal pressure through comparison with previously published data and models in the literature, and produced good agreement with the data. For hyper/hypobaric simulations, the model was first adjusted with explicit implementation of the ideal gas law for conversion of ppm to mass at different pressures. Male and female SD rats were exposed to toluene via inhalation, and the data were compared with model predictions. Initial model predictions underestimated the experimental data. However, the model predicted decreased toluene in tissues with decreasing pressure, which was consistent with the experimental data. Predictions of published hyperbaric exposures slightly overestimated those data by about 30%. Future work will focus on comparison of predictions with additional hypobaric and hyperbaric, and examination of other potential physiological contributors to pressure-dependent kinetic differences.
either 0.4 ppb (EPA’s Provisional Health Advisory Level) or 2.2 ppb (a concentration measured in a private well in the community and considered to be at the high end of plausible drinking water concentrations from private wells). A simple description of PFOA elimination kinetics using a urinary clearance coefficient of 4.0 x 10^-5 L/min/ml was used to simulate serum PFOA concentrations for the predicted dose-response relationship applied to in vivo measurements and then fitted with available rodent pharmacokinetic data. Blood and tissues estimations were consistent for all congeners using the same model structure. Each congener had small changes for a few model parameters (exchange rates between blood lipoproteins, liver tissues and fatty tissues). The model simulations suggest a more rapid uptake in blood lipoproteins compared to the reported PBDE kinetics due to growth dilution and an additional excetration route associated with menstruation may underlie the reported relationship between serum PBDE levels and age at menarche. The model structure was extended using adipose tissues divided into blood lipoproteins, fatty tissues, and depot tissues to 94 A Multiscale Mechanistic Model of TCDD-Induced Toxicity for Assessing Expected Dose-Responses for Adverse Outcome Pathways in Liver

S. Bhattacharya, P. D. McMullen, S. Pendse and M. E. Andersen, Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.

Ongoing efforts to describe and apply adverse outcome pathways (AOPs) for risk assessment require linkage of early molecular interactions to phenotypic responses. New advances in multiscale “virtual tissue” models provide a quantitative framework to link together: (a) tissue disposition of a chemical; (b) molecular initiating events such as receptor binding; (c) explicit models of intraacellular and organism level AOPs; and (iv) phenotypic endpoints like cell proliferation. Here we describe our development of a virtual tissue model of the rodent liver lobule for activation of the aryl hydrocarbon receptor (AhR) toxicity pathway in hepatocytes exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This case study shows the process of data integration and multiscale model development for mechanistic dose-response prediction and application of the AOP framework to risk assessment. First, a modified cluster aggregation algorithm provided a two-dimensional computational representation of the rodent liver lobule. This multiscale lobular model was linked to the ComputCell3D modeling environment that incorporated a network representation of intra-hepatocyte activation of the AhR signaling pathway. A PBPK description of TCDD uptake in the liver through hepatic sinusoids estimated differential cellular dosimetry as an input to the spatial model. Zonal heterogeneity in TCDD-induced cytochrome induction arises from for descriptions of spatial gradients in basal AhR expression across the lobule. Simultaneously, a combination of published gene expression and chromatin immunoprecipitation data served to refine a detailed transcriptional network of the AhR pathway with differential sensitivity ascribed for different liver regions. Overall, the multiscale model accurately simulated the observed dose-responses for various endpoints. Virtual tissue models promise more quantitative linkage to AOPs than possible through simple narrative descriptions of these processes.

92 Evaluation of the Observed Association between the Early-Age Exposure to Polybrominated Diphenyl Ethers (PBDEs) and Altered Age at Menarche on the Basis of Kinetics

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Epidemiological associations between blood PBDEs and earlier menarche in the general population have raised concerns for possible reproductive health effects. However, rapidly changing physiology during puberty that can affect the kinetics of a chemical may underlie the observed associations. To assess the role of pharmacokinetic variability during puberty on the reported PBDE epidemiologic study results, we used a Monte Carlo (MC) Physiologically-based Pharmacokinetic (PBPK) model. A PBPK model was developed based on single pharmacokinetic structure that can predict tissue and blood concentrations for BDE 47, 99, 100, and 153 in rodents. The model structure was extended using adipose tissues divided into blood lipoproteins, fatty tissues, and depot tissues to 94 A Multiscale Mechanistic Model of TCDD-Induced Toxicity for Assessing Expected Dose-Responses for Adverse Outcome Pathways in Liver

S. Bhattacharya, P. D. McMullen, S. Pendse and M. E. Andersen, Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.

Ongoing efforts to describe and apply adverse outcome pathways (AOPs) for risk assessment require linkage of early molecular interactions to phenotypic responses. New advances in multiscale “virtual tissue” models provide a quantitative framework to link together: (a) tissue disposition of a chemical; (b) molecular initiating events such as receptor binding; (c) explicit models of intraacellular and organism level AOPs; and (iv) phenotypic endpoints like cell proliferation. Here we describe our development of a virtual tissue model of the rodent liver lobule for activation of the aryl hydrocarbon receptor (AhR) toxicity pathway in hepatocytes exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This case study shows the process of data integration and multiscale model development for mechanistic dose-response prediction and application of the AOP framework to risk assessment. First, a modified cluster aggregation algorithm provided a two-dimensional computational representation of the rodent liver lobule. This multiscale lobular model was linked to the ComputCell3D modeling environment that incorporated a network representation of intra-hepatocyte activation of the AhR signaling pathway. A PBPK description of TCDD uptake in the liver through hepatic sinusoids estimated differential cellular dosimetry as an input to the spatial model. Zonal heterogeneity in TCDD-induced cytochrome induction arises from for descriptions of spatial gradients in basal AhR expression across the lobule. Simultaneously, a combination of published gene expression and chromatin immunoprecipitation data served to refine a detailed transcriptional network of the AhR pathway with differential sensitivity ascribed for different liver regions. Overall, the multiscale model accurately simulated the observed dose-responses for various endpoints. Virtual tissue models promise more quantitative linkage to AOPs than possible through simple narrative descriptions of these processes.
Dosimetric Analysis of VITROCELL VC10® Smoke Exposure System with Photometer and Quartz Crystal Microbalance


Air liquid interface whole smoke exposure technologies such as the VITROCELL VC10® smoke exposure system (VC10) are applied to assess the effect of cigarette smoke on biological endpoints (e.g. cytotoxicity, mutagenicity and DNA modifications). Presently, dose response evaluations have been primarily determined on an airflow dilution basis following smoke generation under ISO or intense smoking regimes. Correlation of the biological response to dose is important for comparative analyses of products and data from other smoke exposure systems and may be facilitated by dosimetry tools such as photometers, quartz crystal microbalances (QCMs) and carbon monoxide monitors. The goals of this study were to 1) assess the consistency of particle detection via deposition or mass with QCMs and photometers, respectively, across all dilution bars and at airflows spanning the operating range of the VC10, 2) assess consistency of particle detection in multiple exposure modules and 3) correlate photometer readings at set airflows to deposited mass such that estimates of mass can be derived from the VC10 Photometer Monitor Software.

Consistency in particle deposition and mass was generally observed across all dilution bars and between modules at a range of airflows that spanned the operating range of the VC10. Consistency was also observed across all chamber positions within modules from 12 to 1L/min while some variability was observed at 0.5L/min. Linear correlations between particulate deposition and measured particulate mass were observed, and statistical and power curve analyses confirmed the accuracy of the predicted QCM data generated with the VC10 Photometer Monitor Software. Collectively, we report the capacity to assess deposited mass with reproducibility with QCMs and to accurately estimate mass based on area under the curve data from photometer readings. These data will be helpful in correlating biological responses to dose related exposures with the VC10.

Predicting Tissue:Plasma Partition Coefficients: Interindividual and Interspecies Variability

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A tissue composition based mechanistic model for the prediction of steady state tissue: plasma partition coefficients (Kt:p) of organic chemicals in mammalian species was used to assess interindividual and interspecies variability. The approach predicts Kt:p using available chemical properties (lipophility, binding to phospholipid membranes, and pK±K±), together with biological properties of the tissue itself (fraction of water, neutral lipids, neutral and acidic phospholipids, proteins and pH). Biological properties were identified from the biomedical literature for rat, pig, human, guinea pig, monkey, mouse, beagle dog and rabbit species. Property means and coefficients of variation were used in a Monte Carlo simulation to assess variability within and between species. The results show that interindividual and interspecies variability becomes more pronounced as LogPO:W increases and the difference is insignificant for compounds with LogPO:W<2.0. Interspecies variation is shown to be driven by differences in the acidic phospholipid composition. The Monte Carlo composition based mechanistic model can be used to predict Kt:p for organic chemicals across 8 different species and can be an important component in population physiologically based pharmacokinetic (PBPK) modeling, safety evaluation, chemical risk assessment and screening.

Integrating Biological Variability with QSARs in PBPK Models to Simulate Distributions of Internal Dose in Humans

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Existing mechanistic algorithms use point estimates for blood and tissue composition to predict the blood-air and tissue-air partition coefficients (PCs) required for PBPK modeling. The objectives of this study were: i) to develop a framework to predict the probability of distributions of PCs and ii) to incorporate them, along with published information on the variability of cytochrome P450 CYP2E1 content and physiological parameters, within a human PBPK model to simulate the variability of toxicokinetics of inhaled VOCs. Previously published human and rat data on matrixicr PCs of toluene and acetone (in blood, fat, liver, and muscle tissueair PCs), along with data on oilair and waterair PCs were used in a Markov chain Monte Carlo (MCMC) approach to characterize the distributions of human blood and fat tissue composition parameters in the mechanistic algorithm for PCs (i.e., neutral lipid, water and protein contents). The posterior distributions resulting from the MCMC analysis were used along with physicochemical properties predicted with QSARs to compute distributions of chemical-specific tissueblood and tissuefat PCs. Monte Carlo simulations were then carried out with the PBPK model to predict the blood kinetics of VOCs (benzene (25 ppm, 2 h), styrene (80 ppm, 6 h) and trichloroethylene (100 ppm, 4 h)) following inhalation exposures. For these VOCs, the predictions of the PBPK model compared well with the available human volunteer data. Overall, this study demonstrated the feasibility of using PBPK models to simulate the combined impact of the variability of biological and chemical properties on the internal dose of chemicals. (Supported by Natural Sciences and Engineering Research Council of Canada)

PPB Models for Gasoline-Ethanol Biofuels in Adult and Pregnant Rats


As utilization of biofuels (BF) in the commercial marketplace has increased in recent years, so has the need for evaluation of exposure-related health effects, such as developmental neurotoxicity. This research describes the development of inhalation life-stage physiologically-based pharmacokinetic (PBPK) models for vapors of gasoline (E0) and two BF blends, E15 (15% EtOH) and E85 (85% EtOH). Time course blood hydrocarbon concentration (BHC) data were collected from non-pregnant female rats exposed to E0, E15, and E85, at total hydrocarbon concentrations (THC) of 3K, 6K, and 9Kppm (60h exposure, 4-6 time points per dose). Peak (end-of-exposure) BHC data were also collected from pregnant dams exposed to 9Kppm (6-36h, GD9, 16, 20) of E0 or E15. These datasets were used to evaluate and refine PBPK models for each mixture and to compare estimates of BHCs across BF types at comparable THC. PBPK models were constructed using a series of sub-models for prominent aromatic and aliphatic HCs representing ~45-66% of total vapor (depending on BF), while the remaining fractions were lumped. The overall modeling framework allowed for flexibility across blend compositions. The sub-models were parameterized with literature and structure-activity-based estimates; estimated physicochemical and metabolic parameters were used for the lumps. The models adequately simulated BHCs for most aromatic and aliphatic HCs across the various exposure scenarios. Specifically, HCs with robust calibration literature were usually well simulated, while clearance rates for the other aliphatics often did not adequately match model BHC predictions. Simulations for aromatics at high vapor concentrations were improved by including metabolic interactions; HCs at low concentrations were often better simulated without these interactions. The final models may be relevant for simulations of other blend ratios. Abstract does not reflect EPA policy.

Evaluation of Semi-Generic PBTK Modelling for Emergency Risk Assessment after Acute Inhalation Exposure to Volatile Hazardous Chemicals

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Rationale: Physiologically Based Toxicokinetic Models (PBTK) may facilitate emergency risk assessment after chemical incidents, but are rarely used due to their relative complexity and skill requirements. We aimed to evaluating a semi-generic PBTK model built in MS Excel for nine chemicals that are widely-used and often released in a chemical incident.

Methods: The semi-generic PBTK model was used to predict blood concentration-time curves using exposure scenarios from human volunteer studies (low exposure doses), case reports (medium to high exposure doses) and hypothetical exposures at EPA Group-3 Levels (high exposure doses). Semi-generic means the model uses built-in QSARs to set some parameter values. Predictions were compared to measured blood concentrations from volunteer studies or case reports as well as to...
blood concentrations predicted by chemical-specific models. The performances of the semi-generic model were evaluated on biological rationale, accuracy and shape of predicted concentration-time profiles, ease of use and range of application. Results: Our results indicate that the semi-generic PBTK model can be readily used to predict blood levels for 6 out of 9 parent chemicals (benzene, xylene, toluene, styrene, trichloroethylene and tetrachloroethylene), with the exception of methanol, 2-propanol and dichloromethane. The semi-generic model could not cope with the endogenous production of methanol and acetone (metabolite of 2-propanol). The model could not simulate the formation of HbCO, which is one of the toxic end-points of dichloromethane. Conclusion: For some hazardous chemicals, a semi-generic PBTK modelling approach can be just as accurate as a more chemical-specific PBTK modelling approach.

**96d** Interpreting NHANES Biomonitoring Data: Dioxins

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Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), mono-ortho and non-ortho polychlorinated biphenyls (dioxin-like PCBs) are identified as a family of organic compounds known as “dioxins”. Dioxins are persistent endocrine-disrupting chemicals that bioaccumulate following environmental exposure or dietary intake. Additional adverse health effects of dioxin exposure in humans include the development of serious diseases such as diabetes, cancer, deleterious effects such as an altered immunological response and changes in the expression of receptors and metabolic enzymes. As part of translational research to make computerized models accessible to health risk assessors we recorded the Concentration-and-Age-Dependent Model (CADM) for pharmacokinetics of dioxins in Berkeley Madonna simulation language. The recorded model was evaluated by comparing simulation results to published model simulations and to human data sets. Overall, there was good agreement between the estimated and measured values. Our recorded model allows the estimation of dioxin concentrations in breastfed infants. Also presented is an application of the recorded model to interpret biomonitoring data from the National Health and Nutritional Examination Survey (NHANES). The model simulated the NHANES-measured data very well from ages 6 to 60+ years. The model describes TCDD cumulative nature in humans and accommodates the observed variation in exposure/uptake over the course of a lifetime. Hence, this model may be useful as a screening tool that can provide information on interpreting biomonitoring data and risk assessment.

**96e** Physiologically-Based Pharmacokinetic (PBPK) Models Application to Screen Environmental Hazards Related to Adverse Outcome Pathways (AOPs)


PBPK models are useful in estimating exposure levels based on in vitro to in vivo extrapolation (IVIVE) calculations. Linkage of large sets of chemically screened vitro signature effects to in vivo adverse outcomes using IVIVE is central to the concepts of the National Academy of Sciences’ Toxicology in 21st century vision. Except for metabolic clearance, most parameters used to develop PBPK models are readily available in literature, or calculated using chemical structure activity relationships. Lack of computational methods to determine clearance inhibits the application of PBPK models to large sets of chemicals without conducting costly and time-consuming experiments. This problem, however, should not exclude the use of PBPK models to screen a large set of chemicals for possible environmental exposure levels that can lead to adverse outcome effects. This can be accomplished by developing “generic” PBPK models. Metabolic clearance capacity in the generic model is set to zero to estimate maximal exposure level (EXPmax) that will yield parent chemical tissue levels equivalent to in vitro levels identified for the signature effects along an identified adverse outcome pathway (AOP). EXPmax levels are then screened against environmental exposure levels (EXPenv). If EXPenv is less than EXPmax, then parent target tissue levels of the chemical under the environmental exposure situations will be less than toxicologically-effective in vitro levels (EC50s or LELs), indicating that the assumption of zero clearance is health hazard conservative. For chemicals where EXPenv is larger than EXPmax, metabolic clearance of these chemical may be a critical factor in determining their health risks. This abstract does not necessarily reflect EPA policy.

**96f** Evaluation of the Pharmacokinetics of Methylphenidate in Juvenile and Adult Humans and Nonhuman Primates Using a Physiological Model

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Methylphenidate (MPH) is a psychostimulant drug marketed for treatment of attention deficit hyperactivity disorder in children and adults. A physiologically-based pharmacokinetic (PBPK) model was developed to characterize the age- and species-dependent pharmacokinetics of MPH in juvenile and adult humans and rhesus monkeys. The model was first calibrated in adult humans for MPH enantiomers and then extrapolated to children and monkeys. The calibrated MPH PBPK model was then utilized to extrapolate to children the pharmacokinetic MPH serum profiles associated with toxicity observed in juvenile monkeys. MPH is extensively and rapidly metabolized in the liver and gastrointestinal tract, primarily by carboxylesterase 1, with minor contributions from oxidative enzymes. Juvenile monkeys and children appear to metabolize MPH more rapidly than adults, with more rapid metabolism in juvenile monkeys than in children. Juvenile monkeys chronically exposed to oral MPH doses of 2.5 and 12.5 mg/kg (twice per day) showed a temporary delay in puberty (Mattison et al. 2011). Pharmacokinetically equivalent MPH doses for a 15-year-old boy were estimated to be 0.3 and 0.1 mg/kg using Cmax and AUC dose metrics from juvenile monkeys given 2.5 mg/kg of MPH. Therapeutic MPH doses in children ranging from 0.3-0.8 mg/kg, given twice a day, produce MPH kinetic profiles similar to those in juvenile monkeys that experienced delays in puberty. This computational analysis suggests that continued pharmacovigilance is prudent.

**96g** Intra- and Interspecies Extrapolation of Dose during the Developmental Period Using PBPK Models

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PBPK models are increasingly used to extrapolate the pharmacokinetics of a chemical or drug within a species from adult to young (intraspecies extrapolation) and between species, young animals to children (interspecies extrapolation). We developed adult and neonatal monkey and adult human and newborn PBPK models for bisphenol A (BPA) and for methylphenidate (MPH, Ritalin), adult and juvenile monkey and adult human and children PBPK models. Both the BPA and the MPH models relied on intra- and interspecies extrapolation. PBPK models were constructed using pharmacokinetic studies in infant (PND 5 to 77) and adult monkeys and adult humans for BPA and for MPH, juvenile (4-5 years) and adult monkeys and children (boys, 6 to 15 years old) and adult humans. These PBPK models were constructed using literature on the physiology of young and adult monkeys and humans and body weight scaling of chemical- or drug-specific model parameters (intra- and interspecies extrapolation). The acceptance or recalibration of the scaled parameters was determined by comparing predicted serum time courses data with observed data. Scaling of hepatic metabolism (iv dosing of adult monkey and infant monkey) and systemic (urinary) clearance of BPA metabolites (oral dosing of adult monkey and adult human) was not adequate to describe the kinetics of BPA and its metabolites, thus these model parameters were recalibrated. Lack of data precluded the evaluation of the human infant. For MPH, scaling of hepatic metabolism (hydrolysis) was successful for the oral administration of MPH in the child, adult human and adult monkey, but not in the juvenile monkey. Systematic clearance of diarylpropionic acid, the metabolic fate of MPH could be described by scaling for the adult and juvenile monkey and the adult human and child. The successful use of adult scaled parameters in PBPK models to predict pharmacokinetics during the developmental period is a useful method to evaluate age dependencies for both intra- and interspecies extrapolation.

**96h** Assessing Biomonitoring of Bisphenol A in Infants and Pregnant Women Using a Lifestaging Physiologically-Based Pharmacokinetic Model

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Growing concerns about exposure to various contaminants in the environment has led to major efforts at gathering a database of chemicals measured in blood and/or urine. These biomonitoring surveys provide a wealth of information on the levels of chemicals or metabolites detected in the general population and only give an
indication on the possible dose exposure leading to these observed internal levels. A study led by Health Canada scientists, the Plastics and Personal-care Product use in Pregnancy (P4) is a great example of where urine levels of chemicals are being measured in pregnant woman-infant pairs. The objective of this work is to explore biomonitoring and exposure data of Bisphenol-A (BPA) in pregnant women and levels found in their infants using biological models. At various life stages from infancy to adulthood, physiologically-based pharmacokinetic (PBPK) models can be used to describe age-dependent changing biological processes that can affect the fate of a chemical in the body. A lifestyle PBPK model was developed to estimate the changing levels of BPA internally in women and infants. Datasets collected from human and animal studies were used to define these biological processes in the models. Parameters for human pregnancy kinetics, such as mother placental transfer to the foetus, cord blood distribution or neonatal metabolism of BPA, were extrapolated from published pregnancy studies in monkeys. Model simulations of adult women urine levels were compared against data from the P4 study to evaluate study species differences of the biological processes. The PBPK model was also simulated and examined against meconium levels from the P4 study during foetal stages. Model simulations of urine levels were generally consistent, within a two-fold difference, with the pregnancy data. As a tool to support risk assessments of chemicals, the current PBPK lifestyle model will help in the evaluation of exposure in pregnant women as measured in BPA biomonitoring which can affect body burdens in their infants.

Two pancreatic injury models were tested in male Wistar Han rats. (1) Caerulein: animals were dosed by IP injection on day 1 at 0, 15 or 50 μg/kg, x3, one hour apart; (2) CHB: animals were dosed by SQ injection on day 1 at 0, 50 or 150 mg/kg. Serum samples were collected at 1, 6, 24, and 48 hr post last dose. Fasted animal weights were recorded up to 72 hr. Pancreatic injury was observed in rats treated with Caerulein at 15 or 50 μg/kg and CHB at 150 mg/kg. Both mir-216a and mir-217 correlated with acinar cell injury in each rat model however mir-217 was more sensitive with a larger magnitude of response. While lipase and microRNAs correlated well in the Caerulein treated animals, microRNAs remained elevated longer than lipase. In conclusion, our data show that in rats, mir-216a & mir-217 are sensitive indicators of acute exocrine pancreatic toxicity that are equal to, or better than, amylase and lipase. Further validation of these microRNAs as exocrine pancreatic injury biomarkers in rats and other nonclinical species with other pancreatic toxicants is needed.

MicroRNAs (miRNAs) are small, single stranded, non-coding RNAs that function as post-transcriptional regulators. Upon binding, target mRNA transcripts undergo degradation or translational repression. Dysregulation of miRNAs has been implicated in the pathogenesis of many disease states. Extracellular miRNAs have been detected in the blood and body fluids of animal models and patients. These, circulating miRNAs (cmiRNAs) have high potential utility in preclinical toxicology as biomarkers of organ toxicity because they are minimally-invasive, stable in biofluids, and likely translatable to humans due to their high cross-species conservation. We hypothesized that a rat model of pancreatic injury would have circulating miRNA biomarkers of injury. Sprague-Dawley rats were treated with intraepineuronal injection of 0.05 mg/kg caerulein once per hour for three consecutive hours to induce pancreatitis. Microscopic examination of pancreatic slices along with increased lipase and amylase levels confirmed pancreatic injury. 742 miRNA were assayed in plasma by quantitative PCR analysis. There were 17 miRNA which showed greater than a 3-fold difference between treated and control rats. 4 of these miRNAs were increased in rats treated with caerulein when compared with vehicle treated controls. In particular, mir-375-3p was increased 32-fold in treated rats. mir-375 is a relatively well studied miRNA reported to be expressed in pancreatic islets and brain. It is documented to play a role in pancreatic cell development and has been reported as a biomarker of beta cell death and diabetes in mice. The large increase in circulating mir-375-3p and clear link with pancreatic biology suggests that mir-375-3p is a potential candidate biomarker for pancreatic injury. Confirmation and characterization of sensitivity and specificity is underway with a larger study size and other treatment conditions.

Characterization of Urinary microRNAs As Biomarkers of Acetaminophen Toxicity in Children

Acetaminophen (APAP) is the most common drug used for the treatment of pain and fever in children. While generally considered to be safe, APAP is also responsible for 14% of acute liver failure cases in children. Recently, several studies have reported human circulating microRNAs (miRNAs) as novel biomarkers of APAP hepatotoxicity. Elevations of urinary miRNAs have been detected in rats administered toxic doses of APAP. This study examined urinary miRNA as a potential biomarker of APAP toxicity in children. Real-time quantitative polymerase chain reaction (qRT-PCR) arrays were used to detect global expression of miRNAs from three pediatric subgroups: i) healthy children (n=10, ALT median 18, range 10-37 IU/L), ii) hospitalized children receiving therapeutic doses of APAP (n=10, ALT median 26, range 6-177 IU/L) and iii) children hospitalised for APAP overdose (n=5, ALT median 2314, range 25-9909 IU/L). Urinary miR-375 was significantly increased in children with APAP overdose, compared to control subjects and therapeutic exposure subjects (p=0.05). The elevation of urinary miR-375 was further confirmed by qRT-PCR single reaction. Urine levels of miR-375 correlated with serum APAP protein adducts, an indicator of the oxidative metabolism of APAP (r=0.61). In conclusion, this study suggests that urinary miR-375 could represent a non-invasive clinical biomarker of APAP toxicity in children. This study was funded in part by a grant (R01 DK75936 to LJP) from the National Institute of Diabetes, Digestive and Kidney Diseases.
MicroRNAs (miRNAs) are increasingly emerging as promising biomarkers of cardiovascular pathologies and therapeutic response. While cardiac troponins are used as clinical biomarkers of cardiac tissue injury, there is a lack of biomarkers of early events of cardiac cell damage. Using a newly developed chronic cardiotoxicity mouse model, expression profiling of 1179 unique miRNAs was performed to identify early predictive biomarkers of cardiac tissue damage. Male B6C3F1 mice were given a weekly dose of 3 mg/kg doxorubicin (DOX; an anti-cancer drug), or an equivalent volume of saline via tail vein for 2, 3, 4, 6, and 8 weeks, resulting in cumulative DOX doses of 6, 9, 12, 18, and 24 mg/kg, respectively. Mice were euthanized a week after the last dose. Plasma levels of cardiac troponin T indicated cardiac cell injury following exposures to 18 mg/kg DOX and higher, whereas microscopic examination of heart tissues revealed the presence of cardiac lesions at 24 mg/kg. Thirty-three heart miRNAs showed significant (FDR<0.1) perturbation in expression, with the expression of 1, 1, 2, 8, and 24 miRNAs being affected at cumulative doses of 6, 9, 12, 18, and 24 mg/kg DOX, respectively. miRNA-34a was the only miRNA that showed increased expression at all DOX doses and exhibited a significant dose response. Considering the pro-apoptotic effects of miR-34a during myocardial infarction and cardiac aging, up-regulation of miR-34a at 6 mg/kg cumulative DOX dose may indicate apoptosis as an early event in hearts of DOX-treated mice. Increased transcription of apoptotic genes in the heart further support this notion. These data suggest the utility of miRNAs as predictive biomarkers of early stages of cardiac cell injury that may serve as a potential tool in therapeutic risk assessment.

MicroRNAs (miRs) are single-stranded, non-coding RNAs that mediate post-translational inhibition or promote degradation of mRNA targets. miRNA profiles have been shown to be detectable in cell-free body fluids including serum and plasma samples. Moreover, circulating miRNAs have been proposed as attractive blood biomarkers of human diseases and toxicities because of their disease/toxicity-specific dysregulation and their relative stability compared with mRNAs. MicroRNAs (miRs) have the potential to be translatable biomarkers of target organ injury. An ideal miR biomarker would be specific with sequence homology across different species. Identification of such candidates requires generation of a miR tissue atlas and the identification of tissue-specific/enriched miRs with sequence homology across relevant nonclinical species and human. Tissues from 5 male Marshall Beagle dogs were acquired from Bioréclamation, LLC (Hicksville, NY). RNA from 20 individual tissues was analyzed by microRNA-SEQ using the Illumina platform. miR sequences were aligned to the canine genome. MiR counts were quantified, normalized, and analyzed for statistical significance (value < 0.005). Specific miRs indentified in pancreas (miR-216a, miR-216b, miR-802, miR-200), tests (miR-34a) and sciatic nerve (miR-432) have potential as new biomarkers for tissues which currently have limited or no validated biomarkers of injury. In a dog proof of concept study, plasma levels of two liver injury candidate miRs, miR-122 and miR-885, were demonstrated to be elevated following 7-days treatment with Compound X, and correlated with ALT and liver pathology. In collaboration with Eli Lilly and Co., via HESI Genomics Group, we are in the process of comparing rat and dog tissue atlases to pinpoint miRs with sequence homology and tissue specificity in both species. Preliminary results of the atlas comparison suggest good correlation between tissue specific miRs (miR-122, liver) and non-specific miRs (miR-192). Results of this comparison suggest that certain miRs have the potential to be translatable biomarkers of target organ injury.

MicroRNAs (miRs) are expressed in an organ specific manner and are easily detectable in serum or plasma. In contrast to protein biomarkers, many miR sequences are conserved across species and assays are readily available for quantification. This has elicited interest in miRs as sensitive, specific and species translatable biomarkers for organ toxicity. In fact, liver (miR-122), skeletal muscle (miR-133a) and brain (miR-124) have been shown to be increased in the serum of rats with the respective organ toxicity. Interpretation of serum miR changes with respect to organ toxicity necessitates an understanding of the tissue(s) of origin of each miR and its isomiR(s). In order to confirm existing miRs and discover novel miRs we process of comparing rat and dog tissue atlases to pinpoint miRs with sequence homology and its isomiR(s). In order to confirm existing miRs and discover novel miRs we have utilized Illumina deep sequencing to construct a rat miR body atlas which consists of ~20 tissues of toxicologic interest. The data has confirmed many of the known tissue enriched miRs and revealed novel tissue enriched miRs such as liver miR-802-5p, brain 125a-5p, pancreas 148a-3p, adrenal 351-3p and stomach 205-5p. Serum miR profiling in rat and dog with histopathologic evidence of caerulein induced pancreatic injury revealed that miRs-375 and 216a increased in the serum, suggesting they may be translatable biomarkers of pancreatic injury. Takeda Pharmaceuticals created a dog miR body atlas utilizing deep sequencing and, in conjunction with HESI (Health and Environmental Sciences Institute), we have compared the rat and dog atlases for conserved tissue enriched miRs. We believe the combined data sets will be a valuable miR biomarker resource for application in preclinical toxicology studies.
The use of IL2 is limited in the clinic because of its unmonitorable vascular toxicity. Here, we assessed tissue and circulating microRNAs as candidate biomarkers of vascular injury in male Wistar-Han rats administered IL2 for up to 5 days. Vascular changes consisted of endothelial hypertrophy/hyperplasia and/or medial vascular hyalinization and perivascular inflammation, most notably in the liver, kidneys, papimfornx and areas of injection, with perivascular mixed cell infiltrates. These changes were accompanied with increased expression of CD31 as assessed by immunohistochemistry, decreased expression of miR-126 as assessed by in situ hybridization, and increases in circulating miR-145 and miR-155. These changes and the histomorphologic findings were partially reversible after a 7 day recovery period. These observations identify increases in circulating miR-145, a marker of smooth muscle cells, prior to the onset of necrosis of the vascular wall, and support the further evaluation of miR-145 as a marker of acute vascular injury.

While no histological renal injury was evident on D7, significant urinary albumin elevations measured for DOX treated animals, compared to vehicle-treated controls, suggested ongoing glomerular injury at this time point. By D14, following a second drug injection given on D7, rats developed overt glomerular and tubular injury accompanied by further urinary elevation of albumin, along with urinary elevations in tubular injury markers lipocalin-2, kidney injury molecule-1, cystatin c, and β2-microglobulin, compared to vehicle-treated controls. Microarray profiling identified eight miRNA species significantly elevated in urine during time points at which albumin was significantly elevated and six of these species make attractive biomarker candidates based on proposed function. Additionally, three of these miRNAs show suggestive elevations in urine prior to observable changes in albumin concentrations, which may indicate that these miRNA biomarkers may be sensitive for the detection of glomerular injury.
Cardiac troponin I (cTnI) is an established biomarker of cardiotoxicity, but its utility is limited by its short half-life. The present study was conducted to identify potential circulating cardiotoxicity miRNA biomarkers as an alternative to cTnI using a rat isoproterenol myocardial-injury model. In an initial screen, 9 heart or heart/skeletal muscle specific miRNAs were identified out of 768 miRNAs across a panel of tissues from untreated male Sprague-Dawley rats. The same panel of miRNAs was assessed in the serum following cardiac injury. In a subsequent experiment, male Sprague-Dawley rats received a single subcutaneous injection of isoproterenol (0.05 mg/kg) or vehicle. All surviving rats were sacrificed 24 hours post-injection, and cardiotoxicity was confirmed by histopathology. Serum was collected just prior to sacrifice from treated and control rats for analysis of circulating miRNAs. Plasma samples were collected predose and 24 hrs post dose for cTnI measurements. Consistent with its short half-life, plasma cTnI levels at 24 hrs post dose were comparable to baseline despite histologic evidence of cardiac injury. Several miRNAs were only present in the serum from isoproterenol-treated rats relative to controls. However, of the 9 heart or heart/skeletal muscle-specific miRNAs identified in the tissue screen, only miR-208, a potential biomarker of cardiac toxicity was detected in the serum following isoproterenol treatment 24 hrs post-dose. To our knowledge, this represents the first report of tissue expression profiling to identify heart-specific miRNAs. Ongoing work is examining the serum half-life of miR-208 after cardiac injury following isoproterenol treatment.

Cardiotoxicity is one of the critical toxicities and it is important to appropriately detect cardiac toxicity early. However, there are few helpful biomarkers for cardiotoxicity. This study was conducted to obtain possible biomarkers for estimating cardiotoxicity. Isoproterenol (ISO, 5 mg/kg), an adrenergic β agonist which can induce cardiotoxicity, was intraperitoneally administered to male F344 rats at 7 weeks old (3 or 4 animals/group). The heart for the histopathological analysis and the plasma for the metabolomics analysis were obtained from the rats 2, 6, 24 and 48 hours after the administration. Metabolites were measured using an UPLC/QTOF-MS, and a principal component analysis (PCA) was used for screening of potential biomarkers. In the histopathological analysis, focal vascular degeneration and coagulation necrosis at 2 hours, sporadic coagulation necrosis at 6 hours, inflammatory cell infiltration and coagulation necrosis at 24 hours, and fibrotic changes along with the infiltration at 48 hours were observed in the ISO-treated groups. The most severe changes in histopathology were observed at 24 hours during the progress of the heart damage. In the metabolomics analysis, PCA indicated the clear differentiation of the control and ISO-treated groups at 2 and 6 hours, and 9 metabolites including arachidonic acid contributed to the differentiation. These metabolites in the ISO-treated groups were increased more than those in the corresponding control groups at 2 and 6 hours, and the metabolites in the ISO-treated groups were equivalent to the corresponding control groups at 24 and 48 hours. These results showed that the identified metabolites were increased along with the histopathological changes in the heart and these metabolites achieved the peak levels earlier than the histopathological changes. Additionally, the identified metabolites can be the possible biomarkers for ISO-induced cardiotoxicity.

Cardiovascular disease is one of the critical toxicities and it is important to appropriately detect cardiovascular disease early. However, there are few helpful biomarkers for cardiovascular toxicity. This study was conducted to obtain possible biomarkers for estimating cardiovascular toxicity. Isoproterenol (ISO, 5 mg/kg), an adrenergic β agonist which can induce cardiovascular toxicity, was intraperitoneally administered to male F344 rats at 7 weeks old (3 or 4 animals/group). The heart for the histopathological analysis and the plasma for the metabolomics analysis were obtained from the rats 2, 6, 24 and 48 hours after the administration. Metabolites were measured using an UPLC/QTOF-MS, and a principal component analysis (PCA) was used for screening of potential biomarkers. In the histopathological analysis, focal vascular degeneration and coagulation necrosis at 2 hours, sporadic coagulation necrosis at 6 hours, inflammatory cell infiltration and coagulation necrosis at 24 hours, and fibrotic changes along with the infiltration at 48 hours were observed in the ISO-treated groups. The most severe changes in histopathology were observed at 24 hours during the progress of the heart damage. In the metabolomics analysis, PCA indicated the clear differentiation of the control and ISO-treated groups at 2 and 6 hours, and 9 metabolites including arachidonic acid contributed to the differentiation. These metabolites in the ISO-treated groups were increased more than those in the corresponding control groups at 2 and 6 hours, and the metabolites in the ISO-treated groups were equivalent to the corresponding control groups at 24 and 48 hours. These results showed that the identified metabolites were increased along with the histopathological changes in the heart and these metabolites achieved the peak levels earlier than the histopathological changes. Additionally, the identified metabolites can be the possible biomarkers for ISO-induced cardiotoxicity.

Drug-induced vascular Injury (DIVI) is a preclinical toxicity usually characterized by hemorrhage, vascular endothelial and smooth muscle damage and inflammation. Preclinical DIVI findings cause delays or termination of drug candidates due to low safety margins, the absence of non-invasive biomarkers for monitoring vascular injury and the uncertain relevance to humans. Under the framework of the EU-funded IMI SAFE-T consortium, we are qualifying a set of translatable biomarkers associated with the onset of histopathologic damage in various vascular beds in humans. Patients with conditions involving inflammatory in-filrates, a-\vati-\jion and injury to the vascular endothelium and fibinoid necrosis of the media were included, along with age- and sex-matched healthy volunteers. Twenty soluble proteins were measured in serum samples collected in healthy subjects and in patients during the acute and the resolution phases of disease. The panel of markers included inflammatory factors as well as endothelial proteins: CRP, E-Selectin, GROα, IL-6, IL-8, IP-10, I-TAC, NGAL, MCP-1, MIG, MIP-1, P-Selectin, SAA, sICAM-1, sICAM-3, sVCAM-1, thrombomodulin, TIMP-1, TNFRSF1A and VEGF. Univariate analysis showed that no single marker measurement provided a sufficient predictive power with an area under ROC curve (AUROC) above 0.8. In contrast, a multivariate score based on a robust combination of healthy volunteers from patients with vascular injury, and further separated the latter group into those in the acute and quiescent stages of disease. Of the several machine learning approaches used to calculate the performance of combinations of markers, a naïve Bayes classifier based on 10 parameters (MIP-1, SAA, TNFRSF1A, TIMP-1, P-Selectin, sICAM-1, sICAM-3, thrombomodulin, age and sex) achieved the best diagnostic performance (AUROC = 0.88). Studies to further validate these biomarkers are currently on-going. The panel of biomarkers established in this study shows promise as a clinical monitoring tool for compounds that cause DIVI in preclinical studies.

L-type Fatty Acid Binding Protein (L-FABP), which is located in the cytoplasm of renal proximal tubular cells, responses to ischemia and oxidative stress and is excreted in urine. KDIGO (Kidney Disease Improving Global Outcomes) selected L-FABP as one of the major 5 biomarkers (L-FABP, cystatin C, NGAL, Interleukins, KIM-1) for detecting the acute kidney injury. The L-FABP ELISA kit has already been approved as a clinical diagnostic agent for renal injury in Japan and in experimental animals widely used in non-clinical toxicity studies. The purpose of this research was to evaluate L-FABP as a nephrotoxicity biomarker in experimental animals. We used Wistar rats and found that L-FABP was superior to traditional biomarkers such as BUN and serum creatinine in measuring kidney injury which partially recovered over 144 h after injury. LC/MS/MS studies of 24 h urine samples revealed increased urinary ATP5P protein in mice subjected to I/R-induced AKI and rats subjected to gycerol-induced AKI when mitochondrial function was decreased. Consistent with LC/MS/MS data, Western blotting of 24 h urine samples revealed increased ATP5P protein in mice subjected to a range of I/R times and degrees of kidney injury. Also, preliminary LC-MS/MS findings revealed that urinary ATP5P was increased in human subjects with post-cardiac bypass surgery-induced AKI increased when compared to controls. These studies provide evidence that ATP5P is a urinary marker of mitochondrial dysfunction and will permit better understanding of the timing and mechanisms of renal injury and the state of mitochondrial homeostasis during AKI.
both strains in urinary L-FABP excretion. Histological observation revealed that foamy eosinophilic globules slightly existed in proximal tubule on Day 4 and those urinary L-FABP excretion elevated on Day 1 in correspondent rats. These results suggest that the urinary L-FABP may be a very useful sensitive biomarker of nephrotoxicity not only in humans but also in experiment animals.

114 Preclinical Nephrotoxicity Biomarker Analysis Using Toxicoproteomics Approaches

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Increased application of proteomics approaches to toxicological studies has led to the development of a new discipline that is being called "toxicoproteomics". Major research areas of toxicoproteomics include development of biomarkers of toxicity, identification of molecular targets of toxicants, and elucidation of toxicity mechanisms. To understand the molecular mechanisms of toxicity and identify kidney injury biomarkers, quantitative proteomic analyses were conducted on kidneys from aristolochic acid (AA)-treated rats. Animal treatment was conducted according to the protocol approved by the NCTR Institutional Animal Care and Use Committee. Big Blue rats were treated with 0.9% sodium chloride as the control or AA at 10 mg/kg body weight, five times/week, for 12 weeks. Kidneys were collected one day after the last treatment. Kidney tissues from the control and the AA-treated rats were processed for proteomic analysis using stable isotope labeling and two-dimensional liquid chromatography coupled online with tandem mass spectrometry (LC-MS/MS). More than 100 proteins changed their abundance as a result of AA treatment, which may be related to the toxicity and carcinogenicity of AA. Intriguingly, several FDA-qualified preclinical kidney injury biomarkers were identified in this study, and their abundance showed significant changes. A mass spectrometry-based multiplex kidney injury biomarker assay was developed for targeted quantitative measurement of the biomarker candidates discovered in this study, and many of the expression-changed proteins were confirmed. The combined open discovery and targeted proteomics approaches facilitated the identification of kidney injury biomarkers. Additional studies need to be performed in animals and humans to investigate their translational nature. The views presented do not necessarily reflect those of the U. S. Food and Drug Administration.

115 Detailing the Human Exposome: Mass Spectrometry-Based Chemical Profiling of Human Mitochondria

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While most studies have utilized easily accessible bio-fluids to monitor environmental agents and associated metabolic response, few have measured exogenous compounds within sub-cellular compartments. The purpose of this study was to profile the endogenous and exogenous mitochondrial metabolome of human organ tissue, with an emphasis on identifying the presence of environmental agents. Viable mitochondria were obtained from human adrenal glands (n=5) within 36 hours of removal using a differential centrifugation isolation procedure. Isolates adapted to identify OP insecticide adducts on both plasma BChE and RBC acyl-phosphate hydrolase (APH), two known biomarkers of OP exposure. Plasma, RBCs or dried blood spots (DBS) are used for our IMB-MS methods. The methods have been designed for adaptation to high-throughput protocols to facilitate their transfer to clinical laboratories. These methods will be validated using a cohort of Washington State agricultural workers with characterized OP exposures to chlorpyrifos or azinphos-methyl. The IMB-MS protocols purely BChE and APH from as low as 25 μL of plasma or RBCs, respectively, or from DBS. The use of DBS will facilitate sample collection, shipping, and archiving as well as the analysis of already archived samples. The IMB-MS protocols have significant advantages: they do not require a pre-exposure measurement, they can detect low levels of exposure, they can be automated, and they provide information on both the OP and percentage modification of the active site serines. Supported by NIH (P42ES04696, R01ES009883, ES09601/ EPA-R826886, P41GM103533); the PNASH Center (U50CH07544-10); and by financial support from the CDC (Atlanta, GA).

116 Proteomic Analysis of Dried Blood Spots for Biomonitoring Organophosphorus Exposures

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Organophosphorus (OP) compounds are involved in most of the occupational and environmental intoxications reported worldwide. They inhibit cholinesterases and other serine hydrolases, leading to neurological damage and serious health effects. The standard methods used for biomonitoring OP exposures are the Ellman assays, which measure the enzymatic activity of either red blood cell (RBC) acetylcholinesterase or plasma butyrylcholinesterase (BChE). Despite being fast and inexpensive, these assays suffer from several drawbacks. We have developed a rapid immunomagnetic bead (IMB) purification for biomarkers of OP exposure, followed by OP-adduct identification on active site serines by high-resolution mass spectrometry (MS). The IMB-MS methods have been adapted to identify OP insecticide adducts on both plasma BChE and RBC acyl-peptide hydrolase (APH), two known biomarkers of OP exposure. Plasma, RBCs or dried blood spots (DBS) are used for our IMB-MS methods. The methods have been designed for adaptation to high-throughput protocols to facilitate their transfer to clinical laboratories. These methods will be validated using a cohort of Washington State agricultural workers with characterized OP exposures to chlorpyrifos or azinphos-methyl. The IMB-MS protocols purely BChE and APH from as low as 25 μL of plasma or RBCs, respectively, or from DBS. The use of DBS will facilitate sample collection, shipping, and archiving as well as the analysis of already archived samples. The IMB-MS protocols have significant advantages: they do not require a pre-exposure measurement, they can detect low levels of exposure, they can be automated, and they provide information on both the OP and percentage modification of the active site serines. Supported by NIH (P42ES04696, R01ES009883, ES09601/EPA-R826886, P41GM103533); the PNASH Center (U50CH07544-10); and by financial support from the CDC (Atlanta, GA).

117 Toxicometabolomics Approach to Prediction of Hepatotoxicity by Troglitazone/LPS in Rats

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Troglitazone (TGZ) is a thiazolidinedione antidiabetic agent which is the synthetic ligands for the peroxisome proliferator-activated receptor γ (PPAR γ). However, it was withdrawn from the market in 2000 due to liver injury in humans. In this study, we endeavored to discover surrogate biomarkers which are correlated with hepatotoxicity induced by TGZ using urinary proton nuclear magnetic resonance (1H NMR) spectral data. A procedure of 1H NMR urinary and serum analysis using pattern recognition was proposed for early screening of the hepatotoxicity of TGZ with lipopolysaccharide (LPS) in rats. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were the highest in 6 hours and hepatic inflammation, in histopathology, was observed in 2 hours. All treated animals were divided into two groups of responder and non-responder based on the ALT and AST levels because the levels of ALT and AST were not changed dose-dependently after treatment of TGZ (2 g/kg, p.o.). In urinary analysis, 1H NMR spectroscopy did not show different clustering between responder and non-responder in global metabolic profiling through principal component analysis (PCA). However, it was significantly separated in orthogonal projections to latent structures-discriminant analysis (OPLS-DA). In targeted profiling, endogenous metabolites of phenylacetylglycine, glucose, acetate, 2-oxoglutarate, formate, creatine, citrate, 3-indoxysulfate, lippurate, acetone, phenylalanine, glycine, betaine, cis-aconitate, and tyrosine were selected as putative biomarkers for hepatotoxicity by TGZ. In serum analysis, pattern recognition was similar to the results of the urinary analysis, and then we could select putative 6 endogenous metabolites such as ethanol, glucose, alanine, glutamine, lactate, and 3-hydroxybutyrate. According to these results, toxicometabolomics can be used to predict or screen hepatotoxicity caused by TGZ in urine and serum of rats.
Pro-inflammatory signals upregulate expression of cell surface αM integrin CD11b on eosinophils, favoring cell adhesion to airway epithelium in chronic disorders such as asthma. Absence of CD16B (CD16-) is used to differentiate eosinophils from other granulocytes (CD16+B+), in humans. However, in cynomolgus macaques (CM), alternative methods must be used to identify eosinophils due to the absence CD16B expression on granulocytes in this species. To resolve this, we used the inherent autofluorescence of CM eosinophils to differentiate them from other granulocytes, and monitored CD11b up-regulation \textit{ex vivo}, following IV administration of either 1 or 10 mg/kg ACRA. Blood samples were collected on day-1, pre-dose, 2, 8, 15, and 28, and 43 days and exposed, \textit{ex vivo}, to increasing concentrations of a pro-inflammatory cytokine for 30 min at 37°C. Samples were acquired on a flow cytometer following red blood cell lysis and staining with anti-CD11b allophycocyanin-conjugated antibody. CD11b median fluorescence intensity on eosinophils, identified by their granularity and autofluorescence, was reported for each sample. Animals given 10 mg/kg of ACRA had complete suppression of eosinophil activation over 43 days (plasma levels > 10,000 ng/ml). Suppression of eosinophil activation returned to baseline in animals dosed with 1 mg/kg of ACRA after 28 days, when drug plasma levels were ≤ 240 ng/ml, the EC50 derived from \textit{in vivo} eosinophil CD11b activation assays. Thus, although some understanding of target coverage was known from \textit{in vitro} studies, the eosinophil PD assay applied in this PK study provided valuable information about target coverage. The identification of potential hazards is a key outcome of toxicity studies. With the introduction of omics-technologies into toxicological research, questions have been raised concerning the sensitivity of these methods. We have developed the MetaMap® Tox database with toxicity and metabolome data of 500 reference compounds obtained from rat studies. Plasma metabolome analysis was performed after 7, 14 and 28 days. We have evaluated the predictive performance of MetaMap®Tox using 78 substances for which the predicted mode of action based on metabolomics was compared to the actual outcome of the guideline studies. Overall, 85% of the effects observed with classical parameters have also been predicted by the metabolome data. Out of 45 compounds that did show effects in classical parameters in male animals, 36 compounds (80%) showed the same effect in the metabolome and could be considered as correctly positively predicted. Out of 35 not showing any effect in the classical parameters, 30 substances (86%) did not show an effect on the metabolome (correctly negatively predicted), whereas 5 compounds (14%) did show effects in the metabolome (false-positive predictions). The numbers for female animals are 36 out of 42 correct positive predictions (86%, based on 42 compounds showing toxicity in classical parameters) and 6 out of 42 false negative predictions (14%), 30 out of 36 correct negative predictions (83%, based on 36 compounds showing no effects in classical parameters) and 6 out of 36 false positive predictions (17%). In conclusion our analysis indicates that metabolome data can be used to predict toxicological effects in rats. Since metabolome data often provides a deeper mechanistic insight into the effects observed, metabolome analysis gives an added value for systemic toxicity testing.
Global gene expression studies of liver from carcinogen-exposed animals using RNA-Seq can provide insights into regulatory genes and critical pathways that might lead to hepatocellular carcinoma. We recently conducted RNA-Seq analysis on liver RNA from male F344 rats exposed to 1 ppm aflatoxin B1 (AFB1) in feed for 90 days, prior to the appearance of tumors. Among the more than 1,000 differentially expressed genes, we found a total of 49 potentially novel Cufflinks-assembled transcripts of which 21 transcripts had Ensembl annotation and 28 transcripts did not. Ten of these novel AFB1 altered transcripts were validated by PCR and specific reverse transcriptase primer extension for eventual assembly into expression systems. Data showed a 5-fold increase in the stem cell marker, Sox-9, and the increased reads revealed a longer transcript than predicted for the rat Esenbl transcript or mouse and human orthologs. Similarly, AFB1 produced a 50-fold increase in the p53-regulated transcript, Eda2R (ectodysplasin A2 receptor), for which an extensive, unannotated 3’-region became evident. The set of 28 unannotated, novel transcripts showed AFB1 altered expression ranging from a 5-fold decrease to 659-fold increase. We previously reported two unannotated transcripts from this dataset, termed HAFT1 and HAFT2 (hepatic aflatoxin altered transcripts 1 and 2). Here, we selected 8 other unannotated novel Cufflinks transcripts from RNA-Seq data for PCR amplification and cloning for which partial sequence was obtained that correctly mapped back to each chromosomal location. One of these included a low copy transcript increased 281-fold in AFB1 that contained at least 4 exons in an unannotated region of Chr16 flanked by Fgfr1 and Tac1 genes. Rapid amplification of cDNA ends (RACE) procedures was used to determine complete cDNA sequence for cloning, expression and cellular function. Complete sequencing of such transcripts permitted the way for a new set of biomarkers for chemical hepatocarcinogenesis prior to tumor formation.

Adam8 belongs to a disintegrin and metalloprotease family of genes. This protein has been implicated in a various cellular processes including cell-cell adhesion, muscle development, neurogenesis and asthma. Recently a hepatic global gene expression analysis of the liver was undertaken to identify specific genes that mediate 2-aminoanthracene (2AA) toxicity in the liver. Findings from this study were to assess if serum microRNAs could be used as biomarkers of melamine and cyanuric acid-induced kidney toxicity. Male Fischer 344 rats were a fed diet fortified with equal amounts of melamine and cyanuric acid (0, 180, or 240 ppm each) for 28 consecutive days. Nephrotoxicity, as assessed by increased blood urea nitrogen (BUN) and serum creatinine versus control, was observed in the 240 but not 180 or 0 ppm dose group. Serum samples from the three dose groups were prepared from terminal blood and checked for hemolysis prior to evaluating microRNA expression. Equal volumes of nonhemolytic sera were pooled from a minimum of 3 animals per dose group. Using a PCR array, the relative expression of 373 microRNAs was measured. Based on the screening data, 30 microRNAs were modulated by approximately 4-fold compared to the control. To validate these data, the expression of the 30 microRNAs was measured by quantitative real-time PCR in each of the three individual samples per dose group. Circulating levels of rno-miR-144-3p were down-regulated in sera of rats dosed with 240 ppm melamine and cyanuric acid in comparison to the control group. The down-regulation of rno-miR-144-3p was further confirmed using a larger sample size (n = 12 per dose group; P < 0.025). These data suggest that rno-miR-144-3p may be a minimally-invasive biomarker of kidney injury induced by melamime and cyanuric acid. It is at least as sensitive as the currently used biomarkers BUN and serum creatinine. Future studies will evaluate the expression of rno-miR-144-3p following exposures to additional nephrotoxicants.

The liver and kidney are fundamentally important tissues for drug metabolism, disposition, and excretion. Growing evidence suggests epigenetic mechanisms of gene regulation may play a role in susceptibility to certain toxicities and adverse drug reactions. microRNAs continue to show promise as biomarkers. While relatively immature compared to other genomic resources, growing knowledge of individual miRNAs and their putative gene targets allows for large scale inquiry into genome-wide miRNA expression. miRNA expression in liver and kidney tissues was examined in F344 rats during their life cycle. Animals were evaluated at 2, 5, 6, 8, 15, 21, 52, 78, and 104 weeks of age in both sexes (n=5) using Agilent 8x15k rat miRNA microarrays. 263 and 275 miRNAs were found to be expressed in the liver and kidney, respectively, in at least one age and sex. 221 miRNAs were commonly expressed in both liver and kidney, including criteria of 4.5 fold change and ANCOVA (PDR 5%) revealed 249 (94%) differentially expressed miRNAs (DEMs) in the liver, while 210 (76%) were differentially expressed in the kidney. Of the 305 total DEMs, 154 miRNAs were differentially expressed in both tissues. A notable age difference in expression consisted of 28 miRNAs showing 2

**122 Subchronic Aflatoxin B1 Exposure Produces Expression of Novel Liver Transcripts**

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**122a Metalloproteinase-Disintegrin Adam8: A Potential Biomarker for 2-Aminoanthracene Toxicity in the Liver of Fisher-344 Rats**

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Adam8 belongs to a disintegrin and metalloprotease family of genes. This protein has been implicated in a various cellular processes including cell-cell adhesion, muscle development, neurogenesis and asthma. Recently a hepatic global gene expression analysis of the liver was undertaken to identify specific genes that mediate 2-aminoanthracene (2AA) toxicity in the liver. Findings from this study were to assess if serum microRNAs could be used as biomarkers of melamine and cyanuric acid-induced kidney toxicity. Male Fischer 344 rats were a fed diet fortified with equal amounts of melamine and cyanuric acid (0, 180, or 240 ppm each) for 28 consecutive days. Nephrotoxicity, as assessed by increased blood urea nitrogen (BUN) and serum creatinine versus control, was observed in the 240 but not 180 or 0 ppm dose group. Serum samples from the three dose groups were prepared from terminal blood and checked for hemolysis prior to evaluating microRNA expression. Equal volumes of nonhemolytic sera were pooled from a minimum of 3 animals per dose group. Using a PCR array, the relative expression of 373 microRNAs was measured. Based on the screening data, 30 microRNAs were modulated by approximately 4-fold compared to the control. To validate these data, the expression of the 30 microRNAs was measured by quantitative real-time PCR in each of the three individual samples per dose group. Circulating levels of rno-miR-144-3p were down-regulated in sera of rats dosed with 240 ppm melamine and cyanuric acid in comparison to the control group. The down-regulation of rno-miR-144-3p was further confirmed using a larger sample size (n = 12 per dose group; P < 0.025). These data suggest that rno-miR-144-3p may be a minimally-invasive biomarker of kidney injury induced by melamime and cyanuric acid. It is at least as sensitive as the currently used biomarkers BUN and serum creatinine. Future studies will evaluate the expression of rno-miR-144-3p following exposures to additional nephrotoxicants. IAG FDA 224-12-0003/NIH ES12013.

**122b CD1X on Dendritic Cells: A Biomarker of Metallic Allergens and Metallic Nonallergens In Vivo**

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The CD1 family is a large multigene family that expresses multiple members on cell surface of numerous cell types, including dendritic cells (DCs). Some CD1 family members are expressed on dendritic cells and are currently used as a biomodulator to predict allergic responses to medical device materials. The cells were exposed for 24 hr to the following metal allergens and non-allergens for biomarker identification and development by employing flow cytometry. Interestingly, of 16 selected cell surface markers (CD1a, CD1X, CD44, CD54, CD68, CD80, CD86, CD102, CD184, CD197, CD206, HLA-DR, TLR-9), we found a new functionality for the protein CD1X on DC. The expression was down-regulated significantly following exposure to 8 metallic allergens (ammonium hexachloroplatinate(IV), cobalt(II) chloride, nickel(II) sulfate, nickel(II) chloride, mercury(II) chloride, beryllium(II) sulfate, gold(I) chloride, potassium dichromate(VI)) while the expression of CD1X was increased by 4.5 fold change and ANCOVA (PDR 5%) revealed 249 (94%) differentially expressed miRNAs (DEMs) in the liver, while 210 (76%) were differentially expressed in the kidney. Of the 305 total DEMs, 154 miRNAs were differentially expressed in both tissues. A notable age difference in expression consisted of 28 miRNAs showing 2
week-specific expression in the liver but not in the kidney. The genes encoding 26 of these 28 miRNAs are located on rat chromosome 6. Nine miRNAs showed sex differences in both tissues and only 1 miRNA (miR-22) showed a similar expression pattern in both tissues; male-specific up-regulation at 21 weeks of age. miR-22 has been shown to post-transcriptionally inhibit PGC-1α, PPARα, and Sirt1, key regulators in metabolism pathways. These results comprise one of the first large-scale comparisons of global miRNAs in the liver and kidney over the rat life cycle that may impact susceptibility to adverse drug reactions.

122e Next-Generation Sequencing of Plasma, Urine, and Tissue microRNA from Sprague-Dawley Rats Treated with Acetaminophen or Cisplatin


MicroRNAs (miRNA) are short single-stranded RNA sequences comprising of approximately 22 nucleotides that have a role in the post-transcriptional regulation of genes. The identification of tissue specific or enriched miRNAs may be useful as novel safety biomarkers. Recent work has demonstrated that both the presence and magnitude of miRNA in biofluids (e.g., plasma and urine) can be associated with tissue-specific damage. This study is the first to use next generation sequencing to analyze changes in miRNA profiles of plasma, urine and tissue samples of rats treated with either a hepatotoxicant (Acetaminophen [APAP]) or a nephrotoxicant (Cisplatin). To verify tissue-specific damage, an analysis of histopathology and serum chemistry was performed. In animals treated with APAP, eight miRNAs were significantly (p<0.05) altered in the liver, 45 in plasma and none in urine. Consistent with the literature, levels of miR-122 (193-fold) and miR-192 (63-fold) were significantly (p<0.001) upregulated in plasma from these animals. Histopathology and increases in liver enzymes demonstrated liver necrosis for APAP-dosed rats without a concomitant kidney injury. In Cisplatin-treated animals, 20 miRNAs were significantly (p<0.05) altered in the kidney, nine in plasma and six in urine. Also consistent with the literature, levels of miR-34a were significantly (p<0.004) upregulated in the kidney (6.1-fold) and plasma (7.2-fold); a significant (p<0.002) 10.0-fold increase in the expression of miR-378 in the urine has not been previously reported. Histopathology and changes in serum markers for kidney injury confirmed kidney but not liver damage in Cisplatin-treated rats. We next plan to use qPCR to validate the next generation sequencing of urine miRNA. Taken together, comparative analysis of urine, plasma, and tissue miRNA demonstrated the utility of miRNA as biomarkers of organ injury and identified miR-378 as a potential novel urine marker for Cisplatin-induced injury.

122f Circulating microRNAs As Potential Non-Invasive Biomarkers in Patients with Crohn’s Disease


MicroRNAs (miRNAs) are a class of small, non-coding RNAs that negatively regulate gene expression, processes that appear to be involved in the pathogenesis of human diseases and toxicities. Circulating miRNAs offer great potential as biomarkers for disease detection because of their remarkable stability in blood and their characteristic expression in different diseases and toxicities. Differential expression of miRNAs has been described in multiple autoimmune-associated diseases. Recently, unique miRNA expression profiles have been described in the intestinal epithelial cells and in the peripheral blood of patients with Crohn’s disease (CD). We hypothesized that miRNAs may be involved in the homeostatic regulation of CD-associated inflammatory responses and their disease-associated dysregulation could be detected in circulating miRNA signatures. Specific miRNAs with important roles in regulating key pathogenic mechanisms in CD inflammation were selected to assemble a Taqman assay panel for screening of plasma from CD patients (Montreal classification, B1-B3) and healthy volunteers. We found that the profile of a number of circulating miRNAs is capable of distinguishing healthy volunteers (HV) from CD patients. In particular, miR-182 levels were upregulated in the CD subjects (B3 vs HV, p=0.006). Interestingly, tumor necrosis factor (TNF)-like ligand 1A (TL1A) is a direct regulated target of miR-182. Numerous genome wide association studies have linked several polymorphisms of the TL1A gene to CD, and inflamed intestinal tissue from patients with inflammatory bowel disease show high expression levels of TL1A. Additionally, an intestinal enriched miRNA, circulating miR-194, was upregulated in the CD subjects (B3 vs HV, p=0.001). The results described here have implications for the use of circulating miRNA profiles as potential non-invasive biomarkers for CD detection.

122g Novel Kidney Injury Biomarker Panels to Detect Nephrotoxicity in Mouse Models

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Kidney is the primary organ for drug toxicity. The application of kidney injury biomarkers has greatly improved the detection of nephrotoxicity during drug development and will accelerate the speed to bring more effective and safe drugs to market. Over the past decade, a panel of novel urinary kidney biomarkers has been approved by the FDA, EMA and PMDA to detect acute nephrotoxicity in preclinical rat studies. Even though mouse models are widely used in drug development process, lack of reliable assays to quantitatively assess multiple protein markers in small volumes of mouse urine has limited the application of these markers in mouse models.

In the present study, we developed multiplexed immunoassays for two panels of mouse kidney injury biomarkers. Furthermore, we validated the sensitivity and specificity of these assays in two different mouse kidney injury models. To induce kidney injury, BALB/C mice were subjected to either bilateral ischemic reperfusion (I/R) for 30 minutes or one dose of cisplatin treatment. Urine specimens were collected and analyzed for urinary protein markers with MILLIPLEX® MAP Mouse Kidney Injury Magnetic Bead Panels. There was a significant increase in many of the novel markers in both I/R and cisplatin treated mouse models reflecting tubular damage. In conclusion, we have developed novel multiplexed assays for mouse urinary kidney biomarkers and validated them in both ischemia and drug induced kidney injury models in mice. The simultaneous measurement of these proteins with multiplex technology offered a robust and convenient method to study these biomarkers in small volume of mouse urine samples.

122h Validation of an Immunoassay for Analysis of Oxytocin in Sheep Plasma to Support Progress towards a Novel Drug Delivery Strategy for the Treatment of Postpartum Hemorrhage

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Postpartum hemorrhage is the single largest contributing factor to global maternal mortality, with the burden occurring overwhelmingly in low income countries (WHO & UNICEF 2012). Monash University is working to develop a heat stable and non-injectable oxytocin (OXT) product that can be simply administered by all levels of healthcare personnel.

In support of Monash University’s pre-clinical program, we have validated a radioimmunoassay for the detection of OXT (oxRIA) in sheep plasma, and generated preliminary data on the dose pharmacokinetics (PK) of OXT in sheep, after intravenous (IV) delivery. Validation of the oxRIA, and subsequent studies were carried out using OXT Radioimmunoassay Kits (Phoenix Pharmaceuticals, Inc.). The mean lower limit of quantification for the oxRIA, over three assays, was 760 fg. Recovery of OXT in biological matrix was determined by adding standard OXT at three concentrations to sheep serum, with six replicates at each concentration. After correction for extraction efficiency (69.1%, 0.70% CV), the amounts of OXT recovered from serum was >99%. Slope of the regression line was 1.0986 and r-value was 0.9596. Mean intra- and inter-assay coefficients of variations were 4.6% and 7.5% respectively, over six assays.

To assess the PK of IV-dosed OXT, six sheep were administered 10 IU (~160μg) OXT directly into the jugular vein. Following injection, blood was sampled at ten time points, and plasma concentrations of OXT were determined. Half-life (t1/2) of OXT1 was determined to be ~5 min.

In conclusion, we have shown the oxRIA is reliable for analysis of OXT in sheep serum. The assay was used to assess the t1/2 of OXT delivered to sheep IV, and this value agreed favorably with the t1/2 of OXT reported for other species. On-going pre-clinical studies include the evaluation of both the PK and pharmacodynamics of OXT formulations administered by intramuscular (IM) injection, pulmonary and nasal delivery.
Males and females exhibit different morbidity and mortality across a wide range of conditions which suggests basic pathophysiology may control the incidence of multiple disease processes. Women suffer disproportionately higher rates of many of these diseases, particularly autoimmune and inflammatory diseases. Inflammatory processes induce oxidative stress and reduce cellular antioxidant capacity. This investigation examined the relationship between oxidative stress and inflammatory marker expression and gender in addition to determining the effect of confounders such as drug use, lifestyle and medical history. These conditions are linked to physiological changes that may alter the expression of certain biomarkers of inflammation and oxidative stress. Urine specimens (n=62) were assayed for Myoglobin, C Reactive Protein, Myeloperoxidase, Estradiol, Testosterone, Malondialdehyde, 8OHdG, and Interleukins 1α and 6 using ELISA. Laboratory reference ranges for urine, serum and plasma do not typically differentiate between genders. More importantly, gender-based ranges of urinary concentrations have not been determined for many oxidative stress (OS) and inflammatory cytokines linked to common chronic diseases. Multivariate regression analysis suggest predictors of oxidative marker expression may include gender, preexisting disease and drug use. The results suggest significant gender-based differences may exist in the expression of inflammatory and oxidative biomarkers in the urine of drug users. This work has been supported in part by the Agency for Community Treatment and Services of Tampa and the Laboratory of Occupational and Public Health Toxicology.

125 Identification of MHC Haplotypes Associated with Drug-Induced Hypersensitivity Reactions in Cynomolgus Macaques

The occurrence of immune-mediated drug hypersensitivity reactions (IDHRs) can have a significant impact on therapeutic drug use and development. While the mechanism(s) and risk factors are not fully understood, an association between specific human leukocyte antigen (HLA) alleles and IDHR has been described for some drugs (e.g. abacavir and HLA B*57:01). However, whether such an association exists between IDHR and major histocompatibility complex (MHC) alleles from other species has not been described. In this study, genomic DNA samples were obtained from 62 cynomolgus macaques from several preclinical toxicology studies (with and without IDHR) and genotyped using microsatellite analysis to determine the MHC haplotype for each animal. Of the 62 macaques, 9 animals had clinical evidence of IDHR (manifested as skin lesions). The M1, M2, M3, M4, M5, M6, and M7 haplotype frequencies in the MHC regions were identified to be 17%, 8%, 15%, 19%, 2.4%, 7.3%, and 2.4%, respectively in these monkeys. Recombinant MHC haplotypes and undetermined MHC alleles were represented (2-test for trend, P < 0.05) with the occurrence of IDHR (skin lesions) in this population. These data suggest that cynomolgus macaques may be a good preclinical model for studying mechanisms underlying IDHR. Additionally, genetic haplotype analysis is warranted to determine the potential polymorphism(s) that may be functionally important in IDHR in cynomolgus macaques.

126 Defective TGFβ Signaling in Dermal Dendritic Cells Leads to Their Reduced Lymph Node Migration and Contact Hypersensitivity Responses with Ultraviolet B Irradiation
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Transforming Growth Factor-β1 (TGFβ1) is an important immunoregulatory cytokine in the skin critical for the development of epidermal dendritic cell (DC) subset-Langerhans cells (LCs). However, the role of TGFβ signaling for the immunomodulatory function of LCs or other skin-resident DC subsets-CD207- dermal DCs (dDCs) and CD103+ CD207+ dDCs in response to Ultraviolet B (UVB) irradiation is not clear. Mice expressing a dominant negative TGFβ Type II receptor (DNR) specifically in CD11c+ DCs displayed reduced steady-state migration of all three DC subsets compared to WT C57BL/6 mice. Acute UVB-induced migration of dermal DC subsets, CD207- and CD207+ dDCs in DNR to skin-draining lymph nodes (SDLNs) was reduced in DNR compared to WT mice, which correlated with a reduced percentage of p-Smad2+ and CD86high dermal DCs in DNR with UVB. There was no significant difference in UVB-induced migration of LC subset between DNR and WT mice. In addition, the UVB-induced migration of CD207- and CD207+ dDCs in a chronic UVB model as well as migration of CD207- subset in an ear explant assay was suppressed in DNR compared to WT mice with no difference in LCs. Consistent with reduced
steady state and UVB-induced migration of dermal DC subsets, ear thickness response measured in a contact hypersensitivity model (CHS) with and without UVB, was reduced in DNR mice compared to WT with an associated reduction in memory effector differentiation and IFNγ, IL17 and IL2 cytokine profile of CD4+ and CD8+ T cells. Together, these results suggest that TGFβ signaling in DCs is important for UVB-induced skin inflammation and optimal response to hapten sensitization in a CHS model, both mediated through the DC-T cell arm of the immune response.

127 Alteration of Cytoskeletal Molecules in a Human T Cell Line Caused by Continuous Exposure to Chrysotile Asbestos

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We have been investigating the immunological effects of asbestos, since the silicosis patients who had exposed silica, the core chemical of asbestos. SiO2, often complicate the autoimmune diseases such as Rheumatoid arthritis, SSc, and ANCA-related vasculitis. Therefore, we are considering asbestos may possess the immunological effects, although the physical fibrous form is differed from particulated silica. We considered the target of asbestos may be the anti-tumor immunity, since the major complications of asbestos-exposed patients are the cancers. In this report, we investigated alteration of protein expression among MT-2 original cells that had no contact with asbestos, and six chrysotile-continuously exposed independent sub-lines using ProteinChip and two-dimensional gel electrophoresis (2DGE) assays. Further confirmation of the changes in protein expression due to asbestos exposure was obtained after the 2DGE method indicated protein modification of β-actin. β-actin was upregulated in mRNA, as were the levels of protein expression and phosphorylation. Moreover, a binding assay between cells and chrysotile showed that various molecules related to the cytoskeleton such as vimentin, myosin-9 and tubulin-P, as well as β-actin, exhibited enhanced bindings in asbestos-exposed cells. The overall findings indicate that the cell surface cytoskeleton may play an important role in inducing the cellular changes caused by asbestos in immune cells, since fibers are not incorporated to the cells and how the alterations of cytoskeleton determined cell destiny to cause the reduction of tumor immunity is important to consider the biological effects of asbestos. Further studies to target several cytoskeleton-related molecules associated with the effects of asbestos will result in a better understanding of the immunological effects of asbestos and support the development of chemo-prevention to recover anti-tumor immunity in asbestos-exposed patients.

128 Analysis of Blood DcR3 Concentration and Its Correlation to Clinical Parameter of Autoimmune Diseases in Silicosis Patients

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Silicosis patients frequently complicate with autoimmune diseases. Dysregulation of apoptosis, particularly in the Fas/Fas ligand (FasL) pathway, has been considered to play a role in the pathogenesis of autoimmune diseases. DcR3 is known as an intracellular decoy receptor that binds FasL and inhibits FasL-induced apoptosis. Initial studies showed that DcR3 expression is elevated in cancer cells, however, later work showed that DcR3 expression is also increased in inflammatory diseases, where serum DcR3 levels correlate with disease progression (Linn WW & Hsieh SL 2011). Our previous report showed DcR3 mRNA expression increased in PBMC of silicosis patients (Otsuki T et al. 2000). In this study, we analyzed blood DcR3 expression and correlation to clinical parameter of autoimmune diseases.

Plasma samples that included 19 of healthy volunteers (age 42.94±7.95, mean 8: female 11), 20 of silicosis patients (age 74.85±5.35, mean 19: female 1), 25 of systemic sclerosis patients (age 62.32±12.06, mean 3: female 1) were provided from Kawasaki Medical School hospital and Kusaka hospital (Bizen, Okayama, Japan) and Kusaka Hospital, Bizen, Okayama, Japan. All factors were analyzed with ELISA. Statistic analysis was performed using SPSS. DcR3 blood level was remarkably increased in silicosis patients compare to healthy volunteers. This result suggested that blood DcR3 increment may cause complication of autoimmune diseases through impair Fas/FasL pathway in silicosis patients. Furthermore this study showed there are no correlation between plasma DcR3 and any other clinical parameter of autoimmune diseases in silicosis patients. This result indicated plasma DcR3 elevation means impairment of immune system rather than directing particular autoimmune disease.

129 FoxO1 Regulates Apoptosis Induced by Asbestos in the Human T Cell Line MT-2

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Asbestos is known to cause malignant mesothelioma and lung cancer. Recent studies indicate the implication of tumor immunity in development of various tumors including malignant mesothelioma. Thus, human T cell line MT-2 cells were cultured to asbestos for longer than 8 months and resultant cells were designated MT-2Rst. Gene expression analysis revealed that the amount of forkhead transcription factor FoxO1 mRNA decreased after long term exposure to asbestos in MT-2 cells. According to the reduction of FoxO1, pro-apoptotic Foxo target genes, Puma, Fas ligand, and Bim, were down-regulated in MT-2Rst cells. Furthermore, ΔFvRNA-mediated knock-down of FoxO1 reduced number of apoptotic cells after treatment with asbestos in parental MT-2 cells. On the other hand, over expression of FoxO1 does not affect on asbestos-induced apoptosis in MT-2Rst cells. These results suggest that FoxO1 play an important role in regulating of asbestos-induced apoptosis, and the presence of multiple pathways regulating resistance to asbestos in MT-2Rst cells.

130 Contribution of Autoantibodies to Fiber-Induced Fibrosis

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Non-malignant pleural abnormalities arising from asbestos exposure may manifest as pleural plaques, diffuse pleural thickening, or pleural effusions. Clinical observations suggest amphibole, but not chrysotile, exposures are linked to diffuse pleural thickening and progressive loss of lung function. Recent research suggests that factors such as pathogenic autoantibodies and fiber-induced tissue damage contribute to these different clinical outcomes. In order to study the contribution of these factors to pleural disease, we used microscopy to quantify collagen deposition within the pleura and peritoneal cavities of mice exposed to amphibole or chrysotile asbestos or to the carcinogenic fiber erionite. Multiphoton microscopy was used to analyze collagen density of the lung visceral pleura or peritoneal tissue (i.e. diffuse collagen deposition) and the dissection microscope was used to assess localized collagen plaques. Mouse sera were examined for anti-nuclear antibodies (ANAs) to determine potential correlations with collagen density. Intra-tracheal exposure to fibers resulted in an overall increase of ANAs compared with saline control exposures, with the highest ANA level associated with erionite exposure. Erionite exposure was also associated with the highest collagen density within the peritoneal cavity while amphibole was associated with the highest collagen density within the pleural cavity. Collagen density was slightly increased in amphiboile-exposed, ANA-positive mice compared to ANA-negative mice; however, there was no significant correlation between ANA presence and pleural plaques. Further study is needed regarding the possible contribution of anti-fibroblast and anti-mesothelial autoantibodies to collagen deposition, since these antibodies are shown to directly increase collagen production by pleural and peritoneal cells. Overall, these data suggest that fiber exposure increases diffuse collagen deposition within the pleural and peritoneal cavities, with autoantibodies potentially contributing to diffuse, but not localized, collagen deposition.

131 Antinuclear Autoantibodies and IL-17 Are Induced in C57BL/6 Mice Following Amphibole, but Not Chrysotile, Exposure

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Exposure to amphibole asbestos has been shown to increase production of autoantibodies in mice and humans and to increase the risk of systemic autoimmune disease. However, epidemiological studies of chrysotile exposure have not indicated an increased risk for autoimmunity. To explore these apparently different responses to asbestos exposures in mice, and to suggest possible mechanistic explanations for the difference, C57BL/6 mice were exposed intratracheally to amphibole or chrysotile asbestos, or to saline only. Serum antinuclear antibodies (ANA), antibodies to extractable nuclear antigens (ENA), serum cytokines, and immunoglobulin isotypes were evaluated 8 mo after the final treatment. The percentages of FoxO1, pro-apoptotic Foxo target genes, Puma, Fas ligand, and Bim, were down-regulated in MT-2Rst cells. Furthermore, ΔFvRNA-mediated knock-down of FoxO1 reduced number of apoptotic cells after treatment with asbestos in parental MT-2 cells. On the other hand, over expression of FoxO1 does not affect on asbestos-induced apoptosis in MT-2Rst cells. These results suggest that FoxO1 play an important role in regulating of asbestos-induced apoptosis, and the presence of multiple pathways regulating resistance to asbestos in MT-2Rst cells.
overall percentages of T and B-cells in the spleen or lungs of exposed mice. Chrysotile induced a significant increase in the normally rare populations of suppressor B-cells (CD11c+, CD5+, CD11d+) in both the spleen and lungs. Overall, the results suggest that while there may be an inflammatory response to both forms of asbestos, there is an immunosuppressive response in only the amphibole-exposed, but not the chrysotile-exposed mice. These data have critical implications in terms of screening and health outcomes of asbestos-exposed populations.

132 Effect of Perinatal BPA Exposure on Spleen and Serum Pro-Inflammatory Cytokines in Weaned Rats

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Bisphenol A (BPA) is an environmental endocrine disruptor, well known for its reproductive and neurotoxicity, but it can also interact with immunity and inflammation. We have shown that perinatal exposure to BPA and gender affect MHC II levels in spleens of weaned rat. We here report the effect of perinatal BPA exposure (400μg/kg/day) on spleen and serum levels of 3 key pro-inflammatory cytokines (IL-1β, IL-6, TNFα) in both female and male rats. Oral exposure lasted throughout pregnancy and until PND9; rats were then humanely euthanized at weaning. Spleens and sera were collected, snap frozen, and stored at -80°C. Cytokines levels were measured using a multiplex approach. Spleen IL-1β was similar between females and males in both vehicle and BPA groups, but was lower in BPA-treated animals for both genders. Spleen IL-6 was similar between females and males in the vehicle group, but was higher in BPA males compared to vehicle males. Spleen TNFα was low and not affected by BPA. Serum IL-1β was lower in males compared to females in both vehicle and BPA groups, but was higher in BPA males compared to vehicle males. We did not detect any IL-6 or TNFα in these rat sera. Finally, there was no correlation between serum and spleen levels for each cytokine, but spleen cytokine levels correlated with each other. In conclusion, it appears that perinatal exposure to BPA affects peripheral pro-inflammatory cytokines, with some gender-specific effects. In addition, serum did not appear to be an adequate spleen surrogate tissue for IL-1β, IL-6, or TNFα.

133 Perinatal Exposure to Bisphenol A Alters Immune Cell Differentiation at Systemic and Intestinal Level in Young Offspring Rats


Aims: Previous data in rats showed that perinatal exposure to bisphenol A (BPA) altered intestinal immune homeostasis in young offspring, weakening host defenses to intestinal infection. We aimed to investigate whether developmental immunotoxicity during BPA perinatal exposure resulted from early alteration in immune cell differentiation. Methods: Dams were treated with BPA [5 μg/kg/d] or vehicle (corn oil) from day 15 of gestation until weaning (postnatal day 21). Immune cells from spleen, mesenteric lymph node (MLN) or jejunal lamina propria were harvested from female offspring at PND25. Analysis of immune cell frequency for T regulatory (Treg) and T helper (Th) cells, or dendritic cell (DC) was performed by flow cytometry using specific cell markers. Total and E. coli-specific IgA responses in PND25 offspring were also measured. Results: Perinatal BPA treatment decreased frequency of Treg cells (0.33+/−0.04 vs 0.39+/−0.02% of total splenocytes in controls, p<0.02) and of T cells in spleen (p<0.01) and MLN (p<0.04). These data were associated with a decrease in migratory DC frequency at systemic level (0.09+/−0.01 vs 0.16+/−0.04% of total splenocytes in controls, p<0.04), as well as in the gut and associated MLN (p<0.04). Moreover, the decline in DC and Th cells questioned the ability of BPA-exposed rats to induce cell response. As expected, a decrease in E. coli-specific IgA response (p<0.0001) was observed in serum of BPA-exposed rats, while total IgA were not impacted. Conclusion: This study shows that perinatal exposure to BPA at a dose close to the estimated dietary exposure in human (0.3-0.9 μg/kg/day, 95th percentile, European Food Safety Agency 2013) impacted the frequency of antigen presenting cells in young offspring with consequences on T helper differentiation. This led to a decline of T regulatory cells concomitant to altered IgA response toward microbiota, two important actors for systemic and local immune homeostasis.

134 Transmaternal Exposure to Bisphenol A, but Not Phthalates, Accelerates Diabetes Type 1 Development in NOD Mice

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In nodules, two important actors for systemic and local immune homeostasis.

Type 1 diabetes (T1DM) is an autoimmune destruction of insulin producing beta cells in the pancreas with a genetic predisposition that can be triggered by environmental factors. We have previously shown that bisphenol A (BPA) accelerates the spontaneous diabetes development in the diabetes-prone NOD mice. Here, we hypothesized that oral exposure to a combination of the rapidly metabolized endocrine disruptors BPA and phthalates, which is relevant for human mixed exposures, might give a further effect on diabetes development in NOD mice. A possible link between the development of T1DM and exposure through drinking water from before birth and throughout life to BPA (1mg/l), a mixture of phthalates (DEHP 1mg/l, DBP 0.2mg/l, BBP 10mg/l and DiBP 20mg/l) and a combination of BPA and the phthalate mixture was investigated in NOD mice. Presently, BPA exposure resulted in increased prevalence of diabetes and decreased number of tissue resident macrophages. Interestingly, BPA exposure also impaired the phagocytic activity of peritoneal macrophages. None of these effects were observed after phthalate exposure alone or together with BPA. Exposure to BPA alone or in combination with phthalates resulted in decreased cytokine release (TNFα, IL-6, IL-10, IFNγ, IL-4) from in vitro stimulated splenocytes, indicating a systemic change in immune function. We conclude that BPA, but not phthalates or the combination of BPA and phthalates, accelerates the diabetes development in NOD mice, possibly via decreased number and function of macrophages in pancreatic islets.

135 Total Blood Mercury, Fish Consumption, Proximity to Alluvial Arsenic Deposits, and Serum Autoantibodies among the Cheyenne River Sioux Tribe Members

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Mercury (Hg), known to induce autoimmune in rodents, is a ubiquitous toxicant on the Cheyenne River Sioux Tribal (CRST) lands. Mercury exposure happens through fish consumption (FC), a part of Native culture that supports subsistence. Our goals were to ascertain whether total blood Hg levels (THg) reflect Hg exposure via FC, smoking, and to determine if THg is associated with the production of anti-nuclear antibody (ANA) and specific auto-antibodies (sAuAb). We recruited 75 people who consume fish from CRST waters. Hg exposure via FC and smoking were assessed using questionnaires. Blood samples were collected and THg was assessed by mass spectrometry (ICP-MS). Immunofluorescence was used for serum ANA. Native, denatured DNA, chromatin and histone sAuAbs and other diseases sAuAbs were measured by ELISAs. We modeled these immune measures using demographic (age, gender) and exposure predictors. Female gender, age and FC were significant predictors of THg; self-reported smoking was not. 31% of participants tested positive for ANA stained stronger than ≥2+. Even ANA was not significantly associated with THg, the interactions of gender with THg and close proximity to As deposits were statistically significant. Also, female gender, age and FC were significantly associated with elevated sAuAb. Although FC resulted in detectable body burden of Hg, THg alone did not explain ANA or sAuAb in this population. We are working with CRST communities to investigate the relationships among FC, complex metal exposures and the community’s immune responses.
Inorganic mercury (Hg2+) is a potent immunomodulator that has been implicated as a factor contributing to autoimmune disease in animal models as well as in humans. At this time a mechanistic understanding of how Hg2+ depresses immune function remains elusive. Furthermore, while studies clearly demonstrate an association between Hg2+ exposure and increased risk of autoimmune disease on a population level, there are currently no biomarkers that can be utilized to identify Hg-induced autoimmunity. Recently there has been increased appreciation of the involvement of B cells in autoimmune disease. We have employed advanced proteomic and multicolor phosphoflow cytometric approaches to investigate the interaction of Hg2+ with immature (T1 and T2) and mature mouse B cells. Deficits in the B Cell Receptor (BCR) signaling pathway of T1 and T2 B cells is known to be associated with autoimmune disease. Lyn is an Src family protein tyrosine kinase known to be intimately involved in the initiation and regulation the BCR signaling pathway in all B cells. Lyn kinase activity is both positively and negatively regulated by phosphorylation of several strategic tyrosine residues. Results from both the proteomic and phosphoflow approaches have converged on supporting the hypothesis that altered Lyn phosphorylation and functionality consequent to mercury exposure may be key interrelated phenomena mediating immunotoxicity of mercury intoxicated B cells and the emergence of an autoimmune B cell repertoire in mercury intoxicated individuals. This being the case, expression of specific Lyn phosphorylation profiles in B cells may be useful biomarkers of Hg-induced autoimmunity in Hg2+ exposed individuals. Supported by NIH grant ES019228.

**136 Altered Lyn Phosphorylation May Be a Key Mediator of Autoimmunity Associated with Mercury Intoxication of B Cells**

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**137 Modeling Mechanism by Which Trichloroethyleno Inhibits Liver Repair in Mouse Model of Autoimmunity**

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Chronic exposure to industrial solvent and water pollutant trichloroethylene (TCE) in female MRL+/+ mice generates disease commensurate with human autoimmune hepatitis. The current study was initiated to investigate why TCE-induced autoimmunity targeted the liver. The time- and dose-dependent effects of TCE on pro-inflammatory events in liver and macrophages from female MRL+/+ mice were examined. Compared to other tissues the liver has an unusually robust capacity for repair and regeneration that protects against pathology. Consequently, the impact of TCE on these hepatoprotective events were also examined. The addition of TCE to drinking water for 12 weeks induced a dose-dependent decrease in macrophage production of IL-6 at both the transcriptional and protein level. Although often regarded as a pro-inflammatory cytokine, IL-6 is primarily protective in the liver. TCE had little effect on liver expression of pro-inflammatory genes (Tnfa, Saa2 or Ccl2) until the end of a 40-week exposure. Instead, TCE suppressed hepatic expression of genes involved in IL-6 signaling (Il6r, gp130, and Egr1). Linear regression analysis confirmed liver histopathology in the TCE-treated mice correlated with decreased expression of Il6r. A toxicodynamic model was developed to define the effects of TCE on IL-6 signaling and liver pathology under different levels of exposure and rates of repair. This study underscored the importance of longitudinal studies in mechanistic evaluations of immuntoxicants. It showed that later-occurring liver pathology caused by TCE was associated with early suppression of hepatoprotection rather than an increase in conventional pro-inflammatory events. This information was used to create a novel toxicodynamic model of TCE-induced liver inflammation.

**138 Activation of Poly(ADP-Ribose)Polymerase-1 in Trichloroethylene-Treated MRL+/+ Mice: Potential Role in Autoimmunity**

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The mechanisms by which trichloroethylene (TCE) induces/exacerbates an autoimmune response are not well understood. Previously, we have shown that TCE exposure leads to oxidative stress which contributes to TCE-mediated autoimmune. This study was aimed to further assess the role of oxidative stress in TCE-induced autoimmunity by assessing the role of poly(ADP-ribose)polymerase-1 (PARP-1, which is mainly involved in DNA repair, and apoptosis by depleting NAD+ and ATP). To achieve this, groups of female MRL+/+ mice were given TCE, N-acetylcysteine (NAC) or TCE + NAC for 6 weeks (TCE, 10 mmol/kg, i.p., every 4th day; NAC, 250 mg/kg/day through drinking water). TCE exposure led to significant increases in serum levels of anti-nuclear (ANA) and anti-dsDNA antibodies. TCE treatment also led to significant increases in the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in kidney (1.7 fold) and liver (1.6 fold) compared to controls, suggesting increased oxidative DNA damage. TCE-induced DNA damage was also associated with significant increases in PARP-1 levels which were 2.8- and 2.9-fold higher in kidney and liver, respectively. Remarkably, NAC supplementation not only attenuated DNA damage and its repair (reduced 8-OHdG and PARP-1 levels), but also the TCE-mediated autoimmunity as evident from significantly reduced serum ANA and anti-dsDNA. These results suggest that TCE-induced oxidative DNA damage along with increased PARP-1 levels may play a role in TCE-induced autoimmunity. Attenuation of PARP-1 activation in TCE-mediated autoimmunity via increased apoptosis of the cells. Supported by NIH ES016302.

**139 Evaluation of Nitrosative Stress in the Pathogenesis and Prevention of Autoimmunity Mediated by Trichloroethene**

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Exposure to trichloroethene (TCE), a ubiquitous environmental contaminant, has been linked to a variety of autoimmune diseases (ADs) including SLE, scleroderma and hepatitis. Mechanisms involved in the pathogenesis of such ADs are largely unknown. Earlier studies from our laboratory suggested the contribution of oxidative/nitrosative stress in TCE-induced autoimmunity, and N-acetylcysteine (NAC) supplementation provided protection against autoimmunity by attenuating oxidative stress. This study was undertaken to evaluate the role of nitrosative stress in TCE-mediated autoimmunity. Groups of female MRL+/+ mice were given TCE, NAC or TCE + NAC for 6 weeks (TCE, 10 mmol/kg, i.p., every 4th day; NAC, 250 mg/kg/day through drinking water). TCE exposure led to significant increases in serum levels of anti-nuclear (ANA) and anti-histone antibodies. TCE exposure also led to significant induction of iNOS and increased formation of nitrotyrosine in the sera and livers. Proteinomic analysis (2D gel followed by mass spectrometry) identified several nitrated proteins in the liver of TCE-treated mice, also suggesting an overall increase in nitrosative stress. Furthermore, TCE exposure led to decreased GSH levels and increased NF-κB p65 expression. Remarkably, NAC supplementation not only ameliorated the TCE-induced nitrosative stress, reduced GSH levels and NF-κB p65 activation, but also the markers of autoimmunity, as evident from decreased levels of autoantibodies in the sera. These findings provide further support the role of nitrosative stress in TCE-induced autoimmune response. Attenuation of TCE-induced autoimmunity in mice by NAC provides an approach for designing preventive and/or therapeutic strategies. Supported by NIH ES016302.

**140 Docosahexaenoic Acid Consumption Suppresses Silica-Induced Pneumonitis and Glomerulonephritis in Lupus-Prone NZBWF1 Mice**

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Exposure to environmental agents such as silica is suspected to contribute to initiation and exacerbation of autoimmunity in genetically predisposed individuals. In prior work, we observed in lupus-prone mice that intranasal exposure to crystalline silica induced a marked inflammatory response in the lungs characterized by elevated pro-inflammatory cytokines, perivascular lymphocytic infiltration, and secretion of IgG, as well as comparable systemic effects that resulted in early onset of glomerulonephritis. Consumption of the n-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) has been demonstrated to prevent inflammation and progression of autoimmunity in both animal and human studies, suggesting that diet is an important factor in autoimmune disease-prone individuals. The purpose of this study was to test the hypothesis that DHA consumption can prevent silica exacerbated lupus nephritis. Lupus-prone NZBWF1 mice were fed a modified AIN-93G diet supplemented with 0, 1, 3 or 6% DHA-enriched algcl oil for 2 wk prior to intranasal silica exposure (1 mg weekly for 4 wk) and maintained on experimental diets until sacrifice. Diets supplemented with 3% and 6% DHA markedly repressed perivascular lymphocytic infiltration in lungs of silica-treated mice. Repressed pneumonia was temporally related to reduced pro-inflammatory cytokines, IgG secretion, and autoantibodies to dsDNA in both bronchoalveolar lavage fluid and plasma. The anti-inflammatory effects of DHA were
further reflected in the kidneys of lupus-prone mice by delayed onset of proteinuria and reduction of glomerulonephritis. The percent energy intake from 3% and 6% DMA-enriched AIN-93G diet were comparable to 2.7% and 5.5% respectively, of dietary intake or pharmacological supplementation in humans. Taken together, these results suggest that consumption of the n-3 PUFA, DHA, might be effective in preventing silica-induced acceleration and exacerbation of autoimmunity.

141 Early Immunomodulatory Effects of Dermal Triclosan Exposure in Mice

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It has recently been shown that dermal application of the commonly used anti-microbial compound triclosan, enhanced allergic responses in a mouse asthma model. To help elucidate the mechanisms of this augmented allergic response, we investigated the early immune-related effects of dermal triclosan exposure in mice. Triclosan was applied daily to the dorsal surface of the ears of BALB/c mice at concentrations ranging from 0%-3%, and leukocytes were examined in different organs over time using flow cytometry. Examination of the draining lymph nodes revealed a dose-dependent increase in cell numbers and enhanced GATA-3, IL-4, IL-21 and IL-17 expression suggesting enhanced Th2- and Th17-like effector T cell polarization. Effects on antigen presenting cells were also observed including increased expression of MHC class II, CD80 and CD86. In mice sensitized to ovalbumin (OVA) and subsequently challenged with OVA through pharyngeal aspiration on day 7, mice exposed to triclosan had increased cell numbers in the bronchoalveolar lavage and mediastinal LN by 24 hours compared to vehicle-treated mice. Interestingly, the introduction of OVA into the airway of triclosan-exposed mice was associated with increased swelling of the ear tissue along with an influx of CD11b+ cells and dramatic increase in TSLP and TNF-alpha expression. Taken together, the early immunomodulatory effects of triclosan suggest an adjuvant-type effect that involves CD4 T cells and antigen presenting cells which may contribute to the augmentation of allergic responses.

142 An Immunologic Role of microRNA 210 in a Murine Model of Dermal Toluene-2, 4-Diisocyanate Sensitization

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Diisocyanates, such as toluene 2, 4-diisocyanate (TDI) are the principal cause of occupational asthma induced by low molecular weight chemicals. Recently, the study of immune regulation by microRNAs has revealed the importance of these regulatory molecules in allergic disease. Our laboratory has shown that mirRNA 210 (miR-210) expression increases in the draining lymph nodes (DLN) following dermal exposure to TDI in a murine model, however, the role of miR-210 in allergic disease is unknown. These studies were conducted to elucidate the functional role of miR-210 during sensitization to TDI. Female BALB/c mice were dermally exposed to TDI (4%) or vehicle. RNA was isolated from specific cellular subsets (T-cells and B-cells) at four days post exposure and analyzed for miR-210 expression using real-time quantitative polymerase chain reaction analysis. A statistically significant increase in miR-210 expression occurred in the DLI CD4+ cell population of TDI-exposed mice. Confirmed (foxxp3) and predicted (runx1, runx3, smad4, and stat6) miR-210 transcription factor targets were identified using computational algorithms. Augmentations in foxxp3 protein expression and decreases in runx1 and foxxp3 mRNA occurred concurrently with expression of miR-210 following dermal TDI exposure. Understanding the immunologic mechanisms of allergic disease is critical for the development of preventative and therapeutic strategies and these studies suggest a functional role for miR-210 in the regulatory T cell pathway and ultimately in the pathogenesis of TDI sensitization.

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Human exposure to arsenic in drinking water is known to contribute to many different health outcomes such as cancer, diabetes, and heart disease. Several epidemiological studies suggest that T cell function is also altered by drinking water arsenic exposure. However, it is unclear how individual responses differ to various levels of exposure to arsenic. Our laboratory has recently identified differential responses of human peripheral blood mononuclear cell (HPMBC) T cells as measured by polyclonal T cell activation following sodium arsenite exposure. Certain healthy individuals exposed to low concentrations (0.1-100 nM) of arsenic in vitro showed a dose-dependent suppression at low concentrations of arsenite, whereas other individuals were not suppressed at low concentrations. In a series of 30 normal donors, we found that three individuals were sensitive to low dose sodium arsenite-induced inhibition of T cell proliferation produced by phytohemagglutinin (PHA) and anti-CD3/anti-CD28. There also appeared to be a correlation with the amount of IL-2 produced by these cells in culture. Current studies suggest that donors who are sensitive to As+3 may be even more sensitive to monomethylarsonious acid (MMA+3) at concentrations of 0.1-100 nM. Our studies demonstrate for the first time that low doses of As+3 and MMA+3 are immunosuppressive to HPMBC T cells in some individuals.

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144 Association of Pro-Inflammatory and Apoptotic-Related Genes Expression with Lymphocyte DNA Telomere Length (TLT) in a Mexican Population Exposed to Arsenic


Long term exposure to inorganic arsenic (iAs) leads to a sustained inflammatory response. Chronic inflammation is frequently associated with oxidative stress which may cause telomere dysfunction, leading to apoptosis or, on the opposite side, to cells malignant transformation. It has been reported that during acute inflammation telomere length increase temporarily, maybe as a strategy to assure cell expansion and insult elimination. However, telomere length shortening has been reported in peripheral blood lymphocytes as a marker of accumulated effect of oxidative stress and inflammation. In this work we evaluated the expression of inflammatory and apoptotic related genes and its association with leucocyte telomere length (TLT) in a Mexican population chronically exposed to iAs (n=128) in drinking water. Results showed a positive association between iAs exposure and granulocyte and macrophage colony stimulating factor (GM-CSF) gene expression (p=0.00305) and TLT was positively associated with urinary concentration of DMA III-V (p=0.044), but only when variables corresponding to Apaf-1, TGF-β e IL-8 gene expression were included in the multivariate model. These preliminary results suggest that inflammatory responses may contribute to lymphocyte telomere dysfunction and it could represent a mechanism associated with an increased risk for iAs-related diseases development in exposed populations.

145 Inhibition of Early T Cell Cytokine Production by Arsenic Occurs Independently of Nrf2

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Nuclear factor erythroid 2-related factor 2 (Nrf2) is a stress-activated transcription factor that induces the expression of a variety of cytoprotective genes. Nrf2 has also been shown to mediate immunosuppressive effects in multiple inflammatory models. Upon activation, Nrf2 dissociates from its repressor protein, Keap1, and translocates to the nucleus to induce the expression of Nrf2 target genes. This Nrf2-Keap1 interaction can be disrupted by the environmental toxicant and chemotherapeutic agent arsenic trioxide (ATO). The purpose of this study was to determine the effects of ATO on early events of T cell activation and the role of Nrf2 in those effects. The Nrf2 target genes Hmox-1, Nqo-1, and Gclc were all

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found to be upregulated by ATO treatments (1-2 μM) in splenocytes derived from wild-type, but not Nrf2-/−, mice, suggesting that Nrf2 is activated by ATO in splenocytes. ATO was also found to inhibit IFNγ and IL-2 production in wild-type splenocytes activated with the T cell activator, anti-CD3/anti-CD28. However, ATO also inhibited IFNγ and IL-2 production in Nrf2-null mice, suggesting that the inhibition is independent of Nrf2. In contrast, ATO had little effect on either protein or mRNA expression of the cell surface receptors CD25 and CD69. Collectively, this study suggests that although ATO activates Nrf2 in splenocytes, inhibition of early T cell cytokine production by ATO occurs independently of Nrf2. (This work is supported by NIH grant: ES018885)

**145a Cross-Reactivity of Halogenated Platinum Salts**

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Halogenated platinum (Pt) salts are well-known respiratory sensitizers associated with the development of asthma. People may be exposed to a variety of platinum compounds in different contexts (e.g., occupationally, automobile exhaust). Published reports suggest that sensitization to one Pt compound may result in hypersensitivity reactions to other Pt compounds. We investigated the potential for this type of cross-reactivity using a mouse model of Pt hypersensitivity. Mice were sensitized through application of 100 μL 1% ammonium hexachloroplatinate (AHCP) in DMSO to the shaved back on days 0, 5 and 19, and 25 μl to each ear on days 10, 11 and 12. Unsensitized mice received vehicle. On day 24, mice were challenged by intratracheal aspiration (IA) with saline or 100 μg ammonium tetrachloroplatinate (ATCP) in saline. Before and immediately after dosing, airway responses were assessed using whole body plethysmography (WBP). On day 26, changes in ventilatory responses to methacholine (Mch) aerosol were assessed by WBP. All mice dosed with AHCP demonstrated significant increases in total serum IgE, suggesting the animals were sensitized. An immediate airway response (IAR) was observed in mice sensitized and challenged with AHCP. Dose-dependent increases in Mch responsiveness occurred in mice sensitized and challenged with AHCP. Bronchoalveolar lavage fluid (BALF) harvested from mice sensitized and challenged with AHCP contained an average of 5% eosinophils compared to less than 0.5% in control mice (p < 0.05). When challenged with 100 μg ATCP, AHCP sensitized mice also exhibited an IAR. Compared to control mice, BALF harvested from the lungs of ATCP challenged mice also contained elevated numbers of eosinophils and were responsive to Mch aerosol. Our data demonstrate that ATCP can trigger allergic lung responses in AHCP sensitized mice. This abstract does not represent EPA policy.

**145b Contact Sensitizing Potential of 2-Ethylhexyl p-methoxycinnamate in Female BALB/c Mice**

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2-Ethylhexyl p-methoxycinnamate (EHMC) is a common, active ingredient in sunscreens that absorbs in the ultraviolet B range. EHMC has been detected in both the plasma and urine of humans 3-4 hours after topical application. Published reports suggest that EHMC may be a contact sensitizer or irritant in humans, and may modulate immune responses related to hypersensitivity. The objective of this study was to evaluate the direct hypersensitivity potential of EHMC in female BALB/c mice following dermal exposure using the ICCVAM-validated local lymph node assay (LLNA) in combination with a measurement of irritancy (IRR), and the mouse ear swelling test (MEST). EHMC was applied to the dorsa of both ears in exposed insects at concentrations equal or greater than 80 and 120 μg/L/L, respectively. The LC50 values for toluene and thinner at 24 h exposure were 129.66 μg/L/L, respectively. RT-PCR analysis performed on insects exposed to thinner revealed the activation of glutathione-S-transferase, an oxidative stress marker. Toluene alone, in addition to glutathione peroxidase, also overexpressed other genes such as hypoxia-inducible factor 1 α, DNA Ligase IV and delta-9 desaturase. These results suggest T. castaneum is a suitable alternative animal model for the study of toxicological effects of volatile aromatic compounds. This insect may help predicting the toxicity of volatile organic chemicals, as it reproduces some of the effects observed in mammalian model systems. Grant from University of Cartagena. Program to Support Research Groups (2013–2014). K.C. is sponsored by the National Program for Doctoral Formation (Colciencias, 567-2012).

**148 Screening of Compounds That Alter Sleep-Wake State Alteration in Zebrafish**

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The zebrafish is emerging as alternative to mammalian animal models. Although it is more phylogenetically distant from humans than mammals, zebrafish are veritably that display other advantageous characteristics including embryo transparancy, which allows direct visualization and evaluation of internal organs or small size, which allows to manipulate them easily and to use 96- and 384-well plates to

**146 Identification of Chemical-Specific Toxicity Transcript Profiles In Vitro Using a Comprehensive or a Custom-Made Microarray**

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We have demonstrated that it is possible to define chemical mode of action (MOA) by assessing chemical-specific changes in gene expression in a small number of cell types enriched in relevant pathways for such MOA. For looking more cost effective approaches to gain information to define MOA for chemicals of interest, we have evaluated the transcriptional profile elicited by 34 chemicals in Ishikawa (uterine) and MCF7 (breast cancer) cells, using two platforms: a conventional microarray-based method (U219, Affymetrix) and a custom approach which measures the expression levels of approximately 1000 landmark genes, and uses a computational model trained to infer the response of the other genes (L1000; Genometry). Each of the 34 chemicals was tested at 3 doses and at 3 different time points. In order to compare the two platforms, Affymetrix U219 chip ID was converted to U133A chip ID, since the latter array was used to derive the probes for the L1000 array, using Affymetrix best match list. The same number of up or down regulated genes from each platform were used to query the cMap database (Broad Institute). Of the 34 chemicals evaluated, 23 are represented in the database. For those chemicals, data from either platform was specific enough to identify matches in the database for the same chemical or comparable agents acting via the same mechanism or mode of action. For chemicals not in the database, the highest ranking connections are with agents that act via the same MOA. For example, the transcriptional signature of bisphenol A, a weak estrogen, matched the one elicited by other estrogens in the database, most prominently with estradiol and genistein. These results support a conclusion that if the L1000 array may be useful as a cost-low alternative source of cMAP data. We are comparing data obtained with the same 34 chemicals tested in two additional cell types to further test this idea.
array embryos. The model is ideal to be used as a predictive tool in Drug Discovery, being able in a high extent to compare the results with those obtained in mammals. Increasing knowledge in zebrafish gives more support to this alternative model. Physiological, biochemical and behavioral daily rhythms are regulated by circadian clock. The sleep-wake cycle is a well-studied output of the circadian clock. Apart from having many biological advantages, zebrafish are diurnal and present highly conserved circadian and sleep regulation systems, so they have been postulated also as a good model for circadian rhythm studies. Behavioral profiling in zebrafish reveals a conserved vertebrate neuropharmacology and identifies regulators of rest/wake states. For screening purposes, working with zebrafish larvae has many advantages in terms of rapidity and throughput.

The main objective of this study was to validate a protocol to detect compounds with potential to alter rest/wake states in zebrafish. For this purpose, zebrafish larvae were exposed to melatonin and other reference drugs as barbiturates and benzodiazepines directly in 96 well plates. After treatment larvae movement was tracked during 24 hours automatically. Diurnal and nocturnal locomotor activity and responsiveness of larvae are the main endpoints related to sleep states. This study supports the use of zebrafish in order to detect compounds that alter the sleep-wake states.

### 149 Studying Obesogens Using Zebrafish

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There is a growing prevalence in adult and childhood obesity, which leads to medical complications such as heart diseases, diabetes and other metabolic abnormalities. More than 35% of adults and roughly 17% of children in the U.S. are obese, with a projected increase in obesity prevalence in 2030 estimated at 33%. Recently, the American Medical Association officially classified obesity as a disease. Human health has been shown to be affected by the increasing presence of environmental pollutants in forms of industrial products (pesticides, drugs, plasticizers, etc.), some of which have been reported to lead to obesogenic effects in animal models. Indeed, exposure in utero to tributyltin (TBT) has been shown to predispose obesity in mice. Previously, we reported tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA), both commonly used as flame retardants, activate human and zebrafish peroxisome proliferator-activated receptor gamma (PPARγ) in vitro and induce adipogenesis in 3T3-L1 preadipocytes. PPARγ, highly expressed in adipose tissues, promotes adipogenesis and regulates lipid metabolism. To further investigate the link between environmental pollutants and obesity, we have utilized zebrafish as an obesogen model. We describe the metabolic fate of TBBPA and TCBPA to better assess the use of zebrafish as a vertebrate model for mamma- lian endocrine disruption. Additionally, we explore in vivo and in vitro activation of both human and zebrafish PPARγ by pharmaceutical human PPARγ ligands, TBBPA, TCBPA and TBT. We also show that TBBPA, TCBPA and TBT act as obesogens and promote lipid accumulation in zebrafish larvae, as well as induce late-onset obesity.

### 150 Effects of LXR Activation on Lipid Metabolism and Visual Function in Zebrafish

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Liver X receptor (LXR), a ligand-activated transcription factor, is a key regula- tor of multiple pathways involved in lipid, cholesterol and carbohydrate metabo- lism. While there are two LXR isoforms in mammals, LXRα (NR1H3) and LXRβ (NR1H2), only one LXR gene with higher sequence similarity to mammalian LXRα has been reported in zebrafish (ζLXR). LXR has been shown to be expressed at early developmental stages in zf, suggesting a primordial role of LXR. To assess the effects of LXR activation during zf development, zlf larvae were exposed to the LXR agonists T0901317 (2μM) and GW3965 (1μM) from 4 to 6 days post-fertilization (dpf), and RNA was extracted for microarray and qRT-PCR analyses. Microarray data demonstrated several conserved effects of the LXR ligands with mammalian models, including upregulation of known mammalian LXR target genes, and sev- eral genes involved in fatty acid and cholesterol biosynthetic pathways. These pathways could potentially be used to assess the effects of environmental compounds target- ing LXR in zebrafish. Our data shows that LXR plays an important role in lipid metabolic pathways during zf development and supports zebrafish as an alternative model for LXR-related studies involving lipid metabolism. Moreover, ζLXR exposure to the LXR ligands resulted in regulation of genes associated with eye development and visual perception, including photoreceptor and lens-specific genes, suggesting that LXR may also play a role in eye development and visual function in zebrafish.
Effects of Methymercury on Dopaminergic Neuron Development in Zebrafish Larvae

Methymercury (MeHg) is a well known neurotoxin capable of altering the structure and function of the central nervous system. Many signs of MeHg toxicity, including hypoesthesia and tremors, are similar to that of many neurodegenerative diseases, most notably Parkinson’s disease (PD). The disease is pathologically characterized by the progressive loss of dopaminergic (DA) neurons in the midbrain, resulting in the reduction of dopamine secretion and the development of movement disorders. Wild type zebrafish (ZF; Danio rerio) and the transgenic strain (Tg(dat:EGFP)), expressing green fluorescence protein (GFP) in DA neurons of the ventral diencephalon, were used to evaluate the effects of environmentally relevant concentrations of MeHg on the progression of PD-like phenotypes. Three day old ZF larvae were exposed to either 0.06 or 0.3 μM MeHg for 48h. Preliminary data from whole-mount immunostaining for thyrosine hydroxylase and live fluorescence imaging of dopamine transporters suggested disruption of the typical horseshoe pattern of DA neurons in MeHg exposed wild type and Tg(dat:EGFP) larvae. The effects of MeHg on DA neurons appeared to be dose correlated. Preliminarily locomotor assessments also showed a decreased mobility in MeHg exposed fish. There appeared to be a trend of reduced swimming activities in the 0.06 μM MeHg treated larvae, similar to those treated with 250 μM of 6-hydroxydopamine hydrochloride, a positive control for PD-like phenotype. Morphological changes such as endema, curvature of the spine, and hemorrhages confirmed MeHg toxicity in the exposed fish. mRNA expressions of parkin, PINK1, and LRRK2 genes associated with the recessive familial PD, were also determined. The preliminary results suggest that exposure to MeHg may increase the predisposition to neurodegenerative diseases, particularly PD, in a zebrafish model.

The Challenge of Studying Exposure in Zebrafish Embryos

The embryonic and/or larval zebrafish model is rapidly gaining popularity to study toxicity of new compounds. Various characteristics warrant the zebrafish as an ideal non-mammalian whole organism model that could bridge gaps between in vitro cell systems and complex higher animal models. A drawback of the zebrafish model is that the chorion, a shell that protects the embryo in its earliest days, can act as a barrier for test compounds which might result in false negative test outcomes. However, using whole-organism homogenates to analyze total body burden might not give a realistic picture of the internal exposure of the embryo since compounds might stick to the outside of the chorion. Furthermore, in larvae, this approach will not give any information about the biodistribution of compounds. In the present project we studied the uptake and biodistribution of a series of test compounds with different physical and chemical properties. Hereto, zebrafish embryos and larvae were treated with radioactive labeled test compounds for different time periods. By using autoradiography of serial microscopic sections, it was shown that both molecular weight and logP influenced the passage through the chorion, the uptake into the embryo and the biodistribution of the compounds. In general, total body burden analysis of compounds with a high molecular weight suggested considerable uptake by larvae, these compounds did not pass the epithelial cells of the gastrointestinal tract and stayed in the lumen. Consequently, internal exposure to these compounds was limited. By testing the biodistribution of more compounds and taken into account the physical chemical properties of a compound, the predictability of the zebrafish model will be improved.

High-Content Screening Assay for Identification of Chemicals Impacting Cardiovascular Function in Zebrafish Embryos

Targeted assays are needed to better evaluate effects of chemicals on organogenesis and begin classification of chemicals by toxicologically relevant modes-of-action. Using transgenic zebrafish (fli1:egfp) that stably express enhanced green fluorescent protein (eGFP) within vascular endothelial cells, we have developed and optimized a 384-well based high-content screening (HCS) assay that enables us to screen and identify chemicals affecting cardiovascular function at sub-lethal, non-teratogenic concentrations. Following static exposure of one embryo per well from 5-72 hours post-fertilization (hpf), automated image acquisition procedures and custom analysis protocols are used to quantify body length, circulation, heart rate, pericardial area (a biomarker for cardiac looping defects), and intersegmental vessel area within freshly hatched live embryos. After optimizing 72-hpf anesthesia procedures, we evaluated each endpoint across four independent control plates containing 384 initial embryos per plate. Survival and imaging success rates across these plates ranged from 93-99% and 42-74%, respectively. Criteria were then defined for assay success and analysis of treatments, and 10 chemicals were screened for targeted effects on cardiovascular function. Compared to existing zebrafish-based assays, this method provides a comprehensive discovery platform with 1) increased sample sizes; 2) broad concentration-response format; and 3) the ability to identify chemicals that target cardiovascular function at non-teratogenic concentrations.

A New Transgenic Zebrafish Model to Predict Organ Toxicities in Mammals

Zebrafish have many attractions for toxicity testing: they are cheap and easy to breed under laboratory conditions, can readily be exposed to chemical and pharmacological compounds and are susceptible to genetic manipulation. Reference compounds with known or no in vivo liver toxicity were tested on larval and adult zebrafish as well as isolated liver microsomes and the effects compared to available in vitro (hepatocyte) and in vivo (clinical/preclinical) data. Uptake and metabolism of compounds was analysed by LC/MS. To maximize test compound throughput, transgenic reporter lines have been generated based on our analysis of the transcriptional response of zebrafish larvae to a set of reference compounds. One potential limitation of the zebrafish is the functional divergence of its bioactivation and detoxification systems compared to those of preclinical mammalian models used in drug development: testing with human CYP3A4 specific substrates revealed differences in their metabolism between zebrafish and human. To circumvent this limitation we have generated “humanized” transgenic zebrafish using either a human Bacterial Artificial Chromosome (BAC) containing the human CYP3A4 locus or the human CYP3A4 cDNA cloned downstream of a liver specific promoter. Enzyme activity studies with the human CYP3A4 substrate, midazolam,
showed a significant increase in 1-hdyroxy-midazolam metabolite generation in
these transgenic lines compared to wild-type. The metabolic and transcriptional
response of the “humanized” fish to a set of test compounds has been compared to
that of wild-type fish. The results will be presented and discussed in the context of
the utility of the humanized line for pre-clinical safety testing.

158

Novel System for Express Screening of Organ/Tissue-Specific
Toxicity in Live Juvenile and Adult Zebrafish

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Sponsor: A. Lyubimov.
We propose a cost-effective high throughput-screening model for in vivo testing of
organ/tissue-specific toxicity and efficacy in real time in adult zebrafish whose internal organs/tissues are selectively labeled with an endogenously expressed fluorescent
protein, which is released into circulation upon toxic destruction of the organ/
tissue. Thus, the fluorescence can be detected in off-site tissues as a result of the fluorescent protein molecules release from damaged cells followed by the fluorescent
protein diffusion and distribution via circulation from the targeted organ to neighbor and distant tissues. This ectopic fluorescence can be detected and quantitatively
assessed in vivo by a comparison of fluorescence in the non-targeted tissue in untreated/non-transgenic fish vs. treated fish, wherein the change is indicative of the
toxic/pharmacological activity. The accuracy of detection of ectopic fluorescence
has further been enhanced by using optically transparent zebrafish strains (sheer or
casper) expressing fluorescent proteins in targeted organs, e.g. liver, pancreas, heart,
brain etc. With multiple reporter fluorescent proteins the measurements are carried
out in different spectral conditions specific for each reporter. This technology has
been tested using DEN toxicity towards liver and pancreas in transgenic zebrafish
harboring DsRed2- and GFP-tags in liver and pancreas, respectively. Transgenic
zebrafish (i.e., an optically transparent strain) with high levels of a fluorescent reporter in hepatocytes has responded to hepatotoxic compound DEN by releasing
DsRed2 fluorescent protein into circulation. As a result fluorescence has been accumulated in areas distant from the liver, such as tail, fin and others. In contrast,
DEN had no effect of GFP release from pancreas. This assay is non-invasive and
can be carried out on the same fish multiple times for monitoring time-course toxicity. The same strategy will be applied to an assessment of pharmacological effects
of antitumor drugs on syngeneic and allogeneic tumors, growing in fish.

159

Cytotoxicity of Natural Toxins Mediated by the Organic
Anion Transporting Polypeptide (OATP1B3)

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Canada.
Microcystins (MCYSTs) are natural toxins that inhibit serine-threonine protein
phosphatase. The protein phosphatase inhibition assay (PPIA) has been used in our
laboratory as a screening method to detect MCYSTs in lake water since 2007. We
recently developed a novel assay using the real-time cell analyzer (RTCA, Roche
xCELLigence) for detecting MCYST cytotoxicity. The assay is based on the fact
that MCYSTs toxicity requires the active uptake of MCYSTs into the cytoplasma
membrane which is mediated by the organic anion transporting polypeptides
(OATPs). The aims of this study were (1) to investigate differential cytotoxicities
of other natural toxins that possibly presented in water including nodularin, tautomycin, and MCYST analogues and (2) to observe the role of transporters in the
toxicities of these natural toxins.
The wild type Chinese hamster ovarian cells (CHO/WT) and CHO with
OATP1B3 expression (CHO/OATP1B3) were exposed to the toxins using the
RTCA method. After 48h of incubation, no toxic effect was observed in CHO/
WT, while growth inhibition was demonstrated in CHO/OATP1B3. Coincubation of toxins with the known substrates of OATPs, taurocholate (TC) and
bromosulfophthalein (BSP), inhibited the uptake of toxins through the OATP1B3
transporter and decreased the cytotoxic effects. The in vitro analysis of inhibition
on purified protein phosphatase, PP1, was also performed to compare the differential potencies of the toxins. Inhibition concentrations at 50% (IC50) from both
cytotoxicity and PPIA were compared.
In conclusion, we demonstrated that some natural toxins, such as nodularin, tautomycin and MCYSTs, require the active uptake by the OATP1B3 to induce cytotoxicity. The cytoxicity potencies are not necessarily related to the PP1 inhibition
potencies.

160

Development of an In Vitro Assay Measuring Estrogenic
Responses for Use in Chemical Safety Assessment

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To support the vision outlined in the 2007 report “Toxicity Testing in the 21st
Century: A Vision and A Strategy”, our laboratory is evaluating key cell response
networks to develop proof of concept safety assessments for a series of prototype
compounds and networks, including the estrogen receptor (ER)-mediated uterine proliferation pathway. We evaluated the ability of a human uterine epithelial
adenocarcinoma cell line (Ishikawa) to recapitulate in vitro phenotypic responses
to ethinyl estradiol (EE), including proliferation, epithelial-to-mesenchymal transition (EMT), and transcriptional activation of key ER-mediated genes (PGR,
ALPP, GREB1). Cells were treated with vehicle (ethanol; EtOH) or EE (18 doses,
10-14 to 10-6M) for 1-6 days and cell viability was measured. This phenotypic
anchor, an approximate 1.2 fold increase in cell viability, helped inform the dose
range selection of EE that caused no effect, minimal effect, or maximal effect,
which were then used to further examine transcriptional responses encompassing
several earlier time points (6, 12, 24, 72 hours) as an indicator of transcriptionally
mediated precursor events. The lowest concentration at which we observed gene
expression changes was 1pM at 12, 24, and 72 hours, the same dose at which
we observed a phenotypic effect. Interestingly, DEPTOR, a negative regulator
of mTOR, is upregulated at these low doses. EMT, an additional in vivo and in
vitro phenotypic outcome associated with estrogen signaling, occurred at doses and
times similar to those inducing proliferation (day 3; 10pM). Our results suggest
that EE induced increased cell viability, EMT, and transcriptional activation occur
at similar doses and are consistent with in vivo responses providing evidence that
Ishikawa cells are an adequate platform for evaluating estrogen mediated cellular
response networks.

161

A Robotic MCF-7:WS8 Cell Proliferation Assay to Detect
Agonist and Antagonist Estrogenic Activity

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Endocrine disrupting chemicals with estrogenic (EA) or anti-estrogenic (AEA) activity have been extensively reported to possibly have many adverse health effects.
We have developed robotized assays using MCF-7:WS8 cell proliferation (or suppression) to detect EA (or AEA) of 78 test substances supplied by the Interagency
Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
and the National Toxicology Program’s Interagency Center for the Evaluation of
Alternative Toxicological Methods (NICEATM) for validation studies. We also
assayed ICI 182,780, a strong estrogen antagonist. Chemicals to be assayed were
initially examined for solubility and volatility to determine optimal assay conditions. For both EA and AEA determinations, a range-finder assay was conducted
to determine the concentration range for testing, followed by a comprehensive
assay. Test substances with potentially positive results from an EA comprehensive
assay were subjected to an EA confirmation assay that evaluated the ability of ICI
182,780 to reverse chemically-induced MCF-7 cell proliferation. The AEA assays
examined the ability of chemicals to decrease MCF-7 cell proliferation induced by
non-saturating concentrations of 17β-estradiol (E2), relative to ICI or raloxifene
(RAL), also a strong estrogen antagonist. To be classified as having AEA, a saturating concentration of E2 had to significantly reverse the decrease in cell proliferation produced by the test substance in non-saturating E2. We conclude that our
robotized MCF-7 EA and AEA Assays have accuracy, sensitivity, and specificity
values at least equivalent to validated test methods accepted by the US EPA and the
Organisation for Economic Co-operation and Development (OECD).

162

Evaluation of the p53-Mediated DNA Damage Response
Toxicity Pathway Using Chemicals with Distinct
Mechanisms of DNA Damage

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As part of a larger effort to provide proof of concept in vitro only risk assessments,
we have developed a suite of high throughput assays for key readouts in the p53
DNA damage response toxicity pathway: double strand breaks (p-H2AX), fixed
chromosomal damage (micronuclei; MN), p53 activation (p53, p-p53), p53 tran-

38     SOT 2014 Annual Meeting


scripational regulation (MDM2, WIP1, p21) and cell fate (cell cycle, apoptosis). 18 point dose-response curves were generated for protein and cell fate endpoints in a p53 competent cell line (HT1080) for 3 mechanistically-distinct chemicals: etoposide, methylmethane sulfonate, and quercetin. Gene array analysis was also performed for each chemical at 7 doses, and determined those with no effect, minimal effect, or maximal effect on MN response. Evaluation of cellular response across chemicals and doses revealed two important observations. 1) The p53 transcriptional program is chemical dependent. At similar levels of DNA damage, the three chemicals cause very different protein and cellular responses (apoptosis vs. arrest). Together with previously published CHIP-seq studies, our transcriptional data indicate that these different responses are determined very early (i.e., prior to transcriptional activation) and are likely a result of differential kinase activation and augmentation of the general p53 transcriptional response by co-regulators. 2) The p53 transcriptional response does not prevent permanent DNA damage induction at low doses. MN induction occurs at doses equal to, or lower than, doses required to activate p53-mediated gene transcription. This observation is important because it indicates that any protective effect of p53 is likely due to p53’s activity as a recruitment factor for repair proteins at the site of DNA damage. We are currently evaluating the role of post-translational p53 activities (repair center formation) in the prevention of permanent DNA damage at low chemical doses.

163 Embryonic Stem Cells Carrying a Transgenic BMP-Reporter Construct: A Useful Tool for the Identification and Analysis of Teratogenic Compounds In Vitro

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W. Dekant1.

Genesis

Monteiro, R.M., S.M.de Sousa Lopes, M.Bialecka, B.S.de, A.Zwijsen, and

During the differentiation of ESC into cardiomyocytes, we analyzed the differentiation of ESC into various tissues is developed that allow the

We have isolated and characterized embryonic stem cells from transgenic mice carrying a EGFP transgene under the control of a BMP responsive element that has been shown to nicely recapitate in vitro BMP activity (Monteiro et al. 2008). During the differentiation of ESC into cardiomyocytes, we analyzed the differentiation process using quantitative RT-PCR, FACS, quantitative EGFP measurements, and the functional analysis of contracting cardiomyocytes. Our results show that the activity of the reporter gene can be used for the detection of teratogenic activities of valproic acid, retinoic acid, and 6-aminonicotinamide. These cells provide a useful tool to characterize the molecular mechanism un-

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Embryonic stem cells (ESC) are used as a tool for the identification of teratogenic modes of action in the analysis of chemicals or pharmaceuticals. In particular, the embryonic stem cell test (EST) has been scientifically validated previously and is now used for screening purposes. The differentiation of ESC into various tissues is regulated by a set of essential signaling pathways, including the TGF, Wnt, and Shh pathways as well as tyrosine kinase receptors mediating FGF or EGF signaling. For some of these essential signaling pathways, transgenic reporter mice have been developed that allow the in vitro analysis of pathway activity during embryonic development. We have isolated and characterized embryonic stem cells from transgenic mice carrying a EGFP transgene under the control of a BMP responsive element that has been shown to nicely recapitate in vitro BMP activity (Monteiro et al. 2008). During the differentiation of ESC into cardiomyocytes, we analyzed the differentiation process using quantitative RT-PCR, FACS, quantitative EGFP measurements, and the functional analysis of contracting cardiomyocytes. Our results show that the activity of the reporter gene can be used for the detection of teratogenic activities of valproic acid, retinoic acid, and 6-aminonicotinamide in vitro. In addition, these cells provide a useful tool to characterize the molecular mechanism underlying the activity of chemicals or pharmaceuticals on the differentiation process during early embryonic development.


164 Cyclosporin A Pharmacokinetics in In Vitro Systems

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The immunosuppressant Cyclosporin A (CsA) is administered to reduce graft rejections after transplantation in patients, but it is also nephro-, hematopoietic diseases, and neurotoxic. Hepatotoxicity is related to elevated CsA blood levels and CYP3A4/5 activity in patients. Nephro- and hepatotoxicity can be diminished by drug monitoring and adjusted CsA administration. In this work, Cyclosporin A was used as a model compound to assess the kinetic profiles in three liver models (primary human hepatocytes [PHH], primary rat hepatocytes [PRH] and HepaRG cells) after repeated dosing. Cells were treated for up to 14 days with a high (TC10) and a non-toxic concentration (1/10th of TC10) specifically determined for each model. CsA concentrations were measured by LC-MS/MS in the treatment solutions, cell culture media, cell lysates and plastic binding samples at 5 specific time points on the first and the last treatment day. Comparisons across the models revealed three different kinetic profiles. Both PHH and HepaRG cells significantly metabolized CsA and a no bioaccumulation was observed after repeated dosing. Instead, CsA accumulated in PRH. Comparison of kinetics in HepaRG cells and PHH indicated the difference between a cell line and a primary cell culture, with the latter showing biological variability between different human donors. These results underline the interspecies difference for the CYP3A4 metabolism route between human and rat hepatocytes, which can also be observed in vivo.

165 Utilizing Human Population-Based In Vitro Model to Investigate Pesticide Mixtures and Drug/Metabolite Pairs

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A population-based human in vitro model offers exceptional opportunities for evaluating the potential hazard, mode of action, and population variability in response to chemicals. Challenges remain that require further assessment to increase the utility of the information obtained from this model. This study was designed to address two of these challenges: limited metabolic capacity of lymphoblasts and the potential to screen complex mixtures. We selected lymphoblast cell lines from 4 racially and geographically diverse populations based on availability of genome sequence and basal RNA-seq. For the mixture experiment, 146 cell lines were exposed to pesticide mixtures (organochlorine pesticide environmental mixture extracted from a passive surface water sampling device, or a mixture of 36 currently used pesticides). For the drug/metabolite experiment, we exposed 331 cell lines to Carbamazepine, Sulfamethoxazole, or two major metabolites of each drug. Concentration-response cell viability data was used to derive a 10% effect (or no-effect) level for each compound/cell line. We found that a mixture of pesticides currently in-use was less toxic but more variable than a mixture of organochlorine pesticides. A significant correlation between pesticide mixtures indicates that cells that were sensitive to one pesticide mixture were likely sensitive to the other. Significant population differences were found for the currently used pesticide mixture. For drug/metabolite pairs, we observed a wide range of inter-individual variability in cytotoxicity. Significant pairwise correlations of EC10 were found for each drug and its two metabolites. We found statistically significant and/or suggestive associations between genetic variants and cytotoxicity of each drug/metabolite. These data provide the opportunity to establish population-based confidence intervals in cytotoxicity, as well as probe candidate susceptibility pathways.

166 Toll-Like Receptor 4 Signaling Heterogeneity in Different Cell Models Can Impact Data Interpretation and Toxicity Testing

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Toll-like receptor (TLR) mediated pro-inflammatory signaling in response to pathogen-associated molecular patterns (PAMPs) and endogenous ligands has been implicated in a diverse array of destructive immunopathological consequences. Divergence and heterogeneity in TLR4 surface expression and signaling in immortalized cell models may present advantages or limitations in experimental applications. The aim of the present study was to evaluate TLR4 functionality in in vitro models of diverse human tissue origin: THP-1 acute monocytic leukemia, HepG2 hepatocellular carcinoma, Caco2 colorectal adenocarcinoma, and H295R adrenocortical carcinoma. LPS-mediated induction of the TLR4 pathway was assessed by seeding cells into 96-well plates at 10,000 cells/well followed by 24 hr equilibration at 37°C, 5% CO2. Cells were exposed to LPS (0.1, 1, 10, 100, 1000, and 10,000 ng/mL), an antagonist naloxone (1, 10, 100, and 1000 μM), or LPS + naloxone for a period of 24 hr. Media was collected for IL8 ELISA quantitation. The THP-1 cell line exhibited a clear concentration-response to LPS, reaching a maximum of 375 pg/mL IL8 at 10,000 ng/mL LPS. HepG2 cells showed a small increase at the highest LPS exposure (150 pg/mL IL8 at 10,000 ng/mL LPS). Caco2 (>50 pg/mL IL8 at 10,000 ng/mL LPS) and H295R (<5 pg/mL IL8 at 10,000 ng/mL LPS) cells also showed minimal effects. These data indicate concentration-dependence of THP-1 to LPS and attenuation of LPS-mediated activity by simultaneous naloxone exposure (e.g., 60 pg/mL IL8 at 10,000 ng/mL LPS + 1000 μM naloxone). IL8 expression exhibited by the other in vitro models indicates low background production of the cytokine and an apparent lack of LPS concentration-dependent upregulation. Interestingly, naloxone appears to inhibit the expression of IL8 in the absence of LPS stimulation in all cell lines tested, with the exception of the monocytic THP-1. In conclusion, selection of a cell line can greatly influence the data obtained and subsequent interpretations regarding the role of TLR4 in any observed effects.

SOT 2014 ANNUAL MEETING 39
167 Assessing Cellular Stress via Hif-1α and Nrf2 Signaling Using Protein Stability and Transcriptional Reporters
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The signaling pathways involved in the cellular stress response have been identified as useful screening targets for assessing potential compound toxicity that can be studied in a variety of in vitro cell model systems. We have used reporter gene assays to study two specific pathways involved in cellular stress response: hypoxia and antioxidant response as mediated by Hif-1α and Nrf2 transcription factors, respectively. For both pathways we tested the hypothesis that monitoring stability of the transcription factor protein directly using novel protein fusion reporters would accurately reflect changes in transcriptional activity as measured by the expression of a reporter being driven by response element containing promoters. Assessment was performed in transiently transfected human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iCell® Cardiomyocytes) followed by treatment with cobalt chloride or 1,10-phenanthroline (Hif-1α) and D.L-sulforaphane or tert-butylhydroquinone (tBHQ) (Nrf2). Results demonstrate that changes in the signal from the protein reporter consistently reflect the observed changes in transcriptional activity at similar exposure concentrations. In addition, treating HEK293 cells stably transfected with Hif-1α or Nrf2 response element reporter constructs with the chemical inducers described above demonstrate that transcriptional induction can be multiplexed with a cell viability assay allowing for direct measurement of both reporter gene and cell viability from the same assay well. As toxicity assessment efforts continue to move towards utilizing more physiologically relevant in vitro model systems, the ability to monitor cellular stress using reporters closer to the primary signaling event, such as the stability of the transcription factor protein directly, may be a useful complement to more traditional transcriptional reporter assays. In addition, the ability to multiplex reporter and viability assays may be valuable, particularly when utilizing limited cell types such as iPSC or primary cells.

168 Characterization of a 3D Scaffold-Free Culture of Human Breast Carcinoma Cells to Understand Estrogenic Endocrine Disruption
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The use of scaffold-free agarose hydrogels provides a system in which cells form spheroids, initiating contact with other cells and the matrix. We have demonstrated the use of this system to culture MCF-7 human breast cancer cells. MCF-7 cells within this model self-aggregate and develop into differentiated spheroids, possessing a defined luminal space. As shown by transmission electron microscopy, cells in MCF-7 spheroids display tight junctions, desmosomes and apical/basal polarity. MCF-7 cells grown in 3D display increased expression of breast-differentiation markers including cytokeratin 18 (KRT18). When compared to 2D cultures, 3D cultures show a reduced fold induction of mRNA of estrogen responsive genes (GREB, PGR and CTSD) following estradiol exposure in concentration response and time course studies. The expression of e-cadherin (CDH1) was downregulated in 2D cultures following estradiol exposure. Interestingly, CDH1 mRNA was increased in 3D cultures as soon as 2 hours post-exposure as demonstrated by RT-PCR and Western blotting. We propose to use this system to assess the molecular and phenotypic changes associated with exposure to endocrine disrupting compounds (EDCs), including diethylstilbestrol, bisphenol A and genistein. The use of a differentiated scaffold-free 3D culture system offers a unique opportunity to study the phenotypic and molecular changes associated with exposure to EDCs. 3D cell culture models are intriguing due to their ability to bridge the gap between animal models and traditional 2D cell culture. 3D models allow for the growth of human cells in an environment that is closer to the in vivo environment compared to tissue culture plastic. While many 3D models rely on scaffolded matrices for the growth of cells at low densities, scaffold-free models allow cells to aggregate and maximize cell-cell contact, free of the influence of surrounding matrix.

169 Effects of Phenylethylamines on Huh-7 Human Hepatoma Cells Studied with Untargeted Global Metabolomics
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Phenylethylamines such as β-phenylethylamine (PEA) and N,N-dimethylphenylethylamine (DMPEA) are found in products marketed as dietary supplements. While the major safety concern with this class of compounds is cardiovascular toxicity, the hepatotoxicity of some phenylethylamines such as methamphetamine and MDMA is well established. PEA and DMPEA were initially screened in Huh-7 cells at concentrations from 5 to 1000 μg/mL for 48 hours. Significant cytotoxicity was observed at 500 μg/mL and above for both compounds. PEA but not DMPEA showed a significant dose-related increase in reactive oxygen species as concentration increased. LCMS-based metabolomics studies were conducted on cells treated with PEA or DMPEA at 100 μg/mL for 48 hours. Examination of the supernatant culture medium identified several putative metabolites of both PEA and DMPEA. The most prominent metabolite for both compounds was N-phenylacetyl glutamine suggesting that the major pathway for both compounds was through oxidative de-ammoniation by monoamine oxidase to phenylacetaldelyde. Significant (p<0.05, fold change=1.5) metabolic changes in the supernatant medium from PEA treatment included increased release of mevalonic acid from the cells, suggesting increased activity of the cholesterol synthesis pathway. Significant metabolic changes in the supernatant medium from DMPEA treatment included; increased utilization by cells of amino acids from the medium for cellular function and protein synthesis pathways; and, decreased release of creatine and 1-methylhistoimtanide from cells, suggesting decreased flux through intracellular bioenergetic pathways. These findings suggest that PEA and DMPEA are extensively metabolized by Huh-7 cells and that these compounds and/or their metabolites can alter liver metabolism in ways that could lead to liver toxicity.

170 An Integrative Approach for the Prediction of Acute Oral Toxicity: Past and Future

The model presented assesses results of mechanism-based in vitro assays and relevant physical-chemical properties to predict an LD50 value, the standard measure of acute oral toxicity. The uniqueness of such a model is that it can serve multiple purposes, at a multidisciplinary level (Chemistry, Biology and Safety Evaluation): to rank-order compounds and guide the design or identification of hits for further testing, to identify potential subcellular targets and hypothetical mechanisms of acute oral toxicity, and to provide an estimate of acute oral toxicity for labeling purposes. Such a testing strategy has been implemented using a series of 57 compounds in the frame of a long-range research program with CeeTox, Inc. (SOT 2010, 2011, 2012 and 2013). The predictive performance of our model was assessed using a set of 100 public domain compounds. Chemicals were defined as toxic (T) or non-toxic (NT) on the basis of an in vivo LD50 threshold of 500 mg/kg, a value that could be acceptable for early efficacy testing. We showed that the overall concordance was above 85%, the sensitivity and the specificity being 87% and 89% respectively. To expand the applicability domain of the model and anticipate the complexity and diversity of cosmetic products, a set of reactive and non-reactive proprietary compounds was evaluated. The model remained highly predictive of the 39 chemicals categorized as non reactive (sensitivity = 83%, specificity = 94%) while for the 43 reactive compounds, the model could not be applied. This study is a clear demonstration that one way to address acute oral toxicity is to combine multiple, mechanism-based parameters and key chemical properties. However, further efforts are needed to fully handle the complexity of cosmetic ingredients. Incorporating SAR and metabolism for example, could be a way to expand the applicability domain of this model.

171 DNA Damage and Adduct Formation by Prototype Metabolic Activation-Dependent DNA-Reactive Carcinogens in Chicken Embryo-Fetal Liver
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Prototype metabolic activation-dependent DNA-reactive carcinogens and structurally related, mutagenic, but less potent carcinogens or noncarcinogens were evaluated in the chicken egg genotoxicity (CEGA) assay, which provides a non-animal intact and aseptic test organism. In the assay, 3 daily doses, 48, 24 and 3 hr prior to termination, were administered to 9 to 11 day old embryo-fetuses and livers were harvested for measurement of two endpoints, DNA breaks using the alkaline single cell gel electrophoresis (comet) assay and DNA adducts using the N-2-hydroxy-1,4-nucleoside phosphate (NPL) assay. The effects of two carcinogens of different structures requiring distinct pathways of bioactivation, 2-acetylaminofluorene (AAF), aflatoxin B1, benzo[a]pyrene (BaP), and diethylthiuramoxide, were compared to their structurally related noncarcinogens fluorene (FLU) and benzo[a]pyrene (BeP) or...
weak carcinogens, alflaxatin B, and nitrosodiethanolamine. The four prototype carcinogens produced DNA breaks, whereas less poter carcinogens and noncarcinogens yielded borderline or negative results, respectively. AAF and BaP also produced DNA adducts, whereas none was found with FLU or BeP, consistent with comet results. Thus, the CEGA, using comet and NPL, is capable of detect- ing the genotoxicity of diverse carcinogens while not yielding false positives for non-carcinogens.

172 Genetic Deletions in Mitochondrial Cholesterol Transporters Result in Germline Defects in C. elegans

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Basic DNA replication and mitochondrial machinery are conserved in the nem- atode C. elegans. Because of this, it is a prime organism to investigate the contribu- tions of individual mitochondrial cholesterol transporters to normal cholesterol homeostasis within the developing germline. Previous studies performed by our laboratory and others indicate that Bisphenol A (BPA) causes defects in the de- veloping germline of both C. elegans and rodents models. In C. elegans, defects are prevented by the addition of cholesterol to the treatment medium. To investigate whether inhibiting cholesterol uptake affects germline development, we have char- acterized several strains of C. elegans with null deletions in one or more mitochondri- al cholesterol transporters. Preliminary experiments in adult C. elegans lacking either functional Steroid Acute Regulator Protein (StAR) or 18kDa translocator protein (TSPO) orthologs show an increase in the incidence of apoptosis in germ cell nuclei relative to control worms as determined by acridine orange staining. Furthermore, DAPI staining of mutant worms uncovered the presence of gaps in the pachytene region of the gonad, where germ cell nuclei are normally present. Closer examination of StAR mutant worms revealed a reduction in Syp-1 (a synap- tonemal complex protein) immunofluorescence, indicating incomplete synopsis of chromosomes during germ cell meiosis. Overall fertility was also affected in these worms as measured by a decrease in total brood size. Together, the data collected in cholesterol transport mutants show similar defects in the germline as BPA treated worms. Future studies will investigate the relationship between cholesterol and BPA uptake within the germline of C. elegans.

173 Validation of the Yeast Androgen Screen for Identification of Endocrine Active Substances That Interact with the Androgen Receptor

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Humans are occupationally and environmentally exposed to large numbers of chemicals that have not been adequately tested for toxicological effects, including endocrine disrupting potential. The quantitative high-throughput screening (qHTS) paradigm has provided a more efficient and cost-effective alternative to traditional toxicity tests for profiling environmental chemicals for toxicity. The U.S. Tox21 program has screened a library of approximately 10K chemicals in three independent runs for estrogen receptor alpha (ERα) agonist and antagonist activity using two types of ER reporter gene cell lines, one with an endogenous full length ERα (ER-luc; BG1 cell line) and the other with a transfected partial receptor consisting of the ligand binding domain (ER-β; ERTr β-lactamase cell line), in a qHTS format. Concentration-response data were analyzed by three Tox21 partners (NCCG, EPA, and NTP) using different analysis approaches to determine activity. The ability of the two assays to correctly identify ERα agonists and antagonists was evaluated using a set of 39 reference compounds with known ERα activity. Although both assays demonstrated adequate (i.e. >85%) predictivity, the ER-α assay was more sensitive and the ER-β assay more specific. The qHTS assay results were compared with results from previously published ERα binding assay data and showed >80% consistency. Actives identified from both the ER-α and ER-β assays were analyzed for structure-activity relationships (SARs) revealing known and potentially novel ERα active structure classes, e.g. steroid hormones and flavonoids. The results demonstrate the feasibility of qHTS to identify envi- ronmental chemicals with the potential to interact with the ERs signaling pathway and the two different assay formats improve the confidence in correctly identifying these chemicals.

173c Developing Osteoblasts As an Endpoint for the Mouse Embryonic Stem Cell Test


The mouse Embryonic Stem cell Test (mEST) is a promising in vitro assay for detecting potent embryotoxicity; however, the addition of another endpoint may improve the predictive value of the test. Differentiation of mouse embryonic
stem cells (mESCs) to osteoblasts was examined as such an endpoint. A number of variables such as culture conditions, starting cell number and genetic background of the cell lines were investigated. Direct plating of D3 ESCs in the presence of ascorbic acid, β-glycerophosphate and dexamethasone resulted in the most consistent and robust differentiation to osteoblasts which was detectable by alizarin red staining as early as culture day 7. Differentiation continued to increase up to at least culture day 14 as indicated by levels of osteocalcin mRNA and other osteoblast lineage specific markers. The 14 day D3 culture system was then used to test the predictivity of osteoblast differentiation as an endpoint in the mEST; determination of cytotoxicity was performed as in the validated mEST using both the D3 and ST3 cell lines; osteocalcin mRNA was used as the differentiation endpoint in the osteoblast system. We have tested seven compounds used in the mEST validation experiments using the prediction model developed in the validation study. These compounds include 5-fluorouracil (CAS NO. 51-21-8), diphenhydramine hydrochloride (CAS NO. 147-24-6), metformin hydrochloride (CAS NO. 133073-73-1), lithium chloride (CAS NO. 7447-41-8), acetylamide (CAS NO. 79-06-1), valproic acid (CAS NO. 1009-66-5), and penicillin G (CAS NO. 69-57-8). Our results show that this osteoblast EST can correctly classify the embryo toxicity for all of the tested compounds. These results suggest that differentiation to osteoblasts may be used as an additional endpoint for the mEST. Future work will focus on compounds that were misclassified by the validated mEST and compounds which produce limb defects.

**173d Analysis of High-Throughput, High-Content Data in a C. elegans-Based Toxicity Assay**


To quantitatively assess the effects of chemical exposures on transcription, an automated high-throughput in vivo toxicity assay was developed. This assay measures changes in the levels and cell-specificity of expression of individual genes in C. elegans. Transcriptional responses of archetypal stress-inducible target genes were measured by qRT-PCR in response to: juglone, an oxidative stressor; N-methyl-N′-nitro-N-nitrosoguanidine, a DNA damaging agent; cadmium, a heavy metal; chlorpyrifos, an organophosphate neurotoxin; and tunicamycin, an endoplasmic reticulum stressor. Genes were selected that were significantly upregulated in response to at least one toxicant, had large dynamic ranges (i.e., low constitutive levels of expression), and demonstrated a mechanically relevant response. Individual strains of transgenic C. elegans that express fluorescent proteins under the control of cyp-35A2, gst-4, hsp-16.2, hsp-16.4, hsp-4 and mlf-2 were exposed to six concentrations of each toxicant. Fluorescence data were measured from images (N=12 per replicate) acquired using a high content imager and subsequently analyzed using CellProfiler’s Worm Toolbox; high-throughput imaging analysis software designed for use with nematodes. Image analysis yielded a large number of highly variable nematodes at each concentration. A robust Bayesian method was used to compare the fluorescent intensity in control animals to the highest concentration of toxicant for each gene/toxicant combination. Parameters describing the distributions of nematode intensities were estimated using Markov chain Monte Carlo methods, and the difference in the means of the control and treated distributions were compared. A threshold for significance was set based on the 95% highest density interval for the difference in means and visual confirmation of fluorescence. The results obtained using this novel image-based assay were consistent with the density interval for the difference in means and visual confirmation of fluorescence. means were compared. A threshold for significance was set based on the 95% highest density interval for the difference in means and visual confirmation of fluorescence. The results obtained using this novel image-based assay were consistent with the density interval for the difference in means and visual confirmation of fluorescence.

**173f A High-Throughput Microscopy Pathway in Toxicity Reporter Platform for Chemical Safety Assessment**

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Chemicals react or interact with cellular components leading to a perturbation of signal transduction networks as the cells try to reestablish homeostasis. In case these perturbations are detrimental to cells adaptive stress responses are activated. However, if cellular stress is too severe these adaptive stress responses are unable to reestablish homeostasis and a threshold will be reached where the cell activates cell death responses to avoid cell community-level detrimental effects. We anticipate that high content imaging of critical cellular stress response hallmarks in the development of specific toxicities will be instrumental to assess toxicological hazard. To this end we have developed a set of endogenously regulated BAC-based GFP reporter HepG2 cell lines that enable the live cell visualization of dynamic responses of cellular adaptive stress response programs, including reporters for oxidative stress (Keap1, Nrf2 and Srxn1), DNA damage response (P53BP1, p53, p21 and Mdm2) and the unfolded protein response (ATF4, XBP1, BiP and CHOP). We have carefully characterized these reporters with respect to induction of GFP fusion products and dependence on the anticipated transcriptional control by RNAi mediated knockdown. Moreover, each adaptive stress response reporter is preferentially activated by their respective model compounds (oxidative stress: iodoacetamide, diethylmaleate; DNA damage: cisplatin and etoposide; UPR: thapsigargin and tunicamycin). As a next step we applied these HepG2 reporter cell lines to assess the activation of cellular stress responses by 150 compounds that are associated with drug-induced liver injury. All compounds were evaluated at 1, 5, 10, 50 and 100 Cmax and evaluated for the induction of Srxn1-GFP (Nrf2 activation) or CHOP-GFP (UPR activation). We identified specific sets of DILI compounds that strongly activate both, one or none of the stress response reporters. We anticipate that our cellular stress response reporters in combination with HCl may play a key role in future safety assessment of chemicals.

**173e Quantitative Model of Systemic Toxicity Using ToxCast and ToxRefDB**

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EPA's ToxCast program profiles the bioactivity of chemicals in a diverse set of ~700 high throughput screening (HTS) assays. In collaboration with L'Oreal, a quantitative model of systemic toxicity was developed using no effect levels (NEL) from ToxRefDB for 633 chemicals with HTS data, chemical fingerprints, and a subset with reverse toxicokinetic (RTk) data. Floor and ceiling performance based models were generated using HCA to define clusters of similar NEL values using 4.6 OMU, a 1/5th order reduction in model uncertainty based on our performance baselines. HTS data was then incorporated into the model using 74 groups of assays based on biology (e.g. response data, gene families), technology annotation (assay mechanisms, signal directions), and assay confounders (oxidative stress, cytotoxicity). For each assay grouping, a mean activity value was computed and adjusted for confounders. Incorporating HTS data with read-across resulted in a 4.2 OMU, a total reduction in model uncertainty of 2/5th. RTk steady-state concentrations were then incorporated to adjust in vitro concentration (uM) to in vivo dose (mg/kg/day). Although RTk values were only available for a subset of the total chemical set (211), including RTk further lowered the overall model uncertainty to 3.7 OMU, roughly 3/5th of the total model uncertainty we expect to be able to reduce. Herein, we have identified a model that incorporates HTS (dynamics), read-across (chemistry) and RTk (kinetics) to predict systemic NEL, harnessing and incorporating the power of both new and existing data. This abstract does not necessarily represent EPA policy.

**173g Transcriptomic Characterization of Mouse Embryonic Stem Cell Differentiation and Its Modulation by Thalidomide**

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The Tox21 program calls for transforming toxicology testing from traditional in vivo tests to less expensive and higher throughput in vitro methods. In developmental toxicology, a spectrum of alternative methods has been developed including cell line based tests. In particular, embryonic stem cells (ESCs) have received widespread attention as a promising alternative model for developmental toxicity assessment. Here, we characterized gene expression changes during mouse ESC differentiation and their modulation by thalidomide. C57BL6 ESCs were allowed to differentiate spontaneously and RNA of solvent controls was collected at 0, 24, 48, 72, 96, 120 and 168 h after embryoid body (EB) formation; RNA of thalidomide-exposed EBs were collected at 24, 48 and 72 h. Samples were hybridized to Affymetrix Mouse Gene 2.0 ST Array; using a stringent cut-off criteria of Bonferroni-adjusted p < 0.05 and fold change > 2.0, a total of 1501 probesets were found differentially expressed among the solvent controls at different time points. Out of these probesets 1032 were mapped on the DAVID database as annotated genes, and gene ontology (GO) analysis showed these regulated genes were mostly involved in differentiation-related processes such as development, metabolism, morphogenesis, cell differentiation, cell organization and biogenesis, embryonic development, and reproduction. Principal component analysis (PCA) based on these genes showed that the unexposed solvent controls appeared in chronological order in the PCA plot, which when connected by the regression curve formed a differentiation track. The thalidomide-exposed cultures appeared to deviate from this differentiation track, suggesting its modulating effects on the differentiation process.
The differentiation track defined in this study may be further explored as a baseline for developmental toxicity testing, with compounds causing significant deviation from the differentiation track being predicted as potential developmental toxins.

173h Performance of the BG1Luc and ER β-Lactamase Estrogen Receptor Transactivation Assays in Tox21

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Two estrogen receptor (ER) transactivation (TA) assays, the BG1Luc and HEK293 ER β-lactamase (ER-blα) methods, were adapted for use in the U.S. Tox21 high-throughput screening program. Both in vitro assays detect substances with ER agonist or antagonist activity. BG1Luc endogenously expresses full-length ER (α and β) and is stably transfected with a plasmid containing four estrogen responsive elements (ERE) upstream of a luciferase reporter gene. ER-blα is a mammalian one-hybrid system stably expressing a β-lactamase reporter gene under the control of the GAL4 DNA-binding site and a fusion protein consisting of the human ERα ligand-binding domain and the GAL4 DNA-binding domain. Approximately 10,000 chemicals were tested three times in both assays in agonist and antagonist modes. To differentiate true ER agonists from cytotoxic substances, cell viability was determined. Concentration-response data (N=15) were analyzed to evaluate the performance of the two assays. The assay data quality was high in both agonist and antagonist modes as indicated by acceptable signal to background ratio (2.5 to 8), CV (<10.5%), reproducibility (outcome matches across triplicate runs, ≥0.878), and Z’ factor (≥0.4). Sensitivity and specificity of the assays were compared to ER TA performance standards that were developed with the OECD for the BG1 manual method. Sensitivity was 100% for BG1 agonist, 85% for ER-Blα agonist, and 100% for both agonist and antagonist assays. Agonist and antagonist specificity were 100% for BG1 and ER-Blα. Reference standard values were: estradiol EC50 30 pM for BG1 and 275 pM for ER-Blα, and hydroxytamoxifen IC50 71 nM for BG1 and 6 nM for ER-Blα. Understanding the differences behind the performance of these assays is critical to their acceptance and utilization by both regulators and industry. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. N01-ES-35504.

173i Functional Assays and Alternative Species: Using Larval Zebrafish in Developmental Neurotoxicity Screening

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The U.S. Environmental Protection Agency is evaluating methods to screen and prioritize large numbers of chemicals for developmental toxicity. As such, we are exploring a behavioral testing paradigm, which can assess the effects of sublethal and subteratogenic concentrations of developmental neurotoxicants on 6 day larval zebrafish (Danio rerio). This assay simultaneously tests individual zebrafish under both light and dark conditions in a 96-well plate using a video tracking system. By controlling the duration and intensity of light, we are able to detect changes in locomotion during light-dark transitions, and adaptation to both light and dark during the approximate 1.5 hour testing period. Multiple chemicals at several concentrations (≥120 µM nominal concentration) can be tested in large numbers of larvae using this method. We have evaluated a training set of chemicals (22) that are generally considered positive (n=16) or negative (n=6) controls for developmental neurotoxicity in mammals. Many of the developmentally neurotoxic compounds perturbed behavior at subteratogenic doses (e.g., lead, hepta-chlor, chlorpyrifos, chlorpyrifos oxon), while many non-neurotoxic compounds did not (e.g., acetaminophen, saccharin, glycolosate). Exposure to developmental neurotoxicants altered the overall activity level in light and dark conditions, and/or the activity pattern. The zebrafish neurodevelopmental assay using this training set of chemicals had a sensitivity of 0.875 and a specificity of 0.833. The training set results, therefore, indicate that careful evaluation of zebrafish larval behavior is capable of identifying mammalian developmental neurotoxicants. This abstract may not necessarily reflect official Agency policy.

173j Effects of ToxCast Phase I Chemicals on Steroidogenesis in H295R Human Adrenocortical Carcinoma Cells

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A delicate balance of steroid hormones is essential for proper development and reproduction. Disruption of steroidogenesis by environmental toxicants results in altered hormone levels causing adverse reproductive and developmental effects. H295R human adrenocortical carcinoma cells were used to evaluate the effect of chemicals on steroidogenesis. Using a 96-well format, cells were pre-stimulated with 100M forskolin for 48 hr to induce steroidogenesis followed by chemical exposure for 48 hr. Media were removed and 13 hormone analytes were quantified by HPLC-MS/MS including progesterone (PREG), progesterone (PROG), glucocorticoids, androgens, and estrogens. Initially, 31 unique ToxCast Phase I chemicals (primarily pesticides) were tested at a single non-cytotoxic concentration. 220 chemicals were found to alter the levels of at least one hormone analyte. Based on the single concentration analysis, 96 chemicals disrupting 46 hormones were selected for six-point concentration-response evaluation (0.006 – 100 µM). Concentration-dependent disruption of at least one hormone was observed with 68 of the selected chemicals. By evaluating the effects of chemicals on 13 hormones this assay provides valuable mechanistic insight into the possible targets for chemical perturbation in the steroidogenic pathway. For example, ≤10 chemicals altered PREG or 17αOH-PREG while 27 and 35 chemicals had an effect on PROG and 17αOH-PROG levels, respectively. These results demonstrate that the chemicals evaluated likely do not target CYP17a hydroxylase activity. However, 33 chemicals altered testosterone levels and 38 chemicals concentration-dependently altered estradiol levels revealing significant disruption of subsequent dehydrogenation and aromatization steps. Cumulatively, these results suggest CYP17a lase and hydroxysteroid dehydrogenase activity are the most likely targets for the disruption of steroidogenesis by the subset of ToxCast Phase I chemicals evaluated. This abstract does not necessarily reflect USEPA policy.

173k In Vitro Safety Profile of Personal Care Products—Use of an In Vitro Testing Platform Based on a Reconstructed Vaginal Tissue Model

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One of the common goals of this industry is to confirm the safety of their products. Ethical concerns have led to the use of alternative testing methods in lieu of tradi- tional testing methods. Several studies have shown good correlation between alter- native test methods, traditional testing methods and human exposure. In the current study, the safety profile of three products with potential for vaginal exposure was assessed using the reconstructed human vaginal EpiVaginal™ model (MatTek Corporation, USA); the assay negative control (sterile, deionized water) and positive control (1% Triton® X-100) were tested alongside. To increase the confidence in the test outcome, histopathology evaluation was conducted to assess the extent of cellular damage. Two liquid products were directly applied to the EpiVaginal™ tissues, while the wet wipe product was placed in direct contact with the tissue. Vaginal irritation expressed as ET50 values (3.32 and 12.71 hours) showed a higher irritation potential for the liquid formulations compared to the wipes (24 hours). The lower irritation potential of the wipe product may be related to the availability of a rather limited amount of the liquid formulation in the wipes compared to the liquid formulations. Histology evaluations showed good correlation between the ET50 and change in tissue structure. The results of this in vitro test methodology confirmed the safety profile of the products, should vaginal exposure occur during use. This two-endpoint testing platform (viability and histology) provided not only a correlative interpretation of the data, but also indication of the structural changes of the tissues exposed to the test article that are relevant to human exposure. Future plans include further exploring the capability of this in vitro testing platform for screening products before entering clinical trials.

173l Chemotherapeutic Class-Specific Neurotoxic Effects on Rat DRG Cells Using High-Content Analysis

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We exposed primary rat dorsal root ganglion (DRG) to peripheral neuropathy (PN)-causing drugs and evaluated cell-based changes using high content analysis (HCA). Neuron-specific BIII tubulin was stained along with other cell markers

SOT 2014 ANNUAL MEETING 43
to evaluate subcellular changes following DRG culture exposure to PN-causing drugs. Cultures consist of mostly non-neuronal cells (e.g., Schwann and satellite cells), but neuronal and support cell growth is highly interlaced. Nuclear, neu- rite/process (N/P), and Nissl changes were evaluated at 0, 24 or 72 hr following drug exposure (0.003-30 μM, 24 hr). Drug inter- and intra-class-specific changes were identified for anti-tubulin drugs (hyperstabilizers and destabilizers), alkylating agents, a proteasome inhibitor, and other anti-cancer drugs (doxorubicin, lapatinib, birinapant, and thalidomide). Destabilizers all caused varying loss of N/P and Nissl area staining, but drug-specific variations on other marker responses were found. Hyperstabilizers caused mild N/P loss, increased alpha tubulin intensity, de- creased Nissl area, but had no effect on Nissl intensity. At 10 and 30 μM, cisplatin and oxaliplatin (but not carboplatin) caused Nissl marker changes, but N/P and cell loss was delayed. At different concentrations doxorubicin and lapatinib caused similar nuclear, Nissl, N/P, and cell loss. Thalidomide and birinapant caused no significant changes at any concentration tested. Interestingly, different classes of drugs showed differential effects on non-neuronal cells. The use of HCA with specific markers has allowed us to class-rank the drugs tested. Moreover, this complex culture model demonstrates concordance with reported morphological changes induced by these classes of agents in vivo. Funded by NCI Contract No. HHSN26120080001E.

173m Effect of Nicotine on Zebrafish Embryos: High-Content Analysis to Assess Developmental Toxicity

Zebrafish embryos represent one of the only vertebrate model systems amenable to high throughput toxicity screening. Nicotine, a drug of abuse, has been reported to have many adverse effects on the developing nervous system. Several studies have shown that zebrafish adults and embryos are ideal for monitoring nicotine effects. Previously, we reported that nicotine alters the expression of biomarkers of endocrine function in zebrafish embryos. This observation is concordant with mammalian and human data depicting nicotine as an agent that can modulate hormone levels. Additionally, nicotine exposure has been shown to affect the ner- vous system of zebrafish larvae and juveniles generating abnormal arborization of axons, suggesting a defective path-finding signaling process. Here, we report auto- mated fluorescent image acquisition of hhb-GFP transgenic embryos that express green fluorescent protein as a reporter in their motoneurons and axons, to detect nicotine’s effects. The experiment is performed in vivo using embryos arrayed in 384-well plates. Post-acquisition image analysis provides average motoneuron axon lengths for control and experimental groups. The data show no significant dif- ference in the ventral axon length between the control and the nicotine-treated groups when the embryos were treated at 28 hours post-fertilization for 24 hours. However, nicotine-treated embryos were slightly and significantly smaller than the controls. These observations are in agreement with previous studies reporting that nicotine promotes arborization (axon pathfinding) but does not have any drastic effect on axonogenesis. Protocol # E0735901.1

173n 4-Vinylcyclohexene-Induced Changes in Gene Expression and Antioxidant Status Is Mediated via Oxidative Stress in Drosophila melanogaster
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4-Vinylcyclohexene (VCH) is a by-product of pesticides, plastic, rubber, flame-retardants and tyre productions. The ovotoxicity of VCH has been reported in animal models. However, there is dearth of information on the possible involvement of oxidative stress in the toxicity of this occupational chemical. Thus, this study was designed to investigate the effect of VCH on selected genes expressions, antioxidant status and oxidative stress markers in Drosophila melanogaster model. Flies (both genders) of 1- to 3- days old were orally treated with different concentrations of VCH (10 μM-1mM) in the diet for 5 days. Subsequently, the survival and negative geotaxis assays in the flies were determined, as well as the quantification of reactive oxygen species (ROS) generation. In addition, the real time RT-PCR expressions of selected oxidative stress and antioxidant mRNAs genes of HSP27, 70 and 83, SOD, Nrf2, MAPK and catalase were determined. Furthermore, we evaluated selected enzyme concentrations such as catalase, GST, δ-ALA-D and acetyleholinesterase (Ache). Our data showed that VCH oral exposure is accompanied by the reduction in negative geotaxis. Also, VCH caused significant inductions of the mRNA of SOD, Nrf2 and MAPK genes expressions, which was associated with increased ROS production, altered activities of antioxidant enzymes (GST and catalase), and inhibition of δ-ALA-D and Ache concentrations (p < 0.05). These data imply that, VCH mechanism of toxicity is mediated by oxidative damage, as evidenced by the alteration in the oxidative stress-antioxidant balance. Thus, D. melanogaster has provided further insights into the mechanism of VCH-induced toxicity.

173o Similar Toxicity of Three Fluorocompounds to the Nematode Caenorhabditis elegans
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Fluorides are commonly added to drinking water in the United States to de- crease the prevalence of dental caries. However, the use of fluorides in drinking water has been controversial, primarily due to unresolved toxicological questions. Silicofluorides, such as sodium hexafluorosilicate (Na,SiF₆) and fluoroalumic acid (H₃SiF₆), are the compounds most used for fluoridation, although fluoride salts such as sodium fluoride (NaF) are also used. Interestingly, only the toxicity of sodium fluoride has been examined and not that of the more often-used sili- cofluorides. In the present study, the toxicities of sodium fluoride, sodium hexa- fluorosilicate, and fluoroalumic acid were compared in the alternative toxicological testing organism Caenorhabditis elegans using three quantitative, high-throughput toxicological assays: growth, reproduction, and feeding. Within each assay, the toxicities of the three fluoride compounds varied considerably when expressed as millimoles of compound, seemingly indicating that sodium fluoride was less toxic than the silicofluorides. However, both silicofluorides contain 6 fluoride ions per molecule. Thus, when toxicity was expressed in parts per million (ppm) fluoride, all three fluoride compounds produced concentration–response toxicity profiles with almost identical EC50s and LECs, suggesting that silicofluorides have toxicities similar to sodium fluoride.

173p Using Acute Toxicity, Transcriptomics, Metabolomics and Lipidomics to Understand Effects of Chemical Flame- Retardants on Eco-Indicator Daphnia magna
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The effects of legacy flame-retardant (FR) chemicals octa and penta brominated diphenyl ether (PBDE) and pentaBDE replacement formulation Firemaster550® (FM550) were analyzed on freshwater crustacean Daphnia magna, an ecological keystone species used in fresh water quality assessment. Acute 48-hour LC50 values were determined for penta and octaBDE and for five FM550 formulations and related compounds: FM550, bis (2-ethylhexyl) tetraborophosphate (BEH-TEBP), triphenyl phosphate (TPHP), Firemaster® BZ-54 (BZ54) and the non-brominated BEH-TEBP analog bis (2-ethylhexyl) phthalate (BEHP). Microarray transcriptomics, HNMR-based metabolomics, and lipidomics were utilized to investigate mode of toxicity. Nominal LC50 values ranged from 58 μg/L (pentaBDE) to 3.96 mg/L (octaBDE) and were not correlated to LogKow. Microarray gene expression analyses performed at 1/10 LC50 found that BEH-TEBP and BZ54 caused similar gene expression patterns while the responses to other FRs were distinct. OctaBDE, BZ54 and BEH-TEBP caused changes in the expression of signal transduction genes located in the cellular membrane, but affected different signal systems and therefore caused different biological effects. PentaBDE affected protein synthesis and turn-over and FM550 appeared to cause nutritional dysregulation. Results indicate that hydrophobic FR compounds affect Daphnia in unique ways, not via a general narcotic mechanism.

174 Exposure to 2, 3, 7, 8-Tetrachlorodibenzo-p-Dioxin (TCDD) Induces Expression of Prostaglandin E2 Receptor 4 (EP4) Gene in the Developing Anteroventral Perventricular Nucleus (AVP)
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Developmental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) interferes with masculinization of gonadotropin release patterns, most likely by blocking estradiol (E2)-dependent sexual differentiation of the anteroventral periventricular nucleus (AVP). The process of sexual differentiation involves both cell death and alterations in neurite outgrowth, but the underlying molecular mechanisms are unclear. It has been reported that dendritic spine number and shape is modified...
by TCDD. PGE2 induces formation of dendritic spines on preoptic area (POA) neurons through its EP receptor interaction. EP receptors have been implicated in sexual differentiation of dendritic outgrowth and synaptogenesis in other brain regions, and neonatal activation of EP2 and EP4 receptors are both necessary and sufficient for the organization of male sexual behavior in the preoptic area (POA). To date, no work has examined whether similar processes specifically regulate differentiation of the AVPV, a nucleus in the rostral POA. To determine whether these EP receptors are altered by TCDD in the AVPV, we administered vehicle or TCDD 600 ng orally to rat dams on the first post-partum day. Pups were euthanized the following day (PND2) and AVPV regions microdissected from frozen sections. Using QPCR, we found that only the EP2 and EP4 receptors were differently expressed in both sexes and only EP4 receptor was significantly upregulated by TCDD in males. These findings suggest that TCDD may interfere with masculinization of the AVPV by increasing EP4 receptor.

Neonatal Exposure to Pesticides in Mice Alters Neuroprotein Levels Important for the Developing Brain


Pesticides are used for plant protection and are divided into different chemical classes. In this study we investigate developmental neurotoxic effects of three different classes of pesticides; organophosphate (chlorpyrifos), carbamate (carbaryl) and organochlorine (endosulfan). It has been recognized that the developing mammalian brain has critical periods when the brain is more sensitive to toxic insults. When these insults occur during a period of rapid brain growth they can cause developmental neurotoxic effects and may alter adult susceptibility to other toxicants. Therefore, timing of exposure is of significance.

We have recently seen that these pesticides can induce behavioral neurotoxic effects. Therefore the aim of the present study was to explore if neonatal exposure to these pesticides can affect neuroprotein levels e.g. calcium/calmodulin-dependent kinase II (CaMKII), growth-associated protein-43 (GAP-43), glutamate receptor-1 (GluR1), postsynaptic density protein 95 (PSD95), synaptophysin and tau, which are important for normal development, of the mouse brain. Male NMRI-mice were exposed, on postnatal day 10, to a single oral dose of 5 mg chlorpyrifos/kg bw, 0.5-20 mg carbamate/kg bw or 0.1-0.5 mg endosulfan/kg bw and control animals received a 20% fat-emulsion vehicle. The animals were euthanized 24h after exposure and the neuroprotein levels in hippocampus and cerebral cortex were analyzed. Significant increased levels of GAP-43, GluR1 and tau, and significant decreased levels of CaMKII and synaptophysin compared to control was seen. The neurotoxic effects differed between the brain regions. The results from the present study show that a single oral exposure to pesticides, during a critical period of brain development, can cause developmental neurotoxic effects, shown as changed neuroprotein levels. The neurotoxic effects are in line with previous behavioral studies. Further investigations on the developmental neurotoxic effects of pesticides are needed to understand the mode of action and assess the effects of low dose interaction of mixtures.

Arsenic Inhibits Hedgehog Signaling during P19 Cell Differentiation

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Arsenic is a toxicant found in ground water the world, and human exposure mainly comes from drinking water or from crops which are irrigated by contaminated water. It can be leached into a liquid that comes in contact with plastics, suggesting concerns about the health risk of human exposure. Previous animal studies have suggested that maternal exposure to phthalates during fetal and/or neonatal periods may cause reproductive and developmental toxicities in offspring due to their actions as endocrine disrupting chemicals (EDCs). DEHP is converted to the presumed toxic metabolite, MEHP, by lipase enzymes in the gastrointestinal tract. Upon ingestion, DEHP is rapidly metabolized to MEHP by pancreatic lipases in the lumen of the gut in both rodents and humans, before being further converted into oxidative metabolites and glucuronidated for excretion in the urine and faeces. Since DEHP is converted to MEHP in the gastrointestinal tract, it remains unclear whether DEHP is harmful to fetal brain development by parenteral routes of exposure. We therefore gave DEHP to pregnant Sprague-Dawley rats by gavage and to assess whether DEHP have direct impact on the neural cells viability and patterning of dendrites in fetal rat. DEHP treatment results in induction of oxidative stress leading to disrupt the developmental processes of the central nervous system. MTS was used to examine DEHP reduce dose dependent cell viability. With using immunofluorescence microscopy, malformation of neural morphology in fetal brain was detected. Therefore, our data summarized that DEHP is cytotoxic to the fetal neural cells and requires induction of ROS to exert their developmental alternation effects.

Contribution of DEHP in Malformation of Fetal Brain Development

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DEHP, Di(2-ethylhexyl)phthalate, a phthalate plasticizer used most often in the world. It can be leached into a liquid that comes in contact with plastics, suggesting concerns about the health risk of human exposure. Previous animal studies have suggested that maternal exposure to phthalates during fetal and/or neonatal periods may cause reproductive and developmental toxicities in offspring due to their actions as endocrine disrupting chemicals (EDCs). DEHP is converted to the presumed toxic metabolite, MEHP, by lipase enzymes in the gastrointestinal tract. Upon ingestion, DEHP is rapidly metabolized to MEHP by pancreatic lipases in the lumen of the gut in both rodents and humans, before being further converted into oxidative metabolites and glucuronidated for excretion in the urine and faeces. Since DEHP is converted to MEHP in the gastrointestinal tract, it remains unclear whether DEHP is harmful to fetal brain development by parenteral routes of exposure. We therefore gave DEHP to pregnant Sprague-Dawley rats by gavage and to assess whether DEHP have direct impact on the neural cells viability and patterning of dendrites in fetal rat. DEHP treatment results in induction of oxidative stress leading to disrupt the developmental processes of the central nervous system. MTS was used to examine DEHP reduce dose dependent cell viability. With using immunofluorescence microscopy, malformation of neural morphology in fetal brain was detected. Therefore, our data summarized that DEHP is cytotoxic to the fetal neural cells and requires induction of ROS to exert their developmental alternation effects.

Developmental Vulnerability of Rat Hippocampal Neural Stem Cells to the Neurotoxicant Methylmercury (MeHg)

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The developing brain is sensitive to environmental neurotoxicants such as methylmercury (MeHg), to which humans are exposed via tainted seafood. Studies implicate association of perinatal exposure with learning, memory and IQ deficits in children, suggesting hippocampal dysfunction. Using single-cell analysis, we showed that after 24 hours, a single, low exposure (0.05/1g) adversely affects neurogenesis in postnatal day 7 (P7) rat hippocampus (akin to 3rd trimester in humans), a period of major neural stem cell (NSC) proliferation. NSC proliferation continues but wanes during prepubescence (P14) and adolescence (P21). Yet, the period of developmental vulnerability and lasting consequences of exposure on future neurogenesis remain undefined. To define developmental vulnerability, we injected s.c P14 and P21 rats once with a low or high (5ug/g) MeHg dose and sacrificed 24h later, pulsing with S-phase marker EdU 2h before sacrifice. S-phase cells at P14 were not vulnerable to the low dose, but the high dose reduced mitotic cells in the dentate gyrus hilus by 29% without inducing cell death (cleaved caspase-3) or altering the size of the intermediate neural progenitor pool (Tbr2+ cells). These results indicate that P14 rats retain some vulnerability to MeHg. In contrast, P21 exposure (24 or 48 h) had no effect on S-phase cells nor apoptosis at either time point. To determine long-term effects of early exposure, P7 rats were injected once with MeHg and assessed with BrdU analysis. Significant increased levels of GAP-43, GluR1 and tau, and significant decreased levels of CaMKII and synaptophysin compared to control was seen. The neurotoxic effects differed between the brain regions. The results from the present study show that a single oral exposure to pesticides, during a critical period of brain development, can cause developmental neurotoxic effects, shown as changed neuroprotein levels. The neurotoxic effects are in line with previous behavioral studies. Further investigations on the developmental neurotoxic effects of pesticides are needed to understand the mode of action and assess the effects of low dose interaction of mixtures.

Human Embryonic Stem Cells and Their Neuronal Descendants Exhibit Differential Sensitivity to PBDEs

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Polybrominated diphenyl ethers (PBDEs) are ubiquitous environmental pollutants of major concern due to their possible effects on human neurodevelopment. At a cellular level, the potential mechanisms include disrupting proliferation and dif-
fermentation, perturbing thyroid hormone signaling, and/or generating oxidative stress. Additional studies are needed to understand the molecular bases of PBDE-induced neurotoxicity and the potential developmental risks associated with these exposures. Knowledge gaps include: 1) PBDE effects in human model systems; and 2) windows of vulnerability to PBDE exposure. In this study, we used human embryonic stem cells (hESCs; UCSC4) and their progeny, neuronal progenitors (NPGs), to investigate the dose-dependent effects of two commonly used PBDE congeners (BDE-47 and -99). The approaches included a combination of assays for assessing cell viability, death, proliferation, differentiation and thyroid hormone receptor (THRA, THRB) expression. PBDEs had dose-dependent toxic effects on both cell types with NPGs exhibiting greater sensitivity than hESCs. NPG responses included reduced expression of FOXA, a transcriptional regulator of neural development. In line with previous studies using rodent cell models, BDE47 and -99 significantly reduced THRA and increased THRB expression in NPGs. For example, 25 μM BDE47 and -99 exposure for 24 h upregulated THRB mRNA levels by 22- and 18-fold, respectively. In summary, our results imply that human exposures to PBDEs during the early embryonic period may alter neurogenesis and that sensitivity varies during development. Follow-up studies are focusing on the mechanisms underlying differences in sensitivity of hESCs vs. NPGs and the relevance of our results to human in vivo brain development.

180 Alterations of Postnatal Development of Feedback Inhibition and Basic Excitability in the Hippocampus of Rats Prenatally Exposed to Valproic Acid

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There is a concern about the impact of exogenous chemical substances on the neuronal development of children. Current developmental neurotoxicity guideline (TGC426) is mainly based on zebrafish animal studies such as behavior and patholog-ical examinations, without any electrophysiological testing. Valproic acid (VPA), used as an antiepileptic drug and a mood stabilizer, is known to produce animal models of autism spectrum disorder. We aimed to clarify whether prenatal VPA affects early postnatal development of neuronal circuitry with our electrophysiological approach. VPA was orally administered to the pregnant day 15 Wistar rats with different concentrations of 0 (control, saline only), 150, 300 or 600 mg/kg. On the days of PND 13, 14 and 15, field potentials were recorded from the CA1 area of hippocampal slices obtained from the control and VPA groups to test development of the local circuits. Stimulation/response curves of field excitatory postynaptic potential and those of population spike (PS) enhanced at PND14 and 15 in the VPA-300 group, indicating an enhancement of spike generation or glutamatergic synaptic transmission. The ratios of paired-pulse responses of PS were almost equal to 1 in the control group, showing a lack of feedback inhibition, but decreased in the VPA-300 group from PND15 to 15, suggesting a developed feedback inhibition. Our results provide the evidence that prenatal VPA exposure enhances inhibitory circuits and then potentiates basic excitability in the hippocampal CA1 area. The present results were similar to those obtained from prenatal exposure to an industrial chemical 1-bromopropane, suggesting its electrophysiological evaluation may be useful for developmental neurotoxicity to predict producing a neuropathology after growth.

181 Neurobehavioral Effects of Developmental Exposure to 2, 2', 4, 4'-Tetrabromodiphenyl Ether (BDE-47) and Mechanistic Exploration Using Whole-Genome Transcriptional Profiling

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Polybrominated diphenyl ethers (PBDEs) are a class of high production volume flame retardants used in a variety of consumer products that are considered ubiquitous environmental pollutants. 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) is one of the predominant PBDE congeners found in environmental and biological samples. Developmental exposure to PBDEs, including BDE-47, is associated with neuronal and developmental effects in humans and wildlife; however, the exact mechanism is unknown. We utilized the embryonic zebrafish to assess the development and neurobehavioral responses induced by developmental BDE-47 exposures. Zebrafish were exposed at 6 hours post fertilization (hpf) and general mortality and malformation were assessed at 24 and 120 hpf. We subsequently examined the effects of BDE-47 exposure on photo-motor response in 24 hpf embryos using our lab-derived Photo-motor Response Assessment Tool (PRAT), and we investigated effects on 120 hpf photo-induced larval locomotion using the ViewPoint Zebrafish system. We also ascertained the consequences of developmental BDE-47 exposure on adult zebrafish cognitive development using an active avoidance learning assay. Developmental exposure to BDE-47 up to 20μM resulted in no overt toxicity. BDE-47 exposure led to a dose-dependent increase in activity in the locomotor assay. Adult zebrafish cognition was negatively affected by developmental BDE-47 exposure, as measured by a decrease in the learning rate in the active avoidance task. To explore the mechanism by which early life-stage BDE-47 exposure initiates the observed behavioral effects, whole transcriptome RNA sequencing was performed on embryonic zebrafish exposed from 6-48 hpf. We observed distinct gene expression changes induced by BDE-47 compared to control animals, and functional analyses revealed possible mechanisms important in the behavioral effects of BDE-47.

182 An Automated High-Content Screen for Migration of Neural Crest Precursor Cells


Cell motility is fundamental to development of neural-crest-derived structures in the embryo. Using neural crest stem cells (NCSCs) derived from induced pluripotent stem cells (iPS) cells, we developed a scalable high content screening (HCS) assay to measure the effect of chemicals on NCSC migration. The assay format involves “scratching” a confluent cell monolayer with a Teflon-coated pin tool (V&P Scientific, CA). In order for the assay to be routinely performed on hundreds of test chemicals, the pin tool incorporates 384 elements so that each well is scratched in parallel and reproducibly. The pin tool creates a cell-free region in each well that is 600 microns in diameter. Importantly, “scratching” did not appear to damage the culture surface, which might prevent cell migration due to removal of serum proteins or introduction of grooves running perpendicular to the cell borders. We showed that removal of the cells with the pin tool was spatially consistent well-to-well enabling automated image analysis to assess cell migration into the cell-free region. In order to control for false positives (i.e. pro-proliferative chemicals that lead to an increase in cell count within the cell-free region and cytotoxic chemicals (i.e. chemicals that lead to overall cell loss), cell density outside of the “scratched” region was measured and compared to neutral controls. The scalability of this assay enabled us to rapidly screen the NC22 library of FDA-approved drugs as well as several reference compounds such as Cytochalasin D and paclitaxel. Not only did the imaging-based format of this assay enabled large-scale screening via fixed-end-point screening, but it also allowed follow-up screens that incorporated staining for markers such as Sox10, and in some cases live-cell imaging to measure alterations in directional persistence for individual cells.

183 Molecular Analysis of Rat Palatal Development

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Development of the secondary palate involves cell growth, adhesion, differentiation and apoptosis. Cleft palate, which results from failure of these processes, involves interplay between teratogenic and genetic factors. A diverse range of molecules increases the incidence of cleft palate in the rat, data derived from this species are used in human risk assessment during development of New Chemical Entities. A deeper understanding of the molecular perturbations that lead to cleft palate would refine the utility of the rat in assessing risks to human health. Rat palatal shelves initiate from the maxillary processes on embryonic day (E)13 and grow lateral to the tongue during E14 and E15. During E16 the palatal shelves re-orientate to a horizontal position above the tongue and fuse via their medial edge epithelia by E17. While it is believed that the molecules driving palatogenesis in the rat are identical to those underlying mouse palatal development, this assumption has not been verified. To profile the genes expressed during palate development, we performed RNA-seq analysis of palatal shelves dissected from rat embryos at eight developmental stages between E14.5 and E17 and recovered ~90 μg of RNA from each pair of palatal shelves. Sequence analysis generated an average of 54 million reads from each sample, ~85% of which mapped to unique positions in the rat genome. To calculate gene expression levels we mapped reads to the transcriptome and used a Bayesian deconvolution algorithm to infer transcript expression levels. Transcript levels for each gene were combined to obtain gene expression levels which showed a dynamic range of five orders of magnitude. Strong, temporally-coherent signals were observed for thousands of genes. Comparison of the expression profiles of a subset of genes that play an essential role in mouse palate development with their rat orthologues indicated similar expression patterns suggesting that the principal underlying pathways are conserved between mouse and rat.
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Congenital heart defects and cardiovascular disease cause significant morbidity and mortality. Researching heart formation will increase our understanding of the processes that underpin these conditions. Our research has focused on the epicardium, the epithelial layer that covers the heart. Epicardial cells (EPDCs) migrate into the myocardium where they differentiate into fibroblasts and smooth muscle cells essential for the development of the coronary vasculature. EPDCs also provide signals required for myocardial proliferation and functionality. However, the precise mechanisms that regulate epicardial cell biology remain unclear.

The EHC mouse has a recessive embryonic lethal mutation in Myh10, and as a consequence develops multiple cardiac defects, including abnormal epicardial cell morphology and no functional coronary network. The localization of Wilms Tumour 1 (WT1), a marker of both epicardial cells and EPDCs, was explored using immunohistochemistry (IHC). Embryonic day 14.5 EHC embryos were genotyped by DNA sequencing: hearts were frozen and sectioned using a cryostat. Sections were stained using an anti-WT1 rabbit polyclonal antibody and fluorescence labeled anti-rabbit secondary antibody. Abnormal clusters of WT1 positive cells were observed at the interface between the epicardium and myocardium, suggesting EHC and EPDCs have reduced ability to migrate into the subepicardial space. We are currently investigating the cell specific requirement for Myh10 by generating myocardi al Myh10 knock out embryos (MHC Creflox/flox). Initial IHC results show NMIIB protein localisation is reduced in the embryonic myocardium, indicating Myh10 ablation in cardiomyocytes. Preliminary data indicates that Myh10 expression in cardiomyocytes is not required for coronary vessel development. The findings in the EHC mutant will improve our understanding of the complexities of embryonic coronary vessel development and the potential for abnormalities to arise.

Intravenous Infusion in the Juvenile Rat

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Toxicology studies in juvenile animals are part of non-clinical development of pediatric therapeutics and could be conducted in various species, the rat still being the most commonly used species. Most of the administration routes are technically feasible in the juvenile rat but more complex methods of administration, such as intravenous infusion, require challenging design adaptations. A feasibility study was first performed in close collaboration with our animal welfare group to develop continuous intravenous infusion in the juvenile rat surgically implanted with a catheter on postnatal day (PND) 21. Following anaesthesia with isoflurane, a catheter was implanted into the posterior vena cava via the femoral vein. The pups were continuously infused (24 h/day) with sterile physiological saline for 4 weeks. The technical challenges, such as the length of the catheter and the implantation site, associated with infusing a juvenile rat during a phase of rapid growth were investigated. The feasibility study was then followed by a complete juvenile study using a higher number of animals (20 males and 20 females). Half of the rats was necropsied at 7 weeks of age after the end of the 4-week infusion period and the other half was retained without further infusion for up to 11 weeks of age. Standard toxicity and developmental endpoints such as clinical observations, growth, food intake, clinical pathology, sexual maturation, auditory and pupillary reflexes were evaluated throughout the study. Two behavioral tests (open field and water maze) were also conducted during the observation period. The in-life results were compared with background data from non-catheterized animals at a similar age and histology results at 7-week and 11-week sacrifice time-points were compared with those from catheterized rats used in general toxicology studies.

The present evaluation demonstrated that a 4-week continuous intravenous infusion period is feasible using equipment, procedures and welfare practices adapted to the juvenile rat and did not interfere with the growth or development from PND 21 to 11 weeks of age.

Social Housing of Mature Males in a Nonhuman Primate Breeding Program

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The cynomolgus monkey (Macaca fascicularis) is the preferred species for nonhuman primate (NHP) developmental toxicity studies for biopharmaceuticals. Social housing of NHPs has become mandatory in many countries. The revised USDA regulations require social housing of all social species, which includes NHPs. Although social housing of females and juvenile male NHPs is easily achieved, pair-housing of sexually mature adult males is considered challenging and even more so for male breeder animals. We have implemented a Three Pillar Concept for conducting developmental toxicity studies in NHPs, which addresses pen housing, training, and behavioral assessment of animals for selection of compatible cage mates. This Concept has been applied to our male breeder program. The assessment of social ranking by observance of aggressive and submissive behaviors allows the development of a hierarchical structure with ranking of animals as alpha, submissive, or subordinate. Assessment must be done by a trained animal behaviorist with an understanding of social structure of primate societies. Following establishment of ranking on the dominant-subordinate spectrum, a three-phase approach is used to ensure sustainable commingling pairs. An appropriate familiarization period is key to establishing successful pairs. Effects following initial pairing of males, such as delayed body weight gain, are transient. During active mating, the male pair is separated and housed individually. The female is placed in the male cage for up to 48 hours. Following completion of the mating period, the female is removed and the male commingling pair is reunited. Careful monitoring and management is critical to ensure successful commingling pairs and to minimize aggression. Challenges are mainly related to appropriate training, experience, and timing. To date, we have established 16 male breeder pairs with mature males with body weights up to 15 kg. This demonstrates that sexually mature breeding males can be socially housed successfully even during an ongoing mating program.

The Role of the Epicardium in Cardiogenesis: Insights from the EHC Mouse

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The findings in the primate (NHP) developmental toxicity studies for biopharmaceuticals. Social housing of sexually mature adult males is considered challenging and even more so for male breeder animals. We have implemented a Three Pillar Concept for conducting developmental toxicity studies in NHPs, which addresses pen housing, training, and behavioral assessment of animals for selection of compatible cage mates. This Concept has been applied to our male breeder program. The assessment of social ranking by observance of aggressive and submissive behaviors allows the development of a hierarchical structure with ranking of animals as alpha, submissive, or subordinate. Assessment must be done by a trained animal behaviorist with an understanding of social structure of primate societies. Following establishment of ranking on the dominant-subordinate spectrum, a three-phase approach is used to ensure sustainable commingling pairs. An appropriate familiarization period is key to establishing successful pairs. Effects following initial pairing of males, such as delayed body weight gain, are transient. During active mating, the male pair is separated and housed individually. The female is placed in the male cage for up to 48 hours. Following completion of the mating period, the female is removed and the male commingling pair is reunited. Careful monitoring and management is critical to ensure successful commingling pairs and to minimize aggression. Challenges are mainly related to appropriate training, experience, and timing. To date, we have established 16 male breeder pairs with mature males with body weights up to 15 kg. This demonstrates that sexually mature breeding males can be socially housed successfully even during an ongoing mating program.

The Non-Obese Diabetic (NOD) Mouse As A Model for Diabetic Pregnancy

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Diabetes in pregnancy is a well-known risk factor for neural tube defects (NTDs). In previous work, we used streptozotocin induction of diabetes in the mouse as a model of type 1 diabetes during pregnancy. We reported that embryonic gene expression is altered under diabetic conditions, and we also observed a general increase in variability of expression levels. To determine whether this is a general feature in diabetic pregnancies, we employed a model of spontaneous type 1 diabetes occurrence, the non-obese diabetic (NOD) strain of mice. In this strain, litters in diabetic dams exhibit NTDs at a rate of ~40%, with no malformations found in normoglycemic pregnancies. Gene expression profiling by microarrays and next-generation sequencing, at two different developmental time points, also indicated greater variation of gene expression levels within the group of diabetes-exposed embryos. On the basis of these findings, we propose a variation-based theoretical model to explain the incomplete penetrance of the NTD phenotype in diabetic pregnancies of inbred strains in mice. We also illustrate the discovery-advancing power of this paradigm. Beyond genetic models, our concept has broad impact for incomplete penetration of outcomes from teratogen/toxin exposures in general, and for prevention strategies.

Fetal Myocardial Reduction in Hyperglycemic Mouse Pregnancy Is Associated With Dysregulated Expression of the Antiapoptotic Gene BCL-2 in the Developing Heart: Preliminary Results

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A growing number of environmental contaminants may adversely affect fetal development through disruption of glucose homeostasis. We previously detected significant late-gestation ventricular myocardial reduction in fetal mouse hearts collected from pregnant mice dosed with streptozotocin, which is toxic to insulin-producing beta cells of the pancreas and induces hyperglycemia. Flow cytometric analysis of the myocardial cells showed an enhanced rate of apoptosis, suggesting dysregulated cell death as a mechanism associated with the myocardial defect. The present study therefore examined expression of the anti-apoptotic gene Bcl-2 in fetal myocardium from hyperglycemic mouse dams on days 14 and 17 of gestation. The hyperglycemia was induced in female Rockefeller (inbred CD1) mice, 6 to 8 weeks old, by pre-breeding streptozotocin (STZ) injection. Expression of Bcl-2 in the fetal myocardium from diabetic dams was decreased by 53% and 51% at GD14 and 17, respectively. These results suggest maternal hyperglycemia may damage the developing myocardium by altering expression of gene pathways that regulate cell death.
For the functional inhibition studies, BeWo cells and cells overexpressing the wild-type BCRP in the placenta and 2) direct inhibition of glyburide transport by BCRP. Pregnant women that express the common C421A-BCRP variant and/or consume soy may be at an elevated risk for drug-induced neonatal hypoglycemia. Support: ES020522, ES005022, ES007148, and an AFPE Predoctoral Fellowship.

Impact of Fatty Acids on Human UDP-Glucuronosyltransferase 1A1 Activity and Its Expression in Neonatal Hyperbilirubinemia

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While breast milk has been known as a cause of neonatal hyperbilirubinemia, the underlying mechanism of breast milk-induced jaundice has not been clarified. Here, the impact of fatty acids on human UDP-glucuronosyltransferase (UGT) 1A1 — the sole enzyme that can metabolize bilirubin — were examined. Oleic acid, linoleic acid, and docosahexaenoic acid (DHA) strongly inhibited UGT1A1 activity. Forty-eight hours after a treatment with a lower concentration of DHA (10 mg/kg), total bilirubin significantly increased in neonatal hUGT1G1 mice, which are human neonatal jaundice models. In contrast, treatments with higher concentrations of fatty acids (0.1–10 g/kg) resulted in a decrease in serum bilirubin in hUGT1G1 mice. It was further demonstrated that the treatment with higher concentrations of fatty acids induced UGT1A1, possibly by activation of peroxisome proliferator-activated receptors. Our data indicates that activation of peroxisome proliferator-activated receptors would increase UGT1A1 expression, resulting in reduction of serum bilirubin levels in human infants.

Genistein Reduces Human Placental BCRP/ABCG2 Transporter Expression and Function In Vitro: Potential Risk for Fetal Drug Exposure

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Pregnant women with gestational diabetes are often treated with the hypoglycemic agent, metformin. The aim of this study was to determine the effect of the soy isoflavone, genistein, on the placental circulation by the breast cancer resistance protein (BCRP/ABCG2) expressed on placental syncytiotrophoblasts. The purpose of this project was to determine the effect of the soy isoflavone, genistein, on the 1) regulation (protein and function) of BCRP in the placenta and 2) direct inhibition of glyburide transport by BCRP in cell-based systems. Human choriocarcinoma BeWo cells were incubated with genistein (0–10 μM) for 48 h and collected for western blot and functional analysis. For the functional inhibition studies, BeWo cells and cells overexpressing the wild-type (WT) or functionally-reduced variant (C421A) of BCRP were incubated with increasing concentrations of genistein (0–100 μM) for 1 h. Retention of BCRP substrates Hoechst 33342 or BODIPY-glyburide was quantified with an automated fluorescence cell counter. Genistein reduced the protein expression of BCRP and increased the cellular retention of Hoechst up to 40% in BeWo cells at 48 h. In the direct inhibition studies, genistein enhanced the accumulation of Hoechst in placental BeWo cells (50%) and both BCRP-overexpressing cells (WT and C421A, 300%), with a trend for the C421A-BCRP cells to accumulate even more Hoechst in the presence of genistein. Interestingly, genistein increased the accumulation of BODIPY-glyburide up to 70% only in the C421A-BCRP cells. In conclusion, genistein decreases the placental expression and direct transport of glyburide by BCRP. Future studies with the common C421A-BCRP variant and/or consume soy may be at an elevated risk for drug-induced neonatal hypoglycemia.
194 Ethanol (EtOH) Alters the Redox Environment, Histiotrophic Nutrition Pathways (HNP), and the Thiol Proteome during Rat Organogenesis

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Ethanol is a known teratogen involved in the etiology of structural and functional defects by mechanisms that include redox regulation and related disturbance of the actin cytoskeleton, although, modes of action are not well defined. This study uses the rat whole embryo culture (rWEC) model to further elucidate the relationships between embryonic nutrition, redox environment and observed changes in the thiol proteome following EtOH exposures (1.5–6.0 mg/ml). Soluble thiol concentrations (GSH and Cys) and their respective intracellular redox potentials (Eh) were determined using HPLC. EtOH reduced GSH and Cys concentrations in embryo (EMB) and visceral yolk sac (VYS) tissues, and also in yolk sac fluid (YSF) and amniotic fluid (AF). These changes were accompanied by greater oxidation as indicated by increasingly positive Eh values. Disruption of nutrient transport and cytoskeletal dynamics has been previously shown following EtOH exposure. EtOH reduced HNP activity in rWEC as measured by the clearance of FITC-albumin from the culture media. A significant decline in total FITC clearance was observed at all doses, showing an optimal >50% change at the high dose. EtOH-induced changes in protein concentrations within the thiol proteome were measured in EMBs and VYS using isotope-coded affinity tags (ICAT). Decrease for specific proteins involved in cytoskeletal dynamics and endocytosis (α-actin, α-tubulin, cubulin, and actin-related protein) and in proteins involved in nuclear trafficking (Ran and RanGAP) were observed. This study suggests that EtOH can alter redox environments, HNP activities, and concentrations of the thiol-reactive proteins involved with cellular transport systems, which all may collectively contribute to EtOH-induced developmental toxicity during early organogenesis.

195 Two Alleles of Med31 Provide a Model to Study Defects in Cellular Proliferation and Endochondral Ossification

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Over 400 different skeletal dysplasias have been characterised and represent a significant global health concern, with incidence rates estimated around 1 in every 4000-5000 live births. Understanding the mechanisms which govern early skeletal development in the embryo is key to developing potential therapeutics.

We have isolated two separate mouse lines with mutations in the Mediator complex gene Med31 derived from an ENU mutagenesis screen. The first mouse is null for the Med31 protein during development, and displays delays in the development of the limbs. Using a combination of wholemount staining for the mitotic marker phospho-histone H3 on E10.5 embryos and immunohistochemistry on E16.5 parafomaldehyde fixed sections using the Ki-67 antigen, we have confirmed that the mutants have cell proliferation defects which contribute to delays in bone ossification.

The second mouse line has a Med31 missense mutation (Y57C), confirmed by genomic sequencing, which we predict affects the ability of the Med31 protein to interact with Med7N within the Mediator complex. Individual null or Y57C heterozygous mice are phenotypically normal, whilst double mutants, of the genotype ‘Med31 null/ Med31 Y57C’ show delays in limb development similar to Med31 null mice, when stained with Alcian blue and Alizarin red in skeletal preparations.

To further understand the function of Med31 in the context of cellular proliferation in the developing limb, we have developed a cell culture knock out model, in which we reduce Med31 expression using shRNA transfection into the mesenchymal cell line C3H10T1/2. We will use a combination of cell culture and whole embryo models to further elucidate the function of Med31 in limb development.

196 The Effects of Thalidomide on Fgf8, Bmp4 and Hoxa11 Expression in the Limb Bud in Rabbit Embryos


Thalidomide (TM) induces limb defects in humans and some animal species including rabbits. Although the mechanism of TM-induced limb defects has been investigated for a long period, the limb development-related genes expressions have not been vigorously characterized in rabbits. In this study, we investigated the Fgf8, Bmp4 and Hoxa11 expressions in the limb buds in TM-treated Klkl/JW rabbit embryos on gestation day (GD) 10, 11 and 12 by whole mount in situ hybridization. It has been reported that Fgf8 and Bmp4 expressions were changed in the limb bud in TM-treated rabbit and chick embryos, respectively. Hoxa11 was selected because Hoxa11 knockout mice showed morphological changes at the ulna and radius and the radius malformation was observed in TM-induced rabbit fetuses. On GD 10 and 11, growth retardation of the whole body was induced by TM treatment. The expression lengths of Fgf8 on GD 10 and 11 in the forelimb bud were significantly or tended to be decreased in the TM-treated embryos, which was correlated to the growth retardation and was not considered to be directly relevant to the teratogenic effect of TM on the forelimb. Hoxa11 is not expressed on GD 10. The TM-induced characteristic changes in the expression pattern of Hoxa11 on GD 11 and Bmp4 on GD 10 and 11 were not noted. On GD 12, there was no effect of TM on growth retardation. The expressions of Fgf8 and Bmp4 were not changed by TM on GD 12, while Hoxa11 expression was narrowed by TM at the anterior region and was not changed at the middle and posterior regions in the forelimb bud and in all regions in the hindlimb bud. The anterior region was located on the radial side and the radius malformation was observed in the TM-treated rabbit fetuses. Therefore, the decrease in the Hoxa11 expression on GD 12 was related to the limb defects induced by TM and can be a good marker for early prediction of the teratogenic effect of TM on the forelimb.

197 Humoral Immunity and Lymphocyte Immunophenotyping Control Background Data in Mainland and Indonesian Cynomolgus Monkey Infants

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Background: Lymphocyte immunophenotyping (LIP) and T-cell dependent antibody response (TDAR) are the most common tests in assessing potential developmental immunotoxicology in pre- and postnatal developmental (PPND) studies. Background data from Mainland (ML, China and Cambodia) and Indonesian Island (IL) Cynomolgus monkeys were compared in order to accurately assess the presence or absence of test article-related changes in treated monkeys from different sources.

Methods: Control data were analyzed retrospectively from a total of 14 and 10 PPND studies for LIP and TDAR data, respectively. Blood samples for LIP by flow cytometry in infants were collected throughout the postnatal period and analyzed for total CD3+ T cells, CD3+CD4+ Helper T cells, CD3+CD8+ Cytotoxic T cells, CD3+CD20+ B cells, and CD3+CD16+ or CD3+CD159a+ Natural Killer (NK) cells. To evaluate TDAR, keyhole limpet hemocyanin (KLH) was injected twice, and primary and secondary IgM and IgG levels in serum were measured by ELISA.

Results: IL monkeys generally had higher absolute counts and relative percentages of NK cell populations, and lower circulating B cell numbers when compared to ML monkeys. IgM levels elevated rapidly after the 1st and 2nd doses, with peak IgM concentrations in IL 24-27% lower than that observed in ML. IgG levels were determined using HPLC. EtOH reduced GSH and Cys concentrations in embryo (EMB) and visceral yolk sac (VYS) tissues, and also in yolk sac fluid (YSF) and amniotic fluid (AF). These changes were accompanied by greater oxidation as indicated by increasingly positive Eh values. Disruption of nutrient transport and cytoskeletal dynamics has been previously shown following EtOH exposure. EtOH reduced HNP activity in rWEC as measured by the clearance of FITC-albumin from the culture media. A significant decline in total FITC clearance was observed at all doses, showing an optimal >50% change at the high dose. EtOH-induced changes in protein concentrations within the thiol proteome were measured in EMBs and VYS using isotope-coded affinity tags (ICAT). Decrease for specific proteins involved in cytoskeletal dynamics and endocytosis (α-actin, α-tubulin, cubulin, and actin-related protein) and in proteins involved in nuclear trafficking (Ran and RanGAP) were observed. This study suggests that EtOH can alter redox environments, HNP activities, and concentrations of the thiol-reactive proteins involved with cellular transport systems, which all may collectively contribute to EtOH-induced developmental toxicity during early organogenesis.

Discussion: Blood samples were collected throughout the postnatal period and analyzed for total CD3+ T cells, CD3+CD4+ Helper T cells, CD3+CD8+ Cytotoxic T cells, CD3+CD20+ B cells, and CD3+CD16+ or CD3+CD159a+ Natural Killer (NK) cells. To evaluate TDAR, keyhole limpet hemocyanin (KLH) was injected twice, and primary and secondary IgM and IgG levels in serum were measured by ELISA.

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Development and Validation of an Analytical Method for Monobutylphthalate, a Metabolite of Di-n-Butylphthalate, in Harlan Sprague-Dawley Rat Pup and Fetus by Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS)


Di-n-butyl phthalate (DBP) is a common plasticizer used in a variety of consumer products. Exposure to DBP is widespread, and its potential toxicity has been, and continues to be, investigated. The objective of this work was to develop and validate a method to quantitate mono-n-butyl phthalate (MBP), the major metabolite of DBP, in rat pup and fetus. Samples were prepared by spiking 0.5 g of pup or fetus homogenate with 25 mL of water, 25 mL of internal standard solution (MBP-d4 in water), and 300 mL of 0.1% formic acid in water. Analyte extraction was achieved by the addition of 1 mL of acetonitrile, vortex mixing, and centrifugation. The supernatant was filtered, submerged into liquid nitrogen, and centrifuged to facilitate lipid separation. The method was successfully validated over the range 50-5000 ng/g in pup, with cross-validation for fetuses. Validation parameters included linearity (r ≥ 0.99), recovery (mean recovery = 100%), sensitivity (limit of detection = 9.4 ng/g, experimental limit of quantitation (ELOQ) = 50 ng/g), precision (relative standard deviation ≤ 10%), and accuracy (relative error ≤ 10%). Background phthalate interference was detected in both pup and fetus, but MBP peak area ratios for the blanks were ≤ 40% of those for the ELOQ standards, demonstrating acceptable selectivity. Stability was established for frozen matrix samples stored at -20°C for 60 days, matrix samples subjected to freeze-thaw (3 cycles over 3 days), and also ambient and refrigerated extracts for 3 days. It was also demonstrated that pup samples as high as 7,500 ng/g and fetus samples as high as 25,200 ng/g could be accurately quantitated by diluting into the calibration range. The method is being applied for quantitation of MBP in pup and fetus samples from rats administered 0, 300, 1000, 3000, or 10,000 ppm DBP in feed.

Developmental Toxicity Assessment of Multiwall Carbon Nanotubes in Pregnant Mice after Intratracheal Instillation

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In order to evaluate the developmental toxicity of multi-wall carbon nanotubes (MWCNTs) via inhalation exposure, we conducted an intratracheal instillation study of MWCNTs in pregnant mice. MWCNTs (MWCNT-7) dispersions were prepared by ultrasonication using an ultrasonic bath for 30 min. In order to compare the biological effect of dispersion media, we used two different media, mouse serum and 2% (w/v) of sodium carboxymethyl cellose (CMC-Na) solution. The MWCNT dispersions in serum were administered to pregnant Crl:CD1(ICR) mice on gestation day 9 at dosage of 0, 3.0, 5.0 and 7.0 mg/kg bw (mouse serum). The MWCNT dispersions in 2% CMC-Na solution was administered at dosage of 5.0 mg/kg bw. Ten pregnant mice per group were evaluated and dissected on gestation day 17. Decreased body weights in all MWCNT exposed pregnant mice were observed, although there were no clear dose-dependent changes. No statistically significant difference was observed between the control group and MWCNT exposed groups in the numbers of corpora lutea, dead fetuses (early or late resorption), and live fetuses, or sex ratio. Body weight of fetuses and placental weights were significantly decreased in the most MWCNT exposed groups, although no clear dose-dependency was observed. Furthermore, external malformations (i.e., oligodactyly, extensive contractures, kinked tail, cleft lip, and cleft palate) were observed in all MWCNT exposed groups. The fetuses with external malformations were derived from the same litters. The equivocal dose-dependent effects may be due to a litter dependent toxic mechanism. These results suggested that the intratracheal exposure of MWCNT resulted in teratogenic effects in mice in spite of different dispersion media. Further examinations are needed to clarify the mechanism of the developmental toxicity by MWCNTs.

Elucidation of a Novel Molecular Mechanism of Dioxin Toxicity

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exerts a variety of toxicity. Beside the essential role of aryl hydrocarbon receptor (AhR), the molecular basis of TCDD toxicity has been elusive. Recent in vitro experiments showed that TCDD activates cPLA2 via a different pathway from transactivation of AhR target genes. Thus, there is a research need to elucidate the possible involvement of this alternate pathway in the manifestation of TCDD toxicity in vivo. First, we administered TCDD to fetuses, lactational pups, and adults of wild-type or cPLA2 KO mice to find out the manifestation of toxicity. Next, among a variety of dioxin toxicity, we studied the molecular mechanism of toxicity by focusing on the neonatal hydronephrisis. Finally, using AhRα mice, we investigated the role of AhR localization into nucleus with regard to the alternate pathway. Fetal toxicity: cPLA2α (+/-) pregnant mice were administered TCDD on gestational day (GD) 12.5, and onset of hydronephrisis and cleft palate and changes in fetal survival rate were examined on GD18.5. cPLA2α was found to be an important part in the manifestation of neonatal and fetal hydronephrisis, and at the same time in the reduction of TCDD-induced fetal lethality. Neonatal toxicity: cPLA2α (+/-) dams and AhRα(-) dams were orally administered TCDD on postnatal day (PND) 1. Pups were sacrificed on PNDs 7 and 14 to examine the development of hydronephrisis. Hydronephrisis was not developed in either cPLA2α KO or AhRα(-) mice. Adult toxicity: cPLA2α (+/-) and cPLA2α (-/-) mice aged 12 week old were injected i.p. with TCDD. Hepatotoxicity and thymic atrophy were examined on day 8. In adult mice, cPLA2α was found not to be associated with the manifestation of these kinds of TCDD toxicity. In conclusion, using different stages of mice, neonatal hydronephrisis was found to be a good model to study the molecular basis of a novel pathway in the induction of TCDD toxicity. The nuclear localization of AhR was suggested to play an essential role in the activation of the novel pathway.

Evaluation of the Sodium Valproate-Induced Developmental Abnormalities and Its Mechanisms in Zebrafish


Sodium valproate (VPA) is known to induce developmental abnormalities such as spina bifida, craniofacial anomalies and developmental neurotoxicity (DNT) in mammals. In the present studies, we evaluated the VPA-induced developmental abnormalities in zebrafish and analyzed the mechanisms of the abnormalities focusing on the craniofacial anomaly and DNT. [Study 1] Zebrafish (Tupfelf Longfin) were exposed to VPA at 75 μM from 5 to 144 hours post fertilization (hpf), and morphological evaluation was conducted at 144 hpf. Additionally, gene expression analysis of dkie2, a marker for neural crest cells, was performed by in situ hybridization on staged embryos. VPA-treated larvae showed abnormal findings in the notochord and lower jaw, corresponding to the phenotypes observed in VPA-exposed mammals, and the lower jaw anomaly was accompanied with a decrease in dkie2 expression in the hyoid arch. These results imply that the VPA-induced morphological anomaly in the lower jaw is related to morphogenesis failure of the neural crest-derived structures in zebrafish. [Study 2] Zebrafish were exposed to VPA at 40 μM from 5 to 144 hpf. After VPA-free period for 24 hours from 144 hpf, locomotor activity was evaluated under 10 minutes light and dark cycles, and gene expression analysis of tyrosine hydroxylase (th), a marker for dopaminergic neuron, was performed on staged embryos. VPA-treated larvae showed an increase in locomotor activity during the dark period and a decrease in th expression in the hypothalamus. These results imply that the VPA-induced DNT is related to inhibition of the dopaminergic neuron development in zebrafish. In conclusion, our studies revealed that VPA induces some developmental abnormalities in zebrafish and suggested that inhibition of the neural crest-derived cranial and dopaminergic neuron development is related to these developmental abnormalities.

Evaluation of Teratogenicity of IMiD® Compounds Using the Zebrafish Developmental Toxicity Assay

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IMiD immunomodulatory analogues are analogues of thalidomide that represent a promising class of drugs for the treatment of inflammatory, autoimmune, and cancerous diseases. To investigate the usefulness of the zebrafish developmental toxicity
203 Expression of Glyoxylase 1 (glo1) throughout Zebrafish Embryonic Development and Alterations following Atrazine Exposure

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Atrazine, a commonly used herbicide in the Midwest, is an endocrine disruptor and a suspected carcinogen. Although atrazine was recently banned by the European Union for widespread contamination risks in potable water supplies, this herbicide is still commonly used in the United States with a current maximum contaminant level (MCL) of 3 ppb in drinking water. The health risks associated with this MCL are currently being reviewed by the Environmental Protection Agency, however the mechanisms of atrazine toxicity are not well defined. In this study transcriptomics was first used to identify genes with altered expression following exposure to 0, 0.3, 3, or 30 ppb atrazine during embryogenesis with the zebrafish model system. This analysis showed that expression alterations were enriched with genes associated with neuroendocrine development and function, cell cycle regulation, and carcinogenesis. From this list of genes, glyoxylase 1 (glo1) was targeted for further study. glo1 is part of the glyoxylase system which converts methylglyoxal to S-D-lactoylglutathione. Upregulation of glo1 is linked to cell proliferation and is associated with various cancers in humans. To further our understanding of this genetic target, expression was analyzed at five developmental time points (24, 36, 48, 60, and 72 hpf). In situ hybridization was used to characterize spatial gene expression of glo1 throughout development under normal conditions and after exposure to 0, 0.3, 3, or 30 ppb atrazine. glo1 was found to be ubiquitously expressed at each time point with most of the staining concentrated in the brain. A high level of expression was also observed in the liver. Real time quantitative PCR (qPCR) of 35 cycles was used to further investigate the developmental expression of glo1 and its deregulation by atrazine exposure. qPCR was first used to profile expression throughout embryogenesis in control conditions at 12, 24, 36, 48, 60, and 72 hpf. Expression of glo1 was developed in the zebrafish model system. This is a potential carcinogen. Visceral defects were observed with atrazine and pan-caspases were observed during embryogenesis. This study showed that expression during the first 12 hours (2-14 hpf) was critical in producing pectoral fin defects. Development of the pectoral fin shares a similar molecular pathway as those of tetrapod limbs; however pectoral fin defect was only observed with atrazine in the ZFDA. These data with the IMID compounds show a lack of concordance of developmental defects between zebrafish and other species commonly used for EF evaluation because limb malformations have been reported with atralidomeline and pomalidomide in rabbits, and with lenalidomide in non-human primates.

204 Deregulation of miRNA-126 Expression in Developing Zebrafish Exposed to the Herbicide Atrazine

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Atrazine is a commonly used herbicide in the United States that is reported to frequently contaminate drinking water sources. Studies have indicated atrazine to adversely impact the neuroendocrine and reproductive systems and to be a potential carcinogen. The current maximum contaminant level for atrazine in drinking water set by the US Environmental Protection Agency is 3 parts per billion (ppb); however, levels higher than 3 ppb are often reported. Ongoing studies in our laboratory are investigating the immediate and latent adverse health outcomes associated with a developmental atrazine exposure and identifying the generic and epigenetic mechanisms of atrazine toxicity using the zebrafish model system. MicroRNAs (miRNAs) are epigenetic regulators that post-transcriptionally control the translation of mRNA. To identify if an embryonic atrazine exposure would alter miRNA expression, a unique microarray platform containing all known zebrafish and human miRNAs was designed. Zebrafish embryos were exposed to 0, 0.3, 3, or 30 ppb atrazine through 72 hours post fertilization (hpf), and miRNA expression was analyzed using the microarray platform. Expression of 18 zebrafish and 9 human miRNAs were significantly altered in response to atrazine exposure. One of the most robustly changed miRNAs was miR-126, a miRNA associated with angiogenesis and tumorigenesis. To further investigate the developmental expression of miR-126 and its deregulation by atrazine exposure, quantitative PCR (qPCRs) was first used to profile expression throughout embryogenesis in control conditions at 12, 24, 36, 48, 60, and 72 hpf. Expression of miR-126 was measured during six developmental time points specific with an increase in expression after 12 hpf and a peak in expression at 36 hpf. Following a decrease in expression at 48 hpf, expression levels were significantly higher at 60 and 72 hpf. Deregulation of miR-126 following exposure to 0, 0.3, 3, or 30 ppb atrazine at all six developmental time points was also analyzed to further our understanding on the impacts of atrazine exposure on miR-126 expression.

205 Exposure to Chemicals Found in Umbilical Cord Blood Alters Kisspeptin/GPR54-GnRH Pathway Expression and Development in the Zebrafish Embryonic Development Model

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There are numerous positive and negative inputs that affect the development of the hypothalamus–pituitary–gonadal axis during fetal and early-life stages. Therefore, alterations in reproductive function that manifest later in life, such as the earlier onset of puberty could be a result from perturbations or exposures during critical neuronal developmental time-points. Recent work has identified Kisspeptin (KISS), expressed in the arcuate nucleus (ARC) region of the brain, that are vital for the central regulation of gonadotrophin releasing hormone (GnRH) neurosecretory activity and timing of puberty. At present, there is little research examining the effect of chemicals found in the umbilical cord blood on KISS mRNA levels or on the development of KISS positive gonadotropes in any developmental vertebrate model. The goal of this study is to use the developing zebrafish embryo model to determine the impact of individual chemicals and low-level mixtures of compounds that are present in the umbilical cord blood on KISS-NK-DYN-GnRH signaling system. Embryos were exposed to several compounds at concentrations reported in umbilical cord blood. KISS 1 and 2, GPCR54-1 and 2, and GNRH3 mRNA expression was measured at 2 hour intervals for the first 24 hours. We first identified mRNA expression of two orthologs of KISS 1 and 2 and GPCR54-1 & 2 during initial stages of development. This is subsequently followed by GnRH exposure at 23 hpf. Initial exposures to either estradiol or the anti-diabetic drug metforin increased mRNA expression of KISS 1 and 2 as well as shifted the timing of expression. Our results indicate that there is a shift in timing and mRNA expression that may impact GnRH neuronal signaling and development.

206 Induction of Vitellogenin in Thracemyx scripta Exposed to Atrazine

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Due to their ubiquitous presence, persistence, and tendency to bioaccumulate, endocrine disrupting chemicals (EDCs) may cause significant impacts on the health of wildlife. Atrazine, a commonly used pesticide in the United States, is a type of EDC that is causing much concern due to the recent finding that it may cause adverse effects at low doses. This study focuses on the estrogenic effects of atrazine (2-Chloro-4-ethylamino-6-isopropylamino-s-triazine). Since it has been shown that atrazine caused two male American leopard frogs (Rana pipiens) to have oocytes that were vitellogenic, we hypothesized that exposure to atrazine may result in the induction of vitellogenin in hatching red eared slider turtles (Trachemyx scripta). Vitellogenin is the egg yolk precursor protein that is typically found when female oviparous vertebrates are reproducitively active. Because males also carry the gene that codes for vitellogenin, this protein is a reliable biomarker in oviparous vertebrates for exposure to estrogenic compounds. Hatchling male and female T. scripta received intraperitoneal injections of one of four different concentrations of atrazine (0.0001, 0.01, 0.1, or 1 ng/g bm), estradiol-17B in two doses (1 or 1000ng/g bm), or vehicle (saline) only. Plasma vitellogenin concentration was measured using an ELISA developed specifically for T. scripta vitellogenin. Significant production of vitellogenin occurred in turtles in both the estradiol groups as well as the atrazine groups in a dose response manner. Our data shows that exposure to low doses of atrazine causes induction of vitellogenin in the turtle and demonstrates the potential for this compound to act as an EDC. 

Developmental Exposure to Benzo[a]pyrene Affects Behavior and Energetics in Larval and Adult Zebrafish

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants present in urban air, dust, and soil resulting from incomplete combustion of organic materials or fossil fuels. It is widely recognized that PAHs pose risks to human health, especially for the developing fetus and infant, where PAH exposures have been linked to in-utero mortality, lower intelligence, and cardiovascular effects. Using the zebrafish model, we evaluated the developmental toxicity of PAH exposures. Zebrafish embryos were exposed from 6-120 hours post fertilization (hpf) to B[a]P, BaA, BEZO, and 9,10-PHEQ concentrations that caused no observable morphological toxicity. Using the Seahorse Extracellular Flux Analyzer, we measured in vivo respiration in 26hpf embryos exposed to PAHs. Exposure to B[a]P, BaA, BEZO, and 9,10-PHEQ caused decreased oxygen consumption rates, indicative of mitochondrial damage. Developmental B[a]P and BaA-Q exposure also resulted in hyperactive swimming at 120hpf. To determine if behavioral and physiological effects persisted, a subset of exposed animals were raised to adulthood in chemical-free water and assessed for learning and energetic deficits. Preliminary results indicate B[a]P-dependent deficiencies in learning and performance in an active avoidance conditioning test. To determine if adult physiological function is altered by developmental PAH exposure, a swim tunnel respirometer was used to measure total oxygen consumption. Animals developmentally exposed to B[a]P demonstrate a significant increase in oxygen consumption rates. Finally, to explore what transcriptional events are occurring prior to the onset of developmental toxicity, mRNA expression changes in B[a]P-exposed embryos were examined with whole-genome RNA-seq at 48hpf. Collectively, these data demonstrate that developmental PAH exposure results in a number of non-cancer endpoints and we are now positioned to identify the mechanisms underlying these complex responses. This research was supported by NIEHS grants P42ES016465 and P30ES002010.

Effects of Benzo[a]pyrene and Cyp19B Knockdown on Early Zebrafish Development

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Benzo[a]pyrene (BaP) is a ubiquitous environmental contaminant that is an endocrine disrupting and carcinogenic PAH. Previously we found that BaP significantly decreased fish brain aromatase (CYP19B) expression, a key enzyme in steroidogenesis. We hypothesized that BaP deregulates the steroid hormone hypothalamo-pituitary-gonadal feedback loop adversely affecting development and physiology. Here, we consider whether the toxicities observed following BaP exposure are comparable to those following transient CYP19B knockdown during early development. One-cell zebrafish embryos were injected with a CYP19B morpholino or the Genetools control-MO. Other non-injected embryos were exposed to nominal waterborne concentrations of BaP (0, 10 & 50 μg/L) for 96 hours post-fertilization (hpf). BaP and CYP19B knockdown resulted in a number of similar phenotypic endpoints. Cumulative mortality of zebrafish larvae was significantly increased by 5, 6, and 20% for low BaP, high BaP, and CYP19B knockdown, respectively, compared to controls. Hatching efficiency was significantly decreased by all treatments at 48 hpf. In a treatment-blinded morphological assessment of larvae at 96 hpf, the body length, optic vesicle, and swim bladder inflation of larvae were significantly decreased while pericardial and abdominal edema were increased by high BaP and CYP19B knockdown. The incidence of normal larval body, tail and pectoral fin shape was significantly decreased in both treatments. To identify the effects of BaP and CYP19B knockdown on primordial germ cell (PGC) migration, in-situ hybridization was done on embryos at the 25 somite stage; preliminary results suggest that BaP may affect PGC migration. Zebrafish were collected at 2B, 32, 35 & 52 days post-fertilization to determine if gonad maturation was delayed. Results suggest BaP effects in zebrafish embryos are similar to the effects observed by aromatase knockdown. Supported by NIEHS R03 ES018962.

Developmental Exposure to Benzo[a]pyrene Affects Behavior and Energetics in Larval and Adult Zebrafish

K. L. Willett, R. L. Tanguay and L. Truong, Dept. of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants present in urban air, dust, and soil resulting from incomplete combustion of organic materials or fossil fuels. It is widely recognized that PAHs pose risks to human health, especially for the developing fetus and infant, where PAH exposures have been linked to in-utero mortality, lower intelligence, and cardiovascular effects. Using the zebrafish model, we evaluated the developmental toxicity of PAH exposures. Zebrafish embryos were exposed from 6-120 hours post fertilization (hpf) to B[a]P, BaA, BEZO, and 9,10-PHEQ concentrations that caused no observable morphological toxicity. Using the Seahorse Extracellular Flux Analyzer, we measured in vivo respiration in 26hpf embryos exposed to PAHs. Exposure to B[a]P, BaA, BEZO, and 9,10-PHEQ caused decreased oxygen consumption rates, indicative of mitochondrial damage. Developmental B[a]P and BaA-Q exposure also resulted in hyperactive swimming at 120hpf. To determine if behavioral and physiological effects persisted, a subset of exposed animals were raised to adulthood in chemical-free water and assessed for learning and energetic deficits. Preliminary results indicate B[a]P-dependent deficiencies in learning and performance in an active avoidance conditioning test. To determine if adult physiological function is altered by developmental PAH exposure, a swim tunnel respirometer was used to measure total oxygen consumption. Animals developmentally exposed to B[a]P demonstrate a significant increase in oxygen consumption rates. Finally, to explore what transcriptional events are occurring prior to the onset of developmental toxicity, mRNA expression changes in B[a]P-exposed embryos were examined with whole-genome RNA-seq at 48hpf. Collectively, these data demonstrate that developmental PAH exposure results in a number of non-cancer endpoints and we are now positioned to identify the mechanisms underlying these complex responses. This research was supported by NIEHS grants P42ES016465 and P30ES002010.
anti-oxidant pathways. Therefore, we explored the potential for these compounds to produce adverse developmental effects using zebrafish (Danio rerio) as a model vertebrate. We specifically tested the hypothesis that flavonoids exhibit effects on development through ER-dependent and -independent mechanisms. Embryos were exposed to 5 μM of each flavonoid (epigallocatechin, biocianin A, 5-epi-epigallocatechin, epigallocatechin-3-gallate, and kempferol), 1-50 μM, from 6 hours post fertilization (hpf) to 120 hpf. Effects included yolk-sac and pericardial edemas, axial defects, fin dysmorphogeneis and craniofacial abnormalities. Two compounds (S-equol and biocianin A) induced estrogen-responsive genes (vitellogenin, cyc19a1b, lbh) indicative of nuclear ER activation. Co-exposure with the ER antagonist tamoxifen significantly inhibited induction of these genes, but did not rescue against developmental toxicity. We also observed spastic pectoral fin and caudal tail movements for all compounds, which is suggestive of neurotoxicity, and not apoptotic of estrogen action. We therefore assessed acute effects on neurobehavior in naive 120 hpf larvae challenged with a battery of 24 flavonoids. All induced hyperactive swimming behavior suggesting flavonoids have stimulatory properties at these concentrations. These results taken together indicate that although the estrogen receptor mediates some effects on gene expression, developmental toxicity and neurobehavioral effects are likely not a result of nuclear ER action. This research is supported by NIEHS grants P30 ES00210, RC4 ES019764 and T32 ES07060.213 Combined Effects of Selenomethionine and Osmotic Stress on the Unfolded Protein Response and Apoptosis during Embryonic Development

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Pregnancy-Induced Hypertension (PIH) can lead to increased risk of adverse fetal and maternal outcomes by generating hypoxic stresses for the mother and developing fetus. Although osmotic stress may not have direct lethal effects, it may alter the toxicities of xenobiotics during development. Organic forms of selenium, such as selenomethionine (SeMet), are often taken as dietary supplements, yet alter the toxicities of xenobiotics during development. Measuring CYP1A mRNA and protein expression as bio-markers for AHR activation, we determined that mTIP principally interacts with SeMet but may also bind with AHR1A and AHR1B to induce CYP1A expression. However, triple knockdown of all AHR isoforms failed to prevent mTIP-induced cardiac toxicity even though there was no detectable CYP1A expression. These results suggest that mTIP causes AHR-independent toxicity through a pathway that is also antagonized by CH223191.

ATF4, IRE1 and BiP were up-regulated in a time-dependent manner after 4 and 8 hours and BiP continued to increase at 12 hours. Osmotic stress without SeMet induced IRE1 and ATF6 after 24 hours. These results indicate embryo lethality may occur through apoptosis and the unfolded protein response and that osmotic stress may compound these effects.

214 Ethanol Exposure during Medaka (Oryzias latipes) Embryogenesis: Impact on Cannabinoid Receptors

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Developmental ethanol exposure is able to induce Fetal Alcohol Spectrum Disorder (FASD) phenotypes in medaka (Oryzias latipes). This study investigated possible expression of cannabinoid receptor (cnr) mRNAs during medaka embryogenesis and variability to ethanol-induced FASD phenotypes. Data base searching (GenBank, Ensembl) indicates that medaka genome includes three human orthologs CNR (cnr1a, cnr1b and cnr2). A quantitative real-time PCR (qPCR) technique was used to analyze the expression of these three cnr during medaka embryogenesis and their response to ethanol. Embryos at different stages of development and hatchings were utilized for RNA extraction followed by qPCR analysis of cnr1a, cnr1b and cnr2 mRNAs at the message level. Moreover, fertilized medaka eggs (Iwamatsu stage 10) were exposed to ethanol (300 mM) either for 2 days or for 6 days followed by cnr1a, cnr1b and cnr2 mRNA analysis. qPCR analyses indicate that the expression of all three, cnr1a is developmentally regulated and only cnr2 mRNA showed maternal expression. The mRNA concentrations of all cnr were found to be enhanced after 3 dpf and attained maximal level either prior to or after hatching. Embryos exposed to ethanol (300 mM) two days are unable to produce any significant effect in cnr gene expression immediately after ethanol removal; however, if these embryos were maintained in clean hatching solution without ethanol for another four days (6 dpf), only cnr1a mRNA was reduced significantly. Expression of other two cnr mRNAs (cnr1b and cnr2) either in 2 dpf or in 6 dpf remained at the same level as in controls. Six dpf exposure of the embryos to ethanol (300 mM) is also showed significant reduction in cnr1a mRNA content; however, cnr1b and cnr2 mRNAs maintained the same status as in the controls. Among the three, ethanol disrupts the expression of only cnr1a that may probably lead the development of FASD phenotypes in medaka.

215 PAH Exposure in Early Xenopus Embryos Alters Cardiac Function and Aryl Hydrocarbon Receptor (AhR) Signaling


The incomplete combustion of fossil fuels results in deposition of the polyaromatic hydrocarbons (PAHs), pyrene (PYR) and phenanthrene (PHE), into air, soil, and water. These common and persistent contaminants are also present in tobacco smoke, coal tar sealant, and weathered crude oil and have been detected at significant levels in human milk and cord blood. Surprisingly little is known about their toxicity to humans, particularly during development. To gain a better understanding of potential risks to humans, we characterized the effects of PHE and PYR exposure on cardiac function and AhR signaling using embryos of X. laevis, an important model of vertebrate development. We exposed albino embryos, at the onset of gastrulation, to 0.25, 2.5, or 25 μM PHE or PYR for 120 h and recorded the cardiac activity at various stages of heart development using an inverted dissecting microscope, Leica™ video camcorder, and recording and editing software (Camtasia™ and Image™) to determine heart rate, interbeat variability, and contractility. The mRNA levels for the two CYP1A genes, CYP1A6 and CYP1A7, and for the AHR gene (AhR repressor) were quantified for whole embryos exposed for 96 h to PYR at 0.5 and 25 μM. At stage 42 (72 h, during heart valve formation), PHE treatment (2.5 and 25 μM) was associated with decreased heart rate and increased interbeat variability (P < 0.01), a measure of arrhythmia, while exposure to PYR at these same doses led to increases in both heart rate and contractility (P < 0.01). These two PAHs have distinct impacts on cardiac signaling, suggesting the Xenopus heart may serve as an excellent developmental system for investigating PAH-induced alterations in signaling. In addition, exposure to 0.5 μM, but not 25 μM, PYR for 96 h was associated with 2-3 fold increases in CYP1A6 and AHR mRNA levels (P < 0.01), indicating activation of AhR signaling, a pathway essential to normal development, and a possible low-dose effect for PYR.
Innovative toxicity test systems that are translational to effects in humans and that allow reduction, refinement and replacement (3Rs) of vertebrates to assess chemical safety are urgently needed for ethical, scientific and economic reasons. Whereas cell culture systems may have predictive value, they lack the multicellular or multi-organ complexity of whole organisms. In addition, in view of developmental and reproductive toxicology (DART), they lack a complete life cycle. Therefore, we have compared D. discoideum (slime mold), C. elegans (nematode) and embryos of D. rerio (zebrafish) as possible systems to test for DART. These systems have gained attention as highly promising 3R alternatives since they have highly conserved gene-ontologies, tissue types and molecular responses. We have performed a combinatorial knowledge and experimental based comparison to align D. discoideum, C. elegans and D. rerio in order to identify common denominators as translational biomarkers for DART. We exposed the three systems to 4 compounds, the kinase inhibitor H89, antimicrobial agent mofloxicaine, boric acid and valproate. Interestingly, although individual species showed species specific phenotypic effects upon compound application, these phenotypes were indicative of alterations in conserved and expected core molecular pathways, like PKA and inositol signaling. Individual test systems further showed differences in sensitivity to compounds. In all test systems, RNA-sequencing revealed that alterations in gene expression profiles are additive between test species and that they represent genes that have orthologs in humans. We concluded from these findings that the combinatorial use of surrogate systems may be a valuable tool for predictive DART testing. Further validation includes benchmarking of well known DART toxicants and building of a Weight of Evidence platform.

**219 Exposure to Pyrene and Phenanthrene Alters Normal Pigmentation in Xenopus laevis Embryos**


Pyrene (PYR) and phenanthrene (PHE) are among the most abundant of the polycyclic aromatic hydrocarbons (PAHs) deposited into the environment. Despite the observation that both are present in human milk and cord blood, little is known about their developmental toxicity. Our lab uses a well-established amphibian model of vertebrate development, the African clawed frog *Xenopus laevis*, to assess the developmental impacts of PYR or PHE exposure. Melanophores are the amphibian cellular equivalent of human melanocytes that produce the brown-black pigment melanin. Embryos, at the onset of gastrulation, were exposed to 0, 0.25, 2.5, or 25 μM PHE or PYR for 96 hr and melanophore number and dendricity (branched or Dalmatian spot appearance) were observed. By 96 hr, melanophore dendricity was drastically reduced in embryos exposed to 25 μM of either PHE or PYR (P<0.001), effects that were not reversed after return to control conditions for 24 hr. In addition, while there was no effect at 96 hr on melanophore number (or density), the distance migrated by the melanophores was reduced in embryos exposed to either PHE or PYR at 25 μM (P<0.01). Loss of dendricity can disturb the dispersal and transfer of melanin to surrounding keratinocytes, thus interfering with UV protection in the skin. Melanophores could serve as an accessible indicator cell system for potential PAH-induced negative impacts on developing tissues, particularly for other neural- and vascular-relevant receptors, etc. Interestingly, many off-targets involved in cell cycle and spindle apparatus formation, like histone deacetylase, microtubules formation, etc. With these additional models it was possible to significantly improve the ratio of identified DART positive compounds.
Secondary, non-mammalian in vitro assays were used for the prediction of DART, mainly results from the FETAX (Frog Embryo Teratogenesis Assay Xenopus) and ZETA (Zebrafish) assays. The predictivity of these in vitro assays when used as single results were similar to the in silico models. But a combined in silico in vitro approach was able to identify and evaluate compound inducing DART effects with sensitivities up to 75% and specificities around 70%, depending on the in silico models used and available in vitro results. These validation results showed that an integrated testing strategy of in silico models and in vitro assays is able to predict the in vitro DART inducing potential of drug candidates effectively and early in development.

221 Transgenerational Effects of the Endocrine-Disrupting Herbicide Atrazine in Zebrafish
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Atrazine, an agricultural herbicide that is a common contaminant of potable water supplies, is implicated as an endocrine disruptor and potential carcinogen. Ongoing studies are defining the genetic and epigenetic mechanisms of an embryonic exposure to atrazine, assessing the later in life adverse health outcomes in the exposed generation, and transgenerational effects using the zebrafish model system. Embryos were exposed to environmentally relevant concentrations of atrazine shortly after fertilization through the end of embryogenesis. Transcriptional profiles immediately following the exposure identified expression alterations in genes associated with neuroendocrine function, cell cycle regulation, and carcinogenesis. Adults developmentally exposed to atrazine had a significant difference in the number of pairs that successfully bred and was coupled to a distinct alteration in reproductive system. Embryos were exposed to environmentally relevant concentrations of atrazine shortly after fertilization through the end of embryogenesis. Transcriptional profiles immediately following the exposure identified expression alterations in genes associated with embryonic and tissue development and with neurological and ophthalmic diseases. Furthermore, the beta-estradiol pathway was predicted to be inhibited. The F2 were raised to maturity and significant differences in reproductive function outcomes were observed with the number of pairs that successfully bred and was coupled to a distinct alteration in reproductive phenotype in the exposed females. To determine if adverse effects were passed onto progeny, an unexposed generation was created from adults developmentally exposed to atrazine to assess paternal and/or maternal inheritance (F2). RNA-sequencing of the F2 at the end of embryogenesis revealed enrichment of expression alterations in genes associated with embryonic and tissue development and with neurological and ophthalmic diseases. Furthermore, the beta-estradiol pathway was predicted to be inhibited. The F2 were raised to maturity and significant differences in reproductive function outcomes were observed with the greatest impacts in the cross of the males developmentally exposed to atrazine with control females. No significant differences were observed in the F3 larvae in regards to survivorship, hatching or morphological analysis of whole larvae, head, or eye length. Overall these results indicate that transgenerational effects on the female reproductive system are most pronounced in progeny from the males developmentally exposed to atrazine. Overall these results indicate that transgenerational effects on the female reproductive system are most pronounced in progeny from the males developmentally exposed to atrazine.

222 Evaluation of Teratogenic Effect of BMP2-Inducible Kinase Inhibition in Zebrafish by Using Morpholinio Antisense Oligonucleotides

Bone morphogenetic protein 2 (BMP2) has regulatory role in attenuating the program of osteoblast differentiation. BMP2 inducible kinase (BIKE2) is a serine/threonine protein kinase upregulated during BMP2 activation. Since there are no published reports related to BIKE2 inhibition inducing embryo malformation, we evaluated roles of BIKE2 in embryo development in zebrafish using morpholino antisense oligonucleotides (oligos) targeting BIKE2 and p53. (Anti-p53 mitigates morpholino-associated apoptosis/toxicity in embryos). Zebrafish embryos (2-32 cell stage) were micro-injected with BIKE2 antisense, or non-sense control oligonucleotides and subsequently evaluated for developmental malformations 5 days post fertilization/post-injection. Craniofacial, heart, and profound axial malformations, including caudal dygenesis and ectopic tails were observed in groups injected with BIKE2 morpholino antisense oligos. The spectrum of malformations was similar to the “super-dorsalization” phenotype previously reported with BMP2 inhibition. Compared with control non-sense oligos, malformations were significantly higher in embryos containing antisense BIKE2 oligos (5% in control vs 20% using a single BIKE2 antisense oligo, and 37% using a combination of two BIKE2 antisense oligos, respectively). Compounds that bind with high affinity and specificity to BIKE2 also caused teratogenic effects indicating skeletal abnormalities consistent with gene targets downstream of BMP2 and/or involved in dorsalization using RT-PCR. Reductions of the gene products ostepherin (regulating osteocyte precursors for bone cell differentiation), pax2a (required for midbrain–hindbrain boundary formation, which affects dorsal expansion), ev1, a (ventral signal required for normal mesodermal progenitor cells development), and krox-20 (required for hindbrain formation and specification) were observed. Together these studies indicate that inhibition of BIKE2 has teratogenic effect on zebrafish embryo development.

223 Tributyltin (TBT) Exposure Promotes Zebrafish Sexual Differentiation
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Tributyltin (TBT), an antifouling agent, has been implicated in the masculinization of fish species worldwide, however the molecular mechanism is still not completely understood. Our lab has previously examined the actions of TBT as an endocrine disruptor in zebrafish (Danio rerio) and determined, in vitro, that TBT inhibits zFER specific activity in a dose dependent manner and may potentially act through the RXR portion of the PPAR-RXR (peroxisome proliferator-activated receptor gamma - retinoid X receptor alpha) heterodimer. Additionally, zebrafish were exposed to increasing concentrations of TBT and sex differentiation genes were analyzed via qPCR. Results from qPCR focused our experimental efforts on the candidate gene, SRY-box containing gene 9a (Sox9a). Sox9a is a key regulator in mammalian testis differentiation, where it is shuttled to the nucleus upon differentiation; this appears to be a conserved mechanism across fish, marsupials and placental mammals. Developing zebrafish were exposed to 1µM and 2.5µM TBT from 10 days post hatch (dph) to 90 dph and sampling was done at 25, 40, 60 and 90 dph. Fish were treated three times per week with TBT, estrogen, testosterone, or vehicle. Following treatments, tissues were fixed in 4% PFA, processed, paraffin imbedded, and then immunohistochemical (IHC) analysis was performed to assess Sox9a nuclear or cytoplasmic localization.

223a Mitochondrial Uncoupling Disrupts Neurodevelopment in Zebrafish Embryos
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Caloric restriction is a proven method for weight loss and possible lifespan extension. However, caloric restriction is detrimental in early development as malnourished mothers produce offspring with low birth weights, developmental delay and neurodevelopmental defects, which have been linked with diseases in later life. Inhibiting mitochondrial energetics, such as through exposure to uncouplers like 2,4-dinitrophenol (DNP), mimics caloric restriction. DNP disrupts the proton gradient needed for ATP synthesis by oxidative phosphorylation, and is a common environmental toxicant in automobile exhaust, industrial waste and pesticides. DNP is also seen as an illicit weight-loss drug, particularly with women of childbearing age due to its ready availability online. Thus it is important to analyze the effects of exposures to mitochondrial toxins such as DNP on the developing embryo, as a potential cause of some birth defects and neurological disorders. We use zebrafish embryos to study the effects of mitochondrial uncoupling on embryonic development, as breeding pairs produce hundreds of transparent embryos that develop rapidly outside of the mother and hatch within 3 days post-fertilization (dph). Here we show that mitochondrial uncoupling by DNP disrupts embryonic development, causing developmental delay and neurodevelopmental defects. The development of the primary motor neurons and retinal layers were particularly impacted, which may explain the locomotor defects observed in DNP exposed embryos. Biochemically, we observed that mitochondrial ATP turnover was decreased throughout embryonic development. Likewise, protein oxidation and other markers of oxidative stress response were decreased. As reactive oxygen species (ROS) are required for nervous system development, we propose that the neurodevelopmental defects we observe are likely due to decreased ROS signaling in embryos.

223b Relative Developmental Toxicity of Pentachlorophenol and Pentachloroanisole in Zebrafish (Danio rerio)
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Pentachlorophenol (PCP) and pentachloroanisole (PCA) are widely distributed chlorinated aromatic compounds and have been found in the environment and human populations. The objective of this study is to characterize and compare the effects of PCP and PCA on development using a fish model. Zebrafish embryos were exposed to 0.1, 1, 10, 100, 500, 1000 µg/L PCP and PCA respectively for 96 h. The sub-lethal and lethal effects were studied through malformation observation, LC50 testing for survival rate at 96 hpc and EC50 testing for hatching rate at 72 hpc. The mRNA gene expression changes related to brain growth and thyroid
hormone regulating pathways were investigated. LC50 at 96 hpf was 3.04 µg/L for PCP << 336.0 µg/L for PCA, and EC50 for hatching at 72 hpf was 2.74 µg/L for PCP << 339.1 µg/L for PCA, indicating that the developmental toxicity of PCP was at least 100 times higher than that of PCA. Other sub-lethal effects, such as yolk deformation, malformation of tail, scoliosis, hemorrhage and pericardial edema, were observed. Both PCP and PCA showed hyperactivity effects similar to that of triiodothyronine (T3) resulting in increased mRNA expressions of Synaptophysin (SYN), Isothyroid Deiodinase type II (Dio2), Isothyroid Deiodinase type III (Dio3), Thyroid hormone receptor alpha 2 (THRαa) and Thyroid hormone receptor beta (THRβ). Our results on the relative developmental toxicity of PCP and PCA and the possible underlying mechanisms will be useful to support further research in human populations.

The interaction between regulatory pathways is an important mechanism by which organisms can adapt to their environment and respond to endogenous stress. One interaction that recently has been identified in adult mammals, cell culture, and fish embryos is that occurring between the aryl hydrocarbon receptor (Ahr) and members of the nuclear factor erythroid-2 related factor (Nrf) family. Each transcription factor regulates numerous downstream genes involved in the cellular response to toxicants and oxidative stress; they each also play a role in normal development. In zebrafish embryos, where there are six nrf genes, developmental gene expression of all nrf has been shown to be altered in the absence of Ahr1B protein using morpholino knockdown. The mechanism of this altered transcriptional response by Ahr1B has been explored using Chromatin Immunoprecipitation (ChIP) in zebrafish embryos to test the hypothesis that Ahr1B is binding directly to cis-promoter elements to regulate the developmental transcription of nrf genes. Using ChIP coupled with PCR, Ahr1B has been shown to directly bind to XREs on the promoter of each nrf gene in the presence of TCDD, confirming its role as a transcriptional regulator of the entire nrf gene family. This research highlights the importance of the TCDD-Ahr-Nrf gene battery during the most sensitive life stage, the embryo, and provides a better understanding of how combinatorial molecular signaling can protect embryos from potentially embryotoxic events following toxicant exposure.

As part of the chemical screening and prioritization research program of the US EPA, the ToxCast Phase II chemicals were assessed using a vertebrate screen for developmental toxicity. Zebrafish embryos (Danio rerio) were exposed in 96-well plates from late-blastula stage (6hr post fertilization, pf) through day 5pf (1-2 days post-hatch). All exposures were by immersion and renewed daily. The 700 chemicals included food additives, consumer use product ingredients, pesticides, failed pharmaceuticals, and “green” plasticizers (http://epa.gov/ntct/toxcast/chemicals.html). Intra- and inter-plate replicates were included for quality control. Developmental toxicity was initially assessed using a single nominal concentration of 80 µM: positives and a selection of negatives were confirmed by concentration-response determinations. On day 5pf, larvae were moved from exposure solution to a control solution without chemical, and on day 6pf were assessed for overt toxicity (i.e., death, non-hatching and dysmorphology; n=4 embryos per chemical). Dysmorphology was a combined score using both in-life observation and brightfield, high-content image analysis. Overt toxicity was noted with 46% of the chemicals tested compared to 62% positive chemicals when the ToxCast Phase I library, consisting of mostly pesticide active ingredients, was previously tested. As with the Phase I library, the octanol-water partition coefficient (logKow) of the Phase II library chemicals was positively correlated with overt toxicity: there were 18% positive chemicals with logKow 0-4; 41% positive chemicals with logKow 0 to 4; and 67% positive chemicals with a logKow >4. All chemicals positive at the single concentration were further assessed for potency using a Dose-Response Study (8-point, semi-log concentration curve: n=3 embryos per concentration). These data demonstrate the utility of zebrafish in medium-throughput chemical testing programs for detection of adverse developmental outcomes. This abstract may not necessarily reflect official Agency policy.

Polycyclic aromatic hydrocarbons (PAHs) are known to induce developmental defects in vertebrates, and exposures of fish embryos to PAHs have been shown to cause cardiac deformities. However, the mechanisms underlying this toxicity remain unclear. The aryl hydrocarbon receptor (AHR), a transcription factor, is activated by and mediates toxicity of some PAHs. Exposure to a simple PAH mixture consisting of an AHR agonist (benzo(a)pyrene-BaP) and fluoranthene (FL), an inhibitor of cytochrome p50 (CYP1) - a gene induced by activation of the AHR - results in pericardial effusion and an elongated heart. Knockdown of AHR2 prevents this toxicity. In this study, we used a microarray analysis to identify genes involved in this toxicity. To determine the role of AHR2 by knocking down this gene, we used zebrafish embryos injected with a control morpholino and AHR2 morpholino. Embryos at 36hpf were then exposed to DMSO, 100 µg/L BaP, 500 µg/L FL, or 100 µg/L BaP + 500 µg/L FL. We extracted heart tissues at various time points (2h, 6h, 12h, and 18h) for RNA. Overall data showed that gene expression differences between the AHR knockdown and control embryos decreased over time, suggesting that the AHR mediated gene expression due to PAHs decrease within 18 h of exposure. Furthermore, despite BaP + FL resulting in a deformed heart, this mixture resulted in smaller effect on overall gene expression compared to BaP or FL alone. Differentially expressed genes by BaP + FL in AHR knockdown and control embryos were primarily involved in Ca2+ cycling, cardiac muscle contraction, suggesting that PAH affects on cardiac muscle function may underlie the structural deformities. Furthermore, several genes such as Pdlim1 - a gene involved in cardiac looping – that were identified may play a direct role in underlying cardiac abnormality in fish. Ingenuity pathway analysis on transcriptomic data suggested that FOS signaling pathway might be playing a key role in mediating AHR-dependent cardiac toxicity.

Background: Lead exposure has been associated with high blood pressure. Oxidative stress and inflammation have also been independently associated with high blood pressure. Objective: We examined the association of blood lead levels, serum gamma-glutamyl transferase (GGT), and serum C-Reactive Protein (CRP) levels with systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension in adult participants in the National Health and Nutrition Examination Survey (NHANES) 2007-2010. Methods: The outcome of interest were SBP and DBP, hypertension, blood lead levels, serum GGT, and serum CRP. Serum CRP, serum GGT, and blood lead were log normal transformed. Blood lead was also analyzed using weighted quartiles. Linear regression, logistic regression and multilog procedures were used with a priori covariates age, race/ethnicity, gender, education, smoker status, alcohol use, diabetes, body mass index, and serum creatinine. Results: Higher SBP statistically correlated with blood lead and serum GGT in all adults and in male and female subgroups. Blood lead and serum GGT were associated with hypertension when all individuals were entered in the model and in the female subgroup after stratification. Elevated serum CRP was associated with serum GGT and with DBP, but not with blood lead, SBP, or hypertension. Conclusions: This study demonstrates a possible biological pathway through which lead induces hypertension and increases SBP. Our results indicate that lead may work through a direct mechanism as well as through the indirect mechanism of oxidative stress in order to affect these outcomes.

Background: Exposure to environmental chemicals may play a role in the development of obesity. There is evidence that environmental exposures are associated with obesity in children and adults.

223c Transcriptional Regulation of the Nuclear Factor Erythroid-2 Related Factor (NRF) Family by the Aryl Hydrocarbon Receptor (AhR)

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223d Toxicity Screening of the ToxCast Phase II Chemical Library Using a Zebrafish Developmental Assay

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223e AHR-Dependent and AHR-Independent Genes Involved in the Synergistic Cardiac Developmental Toxicity of PAHs

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223f Serum Gamma Glutamyl Transferase Is Associated with Higher Blood Pressure and Lead-Induced High Blood Pressure: A Model Pathway Using NHANES 2007-2010


223g Association of Urinary Dichlorophenols with Increased Body Weight Measures and Obesity in Children and Adults: Analyses of NHANES 2007-2010


Background: Exposure to environmental chemicals may play a role in the development of obesity. There is evidence that environmental exposures are associated with obesity in children and adults.
Objective: To examine the association of urinary levels of the environmental pesticides 2,5-dichlorophenol (2,5-DCP) and 2,4-dichlorophenol (2,4-DCP) and the environmental phenoxytriclosan with body weight outcomes in children, adolescents and adults participating in the National Health and Nutrition Examination Survey (NHANES) 2007-2010.

Methods: We performed multivariate linear and logistic regression to analyze the association of BMI z-score, waist circumference, and obesity with urinary pesticide concentration in children and adolescents. We conducted the same analyses in adults with the outcomes of BMI, waist circumference and obesity.

Results: We found a statistically significant positive association (p<0.05) between 2,5-DCP and BMI z-score and obesity in children and adolescents. In adults, there was a statistically significant positive association (p<0.05) between 2,5-DCP and BMI, waist circumference, and a non-monotonic association with obesity. However, a statistically significant relationship between 2,4-DCP and body weight outcomes was not found in adults. No associations were found between the body weight outcomes and any of the body weight outcomes for either children or adults.

Conclusions: We found an association between dichlorophenols and increased body weight measures in both children/adolescents and adults. Additionally, we found an association between dichlorophenols and obesity in children/adolescents and a non-monotonic response in adults.

223h Airway Inflammation Markers in Exhaled Breath from Children Are Linked with Exposure to Black Carbon

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The current study assessed the associations between airway oxidative stress and inflammation markers and BC exposure in primary school children in a Western European urban area.

In 130 children 6-12 years old, the fraction of exhaled nitric oxide (FeNO), exhaled breath condensate (EBC) pH, 8-iso prostaglandin and IL-1β were measured in two seasons. Acute BC exposure on the sampling day was assessed using BC measurements at a central monitoring site. Land use regression (LUR) models were applied to estimate weekly and seasonal average BC exposure. Associations between biomarkers and exposure were tested using linear mixed effects regression models. Next to single exposure models, models combining multiple exposures were analyzed. Oxidative stress and inflammation markers were associated with BC levels, independent of gender, age, allergy status, parental education level and meteorological conditions on the sampling day. Depending on the considered single or multiple exposure model, IQR increases in acute BC exposure ($100 ng/m^3$) were linked with higher 8-iso prostaglandin ranging from 5.9 (95% CI 0.1-12.0) to 9.4% (95% CI 2.1-17.2). Increased FeNO levels ranging from 12.1 (95% CI 2.5-22.8) to 15.2% (95% CI 4.4-27.0) were associated with IQR increases in weekly BC exposure (1730 ng/m^3). IQR increases in acute and weekly BC were linked with increases in IL-1β between 19.3 (95% CI 1.3-40.5) and 42.8% (95% CI 11.2-83.2).

Acute BC exposure was associated with airway oxidative stress while weekly and acute exposure was linked with airway inflammation. The association between BC exposure and IL-1β in EBC reflects activation of cells involved in first line defense mechanisms of the airways such as airway epithelial cells and macrophages, as was previously suggested by in vitro studies.

223i Arsenic Level in Drinking Water and Lung Cancer Mortality among 127 United States Counties from 1950 to 1979

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Background: Studies from Asia and Latin America found arsenic to be a risk factor for bladder and lung cancers in areas with high levels of arsenic (> 100-200 ug/L) in drinking water. Evidence for cancer risk at the < 100 ug/L arsenic levels found for bladder and lung cancers in areas with high levels of arsenic (> 100-200 ug/L) in drinking water was supported by a study in Diepenbeek, Belgium and 4Provincial Institute of Hygiene, Antwerp, Belgium.

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We hypothesized that greater longevity may influence age-adjusted incidence of two selected mesenchymal cancer types since enhanced robustness in older age groups may more accurately reflect age-related cancer trends. Using SEER 9 registry data we examined age-adjusted pleural mesothelioma (PM) and soft tissue sarcoma (STS) incidence rates for 1973 through 2010 in conjunction with concurrent changes in age distribution of the US population. The overall age-adjusted rate per 100,000 person-years for PM significantly (* p<0.05) increased by 185%* from 1973 to its peak value in 1992, then decreased by 18% from 1992 to 2010. The 75 yr age group dominated the early PM trends, increasing by <600%* for 1973-1992, and again by 19%* for 1992-2010. In contrast, the 0-74 yr age group showed a 96%* increase in age-adjusted PM for 1973-1992, and then declined by 47%* for 1992-2010. By comparison, overall age-adjusted STS rates were increased by 17%* for 1973-1992 and again by 29%* for 1992-2010. The 75+ yr age group dominated the STS trends in both intervals, increasing by 43%* for 1973-1992, and again by 41%* for 1992-2010, while the 0-74 yr age group showed only 11% (n.s.) and 25%* increases in these respective intervals. The SEER 9 registry exhibits significant increases in the 75+ yr population at risk from 1973 (3.7%) to 1992 (7.4%) and again for 2010 (10.1%). We conclude that the continuing shift toward greater longevity since 1992 may help to explain the greater elevation in age-adjusted PM and STS rates among the increasingly robust 75+ yr age group. Regulatory controls on asbestos may explain the 47% decline in PM for the 0-74 yr age group from 1992 to 2010, while the 19% increase in the 75+ yr age group may represent a longevity effect that is apparent for both PM and STS.
nostic methods and changes in disease classification since 1992 also may contribute to these trends, and the issue of longer latency for asbestos-related PM should be examined further.

226 Aflatoxin Exposure Associates with Abnormal Liver Function and Infections with HIV and TB in Uganda

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Aflatoxins (AF) exposure is a serious global health threat. The epidemic of liver disease and infections with HIV and TB was associated with AF exposure in Ghana; however, the correlation between AF exposure and the progression of these epidemic diseases has not been firmly established in other African populations. In this study we assessed AF-lysine adduct level in serum samples collected from a normal human group (n=267), a group with abnormal liver function (n=132), a group with HIV infection (n=536), and a group with HIV/tuberculosis co-infection (n=62) in Uganda. AF-lysine adduct was measured via high-performance liquid chromatography (HPLC)-fluorescence detection with a limit of detection of 0.4 pg/mg albumin. Median serum AF-lysine adduct level was 1.54 pg/mg albumin (range=0.45-253.11) among normal human group; median serum AF-lysine adduct level was 2.88 pg/mg albumin (range=0.54-153.06) among the group with abnormal liver function; median serum AF-lysine adduct level was 3.19 pg/mg albumin (range=0.41-167.04) among the group with HIV infection; and the median serum AF-lysine adduct level was 8.90 pg/mg albumin (range=0.54-70.41) among the group with HIV and tuberculosis co-infection. Groups with abnormal liver function (p<0.01), HIV infection (p<0.01), and HIV/tuberculosis co-infection (p<0.001) had significantly higher AF-lysine adduct levels than that in the normal human group. Patients with severe HIV progression, dependent on CD4+ T cell counts, had a significant higher adduct level than those with minor progression (P-trend=0.0062). In addition, the adduct level was synergistically increased in HIV patients infected with tuberculosis compared to the HIV patients without tuberculosis and non-infected normal group (P-trend=0.0001). This study confirms previous findings and implies that mitigation of AF exposure not only can reduce liver diseases, but also an innovative strategy to reduce the spreading of HIV and tuberculosis in epidemic sub-Saharan African countries.

227 A Systematic Review of the Epidemiologic Evidence on Low-Level Arsenic Exposure and Cognitive Function in Children: Applicability for Risk Assessment


The U.S. Environmental Protection Agency is currently revising their 1988 non-cancer and cancer risk assessments for inorganic arsenic. Cognitive function in children is an endpoint for which several recent studies bear consideration in deriving a non-cancer reference dose (RfD). We conducted a systematic review of the epidemiologic literature for scientific evidence on possible neurobehavioral effects at lower exposure levels in children. Eleven cross-sectional, case-control, and cohort studies were identified that report on the association between low-level arsenic exposure and cognitive function in older children (e.g., >5 years) was observed overall. A population-based randomized trial from Matlab, Bangladesh (Hamadani et al. 2011), provided the best evidence for informing a quantitative risk assessment (results reported separately), with supporting evidence from another region of Bangladesh. In this study, verbal and, to a lesser extent, full-scale IQ scores were inversely associated with log urinary arsenic levels in girls, but not boys, at age 5 years. It remains unclear whether the findings in the available literature are applicable to similar child populations in the United States. Standard cognitive test measures have not been developed for Bangladesh, and issues such as differences in cultural practices, poor nutrition, adequate correction for maternal IQ, presence of other neurotoxicants in some studies, and dietary deficiencies in Bangladesh greatly limit generalizability to U.S. populations.

228 Clinical and Molecular Features of Myelodysplastic Syndrome following Benzene Exposure

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Chronic exposure to benzene is associated with several possibly related and overlapping hematopoietic diseases such as aplastic anemia (AA), myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML). Recent reports indicate that MDS developing subsequent to benzene exposure may represent the main risk of hematologic disease associated with benzene, and that MDS may develop following exposures to benzene at lower concentrations than previously reported. In addition, cytogenetic abnormalities long assumed to be induced by benzene such as aneuploidy or loss of all or part of chromosomes 5 or 7, which are associated with some forms of therapy related MDS/AML, may not be associated with benzene exposure after all. We have compiled MDS cases from the peer-reviewed literature with documented benzene exposure and describe the clinical and molecular features of benzene-associated MDS (BZ-MDS). We believe such analysis will help to clarify the etiology and pathogenesis of the disease. We report that BZ-MDS cases frequently feature hypoplastic bone marrow, clear indications of an autoimmune mechanism, marked eosinophilia, relatively young age at diagnosis, and increased survival relative to de novo MDS. In addition, cytogenetic features frequently seen in therapy-related MDS (tMDS) do not appear to be prominent features of BZ-MDS. These results highlight a need for additional study of BZ-MDS and suggest a picture of BZ-MDS that is distinct from both tMDS and de novo disease.

229 The Biomarkers of Exposure to ARsenic (BEAR) Pregnancy Cohort in Mexico: Arsenic Methylation Linked to Poorer Birth Outcomes

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Exposure to inorganic arsenic (iAs) from drinking water is a global public health problem yet much remains unknown about the extent of exposure in susceptible populations, including pregnant women and children. Higher prenatal iAs exposure has previously been associated with a greater risk of adverse pregnancy outcomes and later life health effects. Our objective for this study was to establish the Biomarkers of Exposure to ARsenic (BEAR) prospective pregnancy cohort in order to better to understand the effects of iAs exposure and iAs metabolism during pregnancy on maternal-fetal health, with the ultimate aim of increasing awareness and reducing potential exposures to iAs. Concentrations of iAs in drinking water (DW-iAs) and maternal urinary concentrations of iAs and its metabolites (U-iAs) were determined. Birth outcomes were recorded and analyzed for their relationship to DW-iAs, U-iAs, and concentrations and proportions of individual urinary arsines. DW-iAs for the study subjects ranged from 0.9 to 236 μg/L. More than half of the women (53%) had DW-iAs that exceeded the World Health Organization’s recommended guideline of 10 μg/L. DW-iAs was significantly associated with U-iAs. Overall, neither DW-iAs nor U-iAs were associated with any birth outcomes. However a significant inverse relationship was observed between urinary concentrations and proportions of MMAs and birth weight with an average decrease of 25 g. Biomonitoring results demonstrate that pregnant women in Gómez Palacio are exposed to potentially harmful levels of DW-iAs. A relationship between iAs metabolism and adverse birth outcomes were established.

230 A Cross-Sectional Study of Blood Level Lead and Attention-Deficit/Hyperactivity Disorder by Parental Socioeconomic Status amongst Korea Children

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Background: It has been reported that blood lead level increases the risk of ADHD of children.

Objective: We estimated the effect of blood lead level on ADHD controlling for parents’ socioeconomic status (SES).
Methods: Whether or not blood lead level was associated with ADHD by parental SES was evaluated using information on 3,666 school aged children in Korea. Associations between parental SES and the blood lead level were examined by estimating fixed effects model.

Result: After adjustments for potential confounding variables, children with mother in the lowest education (Odds Ratio (OR): 2.38, 95% CI=1.40-4.03) and those living in the most deprived area (OR=1.29, 95% CI =1.01-1.66) were significantly more likely to be associated with ADHD.

Conclusion: Our findings suggest that the association between blood lead level and ADHD vary by characteristic of parental SES.

Keywords: ADHD, blood lead level, SES

231 Cumulative Risk Assessment of Urban Air Toxics: A Pilot Study in San Antonio, Texas

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A pilot cumulative risk assessment project was conducted to test a modeling strategy for estimating the community health burden potentially associated with air toxics while simultaneously accounting for background air toxics, criteria air pollutants, and non-chemical stressors in an urban population of San Antonio, TX. Generalized additive models (GAMs) were used to quantitatively evaluate the potential association between airborne exposure to certain air toxics (lead, arsenic, antimony, cholormethane) generated by two coal-fired electricity generating units (EGUs), background levels of these air toxics, PM2.5, ozone, and social stressors on all cancer and heart disease mortality for 34 census tracts within the 10-mile radius of the EGUs. Four demographic and socioeconomic indicators (i.e., percent African American, percent female, age, and poverty) were significantly associated with all cancer and heart disease mortality, accounting for 70.6% and 79.1% of the variance, respectively. Accounting for these four demographic and socioeconomic indicators in the GAMs, concentrations of the four EGU-specific air toxics were not significantly associated with either all cancer or heart disease mortality in the study area. However, arsenic from non-EGU related on-road and non-road sources was significantly associated with all cancer mortality (88.8% of the residualized variance), PM2.5, lead from non-point sources, and ozone were significantly associated with heart disease mortality (88.1% of the residualized variance). With 17.9 and 21.27 degrees of freedom for cancer and heart disease, respectively, the GAMs were highly complex and non-linear. Although this pilot study has several limitations, it utilizes an innovative approach to quantitatively assess cumulative risk from non-chemical and chemical stressors and may serve as a preliminary model for future analysis.

232 Weight-of-Evidence Evaluation of the Respiratory Effects Associated with Diacetyl

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Recent case reports and epidemiologic investigations have concluded that diacetyl inhalation in the workplace may be associated with serious adverse respiratory effects, including the relatively rare and lethal disease bronchiolitis obliterans (BO). The concern stems from a series of cases of fixed obstructive lung disease consistent with BO that were reported in eight former employees from the Gilster-Mary Lee microwave popcorn plant in Jasper, Missouri. However, over 100 volatile organic compounds were identified in air samples collected in the workplace, and the majority of the cohort was non-occupationally exposed to respiratory irritants, including known inducers of BO. A weight of evidence analysis was conducted to determine if the suggested associations between diacetyl and BO and pulmonary function decrements (e.g., fixed obstruction, decline in FEV1 and FVC) satisfy the Bradford Hill criteria of causation. Based on our analysis, none of the criteria were met. Specifically, no statistical estimate for BO has been reported in the literature and inconsistent associations have been reported between diacetyl exposure and various pulmonary function deficits (across studies and within a single cohort). Furthermore, temporality cannot be established because baseline medical evaluations were not performed on any of the worker cohorts, and none of the epidemiology investigations demonstrate the presence of an exposure-response relationship that can be attributed to diacetyl. In addition, the lack of deep lung effects observed in experimental animals exposed to concentrations of diacetyl that are orders of magnitude higher than the occupational setting contradicts the hypothesis that diacetyl is the probable cause for lung disease in these workers. Thus the weight of evidence does not support the hypothesis that exposure to diacetyl causes BO and/or pulmonary function decline.
Identification of heat-related health risks is of critical importance for outdoor workers, particularly in the Deep South, where heat indices often reach high stress levels in the summertime. We hypothesize that significant differences in vulnerability to heat-related health risks exist in urban groundkeeper populations due to the urban heat island effect and that volatile organic compound (VOC) exposure will be heightened for groundkeepers at urban sites in close proximity to roads with high traffic volumes or major point source industries, particularly when temperatures are high. We set out to determine whether a small, inexpensive temperature/sunlight monitor attached to the shoe could be used to estimate heat exposure. We recruited 21 groundkeepers to wear temperature/sunlight and passive VOC monitors for 7 consecutive days during the summer. Urban and rural non-occupationally exposed participants were also recruited (N=60). In response to an exit survey, most participants (86%) found wearing the monitor on their shoe was very comfortable and reported the monitor was not hard to remember to wear (81%). When data from the individual monitors were compared to data from the closest weather station, weather stations overestimated average heat exposure. In contrast, daily maximum temperatures from a nearby weather station underestimated maximum temperatures experienced by urban participants. A small proportion of the samples analyzed suggest elevated time-weighted average toluene and benzene exposure for non-smoking participants when compared to ambient levels measured in the recent Birmingham Air Toxics study and estimated benzene levels for at least two participants are over the EPA chronic inhalation reference concentration for cancer risk. The investigation contributes to exposure research by determining the effect of microclimates within urban settings on assessing the heat and VOC related health risks associated with outdoor work in the summertime.

**235 Measuring Personal Exposure to Heat and Volatile Organic Compounds in Groundkeepers**

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**236 Mechanisms for the Development of Rat Lung Tumors following Inhalation Exposures to MDI**

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Although the endpoint of greatest concern after inhalation exposure to methylene diphenyl diisocyanate (MDI) (101-68-8) is occupational asthma, it has been suggested that evidence from cancer incidence and mortality studies of workers is insufficient to determine if exposure to MDI is causally associated with cancer. Chronic administration (17 h/d) of MDI aerosol results in proliferative and fibrotic responses and low incidence of bronchoulveolar (BA) adenomas in rats. This poster reviews, evaluates and characterizes studies investigating early events of MDI pulmonary toxicity. Evidence indicates compensatory homeostatic mechanisms leading to pulmonary tumors. Key events include continued exposures to respirable MDI aerosols, localized particle deposition at concentrations depleting protective biomolecules in fluid lining the lung surface, and exposure duration. Once deposited in the BA region, MDI particles interact chemically with biological macromolecules, including surfactant and other proteins, reducing their concentrations in the lung lining. To maintain normal homeostasis increased synthesis of secretory protein is induced. As the increased synthesis becomes maximized but demand for protective proteins is maintained there is a secondary, compensatory response characterized by an increase in cell replication resulting in BA hyperplasia in the terminal bronchioles and ultimately, after prolonged exposure/cellular stress, to the development of adenomas. The observation that MDI particulates do not accumulate in the lung at doses producing lung tumors, together with the lack of chronic inflammatory response at lower exposures, supports a non-genotoxic, compensatory response of the lung to maintain homeostasis. In conclusion, while the intrinsic properties of respirable MDI might pose a similar hazard for disease in both rat and humans, for humans the risk situation is entirely different. Rat lung tumors after chronic exposure to MDI are not predictive of the potential for human carcinogenicity.

**237 Is There Evidence of an Increased Risk to Human Health from Low-Level Exposure to Benzene?**

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Benzene is a volatile chemical used in industry and present in petrol, exhaust fumes and tobacco smoke. Several epidemiological studies have demonstrated an association between occupational benzene exposure and increased leukaemia risk, in particular acute myeloid leukaemia (AML). However, there is still some uncertainty as to the risk to the general population from low-dose benzene exposure. The main route of human exposure is via inhalation of benzene present in the environment or tobacco smoke. Smoking can contribute up to 90% of an individual’s exposure. In order to investigate the risk associated with low-dose exposure, potency estimates for benzene have been generated from individual occupational studies and meta-analysis data, and an exposure assessment for three population sub-groups, non-smokers, light smokers and heavy smokers carried out. Subsequently, various techniques including life table analysis have been used to evaluate risk of leukaemia and AML. The use of epidemiological data to generate a margin of exposure (MOE) was investigated and suggested benzene may be a high priority for risk management actions based on lifetime exposure estimates and its potency as a leukaemogen. Further assessment using meta-analyses data resulted in estimates of the excess lifetime risk of leukaemia at up to 0.006% for non-smokers and up to 0.06% for heavy smokers. For AML this was 0.003% for non-smokers and 0.03% for heavy smokers. The contribution of benzene to smoking induced leukaemia was estimated at between 8 and 26% (UCL 12-33%). For AML this contribution was estimated at 15 to 31% (UCL 28-60%). The estimates for AML are subject to some uncertainty due to limitations of the available data and further work would be required to confirm these findings. Future work may also include the use of alternative modelling techniques for epidemiological data, the use of biomarker data and further investigation of benzene metabolites and target tissue dose. It may also be possible to apply these methods to additional tobacco smoke toxicants and other smoking related disease endpoints.

**238 Mode of Action and Lack of Human Relevance of Benzo[alpha]pyrene (Benzo[alpha]pyrene)-Induced Thyroid Follicular Cell Adenomas in Male Han Wistar Rats**

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Benzo[alpha]pyrene (Benzo[alpha]pyrene) is a novel succinate dehydrogenase inhibitor fungicide (FRAC class C2). In a 2-year dietary administration study with Han Wistar rats (10, 25, 100 and 600[µg]/[400µg] ppm), an increased incidence of benign thyroid follicular cell adenomas was observed in males in the 600 ppm group only (17% vs. 2% in controls). There were no treatment-related increases in the incidence of any other tumour type in the rat or mouse. Specific mode of action (MOA) studies were conducted and, in conjunction with the regulatory toxicology studies, demonstrate a MOA for induction of thyroid follicular cell adenomas. The MOA of benzo[alpha]pyrene (BaP) is based on the following key events: 1) Induction of hepatic UDPglucuronosyltransferase. 2) A decrease in circulating T3/T4 levels. 3) A compensatory increase in circulating thyroid stimulating hormone (TSH) via the hypothalamus-pituitary-thyroid (HPT) axis. 4) Increased proliferation in thy-
Arsenite and Methyl Methanesulfonate Co-Exposures Induce Synergistic Cellular Responses Associated with Carcinogenic Pathways

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Formaldehyde (FA) is used in both industrial applications and consumer goods, and exposure is associated with irritation of the nasal epithelium as well as pathologies such as hyperplasia, metaplasia and squamous cell carcinoma. This work presents a mode of action (MOA) analysis, conducted to examine the interdependence and collective contribution of these and other effects to upper respiratory tract (URT) carcinogenesis. Data were sorted by species and endpoint and evaluated for consistency in specificity, plausibility and coherence across multiple data-streams. DNA damage in the nasal passages is evident within days of exposure in animals, consistent with the clastogenicity observed in the nasal/buccal epithelium of humans after months to years of exposure. In conjunction with genotoxicity, FA elicits other effects in the respiratory epithelium, such as changes in cell proliferation rates, and cytotoxicity following cilastasis and mucous flow interruption, which progresses to regenerative proliferation at higher concentrations. As related events, genotoxicity, cytotoxicity and proliferation likely interact in a feed-forward manner, with each contributing to the amplification of initiated clones as a function of increasing FA exposure. For example, at low exposure levels both genotoxicity (DNA-protein crosslinks) and irritation may occur at the cellular level, triggering metaplastic transition and transient proliferation at the tissue level. This proliferative burst may indirectly augment direct FA genotoxicity and trigger an outgrowth of nascent clones. Sustained regenerative proliferation may further enhance cellular genotoxicity and promote the expansion of mutant cells. By evaluating the potential for complementarity, this integrated analysis highlights the relationships and relative contributions of cytotoxicity, proliferation and genotoxicity to URT carcinogenesis. The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.
243 Significant Nonlinearity of Lung Cancer Risk in Relation to Arsenic in Drinking Water in Northeastern Taiwan

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Carcinogenic effects of ingested inorganic arsenic (InAs) have been addressed by ecological studies of increases in skin, lung, and bladder cancer in impoverished villages in southwest Taiwan and elsewhere. More recently, increased lung cancer incidence (LCI) was shown in a cohort of 6,888 residents of 3,901 households located in 18 villages in northeast Taiwan who were followed for 11 years [Chen et al. Environ Res 2010; 110(5):455–462]. That study estimated InAs exposure from thousands of household-well-specific measurements classified into five categories (range: undetectable [≤0.15] to >3,000 μg/L), and adjusted by age, gender, education, and consumption of cigarettes and of alcohol. A parallel cohort study by Chen et al. (2010) showed similar effects for bladder cancer. To refine dose-response analysis, the combined LCI and exposure data were re-binned into six exposure categories using residents who did not drink well water as the reference group (~16% of all residents and all person-years of exposure). Log adjusted relative risk (RRadj) was modeled as log(RRadj) = b * log(1 + X) + c * log(1-X), with exposure (X) defined as InAs concentration or as cumulative InAs exposure. Good fits to the re-binned data (p > 0.7, chi-square test) were obtained using each dose metric, but the estimated initial-slope parameter b is significantly negative in each case (2-tail p = 0.043 and 0.029, respectively), consistent with estimates of RRadj ≤ 1 at InAs levels ≤55 μg/L and <1,720 μg/L, respectively. This appears to be the first reported observation of a statistically significant hormetic dose-response relationship (i.e., with one significantly negative initial slope) for a single chemical carcinogen based on epidemiology data. Further analysis of potential confounding factors affecting non-drinkers of well water in this study is needed to better assess the nature and magnitude of any low-dose nonlinearity of LCI that occurred in this cohort.

244 Arsenic and Cadmium-Transformed UROtsa Bladder Cells Stably Expressing SPARC Will Reduce SPARC Expression during the Formation of a Tumor Heterotransplant

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SPARC, secreted protein acidic and rich in cysteine, is an extracellular matrix protein that can bind to structural molecules such as type I collagen yet SPARC’s role in the matrix is a non-structural one. SPARC functions to regulate cell signaling, migration, angiogenesis, and cell proliferation acting more like a cytokine than a matrix molecule. SPARC clearly has a strong role in cancer however, in some types of cancers SPARC is elevated and in others reduced. Our previous studies showed that in an environmental model of bladder cancer utilizing the UROtsa cell line SPARC was drastically reduced in cells transformed with arsenic or cadmium and that this reduction occurred early in the transformation process upon exposure to low doses of either metal. In this study, SPARC was stably transduced into 2 arsenic and 2 cadmium UROtsa cell lines thereby re-establishing SPARC mRNA and protein levels comparable to those in the UROtsa parent cell line. These SPARC-expressing cells were then injected into nude mice and the resulting tumor heterotransplants were characterized. Results showed there were again non-detectable levels of SPARC protein by immunohistochemistry. In Western analyses SPARC levels were barely detectable if at all in the heterotransplants generated with SPARC-expressing cells. Real time PCR analyses showed that SPARC mRNA was being expressed in the tumors but when compared to the expression of the vector blasticidin transcript (BSD), the ratio of SPARC to BSD was reduced in the heterotransplant tumors compared to the cell lines. The possibility of down regulation of SPARC mRNA via miRNAs is being determined. In conclusion, the results of this study show that despite being stably transfected, SPARC expression is greatly reduced in heterotransplants. Therefore, shutting down SPARC protein expression appears to be strongly advantageous to tumor formation.

245 Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) Inhibits Cell Proliferation and MMP-2-Mediated Tumorigenesis in Human Testicular Embryonal Carcinoma Cells

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Testicular germ cell tumors are most prevalent in young adult men ages 15–40 years, and arise as a result of abnormal testicular development. Peroxisome proliferator-activated receptors β/δ (PPARβ/δ) regulates a variety of biological processes but the function of PPARβ/δ in carcinogenesis remains controversial. Stable human testicular cancer cell lines (NT2/D1 and Ter2a) that constitutively over-express PPARβ/δ were produced to effectively examine the role of this receptor in human cancer models. Over-expression and/or ligand activation of PPARβ/δ increased expression of angiopoietin-like protein 4 (ANGPTL4) mRNA, a known PPARβ/δ target gene. Over-expression of PPARβ/δ inhibited cell proliferation in both NT2/D1 and Ter2a cells compared to controls. Ligand activation of PPARβ/δ both in NT2/D1 and Ter2a cells over-expressing PPARβ/δ caused a significant decrease in clonogenicity compared to controls. Over-expression and/or ligand activation of PPARβ/δ had no effect on either staurosporine-induced or UVB-induced poly(ADP-ribose) polymerses (PARP) cleavage; however, an increase in annexinV-positive NT2/D1 and Ter2a cells over-expressing PPARβ/δ, indicative of increased apoptosis, was found using flow cytometric analysis. Over-expression and/or ligand activation of PPARβ/δ inhibited MMP2 and MMP9 activities, presumably associated with the decreased invasion and migration in NT2/D1 and Ter2a cells. The MMP2/9 inhibitor SB-3CT suppressed the PPARβ/δ antagonist GSK3787-enhanced gelatinolytic activities, invasion, and migration in NT2/D1 control and/or over-expressing PPARβ/δ cells. This finding confirms a definitive requirement of PPARβ/δ in MMP2/9-mediated metastasis in vitro. Combined, these novel observations demonstrate the inhibitory effect of activating or over-expressing PPARβ/δ on tumorigenesis in human testicular cancer cells.

246 Ligand Activation of Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) Attenuates Liver Tumorigenesis in HBV Transgenic Mice

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PPARβ/δ can inhibit pro-inflammatory activities in the liver. Since chronic inflammation often precedes liver carcinogenesis, the role of PPARβ/δ in modulating liver cancer was examined. Male hepatitis B virus (HBV) transgenic mice, which develop hepatocellular injury around 2-3 months of age, followed by hyperplasia and neoplasia by 9-12 months of age, eventually developing hepatocellular carcinoma by 18 months, were used for this study. Whole liver was isolated from 7-month-old mice treated with PPARβ/δ ligand for three weeks (short term study). Ligand activation of hepatic PPARβ/δ in these mice attenuated pro-inflammatory genes, tumor necrosis factor-alpha (Tnfα) and inducible nitric oxide synthase (iNos) mRNA. In addition, serum levels of alanine amino transaminase (ALT) were also attenuated in ligand treated HBV mice. These effects were not observed in the control mice. Prolonged PPARβ/δ ligand treatment (8 months long term study) of HBV mice of the same age showed attenuation of tumor multiplicity and average number of liver foci as compared with the control group. Ligand activation of PPARβ/δ in liver of HBV mice increased expression of the PPARβ/δ target gene, angiopoietin-like 4 (Angptl4) mRNA. Western blot analysis of whole liver from these HBV mice showed reduced NF-kB activation indicated by attenuated expression of phosphorylated p65 and phosphorylated IκB protein levels in ligand activated liver tissues. In addition, protein levels of the cell proliferation marker CYCLIN D1 and the oncogene c-MYC were significantly attenuated in livers of ligand treated HBV mice as compared to controls. Combined, results from these studies suggest that PPARβ/δ inhibits hepatic inflammation, and has the potential to attenuate hepatic tumorigenesis.
Ligand Activation of Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) Inhibits TGFβ1-Induced Epithelial-Mesenchymal Transition (EMT) in a Human Lung Cancer Cell Line

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PPARβ/δ regulates fatty acid catabolism, glucose homeostasis, cellular differentiation and numerous anti-inflammatory activities, but the role of PPARβ/δ in carcinogenesis remains controversial. Surprisingly, the role of PPARβ/δ in metastasis has not been extensively investigated to date in any cancer model. Thus, the present study examined the role of PPARβ/δ in tumor growth factor-β-1 (TGFβ1) stimulated lung cancer cells in stable cell lines over-expressing PPARβ/δ in the presence or absence of a potent PPARβ/δ ligand. Ligand activation of PPARβ/δ in A549 cells over-expressing PPARβ/δ resulted in a significant increase of epithelial markers, (e.g; E-CADHERIN and OCCCLUDIN) with a concomitant decrease in mesenchymal markers, including N-CADHERIN and VIMENTIN without affecting the parent and A549-Mig1 vector control cells. Further, ligand activation of PPARβ/δ increased E-CADHERIN and suppressed VIMENTIN mRNA levels in A549 cells over-expressing PPARβ/δ. Migration and invasion is a known phenotype associated with cells undergoing metastasis. Ligand activation of PPARβ/δ inhibited TGFβ1-induced migration and invasion in A549 cells over-expressing PPARβ/δ, indicating an anti-metastatic effect of PPARβ/δ. Furthermore, MMP-2 and MMP-9 mRNA levels were decreased by ligand treatment in cells over-expressing PPARβ/δ. Ligand activation of PPARβ/δ in A549 cells over-expressing PPARβ/δ not only inhibited TGFβ1-induced morphology but also reversed TGFβ1-induced expression of E-CADHERIN and VIMENTIN protein. Interestingly, the PPARβ/δ antagonist GSK3878 in treatment A549 cells over-expressing PPARβ/δ prevented ligand-induced inhibition of EMT, indicating that PPARβ/δ is required for the inhibition of EMT. Collectively, these results suggest that ligand activation of PPARβ/δ could be a potential therapeutic target for lung cancer metastasis.

Evidence the PPAR Beta/Delta and BCL6 Pathway Is Involved in Pancreatic Cancer Development and Progression

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The nuclear receptor Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) regulates expression of genes involved in inflammation, metabolism, and cancer directly or in concert with the transcriptional repressor B-Cell Lymphoma 6 (BCL6) in the pancreas. Ligand-dependent activation of PPARβ/δ causes the dissociation of BCL6 from the complex, which in turn suppresses genes involved in inflammation and invasion. In this study we have examined the mRNA expression of several cancer biomarkers, as well as PPARβ/δ and BCL6 target genes in human pancreatic tissue of matched normal and tumor biopsies as well as the LSL-Kras(G12D); Pdx-δ/ mice. mRNA expression was higher in tumors than in surrounding tissue in humans and was augmented in Kras(G12D) expressing mice compared to non-recombinant counterparts. There was no change in BCL6 expression. BCL6 target genes, MCP1 and NFkB1 involved in inflammation, and VCA1 mRNA in cell adhesion showed significant increases in both human tumors and in pancreas from Kras(G12D) expressing mice. MMP9, another BCL6 target responsible for regulation of cell migration and metastasis, also showed elevated expression from normal tissue in humans. Increased expression of PPARβ/δ, in tumors and in pre-neoplastic lesions may sequester the transcriptional repressor BCL6 and hence play an important role in cancer development and progression and suggests means for treating pancreatic cancer.

Ligand Activation of PPARβ/δ Inhibits Invasion and Formation of Three-Dimensional Spheroids of the LNCaP Prostate Cancer Cell Line

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Prostate cancer is second to lung cancer in causing death from cancer in American men. The American Cancer Society estimates that about 240,000 new cases of prostate cancer will be diagnosed and about 30,000 men will die of prostate cancer in 2013. Our group has shown that ligand activation of PPARβ/δ plays a role in inhibiting skin cancer and colon cancer. The hypothesis that ligand activation of PPARβ/δ will inhibit prostate cancer progression was examined in these experiments. To study the role of PPARβ/δ, human PPARβ/δ was over expressed in LnCaP cells using the Mig1 retroviral system. Western blot analysis showed about 3 times the expression of the PPARβ/δ compared to the parent LnCaP cell line and the control Mig1 infected LnCaP cells. The LnCaP cells were treated with the highly specific PPARβ/δ agonist GW7042 (0.01 to 10 μM) and the invasion was measured using the xCELLigence system. Invasion was dose-dependently decreased following ligand activation of PPARβ/δ in LnCaP cells over-expressing PPARβ/δ compared to the parent LnCaP cells and LnCaP-Mig1 control cells. To mimic in vivo tumor growth, the LnCaP cells were plated as three dimensional (3D) hanging drops and then plated in poly(2-hydroxyethyl methacrylate) (Poly-HEMA) coated wells and treated with transforming growth factor-β1 (2 ng/mL) and tumor necrosis factor-α (10 ng/mL) for 96 h. Morphological analysis showed that LnCaP cells and LnCaP-Mig1 control cells formed microspheres which were not seen in LNCAp cells over-expressing PPARβ/δ. The initial clonogenic assay results showed a dose dependent decrease in the number of clones formed in the LnCaP cells. Combined, these results suggest that activation of PPARβ/δ may play a role in preventing and/or treating prostate cancer. Analysis of the role of PPARβ/δ activation in inhibiting epithelial to mesenchymal transition by studying the changes in the marker proteins like E-CADHERIN, VIMENTIN and N-CADHERIN in this 3D spheroid model are underway.

Does PCE Cause Non-Hodgkin’s Lymphoma?

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Tetrachloroethylene (PCE) is a volatile, chlorinated hydrocarbon used as a solvent in the dry cleaning and textile-processing industries and as an agent for degreasing metal parts. It is also a widespread environmental contaminant. In 2012, US EPA classified PCE as ‘likely to be carcinogenic in humans by all routes of exposure’ based, in part, on epidemiologic evidence associating PCE exposure and Non-Hodgkin’s Lymphoma (NHL). We critically analyzed human, animal, and mechanistic evidence, including epidemiologic studies published subsequent to US EPA’s analysis, to address the question of whether PCE causes NHL in humans. A number of epidemiologic studies of populations exposed to PCE have been conducted, principally in laundry and dry cleaning workers and populations exposed to chlorinated solvents in drinking water. Most of the studies addressing a potential association between PCE exposure (or imperfect surrogates such as occupation) and NHL do not report statistically significant results, including those with higher quality exposure-assessment methodologies. Limitations (e.g., lack of an exposure-response

Uncoupling-Induced Metabolic Reprogramming Provides Insights into Skin Cancer Resistance

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Metabolic reprogramming is a hallmark of tumorigenesis and malignancy across a variety of cancer types. Reduced oxidative phosphorylation and increased ATP synthesis by glycolytic upregulation is thought to provide both the energy and metabolic precursors necessary to meet the increased biosynthetic and energetic demands associated with malignancy. We hypothesized that since uncoupling protein-3 (UCP3) dissipates the mitochondrial electrochemical proton gradient, it may lead to the consumption or depletion of biosynthetic substrates necessary for carcinogenesis. In support, previous studies from our lab have shown that mice expressing a keratin 5-UCP3 transgene are completely resistant to chemically- and Ras-induced skin carcinogenesis; however the metabolic consequences of UCP3 overexpression in relation to cancer resistance are unclear. To examine the metabolic changes associated with UCP3 expression and this profound skin cancer resistance, we performed metabolomic analysis of more than 350 metabolites in epidermis from K5-UCP3 and wild type FVB/N littermate mice. Results of this analysis show significant UCP3-dependent changes in metabolites indicative of increased energy harvesting via glycolysis, breakdown of phospholipids and increased beta oxidation. Consistent with decreased energy reserve, K5-UCP3 epidermis exhibits increased uncoupled respiration, decreased ATP and increased AMP levels, which correspond to increased AMP Kinase (AMPK) activation (Thr172 phosphorylation). Taken together, these data suggest that uncoupling proteins might provide a means to oppose the metabolic demands of tumorigenesis and suggest that targeting mitochondrial uncoupling may be a novel and effective strategy in cancer prevention and treatment.
gradient, small numbers of cases, internal inconsistencies, and potential exposure misclassification) in many of the positive studies weaken the evidence of a causal relationship. In experimental animals, high doses of PCE have been associated with a number of cancers. Potentially the most relevant of these to NNL is monoclonal cell leukemia (MNCL) in aging F344 rats in more than one study. Though the etiology and mode of action of MNCL in F344 rats is not established, some factors suggest that it should not be considered a reliable indicator that PCE poses a carcinogenic hazard to humans. For example, while MNCL is extremely common in F344 rats, it is rare in other rat strains and not seen in mice. Although F344 rats are genetically predisposed to develop MNCL, there is no such predisposition in humans toward the development of the human leukemia that most resembles MNCL in F344 rats. We conclude that weak epidemiologic data and lack of human-relevant animal data indicate PCE is not likely to cause NHL in humans.

252 Bisphenol A Induces Prostate Cancer Progression and Differential Gene Expression
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Introduction and objectives: Estrogen in combination with androgen has been shown to induce prostate diseases including prostate cancer. Bisphenol A (BPA), a small molecule used to manufacture polycarbonate plastics, is one of the most prominent EDCs and has been identified as a xenoestrogen. Humans are exposed to BPA as it leaches from food and beverage containers made of polycarbonate. Our goal was to determine if BPA could substitute for estrogen in inducing prostate cancer progression in a tissue reconstitution model. Furthermore, we wanted to determine if cells induced from cancers would have different gene expression signatures indicative of hormonal regimen used.

Methods: Tissue recombinants composed of BPH1 + rUGM were surgically grafted to the renal capsule of adult male athymic mice that were subcutaneously inoculated with either 25 mg estradiol (E2) or T+BPA (25 mg) and grown for 4 weeks. Grafts were excised at necropsy and BPH1 cells were isolated. RNA was collected from BPH1-BPA and BPH1-E2 as well as parental BPH1 cells. Gene expression profiles were created using quantitative PCR.

Results: BPA induced tissue recombinants composed of BPH1 + rUGM to develop into cancer similar to that of E2. Changes for INHBA expression increased in BPH1-BPA over BPH1 and BPH1-E2 cells (BPH1: 100%, BPH1-E2: 52%, BPH1-BPA: 190%). Conversely, BPH1-BPA showed lower expression than BPH-1 and BPH1-E2 for PPARY (BPH1: 100%, BPH1-E2: 901%, BPH1-BPA: 83%). Whereas, CTNNB1 expression was higher in BPH1-E2 and BPH1-BPA compared to BPH1 cells (BPH1: 100%, BPH1-E2: 201%, BPH1-BPA: 182%). Lastly, there was minimal change in expression between cell lines for CDH1 (BPH1: 100%, BPH1-E2: 90%, BPH1-BPA: 105%).

Conclusions: These data support the concept that BPA acts as an estrogen to promote prostate cancer progression. Although, BPA induced cancers share some similarities with E2 induced cancer, BPA may have a distinct tumor gene expression profile that may distinguish it from other cancers.

253 hTERT-Immortalized Renal Proximal Tubule Epithelial Cells: Characterizing a Model System for Testing the Role of Cadmium and Benzo[a]pyrene in the Development of Renal Cancer
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In order to elucidate the mechanisms by which the heavy metal cadmium (Cd) acts as a co-carcinogen, we aim to characterize a newly developed cell line derived from renal proximal tubule epithelial cells (RPTEC) of a healthy male donor. The RPTEC/TERT1 cell line has been immortalized with the human telomerase (hTERT) subunit only and does not exhibit chromosomal abnormalities. We have conducted controlled exposure experiments designed to demonstrate toxicological responses in RPTEC and to assess their potential to develop into renal subtypes. urothelial and urothelial like cancer in these cells, which will serve as a model for renal cell carcinogenesis. We are conducting exposure experiments to mixtures of the common environmental contaminants, Cd and benzo[a]pyrene (BaP). Our studies are the first to provide information regarding toxicological responses in this novel RPTEC/TERT1 cell line. A significant increase in the expression of genes coding for BaP metabolizing enzymes (CYP1A1, CYP1B1) occurred in a dose- and time-dependent manner at 3, 6, and 24 hours post exposure. Likewise, a significant increase in the heavy metal responsive gene MT2A was observed following exposure to Cd. The presence of BPDE-DNA adducts confirms that the RPTEC/TERT1 cell line responds to BaP consistent with what is known regarding these cell types in a normal, healthy kidney. Future studies will be conducted to test mutagenesis under conditions of co-exposure to Cd and BaP. We hypothesize that Cd inhibits DNA repair processes, therefore, causing BPDE-DNA mutations to become fixed in the genome which may lead to carcinogenesis.

254 Chronic Alcohol Intake Promotes Tumor Growth in a Diethylnitrosamine-Induced Hepatocarcinogenesis Mouse Model through Increased Wnt/β-catenin Signaling
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Ethanol (EtOH) metabolism is involved in both initiating and promoting mechanisms in hepatocellular carcinoma progression in chronic alcoholics. In this study, we developed a mouse model to test the hypothesis that chronic EtOH consumption promotes tumor growth irrespective of EtOH-related initiating mechanisms. Male mice received a single dose of diethylnitrosamine (DEN) on postnatal day 13, and were assigned to either an EtOH-liquid diet, a control liquid diet pair-fed (PF) to the EtOH group, or a standard chow diet 47 days post-DEN injection. After 16 wks of EtOH feeding (5.0% v/v) we observed a 2-4 fold increase tumor multiplicity, but not tumor incidence in EtOH+DEN treated mice compared to PF+DEN and chow+DEN groups, p<0.05, which corresponded to a 4-fold increase in hepatocyte proliferation in EtOH+DEN non tumor liver tissues, p<0.05. In the EtOH+DEN non tumor liver tissues, we also observed a significant increase in β-catenin expression and changes in β-catenin localization when compared to DEN treated PF and chow controls. More important, in a separate rodent model of alcoholic liver disease, prolonged feeding of EtOH alone significantly reduced hepatic retinol and retinoic acid concentrations, increased cytosolic β-catenin expression, phosphorylated (Ser 21/9) GSK3b expression, and increased nuclear accumulation of β-catenin in rat hepatocytes, p<0.05. In addition, RNA analysis using a targeted Wnt PCR array revealed a significant up-regulation of soluble Wnts, transcription factors associated with a proliferative phenotype, and β-catenin targets associated with disease progression, p<0.05. These findings suggest a link between alcohol-related retinoid depletion, up-regulation of Wnt/β-catenin signaling, and tumor growth and progression in our mouse model of HCC. Supported by NIH AA018282.

255 Orally Administered Nicotine Induces Urothelial Hyperplasia in Rats and Mice
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Tobacco smoking is a major risk factor for multiple human cancers including urinary bladder carcinoma. Tobacco smoke is a complex mixture containing chemicals that are known carcinogens in humans and/or animals. Aromatic amines a major class of DNA-reactive carcinogens in cigarette smoke, are not present at sufficient high levels in tobacco smoke to fully explain the incidence of bladder cancer in cigarette smokers. Other agents in tobacco smoke could be excreted in urine and enhance the carcinogenic process by increasing urothelial cell proliferation. Nicotine is one such major component, as it has been shown to induce cell proliferation in multiple cell types in vitro. However, in vivo evidence specifically for the urothelium is lacking. We previously showed that cigarette smoke induces increased urothelial cell proliferation in mice. In the present study, urothelial proliferative and cytotoxic effects were examined after nicotine treatment in mice and rats. Nicotine hydrogen tartrate was administered in drinking water to rats (52 ppm nicotine) and mice (514 ppm nicotine) for 4 weeks and urothelial changes were evaluated. Histopathologically, 7/10 rats and 4/10 mice showed simple hyperplasia following nicotine treatment compared to none in the controls. Rats had an increased mean BrdU labeling index compared to controls, although it was not statistically significantly elevated in either species. Scanning electron microscopic visualization of the urothelium did not reveal significant cytotoxicity. These findings suggest that oral nicotine administration induced urothelial hyperplasia (increased cell proliferation), possibly due to a mitogenic effect of nicotine and/or its metabolites.
Colorectal cancer (CRC) occurs more prevalently in developed countries where it is the second highest cause of cancer-related mortality. Only 15-20% of CRC cases have a genetic pre-disposition, while the remaining 80-85% are caused by environment and lifestyle. Epidemiological studies have suggested a strong link between diet and CRC. Western-style diets can induce local colonic inflammation, which is also strongly associated with CRC development. Benzo[a]pyrene (BaP) and 2-aminon-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) are dietary pro-carcinogens found in meats cooked at high temperatures common in Western-style diets and require metabolic activation to their genotoxic agents by cytochromes P450 (CYP) 1A and 1B1 enzymes. We previously observed overexpression of CYP1B1 and pro-inflammatory cytokine interleukin-6 (IL6) in malignant tissue retrieved from CRC patients, indicating that inflammation and changes in metabolic competency occur in CRC tissue. To further understand the link between diet, inflammation and CRC, we investigated the effect of IL6, BaP and PhIP on human colon epithelial cell lines HCT116 and SW480 grown as 2D and 3D cultures. We measured DNA damage using the micronucleus (MN) assay and determined CYP1A1 and CYP1B1 expression along with associated micro-RNA (miRNA) changes. In both cell lines, MN frequency was increased dose-dependently with BaP and PhIP treatment while pre-treatment with IL6 further enhanced DNA damage. Induction of CYP1B1 gene expression was shown following IL6 treatment while CYP1A1 expression was not changed. Additionally, treatment with IL6 decreased expression of miR-27b, a miRNA known to target CYP1B1 mRNA. Taken together, these data demonstrate that exposure of colon cells to IL6 alters metabolic competency of the cells through epigenetic changes. Similar events occurring in colorectal epithelial cells could lead to increased dietary pro-carcinogen-induced DNA damage, thereby contributing to colorectal carcinogenesis.

Triclosan is a broad spectrum anti-bacterial agent widely used in many personal care products, household items, medical devices, and clinical settings. Human exposure to triclosan is mainly through oral and dermal routes. Although it has been reported that long-term oral exposure of mice to triclosan induces liver tumors, the dermal carcinogenicity of triclosan in either animals or humans is unknown. In this study, using mouse epidermal JB6 Cl 41-5a cells, triclosan within a narrow range of concentrations (3 - 6 μM), stimulated anchorage-dependent cell growth in a concentration- and time-dependent manner. Higher concentrations of triclosan (8 μM) inhibited cell growth, which was due to necrotic cell death. Although triclosan failed to promote anchorage-independent cell transformation in a soft agar colony formation assay, enhanced cell proliferation was demonstrated by a substantial increase in the percentage of BrdU positive cells and an elevation in the protein levels of cyclin A, even at a concentration that triggered necrosis. Western-blotting analysis revealed that triclosan transiently induced the activation of extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38 MAP kinase (p38). Pre-treatment with the MEK1/2 inhibitor PD184352 blocked triclosan-mediated the phosphorylation of ERK1/2 and substantially suppressed triclosan-stimulated cell proliferation, whereas the JNK inhibitor SP600125 or p38 inhibitor SB203580 had little or no effect on triclosan-stimulated cell proliferation. These data suggest that triclosan stimulated mouse epidermal cell proliferation in an ERK1/2-dependent manner and raises concerns about potential dermal carcinogenicity of triclosan.

There are increasing epidemiological and experimental studies supporting the activity of metformin, the most commonly used anti-diabetic drug, as an anti-tumor agent. However the mechanisms associated with the anti-neoplastic activity of metformin remain unclear. De novo lipogenesis is considered as one of the hallmarks of cancer and fatty acid synthase (FAS), which plays a major role in lipogenesis is a negative prognostic factor for patient survival in pancreatic cancer. A key transcriptional regulator of FAS is SREBP (Sterol Regulatory Binding Protein) which is one of the downstream effectors of mTOR signaling. Treatment of Panc28 and L3.6pl pancreatic cells with 5-15 mM metformin decreased phospho-mTOR (p-mTOR) but not mTOR protein and this was accompanied by decreased expression of SREBP and FAS. Previous reports have linked IGF1 to activation of mTOR in pancreatic cancer cells and knockdown of IGF1 by RNA interference decreased mTOR signaling; moreover, metformin also decreased IGF expression suggesting the mTOR inhibition by metformin is due to IGF1 downregulation. Previous reports suggest that IGF1 expression is regulated by specificity protein Sps (transcription factors (TFs)) and we have shown that metformin decreases expression of Sp1, Sp3 and Sp4 transcription factors in pancreatic cancer cell lines. The role of Sp transcription factor in regulating IGF1 and mTOR was further investigated by knockdown of Sp1 (Sp1), Sp3 (Sp3), Sp4 (Sp4) or their combination (Sp1/3/4) by RNAi and this resulted in decreased expression of IGF1 and inhibited mTOR signaling and its downstream effectors in pancreatic cancer cell lines. These data indicate that metformin blocks lipogenesis in pancreatic cancer cells through downregulation of Sp transcription factors and Sp regulated IGF1, resulting in inhibition of mTOR signaling.

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administration did not further modulate the observed receptor-dependent effects. Ligand activation of PPARβ/δ or PPARγ also reduced cell growth in stable cell lines over-expressing these receptors. An analysis of clonal expansion also revealed that elevated expression of PPARβ/δ reduced plating efficiency; furthermore, ligand activation of PPARβ/δ (but not PPARγ) conferred resistance to a dose-dependent manner. Collectively, these observations demonstrate that both PPARβ/δ and PPARγ decrease human malignant melanoma cell line proliferation. These results also demonstrate the utility of the Mig1G model to dissect the functions of PPARβ/δ and PPARγ, including the targeting of these receptors for potential malignant melanoma therapies.

### 261 The Trichloroethylene-Associated P81S VHL Hotspot Mutation Alters Tumor Microenvironment: Metabolic Reprogramming, DNA Damage Response, and Radiation Resistance

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Occupational exposure to trichloroethylene (TCE), a solvent found in contaminated groundwater, has been associated with the P81S hotspot mutation in the von Hippel-Lindau (VHL) tumor suppressor gene in cases of clear cell renal cell carcinomas. There remain considerable mechanistic gaps in the kidney cancer risk assessment of TCE due to lack of appropriate model systems. We purport that specific mutations in VHL that uniquely alter its protein function may provide a selective growth advantage to somatic cells harboring these mutants, leading to clonal expansion. For this study, we developed a stem cell model system to study the unique biology of the P81S mutation. VHL deficient (Vhl−/−) mouse embryonic stem cells were generated that stably express either wild-type, P81S, or R167Q human VHL protein and were examined in vitro under hypoxic conditions for hypoxia-inducible transcription factor family stabilization and E3-ubiquitin ligase complex formation. To examine the microenvironmental stress response, cell lines grown as teratomas were examined for size, proliferation, and apoptosis, which were subjected to whole genome microarray analysis, and proliferation, and exposure to 5Gy ionizing radiation to quantify apoptotic response. The P81S mutation disrupts VHL-E3 complex formation in vitro, and in vivo, teratomas gained a growth advantage resulting from apoptotic resistance, not enhanced proliferation. Transcriptomic analysis suggests that a key component of this survival advantage is metabolic reprogramming and disabling of the DNA damage response, conferring radiation resistance. This work demonstrates a novel interaction between VHL and the ATM-mediated DNA damage response network, and highlights the potential of the TCE-associated P81S VHL mutation to initiate an adaptive response required for selective tumor growth through effects on metabolic diversification, apoptosis suppression, and alteration of the DNA damage response.

### 262 Hydrolysis of DNA-Protein Crosslinks Forms Formaldehyde-DNA Adducts

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Formaldehyde causes squamous cell carcinomas in the nasal passages of rats and has been classified as a known human carcinogen by IARC. The primary genotoxic effects of formaldehyde include the formation of DNA-protein crosslinks (DPCs) and hydroxymethyl DNA adducts. Formaldehyde induced DPCs showed a rapid loss in lymphocyte cultures and no accumulation was observed in rats after repeated exposures. Previous research indicated that formaldehyde induced DPCs were lost through spontaneous hydrolysis rather than DNA repair. In this study, we investigated hydrolytic degradation of formaldehyde induced DPCs with deoxyguanosine linked to cysteine (dG-Ch2-Cys), glutathione (dG-Ch2-GSH) and N-terminal acetylated 12-mer human O6-alkylguanine DNA methyltransferase peptide (dG-Ch2-AGT). Under physiological pH and temperature conditions, the determined half-life for dG-Ch2-Cys and dG-Ch2-GSH was 11.6 min (R2 = 0.9807) and 87.8 min (R2 = 0.9935), respectively. The degradation kinetics of dG-Ch2-AGT consisted of an initial rapid degradation rate with half-life of 7.4 min followed by a much slower degradation rate with half-life of 90.7 min (R2 = 0.9987). During the hydrolysis of DPCs, dG could be directly formed if bond cleavage occurs between dG and methylene (N2-dG-Ch2-bond). However, no dG was detected in the first 3 min, 20 min and 10 min incubation for dG-Ch2-Cys, dG-Ch2-GSH and dG-Ch2-AGT, respectively. On the other hand, N2-hydroxymethyl-dG (HO-Ch2-dG) was readily formed and increased rapidly after initiating reaction for all tested crosslinks, suggesting cleavage of the Ch2-Cys bond, but not the N2-dG-Ch2 bond. HO-Ch2-dG can be degraded to dG following incubation for longer time periods. Thus, the mechanism of DPCs hydrolysis involved the production of HO-Ch2-dG before forming end product of dG. These results suggest that DPCs are likely to be an important source of formaldehyde DNA monoadducts and that monoadducts represent a biomarker of both direct attack of DNA and a breakdown product of DPC.

### 263 Differential Gene Expression Analysis of TPA/UVC Co-Treated TK6 Cells Reveals a Novel Gene Signature Important in Carcinogenesis by Combining DNA Damage and Tumor Promoting Pathways

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Environmental and chemical carcinogens have been shown to act as both initiators and promoters of cancer. In the present study, we attempted to reveal critical pathways in chemical carcinogenesis that were triggered from converging processes of initiation (via UVC irradiation) and promotion (via 12-O-tetracosanoylphorbol acetate (TPA) treatment) in cultured TK6 cells. We have previously shown that TPA-pretreated TK6 cells undergo a synergistic increase in apoptosis and delayed yH2AX clearance following DNA damage induced by UVC-irradiation. To elucidate the cellular pathways responsible for the synergistic interaction between TPA-pretreatment and UVC-irradiation we analyzed the global transcriptional profile with RNA-sequencing. Differential expression analysis revealed significantly deregulated genes associated with the combination of TPA-pretreatment and UVC-irradiation (TPA+/UVC) compared to either stress alone (TPA+/UVC- or TPA-/UVC+). To focus our analysis on genes specific to the combined exposure, we filter the gene list for those that occurred in a TPA-dependent dose responsive trend and significantly up- or down-regulated in the TPA+/UVC+ cells compared to TPA-/UVC- and TPA-/UVC+. We found a set of 84 genes (46 up, 38 down) specific to the combined exposure that are significantly deregulated and likely responsible for the increased sensitivity to UVC-induced DNA damage. Major up-regulated genes include TNFSF4, GDF15, ATF3, SERPINE1, IL29, LIF, FOS, IFNG, SNAI1, NOXA and AADD34a. Major down-regulated genes include PO2UAF1, PAX5, GBP1, SP1, ASK1, GBP6, LYN, TRAF5 and CLI4C. Overall, most genes were connected to pathways important in inflammatory driven carcinogenesis including TNFG, Interferon-γ, TGFβ, API1 and p53. Here we show that TPA-pretreatment causes an increased sensitivity to UVC-induced DNA damage which is accompanied by an exacerbation of immune/inflammatory pathways that may be important for tumor promotion.

### 264 The Aryl Hydrocarbon Receptor Controls Breast Cancer Stem-Like Cell Development and Function

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In 1940, an American woman’s risk of getting breast cancer was 1 in 14. Since then, the age-adjusted incidence of breast cancer has increased such that 1 in 8 women born this year will be diagnosed with breast cancer in her lifetime. Analyses of known risk factors such as diet, exercise, and hormone replacement therapy do not completely explain the increase in breast cancer incidences or the genesis of breast cancers not associated with inheritance of susceptibility genes. A meta-analysis of over 150 published studies on breast cancer and the environment implicates a number of environmental chemicals, including aryl hydrocarbon receptor (AhR) ligands, in breast cancer risk. Tumor invasion and metastasis following relapse is the cause of death in nearly all breast cancer patients. The cancer stem cell (CSC) theory hypothesizes that cancers and their metastases are driven, to a disproportionate extent, by breast cancer stem-like cells (BCSCLs). BCSCLs are defined by their activity and expression of aldehyde dehydrogenase (ALDH), expression of a set of genes associated with “stemness”, ability to self-renew, expression of genes consistent with epithelial-to-mesenchymal transition (EMT), and resistance to chemotherapeutics. Recent studies have implicated the AhR, a transcription factor involved in tumorigenesis, in the development and/or function of stem cells, which share properties with CSCs. From these studies, we postulated that the AhR may play a role in the development or function of BCSCLs. Here, we present data demonstrating high levels of constitutively active AhR in BCSCLs from aggressive human ER-, PR-, Her2- and inflammatory breast cancers. The results indicate that AhR hyper-activation with exogenous ligands increases and AhR inhibition with pharmacological or molecular agents decreases expression of BCSCL characteristics. Our findings further implicate environmental chemicals in breast cancer risk through increasing breast cancer progression and survival after chemotherapy.
Residual aromatic extracts (RAEs) are petroleum streams derived as by-products during solvent extraction of vacuum tower bottoms (VTB) often used in tire extender oils and specialty petroleum products. Due to process conditions, RAEs may have high levels of polycyclic aromatic compounds (PACs), which are thought to drive carcinogenicity in animal bioassays. To maximize the use of these substances while protecting human health, a more efficient method for separating potentially harmful PACs during vacuum distillation was investigated. This work identifies a correlation between carcinogenicity and boiling point which can identify optimal conditions for vacuum distillation to minimize the toxicity of RAEs. Using the modified Ames assay, samples of VTB, raffinate, and RAE were obtained from five refineries to evaluate relationships between mutagenicity index (MI), PAC content and boiling point by GC distillation (GCD). As expected, a positive linear relationship between MI and PAC content occurred for 3-6 ring PAC (R2=0.72), regarded as the carcinogenic PACs. A negative correlation between MI and boiling point (5% vaporized by GCD, R2=0.72) indicated that lower boiling samples (<470°C) were more likely to be mutagenic. This inverse relationship was further demonstrated by distillation of select samples (MI=0.50±0.07 (n=5)) into low and high boiling range fractions. Mutagenicity and 3-6 ring PAC content were markedly higher in low boiling fractions (MI=2.36±0.55 vs.0.17±0.11, PAC=5.2±0.7 vs. 0.97±0.35%, respectively). These results support the hypothesis that PACs are key contributors to the mutagenic potential of petroleum substances that can be separated by boiling point. Because vacuum distillation can be continuously measured and manipulated at refineries, these findings provide a means to readily monitor and minimize the carcinogenic potential of RAEs in situ to ensure continuous product safety.

In a rodent tumor bioassay, high doses of sodium phenobarbital (NaPB), a constitutive androstane receptor (CAR) activator, produced hepatic tumors. Previous mode of action (MOA) studies have demonstrated that NaPB induces hepatic microsomal cytochrome P450 (CYP) 2B enzymes and hepatic cytoplasmic DNA synthesis in rodents, suggesting that the MOA for rodent liver tumor formation is a mitogenic activity via CAR activation. In this study the effects of NaPB on liver weight and histopathology, hepatic CYP2B activity, hepatic replicative DNA synthesis, and mRNA expressions of CYP2B/3A and selected genes related to cell proliferation, were examined after 1-week treatment with NaPB at 500, 1000, 1500, and 2500 ppm in males of CD-1 mice, Wistar Hannover (WH) rats, and humanized mice (chimeric mice with human hepatocytes). The treatment of humanized mice with 1000-1500 ppm NaPB resulted in plasma levels around 3.5-fold higher than those observed in human subjects given therapeutic dose of NaPB. NaPB produced dose-dependent increases in liver weight, CYP2B activity, and CYP2B3A mRNA in CD-1 mice and WH rats and also produced effects on these parameters in humanized mice. While NaPB produced a dose-dependent increase in hepatocyte replicative DNA synthesis in CD-1 mice and WH rats, no increase in replicative DNA synthesis was observed in humanized mice. In addition, NaPB produced no increases in Ki-67, PCNA, GADD45β, and MDM2 mRNA expression in humanized mice but significantly increased those in CD-1 mice and/or WH rats. Thus, while NaPB could activate CAR/PPARγ, as demonstrated by increased CYP2B3A mRNA levels, NaPB did not increase cell proliferation in human hepatocytes of humanized mice. As human hepatocytes are refractory to the mitogenic effects of NaPB, the data demonstrate that the MOA for NaPB-induced rodent liver tumor formation is not relevant for humans.
265d Integrated microRNA, mRNA, and Protein Expression Profiling Reveals Dysregulated Expression of microRNAs and Their Targets in Rat Kidney Treated with a Carcinogenic Dose of Aristolochic Acid


Aristolochic Acid (AA), is classified as a group 1 carcinogen by the International Agency for Research on Cancer. MicroRNA (miRNA) alterations are involved in the initiation and progression of human cancer, but their roles in AA-induced carcinogenesis remain unknown. In order to search for miRNA regulation and identify their functional targets, we have employed a novel approach integrating miRNA, transcriptome and proteome profiling and a computational target predicting algorithm. We treated rats with 10 mg/kg AA and vehicle control for 12 weeks and eight kidney samples (4 for the treatment and 4 for the control) were used for examining miRNA and mRNA expression by deep sequencing, and protein expression by proteomics. AA treatment resulted in significant differential expres- sion of miRNAs, mRNAs and proteins as measured by both principal component analysis (PCA) and hierarchical clustering analysis (HCA).

Specially, 63 miRNAs (adjusted p value < 0.05 and fold change > 1.5), 6,794 mRNAs (adjusted p value < 0.05 and fold change > 2.0), and 800 proteins (fold change > 2.0) were significantly altered by AA treatment. To investigate the influence of miRNA on these processes, we combined proteomic and transcriptomic data into three groups containing responses indicating translational repression (163), translational activation (236), and transcriptional destabilization (460). Our predicted miRNA targets were further compared and validated from experimentally validated targets in miRTarBase (P = 4.96e-06). Cancer is identified as the top function by ingenuity pathway analy- sis (IPA) for identified 399 targets. Our findings suggest that dysregulated miR- NAs and their targets are involved in kidney carcinogenesis in the AA-treated rats.

266 Refining the Caco-2 Model to Evaluate the Impact of Intestinal Hydrolysis on Parabens Absorption in the Gut

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The oral bioavailability of environmental esters such as parabens can be greatly affected by intestinal hydrolysis, but little is known about this metabolic pathway largely due to lack of relevant experimental systems. The conventional Caco-2 model which overexpresses carboxylesterase-1 (CES-1) is not appropriate to this end, because the CES-2 isosform is predominantly expressed in human enterocytes. In this study, we stably expressed CES-2 in Caco-2 cells, while knocking down CES-1, to appropriately evaluate the impact of hydrolysis on the ab- sorption of parabens. Western blot analysis showed that the expression of CES-2 in the Caco-2/CES-2 cells was comparable to that in human enterocytes. Studies on transport and metabolism of methyl- and butylparabens were performed with the Caco-2/CES-2 cells. The results showed that parabens are rapidly hydrolyzed while the genotoxic absorbed parabens (both butylparaben and WIT butylparaben (10 M) was applied to the apical side, all of the parent compound was converted to p-hydroxybenzoic acid (pHBA) within 30 min, most of which was found in the apical side indicating the location of hydrolysis being at or closer to the apical membrane and the efficient efflux of pHBA into the apical side (i.e., luminal side in the gut). Additional experiments with a general CES inhibitor, bi-par-nitro- phenylphosphate, further supported these findings. An in vitro kinetic model was developed to describe the kinetic behaviors of parabens and pHBA in the Caco-2/ CES-2 cells and extrapolate to humans in vivo. The refined Caco-2/CES-2 system, together with pharmacokinetic modeling can provide accurate estimates of oral bioavailability of parent parabens and their metabolites under different exposure conditions which will contribute to risk assessment and better understanding of human exposure to parabens and other environmental esters (supported by the ACC-IRI).

267 Toxicological Effects of Antibody-Mediated TGF-beta Antagonism in Cynomolgus Monkeys

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Transforming growth factor beta (TGF-beta) is a multifunctional cytokine that has an important role in various processes, including cell proliferation and differ- entiation, angiogenesis, and wound healing. Overexpression of TGF-beta can lead to fibrosis and has been linked to chronic conditions such as chronic kidney disease and TGF-beta by the antibody GC1008 may modify this pathologic process. The toxicological impact of chronic TGF-beta neutralization was evaluated in juvenile cynomolgus monkeys. Repeat dose toxicity studies (28 days, 3 months and 6 months) were conducted with GC1008 at dose levels ranging from 0.1 to 50 mg/kg (dosed every other week or every third day). In all studies, the toxicokinetics of GC1008 were highly variable, but adequate exposure was obtained to allow for interpretation of the toxicological findings. When dosed every third day, diminished systemic exposure was observed following 7 doses in a single animal; this animal tested positive for anti-drug antibodies. Across all studies, consistent findings related to treatment with GC1008 were observed. Clinical observations included bleeding, often asso- ciated with eruping teeth, and hematomas. Alterations in hematology parameters included dose dependent, reversible, decreases in red blood cell counts, hemoglo- bin, and hematocrit values. In the 3 and 6 month studies, epithelial cell hyperplasia was noted at varying locations. The epithelial hyperplasia observed in these studies may have been associated with exacerbation of pre-existing background lesions (i.e. periodontitis, rhinitis, cystitis). Overall, these studies have characterized the potential risks associated with chronic neutralization of TGF-beta and suggest that the majority of homeostatic roles of TGF-beta in these normal juvenile primates were not significantly disrupted by antibody-mediated antagonism of this biologically important growth factor.

268 Safety Assessment of Flt3 Ligand: 28-Day Toxicity Studies in Rats and Monkeys

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Flt3 Ligand (also referred to as Flt3L, fms-like tyrosine kinase 3 ligand, or CDX-301) is a hematopoietic cytokine which stimulates the proliferation and differen- tiation of various blood cell progenitors. Nonclinical safety was assessed in two GLP-compliant toxicity studies where Flt3L was administered subcutaneously for 28 days to Cynomolgus monkeys and rats (5, 25, 200 and 1600 μg kg-1 d-1). For all animals, mortality, clinical signs, body temperature, body weights, and clinical pathology were recorded throughout the study. Detailed necropsies were performed in a subgroup of animals 48 hours after the last administration of CDX-301 and on the remaining animals after a 2-week recovery period. Selected tissues were collected for histopathology evaluation and bone marrow smears were evalu- ated. Blood samples were collected periodically for the determination of pharma- cokinetics, anti-drug antibodies and, in the monkeys, for immune cell analysis. No gender differences were observed in the pharmacokinetics of Flt3L. Many animals developed anti-Flt3L antibodies, which were neutralizing in some rats. An increase in dendritic cells and monocytes, but not regulatory T cells, was seen in the mon- keys following Flt3L exposure. Flt3L was well tolerated in both monkeys and rats. The most significant test article-related findings were bone marrow hyperplasia (considered a pharmacological effect of Flt3L), an increased severity of chronic in- flammation at the injection site, increased severity of mononuclear cell infiltration

265c The Role of Cancer Stem-Like Cells in Cr(VI)-Induced Malignant Cell Transformation and Tumorigenicity

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Hexavalent chromium (Cr(VI)) compounds are well-known human carcinogens. Epidemiological studies have shown that occupational and environmental exposure to Cr(VI) is associated with a high rate of lung cancer. Cancer is a disease driven by cancer stem cells (CSCs). In the present study, we hypothesize that stem-like cells (SCs) play a key role in the Cr(VI)-induced malignant cell transformation, contributing Cr(VI)-induced lung cancer. Our results show that chronically expo- sure to low dose of Cr(VI) up to six months caused the malignant transformation of human bronchial epithelial BEAS-2B cells. To examine the existence and nature of CSCs in those Cr(VI)-transformed cells, SCs were isolated by collecting floating cells and were enriched by forming spheres under anchorage-independent growth in serum-free medium. The results show that cancer epithelial stem cell biomarkers such as Notch1 and ALDH were highly expressed in SCs as compared to normal BEAS-2B cells and Cr(VI)-transformed BEAS-2B cells. The results from hetero-transported animal study show that SCs were able to form tumors in immune-deficient nude mice at 100 cells, while Cr(VI)-transformed cells failed to grow tumor, indicating potent self-renewal and essential for tumorigenesis of those SCs. Further investigation indicates that those CSCs exhibited apoptosis resistance and reduced capacity of generating reactive oxygen species (ROS), in- dicating of potent cell proliferation/growth and capacity against exicot oxidative stress. Notably, oxygen consumption rate (OCR) was significantly decreased in SCs compared to normal BEAS-2B cells or Cr(VI)-transformed cells, indicating a metabolic alteration in SCs associated with augmented tumorigenicity and potent survival characteristics. The decrease in OCR in SCs might be due to the loss of fructose-1,6-biophosphatase (FBP1) which is a rate-limiting enzyme in gluconeo-genesis. In summary, the present study suggests that CSCs are critical in Cr(VI)- induced malignant cell transformation and tumorigenicity.
in various tissues, and thymic atrophy. Adverse findings were observed only in the 200 and 1600 μg kg−1 d-1 dose groups in either species. Based on these results, 25 μg kg−1 d-1 was determined to be a no observed adverse effect level (NOAEL) for both species.

269 Immune-Mediated Hepatotoxicity in Monkeys following Administration of a Highly Conserved Biotechnological Protein


Drug-induced liver injury (DILI) is a frequent adverse event leading to the attrition of drug candidates during development and to the withdrawal of approved therapeutics from the market. There are several causes of DILI, including immune-mediated. We report an atypical, but clearly immune-mediated drug-related hepatotoxicity in monkeys associated with a recombinant human protein (rhApo2L/TRAIL; dulanermin) formerly in development for the treatment of cancer. The cynomolgus monkey was chosen as the pharmacologically relevant species for the safety assessment of rhApo2L/TRAIL because the sequence identity between the human and cynomolgus monkey proteins was approximately 98%; additionally, rhApo2L/TRAIL bound to human and monkey cognate receptors with comparable affinities. During the course of the toxicology program, hepatotoxicity was observed in repeat-dose monkey toxicology studies. There was a strong correlation between hepatotoxicity and the presence of anti-drug antibodies (ADAs). The human and monkey proteins differ only by 4 amino acids, and the X-ray crystal structure of the human protein indicated that 3 of the 4 distinct side-chains were surface-exposed. We hypothesized that these 3 amino acids could serve as epitopes for the development of cross-species ADAs, resulting in ADA-mediated hepatotoxicity in monkeys. In vitro and in vivo investigative toxicity studies supported the ADA-mediated hepatotoxicity hypothesis and demonstrated that the equivalent autologous monkey protein was well-tolerated. The thorough characterization of the ADA-mediated toxicity in monkeys enabled the successful transition of rhApo2L/TRAIL into human clinical trials.

270 Comparison of Three Methods for the Evaluation of Cytokine Storm Risk in Early- and Clinical-Stage Biopharmaceutical Development

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Cytokine Release Syndrome (CRS) and Cytokine Storms (CS) are common and potentially dangerous side effects of anti-T cell antibodies and other immunomodulatory therapies. Therapy-induced CRS occurs quickly following infusion and results in the activation of T cells and the release of a wide range of inflammatory cytokines, including IL2, IL4, IL6, IL8, IL17, TNFα and/or IFNγ. We compared three methods to identify a robust assay that allows for the assessment of potential CRS and CS risk. In Method 1 (Romor, 2011), human PBMCs were pre-incubated for 48 hrs at 1 x 10^6 cells per mL, followed by culture on tissue-culture plastic in the presence of test (anti-CD3 clone OKT3 or anti-CD28 superagonist clone ANC28.1) or control (humanized anti-CD20 or humanized anti-TNFα) antibodies. In Method 2 (Findlay, 2011), human PBMCs were cultured on a HUVEC monolayer in the presence of test or control antibodies. In Method 3, a combination of the first two, human PBMCs were pre-incubated at 1 x 10^6 cells per mL, followed by culture at 1 x 10^6 cells per mL on a HUVEC monolayer in the presence of test or control antibodies. In all studies, supernatants were harvested after 24 hours and evaluated for TNFα and/or IFNγ by ELISA. Using Method 1, no measurable IFNγ production in the presence of any concentration of anti-CD3 or anti-CD28 was detected. Method 2 resulted in the production of ng per mL quantities of IFNγ in the presence of at least 100 ng/mL anti-CD3, but not with anti-CD28 up to 10 μg/mL. Method 3 resulted in the production of ng per mL quantities of IFNγ and high ng/mL quantities of TNFα when > 1 ng/mL anti-CD3 or > 1 μg/mL anti-CD28 was present. Method 2 appears to be the most suitable protocol to assess new antibodies and biologics for stimulation of inflammatory cytokine release and therefore as a potential model for CRS and CS induction. It could be extended to compare cytokine profiles with either human or non-human primates PBMCs and may be amenable to further refinement through lymphocyte subset analysis by flow cytometry.

271 Aerosol and Ocular Toxicity of AERAS-402 in Nonhuman Primates

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AERAS-402 is a novel tuberculosis vaccine comprised of a recombinant, replication-deficient adenovirus, serotype 35 (Ad35), expressing a fusion protein of three mycobacterial antigens. This study assessed the toxicity of aerosol vaccination with AERAS-402 following three doses of 10^11 viral particles (vp) to Rhesus monkeys and evaluated toxicity following a single ocular topical administration. Twelve monkeys received test article via aerosol inhalation on Study Day (SD) 1, 8, and 15, and subjected to a full gross necropsy on SD 18 or 43. Two monkeys were administered test article via ocular topical application on SD 1, and subjected to a full gross necropsy on SD 43. Four monkeys were administered control article via aerosol inhalation and ocular topical administration on SD 1, 8, and 15, and subjected to a full gross necropsy on SD 18 or 43. Test article-related organ weight alterations on SD 18 were limited to apparent increased absolute and relative lung weights that correlated with microscopic findings in the lung, mediastinal lymph nodes, bronchi-associated lymphatic tissue, and the naso-oropharynx. All effects were expected with vaccination via the aerosol route, considered non-adverse, and completely resolved by SD 43. There were no test article-related organ weight or microscopic pathology findings following ocular delivery. All animals receiving aerosolized vaccine generated neutralizing antibodies to Ad35 and elicited strong, multifunctional CD4+ and CD8+ T cells by SD 43 in the lungs (obtained from BAL fluid). In addition, low-level, peripheral blood T cell responses were shown in 9/12 vaccinated animals on SD 18 which diminished by SD 43. Based on these findings the no-observed-adverse-effect level (NOAEL) is at least 10^11 vp when administered via aerosol inhalation or ocular topical application.

272 Evaluation of Paracetamol Toxicity in the Cynomolgus Monkey

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Paracetamol (Acetominophen, APAP) was administered orally in a rising dose protocol to 2 male and 2 female Cynomolgus monkeys to determine the Maximum Toleraed Dose (MTD) for subsequent toxicity investigations. In the subsequent repeat dose study involving 6 monkeys, no dose-limiting toxicity was seen at up to 900 mg/kg/day APAP for 14 days. Toxicokinetic analysis showed that plasma exposure rose, albeit less than proportionally, with increasing dose. There were some fluctuations in clinical chemistry and haematology parameters, but these were not dose-related, and there were no compound-related findings observed microscopically. Both monkeys at 900 mg/kg/day had a 5 cm. gas filled segment in their large intestine that was considered compound-related. Metabolite profiling of the urine showed parent and glucuronidated APAP, but the cysteinyl conjugate which would be anticipated if the monkey has similar metabolism to rats and man was notably absent. This opens the possibility that metabolism in the cynomolgus monkey differs from that in the other species, and shows that the cynomolgus monkey is a poor model for investigating paracetamol-mediated toxicity in humans.

273 Development of a Novel Model of Pseudophakia in Nonhuman Primates to Assess Ocular Particle Movement Risk following Intravitreal Administration of Suspension Formulations

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The development of suspensions for intravitreal (ITV) injection carries a risk for particle movement within the eye, leading to visual disturbances or blockage of Schlem’s canal and increasing intraocular pressure (IOP). The risk of such particle movement may be increased in patients with artificial intraocular lenses (IOLs), specifically patients with artificial intraocular lenses (IOLs), termed pseudophakic patients, and pseudophakic patients who have undergone posterior capsulotomies.
To directly evaluate particle movement in a nonclinical setting, a pseudophakic non-human primate (NHP) model was created to mimic clinical procedures for surgical intervention for cataracts. This model was then validated using Triescence®, an approved IV suspension with a history of successful use in pseudophakic patients. The lenses of the right eye of 11 cynomolgus monkeys were removed by aspiration and replaced with FDA-approved acrylate/methacrylate IOLs in the capsular bag. 5/11 animals then underwent a posterior capsulorhexis in the same eye. Following 4-6 weeks of post-surgical recovery, animals were dosed with IV adalimumab. Following IV adalimumab dosing, no significant particle movement was observed in the control (non-treated) eyes or the pseudophakic eyes. A small amount of test article moved into the anterior chamber of 2/5 pseudophakic + capsulotomy eyes only. Movement of Triescence® did not result in increased IOP or any other clinical complications. These results are in line with the clinical performance of Triescence® in these patient populations, and suggest that this pseudophakic NHP model may be appropriate for the assessment of particle movement with novel IV suspensions.

274 Polysomnography Using Video Electroencephalograph, Electro-Oculogram, and Electromyogram Monitored Continuously by Telemetry in Cynomolgus Monkeys

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Medication-induced sleep disturbances are a major concern in drug development as a multitude of prescription drugs cause abnormalities at polysomnography. Rodent sleep architecture (nocturnal) differs from larger mammals (diurnal) which presents higher translational value. Polysomnography is used in clinical diagnostic but is also applicable to cynomolgus monkeys when using telemetry with continuous electroencephalograph (EEG), electro-oculogram (EOG) and electromyogram (EMG) monitoring with video. Sleep stages in cynomolgus monkeys include wake, N1 (somnolence), N2 (sleep characterized by the presence of sleep spindles and K complexes), N3 (deep sleep with slow waves) and rapid eye movements (REM) and are quantified with automated or manual scoring. Optimal cynomolgus data filters included EOG low pass >25 Hz, EEG band pass 0.7-50Hz and EMG high pass >5Hz. As observed in humans, cynomolgus monkeys present a progressive increase in REM sleep duration with a parallel progressive decrease in N3 sleep during the night. REM sleep was characterized by muscle atonia, high frequency (mostly theta) low amplitude EEG in all animals and EOG activity in most but not all epochs. Total sleep time was 70±2.2% of the 12 hour dark cycle. Sleep stages N1, N2, N3 and REM were typically observed in sequence and represented 1.1±0.8%, 65.2±5.3%, 16.0±5.6% and 17.7±2.4% of total sleep time, respectively. Amphetamine (1 mg/kg, PO) significantly reduced total sleep time and all stages. Spectral analysis revealed a significant increase in higher frequency bands after amphetamine. As expected for benzodiazepine, Diazepam (2 mg/kg, PO) significantly reduced N3 sleep compared to control (340%) but with no effect on total sleep time. Video EEG, EOG and EMG by telemetry is a useful non-clinical model to investigate drug induced sleep disturbances.


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EEG investigations are required to characterize drug induced adverse neurological effects. Seizure liability studies generally aim to: 1) confirm drug-induced seizures are self-limiting, 2) determine plasma level at seizure onset 3) identify prodromal signs which can be monitored in clinical trials 4) confirm that conventional drugs (e.g. diazepam 6 mg/kg, IP for rats and 1 mg/kg, IV for large animals) can treat drug-induced seizures and 5) confirm the NOAEL by absence of paroxysmal activity at lower dose level of the test article. Cynomolgus monkeys, Beagle dogs and Sprague-Dawley rats were instrumented with telemetry implants with EEG electrode placement based on the 10-20 system combined with EMG. After 24 h of continuous video-EEG monitoring, animals received an IV infusion of pentylenetetrazole (PTZ) until convulsions followed by diazepam. A seizure detection protocol with a dynamic spike train threshold was used for the EEG monitoring period (total of 44 h). Spectral analysis quantified absolute and relative amplitudes of EEG frequency bands (delta, theta, alpha, sigma, beta and individual frequencies). The PTZ dose required to induce convolution was 31.2, 35.6, 48.3 mg/kg, in Sprague-Dawley rats, Beagle dogs and cynomolgus monkeys, respectively. Most common prodromal signs included tremors, salivation, emesis, ataxia and nystagmus. Spike trains were detected by computerized analysis in all animals. Seizure peak frequency was 3-6 Hz. Spectral analysis revealed an increase power from higher frequency bands (i.e. theta, alpha, sigma and beta) starting on average at 6 min 9 sec prior to ictus. EEG-video was useful to characterize neurological adverse effects and identify prodromal signs. Computerized EEG analysis was a valuable tool to investigate proconvulsant risks.

276 Comparative Nonclinical Assessments of the Potential Biosimilar PF-06140293 and Adalimumab

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Rationale: Adalimumab (Humira®) is a recombinant fully human immunoglobulin (IgG1) monoclonal antibody targeting tumor necrosis factor (TNFα). Adalimumab is approved for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn’s disease, ulcerative colitis and plaque psoriasis. To support clinical development, nonclinical studies evaluated the structural, functional and in vivo toxicokinetic (TK) and pharmacodynamic (PD) similarity of PF-06140293 and adalimumab. Methods: Structural similarity was determined by peptide mapping. Cynomolgus monkeys were administered PF-06140293 or adalimumab-EU by subcutaneous (SC) injection at weekly dose levels 0 or 157 mg/kg (total 5 doses) for 1-month. Assessments included mortality, clinical parameters, clinical pathology, TK, anti-drug antibodies (ADA), histopathology and pharmacodynamics. Results: Peptide mapping showed similar chromatographic profiles and consistent measured masses for PF 06140293, the US-licensed (adalimumab-US) and EU-approved (adalimumab-EU) products. The results of comparative functional and binding assessments (TNF-induced inhibition of apoptosis in U937 cells and Fc-based functionality) were also similar. PF-06140293 and adalimumab-EU were well tolerated in cynomolgus monkeys. PF-06140293 and adalimumab-EU-related effects were limited to microscopic observations of decreased cellularity of lymphoid follicles and germinal centers in the spleen. There were no biologically or toxicologically relevant differences in the incidence or severity of these microscopic findings between PF-06140293 and adalimumab-EU groups. Systemic exposure appeared similar, with systemic exposure (Cmax and AUC(168) ratios of PF 06140293 relative to adalimumab-EU ranging from 1.0 to 1.2. These studies supported entry of PF 06140293 into clinical development.

277 A Repeat IV Dose Toxicity Study of Gemcitabine Formulations in Male and Female CD-1 Mice

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This study compared the toxicity of Hospira’s Gemcitabine Injection (Test Article) with Gemzar® (Reference Article), when administered to CD-1 mice. Test and Reference Articles were composed of the same cytotoxic active ingredient but Test Article contained additional impurities formed after accelerated degradation. One hundred and sixty mice were assigned to one of eight groups (10 mice/sex/group) and dosed with Vehicle, Test or Reference Article at doses of 0, 100, 500, or 600 mg/kg. Mice were dosed by IV administration via the lateral tail vein on Study Day 1 (SD) and 8. Surviving animals were euthanized on SD 15. Mortality, clinical and post dose observations, body weights, food consumption, ophthalmologic examinations, clinical pathology, gross pathology, organ weights, and histopathology were assessed.

There were no consistent premonitory adverse clinical signs, and no visible lesions at necropsy. There were no adverse effects of the Test or Reference Articles on clinical, cage side, or post dose observations, body weight, food consumption or gross pathology. Treatment with Test and Reference Articles both led to reduced red blood cell count, hemoglobin, hematocrit and absolute lymphocyte counts, increased absolute reticulocyte counts and red cell distribution width, increased absolute and relative spleen weights, decreased absolute and relative thymus and testis weights, increased splenic hematopoiesis and testicular germ cell depletion at doses ≥100 mg/kg. Lymphocytolysis and/or lymphoid depletion of spleen, thymus and lymph nodes were also observed and thymic atrophy was noted at 600 mg/kg. Most observations were of no toxicological significance; however testicular germ cell depletion is adverse due to potential effects on reproductive function. Lymphoid organs and testes are the primary target organs for both articles. In conclusion, the toxicological profile of Hospira’s Gemcitabine Injection containing additional impurities was no different to that of the Reference Article, Gemzar®.
Understanding species differences in placental transfer of Fc-containing biopharmaceuticals (particularly monoclonal antibodies) will improve human risk extrapolation from nonclinical embryo-fetal development toxicity data. Maternal and fetal concentration data from 10, 15, 8 and 34 Fc-containing biopharmaceuticals in the rabbit, rat, mouse and cynomolgus monkey from across 56 species and the associated labor. Additionally, if homogenized tissue is analyzed for ex vivo in rats. Scanners were operated with software validated for the conduct of GLP studies. The coefficients of variation (%CV) were calculated from each series of 4 scans performed on the same animal. X-ray Absorptiometry (DXA) and peripheral Quantitative Computed Tomography (pQCT) were done on four rats and 4 NHPs, 4 times each with repositioning between scans using 2 separate scanners. Legs and arms scans were also performed in NHPs. pQCT scans were performed at the proximal tibia diaphysis for the calf muscle or at the femur diaphysis for the thigh muscle, in vivo and ex vivo in rats. Scanners were operated with software validated for the conduct of GLP studies. The coefficients of variation (%CV) were calculated from each series of 4 scans performed on the same animal. The %CVs obtained for rat WB fat and muscle were below 4% and 1% respectively. The %CVs obtained for NHP WB fat and muscle were 35% and below 2% respectively. Fat mass in NHPs were very low at around 200g explaining the larger variability observed. Similar results were obtained for the two DXA machines. For the right arm, the % CV was below 10% for lean mass (7.3%) and below 11% for fat parameters (g %). For the right leg, the % CV was below 3% for lean mass (2.4%) and below 25% for fat parameters (g %). Additionally, body mass measured by DXA differed by 4% relative to the actual NHP body weight. With pQCT, the % CV was below 4% for lean mass and below 20% for fat parameters. DXA and pQCT provide reproducible non-invasive measurements of fat and muscle mass in NHP which can also be combined with bone densitometry. These data support the use DXA and pQCT densitometry in preclinical studies, to provide comprehensive measures of the body composition in nonclinical drug development programs.

Embryo-Fetal Development (EFD) study designs for risk assessment often include satellite groups for the assessment of toxicokinetics, increasing animal utilization and the associated labor. Additionally, if homogenized tissue is analyzed for ex vivo data, this prohibits a direct comparison of drug levels in the maternal and fetal circulation. Therefore, we have investigated an alternative blood sampling method which allows for collection of fetal blood yet does not hinder visceral or skeletal evaluations for the same fetuses. By collecting from the axillary artery and pooling by litter, sufficiently blood volumes have been obtained from gestation day 21 (GD21) Sprague-Dawley rat fetuses to conduct either toxicokinetic or antibody titer analyses on fetal blood. Clinical pathology and hematologic samples of approximately 1mL were gathered across 24 litters and directly compared. Standard
clinical chemistry (GLU, UN, CREA, AST, ALT, etc.) and hematology (RBC, etc.) parameters indicated that samples collected via the axillary artery were equivalent to blood collected via cardiac puncture. However, cardiac puncture often produces structural damage to at least the thoracic cavity which would negate the possibility of further feline evaluations. It can therefore be concluded that this bleeding procedure offers a way to meaningfully reduce satellite animal requirements without a negative impact on the visceral and skeletal evaluations required of the study design.

283 A Novel Approach for Assessing the Safety of Pharmaceuticals


Too often, prescription medications once thought safe for human use cause adverse health consequences and fail during clinical testing or are recalled after public release. One major problem is the inability of current toxicity assessment methods to detect many toxicities caused by pharmaceuticals during preclinical trials. We have developed a novel toxicity discovery method known as the organismal performance assay (OPA), that is capable of revealing mammalian health consequences. OPAs utilize genetically diverse wild mice (Mus musculus) that compete amongst each other for limited resources in semi-natural enclosures. This assay is sensitive, broad and functional because it demands high performance of most physiological systems in order for individuals to be successful. OPAs have proven powerful in detecting adverse health affects from inbreeding, genetic manipulations and dietary fructose. We tested the safety of Paxil (paroxetine) and Baycol (cerivastatin). Performance of pharmaceutical-exposed mice was analyzed when competing directly against control mice in OPAs. Performance was measured by territoriality, survivorship and reproductive success. We hypothesized that pharmaceutical-exposed individuals will suffer significant fitness declines compared to control individuals. Paxil-exposed breeding pairs took significantly longer to produce their first litter (p<0.05) and these litters were significantly biased towards female offspring (p=0.0021; 39:61 M:F). Paxil-exposed offspring also weighed significantly less than controls (p<0.0001). We detected a significant decline in reproductive success in both sexes and treatments (Paxil F: p<0.001; M: p<0.01, Baycol F: p<0.001; M: p<0.001). Pharmaceutical-exposed males were significantly less dominant than controls (Paxil p<0.0001; Baycol p<0.0001). Baycol-exposed individuals suffered higher mortality (p=0.0116). These pharmaceutical-caused health declines were mirrored by previous animal testing, indicating that OPAs provide superior sensitivity compared to conventional approaches. The data indicate OPAs could be a useful tool in safety testing during pharmaceutical development.

284 Recovery Assessment in Preclinical Drug Safety Testing—A Retrospective Analysis of Value and Timing


We have previously reported the profile of target organ toxicities in first time in man (FTIM) toxicity studies conducted in rodents and non-rodents for 77 AstraZeneca candidate drugs (CDs) across a range of therapy areas (Horner et al., 2013, Reg. Tox Pharm, 65, 334-343). Here, we present a further analysis of which target organ toxicities subsequently recovered after a dose free period and whether that profile differed for CDs that progressed into man and those that did not. We also report which additional target organ toxicities (defined as toxicities in a target organ not previously noted) were observed in chronic (≥3 month) studies conducted to support later stage clinical programmes across the different intended therapy areas (Cardiovascular/Gastrointestinal: CVGI; CNS/Pain: CNSP; Respiratory and Inflammation: RITA; Oncology/Infection: OI). Target organ toxicity was primarily defined as compound-related histopathological changes. Overall, the analyses showed that ≥85% of findings were either fully or partially resolved at the end of the recovery period; lack of recovery in the dog male reproductive tract was the most frequent finding. Interestingly, profiles of recovery were largely similar for those CDs that progressed into man and those that did not and were also similar across therapy areas with the exception of oncology, where more findings persisted at the end of the recovery phase. Compared to observations in chronic studies, >60% of toxicities were identified in the FTIM studies with varying detection rates across the target organs. Overall, these data support inclusion of recovery on the FTIM studies and also demonstrate that chronic toxicity studies provide additional information of great value in risk assessment for longer term dosing in humans.

285 Validation of a CYP1A2 and a CYP3A4 Induction Assay Using Puracyp’s 1A2-DRE™, and DPX2™ Cell Lines: Comparison between the Gene-Reporter Assay and Human Hepatocyte Data

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Induction of Cytochrome P450 drug metabolizing enzymes (DME) can lead to rapid elimination of APIs resulting in reduced efficacy and increased metabolism which can potentially lead to formation of toxic metabolites. The FDA routinely requests data on induction of human CYP450 enzymes CYP1A2 and CYP3A4 by new chemical entities in early development. CYP1A2 expression is regulated via the aryl hydrocarbon receptor (AhR), while CYP3A4 is predominantly regulated via the pregnane X receptor (PXR). Human primary hepatocytes are the preferred in vitro model for studying induction of DME, but their use is limited by unpredictable supply of tissues and interindividual donor variability. In this study, two gene reporter assays for evaluating induction of CYP1A2 and CYP3A4 were validated, using Puracyp’s 1A2-DRE™ (a HepG2 derived cell line stably transfected with human AhR gene and a luciferase reporter gene linked to CYP1A2 promoter and the dioxin response element enhancer), and DPX2™ (a HepG2 derived cell line stably transfected with human PXR gene and a luciferase reporter gene linked to the CYP3A4 promoter and enhancer). CYP1A2 inducers rifampicin, phenobarbital, clotrimazole, erythromycin, and phenytoin increased CYP3A4 luciferase activity in a concentration dependent manner, resulting in EC50 and Emax values of 0.56 μM and 50-fold, 9.7 μM and 24.5-fold, 1.9 μM and 27.4-fold, and 3.3 μM and 37.8-fold, respectively. Similarly, CYP3A4 inducers rifampicin, phenobarbital, clotrimazole, erythromycin, and phenytoin increased CYP3A4 luciferase activity resulting in EC50 and Emax values of 0.85 μM and 7.8-fold, 119.8 μM and 5.0-fold, 0.5 μM and 5.0-fold, 10 μM and 4.2-fold and 17.5 μM and 4.0-fold, respectively. Our reporter gene assay data showed good correlation with data generated using human hepatocytes. Therefore, these validated assays can be used in early development for evaluating induction of CYP1A2 and CYP3A4, providing guidance on compound selection and potential for clinical drug-drug interactions.

286 Acute Kidney Injury Biomarkers following Low-Dose Gentamicin Treatment of Rats

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Rationale

The use of biomarkers as part of the preclinical assessment of kidney injury is now a well established goal. Published analysis of multiple datasets from the toxicology industry has convincingly identified a panel of urine and serum biomarkers which correlate with drug induced kidney injury. Several biomarkers have been shown to be superior to blood urea nitrogen (BUN) and serum creatinine at detecting renal injury. The present study correlates histopathologic evidence of kidney damage with urinary and serum biomarkers after very low dose regimens of Gentamicin.

Experimental procedures

Male Sprague-Dawley rats received daily subcutaneous injections of Gentamicin sulfate for 15 days at dose levels of 5, 15, and 50 mg/kg/day. Urine samples were collected over 16 hours for biomarker assessment at multiple timepoints (Pre-Tx, Days 4, 10, and 16). Blood was also collected at the end of a fasting period and serum preserved. A panel of biomarkers was tested in urine (Albumin, B2M, clustatin, cystatin C, KIM-1, NAG, NGAL, OPN, RBP) and serum (BUN, creatinine, and cystatin C). Kidney histology was assessed on days 4 and 16.

Results

Increased excretion of urinary biomarkers was observed on Days 10 and 16 of gentamicin treatment, and was associated with histologic changes on Day 16. Serum and urine creatinine and BUN remained unchanged over the treatment period.

Conclusions

As previously reported, creatinine and BUN levels were insensitive to low levels of acute kidney injury. Increased urine excretion of a group of biomarkers was associated with histopathological findings despite the normal values of creatinine and blood urea nitrogen. These biomarkers are candidate diagnostics and potential surrogates to histological evaluation of acute kidney injury.
Clinical Pathology parameters are routinely assessed during the conduct of toxicology studies. This study was conducted to see if there were any differences in hematology, coagulation and clinical chemistry parameters among Sprague Dawley rats sourced from Charles River sites based in the USA, China and Taiwan. Control group data from completed and ongoing toxicology studies were used to see if there were any differences between the three sources of animals. The results from the three data sets were analyzed using the Charles River USA-supplied rat as reference control point. Analysis of the hematology data showed for males slightly lower white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC) and neutrophils and increased mean corpuscular hemoglobin (MCH) for animals from both China and Taiwan when compared to the USA. In addition, decreased group mean lymphocytes and monocytes were observed for animals from China only. For the female rats decreased WBC, RBC, HGB, HCT, MCV, lymphocytes, monocytes and eosinophils and mean platelet volume (MPV) were observed for animals from China only. For both males and females from both China and Taiwan, decreased Prothrombin time were observed when compared to the USA-sourced rat. Differences in clinical chemistry were observed for rats from both China and Taiwan and included a reduced total protein due to a reduced albumin, reduced potassium and increased globulin when compared to the USA rat. The results of the analysis indicated that those rats sourced from Taiwan were closer to those sourced from China in terms of clinical pathology profile. These observed differences between the three sources of animals enforce the need to be consistent in the source of animals for a toxicology development program and also the need for maintaining separate back ground data bases.

Lower Susceptibility to Histaminergic Reactions in Rodents Using Continuous Infusion: Advantages for Safety Assessment of Parenteral Peptide/Protein Drugs

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Some drugs elicit histaminergic responses in vitro and in vivo, with the rat shown to be particularly sensitive in toxicology testing. As more parenteral therapeutic peptides/proteins are developed a model less prone to histaminergic reactions would offer improved safety testing. Such a model could be continuous vascular infusion via surgically implanted indwelling catheter in rodents and would allow comparison of histamine release response compared to bolus administration. In this study, the sensitivity of mouse and rat to histamine release after bovine pancreatic trypsin inhibitor drug Aprotinin (Trasylol®) given as a bolus or 6hrs intravenous infusion was investigated.

Rodents with implanted indwelling femoral vein catheter were bolus injected with 120.000 or 300.000 U/kg or infused over 6hrs with 240.000 or 300.000 U/kg. After bolus injection, mice developed mild clinical signs, while rats developed clear histaminergic symptoms (cyanosis, swellings, reddening). Reddening was also seen in 1 rat after 300.000 U/kg infusion over 6hrs. Predose histamine levels in mice were markedly (~15-fold) lower than in rats. Induced histamine release was also seen in 1 rat after 300.000 U/kg infusion over 6hrs. Predose histamine levels in mice were markedly (~15-fold) lower than in rats. Induced histamine release was also seen in 1 rat after 300.000 U/kg infusion over 6hrs. Predose histamine levels in mice were markedly (~15-fold) lower than in rats. Induced histamine release was also seen in 1 rat after 300.000 U/kg infusion over 6hrs.

Methylmercury (MeHg) is well known as human neurotoxic agent whose exposure sources are mainly environmental and aquatic-derived food. MeHg is reported to induce central nervous system (CNS) disability. However, the exact mechanism of MeHg-induced neurotoxic effects is still unknown. In this study, to investigate which cell death signaling pathway is related with MeHg-induced cytotoxicity, the effects of MeHg on apoptosis and autophagy were evaluated in human neural stem cells (NSCs). H1.B1.F3. Human NSCs were treated with 1 μM of MeHg for 48 hr and the effect of MeHg on cell signaling pathway was elucidated. MeHg inhibited Akt/mTOR signaling that led to induction of caspase-dependent apoptosis and autophagy in the NSCs. Furthermore, retinoic acid (RA)-induced neuronal differentiation was inhibited by MeHg. Taken together, these results suggest that MeHg inhibits the differentiation of human NSCs by induction of caspase-dependent apoptosis and autophagy.

Verifying Performance of Cell Viability and Apoptosis Assays Applied to 3D Microtissues

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Multicellular 3D culture systems containing more than one cell type and exhibiting formation of a complex extracellular matrix represent a more physiologically relevant environment for performing in vitro cytotoxicity testing. yet provide a challenge for assay chemistries originally designed for measuring events from 2D monolayers of cells. The approach of this research was to investigate whether increasing detergent concentrations to increase lytic capacity or providing physical disruption of samples would improve the results of cell viability and apoptosis assays applied to microtissues grown using 3D culture models. Results demonstrate that increasing detergent concentration in assay reagents has limits that depend on the source and stability of the luciferase enzyme. Introducing a physical disruption step in the protocol (shaking multi-well plates for 5 minutes at 850 rpm) improves the generation of signal from the luminescent ATP assay applied to microtissues grown in 3D models. Physical disruption also improved signal intensity from measurement of caspase-3/7 activity as a marker of apoptosis. Validation of assays designed for 2D monolayers is necessary prior to application to 3D culture models.
Activation of the Aryl Hydrocarbon Receptor by Endogenous Cinnabarinic Acid Induces Stanniocalcin-2 to Elicit Cytoprotection against ER and Oxidative Stress

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The Aryl Hydrocarbon Receptor (AhR) is a cytosolic ligand activated transcription factor historically known for its role in xenobiotic metabolism. However, endogenous tryptophan catabolites such as cinnabarinic acid possess the capacity to activate the AhR. Our lab has recently identified stanniocalcin 2 (STC2) as a novel AhR target gene that was induced in an AhR dependent manner after adenosiv infection of mouse primary hepatocytes. We hypothesized that AhR induction of STC2 plays a cytoprotective role in the mouse liver. Here we demonstrate for the first time that cinnabarinic acid activation of the AhR induces STC2 to elicit protection against ER stress and oxidative stress-induced apoptosis in mouse primary hepatocytes. We confirmed AhR activation and AhR targeting of the STC2 gene after cinnabarinic acid treatment by conducting immuno-cytocchemistry and chromatin immuno-precipitation experiments, respectively. Our preliminary data demonstrates that cinnabarinic acid treatment of primary hepatocytes causes significant enrichment of the STC2 protein while decreasing caspase 3 activities in a dose dependent manner. We also show that primary hepatocytes pre-treated with cinnabarinic acid for 24 hours exhibited reduced caspase 3 activities and reduced percentages of TUNEL positive cells after treatments with thapsigargin, hydrogen peroxide or ethanol when compared to non cinnabarinic acid pre-treated cells. Using AhR conditional knockout primary hepatocytes as well as STC2 knockdown using siRNA, we were able to confirm the direct involvement of STC2 in the cytoprotection of mouse primary hepatocytes against ER stress and oxidative stress-induced apoptosis. Our data conclusively demonstrates that AhR induction of STC2 in the liver plays a crucial cytoprotective role against ER stress and oxidative stress-induced apoptosis. Therefore, our goal is to elucidate the cytoprotective mechanism of STC2 in mouse primary hepatocytes.

CYP1B1 Inhibits TRAIL-Mediated Apoptotic Pathway followed by Sp1 Induction


Cytochrome P450 1B1 (CYP1B1) belongs to the CYP1 family and shares the feature as an enzyme for drug metabolisms. It has been reported that CYP1B1 expression is higher in the tumor tissues than the normal ones, especially in hormone-related cancers such as breast, ovarian and prostate cancer cells. To explore the role of CYP1B1 on cancer cell proliferation, we investigated whether CYP1B1 blocks apoptotic pathways. Using Western blot and RT-PCR, the expression of anti-apoptotic factors such as Bcl-2, Bcl-xl, XIAP, survivin and Mcl-1 were examined and showed to be promoted followed by CYP1B1 overexpression or treatment with CYP1B1 inducer, 7, 12-dimethylbenz[a]anthracene (DMBA). These promoting results were reversed when cells were transfected with siRNA for CYP1B1 or treated with a CYP1B1 inhibitor, tetrathromthoxynil (TMS). Moreover, AIE, a widely known pro-apoptotic factor, was showed to be translocated to cytosol from mitochondria after treatment with TMS. The expression of TRAIL, a member of tumor necrosis factor superfamily, was also examined followed by alteration of CYP1B1 expression and it showed similar changes in expression level to pro-apoptotic factors. Sp1, a transcription factor, has been reported to suppress TRAIL expression by binding TRAIL promoter. To identify whether Sp1 mediates the suppression of TRAIL, we examined Sp1 expression after changes of CYP1B1 expression level and it showed that Sp1 is promoted by CYP1B1. Moreover, Sp1 knockdown with specific siRNA showed promotion of TRAIL-related apoptotic factors and these results were matched by chromatin immunoprecipitation. Moreover, attenuation of Sp1 DNA binding activity using mimicrycin A, a well known Sp1 binding inhibitor, resulted in recovery of TRAIL expression formerly suppressed by treatment with DMBA. Taken together, these data suggest that CYP1B1 blocks apoptosis via TRAIL suppression and this is mediated by Sp1 induction.

Intact 20kDa Extracellular Domain of APO2L/TRAIL Bioanalysis by HRMS: A Potential Cancer Therapeutic Protein


Recombinant APO2L/TRAIL (Ap2 ligand/tumor necrosis factor-related apoptosis-inducing ligand), typically analyzed by LBA, was successfully quantified as intact protein by High Resolution Mass Spectrometry (HRMS). The chromatographic separation of intact APO2L/TRAIL for mass spectrometric analyses was found to be challenging with silica columns. Whereas, polymer column greatly improved peak shape and reproducibility. Moreover, the column temperature had an important impact on the amount of APO2L/TRAIL recovered from column upon injection: Increasing the column temperature from 22°C to 70°C increased the recovery by at least 4-time and improved peak shape. Data processing method was optimized to avoid interferences, it was possible to quantitate only the most abundant charge state and isotopomers or the sum of each charge state and isotopomers. APO2L/TRAIL signal was found to have charge state distributions from +12 to +27 with the 6 most intense charge states being from +18 to +23. Monitoring only one charge state (+20), with an XIC of 10mDa, led to variability and unacceptable peak shape. After summation, the 6 most abundant multiple charge states with an XIC window of 10mDa around the most abundant isotopomer, a S/N=8 was observed at the LLOQ. The method showed very good results: dynamic range was linear (weighted to 1/x2) with a correlation of r=0.9960. The assay precision was below 5% and with accuracy between 101-108% for the LLOQ QCs and 3 additional QC levels. This data successfully confirmed the possibility to use HRMS for intact protein quantification without tropic digestion.

Preventive Effect of Nonmitogenic Acidic Fibroblast Growth Factor on Diabetes-Induced Testicular Cell Death and Atrophy

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Fibroblast growth factor (FGF) family members in previous studies were shown to prevent various oxidative stress-induced cardiac, neuronal and retinal inquires. Particularly, FGF-1 was found to protect the heart from ischemia-, doxorubicin- and diabetes-induced damage suggesting the potential clinical application. However, long-term use of FGF-1 was restricted for its potential risk of carcinogenesis due to its non-specific proliferating activity. Thus a cluster of amino acids responsible for proliferation were deleted in the native FGF-1 to create a modified non-mitogenic FGF-1 (nmFGF-1). The present study tested if the nmFGF-1 protects male germs cells from diabetes-induced apoptotic cell death and testicular atrophy. Mice were treated with multiple low-doses of streptozocin (STZ) to induce type-1 diabetes and then treated with nmFGF-1 for 6 months. Diabetic mice showed a decrease in testicular weight and an increase in apoptotic cell death (detected by TUNEL staining). Treatment with nmFGF-1 alleviated diabetic effects on testicular weight and apoptotic cell death. Mechanistically, the diabetes induces, the mitochondrial apoptotic pathway due to a significant increase in BAX/Bcl-2 ratio, determined with Western blotting assay. Diabetes also induced mild increases in endoplasmic reticulum stress and associated cell death, reflected by increases in cleaved-caspase 12, CHOP, and GRP-78 expression, and a significant increase in TNF-α-alpha expression as an index of inflammation. All these effects induced by diabetes were attenuated by treatment with nmFGF-1. Therefore, these results suggest that nmFGF-1 is able to prevent the apoptotic-effect of diabetes on the testes via the mitochondrial pathway predominantly, resulting in prevention of diabetes-induced testicular atrophy.

Nelumbo nucifera Leaf Extract-Induced Pancreatic Cancer Apoptosis via JNK/ERK Activation-Regulated Mitochondria-Dependent and ER Stress-Triggered Signaling Pathways

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In this study, we investigated the pharmacological effects of N. nucifera leaf in anti-pancreatic cancer and elucidate its molecular mechanisms. The results found that Nelumbo nucifera leaf extract (NNE) significantly caused cytotoxicity in PANC-1 cells with a dose-dependent manner and induced several features of apoptosis signals in PANC-1 cells. NNE was also capable of increasing the protein phosphorylations of JNK and ERK-1/2, but not that p38-MAPK. Transfection of PANC-1 cells with JNK1- and ERK2-specific si-RNA could inhibit JNK-1 and ERK-2 activation and attenuate caspase-3/-7 activity induced by NNE, respectively. Furthermore, exposure of PANC-1 cells to NNE could trigger endoplasmic reticulum (ER) stress indicating by the increase in ER stress-related molecules. Transfection of PANC-1 cells with GRP-78- and GRP94-specific si-RNA could effectively prevented the NNE-induced caspase-3/-7 activity, respectively. More
importantly, quercetin, not that catechin, was the mainly active component of NNE in NNE-induced cytotoxicity and apoptosis in PAN-C-1 cells. In conclusion, these results demonstrate that NNE induces pancreatic cancer cell apoptosis via the JNK/ERK activation-regulated mitochondria-dependent and ER-stress-triggered signaling pathways. And quercetin is the mainly active component of NNE.

297 Most Bioactivation-Dependent Nephrotoxins Induce Apoptotic Death along with Unique Patho-Morphological Characteristics in Mouse Kidneys


Critical issues in the pathophysiology of toxic renal injury includes drug metabolization within target cells, changes in gene expression, oxidative stress, inflammation and various types of cell deaths. Drug-induced nephropathy is often viewed as an endpoint in animal toxicity studies, and the diagnosis of various forms of kidney injury based on clinical data remains very challenging because of the difficulty in interpreting histopathology. Our earlier studies showed that most bioactivation-dependent nephrotoxins cause oxidative stress and apoptotic death. Therefore, this study analyzed the histopathological changes induced by different bioactivation-dependent nephrotoxins [chloroform (CHCl3), diclofenac (DCF), salicyclic acid (SA) and furosemide (FUR)] to profile common and uncommon pathological features to hypothesize whether a subset of changes can be used to categorize nephrotoxins. Mice were administered CHCl3 (1 ml/kg orally), DCFL (300 mg/kg, ip), SA (300 mg/kg, ip) and FUR (500 mg/kg, ip) and sacrificed 24 hours later. PAS or H&E stained kidney sections were examined under a brightfield microscope to assess changes in proximal (PT) and distal tubules (DT). Bowman's capsule, ureter, brush borders, Bowman's space, glomerular congestion and interstitial fibrosis. Results indicated that all the toxins induced nephrotoxicity as judged by elevated serum BUN (5 fold or higher by all toxins), increased BUN/creatinine ratios and tissue MDA levels (fold or higher). Pathomorphologically, the most common features were distorted PTs and DTs along with brush border injury, scattered apoptotic nuclei and abundant inflammatory cells. DCFL showed hypertrophied tubular cells with massive induction of apoptotic nuclei, SA showed degeneration of brush borders with tubular dye accumulation, whereas FUR showed global injury throughout the tissue. Thus, this study may have discerned a novel way of profiling xenobiotic-induced nephrotoxicity [Suppo, by Dept. of Pscs of Manch. Univ. Coll. of Pharm. & AMS Coll of Pharm & HScs, NV]

298 Anthracenyl Isoxazole Amides (AIsMs) As Mitochondrial Modulators of Apoptosis

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Development of G-quadruplex ligands for the treatment of solid tumors is an emerging field of research. G-quadruplex structures within the nuclei of cells are the target for these ligands with the goal of inhibiting telomerase and causing replicative senescence or selective apoptosis of tumor cells. We have previously shown that novel anthracenyl isoxazole amides (AIsMs), such as N5C 748994 (A1M), bind telomeric G-quadruplex DNA and exhibit single digit micromolar IC50 values in SNB-19 glioblastoma cells, suggesting their potential as G-quadruplex-directed antitumor agents. However, confocal microscopy with MitoTracker Red showed that A1M was primarily localized within the mitochondria of cells, with less distribution to the nucleus. As a result, the present study was conducted to determine if the AIsMs modulate mitochondrial function in SNB-19 glioblastoma cells. Our hypothesis was that the AIsMs produce cell death via mitochondrial-mediated apoptosis. A1M significantly increased both early and late apoptosis at 5 μM compared to control after 24 hours as measured by Annexin V/flow cytometry. JC-1 staining indicated that mitochondrial membrane potential was significantly reduced by 5 μM A1M while reactive oxygen species generation was increased at the 1 μM level using MitoSox Red, a mitochondrial indicator of superoxide anion. In addition, exposure to 5 μM A1M resulted in caspase-9 activation, an indication of mitochondrial-mediated apoptosis. Although these findings suggest that the mitochondria is one possible target of A1M-associated toxicity, these effects could also be triggered by A1M binding to G-quadruplex in nuclear DNA (via human telomerase catalytic subunit hTERT shuttling) or directly to G-quadruplex in mitochondrial DNA. Further studies on the mechanisms of A1M toxicity are ongoing. Supported by NIH Grant P20RR017670 (HDB).

299 Assessment of an In Vitro Model for Evaluating the Role of PARP in Ethanol-Mediated Hepatotoxicity

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This investigation assesses the role of poly(ADP-ribose) polymerase in ethanol-mediated hepatotoxicity using the untransfected HepG2 hepatocellular carcinoma line, an established, well-characterized toxicological model. HepG2 cells were treated with ethanol at concentrations between 100 mM and 800 mM, and assessed for markers of cytotoxicity. PARP-1 activity in total cell protein lysates was quantified as a proxy of apoptotic induction at six hours. Our results demonstrated a 1.43-fold AST activity increase in culture medium isolates of cells exposed to 800 mM without significant effect on cellular viability. PARP-1 activity varied greatly and results for enzyme activity remained inconclusive. The results suggest a high degree of insensitivity to ethanol toxicity and nuclear enzyme activity, demonstrating the metabolic unrelevance of untransfected HepG2 in ethanol toxicosis. There is a need to characterize phase 1 metabolic enzyme expression profiles relevant to ethanol for CYP2E1 and ADH pathways to facilitate constructing toxicological models using transfected, as well as the untransfected HepG2 model.

300 Genotoxicity of DEHP and Its Metabolite

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DEHP, Di-(2-ethylhexyl) phthalate, the most common phthalate plasticizer used in manufactures. It can be leached into a liquid that comes in contact with plastics, suggesting concerns about the health risk of human exposure. Several in vivo studies have shown that DEHP can be metabolized in the intestine and converted to MEHP, mono-ethylhexyl phthalate. The metabolite can be absorbed through the vili and travel in the circulation system then causes series damages in endocrine and reproductive system. In addition, investigations also demonstrated an association with elevated induction of rat hepatic cancer, testicular cancer, and developmental toxicity in reproductive system under MEHP exposure. However, the mechanism regarding to carcinogenicity induced by DEHP or its metabolite MEHP remains unclear up to date. We therefore are investigating the cell viability and mutagenicity of DEHP and MEHP in Chinese hamster ovary (CHO) cells. Cytotoxicity and DNA damages induced by these chemicals in nucleotide repair-proficient A549 and repair-deficient UV5, and base excision repair-deficient EM9 cell lines. Results show that the parental chemical, DEHP, as well as MEHP derivatives caused dose-dependent decreases in cell survival, but with very different potencies: MEHP has the high potency; and DEHP lower. Exposure to these compounds induced dose-dependent production of ROS, as determined by cm-H2DCF-DA Fluorescence. Comet assay shows the DNA damages in the trend as same as cytotoxicity, but no difference observed between NER and BER deficient cells exposed to these treatments, even at highly toxic doses. It was noted however that simultaneous exposure of ROS scavenger N-acetylcycteine (NAC) shows the protection against DEHP and MEHP induced DNA damages. In summary, DEHP and MEHP are cytotoxic to the cells and require induction of ROS to exert their genotoxicity effects.

301 Exposure of V79 Hamster Cells to Cadmium Chloride Results in the Production of Double-Strand Breaks and Cell Cycle Changes

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Cadmium is a ubiquitous metal used in many industrial applications, resulting in widespread environmental pollution. Exposure to this metal occurs through inhalation and can result in numerous respiratory disorders, as well as cancer. Non-occupational exposure occurs through inhalation and the ingestion of cadmium from polluted food and water. This study investigates the effects of cadmium exposure on cytotoxicity, genotoxicity and cell cycle progression in V79 hamster cells. Western-blot analysis of caspase and PARP cleavage were used to determine cytotoxicity while genotoxicity and cell cycle changes were analyzed using H2AX, cyclins A and B, and cyclin dependent kinases 1 and 2. To determine if cadmium damage was cell cycle specific propidium iodide analysis was performed on cells synchronized in G2/M, G0, and S phases, using nucodazole, serum starvation, and aphidicolin treatments, respectively. Genotoxicity was measured following analysis of mutations at the HPRT gene. Results determined that cadmium expo-
sure resulted in a dose-dependent increase in apoptosis and double strand break formation which correlated with a greater uptake of cadmium as measured by ICP/MS. Downregulation of cyclins A and B and cdk1 and 2 occurred indicating there was a cell cycle arrest following cadmium exposure. Cells arrested in G2/M by nocodazole prior to exposure demonstrated increased G0/G1 arrest which was p53-dependent. Collectively, this study indicates that cadmium induces genotoxic lesions and causes alterations in normal cell cycle progression.

**302 Induction of Apoptosis by PQ1, a Gap Junction Enhancer That Upregulates Cx43 and Activates the p38-MAPK Signaling Pathway in Mammary Carcinoma Cells**

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Cell death induced by the gap junction enhancer, PQ1, was studied in mammary carcinoma cells (FMC2u) derived from a malignant neoplasm of a female Pyt transgenic mouse. PQ1 was determined to have an IC50 of 6.5 μM in FMC2u cells, while inducing an upregulation in Cx43 expression. The mechanism behind PQ1 induced cytotoxicity in mammary carcinoma cells is thought to be attributed to the change in Cx43 expression; therefore, the effects of Cx43 modulation in FMC2u cell survival was determined through transfection experiments with Cx43 cDNA, which induced an elevated level of protein expression similar to that seen with PQ1 exposure, or siRNA to silence Cx43 protein expression. Overexpression of Cx43 led to a reduction in cellular viability, while an increase in viability was observed after silencing of Cx43 in FMC2u cells. The mitogen-activated protein kinase (MAPK) family has been implicated in the regulation of cell survival and cell death; therefore, the GJIC-independent function of PQ1 and Cx43 in the MAPK-dependent pathway of apoptosis was explored in mammary carcinoma cells. Both the overexpression of Cx43 and PQ1 treatment stimulated an increase in the phosphorylated form of p38-MAPK, reduced levels of the anti-apoptotic protein Bcl-2, and increased the cleavage of pro-caspase-3. Silencing of Cx43 protein expression led to a reduction in the phosphorylation of p38-MAPK and an increase in bcl-2 expression. These results suggest that PQ1 affects not only GJIC, but also cellular survival via a MAPK-dependent pathway. Cx43 may be the key element in the mechanism of PQ1 induced apoptosis.

**303 Zebrafish Assay Format for Determining Compound-Induced Apoptosis Inhibition and Activation**

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During development, naturally occurring apoptosis plays an important role in tissue modeling. However, uncontrolled apoptosis is associated with numerous diseases as well as compound toxicity. Here we describe an assay format used to assess compound induced apoptosis inhibition or activation that relies on the presence of developmentally regulated cell death in live 2 day post fertilization (dpf) zebrafish. At 2 dpf, developmental apoptosis present in the hatching gland and nasal placode can be assessed by acridine orange (AO) live dye fluorescence staining. In contrast, at this stage only a few dispersed apoptotic cells are present in the zebrafish tail. By 3 dpf, essentially no apoptosis is visible at any site. Using these inherent assay parameters, in this study, we assessed 4 characterized inhibitors: Ac-DNLD-CHO, Ac-DEVD-CHO, Z-VAD-fmk, and Q-VD-Oph and 3 activators: Staurosporine, Borrélidin, Gambogenic acid, and 1 negative control compound, Buthionine sulfoximine, shown not to affect apoptosis in mammals. Zebrafish (N = 10) were exposed to the MNLC of each compound for 24 hrs, incubated with AO (1 mg/ml), washed and anesthetized. Effects were initially assessed visually followed by quantitative morphometric image analysis. Compared to the DMSO control, significant inhibition (P < 0.05) of developmentally regulated apoptosis in the hatching gland and nasal placode was observed for Ac-DNLD-CHO (73.7 + 22.6%), Ac-DEVD-CHO (99.60 + 0.4%), Z-VAD-fmk (70.9 + 27.7), and Q-VD-Oph (68.60 + 23.5%), and significant activation (P < 0.05) of apoptosis in the tail was observed for Staurosporine (933 + 701%), Gambogenic acid (2692 + 1205%), and Borrélidin (9227 + 6017%). Buthionine sulfoximine, the negative control, did not significantly affect AO staining. Student’s t-test was used to determine significance (P < 0.05). Effects in zebrafish were then compared with results in mammals. The overall correct prediction rate was 100%, considered excellent by ECVM. These data support use of zebrafish as a predictive model for assessing cell death for safety, toxicity and efficacy studies.

**304 Non-Tubulin Mitotic Disruptors Induce Apoptosis in Cancer Cells**

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Disrupting mitosis is a validated approach for cancer chemotherapy. Mitotic disruptors (e.g. taxanes) are common components of combination chemotherapy used to treat cancers of lung and other organs. These agents induce mitotic arrest and apoptosis in cancer cells. Chemotherapeutic success is limited by development of resistant and dose-limiting toxicity. Mitotic disruptors currently in use, and most of the new agents in development, target tubulin. An alternative approach is to target the regulators of mitosis. The anaphase promoting complex/cyclosome (APC/C) is the master regulator of mitosis and cell cycle, and is a novel anti-cancer target. Using an in silico approach, candidate compounds were identified to disrupt APC/C activity. Compounds were tested for ability to induce cytotoxicity in colony forming and AlamarBlue assays and to induce increases in mitotic index in a variety of human cancer, SV40-transformed and diploid cell lines. Compounds containing a 1-amino-propan-2-ol core were found to induce mitotic arrest and cell death as predicted in lung and ovarian cancer cells, malignant melanoma and HeLa cells and SV40-transformed fibroblasts and bronchial epithelia, while sparing normal diploid cells. These results indicate that targeting the disruption of the APC/C is a viable approach for developing a new class of mitotic disruptors useful for cancer chemotherapy.

**305 Salubrinal Differentially Modulates Mitochondrial Dysfunction-Induced by Halogenated Analogs of 3, 3’-diindolylmethane (DIM) in Prostate Cancer Cells**

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We have previously shown that halogenated analogs of 3,3’-diindolylmethane (DIM), termed ring-DIMs, induce apoptosis and necrosis in human prostate cancer cells. We now show that ring-DIMs at toxic concentrations induce endoplasmic reticulum (ER) stress in human prostate cancer cells, and that cell death induced only by 4,4’-dihaloDIMs is abrogated by pre-treatment with the ER stress inhibitor, salubrinal. Salubrinal reduced phosphorylation of eIF2alpha in cells treated with 4,4’-dibromoDIM and did not increase phosphorylation of eIF2alpha in cells treated with 7,7’-dichloroDIM or DIM. We also found that neither CHOP nor ATF4 levels, both indicators of ER stress, were decreased after pre-treatment with salubrinal. The effect of salubrinal appeared to be linked to mitochondrial stability as pre-treatment with salubrinal negated the decrease in mitochondrial membrane potential (MMP) and mitochondrial abundance in cells treated with 4,4’-dibromoDIM. In contrast, salubrinal exacerbated the cytotoxic effects of 7,7’-dibromoDIM, 7,7’-dichloroDIM and DIM by further increasing the loss of MMP and mitochondrial abundance induced by these compounds. We found that an inhibitor of the mitochondrial permeability transition pore, cyclosporine A, prevented the loss of cell viability, MMP and mitochondrial abundance caused by co-treatments of 7,7’-dihaloDIMs with salubrinal. An in silico 3D docking analysis to find binding partners for salubrinal and the ring-DIMs point to targets other than ER stress and eIF2alpha as mediators of salubrinal’s apparently contradictory modulation of cell death induced by the ring-DIMs.

**306 Targeting CamKII in Prostate Cancer Cells: Induction of Mitochondrial Dysfunction and ER Stress by Halogenated Analog of 3, 3’-Diindolylmethane**

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We have previously shown that a series of halogenated analogs of 3,3’-diindolylmethane (DIM), termed ring-DIMs, induce apoptosis and necrosis in androgen-dependent and androgen-independent prostate cancer cells, with 4,4’-dibromoDIM having the greatest anti-cancer activity. To understand the upstream events leading to the activation of caspases in response to treatment with dibhalo-DIMs in androgen-dependent LNCaP and androgen-independent LNCaP C4-2B cells, we monitored the onset of endoplasmic reticulum (ER) stress and the dysregulation of mitochondrial apoptosis. We found that 4,4’-dibromo- and 7,7’-dichloroDIM and DIM itself caused ER stress-dependent upregulation of CHOP and ATF4, while only 4,4’-dibromo- and 7,7’-dichloroDIM increased phosphorylation of eIF2alpha.
Hepatocytes are the primary cell type of the liver providing the majority of the detoxification which increases the potential for cellular dysfunction and death. Though the source of the insult may be caused by several factors, exposure to drugs represents a significant concern warranting FDA guidance on drug-induced liver injury (DILI). In vitro studies are still the gold standard; however, in vitro screening has gained importance for reducing animal exposure, amenable to high-throughput platforms and better equipped to study cellular mechanisms of action. Typically in vitro screening has incorporated primary hepatocytes cultured in a two dimensional (2D) format where the cells form a monolayer across the bottom of a well. However, when cultured and studied in this fashion they rapidly lose their key functions and de-differentiate over the course of only a few days. The ability to culture, characterize and challenge primary cells in a biomimetic 3D environment enables the performance of longer term studies.

Here we present data demonstrating the differences in response between human primary cells cultured in 2D and in the RAFT 3D cell culture system which has the benefits of a collagen hydrogel with tissue-like properties. Camptothecin and pyocyanin were tested for their ability to cause short-term oxidative stress, as well as long-term induction of apoptosis and necrosis. Kinetic live cell imaging was performed using multiple fluorescent non-perturbing probes to monitor the various effects in real time with incubations up to 48 hours which enabled a thorough assessment of the toxic profile. Image overlay allowed for discrete cellular analysis. Variations in cytotoxicity levels were observed after a 24 hour 800 nM camptothecin treatment (75%/2D; 28%/3D). ROS induction was less in 3D than 2D system. Overall, cells exhibited greater viability using the RAFT system, and were less sensitive to toxins than observed in traditional 2D culture for the endpoints which may indicate a more robust cell culture system.

### 307 Validation of a Novel Cell Culture System to Perform 3D In Vitro Cytotoxicity Analyses Using Primary Hepatocytes

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More aggressive or invasive prostate cancer cells (PCCs) are often resistant to chemotherapy and have poor prognosis. Differences exist in redox status and mitochondrial metabolism that may help explain this phenomenon. We compared two immortalized human PCC lines, PC-3 cells (more aggressive) and LNCaP cells (less aggressive), with regard to cellular glutathione (GSH) levels and susceptibility to oxidants (tetr-butyl hydroperoxide [tBuH] or methyl vinyl ketone [MVK]) or GSH depleters (diethly maleate [DEM] or diame). We tested the hypothesis that cells resistant to less aggressive PCCs, more aggressive PCCs exhibit higher GSH concentrations and are relatively resistant to cytotoxicity from exposure to various oxidants. PC-3 cells exhibited 4.2-fold higher GSH concentration than LNCaP cells. PC-3 cells only exhibited lower lactate dehydrogenase (LDH) release after toxicant exposures than LNCaP cells at some time points but only LNCaP cells underwent diamide-induced apoptosis. Expression of several proteins involved in stress response and regulation of cell growth and proliferation were measured to determine the possible role of altered levels of certain signaling molecules with differential redox state and susceptibility to toxicants. PC-3 cells exhibited higher levels of Bax and caspase-8 cleavage product and lower levels of Bel-2 than LNCaP cells. PC-3 cells exhibited with higher susceptibility to Fas-induced apoptosis than LNCaP cells exhibited higher expression of FasR than PC-3 cells. Consistent with a compensatory response to lower GSH concentrations and generally greater susceptibility to oxidative stress, LNCaP cells exhibited higher levels of GADD153, p53, Hsp27, Cyclin A, SOD2, Trx2, and mitochondrial GSH transporters than PC-3 cells. Thus, significant differences in redox status and expression of proteins involved in apoptosis and stress response may contribute to PCC aggressiveness.

### 308 Heat Shock Response Antagonism: From Toxicological Liability to Cancer Chemotherapeutic Opportunity

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The cellular heat shock response plays an essential role in cytoprotection that involves maintenance of proteinostasis and suppression of apoptosis. Based on an increased vulnerability of cancer cells to proteotoxic stress, small molecule heat shock response inhibitors are now an emerging class of cancer-directed experimental therapeutics. Guided by a phenotypic screen, we have interrogated a focused compound library for functional heat shock response antagonism targeting a panel of malignant melanoma cell lines (G361, LOX-IMVI, A375). From a collection of pro-oxidants 3,7-diamino-phenothiazinium derivatives [methylene blue (MB), azure A, azure B] we have identified the redox-drug MB, used clinically for the intestinal treatment of methemoglobinemia, as a negative modulator of heat shock response gene expression in human A375 melanoma cells. Stress and toxicity response gene array analysis using the RT2 Profiler™ technology (SuperArray, Frederick, MD; MB: 10 µM, 24 h) revealed that MB-treatment selectively suppressed expression of Hsp70 family members (HSPA1A, HSPA1B) and Hsp22 (HSPB1), a finding confirmed at the protein level. Importantly, MB sensitized melanoma cells to the apoptotic activity of geldanamycin, an Hsp90 antagonist that induces the counter-regulatory upregulation of Hsp70 expression underlying cancer cell resistance to geldanamycin chemotherapy. Similarly, MB-co-treatment sensitized melanoma cells to other apoptogenic chemotherapeutics. Taken together, these data suggest feasibility of repurposing the non-oncological redox drug MB as a proteotoxic heat shock response antagonist for chemotherapeutic intervention. Supported in part by grants from the National Institutes of Health [R01CA122484, R03CA167580, R21CA166926, E0007091, E0066944].

### 309 Apoptosis Modulates Hepatotoxic Effects of 2-Aminoanthracene in Fisher 344 Rat

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The arylamine 2-amino-anthracene (2-AA) is an aromatic hydrocarbon employed in manufacturing of chemicals, dyes, inks, and as curing agents in epoxy resins and polyurethanes. 2-AA has been detected in tobacco smoke and cooked foods. The modulation of the toxic effects of 2-AA on the liver by apoptosis was investigated on twenty four post-weaning 3–4 week old F-344 male rats exposed to 0 mg/kg-diet (control), 50 mg/kg-diet (LD), 75 mg/kg-diet (MD) and 100 mg/kg-diet (HD) 2-AA for 14 (2WK) and 28 days (4WK). Analysis of total mRNA extracts from liver for apoptosis-related gene expression changes in AEN, BAX, CASP3, JUN, MDM2, P53, and GAPDH genes by qRT-PCR was coupled with liver tissue histology (H&E staining), TUNEL (Terminal deoxynucleotidyl transferase DUTP nick end-labeling) assay and Caspase (Caspase Glo assay) activity. Dose-related histological changes in liver cell architecture were observed at the highest doses in both 2WK and 4WK exposures. Dose-related increases in TUNEL positive staining were also observed. Caspase3 assays showed dose-dependent increases (2WK) but suppression in LD and MD (4WK) rat livers and an increase in HD rats. Dose-related increases in expression were observed in all genes measured.

### 310 Glutathione Levels and Susceptibility to Chemically-Induced Injury in Human Prostate Cancer Cells

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More aggressive or invasive prostate cancer cells (PCCs) are often resistant to chemotherapy and have poor prognosis. Differences exist in redox status and mitochondrial metabolism that may help explain this phenomenon. We compared two immortalized human PCC lines, PC-3 cells (more aggressive) and LNCaP cells (less aggressive), with regard to cellular glutathione (GSH) levels and susceptibility to oxidants (tetr-butyl hydroperoxide [tBuH] or methyl vinyl ketone [MVK]) or GSH depleters (diethyl maleate [DEM] or diame). We tested the hypothesis that cells resistant to less aggressive PCCs, more aggressive PCCs exhibit higher GSH concentrations and are relatively resistant to cytotoxicity from exposure to various oxidants. PC-3 cells exhibited 4.2-fold higher GSH concentration than LNCaP cells. PC-3 cells only exhibited lower lactate dehydrogenase (LDH) release after toxicant exposures than LNCaP cells at some time points but only LNCaP cells underwent diamide-induced apoptosis. Expression of several proteins involved in stress response and regulation of cell growth and proliferation were measured to determine the possible role of altered levels of certain signaling molecules with differential redox state and susceptibility to toxicants. PC-3 cells exhibited higher levels of Bax and caspase-8 cleavage product and lower levels of Bel-2 than LNCaP cells. PC-3 cells exhibited with higher susceptibility to Fas-induced apoptosis than LNCaP cells exhibited higher expression of FasR than PC-3 cells. Consistent with a compensatory response to lower GSH concentrations and generally greater susceptibility to oxidative stress, LNCaP cells exhibited higher levels of GADD153, p53, Hsp27, Cyclin A, SOD2, Trx2, and mitochondrial GSH transporters than PC-3 cells. Thus, significant differences in redox status and expression of proteins involved in apoptosis and stress response may contribute to PCC aggressiveness.

### 311 Coupling of PARP-1 and Store-Operated Calcium Entry Is Independent of PARB Activity in TGHQ-Induced Cell Death

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Many pathological conditions, including renal disease, are associated with oxidative stress. 2,3,5-tri(Glutathion-yl)hydroquinone (TGHQ), a potent nephrotoxic metabolite of benzene and HQ, generates reactive oxygen species (ROS). ROS generation results in damage to cells, including DNA strand breaks, which activate DNA repair enzymes such as poly(ADP-ribose) polymerase (PARP)-1. Under robust oxidative damage, PARP-1 is hyper-activated, leading to increases in intracellular calcium concentrations (Ca2+) as well as NAD+ and ATP depletion, and ultimately cell death. We are determining the relationship between Ca2+ and PARP-1 during cell death of human renal proximal tubule epithelial cells (HK-2). We have shown that extracellular Ca2+ is responsible for coupling PARP-1 activation to increases in Ca2+ during TGHQ-induced cell death. PARP-1 inhibition attenuates Ca2+ increases induced by TGHQ, and treatment with 2-APB, a store-operated Ca2+ channel (SOC) inhibitor, restored levels of NAD+, with simultaneous de-
creases in PAR protein-ribosylation. Since SOC activation has a direct effect on PARP-1 activity, and PARP-1 inhibition attenuates increases in iCa²⁺, the results show that PARP-1 and SOCs are coupled in TGHQ-induced cell death. We further elucidated the relationship between SOC activation and PARP-1 downstream of PARP-1 activity. Poly(ADP-ribose) glycohydrolase (PARG), which catalyzes the degradation of PARs to yield free ADP-ribose (ADPR), is known to activate SOCs. siRNA knockdown of PARG partially increases PAR ribosylation, but does not restore cell viability in the presence of TGHQ, indicating that free ADPR is not responsible for SOC activation in HK-2 cells. Overall, we show that PARP-1 and Ca²⁺ are coupled through SOC entry, and that the relationship may involve alternative PAR metabolic pathways. We are further investigating the connection between SOCs and PAR metabolism, and other potential mechanism(s) by which PARP-1 and Ca²⁺ interact to contribute to cell death.

**312 Flavonoids Kaempferol but Not Morin Showed Toxic Effects to HepG2 Cell Line**

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**INTRODUCTION:** Kaempferol and Morin are polyphenolic compounds belonging to a class of chemicals called flavonoids. Both substances have very similar structures, which differ only in the presence of an extra hydroxyl substituent in Morin. Although flavonoids are widely known due to their anti-oxidant and anti-inflammatory properties, toxic effects were already shown in literature. Therefore, this study was aimed to assess the effects of Morin and Kaempferol in HepG2 cells to check for induction of cell death evidences and to verify if the structural difference influences on toxic effects. METHODS: HepG2 cells were incubated at 37°C in an atmosphere containing 5% CO2 and 96% relative humidity for 24 hours before treatment and the flavonoids were tested in concentrations ranging from 1μM to 100μM for 24, 48 and 72 hours. Induction of cell death was evaluated by phosphatidylserine exposure at the outer cell membrane using Annexin V and propidium iodide (PI), followed by the assessment of nuclear fragmentation using Hoechst 33342 fluorescent dye. RESULTS: Kaempferol caused toxic effects in HepG2, since at the highest concentration and all times it increased significantly the phosphatidylserine exposure. Moreover, it showed significant increase in nuclear fragmentation at 50 and 100μM in 24, 48 and 72h. On the other hand, Morin did not show any toxic effects in both experiments at same conditions. CONCLUSIONS: Kaempferol showed a potential cytotoxic effect in HepG2 cells at the highest concentrations tested. The cell death observed may occur by apoptotic pathway, since the increase in nuclear fragmentation and in phosphatidylserine exposure are evidences of this mechanism. However, Morin did not present any cytotoxic effects at same conditions. These results show the importance to do a structure-activity analysis when performing the risk assessment for flavonoids uptake. Acknowledgments: CNPq - Brazil

**312a Diesel Exhaust Particle Exposure Causes Endothelial Cells Apoptosis via Autophagy Pathway**

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It is well known that inhaled diesel exhaust particles (DEP) injure human endothelial cells. To elucidate the mechanism, we used an in vitro monolayer HUVEC culture model system. 25000 HUVECs were plated in the well of 24-well dishes overnight. Automobile DEP collected from Japan (courtesy of Dr. Sagai, Tokyo) were sonicated in 0.05% Tween80 with PBS for 1 min, then added immediately at 25 μg/mL and 50 μg/mL medium-diluted DEP solution to the dishes. The Cell Titer 96 Aqueous One Solution Assay (Promega) was applied to detect viable cells after at 1, 2, 4, 8, 16, and 24 h after the treatments. We found the cell viability decreased in both time and dose dependent manner, and only 40% and 20% cell survive respectively at 24 h of 25 and 50 μg/mL DEP. For more detail in sample of 50 μg/mL DEP, TUNEL method was used and demonstrated that those 80% dead cells were killed with apoptosis induction. In order to determine by what mechanism that DEP exposure results in apoptosis, the treated cells were harvested for immunofluorescence microscopy and immunoblotting. Interestingly, autophagy markers LC3-II and Arg1 appeared at 2 h and disappeared at 8 h after exposure to DEP. Their expression both reached the maximal level at 4 h. Furthermore, the up-regulated expression of caspase3, caspase7, caspase9 were confirmed at various time point as well to indicate DEP induce apoptosis in HUVECs. And, ELISA analysis suggested that caspase 3 and caspase 7 expressions increased in a time dependent manner from 8 h to 24 h, while caspase 9 was initiated to be up-regulated from 16 h. In summary, the upregulation of LC3-II and Arg1 unveiled that DEP might induce HUVEC apoptosis via autophagy pathway.

**312b Presence of EndoG in Apoptotic Nuclei during Cisplatin Kidney Injury: A Sign of What?**

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Endonuclease G (EndoG, EG) is a mitochondrial endonuclease that translocates to nucleus during apoptosis. However, it is unknown whether EG directly causes apoptotic DNA fragmentation and cell death or it is anti-apoptotic. Unlike other apoptotic endonucleases, EG is the only endonuclease found in nucleus during apoptosis, has RNase activity, and induces inactive truncated isoform of another endonuclease, DNase I (DI). We hypothesized that the role of EG in apoptosis depends on the presence of DI in the cell, and used cisplatin kidney injury in vitro and in vivo models to test this. Our pilot observations were that although EG sometimes was present in TUNEL-positive nuclei of cisplatin-treated kidneys, overall it did not coincide with TUNEL. Unlike other endonucleases including DI, EG did not colocalize with DNase activity measured by DNase activity probe in pyknotic nuclei in the cultured kidney tubular epithelial NRK-52E cells. EG overexpression in DI-positive NRK-52E cells showed EG was not cytotoxic but instead protective against cisplatin induced cell death. Further study showed that EG was induced but was not cytotoxic in cisplatin kidney injury in vivo. After injection of cisplatin (20 mg/kg), no protection in EG null mice versus wild-type mice was observed by BUN, SCr or histology. EG induction by cisplatin in WT mice was associated with the decrease of native DI expression and appearance of inactive truncated DI. In conclusion, this study showed that EG acts as a proapoptotic enzyme in the absence of DI and it is anti-apoptotic in the presence of DI in kidney cells and the kidney during cisplatin injury.

**312c Neuroprotective Effects of Natural Compounds against Dopamine Toxicity**


Natural dietary supplements are being hailed as age defying and may, in some cases, present a sustainable medical solution for people around the world. Within the next few decades the aged population in the U.S. will be greater than ever before, increasing the demand for cost-effective medicine for the elderly. One of the major health problems in the elderly are motor deficits, caused, in part, by a loss of dopaminergic neurons. Oxidative stress is known to play a part in this neuronal loss. Resveratrol, a natural compound found in grapes and wild blueberries, has antioxidant, anticancer, and anti-inflammatory properties. Our lab has shown that resveratrol protects dopaminergic-like cells (SH-SY5Y) against oxidative stress produced by exposure to dopamine, as seen by cell viability assays (CellTiter-Glo® and Dead protease fluorescence assay). Interestingly, RES activates the MAP kinases ERK1/2 and ERK5; as seen using western blot analysis. Following inhibition of the ERK1/2 or ERK5 pathways, the neuroprotection afforded by RES is lost. In order to test if apoptotic pathways are also involved in RES-mediated neuroprotection, we examined the expression of Bcl-2, an anti-apoptotic protein, following dopamine exposure. Following dopamine-mediated apoptosis, RES treatment abolished Bcl-2 expression. This suggests that RES can mediate the neuroprotective effects of RES against dopamine exposure.
Oxidative stress plays crucial roles in exerting a variety of damages upon arsenic exposure, and NF-E2-related factor 2 (Nrf2) is a crucial transcriptional regulator protecting cells and tissues from oxidative injury. Here, we tested the antagonistic effects of tert-butylhydroquinone (tBHQ), a well-known synthetic Nrf2 inducer, on arsenic-induced oxidative injuries and apoptosis using cultured human HaCaT cell line. Cells were exposed to sodium arsenite (25 μM) with or without tBHQ (50μM, 12 h) pretreatment for 24 h, and cell proliferation activity, oxidative injury, apoptosis, as well as expression of relevant genes and proteins were examined. Arsenic-induced cytotoxicity, the enhancement of reactive oxygen species generation and lipid peroxidation, and the impairment of anti-oxidative enzymes activity, were all suppressed by tBHQ administration. Arsenic was also found to induce phosphorylation externalization, disrupt the mitochondrial membrane potential (Δψm), release cytochrome c into the cytosol, and trigger the cleavage of caspase-3, typical characteristics of mitochondria-dependent apoptosis. tBHQ co-treatment effectively rescued the cells from arsenic induced apoptosis and related alteration of mitochondria. tBHQ pretreatment resulted in a significant accumulation of nuclear fraction of Nrf2 proteins (155% of control), and Nrf2-regulated downstream genes, heme oxygenase-1 (HO-1) and NADPH:quinone oxidoreductase 1 (NQO1) mRNA expressions were overexpressed. In addition, tBHQ released anti-apoptotic Bcl-2 protein (130% of control), increased Bcl-2:Bax heterodimers and reduced cellular apoptosis. Thus arsenic induces cell apoptosis through the mitochondrial pathway, and the protection of tBHQ appears to include both Nrf2 anti-oxidation and Bcl-2 anti-apoptosis in human keratinocytes.
analysis. Unmetabolized FB1, accumulated in kidney in a dose-related manner at concentrations of up to 10 nmol/g tissue. Its concentrations in liver were 12 to 20-fold lower. In contrast, more HFB1 (1.8 nmol/g) was found in liver than in kidneys (0.3 nmol/g). Low levels of NAFB1 and N-acylated HFB1 species (NAHFB1) were found in both tissues. Metabolites having longer fatty acyl chains predominated in liver whereas those with shorter chains were prevalent in kidney; the different chain lengths likely reflecting differences in tissue-specific ceramide synthase isoforms. Metabolite concentrations decreased in the order: NAHFB1, in liver > NAFB1, in liver > NAFB1, in kidney. Recoveries (% of dose) of total FB1 species from the tissues were < 1%. Recovery of HFB1 species was more variable ranging from about 0.1% in kidney to about 2% in liver. Except for FB1, in kidney, where unmetabolized FB1 was predominant, recoveries (µmol basis) of parent and metabolites from the tissues were similar. The results show that FB1 is metabolized by ceramide synthases in vivo to N-acylated ceramide-like compounds. Additional studies are needed to determine the toxicological significance of the NAHB1 and NAHFB1, metabolites.

Presence of Aflatoxins and Fumonisins in Nixtamalized Masa from Mills in the Metropolitan Area of Monterrey, México

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Aflatoxins (AFs) and fumonisins (FBs) are toxic metabolites produced by Aspergillus and Fusarium species that frequently contaminate maize, generating a public health risk due to their hepatotoxic and carcinogenic effects. Traditional nixtamalization (treatment of maize with calcium hydroxide) is partially effective in reducing these toxins but under certain circumstances the toxins can prevail in the masa. The aim of this study was to determine the concentration of AFs and FBs in samples obtained from corn mills in the metropolitan area of Monterrey, México. In total 62 samples of masa were dried, ground and sieved before methanolic extraction of mycotoxins. Samples were analyzed by competitive ELISA (AgraQuant®) for detection of total AFs and FBs. In the results, AFs values ranged from 0 to 22.6 µg/kg, while FBs values ranged from 0 to 16.68 mg/kg. Results showed that some samples had AFs values above the permissible limit (12 µg/kg) according to the official regulation in México (NOM-247-SSA1-2008), while other samples had FBs values exceeding the permissible international limits (4 mg/kg), since México does not have specific limits for FBs. Therefore, we concluded that consumption of nixtamalized masa from some of the mills included in this study may represent a risk for human health.

The Effect of Aflatoxin-B1 on Red Drum (Sciaenops ocellatus) and Assessment of Dietary Supplementation of NovaSil for the Prevention of Aflatoxicosis

K. Zychowski1, A. Rodrigues Hoffmann1, H. J. Ly1, C. Pohlenz1, A. Buentello1, 2, A. A. Romoser1, D. Gatlin1 and T. D. Phillips1. 1Schillinger Genetics, East Texas. AFB1-induced histopathological changes in the liver and decreased Proliferating Cell Nuclear Antigen (PCNA) staining. Importantly, NS supplementation improved overall health of AFB1-exposed red drum.

Ginkgo biloba has been used for many thousand years as a traditional herbal remedy and its extract has been consumed for many decades as a dietary supplement. Ginkgo biloba leaf extract is a complex mixture with many constituents, including flavonol glycosides and terpene lactones. The National Toxicology Program 2-year bioassays found that Ginkgo biloba leaf extract targets liver, thyroid gland, and nose of rodents. However, the mechanism of Ginkgo biloba leaf extract-associated carcinogenicity remains unclear. In the current study, the in vitro genotoxicity of Ginkgo biloba leaf extract and its eight constituents were evaluated using the mouse lymphoma assay and the Comet assay, and the underlying mechanisms of Ginkgo biloba leaf extract-associated genotoxicity were explored. Ginkgo biloba leaf extract, quercetin, and kaempferol resulted in a dose-dependent increase in the mutant frequency and DNA double-strand breaks (DSBs). Western blot analysis confirmed that both quercetin and kaempferol activated DNA damage signaling pathway with increased expressions of γ-H2AX and phosphorylated Chk2 and Chk1. In addition, Ginkgo biloba leaf extract produced reactive oxygen species and decreased glutathione levels in L5178Y cells. Loss of heterozygosity analysis indicated that Ginkgo biloba leaf extract, quercetin, and kaempferol treatments resulted in extensive chromosomal damage. These results suggest that Ginkgo biloba leaf extract and its two constituents, quercetin and kaempferol, are mutagenic to the mouse L5178Y cells with induction of DSBs. Quercetin and kaempferol may be responsible for Ginkgo biloba leaf extract-induced genotoxicity. More studies on the mechanism of genotoxicity and carcinogenicity of Ginkgo biloba leaf extract warrant for its risk assessment.
The majority of samples (both foodstuffs and wrappers) analysed proved negative. However, all batches of industrially prepared hamburgers (but not those obtained from a hamburger restaurant) as well as pepper salami significantly induced luciferase activity in the test system, indicating the presence of xenobiotics, with estradiol equivalents of these products ranging from 0.2 to 44 pg/g. All three products contained soy-based ingredients which apparently accounted for, or at least contributed to, their high estrogenic activity, since no signal was observed with extracts of the packaging material, while two different soy sauces tested yielded an intense signal (28 and 394 pg/ml estradiol equivalent). These findings imply that by and large chemicals arising in the processing or packaging of foodstuffs in Finland constitute an insignificant source of xenobiotics to consumers. However, soy-derived ingredients in certain food items might render the entire products highly estrogenic. The estrogenic activity of soy is attributed to isoflavones whose health effects – though widely considered beneficial – are controversial. As hamburgers are a popular type of food among children, our findings are noteworthy and possibly of concern.

### 324 Genotoxicity Testing of Genipin, a Precursor in the Derivation of the Natural Food Colorant Gardenia Blue


Terrorism Risk Assessment (CTRA) Desktop Consequence Calculator, a tool developed by CSAC that incorporates existing models and data inputs from the 2012 Terrorism Risk Assessment (CTRA desktop tool) methodologies used to examine food process systems and provides examples of potential public health consequences that could result from chemical food contamination. The assessments demonstrate the wide range of hazards posed by varying the chemical, varying the contaminated food product, and, in some cases, varying the point of contamination in a food processing system.

### 326 Inactivation of Pathogens on the Surfaces of Sterile Swabs, Fruits, and Vegetables Using Sanitizing Substances Produced by Radiant Catalytic Ionization


Foodborne illness outbreaks linked to fresh products are becoming more frequent and widespread. The United States Department of Agriculture has estimated the costs associated with foodborne illnesses to be between $2.3 billion and $4.6 billion a year. The areas impacted include fruits and vegetables, meats, seafood, poultry, baking, canning, and dairy industries. Reducing pathogens and additional microbially contaminated on food contact surfaces will improve the quality and shelf life of many food products. New sanitizing technologies have emerged in recent years and are being widely used in a multitude of places to better decontaminate contact surfaces. Historically, both ozone-and peroxide-based technologies have been used as disinfectants in numerous applications. Radiant Catalytic Ionization (RCI) technology, particularly ozone, is thought to be safe to humans and the use of ozone is now considered to be an organic form of treatment to disinfect food contact surfaces. RCI technology has been widely accepted within the food processing industry during recent years. Ozone and hydrogen peroxide, generated by RCI, has countless applications for reducing the number of bacteria. This study has focused on the potential use of oxidative gases, including ozone and peroxide, generated by an RCI photocell for the inactivation of Escherichia coli, Listeria innocua, Pseudomonas aeruginosa, Salmonella typhimurium, and Staphylococcus aureus, introduced on the tips of sterile cotton swabs, and a number of fruits and vegetables which include apples, bananas, spinach, strawberries, oranges, and lettuce. Our results indicate a 99.9% killing of bacteria with a 90 minute exposure to RCI, demonstrating that the low level of oxidative gases produced by RCI has the potential to be an effective surface disinfectant tool for use in food processing.

### 327 Toxicological Evaluation of Oleic-Rich Algal Oil


The advancement of algae-based food ingredients is ideal for enriching the nutritional profile and functionality of food products. As a prospective new food ingredient, oleic-rich algal oil has been investigated for its potential to induce gene mutations as well as its toxicological potential in a subchronic study in rats. According to a plate incorporation and pre-incubation test by Salmonella typhimurium strains and an E. coli tester strain at concentrations of 3.1, 100, 316, 1000, 2500 and 5000 μg/plate, no toxic effects, precipitation, or biologically relevant increases in revertant colony numbers of the test item were noted in any of the tester strains used up to the highest dose group evaluated. Therefore, oleic-rich algal oil did not cause gene mutations by base pair changes or frameshifts in the genome of the strains used and was considered to be non-mutagenic in the bacterial reverse mutation assay. In a subchronic study in rats, CD IGS Sprague-Dawley rats consumed 2.5, 5, and 10% test substance in the diet for 90 days and were evaluated. There were no test substance-related changes in viability, behavior, clinical signs, body weight, organ weight, and relative organ weight values between control and treated animals. Decreases in high dose male food consumption throughout the study were not accompanied by decreases in body weight, body weight gain, and food efficiency, and were therefore considered non-adverse and toxicologically insignificant. There were no hematologic, clinical chemistry, or urinalysis, as well as macroscopic or microscopic observations, post-mortem that were attributed to the administration of oleic-rich algal oil. Based upon these toxicological endpoints, it was concluded that the no-observed-adverse-effect-level (NOAEL) for oleic-rich algal oil is 10% in the diet with no toxicity observed, a level calculated to provide a dietary intake of 5200 and 6419 mg/kg/day in males and females, respectively.

### 328 Predicting Public Health Consequences of Toxic Chemical Contamination of Food

J. Moser1,2 and R. Jablonski1.

To assess the risks (likelihood x consequence) associated with chemical contamination within the U.S. food supply system, it is essential to evaluate potential public health consequences resulting from the dissemination of toxic chemicals through food. The Chemical Security Analysis Center (CSAC), a knowledge analysis and management center under the Department of Homeland Security, developed several hazard and risk assessments related to chemical contamination of food. For the hazard assessments, 12 different food scenarios were evaluated. In each scenario, food was contaminated at a particular point in the manufacturing process; a total of 10 chemicals were analyzed. The CARVER plus Shock method was used as the primary tool to assess the hazard within a specific food processing system. For the risk assessments, 103 chemicals were individually evaluated as threats to human health through contamination and ingestion of 11 different foods. The Chemical Terrorism Risk Assessment (CTRA) Desktop Consequence Calculator, a tool developed by CSAC that incorporates existing models and data inputs from the 2012 CTRA into an easy-to-use platform, was used to determine consequences. This presentation compares the hazard assessment (CARVER plus Shock) and risk assessment (CTRA desktop tool) methodologies used to examine food process systems and provides examples of potential public health consequences that could result
TCT Catalase is an enzyme used for the production of gluconates, which are used as dietary supplements. The enzyme is produced from a recombinant strain of *Trichoderma reesei*. A battery of toxicology studies was conducted to investigate its potential to cause adverse effects in humans. All studies were conducted according to OECD Principles of Good Laboratory Practice and in compliance with OECD test guidelines. Catalase (from a different microbial host) was not an eye irritant and not toxic by oral gavage, with an oral LD50 in rats greater than 5000 mg/kg body weight (bw). In an *in vitro* cytogenetic test using human peripheral blood lymphocytes, TCT Catalase did not induce structural or numerical chromosomal aberrations in the presence or absence of metabolic activation (S-9 mix) up to the highest concentration tested (5000 μg total protein (TP)/ml). No mutagenic activity was noted in the bacterial reverse mutation (Ames) assay in the presence or absence of S-9 mix, when tested up to 5000 μg TP/plate. In a subchronic 90-day oral gavage study in CrI:CD(SD) rats, no biological or statistically significant differences were observed on in-life, neurobehavioral, clinical pathology (hematology, coagulation, clinical chemistry, urinalysis), or anatomic pathology (organ weights, histopathology) endpoints. The NOAEL was established as dietary supplements. The enzyme is produced from a recombinant strain of *Sceletium tortuosum* with a long history of traditional use, is marketed under the trade name Zembrin® as an ingredient for use in functional foods and dietary supplements. It is standardized to contain 0.35–0.45% total alkaloids (mesembrenone and mesembrenol as60%, and mesembrine <20%). A 14-day repeated oral toxicity study was conducted at 0, 250, 750, 2500, and 5000 mg/kg bw/d. A 90-day subchronic repeated oral toxicity study was conducted at 0, 100, 300, 450, and 600 mg/kg bw/d. Because *Sceletium tortuosum* has a long history of human use for relaxation, a functional observation battery (FOB), including spontaneous locomotor activity measured using LabMaster ActiMot light-beam frames system was employed. Several parameters such as: locomotion (time resting, moving, and hyperactive in seconds); rearing behavior (time in rearing, number of rearing); spatial parameters; and turning behavior were investigated in the final week of the study. No deaths or treatment-related adverse effects were observed in male or female CrI:W(BR) Wistar rats in the 14- or 90-day studies. In the 14-day study, the MTD and the NOAEL were concluded as 5000 mg/kg bw/d. The NOAEL from the 90-day study was determined to be 600 mg/kg bw/d, the highest dose tested. Toxicological Safety Assessment of a *Sceletium tortuosum* Extract T. Murbach, A. Clewell and J. R. Endres, AIBMR Life Sciences, Inc., Puyallup, WA. A standardized hydroethanolic extract of *Sceletium tortuosum*, a South African plant with a long history of traditional use, is marketed under the trade name Zembrin® as an ingredient for use in functional foods and dietary supplements. It is standardized to contain 0.35–0.45% total alkaloids (mesembrenone and mesembrenol ≥60%, and mesembrine <20%). A 14-day repeated oral toxicity study was conducted at 0, 250, 750, 2500, and 5000 mg/kg bw/d. A 90-day subchronic repeated oral toxicity study was conducted at 0, 100, 300, 450, and 600 mg/kg bw/d. Because *Sceletium tortuosum* has a long history of human use for relaxation, a functional observation battery (FOB), including spontaneous locomotor activity measured using LabMaster ActiMot light-beam frames system was employed. Several parameters such as: locomotion (time resting, moving, and hyperactive in seconds); rearing behavior (time in rearing, number of rearing); spatial parameters; and turning behavior were investigated in the final week of the study. No deaths or treatment-related adverse effects were observed in male or female CrI:W(BR) Wistar rats in the 14- or 90-day studies. In the 14-day study, the MTD and the NOAEL were concluded as 5000 mg/kg bw/d. The NOAEL from the 90-day study was determined to be 600 mg/kg bw/d, the highest dose tested. Oral Toxicity Assessment of siRNAs and Long Double-Stranded RNAs J. S. Petrick, J. H. Sherman, W. F. Hovden and S. L. Lemke, Toxicology, Monsanto Company, Saint Louis, MO. New crop traits harnessing RNA interference are being developed using agricultural biotechnology. For example, when expressed in corn plants, double stranded RNA (dsRNA) targeting corn rootworm vacuolar ATPase (vATPase) provides protection against corn rootworm. Although ingested RNA is not known to be toxic, we conducted oral proof of concept toxicity studies in CD-1 mice with dsRNA targeting the mouse ortholog of vATPase. Mice were gavaged with a 218 bp dsRNA or a pool of four 21-mer small interfering RNAs (siRNAs) in an acute oral toxicity study at 2000 mg/kg and in a 28 day oral toxicity study at 1, 10, and 100 mg/kg. Test substances did not affect in-life or necropsy endpoints in either study (e.g. body weight, food consumption, clinical observations, or gross pathology). There were no treatment-related effects on clinical chemistry, hematology, histopathology, or vATPase gene expression in selected tissues (qPCR) in the 28 day study. The NOAELs for both test materials in the acute and 28-day studies were 2000 mg/kg and 100 mg/kg, respectively, the highest doses tested. The proof of concept toxicity assessment for dietary double stranded RNAs presented herein support the conclusions that ingested RNA is not toxic to mammals at large multiples of anticipated human exposure and that toxicological studies with dietary RNAs will contribute little, if anything, to mammalian/human risk assessment. Nanostructured Synthetic Amorphous Silica—Absence of Disintegration in Intestinal Environment M. Maier1, P. Albers2, R. Retamal Marin3, F. Babick3 and M. Stintz3. 1Inorganic Materials, Evonik Industries AG, Hanau, Germany, 2Aqura GmbH, Hanau, Germany and 3Technical University, Dresden, Germany. Synthetic amorphous silica (SAS) is a nanostructured material formed by flame hydrolysis or precipitation. In commercial SAS products, basic structural elements (fused nanosized primary particles) are submicron aggregates that themselves form micrometer (or even larger) agglomerates. As SAS is employed in processed food, e.g. as a free-flow agent, we aimed to investigate potential structural changes following oral uptake. In a previous study no significant changes in the structure and size distribution of SAS during heating in water (e.g. food processing) and in acid environment (pH of gastric juice) were observed. We now investigated potential structural changes of SAS during the intestinal passage. Intestinal milieu is quite different from gastric conditions with respect to pH as well as the presence of natural solubilizers and residence time is significantly higher. Food grade precipitated and pyrogenic SAS were studied in an in vitro model. FeSIFS is a fed-state simulated intestinal fluid (acetate buffer, pH 5.0) containing the natural solubilizers sodium taurocholate (bile salt) and phosphatidylcholine (lecithine) in physiological amounts. Following suspension in FeSIFS, SAS was slightly agitated for up to 48 hours (37°C). To check for the presence or absence of changes in particle size distribution and potential surface degradation samples were analysed by laser diffraction (volume weighted size distribution) and transmission electron microscopy. Simulated intestinal conditions did not lead to an increase of fine, nanosized SAS particle fractions. There was no indication of chemical reactions or dissolution processes at the surface of SAS particles. In contrast, partial agglomeration of dispersed SAS aggregates was observed shifting the size distribution to its coarse end with large flocculates of several hundreds of micrometers. In conclusion, SAS was shown not to experience additional dispersion or any other type of degradation during the simulated intestinal passage.
In the treatment of prostate disorders, Q, similarly to Tams, induces vasorelaxation, thereby increasing the risk for orthostatic hypotension. This overlapping profile prompted us to investigate the interaction of Q with Tams. Since Q is extensively metabolized, the effects of the metabolites Q-3-glucuronide and 4'O-methyl-Q on Tams effects are also included. We have determined the potentiating effects Q and metabolites on Tams-induced vasorelaxation. This was tested on rat mesenteric arteries constricted by the α1-adrenergic agonist phenylephrine. Tams (0.1 mM) decreased phenylephrine-induced vasoconstriction 16-fold. Q (5, 10 and 20 μM) caused a substantial concentration-dependent (of 5, 7 and 9-fold respectively) decrease of phenylephrine-induced vasoconstriction, while Q-3-glucuronide (10 μM) and 4'O-methyl-Q (10 μM) only slightly decreased this activity (3 and 2-fold). The combination of Tams with Q or Q metabolites proved to be very potent. Q, Q-3-glucuronide and 4'O-methyl-Q synergistically increased the Tams effects to 275, 57 and 169-fold respectively. Our results show that there is a drastic pharmacodynamic interaction between the drug Tams and the supplement Q. Tams effects are synergistically amplified by Q, 4'O-methyl-Q or Q-3-glucuronide. The interaction of Q and Tams with potentially serious consequences draws attention to drug-supplement interactions that have been neglected thus far.

The Unites Arab Emirates is currently evaluating external sources of forage, including perennial ryegrass (Lolium perenne) straw, for its rapidly growing livestock industry. Unfortunately, perennial ryegrass may be infected with the endophyte Neotyphodium lolii which produces lolitre B, a toxic alkaloid responsible for the neurological syndrome ryegrass staggers. Thus, a range-finding study was conducted in 24 female camels fed four doses (0, 1111, 1478 and 2273 ppb) of lolitre B-containing perennial ryegrass straw over 56 days to establish a threshold of toxicity in camels so that perennial ryegrass straw can be safely fed as part of their dietary ration. Morphometric and blood chemistry values were evaluated. Body weight was adversely affected by consumption of lolitre B-camels fed a diet free of lolitre B gained more (7.4%) than the other three groups (-2.5%, 0.5% and -8.8% for 1111 ppb, 1478 ppb and 2273 ppb, respectively (p < 0.001)). Blood urea nitrogen increased significantly in the three treatment groups (p < 0.001), which is indicative of kidney damage. Brain edema, degenerative renal and hepatic lesions as well as Purkinje cell vacuolar degeneration were observed in two necropsied camels receiving the highest dose of lolitre B. Neurological deficits were assessed by videotape and scored using an established scale for ryegrass staggers. Mean date of onset of ataxia was 35.8, 43.7 and 19.8 days (1111, 1478 and 2273 ppb, respectively). AF99 V. aste was also seen in affected camels in which the lower lip was amyotonic and fell ninety degrees perpendicular to the lower jaw, similar to a partial facial nerve paralysis; this has not been reported in other ryegrass toxicosis studies. Based on these observations, we recommend that endophyte-infected perennial ryegrass straw be fed at 500 ppb or lower lolitre B to avoid clinical disease in camels.

Steviol glycoside sweeteners were permitted for use in the United States by the FDA beginning in late 2008. Prior to that, they were only allowed as dietary supplements, and most stevia products before 2008 were not highly purified (≥ 95% steviol glycosides). Steviol glycoside sweeteners are extracted from the plant Stevia rebaudiana (Bertoni), a member of the Asteraceae (Compositae) family. A number of member plants from this family can induce hypersensitivity reactions via multiple routes of exposure: dermal, inhalation, and ingestion. Well-known members of this plant family include the common herbs, widely consumed foods, and the allergens ragweed, goldenrod, and Chrysanthemum. Based on this common taxonomy, food allergy warnings for stevia are given in some popular food information sources. Not surprisingly, many non-scientific media reports and resources allege the potential for stevia allergy. To determine if such allergy warnings are warranted on stevia products, a comprehensive literature search was conducted to identify all available data related to consumption of stevia extracts or highly purified steviol.
glycoside products and allergic responses. Based on the peer-reviewed literature, hypersensitivity to stevia is rare, and the few cases documented in the literature were reported prior to the introduction of high-purity products to the market, which was considered when global regulatory authorities affirmed the safety of steviol glycosides. Additionally, neither stevia manufacturers nor food allergy networks have reported significant numbers of any adverse events related to ingestion of this sweetener. Diligently maintained manufacturer post market surveillance data indicate a normalized (to sales) incidence rate of <1.1 incident/10 million servings. There have been no reports in the scientific literature since 2008 of stevia-related allergy. Therefore, there is little evidence to support warning statements to consumers about allergy to highly purified stevia extracts.

338 A 28-Day Gavage Toxicity Study in Fischer 344 Rats with 3-Methylfurans
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1Toxicology Research Division, Health Canada, Ottawa, ON, Canada and 2Chemical Health Hazard Assessment Division, Health Canada, Ottawa, ON, Canada.

Sponsor: R. Meehan.

3-Methylfuran is produced in foods during food processing and preservation techniques that involve heat treatment such as cooking, jarring, canning, and pasteurization. Currently there are no studies available on the toxicity of 3-methylfuran. We conducted a 28-day gavage toxicity study (7 days per week) using doses of 0.0, 0.1, 0.3, 1.5, 3.0, 6.0, 12.0 and 25.0 mg/kg bw/day to in order to determine the dose range needed to establish a No Observed Adverse Effect Level (NOAEL) and to better characterize non-neoplastic effects including those affecting hematology, thyroid, clinical biochemistry, gross morphology and histopathology. Histological changes of the liver were noted in all treated animals and gross changes were noted beginning at 3.0 mg/kg bw/kg. Alterations in the activity of serum enzymes indicative of effects on the liver were observed, including increases in levels of ALT and ALP at the highest dose. There was a significant increase in serum thyroxine (T4) and triiodothyronine (T3) which was not accompanied by histological changes in the thyroid. For the most part, statistically significant changes were seen only at the highest dose for hematology and at the two highest doses for clinical chemistry parameters. In contrast, mild histological lesions in the liver were observed even at the lowest dose of 0.1 mg/kg bw/day.

339 Safety, Efficacy, and Toxicological Evaluation of a Novel, Patented Antidiabetic Extract of Trigonella foenum-graecum Seed Extract (CR00010810)
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1Dr. Herbos LLC, Concord, CA and 2ReD, Cepham Inc., Picataway, NJ.

This study was conducted to examine the safety and efficacy of a novel, patented Trigonella foenum-graecum seed extract (Fenugreek, CR00010810). The project also evaluated the safety of CR00010810 in a variety of toxicological assays including acute oral toxicity studies in male and female Sprague-Dawley rats and Swiss albino mice, as well as repeated dose 28-day oral toxicity study in rats. Four groups of male and female rats and mice (n=5) were orally administered a single dose of either 0, 500, 1000 or 2000 mg/kg body weight. Rats and mice were euthanized and gross necropsy examinations were carried out on the 15th day. No observable toxic effects were observed in rats or mice. In another set of experiment, Sprague-Dawley rats were orally administered either 0, 250, 500 or 1000 mg/kg/day over a period of 28 consecutive days. No toxicological or clinical abnormalities were observed. The anti-diabetic potential of CR00010810 (150 and 450 mg/kg p.o.) was chronically evaluated for 30 days, once daily orally for first 20 days and later administered twice for the last 10 days, in type 2 diabetic rat model (streptozotocin 50 mg/kg i.p.). CR00010810 (450 mg/kg) treatment in streptozotocin-induced diabetic rats exhibited significant hypoglycemic activity (approximately 31.5%) as compared to insulin (48.2% with 1 U/kg i.p.). Further studies in animals and humans are in progress to unveil the broad spectrum safety and anti-diabetic potential of this novel fenugreek extract CR00010810.

340 Effect of Omega-3 Fatty Acid Oxidation Products on the Cellular and Mitochondrial Toxicity of BDE 47
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High levels of the toxic PBDE flame retardant 2,2’,4,4’-tetrabromodiphenyl ether (BDE 47) detected in fish species such as salmon have raised concern over the safety of salmon consumption. However, salmon are also rich in omega-3 polyunsaturated fatty acids, specifically, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are potent antioxidants. In particular, recent studies report the oxidation products of omega-3 fatty acids as critical mediators of the beneficial cellular antioxidant responses associated with dietary intake of omega-3s. In the current study, we used an in vitro approach to test the hypothesis that oxidized omega-3s can ameliorate the cellular and mitochondrial toxicity of BDE 47. HepG2 cells were treated with a mixture of oxidized EPA and DHA (oxEPA/oxDHA) to induce a maximal cellular antioxidant response as evidenced by glutathione (GSH) biosynthesis, followed by exposure to BDE 47. Significant induction of GSH by the oxEPA/oxDHA mixture was associated with a small but significant protective effect against BDE 47-induced loss of cell viability, and with significant protection against loss of mitochondrial membrane potential. Examination of mitochondrial respiration in permeabilized cells revealed that oxEPA/oxDHA pretreatment partially but significantly protected against loss in both State 3 mitochondrial respiration and maximum respiratory capacity (uncoupled respiration). Pretreatment with oxEPA/oxDHA also tended to protect against loss of proton leak respiration (State 4), and of flux capacity through complexes II and IV. Our results support a chemoprotective effect of dietary antioxidants against co-consumed persistent contaminants. This study also provides some biochemical insight into assessing the safety of, and risks associated with, seafood consumption.

341 Safety of Carrageenan in Infant Formula: A 4-Week Toxicity Study in Preweaning Piglets
1TOXpertise, LLC, Princeton, NJ, 2Developmental & Reproductive Toxicology, MPI Research, Mattawan, MI, 3Pathology, MPI Research, Mattawan, MI, 4Abbott Nutrition, Columbus, OH and 5Celtic Colloids, Topsham, ME.

Carrageenan (CGN) is a sulfated galactose polymer extracted from seaweed widely used in the food industry. The potential effects of κ-/CGN (Mw 700 kDa, viscosity 80cps) fed to preweaning Yorkshire crossbred piglets (6/sex/group) 2 days after birth for 28 days were evaluated at concentrations of 0, 300, 1000 & 2250 ppm in infant formula, offered 6x/day, at a volume of 500 mL/kg/day. Clinical observations, body weight & food consumption were recorded daily on each animal. Food efficiency & compound consumption were calculated. Blood was collected for hematology, clinical chemistry & coagulation parameters. Urinalysis was performed via cystocentesis. At scheduled necropsy, piglets were evaluated macroscopically and a complete list of tissues was examined microscopically. There were no deaths from CGN. The CGN formulations were well tolerated by the piglets with clinical findings, body weight & food consumption comparable among all groups. No effects seen on hematology, clinical chemistry & coagulation due to CGN, compared to control animals. Glucosuria was noted in 1 male & 3 female piglets at 2250 ppm on day 29, but blood glucose & renal histopathology were normal. Thus, glucosuria was not considered toxicologically meaningful. No adverse tissue histopathology was noted related to CGN treatment. Toluidine blue staining of gastrointestinal tissues for mast cell assessment showed no differences due to CGN. Assessment of Periodic Acid-Schiff staining of the jejunal goblet cell showed no differences due to CGN. In conclusion, the results of this study verified that dietary exposure to CGN at concentrations of 2250 ppm in infant formula (30-446 mg/kg/day) produced no adverse or treatment-related changes in the growth & development of preweaning piglets or any adverse changes to the parameters evaluated. Funded by FMC Corporation & the International Formula Council.

342 Safety of Carrageenan in Infant Formula: A 4-Week Study of the Potential Immune System Effects in Preweaning Piglets
1TOXpertise, LLC, Princeton, NJ, 2Developmental & Reproductive Toxicology, MPI Research, Mattawan, MI, 3Pathology, MPI Research, Mattawan, MI, 4Abbott Nutrition, Columbus, OH and 5Celtic Colloids, Topsham, ME.

Exposure to κ-/carrageenan (Mw 700 kDa, η 80cps) at concentrations of 0, 100, 300 & 2250 ppm in infant formula was evaluated for potential effects on the immune system in preweaning Yorkshire crossbred piglets, using validated methods
for immunophenotyping, circulating cytokines & immunohistochemistry (ICH) of the stomach, duodenum, jejunum, ileum proximal & distal colon, cecum, rectum. Piglets (6/sex/group) were offered infant formula 6 times/day at a volume of 500 mL/kg/day from 2 days of age for 28 days. Whole blood was collected from animals on days 14 & 19 & evaluated for peripheral blood leukocytes by flow cytometry. All cells types analyzed (lymphocytes, monocytes, B cells, helper T cells, cytotoxic T cells, mature T cells) did not show a biologically significant effect in relative cell percentage or absolute cell counts after carrageenan administration at 2250 ppm. No significant effects across gender or time interval (Day 14 or 19) were observed. Sandwich immunoassays were used for cytokine evaluation. Serum samples were evaluated for IL-6 & IL-8, and plasma samples were evaluated for IL-1p & TNF-α, using commercial, validated ELISA kits. There were no test article effects on levels of any of the cytokines. A validated ICH method was used to evaluate gastrointestinal tissue samples for IL-8 & TNF-α. H&E staining was used to complement the ICH. The results of the ICH evaluation were negative for all treated groups; less than the positive control (Dextran Sodium Sulfate (DSS), 3% w/v). The purpose of this IRB approved study was to validate recent findings in Guatemala showing a significant direct correlation between urinary FB1 and the SaIP/SoIP ratio in blood spots collected from 1240 women in 2011 and 2012 from three separate departments, Chimaltenango (low FB intake), Escuintla (low FB intake) and Jutiapa (high FB intake). Based on a survey of FB in corn conducted in May to October 2012, three different departments were selected for the validation study: Sacatepéquez, Chiquimula and Santa Rosa. The average total FB in the corn from the three departments, respectively, was 1.0 mg/kg (n=48), 4.6 mg/kg (n=53), and 3.8 mg/kg (n=42). Approximately 300 urine and blood spot samples were collected in March and April 2013 from the three departments. Corn samples (n=30) from the same locations and times were collected and analyzed for FB to confirm low and high contamination. The level of FB in corn collected from local markets was significantly higher in Chiquimula and Santa Rosa compared to Sacatepéquez. The urinary FB1, the SaIP/SoIP ratio, and SaIP/mg protein in blood spots were also significantly higher in Chiquimula and Santa Rosa compared to Sacatepéquez. This study confirms the findings of the earlier study. The results are consistent with the hypothesis that FB1 disrupts sphingolipid metabolism in humans consuming large amounts of FB contaminated corn. Supported by NIH grant # 1R4C HD067971-01.

### 343a Evaluation of Concentrations of Nickel, Lead, Cadmium, and Vanadium in Marine Species of the Market “La Nueva Viga.” Using ICP-MS


Toxic metals adversely affect people's health. In very small quantities, many of these metals are necessary to keep good health. However, in larger amounts, they become toxic. The toxicological and environmental studies have focused interest in the determination of toxic metals in foods, particularly fish in the marine environment. In this work cadmium, vanadium, lead, and nickel were analyzed in fish: “mojarra tilapia” and “sierra” sampled in the largest seafood market of Latin America “La Nueva Viga” located in Mexico City. The best sold fish species are the “mojarra tilapia”, “sierra”, shrimp, octopus, crab, sea bass and red snapper. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used, because it presents wide spectral coverage, good accuracy and precision, high sensitivity and selectivity. This technique permits the simultaneous detection of elements at low concentration and very small samples. The sample of “mojarra tilapia” and “sierra” was collected in 6 sectors of the market. Samples were dried, grinded and homogenized. The chemical treatment was performed using a Microwave Digestion System prior to the quantification. Analytical parameters were obtained with certified reference material (Dogfish Muscle DORM-2 and Lobster Hepatopancreas TORT-2 (limit of detection, limit of quantification, linear and working range, accuracy and precision). A significant amount of data is necessary for the method development was the selection of the analytic isotope and the internal standards. The mentioned elements were determined finding the following concentrations: “mojarra tilapia” muscle: Ni (0.2-1.2) mg/kg; Pb (0.03-0.12) mg/kg; Cd (0.19-0.22) mg/kg and V (0.043-0.048) mg/kg “sierra” muscle: Ni (0.02-0.05) mg/kg; Pb (0.03-0.16) mg/kg; Cd (0.20-0.22) mg/kg and V (0.010-0.012) mg/kg. The results were compared with national and international norms and all the values were below the maximum allowable limit.
434d β-Alanine: Absorption, Distribution, and Elimination Following Single or Repeated Administration to Rats

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β-alanine (BA) is a nutritional supplement used to enhance workout capacity and delay fatigue. To ensure the safety of BA when used as a dietary supplement, a complete understanding of the absorption, distribution and elimination (ADE) of BA and its metabolites is necessary. These studies assess the ADE of (14C)BA and its radiolabeled metabolites following single or daily oral administration of BA. A single, oral dose of 2-[14C]BA was given to groups of adult, male, Sprague-Dawley rats once, or following 28 days of daily oral gavage administration at 1.000 or 2,000 mg BA/kg body weight. No adverse effects of either single or daily administration of BA for 28 days occurred. No quantitative or qualitative differences in the ADE profiles between animals receiving [14C]BA once and animals that received 28 days of BA prior to receiving a single dose of [14C]BA. BA was absorbed into and disappeared rapidly (T1/2 < 4hr) from the plasma. Total radioactivity in the plasma persisted, however (T1/2 > 24 hours). Excretion in urine predominated (~40% of dose), followed by exhalation of [14C]O2 (26.7% of dose); 5.8% of the dose was excreted in the feces. The results of Quantitative Whole Body Autoradiography (QWBA) showed that BA and its radiolabeled metabolites were widely distributed. On average, only 6% of the administered dose remained in the body 168 hours following [14C]BA, uniformly distributed in skeletal muscle. In summary, there were no adverse effects caused by repeated, high-dose administration of BA. Excretion was predominantly in the urine, followed by exhaled [14C]O2, and feces, during the 168 hours following [14C]BA. Daily administration of BA for 28 days had no effect on the ADE of BA and its metabolites. Approximately 6% of the administered dose remained in the residual carcasses, on average, 168 hours following administration of the radiolabeled dose.

434e Oral Repeated 28-Day Study of Moniliformin in Sprague-Dawley According to OECD 407 Rats

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Moniliformin, a Fusarium mycotoxin commonly present in grains in Northern Europe was tested in an oral repeated 28 days study conducted according to OECD 407 guideline. We have shown earlier that a LD50 cut off value of moniliformin is 25 mg/kg bw in Sprague-Dawley rats. In this study five exposed (3, 6, 9, 12 and 15 mg/kg bw) and two satellite groups (12 and 15 mg/kg bw) were exposed 25 mf/kg bw in Sprague-Dawley rats. In this study five exposed (3, 6, 9, 12 and 15 mg/kg bw day) and two satellite groups (12 and 15 mg/kg bw day) were exposed for most oils were 1.5 - 10% (if tested). For some oils, adverse effects were observed at concentrations ranging from 12.5 - 25%. The highest dose used in most of the studies was 10% or 15%. NOAELs for most oils were 1.5 - 10% (if tested). For some oils, adverse effects were observed at the highest doses. In general, lipidosis and toxicity to the liver were observed at concentrations ranging from 12.5 - 25%. Toxicological effects that were oil-specific were identified using concentrations ≥ 5%. The results support use of 5 - 10% as the highest concentration, unless particular oil characteristics prompt concerns that use of such a dose would be expected to cause harm to the animal. Use of higher concentrations may cause nonspecific or secondary effects due to administration of large amounts of oil, which may confound interpretation and have no relevance for humans consuming oils at the levels used in food. The lack of reports of toxicity in humans of oils used in food supports the hypothesis that the maximum doses of oils that have been used historically in 13-week dietary studies in rats are sufficient for risk assessment purposes.

434g Safety Evaluation Methods for Supplemental Nutrients

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With increasing use of dietary supplements, including those containing nutrients, it is important to determine levels that may be safely consumed. Several regulatory and authoritative bodies have employed the Tolerable Upper Intake Level (UL) method for assessing their safety. For most nutrients, the UL applies to total intake from all sources rather than to supplemental sources. The Council for Responsible Nutrition (CRN) has developed safety evaluation methods to identify an Upper Level for Supplements (ULS). Using these methods, an UL is set if evaluation of the data shows risk. If no risk is identified, a Highest Observed Intake (HOI) can be established as the highest intake as reported within (a) study(ies) of acceptable quality. When appropriate data on supplemental intake are available, the UL may be determined by risk assessment directly (direct safety method). If such data are not available, an indirect/difference method may be used by determining the UL or HOI of a nutrient from all sources, identifying usual intakes from foods, and calculating the UL as a difference.
extensive the dataset, carries a large uncertainty. Application of the CRN methods allows for the derivation of an ULS based on a HOI value for nutrients with no established adverse effects (e.g. vitamin B12), whereas the decision by some authoritative bodies not to set an UL for these nutrients may not adequately communicate the abundance of data available, leading to a perception that there is insufficient evidence to evaluate safety. Additionally, the CRN methods only consider effects that represent a true hazard rather than nuisance effects (e.g. skin flushing associated with niacin). Moreover, these methods conservatively select human No-Observed-Adverse-Effect Levels that do not require adjustment by an uncertainty factor (e.g. vitamin D). The CRN methods are a valuable tool for consumers and their health care providers to make informed decisions about safe and appropriate supplementation levels.

343i Assessing Potential Interactions of “Active” Ingredients in Food
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Over the past few decades, many foods have come to contain “active ingredients”, often marketed to perform a functional role in maintenance of health or improvement in athletic and/or cognitive performance. Safety concerns about potential interactions of these active ingredients have been raised, a case in point being that of “energy” or “sport” drinks. Currently there is no consensus on methodology by which biological interactions of ingredients can be assessed. Also, there are generally few studies available specifically designed to assess interactions. We describe a paradigm, using “first principles” of pharmacokinetics, physiology, pharmacology, and toxicology, for assessing interactions of biologically active food ingredients. The method involves assessment of pharmacokinetics and physiological activity, and of the toxicological outcomes, of the ingredients individually, then using this information to predict potential for additive, synergistic, or antagonistic effects at doses encountered in food. As a case study, the potential for adverse effects from the interaction of common “active” ingredients in energy drinks was evaluated.

Caffeine is ubiquitously present in energy drinks. Other ingredients commonly found in these products include: guarana seed extract, d-glucuronolactone, taurine, L-carnitine, inositol, quercetin, and ginseng extract. Typically, caffeine and taurine are present at about 200 and 1000-2000 mg/container, respectively, while the concentrations of the other ingredients generally ranges from 25 to 200 mg/container. Following compilation of the available data for each ingredient, and application of the “first principles” analysis, we found little to no potential for interaction for all but caffeine and taurine. In fact, it is likely that taurine may ameliorate some of the cardiovascular and neurological stimulant properties of caffeine. The results of our analysis are consistent with previously published opinions with respect to the effects of caffeine, taurine and other ingredients present in energy drinks.

343j Assessment of Human Exposures to Fumonisin B1 in Ghana Using LC-MS/MS
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Humans can be exposed to fumonisin B1 (FB1) through ingestion of contaminated grain products. FB1 has been shown to co-occur with other mycotoxins including aflatoxin, and may act as a pro-carcinogen. Evaluating toxin exposures through the use of biological markers in vulnerable populations is essential for risk determination and the development of food safety strategies. An ultra-performance liquid chromatography/electrospray ionization tandem mass spectrometry method (UPLC/ESI-MS/MS) has been developed to detect FB1 in human urine following purification and concentration with a fumonistest immunoaffinity column. Cleanup conditions were optimized to obtain maximum analyte recovery and sensitivity. Validation was performed in the range of 2-200 µg/L based on expected urinary concentrations. LOD for the method was equal to 0.01 µg/L with an LOQ of 0.1 µg/L. A mean apparent recovery of 71.5% with an inter-day precision of 24.69% relative standard deviation (RSD) was achieved. In this study, 40 urine samples were collected from three Regions in Ghana (i.e., Central, Ashanti, and Greater Accra), and evaluated for the presence of FB1. Our results indicated that 16 out of 40 (40%) of the samples were positive for FB1 (37.5% in the Central, 40% in the Ashanti, and 50% in the Greater Accra Regions). These samples were previously shown to be positive for aflatoxin M1, indicating that Ghanaians exposed to aflatoxins may be co-exposed to fumonisins and that mitigation strategies for multiple mycotoxins are needed in high-risk populations (Research supported by NIH1R01MD005819-01).

344 Chronic Intrathecal Infusion/Sampling via a Surgically Implanted Access Port with Functional Observation Battery Evaluation in Cynomolgus Monkeys
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Intrathecal administration (IT) and sampling continues to be critical in non-clinical pharmaceutical development for drugs that must be delivered across the blood-brain barrier or to allow sampling of cerebral spinal fluid (CSF) to investigate pharmacokinetics. In alignment with the 3Rs (reduction, replacement, refinement) we have refined a method to allow long term intrathecal administration of candidate drugs and/or sampling of CSF. In addition, a modified function observation battery (FOB) often conducted as part of the ICH S7A safety pharmacology core battery, was developed for the evaluation of neurological changes in non-human primates. A cerebrospinal fluid catheter was inserted into the intrathecal space in the vertebral lamina (L3) and connected to a vascular access port. Implanted animals were first used for the evaluation of the cerebrospinal pharmacokinetics of a pharmaceutical, with CSF sampling of 10 timepoints over a period of 36 hours. Following the end of the first phase the animals were fitted with a jacket and tether system to evaluate the feasibility of continuous infusion for 28 days with PBS. Following the 28 day period, the animals were assessed using a FOB evaluation and euthanized for histopathological evaluation. No neurological abnormalities were observed and minimum to mild catheter-associated chronic granulomatous inflammation was associated with the catheter in the subarachnoid space at the injection/sampling site, next to the spinal cord and spinal nerve roots. Similar moderate catheter-associated chronic granulomatous inflammation was also noted along the catheter track beside the vertebral body and dorsal spinal process below the lumbar muscles. In conclusion, the port/catheters were patent for the duration of the experiments (over 100 days in total), greater than the duration of a chronic study. Therefore the improved method was deemed to be appropriate for long term toxicological, neurological and pharmacokinetic evaluations in Cynomolgus monkeys.

345 Successful Conduct of Continuous Intravenous Infusion Combined with Intrathecal Implantation and Administration in the Juvenile Monkey

Early childhood onset of neurological diseases present several challenges and often these diseases represent rare, unmet medical needs. Potential drug candidates may need to cross the blood-brain barrier to reach their target, which may or may not be feasible via systemic administration depending on the nature of the compound. To mimic the clinical scenario as closely as possible, intravenous (IV) infusion and/or cerebrospinal administration in juvenile animals would be indicated. Undertaking such surgical procedures and achieving a viable model in juvenile animals also presents significant challenges. Herein, we show that concomitant surgical implantation of a femoral IV catheter and an intrathecal (IT) catheter at the lumbar level (comparable to clinical site of injection) in juvenile cynomolgus monkeys (18-23 months old) can be successfully achieved. The IT catheter was implanted via a microlaminectomy procedure of the L5 vertebra followed by a durotomy and catheter insertion into the IT space. A subcutaneous pocket was created in the lumbar region, cranial to the catheter site, and the access port was secured to the muscle and the catheter connected to the port. For the IV cannulation, the femoral vein was isolated and the catheter was inserted; the tip of the catheter was placed in the vena cava. The catheter was secured in place with appropriate suture material and brought subcutaneously to an exteriorization point on the animal’s back. Post surgical recovery was completed over a 7-day period. Animals were administered saline by continuous IV infusion and by IT injection every other day over 14 days. Endpoints evaluated included body weights, clinical observations, hematology, coagulation, clinical biochemistry, neurological examinations, gross observations at necropsy as well as histopathology. The results obtained are comparable to historical data obtained in animals of 2 to 5 years undergoing similar experimental procedures and as such feasibility of continuous IV infusion combined with IT implantation at the lumbar level for injection was confirmed.
The objective of this study was to establish and validate a functional observational battery (FOB) at the Center for Drug Safety Evaluation & Research (CDSER) that provides a basic neurological and behavioral assessment in group housed Cynomolgus Monkey. Four female cynomolgus monkeys were assigned to this study and were given vehicle, chlorpromazine, (s)MK-801 or caffeine in 4 separated days. A FOB was performed and recorded by two independent observers for each animal at designated time points in order to minimize the subjective biases. The administration of 5 mg/kg chlorpromazine resulted in sedative effect between 0.5 and 3 hrs and decreased body temperature at 1 hr post dose. The dose of (s)MK-801 (0.1, 0.5, 1.25 and 2.5 mg/kg) resulted in suppressed behavior (low arousal, infrequent vocalization, hypoactive), autonomic responses (salivation, decreased respiration and rectal temperature) and sensorimotor/neuromuscular alterations (un-coordinated, lack of auditory response, tremor/convulsion) at all doses, and unconscious/pupil dilation at high doses (1.25 and 2.5 mg/kg). Caffeine resulted in stimulant behavioral (high arousal, hyperactivity and intermittent circling behavior) at dose of 30 mg/kg and one of two monkeys at dose of 45 mg/kg resulted in stimulant behavioral (high arousal, hyperactivity and intermittent circling behavior) at dose of 45 mg/kg (between 0.5 and 4.5 hr post dose). At higher doses (one at 45 mg/kg and one at 60 mg/kg), caffeine resulted in decreased arousal and hypoactive between 0.5 and 3 hrs post dose. Autonomic alteration was evidenced by vomiting/retching seen in 45 and 60 mg/kg monkeys between 0.5 and 3 hrs post dose. In conclusion, the observations recorded in this study were consistent with expected pharmacological effects of the neurosedative or neurostimulant reference substances. A FOB in group housed cynomolgus monkeys has been successfully established at CDSER for safety pharmacology and/or toxicology studies to support regulatory submission.

A Validation Safety Pharmacology Study of the Central Nervous System, Employing a Functional Observational Battery (FOB) in Female Cynomolgus Monkey

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The purpose of the current investigation is to examine available mechanistic data with n-butanol and explore potential mechanisms that might lead to the observed neurotoxic effects. Acute mechanistic studies with n-butanol have suggested a link between the observed neurotoxic effects and alterations in neuronal receptors (e.g. ion channels, G-protein coupled receptors), transport systems, and direct interaction with phospholipids and cellular adhesion molecules. These findings from the mechanistic data suggest that there may be some similarities with n-butyrate to other known neurotoxins such as butyl acetate, a metabolic precursor to n-butanol, have reported no neurobehavioral changes with repeated exposure in rodent studies. However, significant decreases in absolute brain weight in male rats have been observed following n-butyrate exposure.

Potential Mechanisms Leading to Neurotoxicity following N-Butanol Exposure

A. S. Bale, National Center for Environmental Assessment, US EPA, Washington, DC.

There is growing interest with respect to potential widespread human exposure of n-butanol toxicity due to consideration of usage as a fuel additive. However, limited evidence of toxicities, including neurotoxicity, following exposure to n-butanal is available. Neurotoxicity effects resulting from either an acute or subchronic exposure to n-butanol primarily consist of disruption in motor movement and coordination in experimental animal studies conducted to date. In humans, one study reported that exposure to n-butanol exacerbated hearing impairment in workers chronically exposed to high noise conditions. Studies with n-butyrate, a metabolic precursor to n-butanol, have reported no neurobehavioral changes with repeated exposure in rodent studies. However, significant decreases in absolute brain weight in male rats have been observed following n-butyrate exposure.

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Potential Mechanisms Leading to Neurotoxicity following N-Butanol Exposure

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were exposed to 10mg/kg DNB, i.p., and mitochondria (mt) isolated from cortex and brainstem by differential centrifugation. Mitochondrial proteins were separated by SDS-PAGE and analyzed by LC-MS/MS to identify protein modifications. Database searches were performed using the Mascot search engine and NCBI & UniProt databases. Results were displayed in ScaffoldTM software and the Normalized Spectral Abundance Factor (NSAF) and %Oxidation were calculated. Proteins were selected for pathway analysis in the Database for Annotation, Visualization and Integrated Discovery (DAVID) based on <0.25 or >4.0-fold changed by NSAF or %Oxidation. Brains from 1mo control rats had significantly higher levels of native proteins in several key metabolic pathways, whereas brains from older control animals showed lower levels of native protein. While cortical %Oxidation was not affected by age, specific brainstem mt-proteins were more prone to oxidation, i.e., the number of oxidized brainstem mt-proteins increased with age. Pathway analysis revealed that DNB caused age-related oxidation specific for protein and nucleotide binding (catalyt transmembrane transporters, nucleotide-oxide-triphosphates, and phosphates, (p<0.05)). These data suggest that oxidation of the brain mitochondrial proteome is region, age and pathway specific and that brainstem mitochondria are more susceptible to oxidative xenobiotic injury as a function of age than their cortical counterparts. This work was supported by NIH 2R01 ES08846 (MAP) and NIH 2T32 ES007062 (LLK).

### 351 Dioxin-Like and Non-Dioxin-Like Polychlorinated Biphenyls (PCBs) Modulate Basal and Activity-Dependent Dendritic Arborization in Primary Neuronal Cell Cultures

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PCBs are ubiquitous environmental contaminants that have been linked to cognitive and behavioral deficits in children and experimental animals. We previously demonstrated that exposure to the non-dioxin-like (NDL) PCB congener 95 increases dendritic arborization of primary hippocampal neurons in vitro via activation of the ryosyndrome receptor (RyR) calcium channel. However, whether other PCBs similarly trigger dendritic growth via RyR-dependent mechanisms is unknown. To address this question, we measured the effects of NDL PCB congeners 95 and 52 and dioxin-like (DL) PCB congener 77 on RyR activation and on dendritic arbor complexity. Radioilgand-receptor binding analysis with trinitated ryosyn-dine (3H-Ry) showed that PCBs 95 and 52 increased specific receptor occupancy, indicating RyR sensitization; however, the concentration-effect relationships differed. In contrast, PCB 77 did not affect 3H-Ry binding. To determine whether RyR sensitization predicted effects on dendritic growth patterns, primary cultures of perinatal rat hippocampal neurons were exposed to varying concentrations of each congener for 48 h, and the number of dendritic termini was quantified as a measure of dendritic arbor complexity. Each congener enhanced dendritic growth, but exhibited a unique concentration-effect relationship that did not correlate with potency of RyR activation. PCB exposure reduced arborel complexity in neurons treated with bicusculin, a GABA receptor antagonist that stimulates dendritic arborization, leading to an antagonistic interaction between PCBs and activity on dendritic growth. These data demonstrate that dendritic arborization may be a convergent cellular outcome in PCB developmental neurotoxicity, but that NDL and DL PCBs may stimulate dendritic growth via RyR-dependent and/or -independent pathways. This work was supported by NIH (grants R01 ES014901, R01 ES017425, T32 ES007059), the ARCS Foundation, and the Superfund Research Program (P42 ES04699).

### 352 Perfluorobutrate (PFBa) Produces Dose-, Time-, and Sex-Dependent Pupal Light Reflex (PLR) Deficits and Retinal Degeneration

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During toxicological evaluation of PFBa, we saw a delayed direct PLR in Sprague-Dawley male, but not female, rats exposed to 30 mg/kg PFBa by oral gavage for 90 days. No other signs of neurotoxicity were seen (Reprod Toxicol 2012). To characterize this unusual deficit, retinas from males and females were stained and examined. To determine time course and dose-dependence, the PLR was assessed prior to and weekly during a 28 day exposure of male rats to 0, 30 and 150 mg/kg PFBa (n=18-40/group) followed by retinal histology and immunocytochemistry (n=6-10/group). Staining of the 90 day PFBa retinas with the fluorescent nuclear dye DAPI revealed significant decreases in outer (ONL), inner (INL), ganglion cell layer (GCL) and nuclear detectable (TR) thicknesses for males, but not females, relative to controls. PLR studies in dark-adapted 0, 30 and 150 PFBa groups showed significant dose- and time-dependent delays beginning 1 week after exposure. DAPI staining revealed significant dose-dependent decreases in ONL, INL, GCL and TR thickness. In the 150 PFBa group compared to controls, single- and double label confocal studies demonstrated a loss of bipolar cells and retinal ganglion cells (RGCs), with no increase in GFAP immunoreactivity (no reactive gliosis). Consistent with the loss of photoreceptors and inner retinal cells, mitochondrial and structural retinotoxic effects in male, but not female, rats: consistent with the shorter PFBA half-life in the latter. Although the molecular mechanism is unknown, we suggest that the synaptic connections and somas of the intrinsically photosensitive RGCs mediating the PLR were lost. Supported by 3M and NIH grants EY07024 and EY07551.

### 353 The Toxicity of Low Doses of Ultrafine Diesel Exhaust Particles on Bovine Brain Microvascular Endothelial Cells

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Diesel exhaust particles (DEPs), originated from combustion of diesel fuel, are one of major pollutant in urban area. DEPs are composed of various sized of particles, the most of them are ultrafine particles, chemicals, and gases. DEPs are known as an important risk factor for various health problems including brain diseases. However, we have limited evidence for the toxicity of low doses of ultrafine DEPs on brain. This study was performed to evaluate the neurotoxicity of low doses of ultrafine DEPs with bovine brain microvascular endothelial cells (bBMEC) as a blood-brain barrier (BBB) model. Various concentrations of DEPS (from 1.28 ng/ml to 20 ng/ml) were exposed to bBMEC for 24 h in vitro. After exposure, we evaluated cytotoxicity with lactate dehydrogenase (LDH) activity, reactive oxygen species (ROS) generation with DCFH-DA method, total antioxidant capacity (TAC) with bathocuproinedisulfonic acid disodium salt, and permeability by measuring the flux of fluorescein. As results, low doses of DEPs decreased cell viability and induced oxidative stress by increasing ROS generation and decreasing TAC with dose-dependent manner. Additionally, BBB permeability was shown to increase by DEPs exposure. This study showed that exposure to low doses of ultrafine DEPs might disrupt BBB function accompanied with oxidative stress generation. This suggests a possibility that exposure of DEPs, even if the dose is extremely low, may induce adverse effects on CNS by altering the integrity of BBB.

### 354 Role of Oxidative Potential versus Nicotine in Tobacco Smoke-Induced BBB Toxicity: Genomic and Proteomic Assessment of BBB Endothelial Dysfunction

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Until recently toxicological studies on tobacco smoke (TS) have preferentially focused on lung and cardiovascular physiology leaving out the blood-brain barrier (BBB) and the cerebrovascular system. Previous studies from our group have shown that BBB impairment by TS is mediated through induction of vascular adhesion molecules, pro-inflammatory cytokines and matrix metalloproteinases from activated leukocyte and endothelial cells (EC). These TS-dependent inflammatory stimuli are suppressed by antioxidants. Thus, we hypothesized that TS-induced oxidative stress (including nitric oxide release) is a major determinant for BBB toxicity of cigarette products. For this purpose, we assessed the impact of soluble cigarette smoke extract (CSE) on gene and protein expression levels in BBB EC. Herein we report the specific impact of nicotine (100ng/ml) versus CSE from full flavor (FF) and ultralow nicotine (ULN) cigarettes. Initial profiling showed that levels of reactive oxygen (ROS) and nitrogen species (RNS) were significantly higher in ULN product compared to the FF and nicotine alone thus indicating a higher oxidative potential. Down regulation of BBB endothelial tight junction (TJ) proteins such as ZO-1 was observed in both ULN and FF cigarettes but not in nicotine-exposed EC. In addition, the membrane expression of VE-cadherin was up-regulated; perhaps a safe fail (compensatory) mechanism to inhibit leukocyte transmigration across the BBB. Nrf2- a transcription factor involved in anti-oxidant response signaling was also increased in CSE-exposed endothelial cells substantiating the role of the ROS generation in BBB toxicity. In summary, our results suggest that BBB toxicity of tobacco products correlates to their relative oxidative capacity which also implies that labeling low-nicotine product as light or less harmful in misleading.


535 Neurotoxic Effects of the Global Smokeless Tobacco Product, Gutkha

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Gutkha, a smokeless tobacco (ST) product manufactured in India and readily available in the U.S. (used extensively by South Asian immigrant communities), is composed of powdered tobaccos, areca nut, and a variety of spices. To assess the neuropharmacological effects of subacute gutkha usage, adult male mice were exposed via daily “painting” of the oral mucosa with either water (control), gutkha, or nicotine for 7 d. Serum cotinine levels for all groups were analyzed upon sacrifice within 1 hr post-exposure; gutkha and nicotine alone had comparable cotinine levels ranging between 18-50 ng/mL and 20-60 ng/mL respectively. Evaluation of methylation status of genes in the frontal cortex demonstrated that gutkha treatment increased methylation state of genes known to be associated with specific mental disorders (e.g. Bdnf, Atp1a, and Comt1). In follow-up experiments, gutkha’s effects on local dopamine (DA) regulation in the striatum, important for executive function and reward-related events, were evaluated using ex vivo voltammetry. Brain slices were exposed ex vivo to varying concentrations of nicotine or gutkha and the lowest concentration causing nicotinic acetylcholine receptor (nAChR) desensitization. These studies demonstrated that a >10-fold higher concentration of gutkha (concentration determined by nicotine content in gutkha mixture) than nicotine was needed to cause nAChR desensitization (p<0.05). The results suggest that: (1) effects of gutkha on certain brain parameters may be due to gutkha-associated toxins other than nicotine; (2) gutkha causes changes in the epigenome that could be associated with development of certain mental disorders; and, (3) higher doses of gutkha may need to be consumed in order to desensitize nAChRs associated with nicotine dependence. Thus, gutkha, and possibly other ST products, poses a public health threat that requires further investigation and ultimately enhanced regulation. Supported by Memorial Sloan Kettering Cancer Center and NYU NIEHS Center of Excellence Grant No. ES000260.

536 Assessment of the Neurotoxic Potential of 50 Hz Extremely Low-Frequency Electromagnetic Fields in Naïve and Chemically-Stressed PC12 Cells

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The increasing exposure to extremely low frequency electromagnetic fields (ELF-EMFs, 50 Hz), generated by power lines and electric appliances, is associated with childhood leukemia. This raised public and scientific concern about (other potential adverse) health effects of ELF-EMFs. The central nervous system is expected to be particularly vulnerable to ELF-EMFs as its function strongly depends on electrical excitability and ion channel activity. Moreover, potential neurotoxic effects more likely occur in developing or aged/stressed neuronal cells.

We therefore investigated effects of ELF-EMFs on stressed/aging neuronal cells as these have higher levels of iron and reactive oxygen species (ROS) and are thus more sensitive to environmental insults. We exposed naïve and chemically-pre-treated pheochromocytoma (PC12) cells acutely (30 min) and chronically (48 h) to 50 Hz ELF-EMFs (0.1 – 1000 μT) and measured changes in Ca2+-homeostasis using Fura-2 single cell fluorescent microscopy. Additionally, effects on ROS production and cell viability were assessed using H2-DCFDA and a combined Alamar Blue/Fura-2 single cell fluorescent microscopy. However, both acute and chronic exposure of the different PC12 models to 50 Hz ELF-EMFs up to 1000 μT, i.e. 10,000 times above background exposure, failed to affect basal or K+-evoked [Ca2+]i cell viability and ROS production. This suggests that 50 Hz ELF-EMFs do not induce neurotoxic effects in vitro. Moreover, stressed neuronal cells do not appear more vulnerable to ELF-EMFs. Our future experiments in vitro for developmental neurotoxicity aim to elucidate the toxic potential of ELF-EMFs to developing neuronal cells.

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537 Bisphenol A and 4-Methyl-2, 4-bis(4-hydroxyphenyl)pent-1-ene Induces Neuronal Cell Apoptosis via Akt/Endoplasmic Reticulum Stress-Regulated Signaling Pathway

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Objectives: Bisphenol A (BPA) and 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), an active metabolite of BPA, are an endocrine disrupting chemicals (EDCs) or environmental estrogenic property which can disrupt the hormones produced or secreted in mammalian. Several studies have been reported BPA and MBP can cause severely toxicological effects, including neurological dysfunction. However, effects and action mechanisms of BPA and MBP-induced neurotoxicity remained unclear.

Results: BPA significantly decreased cell viability (the LD50 was determined to be approximately 75 μM) and induced the increase in sub-G1 hypodiploid population, annexin V-FITC binding, and the protein expressions of caspase-3/-7 in Neuro-2a cells, indicating that BPA could induce neuronal cell apoptosis. Exposure of Neuro-2a cells to 75 μM BPA also could trigger ER-stress as indicated by several key markers (GRP-78, GRP-94, CHOP, and XBP-1) and caspase-12 cleavage. Moreover, treatment of Neuro-2a cells with MBP more markedly reduced the number of viable cells (the LD50 was determined to be approximately 10 μM), which was associated with inducing apoptotic events and the expression of ER stress-related key molecules and decreased the phosphorylation of Akt. Transfection with specific si-RNA (GRP-94, CHOP, and XBP-1) and over-expression of constitutive activation of Akt (i.e. Akt), respectively, effectively attenuated MBP-induced neuronal cells apoptosis.

Conclusions: These results indicate that Akt/endoplasmic reticulum (ER) stress-regulated apoptotic pathway plays the crucial role in BPA and MBP-induced neuronal cell death.

538 Quinone-Induced Protein Handling Changes: Implications for Major Protein Handling Systems in Quinone-Mediated Toxicity

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Para-quinones such as 1,4-benzoquinone (BQ) and menadione (MD) and ortho-quinones including the oxidation products of catecholamines, are derived from the metabolism of xenobiotics as well as endogenous molecules. Both BQ and MD have been shown to perturb protein-handling systems and we have demonstrated that the dopamine-derived quinone aminochrome (AC) could inhibit pro tease activity. Since the potential hazardous effects of quinones on protein handling systems, including the 20/26S proteasome, the ER stress/unfolded protein response (UPR), autophagy, heat shock proteins (Hsps) and aggresome formation have not been investigated in detail, we conducted this study to systematically examine quinone-induced protein handling changes. In addition, the potential importance of NAD(P)H:quinone oxidoreductase 1 (NQO1) in modulating protein handling changes and toxicity was investigated using stable transfection to generate an isogenic NQO1-overexpressing neuronal cell line. Our data showed that both BQ and AC could inhibit proteasomal activity and activate the ER stress response in rat dopaminergic N27 cells as indicated by increased phosphorylation of eIF2α. While menadione had no significant effect on any protein handling systems, AC was able to stimulate autophagic flux and induce formation of aggresomes as determined by increased LC3 I/II turnover and the formation of ubiquitin positive inclusion bodies. By modulating quinone-induced ER stress responses, NQO1 protected against BQ toxicity but potentiated AC and MD toxicity. This suggests that NQO1-mediated reduction to unstable hydroquinones and subsequent redox cycling was important for the activation of the ER stress response and toxicity of AC and MD in N27 cells. In summary, our data demonstrates that quinone-specific changes in protein handling are evident in N27 cells and the induction of the ER stress response is associated with quinone-mediated toxicity (supported by R01ES018943).
Scavenging Seizure-Induced Reactive Oxygen Species with a Catalytic Antioxidant Attenuates Oxidative Stress and Neuroinflammation in the Pilocarpine Model of Nerve Agent Toxicity

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Seizure activity is a critical injury response following exposure to nerve agents. Reactive oxygen species (ROS) have been identified as key mediators of seizure-induced neuronal damage, along with aberrant inflammation. One potential avenue of controlling pro-inflammatory responses is by scavenging seizure-induced ROS and thereby improving the redox balance. Adult male rats were subjected to pilocarpine-induced status epilepticus (SE) and proinflammatory cytokine production was assessed in the hippocampus and pitiform cortex which revealed acute increases in their levels. To determine the role of ROS in SE-induced cytokine production, a separate cohort of rats were treated with AEOL10150 [Mn(III) tetrakis(N,N-diethylimidizolium-2-yl)porphyrin], a catalytic antioxidant with high superoxide dismutase and catalase activities and sacrificed after 24h. Pilocarpine-induced SE significantly increased 3-Nitrotyrosine/Tyrosine (3NT/Ty) ratio, decreased glutathione redox status (GSH/GSSG), decreased mitochondrial respiration and increased proinflammatory cytokine production, which were significantly attenuated by AEOL10150. Treatment with AEOL10150 also decreased microgliosis and neuronal death, without altering pilocarpine-induced SE. These results demonstrate that scavenging ROS can decrease indices of seizure-induced neuroinflammation and oxidative stress and highlight the importance of redox mechanisms in controlling inflammation in nerve agent-induced toxicity.

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Combined Therapy with Allopregnanolone and Diazepam Mitigates TETS-Triggered Hyperexcitability of Neuronal Networks In Vitro and Rescues Mice from TETS-Induced Seizures and Death

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Tetramethylenedisulfotetramine (TETS) is a potent convulsant GABA A receptor antagonist considered to be a chemical threat agent. Mice that receive a lethal dose of TETS (0.15 mg/kg, i.p.) can be rescued by the GABA A receptor positive allosteric modifier diazepam (DZP) when administered at a high dose (5 mg/kg, i.p.) immediately after the second clinical seizure (~20 min after TETS injection). However, at this dose, DZP significantly reduces blood pressure and mobility but does not suppress TETS-induced neuroinflammation in the brain. To identify alternative medical countermeasures, we used a primary culture cell model to screen GABAA receptor agonists, singly or in combination. Acute exposure of cultured mouse hippocampal neurons to TETS reversibly alters the pattern of spontaneous Ca2+ oscillations and increases discharge frequency and synchronicity as determined by Fluo-4 fluorescence and extracellular microelectrode array recording. Low concentrations (0.1 μM) of DZP and allopregnanolone (Allop) that are minimally effective when added singly to cultures, show synergy in suppressing TETS-induced alterations of Ca2+ dynamics and electrical discharges when added in combination 20 min after cultures are exposed to TETS. As predicted by the in vitro experiments, doses of DZP (0.05 mg/kg) and Allop (0.03 mg/kg) that are minimally effective when administered singly, increase survival to nearly 100% and reduce the total number of seizures when given in combination immediately after the second clinical seizure. In contrast to high dose DZP, this combinatorial therapy has no effect on blood pressure or mobility, and mitigates TETS-induced activation of microglia but not astrocytes. These studies indicate that in vitro studies can be used to predict effective drug combinations for post-exposure prevention of TETS-induced death. This work is supported by NINDS CounterACT Program grant U54 NS079202.
participant with an age of 65 years and older into T-pos (IgG ≥ 1:100) and non-infected individuals (T-neg; IgG < 1:100). Their cognitive status was evaluated by standardized quantitative tests of memory-based and non-memory-based cognitive functions. The depressive state and the quality of life were assessed by questionnaires. Infected individuals reported different aspects of these functions. In contrast, executive functions were not affected. In particular, lower performance in T-pos seniors was found in a short-term verbal memory test, both regarding immediate recall (P=0.022) and delayed recognition (P=0.037) and in recall from long-term memory assessed by the word fluency tests (P=0.029). Moreover, the rate of correct identified target symbols in a 2-back task was decreased (P=0.048), indicating a reduced working memory capacity. Decreased memory functions in T-pos seniors were accompanied by a decreased self-reported quality of life. The observed association is relevant for public health considering the high prevalence of toxoplasmosis. The relatively large effects on some of the here reported memory functions correspond to the mean decrease usually observed between year 60 and 70.

Changes in gene and protein expression of liver ABC transporters have been previously described during acute APAP intoxication. However, the effect of hepatoxic doses of APAP on brain ABC transporters has never been studied. The aim of this study was to evaluate the effect of APAP on brain ABC transporter expression and the role of the translation factor eIF2α in Nrf2 activation.

For this purpose, male C57BL/6 mice received APAP (400mg/kg) or vehicle. 24h later, brains were removed and protein expression of Mrp1-Mrp5, Bsep and P-gp was determined by Western blotting from total brain homogenates. mRNA expression for these ABC transporters was also assayed by q-RT PCR between 6 and 24 h after APAP. The results show that APAP treatment significantly increases the expression of brain P-gp (159%), Mrp2 (293%) and Mrp4 (38%) proteins relative to controls, with no changes in Bsep, Mrp1 and Mrp5 detected. Mrp3 protein was not detectable in either group. The increased protein expression for P-gp, Mrp2 and Mrp4 correlated with changes in mRNA expression. In agreement with protein content, mRNA expression for Bsep, Mrp1, Mrp3 and Mrp5 did not change with APAP treatment. Indicative of Nrf2 activation, mRNA and protein expression of NAD(P)H:quinone oxidoreductase 1 increased significantly in brain tissue with APAP treatment. Furthermore, nuclear content of Nrf2 protein was found to increase by 50% at 12 h after APAP. In conclusion, acute APAP intoxication induces protein expression of several brain ABC transporters through a transcriptional mechanism. This study also suggests that these changes may be due to in situ Nrf2 activation by APAP. The functional consequences of these changes in brain ABC transporters by APAP deserve further attention.

Mesencephalic dopaminergic neurons are heavily involved in the development and maintenance of drug dependence. Therefore, the morphological changes in dopaminergic neurons of the substantia nigra (SN) and ventral tegmental area (VTA) in morphine dependent rats were investigated. Models of morphine dependence were established in rats, and paraffin-embedded sections, immunohistochemistry and immunofluorescence were used to observe the changes in expression of tyrosine hydroxylase (THI) protein, a specific marker of dopaminergic nerve cells. Fluoro-Jade B staining was used to detect degeneration and necrosis, and terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) detected apoptosis of mesencephalic dopaminergic nerve cells. The number of TH positive cells significantly decreased in rats that were morphine dependent for a longer period of time. With prolonged morphine exposure, SN and VTA dopaminergic nerve cells showed degeneration and necrosis, while apoptotic cells were not observed. The number of SN and VTA dopaminergic nerve cells decreased with increasing periods of morphine dependence, which was most likely due to degeneration and necrosis of nerve cells induced by morphine toxicity.
differentiation. We manipulated mES cells in culture to differentiate in 3 stages: cell transformation to embryoid bodies (EBs); EB differentiation to neural progenitor cells (NPC); and, mES cell differentiation to neural/glial cells. Cell types are confirmed by monitoring expression of Oe4, nestin, mtap-2, and GAP43, as cell-specific markers for stem cells, NPC, neurons, and astrocytes, respectively, using RT-PCR and flow cytometry analysis. Similarly, opioid receptor gene expression for mu (μ), kappa (κ) and delta (δ) receptors, was confirmed in each cell type. Our results demonstrate that 100 μM morphine sulfate (MS) for 24-hrs does not alter cell viability or cell cycle kinetics in mES cells. In addition, MS does not alter μ-opioid receptor gene expression nor receptor protein expression after 24-hrs exposure. However, MS causes internalization of μ-receptors in a concentration-dependent manner, and down regulation of κ-receptor gene expression in stem cells. Chronic exposure of stem cells during transformation to EBs and NPC results in down regulation of both μ-receptor gene expression and NPC-specific marker expression of nestin, respectively. Overall, these results indicate that acute MS exposure does not alter self-renewal of mES cells; however, chronic treatment inhibits cell differentiation to NPC cells. Consequently, the project focuses on the significance of the relationship between MS administration in early and late stage differentiation, and opioid receptor expression and receptor activity. Understanding this mechanism has particular clinical applications for the prevention or treatment of fetal and childhood narcotic addictions.

369 Effect of Prenatal Methadone on Reinstated Behavioral Sensitization Induced by Methamphetamine in Adolescent Rats

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The present study was to investigate the effect of prenatal exposure to methadone on methamphetamine (METH)-induced behavioral sensitization as an indicator of drug addiction in late life. Pregnant rats received saline or methadone (7 mg/kg, s.c.) twice daily from E3 to E20. To induce behavioral sensitization, offspring (5 weeks old) were treated with METH (1 mg/kg, i.p.) or saline once daily for 5 consecutive days. Ninety-six hours (day 9) after the 5th treatment with METH or saline, animals received a single dose of METH (1 mg/kg, i.p.) or saline to induce the reinstated behavioral sensitization. Prenatal methadone treatment enhanced the level of development of locomotor behavioral sensitization to METH administration in adolescent rats. Prenatal methadone treatment also enhanced the reinstated locomotor behavioral sensitization in adolescent rats after the administration had ceased for 96 hours. These results indicate that prenatal methadone exposure produces a persistent lesion in the dopaminergic system, as indicated by enhanced METH-induced locomotor behavioral sensitization (before drug abstinence) and reinstated locomotor behavioral sensitization (after short term drug abstinence) in adolescent rats. These findings show that prenatal methadone exposure may enhance susceptibility to the development of drug addiction in later life. This could provide a reference for drug usage such as METH in their offspring of pregnant woman who are treating with methadone.

370 Inhibition of the Vesicular Monoamine Transporter 2 Alters the Acute and Long-Term Effects of 3,4-(2)-Methylenedioxymethamphetamine

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3,4-(2)-Methylenedioxymethamphetamine (MDMA, Ecstasy) exerts a biphasic pharmacological response on the brain resulting in contrasting acute and long-term effects. During the acute phase, MDMA causes major monoamine release into the striatum of SD rats. As expected, MDMA significantly increased DA and 5-HT release in the caudate putamen. Ro 4-1284 pretreatment had little effect on MDMA-mediated increases in dialysate monoamine concentrations. However, increases in dialysate concentrations of DA and 5-HT metabolites (DOPAC and 5-HIAA, respectively) were indicative of increased monoamine turnover. Immunohistochemistry revealed that Ro 4-1284 prevents striatal depletion of the 5-HT reuptake transporter in MDMA-treated rats, consistent with findings that inhibition of VMAT2 protects against long-term MDMA serotonergic neurotoxicity. In conclusion, we have demonstrated that VMAT2 inhibition by Ro 4-1284 has significant effects on the acute and long-term effects of MDMA. Further studies are required to decipher the mechanisms by which Ro 4-1284 protects against MDMA neurotoxicity. (DA023525, P30ES006694, & T32ES007091).

371 Enhanced Vesicular Function and Reduced Neurotoxicity in Mice Overexpressing the Vesicular Monoamine Transporter

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The vesicular monoamine transporter 2 (VMAT2) packages monoamine neurotransmitters into vesicles for rapid release at the synapse. In addition to its role in neurotransmission, VMAT2 also sequesters toxins away from their sites of action in the cell. Our laboratory has previously characterized mice with reduced vesicular capacity, which results in progressive degeneration of dopamine and noradrenaline neurons and motor and non-motor deficits (J. Neurosci., 2007; J. Neurosci., 2009; Neuropharmacology, 2013). However, it was unknown if an increase in VMAT2 level would result in increased vesicular function in an animal model. We created a BAC transgenic VMAT2 overexpressing (VMAT2-HI) mouse line to determine the effects of increased vesicular monoamine filling on associated neurochemical, behavioral, and toxicological outcomes. The increased vesicular filling in the VMAT2-HI mice results in an enhanced vesicular storage capacity for dopamine as shown by increased vesicular uptake and increased vesicle size. This corresponds to a 21% increase in striatal dopaminergic content, suggesting a sustained enhancement of the dopaminergic system in the VMAT2-HI mice. This increased dopamine storage also results in an 80% increase in synaptic dopamine release as measured by fast-scan cyclic voltammetry. Further, VMAT2-HI mice show behavioral changes, including increased locomotor activity and an enhanced response to amphetamine. VMAT2-HI mice also show improved outcomes on measures of anxiety and depressive-like behaviors, including a 42% reduction in marble burying and a 22% decrease in immobility time on the forced swim test. Finally, since VMAT2 level is a neuroprotective mechanism in monoaminergic systems, we tested the vulnerability to the parkinsonism-inducing neurotoxin MPTP and found that the VMAT2-HI mice had reduced dopaminergic toxicity. These data suggest that monoamine vesicular capacity can be increased and this increased capacity corresponds to reduced toxicity.

372 Diverse Models of Neurotoxicity Link Induction of Astroglial Activation to Activation of STAT3

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Dr. Paul Greengard received the 2000 Nobel Prize in Physiology or Medicine for identifying the dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) signaling pathways in medium spiny striatal neurons. Identification of multiple phospho-sites on DARPP-32 revealed upstream effectors regulating the activation of this specific neuronal cell type. We adopted the same approach to map signaling pathways upstream of the key astrocyte damage marker, glial fibrillary acidic protein (GFAP). In a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of dopaminergic neurotoxicity, we demonstrated the development of neuroinflammation (upregulation of proinflammatory cytokines/chemokines) and activation of the janus kinase and signal transducer and activator of transcription (JAK2-STAT3) pathway preceding the up-regulation of GFAP in astrocytes in the brain. Here, we show that multiple mechanistically distinct models of neurotoxicity (MPTP, amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDA), kainic acid (KA) and trimethyltin (TMT)) engender the same neuroinflammatory response and STAT3 activation in target brain regions of the respective neurotoxins. Pharmacological antagonism of neurotoxic effects of MPTP, METH and KA with nomifensine, ethanol and diazepam, respectively, blocked the neuroinflammation, phosphorylation of STAT3 and GFAP induction, indicating neuronal damage as a component of the initiation of neuroinflammation. Deletion of astrocyte STAT3 in conditional knockout mice prevented the induction of GFAP in MPTP-treated mice. Double immunostaining of GFAP and STAT3 showed...
enhanced nuclear staining localized to astrocytes in association with the induction of astrogliosis. These findings strongly implicate the STAT3 pathway in astrocytes as a key signaling pathway for astrogliosis.

373 BAC-TRAP Technology in Neurotoxicology: The ALDH1L1 BAC-TRAP Mouse As a Tool to Assess Astrocyte Specific Responses to Neurotoxic Injury

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A central problem in neurotoxicology is detecting the selective and unpredictable damage to specific cells produced by toxic agents and mixtures. Evaluating astrogliosis overcomes this problem because reactive astrocytes show the location of toxicant-induced damage occurring anywhere in the CNS. Enhanced expression of GFAP is a hallmark of reactive astrocytes; however, few other astrogliosis biomarkers are known. Thus, determining the specific in vivo transcriptional profile of astrocytes under control and reactive conditions will allow for the identification of additional astrogliosis biomarkers. Heintz and Greengard (2008) introduced BAC-TRAP (translating ribosome affinity purification) technology as an approach for identification of cell-type-specific responses in vivo. Here, we implemented this approach for the assessment of mRNA translation specific to astrocytes responding to damage caused by known neurotoxins. ALDH1L1 is an enzyme thought to serve a housekeeping function in astrocytes. Using hippocampal and striatal damage due to TMT and MPTP, respectively, we evaluated the localization and response of ALDH1L1 compared to astrogliosis seen by GFAP immunohistochemistry and ELISA. Staining of ALDH1L1 revealed localization to astrocytes after TMT and MPTP, while immunoblots of ALDH1L1 revealed basal expression of this protein but little enhanced expression after MPTP and TMT, confirming to be an astrocyte “housekeeping” gene/protein. We then used the ALDH1L1 BAC-TRAP mouse to evaluate astrogliotic-specific mRNA with expression analyses by gene array in control conditions. Tissue was subjected to TRAP utilizing an eGFP antibody that only binds to actively translating RNA in astrocytes. This revealed numerous genes in “resting” astrocytes, including genes previously localized to astrocytes (e.g., GFAP), as well as novel genes to this cell type (e.g., PHOX2A). MPTP expression data are reported in accompanying poster. The ALDH1L1 BAC-TRAP mouse represents a promising tool to investigate astrogliotic responses to neurotoxic injury.

374 BAC-TRAP Technology in Neurotoxicology: Assessing the Astrocyte Response to MPTP-Induced Damage in the ALDH1L1 BAC-TRAP Mouse

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A central problem in neurotoxicology is detecting the selective and unpredictable damage to specific cells produced by toxic agents and mixtures. Evaluating astrogliosis overcomes this problem because reactive astrocytes show the location of toxicant-induced damage occurring anywhere in the CNS. Enhanced expression of GFAP is a hallmark of reactive astrocytes; however, few other astrogliosis biomarkers are known. Heintz & Greengard (2008) introduced BAC-TRAP (translating ribosome affinity purification) technology that allows the characterization of the actively translating RNA in astrocytes. Using hippocampal and striatal damage due to TMT and MPTP, respectively, we evaluated the localization and response of ALDH1L1 compared to astrogliosis seen by GFAP immunohistochemistry and ELISA. Staining of ALDH1L1 revealed localization to astrocytes after TMT and MPTP, while immunoblots of ALDH1L1 revealed basal expression of this protein but little enhanced expression after MPTP and TMT, confirming to be an astrocyte “housekeeping” gene/protein. Thus, ALDH1L1 BAC-TRAP mice can be used to characterize the transcriptome of astrocytes under various conditions. To begin to characterize additional biomarkers of astrogliosis occurring in response to neurotoxic damage ALDH1L1 BAC-TRAP mice were given a single 12.5 mg/kg s.c. dose of MPTP, a well characterized dopaminergic neurotoxicant that induces significant astrogliosis. Striatal tissue was obtained at 12, 24, and 48 hrs following a single s.c. dosage of saline or 12.5 mg/kg MPTP. Striatal tissue was subjected to TRAP utilizing an eGFP antibody that only binds to actively translating RNA in astrocytes. Changes in the actively translating RNA induced by MPTP damage were determined by microarray (illumina MouseWG-8 v2 Expression BeadChip) and the dataset interrogated using Ingenuity Pathway Analysis (IPA). MPTP induced robust transcriptome changes in genes previously identified as astrocyte specific (e.g., 405, 399, 804 fold increases in TIMP1 at 12, 24, 48 hrs, respectively) as well as others not previously considered astrocyte-specific (e.g., 219, 203, 3,69 fold increases in PHOX2A at 12, 24, 48 hrs). Our data indicate the BAC-TRAP technology can be used to identify additional biomarkers of astrogliosis and will aid in characterizing various astrocyte phenotypes.

375 Human iPSC Neurons: In Vitro Models to Predict Clinical CNS Toxicity

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A major cause of drug attrition from clinical trials is neurotoxicity, a problem stemming from the lack of a relevant model to predict human CNS safety. Current neurotoxicity assessment depends on in vivo animal models, which are time-consuming and expensive. In vitro models consist of immortalized cell lines and primary rodent cultures, which are often a poor representation of human physiology. There is need for high throughput, human-based assays that can narrow down drug candidate panels for in vivo animal studies, and augment those studies with human data before initiation of clinical trials. We chose to establish such assays with human induced pluripotent stem cells (iPSCs) that have been differentiated into mature neurons. These iPSC neurons offer a renewable source of mature cells, relevant to human drug safety, and free from ethical concerns. CNS toxicity results from disruption of complex neural networks, which is why our test panel addresses neuronal activity, in addition to neurite outgrowth, cytotoxicity and mitochondrial stress. Few studies tackle neuronal activity in a high throughput system, and fewer still integrate changes in activity with other functional toxicological endpoints. Our novel platform will combine a semi-high throughput multi-electrode array (MEA) with high throughput neurotoxicity assays for potential screening of drug candidates. By using a panel of test compounds known to cause CNS toxicity in vivo we put forward a panel of in vitro human physiological-based assays to evaluate the translatableity of drug safety.

376 Live Cell-Based Assay Using GFP+ Human Stem Cell-Derived Neurons


Adverse outcome pathways (AOP) are conceptual frameworks that portray existing knowledge concerning the link between a molecular initiating event and an adverse outcome. High throughput screening (HTS) and high content screening (HCS) approaches seek to gain a greater perspective of the AOP, leading to more efficient and reliable identification of adverse outcomes of compounds and chemicals. However, HCS assays often utilize primary or stem cell sources which are not amenable to large scale screening and can require extensive cell culture, fixation and permeation, and immunocytochemistry prior to imaging. Thus, major costs are associated with 96-well based high content neurotoxic screening. In addition, preserving or fixative steps can alter cell architecture and a new plate of cells is required for each time point, thus introducing variability to data. This new human cell-based assay provides a scalable solution to the previously labor intensive, single-end-point, and hence limited nature of neurotox assays, while preserving the breadth and quality of data. The assay utilizes clonal, embryonic stem cell derived, human neural cells that have been genetically modified with a non-viral vector encoding a Green Fluorescent Protein (GFP) reporter gene driven by a ubiquitous promoter. Previously, hN2TM human neuronal cells faithfully reproduced early brain development, providing a cell model > 95% positive for neuronal markers while demonstrating higher sensitivity to neuronal toxins than mouse cortical neurons, and our new GFP+ neurons are equally sensitive. Additionally, previous studies using rodent primary cells examined mixed and unknown ratios of neuronal cells, often at various stages of proliferation and differentiation. These new GFP+ neurons are differentiated from a clonal GFP+ neural progenitor population, thus removing variability from cultures while maintaining an adherent monolayer amenable to high content imaging. By eliminating the need for fixing and staining cells, these GFP+ human neurons provide a scalable means to analyze neurite outgrowth in live cells spanning the course of hours to days following exposure to test compounds.

377 A Functional Phenotypic Screen for Synapse Formation in Human iPS-Derived Neurons


There is need for high throughput, human-based assays that can narrow down drug candidate panels for in vivo animal studies, and augment those studies with human data before initiation of clinical trials. We chose to establish such assays with human induced pluripotent stem cells (iPSCs) that have been differentiated into mature neurons. Therefore, we used antibodies to measure expression levels of the pre- and post-synaptic proteins, Synapsin I and post-synaptic protein 95 (PSD95), respectively. Furthermore, using advanced image analysis algorithms we masked neurite regions demarked by enhanced nuclear staining localized to astrocytes in association with the induction of astrogliosis. These findings strongly implicate the STAT3 pathway in astrocytes as a key signaling pathway for astrogliosis.

SOT 2014 Annual Meeting 95
expression of TuJ1 (beta-III tubulin) and measured colocalized signals for the pre- and post-synaptic markers only in these functionally-relevant regions. Additional information that may be related to the mechanism of action for chemicals that showed an effect in the assay included the amount of neurite outgrowth (TuJ1) and alterations in nuclear texture (Hoechst 33342). Our high content screening (HCS) approach enabled us to screen a library of FDA-approved drugs (the NCC2 library) as well as other agents such as forskolin, estrogen, and tamoxifen known to modulate neuronal function. Using this approach we have developed a robust platform for large-scale screening of chemicals that affect synapse formation, the basic unit of neuronal function in humans.

378 eCiphrNeuro: A High-Throughput Assay to Detect Neurotoxic Compounds in Early Drug Discovery


Seizuregenic neurotoxicity produces significant drug attrition during drug discovery. Currently available in vitro assays cannot predict this toxicity due to the failure of general cytotoxicity assays to predict subthalamic target specific electrophysiological liabilities. Ion channel and receptor activity assays can be used to predict some seizure potential, but these only focus on specifically measured targets for prediction and may miss a response which relies on a combination of endpoints. Most evaluation of seizure inducing compounds occurs later in preclinical development in vivo studies which have much higher costs. Therefore, the development of a high-throughput in vitro assay to screen compounds for seizuregenic potential would lead to evaluating compounds earlier at lower cost and greater reliability. Here we demonstrate the use of a 48-well microelectrode array (MEA) to screen for seizuregenic compounds using rat cortical neurons. Spikes were measured and the results were computed for mean firing rate, synchrony, and spike train organization (inter-spike and burst statistics) using custom Matlab scripts and NeuroExplorer. All of the seizuregenic compounds, including GABA-A antagonists, showed significant changes in MFR, synchronization, and spike train and burst organization, while all of the negative controls were ineffective. Glutamate, the excitatory neurotransmitter, showed a robust increase in activity. Neuroinhibitory molecules such as Domoic Acid, a neurotoxin, GABA (GABA agonist), Tetrodotoxin (sodium channel blocker) and TEA (potassium channel blocker) were also tested and found to block activity. These results illustrate the power of a high-throughput rat cortical neuron MEA assay for predicting compound induced neural toxicity.

378a Low Frequency Activity of Cortical Networks Is Differentially Altered by Bicuculline and Carbaryl

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Thousands of chemicals need to be characterized for their neurotoxicity potential. Neurons grown on microelectrode arrays (MEAs) are an in vitro model used to screen chemicals for functional effects on neuronal networks. Typically, after removal of low frequency components, effects on high frequency events (neuronal action potentials, or “spikes”) are used to differentiate chemical effects. However, such approaches discard information and low frequency activity has yet to be examined for its utility in screening chemicals. In this pilot study, spontaneous network activity in primary cultures of rat cortex was collected in MEAs and differential expression of Tuj1 (beta-III tubulin) and measured colocalized signals for the pre- and post-synaptic markers only in these functionally-relevant regions. Additional information that may be related to the mechanism of action for chemicals that showed an effect in the assay included the amount of neurite outgrowth (TuJ1) and alterations in nuclear texture (Hoechst 33342). Our high content screening (HCS) approach enabled us to screen a library of FDA-approved drugs (the NCC2 library) as well as other agents such as forskolin, estrogen, and tamoxifen known to modulate neuronal function. Using this approach we have developed a robust platform for large-scale screening of chemicals that affect synapse formation, the basic unit of neuronal function in humans.

378b Evaluation of the Neuroactivity of ToxCast Compounds Using Multiwell Microelectrode Array Recordings in Primary Cortical Neurons

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Assessment of spontaneous activity in neuronal cultures using multi-well microelectrode arrays (MEA) systems is a rapid, efficient and sensitive method to detect neuroactivity of drugs, chemicals, and particles. Effects of 68 ToxCast compounds (biased for neuroactive compounds) on neuronal activity using 48 well MEA plates were examined previously. In this experiment, effects of 20 additional ToxCast compounds, with a bias towards those inactive in ToxCast ion channel assays, were examined. Five additional compounds, known to be without effect on neuronal function in MEAs (negatives) were included. Further, these experiments refined data analysis approaches by considering recording stability post-hoc and establishing criteria for data inclusion. On day in vitro 14, 1 hr of baseline activity was recorded prior to exposing the cortical networks to 40 μM of each compound for 1 hr; the percent change in mean firing rate (MFR) was determined in the absence and presence of each chemical. Post-hoc analysis determined that the first 20 min of baseline recording was a period of activity stabilization and thus was omitted from analysis. A hit detection threshold of a 39.1% change in weighted (wMFR) was determined based on 2 standard deviations beyond the mean response to DMSO treatment; none of the 5 known negatives altered wMFR beyond this threshold. However, of the 20 ToxCast chemicals, 12 altered wMFR beyond the hit threshold. Hits included tetraethin and fenitin, 4/9 conazoles, piperonyl butoxide, vinclozolin, lactofen, prochloraz, butyl benzyl phthalate and genistein. None of the 25 chemicals were cytotoxic. Concentration-response assessments were concordant with the single point screen result for 7/8 chemicals. These results indicate that spontaneous activity of cortical neurons measured with MEAs may be sensitive to chemicals with actions on endpoints other than ion channels. This abstract was supported by CRADA #644-11 with Axion Biosystems and does not reflect EPA Policy.

378c Effects of Organophosphorus Flame Retardants on Spontaneous Activity in Neuronal Networks Grown on Microelectrode Arrays

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Organophosphorus compounds are increasingly used as alternatives to brominated flame retardants (BFRs) and thus are commonly found in house air and dust. However, there is limited information on their potential health effects. Assessment of spontaneous neural network activity using microelectrode arrays has been shown to be a sensitive method to screen chemicals for potential neuroactivity/toxicity. Therefore, this study compared the effects of nine organophosphorus flame retardants (OPFs) with the BFR tetrabromobisphenol A to determine their ability to alter network function in primary cultures of rat cortical neurons grown on 48 well microelectrode array (MEA) plates. On day 13 or 14 in vitro, 1 hr of baseline activity was recorded prior to exposure to flame retardants (0.1 to 30 μM). Changes in spontaneous mean firing rate (MFR) relative to the vehicle control were assessed 1 hr after exposure. The BFR tetrabromobisphenol A and the OPF tris (2-chloroethyl) phosphate did not alter the spontaneous activity at any concentration tested. The remaining 8 OPFs decreased spontaneous activity in a concentration-dependent manner, with EC50 values ranging from 6.6 to 18.6 μM in the following order: 3-butylyphenyl diethyl phosphate ≥ tricresyl phosphate ≥ 2-ethylhexyl diphenyl phosphate ≥ isopropylated phenyl phosphate ≥ isodecyl diphenyl phosphate ≥ ortho-tricresyl phosphate ≥ triphenyl phosphate ≥ tris (1,3-dichloroisopropyl) phosphate. None of the flame retardants decreased the viability of cortical cells at any concentration tested. This data indicates that some OPFs have the ability to alter the spontaneous activity in neuronal networks in vitro at micromolar concentrations. This abstract does not necessarily reflect U.S. EPA policy.

378d Neurotoxic Effects of Tri-Ortho-Cresyl Phosphate In Vitro

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Tri-ortho-cresyl phosphate (TOCP) provokes organophosphate-induced delayed neuropathy (OPIDN) in the peripheral nervous systems of humans and sensitive animals. On the cellular level axon swelling and disturbance of axonal transport

SOT 2014 ANNUAL MEETING
are the initial events of OPIDN. Low-dose effects of TOCP on central nervous system (CNS) functions on a cellular level have only been rarely studied yet. In the present study, we investigated neurotoxic effects of TOCP on neurons of mice cerebral cortices in vitro. We evaluated cell viability, neurite outgrowth, and the functionality of neurochemical processes. A 24 h exposure of mouse primary cortical neurons (pCNs) to TOCP yielded dose-dependent cytotoxic effects. 89 µM TOCP were associated with significant reduction of about 50 % in cell viability. In addition, TOCP impaired neurite outgrowth and caused degeneration of neurite networks with a lowest effective dose of 1 µM. Using fluorescence-based life-cell imaging techniques we showed that TOCP-induced alterations in cellular ion currents were associated with altered excitatory neurotransmitter glutamate. Glutamate-evoked signals in pCNs were significantly decreased after 24 h exposure to TOCP 100 nM. Both, the percentage of glutamate-responsive neurons and the mean response amplitudes were decreased. In addition to these results, we observed a direct inhibition of glutamate receptor-mediated responses by TOCP. In detail, the simultaneous application of TOCP (100 µM) together with the glutamate stimulation resulted in a block of glutamate-induced responses by 70 %. In summary, we showed here low and lowest-dose effects of TOCP on functional parameters of CNS neurons. Further research aims at investigating the mode of action of TOCP on glutamate receptors and the identification of receptor subtypes that might be affected. Possibly, the effects described here are associated with some neurobehavioral symptoms related to the acrylotic syndrome.

**378e** Rat Brain Neurolemma Microtransplanted into Xenopus Oocytes Is a Useful Tool to Examine the Effects of Environmental Toxins on Endogenous Voltage-Sensitive Ion Channels

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Microtransplantation of mammalian neurolemma is a tool to study the structure and function of transmitter receptors and ion channels associated with the central nervous system. Microtransplanted neurolemma can originate from a variety of sources, possess ion channels and receptors in their native configuration and lipid matrix, and are applicable to examine diseases associated with different channelopathies. This functional system makes neurolemma-injected Xenopus oocytes a viable method for detailed investigations of toxins on ion channel function. Our preliminary results indicate that Xenopus oocytes, injected with rat brain neurolemma are capable of reconstituting native ion currents into their plasma membrane. Expressed ion currents were sensitive to tetrodotoxin, t-conotoxin MVIIIC, and TEA; indicating the presence multiple voltage-gated ion channels (voltage-gated sodium channel, calcium channel and potassium channel, respectively). Furthermore, neurolemma-injected oocytes, exposed to increasing concentrations of permethrin, exhibited a concentration-dependent increase in TTX-sensitive current. Once completely characterized, this procedure will also be amenable to the study of the developmental and reproductive toxicity of various drugs and other environmental contaminants and for the study of additional agents causing acute, chronic, and developmental neurotoxicity in mammalian neuronal tissues, including those from knockout mice and humans.

**378f** Noninvasive Magnetic Resonance Imaging Marker of White Matter Neurotoxicity Caused by Hexachlorophene in the Rat Brain

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Neurotoxicity assessment in drug development is accomplished using microscopic analysis, which may require different types of histological staining depending upon the type of damage to be assessed. Histopathology studies can be time consuming and easily miss lesions if the wrong stain or locus for tissue sample is selected. In this study we used magnetic resonance imaging (MRI) to detect presumably neurotoxic changes in the rat brain caused by hexachlorophene (HC). Adult male Sprague-Dawley rats (N = 7, 347 ± 20 g) were given either HC (30 mg/kg, N = 5) or pure vehicle (corn oil, 1 ml/kg, N = 2) orally once a day for 5 consecutive days. Animals were imaged using MRI T2 mapping before the treatment and at 4 and 6 days after the start of the treatment. At the time of imaging, animals were anesthetized with isoflurane and positioned inside a 7 tesla MRI scanner. T2 relaxation mapping of the whole brain was performed at a resolution of 0.2 x 0.2 x 1.0 mm per pixel. All rats were perfusion-fixed at the end of the observation and their brains underwent histopathological assessment using aminocumarin silver (classic neurotoxicity) and black gold (white matter damage) stains. MRI revealed significant increases in T2 values in all HC-treated animals in areas which correspond closely to white matter. Histological sections stained with aminocumarin silver did not show any damage in HC-treated rats and were classified as 'normal' by a neuropathologist. However, black gold-stained sections revealed the vast damage to white matter, which was seen in the non-invasive MRI scans. Controls treated with corn oil did not exhibit any changes in MRI scans or histology. These data suggest that MRI can be used to detect neurotoxic changes of the brain and could be used to guide not only the loci for histopathology sectioning but also the type of stain used for histopathological studies. (Supported by NCTR and CDER, FDA, #E0741801).

**378g** Mitochondrial Bioenergetics in Young, Adult, Middle-Age, and Senescent Brown Norway Rats

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Mitochondrial and central regulators of energy homeostasis and may play a pivotal role in mechanisms of cellular senescence and age-related neurodegenerative and metabolic disorders. However, mitochondrial bioenergetic parameters have not been systematically evaluated under identical physiological conditions within multiple organ samples in diverse age-groups. In the present study, we used the Seahorse Extracellular Flux Analyzer (SeaKem Bioscience XF-24) to measure four different life-tasks [i.e., 1 Month-Young (Y), 4 Month-Adult (A), 12 Month-Middle-Aged (M) and 24 Month-Old-Aged (O)] of Brown Norway rats (n=5 animals/group). Mitochondrial (15-40 µg/ml) bioenergetic parameters were evaluated together from five brain regions [brain stem (BS), frontal cortex (FC), cerebellum (CER), striatum (STR), hippocampus (HIP)] and three peripheral organs [heart (HRT), liver (LVR), lung (LNG)]. In general, all the regions of the brain followed identical patterns where the maximal respiratory capacities (State V and State V叔) were reduced with age (Y>A>M-O). The State III respiration in BS, CER and HIP demonstrated a similar pattern like State V (Y>A>M-O); whereas the FC and STR displayed highest State III rates in adult group (A>Y>O). The proton leak (State IV) remained unaffected in peripheral organs. In cerebral cortex, the State V and State III rates were highest in younger animals followed by gradual decline with aging as evident in both HRT (Y>A> O>M) and LNG (Y>A> O>M). In LVR, the NADH-linked bioenergetics remained unchanged whereas the FADH-linked maximal respiratory (State V叔) rates increased gradually as a function of age (Y>A>M-O). In summary, the comparative data analysis of this study gives valuable insight into the metabolic status of various organs that could potentially lead to age-associated changes in neurodegenerative or metabolic disorders. Additionally, the observed changes in mitochondrial bioenergetics will serve as a basic platform to elucidate chemically-induced life-stage susceptibility mechanisms important in community health-related research. (This abstract does not necessarily reflect USEPA policy).

**378h** Optineurin Expression in Dopaminergic Neurons and Response to Parkinson’s Disease Relevant Insults

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Optineurin (OPTN) is a protein that was first identified in 1998 as a genetic factor in normal tension glaucoma. Since then, multiple functional roles have been identified for OPTN, including roles in vesicle trafficking, Golgi organization, induction of macroautophagy and cell cycle. Recent data has also shown that OPTN aggregates in sensitive neurons in several neurodegenerative diseases, including Parkinson’s disease (PD). The characteristic pathology of PD includes the aggregation of α-synuclein into Lewy bodies and the progressive loss of DA neurons in the substantia nigra. Macroautophagy is a primary route for degradation of α-synuclein but is dysregulated in PD. Currently, data on potential roles for OPTN in the pathogenesis of PD is very limited. Given OPTN’s roles in many cellular pathways implicated in PD pathogenesis, we wished to characterize basal brain expression and response to dopaminergic (DA) neurotoxins. Here, for the first time, we show immunohistochemical evidence that OPTN is enriched in the pars compacta region of the substantia nigra of untreated rats, in cells with a morphology characteristic of dopaminergic neurons. In primary rat mesencephalic cultures containing dopaminergic and nondopaminergic neurons, OPTN was primarily localized in DA neurons. We also demonstrate increased OPTN expression in DA neurons after methamphetamine exposure, compared to control, assessed by quantitative immunofluorescence. These data demonstrate that OPTN has high expression in
future directions of research, prioritizing a multidisciplinary approach. Dietary factors, obesity, and dysregulation in puberty onset have been reported. The study of these factors is crucial for understanding the determinants of pubertal timing. It has been demonstrated in animal and human studies that a highly heterogeneous group of exogenous substances interfering with the hypothalamic-pituitary-gonadal axis can alter pubertal timing. This phenomenon has been observed in various populations around the world and is known as the “pubertal transition.”

Pubertal timing significantly varies among individuals, and recent studies have demonstrated a progressive decrease in age of onset of puberty in children around the world. This is generally accepted to be due to a complex interaction between genetic, neuroendocrine, and environmental factors, acting in concert with one another in each individual. Determining the underlying mechanisms has been demonstrated in animal and human studies, and understanding these mechanisms can lead to the identification of new biomarkers for the early detection of pubertal disorders.

The purpose of this informational session is to broaden the dialog around the necessary steps for translating DIDI biomarkers for use in drug discovery and development, as well as highlighting ongoing research related to the discovery and validation of DIDI biomarkers across species. Traditional parameters used to detect DIDI include serum creatinine (sCr) and blood urea nitrogen (BUN). In all species, sCr and BUN lack sensitivity and/or specificity in detecting early stages of renal tissue injury identified by routine histopathology. The Critical Path Institute’s Predictive Safety Testing Consortium, the Foundation for the National Institutes of Health, the International Life Sciences Institute-Health and Environmental Sciences Institute (ILSI-HESI), and the European Union Innovative Medicines Initiative (IMI) Safer and Faster Evidence-Based Translation program are in the process of identifying and validating novel biomarkers of kidney injury and dysfunction.

Eight novel urinary protein kidney biomarkers have already been qualified for specific “context of use” in rat preclinical safety studies. Ongoing qualification of additional biomarkers in rats will be discussed. The potential utilities for the qualified rat renal biomarkers in canines and nonhuman primate preclinical toxicology studies will be reviewed. Paramount is the need for qualified renal biomarkers that allow for the monitoring of DIDI in the clinical setting. Efforts have been focused on bridging the gap between biomarkers being qualified in rats to nonrodent species and humans. During this informational session, selected data and other relevant information will be presented that underpins our overall translational strategy for the qualification of biomarkers in rodents leading to the qualification of safety biomarkers for use in the clinical trials.
383 Effects of Nitrated Fatty Acids on Phthalate-Induced Inflammation in Neonatal Neutrophils

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Rationale: Hospitalized infants are exposed to numerous devices that contain the plasticizer di-(2-ethylhexyl)phthalate (DEHP). Metabolites of DEHP, including mono-(2-ethylhexyl) phthalate (MEHP), are markedly elevated in the urine of premature infants and have the ability to alter neutrophil function. MEHP binds PPAR-γ, a nuclear transcription factor that mediates the resolution of inflammation, and is a process that is developmentally impaired in neonatal neutrophils. Nitroalkene derivatives of fatty acids (NO-FA), such as linoleic acid (LA, LNO), and oleic acid (OA, OA-NO3), are formed naturally in the body and are potent PPAR-γ ligands. We hypothesized that NO-FA would suppress the effects of MEHP by competitively binding to PPAR-γ. Methods: Neutrophils from umbilical cord and adult peripheral blood were isolated by density centrifugation and treated with LA, OA, LNO3, OA-NO3 (1μM) or medium control, in the presence or absence of MEHP (100-500 μM). H2O2 production was measured by Amplex Red assay. Cytokines were measured by cytomtric bead array analysis, and gene expression was measured by qPCR.

Results: MEHP induces dose dependent increases in H2O2 production and Cox-2 expression in neonatal neutrophils. Both OA-NO3 and LNO3 inhibit MEHP-induced hydrogen peroxide production and cyclooxygenase-2 (Cox-2) expression in neonatal neutrophils. While OA and LA did not inhibit hydrogen peroxide production, they were effective inhibitors of Cox-2 expression. OAN3 inhibited both basal and MEHP-stimulated IL-1β production.

Conclusions: These data demonstrate that nitrated lipids are capable of down-regulating MEHP-induced effects on neonatal neutrophil activity. Nitrated fatty acids are generated physiologically from dietary lipids. Our findings suggest that specific dietary alterations may help reduce the burden of inflammatory diseases in hospitalized neonates.

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385 3, 3-Diindolylmethane (DIM) Ameliorates SEB-Induced Acute Lung Injury: A Role for microRNA

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are characterized by pulmonary infiltration, hypoxemia, and edema. Among other etiologies, inhalation of toxic substances can lead to this serious condition. Staphylococcal enterotoxin B (SEB), a CDC select agent and public health threat during nosocomial infections, can be used to model ALI. Current clinical therapies for ALI and ARDS remain unreliable and mortality rates are still high. 3,3-diindolylmethane (DIM), a dietary compound found in cruciferous vegetables, has been recently shown to have anti-inflammatory properties and provides a potential therapeutic for ALI. We studied the mechanism underlying the ameliorative effects of DIM on SEB-induced ALI. Mice were exposed to SEB [50 mg; intranasal] and administered DIM [100 mg/kg] via oral gavage. Forty-eight hours after SEB exposure, serum, lung tissue and lung infiltrating mononuclear cells were collected. DIM treatment led to a decrease in lymphocytic infiltration, specifically CD4+, CD8+ and αβ+ T cells. DIM also led to a reduction of vascular leakage in lungs. Isolated mononuclear cells were subjected to high throughput microarray analysis to ascertain global microRNA expression levels. SEB led to dramatic microRNA dysregulation; in particular, modulating miRNAs such as miR-34a, -13a [decreased] and miR-21, -222 [increased], which were reversed in DIM treated mice. These microRNA have various gene targets dispersed throughout the apoptotic and cell cycle pathways, including SIRT1 and Bcl-2, as well as p27kip1, PUMA, CCND1, and CDK6, respectively. Moreover, DIM [10, 25, 50 μM] induced apoptosis in splenocytes and CD3+ T cells treated in vitro with SEB in a dose-dependent manner. Together, these studies demonstrate that DIM treatment may suppress SEB-mediated ALI by induction of apoptosis in T cells via alteration in expression of miRNA.

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384 Lipidomic Changes in the Pancreas and Plasma of Hepatic Alcohol Dehydrogenase-Deficient Deer Mice after Long-Term Ethanol Feeding

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Alcoholic pancreatitis (AP) is a major disease causing significant morbidity and mortality among chronic alcohol abusers. Disregulation of lipid metabolism is one factor that is involved in the early stage of AP can lead to inflammation and fibrosis and may be pancreatic cancer in some cases. However, the mechanism of AP is not well understood due to the lack of a suitable animal model. Earlier, we reported pancreatic injury and a significant endogenous formation of fatty acid ethyl esters (FAEEs) in the pancreas in hepatic alcohol dehydrogenase (ADH)-deficient (ADH-) deer mice fed 3.5 % ethanol daily for 2 months. Therefore, we evaluated the lipidomic changes and FAEE levels in the lipids extracted from the pancreas and plasma of ADH- deer mice fed ethanol for 3 or 6 months using 800 MHz proton (1H)-NMR spectroscopy and GC-MS. NMR data was analyzed by hierarchical clustering and principal component analysis. Pancreatic and plasma total lipid levels increased in mice fed ethanol daily for 3 months, but decreased for those fed daily for 6 months. Decreases were found for phosphatidycholine (PC) related protons in plasma of mice fed ethanol for 3 or 6 months while this change was significant only at 3 months in the pancreas. However, peaks corresponding to the protons of the dialylic group were increased in the pancreatic lipids as compared to a decrease at 3 months and an increase at 6 months in the plasma. The increased signals of FAEEs in the pancreas were confirmed by GC-MS analysis. Our findings suggest that metabolism of ethanol under hepatic ADH deficiency (a metabolic condition found during chronic alcohol abuse) generates FAEEs and decreases PC resulting in mild fatty deposition in the pancreas and its progression to pancreatitis with increasing the duration of ethanol feeding.

In response to excessive nucleotide-binding oligomerization domain–containing protein 2 (Nod2) stimulation caused by mucosal bacterial components, gut epithelia need to activate regulatory machinery to maintain epithelial homeostasis. Activating transcription factor 3 (ATF3) is a representative regulator in the negative feedback loop that modulates TLR-associated inflammatory responses. In the current study, the regulatory effects of ribosomal stress-induced ATF3 on Nod2-stimulated pro-inflammatory signals were assessed. Ribosomal inactivation caused persistent ATF3 expression that in turn suppressed pro-inflammatory chemokine production facilitated by Nod2. Decreased chemokine production was due to attenuation of Nod2-activated NF-κB and early growth response protein 1 (EGR-1) signals by ATF3. However, the underlying molecular mechanisms involve two convergent regulatory pathways. Although ATF3 induced by ribosomal inactivation regulated Nod2-induced EGR-1 expression epigenetically through the recruitment of histone deacetylase 1, NF-κB regulation was associated with posttranscriptional regulation by ATF3 rather than epigenetic modification. ATF3 induced by ribosomal inactivation led to the destabilization of p65 mRNA caused by nuclear entrapment of transcript-stabilizing human Ag R protein via direct interaction with ATF3. These findings demonstrate that ribosomal stress-induced ATF3 is a critical regulator in the convergent pathways between EGR-1 and NF-κB, which contributes to the suppression of Nod2-activated pro-inflammatory gene expression. (This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008405032012)” Rural Development Administration, Republic of Korea.)
Role of TRIF and MAP Kinases in TLR3 & 4-Mediated Regulation of Drug Metabolizing Enzymes and Transporters

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Purpose: The expression and activity of hepatic drug metabolizing enzymes and transporters (DMET) is altered on activation of an inflammatory response by bacterial or viral components. Inflammation in the liver is mediated primarily by the Toll-like receptors (TLR). TLR3 and TLR4 mediate responses by viral and gram -ve bacterial components respectively, through adaptor molecules Toll/interleukin (IL)-1 receptor (TIR) domain containing adaptor protein and TIR domain containing adaptor inducing interferon (IFN)-β (TRIF). We have shown TLRs regulate gene expression of DMET however, the mechanism by which TLR signaling regulates these genes is not known. We hypothesize that TRIF and MAP kinases (MAPK) play a role in down-regulation of DMET through TRIF and TLR4.

Methods: Male, TRIF+/+ or TRIF−/− mice (6-8 weeks) were treated with saline, Poly I:C (5mg/kg) or LPS (2mg/kg) for 8 and 16h respectively. Liver tissues were harvested and stored at -80°. To assess the roles of MAPK, primary hepatocytes were isolated from C57BL/6 mice and treated with and without the following inhibitors: JNK (SP600125, 10µM), ERK (PD98059, 20µM) and p38 (SB203580, 25µM) for 30 min followed by treatment with Poly I:C (50µg/ml) or LPS (1µg/ml). RNA was extracted from livers/cells and gene expression was analyzed by qPCR analysis.

Results: In TRIF+/- mice, Poly I:C treatment led to down-regulation of Cyp3a11, Cyp2a4, Cyp1a2, Cyp2e1, Ugt1a1, Mrp2 genes where as LPS treatment led to down-regulation of Cyp3a11, Cyp2a4, Ugt1a1 and Mrp2 genes. Surprisingly, this down-regulation was not attenuated in TRIF−/− mice. In hepatocytes, Poly I:C and LPS down-regulated the gene expression of DMET in the absence of inhibitors. While JNK inhibitor completely attenuated the down-regulation, p38 and ERK inhibitors did not affect down-regulation of DMET through TLR3 or TLR4.

Conclusion: These results show that down-regulation of DMET through TLR3 & 4 is dependent on JNK where as it is independent of the adaptor protein TRIF.

Regulation of Drug Metabolizing Enzymes and Transporters

P. 387

Coculture of Hepatocytes and Kupffer Cells As an In Vitro Model of Inflammation and Liver Hepatotoxicity

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Immune-mediated chemical-induced hepatotoxicity, i.e. indirect hepatocellular toxicity resulting from immune cells activating liver inflammatory responses, is often overlooked as a potential mode of action due to unavailability of appropriate in vitro models. We have established an in vitro rat co-culture model using Kupffer cells (KC) and hepatocytes (HC) to study how inflammation impacts chemically induced effects on liver. Optimal cell culture conditions have been identified to maintain HC, KC, and KC/HC co-cultures for 48 hours with varying doses of chemicals +/- LPS, representing diversity in the cytokine profiles that may be reflective of compound-specific mode of action in vivo.

Intratracheal Bleomycin (ITB) induces pulmonary injury, inflammation, resolution and subsequent fibrosis. We hypothesize that recruited cells are critical for the inflammatory process, while resolution is dependent upon resident cells. To study the cell populations involved in ITB we used green fluorescent protein (GFP) chimeric mice. Recipient bone marrow was ablated with X-ray radiation and subsequently replaced with GFP+ bone marrow cells. Following 6 weeks of recovery, mice were either intratracheally instilled with saline or 3units/kg bleomycin. Mice were sacrificed at (inflammation phase) or 14 days post ITB. Bone marrow cells and BAL cells were collected and analyzed by flow cytometry. The mean bone marrow replacement was 85% ± 5% GFP+. While resident alveolar macrophages were GFP-, 8 days post injury, GFP- populations were similar between groups in number and appear as mature macrophages (F4/80+ CD11c+). The recruited populations, GFP+, are also primarily F4/80+ CD11c+. In saline treated mice the GFP+ cells are unactivated (CD11b(low)Ly6C−). In ITB treated mice these cells are activated (CD11b(high)Ly6C+). At 15 days there is minimal change in either resident or recruited cell populations with respect to 8 days for saline treated mice. However, in ITB treated mice GFP+ F4/80+ CD11c+ cells are reduced in number, while GFP+F4/80+ CD11c− Ly6C+ are increased in relative quantity. In addition, a novel population of F4/80+ CD11c− is seen in both recruited and resident cells. Recruited but not resident F4/80+ CD11c− cells are activated as they are Ly6C+. We propose that the GFP+ F4/80+ CD11c− cells are resident macrophages that are mediating the resolution process. These studies show that only recruited cells display an acute activation profile in response to ITB at either 8 or 15 days. However, resident cells do differentiate their phenotype by 15 days post ITB, possibly as a mechanism to mediate resolution. Supported by NIH grants F31GM108463, HL086621, TESS05022

Inhalation Exposure to the Tobacco Smoke Component Acrolein Enhances Allergic Responses to Ovalbumin

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Acrolein, an α,β-unsaturated aldehyde generated in part through cooking oils and smoking tobacco, is a highly reactive electrophilic respiratory irritant implicated in asthma pathogenesis. However, few studies have directly investigated the influence of acrolein exposure on allergic sensitization and pulmonary inflammation. This study uses a murine inhalation model to examine whether inhaled acrolein alone or in combination with inhaled ovalbumin (OVA) enhances allergic sensitization to subsequent OVA challenge. Adult male C57BL/6 mice were sensitized to inhaled OVA (1%, 30 min/day, 4 days/week) and/or inhaled acrolein (5 ppm, 4 h/day, 4 days/week) over 2 weeks before challenge with aerosolized OVA (1%, 30 min/day) over three days. Sera, bronchoalveolar lavage (BAL) fluids, and lung tissues were collected after OVA challenge to assess pulmonary inflammation and allergic sensitization. Serum anti-OVA IgG1 levels were increased 4-fold in animals sensitized to both OVA and acrolein, compared to animals sensitized to OVA alone or acrolein alone. In addition, differential cell counts revealed an increase in BAL neutrophils among animals sensitized to OVA and acrolein. Acrolein sensitization was also observed to increase lung mRNA levels of Il13 and Il1a. Interestingly, exposure to both OVA and acrolein did not influence Il13 and Il1a mRNA levels, though it did significantly increase Ccl20 mRNA levels. This study suggests that prolonged inhalation can enhance allergic sensitization and lead to worsened airway inflammation.

Effects of Current Tobacco Exposure on T Cell Immune Responses

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Epidemiological studies have shown that smokers suffer from more frequent infections and infectious complications. The scope of the present study was to test the hypothesis that tobacco smoke exposure (TSE) modulates T cell function, thereby leading to impairment of cellular and humoral responses to the influenza vaccine. Monozygotic twins (MZT) ages 3 to 55 yrs were studied. Each participant’s PBMC immune profile was determined 7 days after flu immunization by measuring expression of 37 different cell surface markers using CyTOF analyses and 51 different serum proteins using Luminesix technology. Influenza vaccine responses were quantified by hemagglutination inhibition (HAI) 28 days after immunization. Associations between TSE and a selection of T cell subsets, some related serum cytokines and vaccine responses were investigated by comparing twins discordant for TSE (i.e. ever smoked vs. never smoked) and concordant for TSE (i.e. both ever smoked or both never smoked).
The percentage of T cells, CD4+, CD8+ and regulatory T cells (p<0.001), as well as serum levels of IFNγ, IL-5 and TNFα (p<0.05), were significantly lower in ever smoking individuals compared to their non-smoking twins (n=16 pairs). If restricted to only current smokers (n=12), stronger significances were seen (p≤0.0002). No differences were seen between TSE concordant twins. Preliminary results indicate no significant differences (p>0.94, n=4 pairs) in vaccine responses between smoking and non-smoking twins, but a larger number of current smokers is needed to draw conclusions.

Overall, our preliminary data suggest that current tobacco smoke exposure may affect T cell immune responses and related cytokines, which may have implications for the development of immune diseases and infection resistance.

**392 Exposure to Sidestream Tobacco Smoke Induces Airway Hyperresponsiveness and Inflammation in Early Postnatal Mice**


There are abundant epidemiological studies linking early environmental tobacco smoke (ETS) exposure with childhood asthma. The present study was designed to determine changes on airway responsiveness and innervation after ETS exposure during early postnatal period. The postnatal day (PD) 2 mice were exposed to either ETS or filtered air (FA) for 10 consecutive days. Lung function, substance P (SP) airway innervation, and nerve growth factor (NGF) and cytokines in bronchoalveolar lavage (BAL) fluid were measured 1, 3 and 7 days after last ETS exposure. 1 day after the last ETS exposure, airway responsiveness and SP innervation in airway smooth muscle were significantly increased, as well as altered airway inflammation, which include significant elevated levels of IL-1β, IL-4, IL-6, IL-10, TNFα and INFγ in BAL compared with FA exposure. Interestingly, 3 days after ETS exposure, airway hyperresponsiveness and SP innervation in airway smooth muscle remained elevated, parallel with high levels of IL-1and NGF. These results indicated that the change in SP airway inflammation play an important roles in airway inflammation and hyperresponsiveness induced by ETS exposure during early in life.

**393 Comparison of Proinflammatory Cytokine (IL-6 and IL-8) Release from Two Types of Cells Exposed to Mainstream Cigarette Smoke Total Particulate Matter**

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Mainstream cigarette smoke is a complex aerosol consisting of thousands of chemical compounds and oxidants, which can cause cellular oxidative injury and inflammation involved in the development and progression of human respiratory diseases such as chronic obstructive pulmonary disease (COPD). Many cell lines are available to be used as the *in vitro* models for investigating the mechanisms of cigarette smoke-induced diseases. Different types of cells can respond to cigarette smoke-induced toxicity differently. AS49 cells (human adenocarcinoma cells) and BEAS-2B cells (human bronchial epithelial cells) widely used in many laboratories are representative two types of cells. The purpose of this study was to compare the difference in proinflammatory cytokine release from two types of cells in response to cigarette smoke total particulate matter (TPM). Cells were exposed to TPM for 24 h. The neutral red uptake (NRU) assay was employed to assess the cytotoxicity of cigarette smoke, and the levels of proinflammatory cytokine (IL-6 and IL-8) were measured by ELISA. The results showed that BEAS-2B cells were more sensitive to smoke-induced cytotoxicity than AS49 cells. TPM of mainstream cigarette smoke caused dose-dependently increase of cytokine IL-6 and IL-8 release from BEAS-2B cells. While the induction of IL-6 and IL-8 release from AS49 cells was not impacted after TPM exposure, and in contrary, the release of IL-6 and IL-8 decreased with the concentration of TPM solutions increased, compared to control cells. These results indicate that the inflammatory response to cigarette smoke can be different depending on the characteristic of cells, and non-tumor cells might be an appropriate model for investigating the smoke-induced inflammatory mechanisms.

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**394 The Effects of Ozone Exposure on Inflammasome Activation and Heart Failure during CVB3 Myocarditis**

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Ozone (O3) is a common secondary air pollutant that contributes to increased morbidity and mortality. Studies have shown a positive correlation between O3 exposure and emergency department visits for the exacerbation of pulmonary conditions. Ozone is also linked to cardiovascular conditions such as cardiac ischemia and heart attacks. In this study, we explored the effects of O3 on heart failure (HF) during coxsackievirus B3 (CVB3) myocarditis. Myocarditis is an autoimmune disease that is characterized by inflammation of the myocardium with or without cardiomyocyte damage. Viral infections, such as CVB3, are the most common cause of myocarditis in the US. Myocarditis is a very important public health issue as it goes largely undiagnosed, and can progress to other conditions like HF and dilated cardiomyopathy (DCM). The Fairweather lab found marked sex differences in the incidence and severity of disease; and has established that testosterone increases myocarditis and DCM in mice with CVB3 myocarditis by increasing IL-1β and the inflammasome. Air pollution can exacerbate the underlying inflammation in myocardium and drive HF. O3 has been shown to increase this pathway in the lung.

We hypothesized that O3 exposure during CVB3 myocarditis activates the inflammasome via TLR4 and increases IL-1β, which may drive HF in males. We explored this hypothesis by injecting 8-10 week old BALB/c mice with CVB3 or PBS intraperitoneally at day 0, and then exposing them to either room air (RA) or ozone (O3) at 0.3 ppm, continuously for 48 hours at days 8 and 9. Mice were subsequently sacrificed at day 10. The interaction of CVB3 and O3 increased protein expression of IL-1β. Furthermore, pressure-volume loop data showed that simultaneous exposures to O3 and CVB3 increased the risk for HF in mice. To our knowledge, this is the first study to explore the interaction between CVB3 infection and O3 exposure in HF. Through this study we can provide a mechanistic explanation for how air pollution increases the risk for cardiovascular events.

**395 Role of Spleen Monocytes (Mo) in Ozone-Induced Lung Inflammation and Injury**

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Ozone is a ubiquitous air pollutant known to damage the lung. Activated macrophages (MP) have been implicated in ozone toxicity; however, the origin and phenotype of these cells is unknown. Evidence suggests that the spleen functions as an extramedullary source of inflammatory monocytes (Mo). In these studies we used spleenectomized (SPX) mice to assess the contribution of spleen Mo to the pulmonary inflammatory response to inhaled ozone. Lung, spleen, and bone marrow were collected 24-72 h after exposure of mice to ozone (0.8 ppm, 3 h) or air and analyzed by flow cytometry. Ozone caused increases in bronchoalveolar lavage (BAL) protein and MP, markers of lung injury and inflammation. Resident alveolar MP from control animals expressed F4/80 and Cd11c+, but not Cd11b+. Following ozone exposure, increases in immature Cd11b+F4/80+ and mature Cd11b+F4/80+ MP were observed in the lung. Subpopulations of these cells were identified that expressed high and low levels of the proinflammasome Mo/MP marker, Ly6C+. Both immature Cd11b+F4/80-Ly6Chi and mature Cd11b+F4/80+Ly6Chi proinflammatory MP increased in the lung after ozone, reaching a maximum at 48 h. This correlated with a decrease in Cd11b+Ly6Chi Mo in the spleen. Conversely, Cd11b+F4/80-Ly6Chi proinflammatory Mo increased in the bone marrow. Splenectomy resulted in a decrease in the total number of inflammatory cells in the lung after ozone, and reduced tissue injury. Flow cytometric analyses revealed a reduction in immature (Cd11b+F4/80-Ly6Chi) and mature (Cd11b+F4/80+Ly6Chi) proinflammatory MP in lungs of SPX mice, but increases in mature Cd11b+F4/80+Ly6Chi anti-inflammatory MP. Cd11b+F4/80-Ly6Chi immature proinflammatory Mo in bone marrow of ozone-treated SPX mice were also decreased relative to sham control. These data suggest that the spleen is a source of proinflammatory/cytotoxic MP that contributes to ozone-induced lung injury. Moreover, in the absence of this splenic reservoir, there is a compensatory increase in inflammatory cells generated in the bone marrow. Supported by NIH ES004738, CA132624, AR055073, ES007148 and ES005022.
Subsequent Ozone Exposures In Vitro Result in Attenuation of Inflammatory Response
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Approximately 133 million residents of the United States live in areas where ozone levels exceed the National Ambient Air Quality Standards. Some of these regions may experience such exceedances on a daily basis. While the health effects of acute ozone exposure are well documented, the effects of chronic exposures are less understood. Given that many people are exposed to harmful levels of ozone on a chronic basis, it is important to characterize the physiological effects of subsequent, daily exposures. Controlled human exposure studies have shown that response to ozone varies between acute and chronic exposure conditions. If only exposed to ozone for one to two days, subjects present with increased airway inflammation and decreased lung function. However, additional exposures up to six days result in the attenuation of these effects. We were interested in investigating the mechanism underlyng this phenomenon using an in vitro model. To accomplish this we used primary human bronchial epithelial cells from healthy volunteers grown in air-liquid interface culture and exposed them to 0.3 ppm ozone or clean air for two hours per day for seven consecutive days. Because inflammatory state varied between chronic and acute exposures, we chose to measure the expression of IL-8, IL-6, and IL1-β, as these are key mediators of inflammation. The expression of these genes peaked following a single day of ozone exposure. During subsequent days of exposure the ozone-mediated induction of pro-inflammatory mediators declined and eventually approached baseline levels. Thus daily ozone exposure in vitro produces a similar suppression of the pro-inflammatory response as observed in clinical studies. Using this model, we plan on identifying the central mechanisms responsible for this effect, which may include altered signaling pathways and epigenetic modifications. Understanding the health consequences of this effect may help identify and treat vulnerable populations.

Development of an In Vitro Functional Assay for Human Macrophages
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Macrophages play a critical role in the defense against pathogens. Drugs being developed to treat inflammatory diseases often target macrophages, which are potent producers of cytokines and other inflammatory mediators. These drugs have the potential to compromise host resistance by inhibiting other macrophage functions (e.g. oxidative burst and phagocytosis) besides cytokine production. The objective of these studies was to develop an in vitro method to measure oxidative burst and phagocytosis in human monocyte-derived macrophages. CD14 positive monocytes were isolated from whole blood using magnetic beads and then stimulated with granulocyte macrophage colony stimulating factor to generate macrophages, and cultured for various incubation periods (from 2-9 days). The macrophage phenotype was verified using the CD206 and -HLA-DR antigens. The expression of microbeigen species (ROS) generation and phagocytosis were measured using FcOxyburst and pHrodo® Bioparticles, respectively. Assay conditions (4 days of culture and 50,000 cells per assay tube) and length of pre-incubation time with inhibitors were optimized for maximal viability and function of the macrophages. Typical assay results show that more than 50% of the CD206-expressing macrophages are phagocytic and more than 90% produce ROS. Cytochalasin D and N-acetyl cysteine were used to inhibit phagocytosis and ROS generation, respectively. Results showed that macrophage immune functions could be measured and inhibited in this assay. These data suggest that the macrophage function assay can be used as a tool for determining the potential of drug candidates to inhibit phagocytosis and oxidative burst.

Evaluation of Sex Sensitivity in Local Lymph Node Assay Using Acetophen and α-Hexylcinnamaldehyde
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The current OECD Test Guideline for the conduct of the Local Lymph Node Assay (LLNA) recommends the use of only female mice for the assessment of skin sensitization potential for a given chemical. The NIH publication No. 99-4494 recommends that only female CBA mice be used, as they reportedly develop a greater tendency to fight and to be involved in ‘social ranking’ behavior when group housed. However, there are several advantages to consider with the inclusion of male mice in LLNA testing including a more refined and responsible use of animals. Therefore, to begin to systematically assess the appropriateness of using male mice in the LLNA a comparative guideline study was conducted with individual housing of mice using the sensitizer, Butachlor 50% EW (BU50) and α-hexylcinnamaldehyde (HCA) respectively. The study compared 5 male and 5 female CBAJ mice/group, at three BU50 concentrations [10%, 25%, 50% (w/v)] and a single HCA concentration [25%] using 1% lotic as the vehicle. Stimulation indices (SI) for the three BU50 treatment groups were 1.16, 1.58 and 1.59 for male and 0.39, 1.55 and 1.56 for females, respectively and SI for the HCA treated group were 4.31 for male and 5.44 for females. The results revealed no significant difference between samples means or variability between the sexes and the SI values observed for both male and female mice of positive control were within the range of historical control data for female mice. These data provide initial support for the use of male mice in the LLNA and will be followed by further experimentation.

Impact of Collection Site and Method on Blood Lymphocyte Subsets in Sprague-Dawley Rats

Immunotoxicology studies in rats often include peripheral blood collection from multiple anatomical sites, and can vary due to non-terminal or terminal collection. Differences in lymphocyte subset counts based on collection site in humans and mice have been recently reported. In order to better control for potential cell count differences between multiple anatomical blood sampling sites in rat research studies, lymphocyte cell counts were performed for samples from the lateral tail or saphenous veins (non-terminal) or inferior vena cava (terminal) of Sprague Dawley rats. Non-terminal samples were collected without anesthesia, and terminal samples were collected after CO2 overdose. Additional lateral tail vein samples were drawn after animals were heated to dilate the vein, for comparison to samples from non-heated animals. T, B, and natural killer (NK) cell subsets were evaluated using a flow cytometer.

In two separate studies, it was found that NK cell percentage and counts were increased, while T cell counts and percentages were decreased in inferior vena cava terminal samples compared to non-terminal samples. Cytotoxic and helper T cell counts were concomitantly lower in inferior vena cava samples relative to...
non-terminal samples. B cell and total CD45+ lymphocyte counts were not impacted by collection site. Pre-heating animals to dilate the lateral tail vein did not impact lymphocyte subset counts.

These results highlight the importance of understanding the possible impact of non-terminal and terminal blood collection, or collection from peripheral and central anatomic sites, on study design and data interpretation.

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**401 The Guinea Pig Systemic Anaphylaxis Model Revisited**

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The guinea pig systemic anaphylaxis model was introduced decades ago to evaluate the sensitizing potential of pharmaceuticals and chemicals. After years of use, it was nearly abandoned, but is attracting renewed interest for the evaluation of pharmaceutical macromolecules or medical devices. As standardization of this model has been meager, the aim of this study was to analyze data obtained with the positive reference compound ovalbumin (OVA) using various experimental modalities. A total of 14 studies including 285 male Dunkin-Hartley guinea pigs were selected. Groups of 5 animals received 1, 2, 3 or 6 sensitizing injections of 0.2, 2 or 20 μg OVA by the intraperitoneal (0.5 ml), subcutaneous (0.1 ml) or intradermal route (0.1 ml). Few animals received aluminum hydroxide with the first sensitizing injection. The delay between the injections was 7 days, except in some animals (2 injections, 14 days apart). After a rest period of 7 or 14 days, the animals were challenged with 0.2 or 20 μg OVA (0.1 ml) via the penile vein (or the ear vein in rare animals). Controls received injections of saline using the same route, volume and injection schedule. Sensitization was evaluated from signs of anaphylaxis observed during a one-hour follow-up. The retrospective analysis of the results shows that the optimal sensitizing dose of OVA was 2 μg. Inconsistent results were obtained with 0.2 μg. Results were similar with the subcutaneous and the intradermal route, but were more variable with the intraperitoneal route. Similar clinical signs were observed after 2, 3 or 6 sensitizing doses with the same challenging dose, but were less consistent after 1 sensitizing dose. The optimal challenging dose of OVA was 20 μg and no overt differences were seen in animals given aluminum hydroxide. The conclusion of this retrospective analysis is that an optimal study plan consists of 2 sensitizing subcutaneous injections of 2 μg OVA with a 7-day interval followed by a challenging intravenous injection of 20 μg OVA after a 14-day rest period.

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**402 Transferability and Reproducibility of the Myeloid U937 Skin Sensitization (MUSST) Test for Skin Sensitization Testing**


Skin sensitization is a disturbed immunological process in which a sensitizer is a substance that triggers an immunological cellular response. Initially, the substance penetrates the epidermis prior to the formation of a stable conjugate with skin proteins. This stable conjugate is then processed by the epidermal dendritic cells, which subsequently mature and migrate out of the skin to the local lymph nodes. The MUSST test method is modeling the dendritic cell activation upon exposure to sensitizers by means of measuring phenotypical changes in the U937 human myeloid cells. Like the dendritic cells, upon contact with sensitizers, U937 cells induce CD86 expression as they are activated. Cell viability is assessed using propidium iodide exclusion. Both parameters are measured by flow cytometry. The prediction model is defined by a stimulation index above 150 with a dose response relationship and at least 2 concordant experiments, for identifying a substance as Sensitizer S/Non-Sensitizer NS.

The aim of this study is to demonstrate the transferability and reproducibility of the MUSST in 4 laboratories and define its predictivity.

The main determinant of the test method reliability assessment was the concordance of classification. S/NS. The intra-laboratory reproducibility (WLR) study was assessed with 21 chemicals tested in 3 independent experiments within L’Oréal. Similar prediction was obtained resulting in a WLR of 95%. Eleven out of the 14 substances were consistently classified (S/NS) by the 4 laboratories resulting in an inter-lab reproducibility (BLR) of 79%. The BLR for the pair-wise comparisons was comprised between 86-93%.

To determine the predictive capacity of the MUSST, a set of 123 substances has been evaluated. The predictive capacity of the MUSST is 86.2% with a sensitivity of 86.1% and a specificity of 86.4%.

The MUSST is an efficient transferable and reproducible assay for the skin sensitization hazard characterization, promising as a tool to be integrated within a battery of assays to perform a skin sensitization risk assessment.

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**403 Time Course Difference in Sensitivity and Sensitivity of Immunotoxicity of Cyclophosphamide in Female Swiss Albino Mice**

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The study was conducted to evaluate the time course difference in sensitivity and sensitivity of in-house bred female Swiss Albino mice to the immunosuppressive effect of Cyclophosphamide Monohydrate (CPM). The mice were treated with CPM by intraperitoneal administration for 4 consecutive days at dose levels of 5, 25 and 50 mg/kg body weight/day, dissolved in Phosphate Buffer Saline (PBS). Concurrent control group animals were dosed with vehicle (PBS) alone. On the first day of treatment, all animals were treated with SRBCs (Innovative Research, USA) in normal saline (8 x 107 RBCs) at fixed dose volume of 0.2 ml/animal intravenously. Eight animals from each group sacrificed on day 5 and four animals per group were sacrificed on day 15. Significant reductions in anti-SRBCs IgM antibody level, WBC count, neutrophil count and absolute and relative weight of spleen and thymus were observed in animals sacrificed on day 5 from groups treated with 25 and 50 mg of CPM/kg body weight whereas significant changes were not observed in animals treated with 5 mg/kg b. wt. dose. Animals sacrificed on day 15 showed no significant immunotoxic changes when compared to control group suggesting recovery. The effects observed in the 25 and 50 mg/kg dosed animals sacrificed on day 5 were consistent with immunotoxic effect of CPM seen in previous immunotoxicity studies in mice. Based on these results, it can be concluded that the time course for treatment and sacrifice of Swiss albino mice in immunotoxicity studies is critical and also demonstrates that in house Swiss Albino mice give responses similar to other recommended strains (e.g. B6C3F1 hybrid mouse).

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**404 Quantitation of Leukocyte Subsets and Leukocyte Activation in Canine Blood Using a 7-Colour Flow Cytometry Assay**

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We developed and validated a 7-colour flow cytometry assay to identify lymphocytes, monocytes, granulocytes, T cells, T cell subsets, B cells and HLA-D expression in canine whole blood. Six commercial antibody conjugates recognizing canine leucocyte antigens were used: CD3FITC, CD21PE, HLA-DP/EC5, CD4ECD7, CD8AFE674 and CD14AF647, in combination with the DNA-binding dye DAPI and the LiveDead viability dye. Whole blood (EDTA, 100 μl) was incubated with lysing buffer, washed and labeled with the viability dye. Cells were stained with CD3/CD21/CD4/CD8,CD14 and HLA or control, washed, fixed and incubated in DAPI. Counting beads were added and samples analysed on the flow cytometer. Data for frequency (%), absolute number (cells/μl) and HLA-D mean fluorescence intensity (MFI) were reported. Viable leucocytes were identified with DAPI and LiveDead stain. Monocytes were gated as the CD3-CD64-+ cluster from a plot of CD3/CD14 versus CD3. Lymphocytes were identified by light scatter and Boolean Logic to exclude monocytes. The T and B cells were identified by expression of CD3 and CD21 respectively and T cell subsets by expression of CD4 and CD8 antigens. Granulocytes were identified from a plot of side scatter (SSC) and CD4 with the granulocyte cluster expressing the CD4+ phenotype and a granulocyte subpopulation with CD4- phenotype. In naïve animals, differential MHC class II expression was observed on lymphocytes but not on granulocytes. Intra-assay precision of ≥ 20 % CV was demonstrated for all major cell types for frequency, absolute values and MFI. Whole blood was stable up to 24 h post-collection and stained samples stable up to 24 h when stored refrigerated. This assay was validated with acceptable precision, stability and robustness and is applicable for assessing the impact of test articles on leucocytes and leucocyte activation in canine peripheral blood.

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**405 Comparison of the Lymphocyte Subpopulations from Peripheral Blood of CD1, CBA/J and Swiss Albino Female Mice following Administration of Cyclophosphamide Monohydrate**

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The prevalent guidelines (OCSPP, 870.7800) for determination of immunotoxic potential recommend use of CD1 mice. Considering the diversity of strains existent with different toxicology labs, a comparative study of immunosuppressive activity induced by cyclophosphamide monohydrate (CPM) was evaluated in CD1, CBA/J and Swiss albino female mice.
The immunotoxicity of a compound is ideally determined by assessing all three components of an immunotoxicity evaluation. Future studies will include development of an assay to measure CTL function by direct assessment of antigen-specific target cell killing by non-human primate CD8+ T cells.

**Quantitative Immunophenotypic Analysis of Rat Thymocytes**


Quantitative immunophenotypic analysis of rat thymocytes is often required for the evaluation of test article effects; changes of thymocyte subsets reveal the test article effects and the severity of the effects. In this study, two methods for assessment of absolute thymocyte numbers were evaluated. Thymuses were harvested and weighed from 15 male and 15 female rats at 12 to 13 weeks of age. Single cell suspension was prepared and thymocytes enumerated for FACs analyses. Thereafter, two ways of absolute cell number evaluation were compared: cell number per thymus and cell number normalized by thymus weight. Cell number per thymus was positively correlated to thymus weight; heavier thymuses yielded more cells, thus male rats had more thymocytes per thymus compared to female rats (Mean±SD: 1136.7±296.4 million cells/thymus and 990.3±218.1 million cells/thymus for male and female rats, respectively). These variations (CV=25%) indicate that cell number per thymus is not a valid basis for calculation of absolute numbers for thymocyte subsets. However, when thymocyte numbers were normalized by thymus weight, results were more comparable among all rats (CV=14%) and between sexes (Mean±SD: 2714.1±359.8 million cells/g and 2712.0±421.5 million cells/g for male and female rats, respectively). Based on the weight normalized thymocyte number, absolute number could be calculated for thymocyte subsets: CD3- cells, CD3-CD4+CD8- cells, CD3-CD4-CD8+ cells, CD3-CD4+CD8+ cell, CD3+ cells, CD3+CD4+CD8- cell, CD3+CD4+CD8+ cells, and CD3+CD4+CD8+ cells. In conclusion, weight normalized thymocyte numbers are a valid basis for calculation of absolute numbers of thymocyte subsets. These absolute numbers can be used for statistical analyses to assess the effects of test articles on the thymus. Furthermore, because the variation of weight normalized thymocyte numbers is small between male and female rats, the two sexes can be combined for statistical analyses when animal numbers/sex/group are inadequate.

**Application of the KeratinoSens™ Assay for Assessing the Skin Sensitization Potential of Crop Protection Active Ingredients and Formulations**

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Recent regulations have advocated for the development of non-animal approaches that can reliably predict the skin sensitization potential of low molecular weight chemicals. The KeratinoSensTM assay has emerged as one of the most promising in vitro assays for predicting skin sensitization of single chemicals, however its performance with formulations and mixtures has not been previously addressed and is a significant need. To assess this potential application, the KeratinoSensTM assay was conducted for 8 crop protection active ingredients including 3 sensitizers (acetochlor, metyldinocap, triclopyr butoxyethyl ester), 5 non-sensitizers (aminopyralid, clopyralid, florasulam, methoxyfenozide, oxyfluorfen) and 10 correspond-
For immunotoxicology testing, there is a big demand to replace animal testing by in vitro experiments. Whole genome miRNA expression analysis on cells exposed to test chemicals in vitro promises to be a valuable option for this aim. The immunological response involves many different cell types. It is therefore likely that multiple immune cell types have to be included to enable adequate evaluation of immunotoxic hazard.

In this study, the value of the mouse thymoma EL-4 cell line as an in vitro screening model for immunotoxicity was assessed using two immunotoxic model compounds, the mycotoxin deoxynivalenol (DON) and the organotin compound tributyltin oxide (TBTO). EL-4 cells were exposed to 0.25, 0.5 and 1 mM DON for 3, 6 or 11 h or to 0.5 and 1 mM TBTO for 3 or 6 h. Biological interpretation of the microarray data revealed that part of the previously detected modes of action of TBTO and DON in the Jurkat T cell line were not observed in the EL-4 cell line. TBTO induced genes involved in calcium signalling and ER stress but did not induce genes involved in T cell activation and apoptosis. DON induced RNA related processes and ribosome biogenesis. Furthermore, DON downregulated ER stress, T cell activation and apoptosis which is opposite to the mechanism of DON observed in the mouse thymus in vivo and in Jurkat T cells in vitro. Apparently, EL-4 cells lack factors that are important to link ribotoxic stress to ER stress and cannot elicit a T cell activation response. Based on the results obtained for TBTO and DON, it can be concluded that the EL-4 cell line has no added value for immunotoxicogenomics based screening.

The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is a free resource that forms hypothesis development about environmental effects on human health. CTD content consists of manually curated chemical-gene-disease interactions, which are integrated with functional information, canonical pathways, and novel tools to infer relationships between chemical exposures and biological events. Here, we highlight new analysis and data visualization features of CTD, including the ability to generate novel pathways associated with chemicals and diseases. We also report results from a pilot project in which we expanded our curations to include relationships between chemicals and non-disease phenotypes (e.g., cell proliferation, adipogenesis). The goal of this project was to determine if including chemical-phenotype data in CTD could advance: a) understanding of environmental disease progression, b) identification of exposure biomarkers, and c) the capacity to conduct and interpret studies across species and experimental systems. We integrated chemical-phenotype and disease data from this pilot project and performed a two-dimensional hierarchical clustering that revealed 18 disease clusters with distinct phenotype profiles. Our results suggest the potential for this strategy to generate meaningful links between chemicals, early cellular phenotypes and diseases. We continue to expand the depth and functionality of CTD to meet the evolving needs of the environmental health research community.

Drug-induced liver injury (DILI) is one of the leading causes of drug development failures and drug withdrawals. DILIsym® is being developed to identify and mitigate DILI risk through in silico analysis of compounds. The DILIsym® representation of the innate immune response was initially based on acetaminophen (APAP) data. Carbon tetrachloride (CCl4), a compound with hepatoxic similarities to APAP, was simulated for further evaluation of the innate immune response to liver injury. DILIsym® simulation results for CCl4 pharmacokinetics were consistent with published PK data. CCl4 is generally thought to induce hepatotoxicity via a free radical metabolite which drives lipid peroxidation. However, when CCl4 was simulated via DILIsym®, free radical generation and lipid peroxidation to levels consistent with the public literature were insufficient to drive hepatotoxicity. Analysis demonstrated that saturation of the metabolic pathway generating the free radical limited the extent of lipid peroxidation and thus the extent of cell death. In comparison, the APAP metabolic pathway had a greater dynamic range, resulting in higher levels of lipid peroxidation and cell death. Papers were identified in which lipid peroxidation independent mechanisms of cell death were suggested, including lipid peroxidation independent mitochondrial toxicity. The CCl4 representation was modified to induce mitochondrial electron transport chain (ETC) inhibition. Concurrent induction of lipid peroxidation and ETC inhibition mechanisms permitted reconciliation of simulated hepatotoxicity with published data. For exam-
ple, >3x ALT elevations were simulated at doses >50 mg/kg, with the highest ALT elevations (>1000 U/L) at doses >100 mg/kg in mice. Simulated CC14 was then used to evaluate and further refine the innate immune response. In summary, quantitative analysis of CC14 in DILysm® not only permitted evaluation of the innate immune response, but also suggested a limitation in the putative primary mechanism of hepatotoxicity.

**413 Organotypic Tissue Cultures As Models to Study the Impact of Cigarette Smoke on Human Upper and Lower Respiratory Tract**

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Cigarette smoke (CS) is etiologically linked to fatal respiratory diseases. To enable the development of modified risk tobacco products (MRTPs) to reduce the risk of smoking-related diseases, there is a need to understand the mechanisms whereby CS causes damage. As an alternative to animal experimentation, the principles of 3 R’s, necessitate the establishment of reliable in vitro models, which may also provide a better predictability of exposure response, when derived from human cells. Among the in vitro models, the three-dimensional organotypic pseudo-stratified culture systems better mimic the morphological, physiological, and molecular attributes of human respiratory tract than primary or immortalized cells cultured as a submerged monolayer. The bronchus and lung parenchyma are the primary sites that manifest smoking-related diseases, however, nasal epithelium has been proposed as a surrogate tissue to study smoking effects on the respiratory tract. We are evaluating the utility of organotypic culture systems derived from upper and lower respiratory tract in the assessment of exposure response to CS and prototypic MRTPs. Repeated whole CS exposure showed that both bronchial and nasal organotypic cultures tolerated in a similar manner the two CS concentrations (10% and 16%), measured by tissue integrity (trans epidermal electric resistance) and cellular toxicity (lactate dehydrogenase) assays. By combining the transcriptomic data with computable biological network models, we further showed that several biological processes, including those related to cellular stress response and proliferation, were perturbed in both nasal and bronchial tissues in response to smoke exposure. In summary, the study gives valuable insight into the effect of repeated whole CS exposure on organotypic models derived from both the upper and lower respiratory tract.

**414 Integration of the Hepatic and Urinary Metabolome with Hepatic Differential Gene Expression in C57BL/6 Mice following Repeated TCDD Dosing**


**415 Cigarette Smoke-Induced Perturbations of Molecular Pathways in Human Organotypic Cultures of Buccal and Gingival Mucosa**

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Smoker’s oral mucosa is subject to cigarette smoke (CS)-induced cytopathological, genomic, and transcriptional changes that could potentially lead to the development of mouth disease. To characterize CS effects, we used two human organotypic in vitro models of the buccal and gingival epithelia (MatTek®). Both in vitro models were exposed at the air-liquid interface to fresh, diluted CS (one reference cigarette smoke, 3R4F at a time, with Health Canada smoking regimen and nicotine doses of 0.279mg/ml or 0.575mg/ml) on 4 occasions with 1h intervals between exposures where the tissues were kept in the incubator under appropriate culture conditions. Systems biology endpoints (gene and miRNA expression) were determined at time 0 following the 4th exposure and 4h, 24h, 48h after exposure to investigate time- and dose-dependent CS effect on both tissues. Other endpoints (e.g. cytotoxicity, pro-inflammatory marker release, cytochrome P450 (CYP) activity, histology) were also measured for some time points. The release of pro-inflammatory markers such as VEGF and MMP-1 was up-regulated in both CS-exposed buccal and gingival tissues 24h after exposure. However, only gingival tissues showed a significant up-regulation of CYP1A1/CYP1B1 activity 48h after exposure. By using computational approaches and capturing systems biology endpoints, various perturbations of biological processes (e.g. inflammation, proliferation, cellular stress) triggered by repeated CS exposure were observed in both buccal and gingival in vitro models.

We describe here for the first time the impact of CS exposure on human buccal and gingival organotypic models using various approaches: a combining systems biology, biological network models, computational methods and standard endpoints.

**416 Tobacco-Specific Gene Profiles in Korean Male Smokers**

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Tobacco smoking-related cancers can be protectable with well controlled cancer prevention strategies. Particularly, the prevalence of male smoking in Korea belongs to the world’s highest levels. Therefore, we performed microarray to find our Korean-specific genetic factors associated with tobacco smoking among age- and sex-matched healthy male smokers and non-smokers (age=38.9±3.7, N=12) with Illumina human HT-12 platform with 47,231 transcript probes. Subjects were evenly divided into tobacco-high and low exposed groups, according to levels of urinary cotinine, a major metabolite of nicotine, which was measured with HPLC-UVD. We also performed qRT-PCR to confirm microarray results, and found that we could express the expression patterns of blood to those of buccal cells. Finally, we performed gene network analyses using DAVID Bioinformatics and KEGG pathways to integrate tobacco smoking-responses. As results, smoking-upregulated genes were 16 genes including ARPC3, CXCR4, ACTG1, and CYP1B1 (p<0.01 and >1.5-fold change). On the other hand, smoking-downregulated genes were 22 genes including DEFA4, VAV3, and FCGR3A. The expression levels of FCGR3A in blood were correlated with those in buccal cells (r2=0.85, p<0.001). After network analyses, we found that Korean male smokers differentially expressed immune responsive pathways such as Fcy-receptor mediated phagocytosis and leukocyte transendothelial migration, compared to non-smokers. In conclusion, our findings provide new insights in understanding of ethnic specific toxic mechanisms and health endpoints of tobacco smoking.
drial ATP, cytosolic GSH depletion, LDH release, and oxidative stress at 1-10mM. APAP induced toxicity was minimal at 4h, moderate at 8h, and substantial after 24h of exposure, suggestive of early, middle, and late stage of injury. A LC/MS/MS based proteomic analysis was performed to investigate alterations of molecular signature in the liver model. Differential expression proteomic signatures were identified by linear model with Empirical Bayes Method (FDR-adjusted p < 0.05). Total of 306, 323, and 485 proteins were found to be differentially expressed at 4h, 8h, and 24h, respectively. Pathway analysis demonstrated that the proteome of the APAP liver toxicity model was enriched in ribosomal proteins, mitochondria content, and stress response proteins. Among the proteins that differentially expressed, 26, 21, and 26 proteins were exclusively altered at 4h, 8h, or 24h by lethal dose acetaminophen (10mM), with potential utility as stage specific markers of APAP toxicity. In addition, two specific proteins were dose-dependently induced or suppressed at 24h, suggesting their potential utility as biomarkers to assess the severity of liver injury. Efforts are underway to validate these proteins in rodent and human from samples. Validation of these biomarkers will aid in shaping clinical intervention in APAP overdose.

418 Use of Mummichog to Map the Exosome: Associating Environmental Chemicals with Perturbed Metabolic Pathways

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High-resolution metabolomics (HRM) using liquid chromatography and high-resolution mass spectrometry has routinely captured >20,000 chemicals in biologic samples. In this study, 3170 compounds were identified and the majority appears to represent low-abundance metabolites and environmental chemicals, which have not been fully characterized. How to interpret such data in the appropriate biological context remains a great challenge, and the identification of chemicals is often the rate-limiting step. This is especially problematic for the >10,000 chemicals used in commerce, where systematic analytic methods for biomonitoring are not available and potential biologic impacts of environmental products have not been fully explored. We have recently described a set of computational algorithms that directly predict pathway and network activities from high-resolution metabolomics, by leveraging the collective power in genome scale metabolic models. This approach greatly speeds up the analysis of metabolomics data, since metabolite identification can be deferred to a posterior confirmation step. In a case study using in vitro models of Parkinson disease, we extended these algorithms to integrate metabolomics and transcriptomics. Our results revealed that a metabolic network of oxidative phosphorylation and carnitine shuttle is dysregulated by the herbicide paraquat. The transcriptomics. Our results revealed that a metabolic network of oxidative phosphorylation and carnitine shuttle is dysregulated by the herbicide paraquat. The.

418a Computational Embryology and Predictive Toxicology of Hypospadias


Hypospadias, one of the most common birth defects in human male infants, is a condition in which the urethral opening is misplaced along ventral aspect of the penis. We developed an Adverse Outcome Pathway (AOP) framework and computer simulation that describes the pathogenesis of hypospadias. We first utilized semi-automated literature mining to identify 511 PubMed hypospadias articles based on MeSH annotations with 'chemicals' and 'proteins'. This information was used to develop an AOP framework linking potential molecular initiating events, altered cellular behaviors, tissue morphogenesis, and abnormal organogenesis. We selected vinclozolin (hypospadias in rats at 96 mg/kg/day in ToxRefDB, AC50 = 0.307 μM for inhibition of the human androgen receptor binding in ToxCast) as a reference for proof-of-concept. A multicellular computer simulation model capable of rendering key events in GT development was built in CellDesigner for simulation using a cellular agent-based model (CompuCell3D.org) by incorporating key signals (e.g., SHH, FGF10, EphB2, and androgen) and cellular behaviors (e.g., selective adhesion, motility) to control urethral tubulogenesis. Overall, the AOP approach and computer simulation: 1) provide a platform for integrating available biological information to predictively model the complex pathogenesis of hypospadias; 2) enable the generation of new research hypotheses; and, 3) contribute to better mechanistic understanding of this adverse developmental outcome. (This work is approved by EPA but does not necessarily reflect official Agency policy).

418b Using High-Content Screening Data from ToxCast to Analyze Cell State Dynamics


We developed a novel approach for analyzing chemical-induced dynamic cell ‘state’ changes using in vitro data. The ToxCast project conducted a high content screening study in which HepG2 cells were treated with 10 concentrations (ranging from 0.4 to 200 μM) of 976 diverse chemicals, and 10 cellular end points were measured after 1, 24 and 72 h of exposure. After analyzing the concentration response data we found 669 chemicals significantly changed one or more end points including: mitochondrial functions (435), cell cycle (412), cytoskeletal stability (332), JNK (253), p53 (253), and cell number (510). In order to capture the overall response to each chemical at a single time point, we constructed a bioactivity vector based on the magnitude and the direction of the change in each end point. The bioactivity vectors for all chemicals were analyzed using the Euclidean distance metric. K-means and cluster quality analysis to identify 15 clusters. We assumed that these clusters capture a range phenotypic ‘states’ of the HepG2 system as it responds to different perturbations ranging from normality to adversity (defined by significant cell loss). By translating bioactivity vectors to cellular states, the temporal effects of each chemical can be described as a sequence of states, called a trajectory. A trajectory describes the dynamic sequence of changes in the HepG2 system as it adapts to chemical exposure. Chemicals with similar mechanisms such as, mitochondrial disruptors, cytoskeletal disruptors and stress kinase activators, produced similar trajectories. On the other hand, structurally similar chemicals, such as thiazolidinediones, produced different trajectories that were consistent with their known toxicity. By analyzing trajectories, it is possible to characterize chemicals that cause adaptive changes that lead to recovery, or those that lead to adversity. Further work is necessary to evaluate the relevance of this work to in vivo outcomes. This abstract does not reflect EPA Policy.

418c Using Targeted Proteomics and Next-Generation Sequencing Approaches to Identify New Plasma Biomarkers and Assess Systematic Drug-Induced Adverse Effects


Adverse effects caused by therapeutic drugs are a serious and costly health concern. Despite the body’s systemic responses to changes in the chemical environment, the liver is often the focus of damage and it is usually the focus of studies of toxic effects due to its active roles in the metabolism of xenobiots. Applying sensitive and high throughput proteomics and transcriptomics technologies including Selected Reaction Monitoring (SRM) and next generation sequencing (NGS) on body fluid samples open a window to identify new biomarkers and assess adverse effects at systems level. To explore this, we utilized SRM and NGS technologies to quantify and profile circulating protein and cell-free RNA in plasma using a well-characterized acetaminophen (APAP) overdose mouse model. As a proof principle study, we initially focused on the levels of 85 liver-enriched proteins. Among them, 52 showed concentration changes in plasma after APAP overdose. Some of the proteins showed better performance than the existing blood markers for detecting and monitoring acute drug-induced injury. From NGS profiling of circulating RNA, the levels of a number of transcripts, both protein-coding and noncoding RNAs, showed changes in plasma. The results are in agreement with our previous report on the association of blood miR-122 levels with liver injury. Detailed analysis revealed the changes of circulating RNA reflected not only the liver injury induced by APAP overdose, but also damage in tissues other than the liver. Besides endogenous RNAs, we also identified a significant amount of exogenous RNAs (exRNAs). The changes in exRNAs also reflect alteration of dieting behavior after APAP overdose. Besides the identification of new blood biomarker candidates, this study illustrates the possibility of using SRM and NGS to assess global effects of therapeutics. This could lead to a new approach for a more comprehensive assessment of the efficacy and safety of therapeutics.
Converging scientific opinion from both the regulatory and scientific community recognize the importance of developing a new mechanism driven toxicity assessment paradigm. We use functional profiling in yeast, Saccharomyces cerevisiae, to identify the fundamental adverse outcome pathways involved in cellular toxicity. The ~4500 viable deletion barcoded strains are pooled, grown for multiple generations in the presence of a toxicant, and sensitivity/resistance of each strain to the toxicant is individually quantified. We screened a diverse variety of industrial chemicals at multiple doses and determined the functional importance of each gene for optimal growth in the presence of each chemical. We used high-resolution profiling dataset for diverse toxicants and the network inference ARACNE algorithm to infer a network representing toxicity related functional linkages between non-essential genes in S. cerevisiae. We identified key network modules enriched in different functional properties impaired upon toxicant exposure which we hypothesize represent common cellular responses to adverse outcomes hubs. We subsequently identified adverse outcome hubs associated with each individual toxicant. Additional dose response analysis enabled us to identify candidate adverse outcome pathways linking early cellular response through to adverse outcomes for each toxicant. We suggest that this functional approach in yeast and development of functional networks provides a unique insight into both the shared and unique molecular responses to toxicant exposure.

Potential use of ToxCast assays for assessing drug-induced liver injury in humans

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Predicting a drug’s potential to cause drug-induced liver injury (DILI) is still a major challenge with the animal models. A paradigm shift from in vivo animal testing to in vitro systems has been proposed, but met with challenges. The EPA has assayed many chemicals with an array of mechanisms-based in vitro systems, providing an opportunity to develop in vitro prediction system for DILI. We developed a robust quantification of dose response for the ToxCast assay data coupling with the DILI annotation by the FDA’s Liver Toxicity Knowledge Base (LTKB) to investigate the potential use of the ToxCast data to predict DILI in humans. Specifically, the LTKB developed 287 benchmark drugs and some of which were assayed by ToxCast, which was used in this study. Area under the dose-response curve, with the median value from each concentration, was calculated for each drug/assay combination and the assay potential for predicting DILI was evaluated by area under Receiver Operating Characteristic (AUROC). With the criteria of AUROC > 0.6 and p-value < 0.05, 42 endpoints out of 451 assayed by ToxCast were identified with the prediction potential. Some of these endpoints associated with key mode of actions underlying DILI mechanisms including mitochondria damage, oxidative stress, PPAR and NURR pathways. The findings are encouraging and indicate the potential utility of the ToxCast system for DILI assessment in humans. The future study will be focused on applying the identified assays to rank and prioritize the ToxCast chemicals according to the DILI potential and improving mechanistic understanding on DILI.

Development of a Novel Method for Deriving Thresholds of Toxicological Concern (TTCs) for Vaccine Constituents

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Safety assessment of impurities, residual materials and contaminants in vaccines is an important risk assessment process for evaluating public health risks associated with new and existing vaccines. Defining thresholds of toxicological concern (TTCs) is a concept that can be applied for screening vaccine constituents and evaluating the toxicity of specific vaccine constituents with little or no toxicity data. TTCs are mathematically modeled and extrapolated levels, below which adverse human health effects are not expected to occur. Probist and lowest-toxic-dose (TDLo) data were obtained from INCHEM, RepDose, RTECS and TOXNET. ToxTree software using three different algorithms (The Cramer Extended, the In Vivo Rodent Micronucleus Assay algorithm, and the Benigni-Bossa rule base for carcinogenicity by IJS) was assigned to assign the chemical test set (n = 197) into structural families based on structural alerts (SA). This resulted in six potential methods for elucidating TTCs: In Vivo Rodent Micronucleus Assay/ probit, Benigni-Bossa/ probit, Cramer Extended/ probit, In Vivo Rodent Micronucleus Assay / TDLo, Benigni-Bossa/ TDLo, and the Cramer Extended/ TDLo. These were subjected to 10-fold cross validation in a Preliminary algorithm (SOT), resulting in two TTC methods for further consideration: Benigni-Bossa/ probit and the Cramer Extended/ probit. The Benigni-Bossa/ probit method outperformed the Cramer Extended/ probit method in terms of specificity (87.2 vs. 48.1%), accuracy (65.2 vs. 52.9%), positive predictivity (66.6 vs. 50%), negative predictivity (64.8 vs. 56%), positive operating characteristics (ROC+) (2 vs. 1) and ROC- (1.84 vs. 1.3). Our results indicated that the Benigni-Bossa/ probit was the most appropriate method. These comments are an informal communication and represent our own best judgment. These comments do not bind or obligate FDA.

Comparison of Cramer Class Prediction between Toxtree, OECD QSAR Toolbox, and Expert Judgment—Strategies for Refinement

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The Threshold of Toxicological Concern (TTC) approach is used to predict the toxicity of compounds with low oral exposure. The Cramer classification decision tree is used extensively for TTC estimation. Assigning an accurate Cramer class to a material is a crucial step to preserve the integrity of the risk assessment. The Cramer class of over 1000 fragrance materials across diverse chemical classes were determined by using the OECD QSAR Toolbox (TB), Toxtree and expert judgment. Concordance between Cramer class predictions by Toxtree, predictions by OECD Toxubs and expert judgment are presented. The overall concordance between expert judgment and Toxtree was 76%, Toxtree (with extensions) 72%, TB (with extensions) 65%, TB 72%. The concordances between predictions and expert judgment for different chemical classes were also analyzed. The concordance between Toxtree (with extensions) and expert judgment for esters, alcohol, ketones and aldehyde chemical classes was 77%, 58%, 54% and 81% respectively. The concordance between TB and expert judgment for esters, alcohol, ketones and aldehyde classes was 70%, 61%, 51% and 61% respectively. The concordance between Toxtree (with extensions) and expert judgment for esters, alcohol, ketones and aldehyde classes was 82%, 50%, 54% and 91% respectively. More detailed evaluation of chemical categories (subgroups) suggested that the lowest concordance between expert judgment and in silico tools (Toxtree and TB) to be among Heterocycles/Oxygen Containing/Furan/Monocyclic/Saturated (<30%), Alcohol/Aryl alkyl (<55%) and Ketone/Cyclic/Monocyclic/Saturated (<55%). Strategies and guidance on determining the Cramer class for various chemical classes are discussed. The suitability of software for specific chemical classes and possible re-coding of rules are presented.

xMSanalyzer and xMSannotator: R Packages for Systematic Study of the Exposome

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Improved analytical technologies and better data extraction algorithms for high-resolution mass spectrometry have enhanced the coverage of chemical detection for metabolic profiling and environmental biomonitoring, including detection of low abundance environmental chemicals and their metabolites. Our recently published software, xMSanalyzer (Uppal et al. 2013), includes utilities that allow quantitatively reliable measurement of metabolites detected in individual biological samples. A number of databases and online tools are available to perform mass spectrum searches, but most of the web-based tools allow query of only few hundred mass-to-charge values in a single search. No convenient interface exists to query including data sources to be searched within ChemSpider and can be used in combination with existing R packages for processing environmental metabolomics data. Our results demonstrate that together, xMSanalyzer and xMSannotator allow reliable detection of environmental chemicals in individual biological samples and facilitate the ability to gauge the physiological relevance of the environmental chemicals and their metabolites.
The exposome is the array of environmental influences and cumulative biological responses throughout an individual’s lifespan, including exposures from the environment, diet, behavior, and endogenous processes. To operationalize exposome research, an “open” database structure is needed in which data from newly acquired samples can be added to existing data in a sufficiently rigorous manner to allow time-dependent studies of an individual over years to decades. Liquid chromatography-high-resolution mass spectrometry with triplicate analysis, improved data extraction and automated 24/7 operation now provides capability to measure >100,000 ions, representing >20,000 chemicals, in a few microliters of biological samples. The number of ions and presence of many unidentified low-abundance chemicals (m/z range 85-2000) makes commonly used analytic procedures, such as MS/MS with calibration against authentic standards, impractical for routine measurement. Consequently, we have devised and begun to test standard operating procedures for exposome research. Analysis of samples in triplicate facilitated detection of chemicals present at low abundance and present in only a small percentage of individuals. Inclusion of a mixture of stable isotopic standards in each sample provided authentication of instrument calibration and consistency of response; single internal standards were insufficient for this goal. The standard also allowed direct determination of consistency of elution characteristics. With daily Quality Control (QC) using apLCMS with xMSAnalyzer (Uppal et al BMC Bioinformatics 2013) and routine replacement of columns and instrument cleaning after 25 days of continuous operation, the results are sufficiently robust to warrant evaluation as a routine pipeline for combination of data in a cumulative exposome research database.

**423 Exposure Data and the Comparative Toxicogenomics Database (CTD)**

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Exposure science studies and characterizes the interactions between environmental stressors and human or ecological receptors. This research plays a critical role in the translation of toxicity data to prioritize research, assess human health risks, and inform public health decisions. To facilitate the characterization of stressor-receptor interactions (including identification of connections between real-world exposures, chemicals, genes and proteins, diseases, and molecular pathways), we initiated a project to curate and integrate chemical exposure data into the publicly available Comparative Toxicogenomics Database (CTD; http://ctdbase.org). CTD is a manually curated database containing over 21 million toxicogenomic relationships for chemical-gene-disease interactions. Gene Ontology (GO) annotations, and molecular pathways that are integrated with analysis tools to promote understanding of the molecular mechanisms underlying environmental diseases. In our exposure curation paradigm, the peer-reviewed literature is manually curated using several controlled vocabularies and free text to capture details in more than 50 data fields characterizing the four major exposure concepts defined by the Exposure Ontology (ExO): Stressor, Receptor, Event, and Outcome. To date, over 600 articles have been curated, resulting in more than 21,000 exposure statements for 550 unique chemicals, 95 distinct receptor populations from 70 countries, 1,545 diseases, and 70 phenotypic outcomes. Integrating this information with core CTD data will allow exposure science data to be queried, retrieved, and contextualized within CTD’s broader biological framework. This integrated platform will help promote mechanistic understanding of environmental influences on human health.

**424 LocaTox: An Interactive Tool Linking Known Environmental Contaminants, Associated Chemical-Human Gene Interactions, and Birth Defect Statistics in Specific Locales**

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Several areas in the US have higher incidences of birth defects than the national average, yet do not display clusters of a single type of congenital abnormality where an association with a specific chemical contamination can be made. To aid in current research to quantify specific chemicals and birth defects, we hope to develop an interactive tool, LocaTox, where an extensive array of data can be integrated on specific locales including topography, local infrastructure, specific industries and levels of air, water, and ground pollution. These environmental factors are then associated with specific birth defect statistics and are paired with chemical-gene and gene-birth defect interactions. A user can actively explore potential connections as an opening to developing research questions. As a prototype, we selected Fresno County California where statistics show a higher rate of birth defect-related infant deaths compared to the cumulative rate in California and where the causes of many birth defects remain unknown. In this study we identified some of the most prominent environmental pollutants in Fresno and their associations, if any, with a wide array of birth defects. Resources used included DART, CTD, ChemIDPlus, t3db, Scorecard and websites such as Pesticide Action Network (PAN) and several US EPA resources. Chemicals prominent in Fresno County previously associated with birth defects were identified and specific chemical-disease-gene relationships were identified. One valuable result from the searching capability is the possibility of linking several exposures rather than individual chemical to various birth defects which possibly expands the overall environmental risk assessment. The content-design of the tool can be adapted for any specific locale of interest.

**425 iLINCS—A Web-Based Portal for Integrative Analysis of Multidimensional Data**

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New technologies and systems approaches are being applied to identify biological signatures that are common across chemicals sharing similar modes of action or predict adverse outcome exposures. However application of systems data for risk assessment requires integration of multidimensional endpoints (readouts) acquired from multiple “omic”, network and biochemical platforms. The effective application of these distinct readouts to risk assessment requires advances in biostatistics, bioinformatics, and computational methods. The NIH Library of Integrated Network-based Cellular Signatures (LINCS) program aims to create a network-based understanding of biology by cataloging changes in gene expression and other cellular processes (http://www.lincsproject.org/). Previously, we demonstrated that integration of transcriptomic and transcription factor RNA-binding profiles significantly improves detection of otherwise obscured chemical signatures. Here, we introduce the web-based iLINCS [Integration of Interactive LINCS Gene Expression Portal; a computational biology tool integrating various types of systems biology readouts (http://eh3.uc.edu/GenomicsPorts/viewLincs.jsp)]. Currently, these readouts include publicly accessible data from Broad Institute (LINK signatures, Connectivity Map signatures), ENCODE transcription factor binding signatures, and disease-related signatures (NCBI GEO GDS collection). The objective is to leverage publicly available libraries of the signatures, meta-signatures and regulatory network signatures to evaluate and identify chemical-related network signatures shared or common to diseases, chemical exposures and transcription factor pathways. The iLINCS web-based portal enables the identification of functional connections between chemicals, genes and diseases. Integrative analysis based on multidimensional data will vastly improve in vitro prediction of apical toxicological outcomes for chemicals lacking in vivo toxicological data. Supported by: U01HL111638.

**426 Harnessing the Wealth of Pharmaceutical Preclinical Toxicity Data—The eTOX Database (eTOX Part I)**

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Most preclinical data acquired during drug development are not available in the public domain due to attrition of projects or IP issues. Lack of data accessibility was identified by the European Federation of Pharmaceutical Industries and Associations (EFPIA) as an obstacle to read-across approaches, development of new in silico tools and data mining for biomarkers.

In the framework of the European Innovative Medicines Initiative, 13 pharmaceutical companies and 12 public partners initiated the eTOX project to collect un-published preclinical safety data and build a database covering multiple endpoints. The participating EFPIA companies identified archived reports on systemic tox studies (paper or pdf) and manually extracted them. In parallel a trusted organisation (“honest broker” - Lhasa Ltd., Leeds) set up the database. The data sets were sent to the honest broker and incorporated into the database after quality check. Due to data heterogeneity a major effort was undertaken for data harmonization and ontology work (see abstract eTOX Part II).

The steadily growing database currently contains 2127 reports (status: Aug 2013) of systemic toxicity studies with more than 800 different chemical structures. The database was populated with virtually all endpoints investigated in systemic toxicity
studies according to guidelines, i.e. clinical observations, clinical chemistry, hematology, macroscopic findings and histopathology. The majority of datasets pertain to rats (72%), followed by dogs, primates and mice. Reported findings are strictly attributed to treatment-related or non-treatment based on the original report. Liver was the most frequently (30 % of the findings) affected organ, followed by spleen (19 %), kidney (17 %) and thymus (16 %). The eTOX database should become one of the largest and most granular preclinical databases for pharmaceuticals. It will facilitate comparison of early candidates ("read-across") and pave the way for the development of new in silico tools (see abstract eTOX Part III).

427 eTOX Ontologies: Enhancing Standards for Better Knowledge Management of Animal Study Data (eTOX–Part II)

Switzerland, 1GlaxoSmithKline, Ware, United Kingdom, 2Pfizer, Groton, CT, 3Sanofi, Paris, France, 4Novartis, Basel, Switzerland, 5GlaxoSmithKline, Ware, United Kingdom, 6Pfizer, Groton, CT.

The eTOX consortium has collected data from more than 5,000 toxicology reports from 13 companies in order to enable data mining and structure-based prediction. Data from these reports were manually extracted and verbatim findings were stored as "is". In order to make that collection of diverse data and terminologies useful, relevant code lists had to be identified and the 1.5 million extracted finding verbatim terms mapped to the appropriate locations. Developments delivered by eTOX include OntoBrowser, an open source tool dedicated to the mapping of verbatim term to preferred terms and maintenance of created ontologies. On top of existing code lists from SEND, a complete ontology was also developed, which included dependencies of terms in order to facilitate endpoint definition and database queries. The most challenging ontology was derived from standard pathology texts, includes the evolving INHAND terms and describes the microscopic anatomic pathology findings. All these ontologies will be shared in the public domain in order to increase interoperability of data in the preclinical safety world.

428 New Frontiers in the In Silico Prediction of In Vivo Drug Toxicity: The IMI eTOX Project (eTOX Part III)

The IMI eTOX project [1] is a European-wide R&D initiative that, for the first time, is collecting and integrating information from in vivo preclinical toxicity reports buried in the archives of the pharmaceutical companies. The resulting repository, complemented with information obtained from public sources, is being exploited using methods as read-across and a fully integrated in silico prediction system.

The project, started in 2010, begins to yield valuable outcomes. So far the 13 participating pharmaceutical companies have already donated more than 5,000 toxicology reports (see eTOX Part I), the consortium has developed ad hoc ontologies to facilitate data integration (see eTOX Part II) and nearly a hundred models have been developed and implemented. With respect to methodological outcomes, the project has developed an integrated prediction system using an original archi-tecture based on independent virtual machines developed by academic partners and interconnected using web services. This solution allows efficient academic-industry collaboration and a safe deployment of the software within the pharmaceutical companies. Predictive models are being developed using a new integrated modeling framework that facilitates their development and maintenance and incorporates functionalities such as structure normalization or assessment of the predictions applicability domain. The predictive models already developed address most of the endpoints classically studied in toxicology. Moreover, since the project is specifically aimed to the prediction of in vivo and organ level toxicological endpoints, some of these models implementing multilevel and multiscale approaches [2].

1. Integrating bioinformatics and chemoinformatics approaches for the development of expert systems allowing the in silico prediction of toxicities (eTOX). IMI-JU project 115002. http://www.etoxproject.eu

429 An Ensemble Model of In Silico Tools to Improve Toxicity Prediction
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Quantitative structure activity relationship(QSAR) are theoretical models that relate a quantitative measure of chemical structure to a physical property, or a biological effect. Such models are of particular interest in regulatory settings for toxicity (adverse affect) profiling of drugs or compounds that may be released from medical devices. Free/proprietary in silico tools are gaining wider acceptance as a faster alternative to otherwise time-consuming clinical and animal testing methods. However, different in silico tools often make contrasting predictions for a given chemical and also vary in the accuracy of prediction across different chemical datasets. The objective of this study is to apply the ensemble learning approach in machine learning paradigm for combining predictions from different models, based on the understanding that consensus predictions are better than an individual prediction. We have developed a novel hybrid QSAR model based on a decision tree framework using Bayesian classification. We compare the predictive performance of in-silico tools (Toxtree, Lazar, OECD Toolbox and Danish QSAR) and the ensemble model for carcinogenicity prediction for a dataset of 332 compounds), medical device leachables (84 compounds) and the gold carcinogenic potency database (480 compounds). The results show that the ensemble model has improved overall performance (Accuracy: 83.81%, 88.10% and 81.04%) and highest inter-rater agreement, (kappa (κ): 0.6322, 0.759 and 0.6164). The study suggests that the ensemble model significantly improves the quality of predictions from a regulatory standpoint.

430 DIAMONDS: Data Infrastructure for Applying Models ON Design and Safety

Innovations in many industrial sectors involve the development of new chemical entities with improved properties. Regulatory frameworks in place may differ per industrial sector, but all have the avoidance of harmful effects to exposed humans as major objective. Safe limit values are often driven by complex endpoints like repeated dose toxicity, carcinogenicity, or reproductive toxicity. Until recently, these limits could only be established by animal studies on a compound by compound basis. Today, developments in toxicological sciences, systems biology and computational chemistry, provide opportunities to predict toxicological profiles of chemicals with highly reduced in vivo testing. At TNO, we are developing 'DIAMONDS', a data infrastructure with statistical and computational tools that achieves and visualizes exactly this goal: the efficient prediction of complex toxicological hazards of new molecules. The applied methodology, detailed in this poster, relies on the combined knowledge of known toxicologically characterized toxicants. By extracting the determinants of a specific toxicological outcome, the toxicological profile of a novel compound can be predicted and its probability specified. In addition, DIAMONDS encompasses the use of (in vitro) screening models for 'biological verification', as structural similarity does not guarantee similar toxicological profiles, and to use that screening model outcome for prediction of the potential toxicity of novel molecules. Our visualization tool allows a rapid interpretation of toxicity prediction probability on the basis of all available data. We have applied this tool on TG GATEs, and ToxCast/ToxRef databases and found associations between structural properties, Omics pathway signatures and toxicity/pathology profiles. We found a strong diversity in predictive performance depending on the chosen endpoint. ToxCast assay data did not provide additional predictive value compared to structural fingerprint-based data (Grants NIGIL-NTC, Celtic-LRI-AIMT-2&3, ZonMW-ASAT).
**431** Collaborative Interoperability between Public Projects to Support Replacement of *In Vitro* Repeated Dose Toxicity Testing  
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Tohoku University, Sendai, Japan, ⁴National Institute of Health Sciences, Tokyo, Japan and ⁵BioSafety Research Center, Iwata, Japan.  
Sponsor: D. Bury.

**432** Hazard Evaluation Support System Database (HESS DB) for Repeated-Dose Toxicity of Chemical Substances: A Multifunctional Tool for Hazard Assessment and Predictive Toxicology  
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Repeated-dose toxicity (RDT) is one of the key regulatory endpoints in the hazard assessment of chemical substances. We have recently released Hazard Evaluation Support System (HESS) and the attached database (HESS DB). HESS is compatible with OECD QSAR Toolbox and has a supportive function to group structural analogs with toxicity data. HESS DB contains test data of RDT studies for currently about 600 industrial chemicals, most of which were conducted in accordance with GLP principles. The DB also includes reference information on ADME and toxicity mechanism for some of those chemicals. Here we demonstrate that HESS DB is very useful for hazard assessment and predictive toxicology. HESS DB has full data set at all tested doses. That allows us to calculate benchmark dose (BMD) and find a parameter useful for hazard assessment. Moreover, chemicals causing similar toxicity can be efficiently searched with the DB, facilitating to find marketed toxic chemicals and new structural alerts whose mechanistic information is limited. These are new targets of toxicological research. Finally, HESS DB provides information on predictable or measurable key events identified on adverse outcome pathways. The combination use with HESS supports to perform category approach or integrated testing strategy to predict the primary toxicity of untested chemicals in a transparent and interpretable manner. Both HESS DB and HESS are downloadable from the website of NITE at no charge (http://www.safe.nite.go.jp/english/kasinn/qsar/ress-e.html).

**433** A Web Application for Estimating Provisional Health Guidance Values of Diverse Environmental Chemicals Using Reliable QSAR Models  
A. Prussia, N. M. Khalil and E. Demchuk. Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, Chamblee, GA. Sponsor: A. Prussia.

ATSDR is congressionally mandated to develop health guidance values, defined as minimal risk levels (MRLs), to be used for assessing the risk of exposure to hazardous environmental chemicals. Epidemiological data and/or toxicity data from animal studies are the preferred sources of information for developing MRLs. However, such in vivo data exist for only a few hundred chemicals compared to the many thousands present in the environment. Through quantitative structure-activity relationship (QSAR) models for predicting these properties exist in the literature, the models generally lack defined measures of reliability and are difficult to apply to current chemicals of interest. To address these issues, a web application was developed for prediction of rat chronic oral toxicity endpoints known as lowest-observed-adverse-effect-levels (LOAELs). The user need only supply a CAS number for an organic compound of interest, which is interpreted and applied to Q SAR models developed from 159 environmental chemicals with experimental LOAELs. The general reliability of these models is confirmed through application of an external test set (Q2 = 0.53); the specific reliability for the chemical of interest is determined through a distance metric to the models’ descriptor space. In the future, web-based use of further validated QSAR models may provide quick and reliable toxicity predictions to guide Agency decisions on risk assessment, provisional exposure levels and investigational prioritizations.

**434** Toxicophores: Identification, Validation, and Application  
B. M. Pailey, C. M. Zwickel and T. K. Baker. Investigative Toxicology, El Lilly, Indianapolis, IN.

Growing pressure to move away from animal testing, forge a need for more predictive *in vitro* and computational tools. Cheminformatics is an attractive approach as it is inexpensive and enables early safety assessment. Our goal was to identify toxicophores, substructures with an increased potential for specific toxicological outcomes within a defined chemical and/or biological context. Over 1000 internal compounds with 4 to 14 day rat and dog histopathology data from toxicology studies were included. The compounds comprise diverse pharmacological (NHRs, kinases, GPCRs, ion channels), physicochemical and toxicological properties. Leadscope® was utilized to identify substructures of compounds within the dataset with statistical significance at any of 50 toxicological end points we considered. These “substructure correlates” were evaluated, prioritized and validated against an external test (Pharmacopendium®) and an internal test set. *In vivo* and *in vitro* ADMET data paired with structural, pharmacological and other data were used to determine the context in which each toxicophore has an increased likelihood of an adverse event in rat or dog 4 to 14 day tox studies. Eight toxicophores for 3 target organ toxicities have been identified to date. Current chemistry containing these substructures are evaluated visually and screened through the relevant *in vitro* surrogate assays to assess relevance and to determine potential for preclinical toxicity risks. Despite the high predictive power of QSAR models, complex molecular descriptors typically do not provide information on what to modify to mitigate risk. The toxicophore approach is a combined knowledge- and judgment-based method with lower applicability compared to QSAR; however, for compounds containing these toxicophores, it can provide strong chemically and biologically relevant hypotheses to interrogate risk. Our data demonstrate an inexpensive, ethical and informative approach to identify molecules with potential preclinical *in vivo* toxicity risks.

**435** Mechanistic Modeling of Acute Oral Toxicity for Rat  
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Our aim is to develop a structure-activity model for acute oral toxicity (AOT) of chemicals to rats. The presented AOT model is based upon a category approach: chemicals are grouped in the same category based on their common interaction mechanism and toxicological mode of action. The formed categories include two types of toxicity: basic and excess. Basic toxicity results from the chemical affecting the most basic function of the cells. Most of the chemicals in this category are non-reactive chemicals acting by narcissism mechanism, which alter reversibly the physiological properties of cell membranes. Chemicals having reactive groups could also reveal basic toxicity because of their limited bioavailability. Excess toxicity is a result of destroying some critical processes for the cells or the whole organism.
Chemicals of this category have highly reactive groups acting by specific interaction mechanisms, which result in an enhanced toxicity compared to the basic toxicity. Two types of excess toxicity were defined: bioavailability dependent and invariant excess toxicity. Bioavailability dependent excess toxicity of chemicals is conditioned by two factors: bioavailability and reactivity. The solubility and partitioning parameters are considered to be adequate descriptors for bioavailability of toxicants. After reaching a certain threshold of water solubility the reactivity factor is starting to play role increasing acute toxicity. The toxic potency of chemicals revealing invariant excess toxicity does not depend on their bioavailability and they possess a constant toxicity within a certain studied range of solubility or partitioning parameters. The current version of TIMES-AOT model contains 2500 training chemicals. Some data were provided with thanks to the Dow Chemical Company. Based on the structure analysis, mechanisms of interaction and toxicological modes of action, 64 AOT categories were already determined. For each of them, local models for predicting AOT were derived.

436 Computational Analysis of the Combined Therapeutic Effects of Traditional Chinese Medicines and Western Therapeutics in Breast Cancer
A. Chu, M. Lin, D. Loomba, J. Ma and D. Johnson, Nutritional Sciences & Toxicology, UC Berkeley, Berkeley, CA.

Allleviating the side effects of chemotherapy drugs with herbal remedies is increasing in popularity. In this research, we used a computational systems pharmacology/toxicology approach to study Taxol and Traditional Chinese Medicine (TCM) recipes used in conjunction to treat and alleviate side effects in breast cancer treatment regimens. Phytochemicals from TCM recipes were determined from the TCM Database®Taiwan and the mechanism of action, potential molecular targets, metabolic pathways, herb-herb and herb-drug interactions, and potential toxicity of individual phytochemicals were elucidated using several resources including the Comparative Toxogenomics Database (CTD), DrugBank, ToxNet, Toxin and Toxin Target Database (t3db), Side Effect Resource (SIDER), Search Tool for Interactions of Chemicals (STITCH), and Pathwaycommons. Phytochemical findings were integrated into Taxol/breast cancer pathways to elucidate potential synergistic and inhibitory effects. This study provides insight on the potential benefits – and some potential negative effects – of commonly used TCMs and Taxol in the treatment of breast cancer. Relevant findings include the induction of gshelin by atracyclins, inhibition of VEGF and cMyc by baicalin and baicalein, and inhibition of cyclin D by tashinones. This research supports the continued search for isolated phytochemicals (and structural analogues) from TCM recipes for use in breast cancer treatments.

437 Computational Analysis of Potential Interactions from the Combined Use of Western Therapeutics and Traditional Chinese Medicines in Postmenopausal Osteoporosis
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Osteoporosis is a disease that significantly decreases bone mineral density and increases the risk for fractures. Boniva® and BoneVigor® are two medications used to treat osteoporosis in postmenopausal women. Boniva® contains ibandronate sodium (IS), while BoneVigor® is a Traditional Chinese Medicine (TCM) that contains the herb Psoralea corylifolia. Bakuchiol (Bk) is one of the 35 active phytochemicals found in the herb. Many osteoporotic patients choose to use a combination of these therapies, which presents a more significant focus for chemical toxicity testing, examination of protein acetylation, may constitute a stratified cellular defense program, allowing cells to handle threshold stresses are unable to activate the transcriptional program, whereas supra-threshold stresses can transcriptionally induce stress genes, with concomitant alterations of expression of genes regulating cell metabolism, proliferation and apoptosis. These transcriptional alterations may be associated with adverse cellular outcomes. Posttranslational and transcriptional control pathways, acting in concert, may constitute a stratified cellular defense program, allowing cells to handle stressors of wide-ranging intensity coherently. As cell-based in vitro assays become a more significant focus for chemical toxicity testing, examination of protein activity changes in the absence of transcriptional changes deserves more attention. Biomarker monitoring for safety assessment at low stress levels may also need a shift in focus to posttranslational pathways.

438 Network Motif Basis of Threshold Responses
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There has been a long-running debate over whether or not environmental toxicants exhibit thresholds for adverse health effects. The difficulty in answering the question stems from two fundamental challenges: (i) statistical analysis by itself cannot prove the existence of a threshold; and (ii) there has been little progress in developing a more mechanistic understanding of how threshold phenomena might arise in biological systems. We believe that mechanistic evidence for the existence of thresholds has to come from studying the underlying biological networks as nonlinear dynamical systems. We computationally analyzed the abilities of several intracellular biochemical network motifs to generate threshold responses. These motifs include proportional and integral feedback control, incoherent feedforward control, saddle-node, pitchfork, and transcritical bifurcations, and ultrasensitivity. For each motif, we present mathematical models to illustrate the kinetic basis for threshold responses. We conclude that integral feedback, feedforward and transcritical bifurcations can generate well-defined thresholds below which the response does not deviate from non-stressed control. Other motifs, such as proportional feedback and ultrasensitivity, can give rise to “threshold-like” responses where the low-dose region rises slowly staying close to the baseline. Feedforward control can also produce nonmonotonic/hormetic responses. In conclusion, threshold behaviors may be understood at the level of network motifs, the study of which is necessary for the emerging, toxicity pathway-based approach for chemical safety assessment. This is an abstract or a proposed presentation and does not necessarily reflect USEPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

439 Post-Translational Control of Adaptive Cellular Stress Responses and Its Implications in Toxicity Testing and Risk Assessment
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Transcriptional induction of stress genes constitutes a major cellular defense program against a variety of stresses arising from exposures to environmental toxicants. Besides these transcriptional programs, which are slow to activate, fast-acting, posttranslational control mechanisms are also at work in the face of cellular stresses. Through computational systems biology pathway models, we are examining processes that would lead to a dose-dependent transition from posttranslational control to transcriptional control with increasing stress levels. These models indicate that posttranslational control serves at least two functional purposes: (1) it handles small and/or transient stress in a timely manner and alleviates the initial impact of persistent stress; and (2) it stabilizes the transcriptional network, which by itself is prone to oscillation due to its delayed negative feedback nature. Posttranslational control, in a feedback or feedforward manner, can underpin perfect adaptation and thus produce thresholds in the responses against external perturbations. Subthreshold stresses are unable to activate the transcriptional program, whereas supra-threshold stresses can transcriptionally induce stress genes, with concomitant alterations of expression of genes regulating cell metabolism, proliferation and apoptosis. These transcriptional alterations may be associated with adverse cellular outcomes. Posttranslational and transcriptional control pathways, acting in concert, may constitute a stratified cellular defense program, allowing cells to handle stresses of wide-ranging intensity coherently. As cell-based in vitro assays become a more significant focus for chemical toxicity testing, examination of protein activity changes in the absence of transcriptional changes deserves more attention. Biomarker monitoring for safety assessment at low stress levels may also need a shift in focus to posttranslational pathways.

440 Computational High-Throughput Quantitative Analysis of Dose-Dependent Histological Features
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Manual quantitation of stained features can be onerous and subjective, yet valuable when integrated with complementary molecular responses. Alternatively, computational approaches such as image segmentation can be used to quantify features.
We have developed a high throughput Matlab®-based tool that runs within the MSU High Performance Computing Center to quantify the level of vacuolization (Oil Red O), immune cell infiltration (H&E or F4/80), and fibrosis (picrosirius red). To test the tool, volume density (Vv) was manually estimated by point counting on representative H&E slides from 2,723 Oil Red O stained hepatic sections from mice orally gavaged with 9 doses (0 to 30 µg/kg) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) every 4 days for 28 days. Significant correlations were calculated between the manual and computational Vv, demonstrating the reliability and accuracy of the tool to identify and quantify histopathological features. Furthermore, unlike manual approaches, the tool was also used to simultaneously quantify vacuole number and size revealing both an increase in number of vacuoles at 30 µg/kg TCDD as well as a dose-dependent increase in size, consistent with a change from micro- to macro-vesicular steatosis noted following visual assessment. Dose-response modeling of Oil Red O stained features estimated ED₂₀, BMD, and BMDL values of 10, 0.26, and 0.12 µg/kg TCDD, respectively, using manual counting and 27, 1.40, and 1.01 µg/kg TCDD, respectively, using our computational tool. In summary, high throughput image segmentation dramatically reduced analysis time to ~1h, facilitated large-scale assessment of histopathological features, and is a viable alternative that is consistent with results from the ≥ 60h manual assessment. Funded by SRP P42ES049111.

440a Deriving Points of Departure and Performance Baselines for Predictive Modeling of Systemic Toxicity Using ToxRefDB
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A primary goal of computational toxicology is to generate predictive models of toxicity. An elusive target of alternative test methods and models has been the accurate prediction of systemic toxicity points of departure (PoD). We aim not only to provide a large and valuable resource of PoD, but also to scope the problem by generating floor and ceiling baseline uncertainty bounds for which to judge future models. EPA’s ToxRefDB, originally populated with pesticide registration data, has grown to incorporate guideline-like studies from the pharmaceutical industry, National Toxicology Program, and publicly available research literature. Over 6000 high quality animal studies on 1071 chemicals were captured using standardized study design, treatment and effect vocabulary. Systemic lowest and no effect levels (LEL/TEL) were obtained from each study across a diverse set of study types including systematic review (SAC), subchronic (CHR), and short-term (ST) studies as well as systemic adult effects observed in developmental (DEV) and reproductive (MGR) studies. Species and study type adjusted chemical-level NEL were derived demonstrating a floor baseline of roughly 5 orders of magnitude uncertainty (OMU; 95% CI) based on the default distribution of NEL. Using SUB to predict CHR rat and mouse to predict rat CHRISTEL, baseline ceilings were established as 3 and 3.5 OMU, respectively. Further classification of study types based on exposure duration (short = SAC, DEV; medium = SUB; long = CHR), established a ceiling baseline for short vs medium, and long vs medium to be 3.3 and 3.9 OMU, respectively. Thusly, the goal of any predictive model of systemic toxicity is to be able to identify the approximate 5 OMU and approach 3-3.5 OMU, but cannot be expected to exceed the inherent uncertainty in toxicological testing and evaluation. This abstract does not necessarily reflect US EPA policy.

440b Dynamic Interaction between Human Serum Albumin and PFOS: A Theoretical Study
A. Zhang, H. Cao and J. Fu, Research Center for Eco-Environmental Sciences, CAS, Beijing, China. Sponsor: B. Zhao

Numerous studies have focused on whether or not PFOS may displace fatty acids bound to human serum albumin (HSA) since the famous persistent organic pollutant exhibited a long half-life of elimination in human serum, approximately 5-4 years. However, there are insufficient information on both PFOS binding sites with HSA and the potential biological function induced by the PFOS-HSA interaction. Recent study of PFOS-HSA complex structure at extremely high PFOS concentration revealed two PFOS binding sites in HSA which overlapped with fatty acid binding sites FA3/4 and FA6, respectively. Unfortunately, such still can not elucidate which site acts as the preferable binding cavity at rational human exposure concentration. Therefore, molecular simulation was adopted to answer the question in the present study. The allosteric modulation associated with PFOS-PFOS interaction of 2 binding sites of HSA, FA3/4 and FA6, was analyzed using molecular dynamics simulations and binding free energy calculations. FA6 site was firstly recognized by PFOS binding to HSA although PFOS exhibit higher binding affinity with FA3/4 site. Single binding PFOS at FA6 site can generate allosteric modulation of subdomain 3A through increasing cavity volume of FA3/4 site. Key residues R410 and Y411 locating at entrance of FA3/4 site controlled the allosteric effect and the binding procedure of second PFOS molecule in FA3/4 site subsequent to the first PFOS in FA6. Besides, FA6 may act as only binding site for PFOS at environmental exposure dose, while both crystallographic structures of ligands-HSA complex and binding free energies analysis excluded the possibility that PFOS binding HSA at FA6 site would significantly affect blood delivery of fatty acids in normal physiological conditions. Nevertheless, PFOS binding at FA6 site can still lead to the potential disturbance in natural transport function of HSA due to conformation alterations induced in other subdomains.

440c Utilization of Gene Expression Marker Data to Computationally Generate Nested Networks Underlying Embryonic Stem Cell Differentiation

Predictive tools which model the differentiation of stem cells under a variety of stresses are an area of current research interest. The biological stem cell differentiation network delineates which cell differentiates into which cell. However, the underlying rules which determine where cells differentiate are not fully understood. We suggest there exists a nested regulatory network that governs, controls, and modifies the probabilities of lineage specification. Pluripotent J1 mouse embryonic stem cells (mESCs) were maintained on a mouse embryonic feeder (MEF) cell layer supplemented with LIF. mESC differentiation was initiated on culture day zero using an adherent cell model. MEF-depleted pluripotent mESCs were seeded onto 96-well gelatin-coated plates in the presence of fetal bovine serum without LIF. Cultures were maintained through day nine (cardiomyocyte formation). On days zero through nine, RNA was isolated from the cell population and analyzed using the MouseRef-8 v2.0 Expression BeadChip (illumina). To detect the regulatory network, genes were selected as biomarkers of specific cell types (pluripotent, ectoderm, primitive streak, mesoderm, and endoderm cells). A novel model was used to calculate plausible daily cell counts for each cell type using biomarker expression data, supplemented with cell number counts on each culture day, and computationally generated daily counts for each cell type were used to generate possible nested regulatory networks. Networks were generated using a Bayesian optimization approach over a stochastic model describing differentiation and proliferation. We will demonstrate the model in controlled and perturbed systems of differentiation. The methodology is scalable to larger systems and modular so that improvements to any feature may be applied independently. This abstract does not necessarily reflect EPA policy.

440d Development of Quantitative Adverse Outcome Pathways Using Health-Protective Assumptions to Fill Data Gaps
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In an adverse outcome pathway (AOP), the target site dose participates in a molecular initiating event (MIE), which in turn triggers a sequence of key events leading to an adverse outcome (AO). Quantitative AOPs (QAOPs) are needed if AOP characterization is to address risk as well as hazard. A QAOP is based on data gaps, and as its mature form, has the ability to predict the dose response and time course (DRTC) behaviors linking toxicant dosimetry, the MIE, and the key events with each other and with the AO. Identification of data gaps during QAOP development is common and, typically, some of the gaps cannot be filled given constraints of time and resources. In such situations, alternative assumptions can be identified about how the missing data would affect DRTC behaviors and the more risk-conservative assumptions incorporated until the missing data become available. We are developing a QAOP for fetal minnows. The MIA is inhibition of CYP19A, which converts testosterone to estradiol (E2). Depression of plasma E2, upregulation of CYP19A mRNA, and reduced levels of the glycoprotein egg yolk precursor vitellogenin (VTG) are key events. Depression of VTG is associated with decreased fecundity, the AO. Available data include time-course studies with 3 doses of the CYP19A inhibitor fadrozole, plasma E2 and VTG levels, ovarian CYP19A mRNA levels, and fecundity. In this presentation we show how quantitative dose-response relationships between key events, such as expression of CYP19A mRNA as a function of decreased plasma E2 levels, while constrained by data, can be described by alternative functions with different low dose behaviors. These alternatives assumptions provide different DRTC behaviors for the overall relationship between the MIA and the AO and can be selected to bias the QAOP to provide risk conservative predictions. This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
The aromatase inhibitor fadrozole inhibits CYP19A, a key enzyme that converts testosterone to estradiol (E2). In fish, E2 levels control synthesis of the glycolipoprotein vitellogenin (VTG), an egg yolk precursor protein essential to oocyte development and larval survival. Fathead minnows were exposed to various concentrations of fadrozole continuously for 8 days and then held in control water for an additional 20 days. Plasma E2 and plasma VTG concentrations were reduced during exposure. While E2 concentrations recovered to control levels post-exposure, VTG concentrations did not fully recover, even after 20 days of deputation. We developed alternative hypotheses to explain the effect of fadrozole on VTG levels. First, it is possible that hepatic production of VTG returned to normal as the E2 concentration recovered. However, plasma VTG could remain depressed for an extended period while oocyte VTG is being replenished. A second hypothesis is that the plasma VTG concentration is not simply proportional to the level of E2 but rather, involves a more complicated biological circuit with alternative setpoints, or attractors, to which it would move after the initial perturbation by fadrozole (i.e., allostasis). Computational modeling cannot by itself identify the actual biological structure of this circuit but it can identify candidate structures that could be investigated in the laboratory. We modeled a candidate circuit that reproduces the VTG data. Our approach is intended to identify the underlying biological explanation for the dynamics of VTG perturbations by fadrozole. This work illustrates the application of computational modeling to evaluate alternative hypotheses and to thereby identify the most appropriate hypothesis for follow-up experimental work. This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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A deterministic biologically based dose response (BBDR) model was developed for the hypothalamic-pituitary-thyroid (HPT) axis to evaluate the effects of iodide intake and perchlorate exposure in the near-term pregnant woman and fetus. The goal of the current study is to extend the model to a probabilistic framework evaluating the response of pregnant population to thyroid disturbances. Maternal thyroid hormones play a crucial role in the fetal neurodevelopment. Perturbation of serum levels of maternal free thyroxine (fT4) following inhibition of thyroperoxidase (TPO) activity by JZL 184. The modeling also was able to predict reduced 2-AG levels mediated metabolism of AA and 2-AG (via neuronal CB1 activation). Our model had a high AC50 (66.5 nM) for cannabidiol (CBD) and its subsequent influence on maternal fT4 levels. Traditional toxicology tests required by US regulatory agencies are expensive and time consuming especially in light of the many chemicals that require such testing. An alternative vertebrate model for developmental toxicity is the zebrafish embryo (Danio rerio). In this study, 309 environmental chemicals were screened, mainly pesticides and antimicrobials from the ToxCastTM Phase I chemical library. Embryos were immersed in media containing chemical concentrations from 0.001 to 80 μM and the half-maximal activity concentration (AC50) for toxicity (lethality, non hatching, or dysmorphology) determined. To extrapolate to mammalian toxicity, the demonstrated relationship between lipophilicity (LogP) and bioconcentration was used to estimate a body burden associated with developmental toxicity (EC50). Toxicity potency rankings derived from AC50 and EC50 calculations for most toxic chemicals with a mean AC50 of 4.01 μM. To further assess extrapolation to mammalian systems, oral equivalent doses from regulatory toxicological studies were used to correlate in vitro concentrations with potential in vivo effects. The ability of the zebrafish developmental screen to predict mammalian toxicity was then assessed by examining the correlation between chemical potencies based on EC50, chemical class, and known toxicity, non hatching, or dysmorphology. The ability of the zebrafish developmental screen to predict mammalian toxicity was then assessed by examining the correlation between chemical potencies based on EC50, chemical class, and known toxicity, non hatching, or dysmorphology. Traditional toxicology tests required by US regulatory agencies are expensive and time consuming especially in light of the many chemicals that require such testing. An alternative vertebrate model for developmental toxicity is the zebrafish embryo (Danio rerio). In this study, 309 environmental chemicals were screened, mainly pesticides and antimicrobials from the ToxCastTM Phase I chemical library. Embryos were immersed in media containing chemical concentrations from 0.001 to 80 μM and the half-maximal activity concentration (AC50) for toxicity (lethality, non hatching, or dysmorphology) determined. To extrapolate to mammalian toxicity, the demonstrated relationship between lipophilicity (LogP) and bioconcentration was used to estimate a body burden associated with developmental toxicity (EC50). Toxicity potency rankings derived from AC50 and EC50 calculations for most toxic chemicals with a mean AC50 of 4.01 μM. To further assess extrapolation to mammalian systems, oral equivalent doses from regulatory toxicological studies were used to correlate in vitro concentrations with potential in vivo effects. The ability of the zebrafish developmental screen to predict mammalian toxicity was then assessed by examining the correlation between chemical potencies based on EC50, chemical class, and known toxicity, non hatching, or dysmorphology. The ability of the zebrafish developmental screen to predict mammalian toxicity was then assessed by examining the correlation between chemical potencies based on EC50, chemical class, and known toxicity, non hatching, or dysmorphology.
research effort which is the goal of International transporter consortium (ITC). In this study, based on public sources, we developed a panel of CASE Ultra modules for 14 human drug transporters expressed in intestine, liver, kidney and brain (MDR1, MRPI-5, BCRP, ASBT, NTCP, BSEP, OATP2B1, OCT1, MCT1, PEPT1). These modules provide evidence of active transport or its inhibition and are based on datasets that range in size from 34 molecules (BCRP substrates) to 1,585 chemicals (MDR1 inhibitors). Performance of the modules was evaluated by 10% bootstrapping and 10-fold external cross-validation. All modules achieve good sensitivity (60-100%), specificity (60-100%), balanced accuracy (70-100%), as well as positive (60-100%) and negative (60-100%) prediction rates. The panel of transporter modules was then applied to 1,543 FDA approved drugs. Only 20% of the drugs were predicted to have no active transport, while over half were predicted as substrates of two or more transporters. The developed panel of modules can be used for assessing transporter interactions at early stages of drug development or chemical hazard evaluation.

**440j** Computational Modeling of OATP1B1 Inhibitors


OATP1B1 (organic anion transporting polypeptide 1B1) is a member of the solute carrier family of transporters. It is expressed predominantly in the liver where it occupies the basolateral side of hepatocyte cell membranes and transfers molecules from the blood into the liver. OATP1B1 substrates include natural products such as bile salts as well as pharmaceuticals such as statins and sartans. OATP1B1 is inhibited by several compounds including cyclosporine A and rifampicin. Drug-drug interactions can occur when an inhibitor of the transporter is coadministered with a substrate drug. For example, when orally administered pravastatin is taken with cyclosporine A, the area under the curve of pravastatin increases almost 900%, potentially leading to toxicological effects. The first International Transporter Consortium report identified OATP1B1 as one of the seven most important transporters in drug disposition. A data set of 336 compounds was compiled from recent publications. The data consisted of single-concentration, percent inhibition data with either estradiol-$\beta$17-glucuronide or estrone-$\beta$-sulfate as the substrate. These values were first converted to IC50 values and then to inhibition constants (Ki) using the Cheng-Prusoff equation. Compounds with Ki values less than 20 mM were classified as inhibitors. The overall data set contained 74% noninhibitors and 26% inhibitors, so the population was skewed towards noninhibitors. An artificial neural network classification ensemble (ANNce) model was trained on 80% of the compounds with the remaining 20% set aside as an external test set. The best model consisted of seven descriptors and six hidden neurons. The model includes lipophilic, atomic charge, and sigma Fukui index descriptors. It has high specificity (90%), sensitivity (89%), and overall concordance (90%) when applied to the external test set. The model was used to predict OATP1B1 inhibition of a subset of about 2,300 compounds from the World Drug Index (WDI). The model predicted 26.5% of the compounds to be OATP1B1 inhibitors with less than 1.5% of the molecules outside the model’s applicability domain.

**440l Global Phosphoproteome Dynamics of Zebrafish (Danio rerio) Embryos**

O. Kwon1, S. Kim2, J. Sim1, M. Song1, K. Yun1, J. Kim2, T. Jeong3 and S. Lee1.1 College of Pharmacy, Kyungpook National University, Daegu, Republic of Korea, 2 Mass Spectrometry Research Center, Korea Basic Science Institute, Ochang, Republic of Korea and 3 College of Pharmacy, Yeungnam University, Gyeongsan, Republic of Korea.

The zebrafish (*D. rerio*) is a popular animal model in studies of vertebrate development and organogenesis. Recent research has shown a similarity of about 70% between the human and zebrafish genomes and of 84% of human disease-causing gene. In particular, zebrafish embryos show a number of desirable features, such as, transparency, a large size, and rapid embryogenesis. Protein phosphorylation is a well-known post-translational modification (PTM), and performs various biological functions. Recent mass spectrometry (MS) developments have been used to study global phosphorylation using MS-based proteomics coupled TiO2 phosphate enrichment. In the present study, we identified 3,500 non-redundant phosphorylation sites on 2,166 phosphopeptides and 1,564 quantified phosphopeptides in zebrafish embryos. The distribution of Ser/Thr/Tyr phosphorylation sites in zebrafish embryos was found to be 88.9%, 10.2%, and 0.9%, respectively. Using Motif-X analysis, a potential kinase motif was predicted for 80% of the identified phosphorylation sites, motif related to MAPK has been found the most frequently appearing. In particular, we found that the pSF motif of 35 phosphorylation sites has not been previously reported in vertebrates. In addition, we created 6 of phosphopeptides cluster pattern during developmental stages in zebrafish embryos. Throughout these results, we reported the largest data set for phosphoprotein in zebrafish embryos and the pSF motif at the first time in vertebrate found. Finally, our zebrafish results can be used the further studies of phosphorylation site or dynamic phosphopeptides in zebrafish embryos.

**440k Interpretable QSAR of Skin Sensitization for Screening Cosmetics and Environmental Chemicals**

R. D. Saiakhov, S. Chakravarti and A. Sedykh. *MultiCASE Inc, Beachwood, OH.*

Computational models of skin sensitization provide important alternative to animal testing of chemicals. However, many of reported models have limited applicability due to small training sets, insufficient validation, and unclear interpretation of results. To address this, we have compiled from scientific literature a harmonized dataset of 374 small molecules with categorical data from local lymph node assay (LLNA). We used CASE Ultra expert system (v.1.4.7.0) to derive two types of models. Model 1 defined strong and extreme sensitizers as active group (73 cmpds), moderate sensitizers as marginally active (103 cmpds), and treated weak and non-sensitizers as inactive (198 cmpds). Model 2 defined strong, extreme, and moderate sensitizers as active (173 cmpds), weak sensitizers as marginal (108 cmpds), and non-sensitizers as inactive (93 cmpds). External cross-validation (10-fold) for Model 1 yielded sensitivity of 50% and specificity of 90%, while for Model 2 - sensitivity of 80%, and specificity of 52%. The developed models have distinct advantages (lower false positives vs. lower false negatives) and can be used jointly for early prioritization of skin care products, topical drugs and environmental chemicals. Major chemical features found by these models to be associated with sensitization were various aromatic, phenolic and ketone groups. Exemplary screening with these models 298 skin application drugs from DrugBank and Drugs@FDA databases suggested that 15 to 30% of them may have some skin sensitizing effect.

**441 Genotoxic Compound Profiling Using MILLIPLEX® MAP DNA Damage/Genotoxicity 7 Plex Panel**

J. Hwang1, N. Scotti2, T. Warrmke1, R. Maheshwari1 and R. Wiese1. 1 R&D Immunooassay, EMD Millipore Corp., St. Charles, MO and 2 Biomedical Engineering, Washington University, St. Louis, MO.

A cell’s response to DNA damage involves many complex pathways and mechanisms, collectively called the DNA damage response. Once initiated, these pathways ultimately lead to the repair of the damage or the initiation of apoptosis. The DNA damage response plays a crucial role in maintaining the function, genomic stability, and viability of the cell and organism at large. Dysfunctions in the response are implicated in many disease states including cancer, premature aging, and neurodegenerative disease. We have developed a Luminex® bead based multiplex immunoassay that allows for the simultaneous detection of multiple DNA damage proteins in a single well, including phosphorylated Chk1, Chk2, H2AX, and p53, and total protein levels of ATR, MDM2, and p21. Using the DNA Damage/Genotoxicity 7 Plex panel, we analyzed the levels of phosphorylated and total proteins in HEPG2 and HEK293 cells treated with genotoxic compounds (ethynitrosourea, methyl methansulfonate, cisplatin, p-chloroaniline, etoposide, hydroquinone, sodium arsenite, paclitaxel and chloramphenicol) and non-genotoxic carcinogens (ethylnitrosourea, methyl methansulfonate, cisplatin, p-chloroaniline, etoposide, hydroquinone, sodium arsenite, paclitaxel and chloramphenicol) and non-genotoxic carcinogens (D-limonene and diethanolamine). Genotoxic compounds, but not non-genotoxic carcinogens, resulted in changes in DNA damage response in a dose and time dependent manner. However, the profile of the genotoxic compounds showed differential regulation of the seven analytes in the DNA Damage/Genotoxicity panel, which implies different modes of action. Cell toxicity was also confirmed using the Human Kidney Toxicity Panel 2 on HEK293 cells. These studies demonstrate the validity of using the DNA Damage/Genotoxicity 7 Plex panel in screening genotoxic compounds.

**441l Global Phosphoproteome Dynamics of Zebrafish (Danio rerio) Embryos**

O. Kwon1, S. Kim1, J. Sim1, M. Song1, K. Yun1, J. Kim2, T. Jeong3 and S. Lee1.1 College of Pharmacy, Kyungpook National University, Daegu, Republic of Korea, 2 Mass Spectrometry Research Center, Korea Basic Science Institute, Ochang, Republic of Korea and 3 College of Pharmacy, Yeungnam University, Gyeongsan, Republic of Korea.

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**Ochratoxin A Induces DNA Double-Strand Breaks and Large Deletion Mutations in the Carcinogenic Target Site of Gpt Delta Rats**

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Institute of Health Sciences, Tokyo, Japan, 2Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan, 3Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan, 4Department of Food Life Science, Azabu University, Kanagawa, Japan and 5Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan.

Ochratoxin A (OTA) is a carcinogen targeting proximal tubules at the renal outer medulla (ROM) in rodents. We previously reported that OTA increased mutant frequencies of the red/gam gene (Spi), suggesting deletion mutations. In the present study, Spi assays and mutation spectrum analyses of the Spi mutants were performed using additional samples collected in our previous study. Spi assay results were similar to those in our previous study, revealing large (over 1 kb) deletion mutations in the red/gam gene. To clarify the molecular progression from DNA damage to gene mutations, in vivo comet assays and analysis of DNA damage/repair-related mRNA and/or protein expression was performed using the ROM of gpt delta rats treated with OTA at 70, 210, or 630 µg/kg/day for 4 weeks. Western blotting and immunohistochemical staining demonstrated that OTA increased γ-H2AX expression specifically at the carcinogenic target site. In view of the results of comet assays, we suspected that OTA was capable of inducing double-strand breaks (DSBs) at the target sites. mRNA and/or protein expression levels of homologous recombination repair (HR)-related genes (Rad51, Rad18, and Brip1), but not nonhomologous end joining-related genes, were increased in response to OTA in a dose-dependent manner. Moreover, dramatic increases in the expression of genes involved in G2/M arrest (Chk1 and Wee1) and S/G2 phase (Cen2a and Cdk1) were observed, suggesting that DSBs induced by OTA were repaired predominantly by HR, possibly due to OTA-specific cell cycle regulation, consequently producing large deletion mutations at the carcinogenic target site.

**Identification of Genotoxic Compounds Using Isogenic DNA Repair Deficient DT40 Cell Lines in a Quantitative High-Throughput Screening (qHTS) Platform**

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DNA repair pathways play a critical role in cellular homeostasis by repairing DNA damage induced by endogenous processes and xenobiotics. Isogenic chicken DT40 cell lines deficient in different DNA repair pathways can be used to identify genotoxic compounds and aid in characterizing the nature of the induced DNA damage. As part of the U.S. Tox21 program, we previously optimized several DT40 isogenic clones for qHTS in a 1536-well format and confirmed the utility of this screening system, which is based on measuring differential cytotoxicity in wild type and DNA repair deficient cell lines following exposure to chemicals. In this study, we screened the Tox21 10K compound library against two isogenic DNA repair deficient DT40 cell lines (ku70/ rad54 and rev3) and one wild-type cell line using a cell viability assay based on measuring ATP levels. Ku70 and rad54 are associated with DNA double-strand break repair, and rev3 is associated with translesion DNA synthesis pathways. From the primary screening, we identified several well-known genotoxic compounds (e.g., melphalan, aclotiam) that induced significantly greater cytotoxicity in the rev3 deficient cell line compared to the wild type cell line. Moreover, several previously unknown compounds were identified as potential direct-acting genotoxic chemicals in our assay. Our results demonstrate the utility of this approach for evaluating the genotoxic activity of chemical compounds using 1536-well based qHTS and for acquiring detailed information on the type(s) of DNA damage induced by these compounds. Supported by NIEHS Interagency Agreement Y3-ES-7020-01.

**A 6-Week Inhalation Protocol to Measure Cigarette Smoke-Induced Damage in the Rat Lung by Ex Vivo Comet and Histopathological Analysis**

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Guidelines for the provision of genotoxicity data in mammalian cells have been re-defined such that an ex vivo study is preferable to a second in vitro mammalian study. We have explored the potential of the Comet assay to monitor DNA damage in rat lung alveolar type II epithelial cells (AECII) isolated from rats exposed to cigarette smoke (CS) via inhalation for 6 weeks. Concurrent histopathology was undertaken to monitor characteristic lesion formation following CS exposure. Female and male rats were exposed to sham air or 0.6 mg/L 3RF4 reference CS for 1 h or 2 h a day for 6 weeks. Lungs were collected, the left lobe processed for histopathology and AECII isolated from the right lobe. Histopathological evaluation demonstrated that goblet cells in the main bronchus, plus unpigmented macrophages and pigmented macrophages in the alveolar lumina were statistically significantly increased in CS exposed rats when compared to sham air. Values obtained (mean severity scores ± SEM) were 0.45±0.16, 1.47±0.30 and 1.38±0.26 for goblet cells, 0.45±0.15, 1.85±0.18 and 2.42±0.19 for unpigmented macrophages and 0.00, 0.45±0.14 and 1.11±0.17 for pigmented macrophages following exposure to sham air, 1.3RF4 CS or 2h 3RF4 CS respectively. The level of DNA damage in isolated AECII was statistically significantly increased in CS exposed rats when compared to sham air. Values obtained (mean % tail DNA ± SD) were 3.19±7.29, 13.43±14.54 and 13.83±16.20 for the Alkaline Comet assay and 18.58±15.52, 37.75±16.17 and 39.84±17.36 for Modified Alkaline Comet following exposure to sham air, 1h 3RF4 CS or 2h 3RF4 CS respectively. In conclusion, we have developed a 6 week inhalation protocol with an ex vivo endpoint. This protocol may have potential use to monitor characteristic lesion formation alongside DNA damage induced by CS.

**Integration of Pig-a Mutation and Micronucleated Reticulocyte Frequencies in a 28-Day Repeat Dose Study**


Genotoxicity of N-ethyl-N-nitrosourea (ENU) was evaluated for Pig-a gene mutation and micronucleated reticulocyte (MN-RET) endpoints in rat peripheral blood using high throughput flow cytometry-based methods. Male Sprague Dawley rats (7-8 weeks age, 6 animals/group/dose) were treated via oral gavage for 28-consecutive days with several doses of ENU (0.625, 2.5, or 10 mg/kg/day) or the vehicle (Phosphate Buffered Saline, pH 6.0). Blood samples were collected from the tail vein on days 0, 15, 29 and 46 for measuring Pig-a gene mutation frequencies in total red blood cells (RBCs) and reticulocytes (RETs) using MutaFlow-PLUS kit reagents. On day 4 and day 29 of the study, blood samples were processed for measurement of MN-RET frequencies with MicroFlow-PLUS kit reagents. Mutant RBCs and RETs exhibited significant dose and time-dependent increases on days 15, 29 and 46 at all the dose levels tested except at the lowest dose (0.625 mg/kg) on day 15. This 0.625 mg/kg dose induced a small but significant increase only in mutant RETs frequencies at this early time point, whereas both mutant RBCs and mutant RETs were elevated on days 29 and 46. With regard to MN-RET frequencies, a significant increase was found on day 4 and day 29 at the highest dose level (10 mg/kg). The mid dose (2.5 mg/kg) was significantly elevated on day 29. In conclusion, the lowest dose of ENU tested in this study (0.625 mg/kg) was observed to cause marked increases in Pig-a mutation despite the fact that it is lower than the threshold dose of 0.88 mg/kg estimated by Dobo and colleagues who also studied the Pig-a endpoint (Mutat. Res., 725:13-21). This increased sensitivity may be attributable to our use of immunomagnetic column separation step that allowed scoring of many times more cells per sample. In addition, the results validated the simultaneous scoring of two genotoxicity endpoints as well as provided a statistical robustness to the data collected. This approach of analyzing more than one endpoint in a single study results in reducing the number of animals used and proves highly cost-effective.

**In Vivo Genotoxicity of 1, 4-Dioxane in Rats**


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1,4-Dioxane is used widely as a solvent in the manufacture of chemicals, and as a laboratory reagent. 1,4-Dioxane induces liver adenomas and carcinomas in mice and rats, and is categorized by IARC Group 2B chemicals. 1,4-Dioxane has been reported to be non-genotoxic in in vitro studies, e.g. Ames, mouse lymphoma TK, chromosomal aberration, in vitro micronucleus and sister-chromatid exchange assays. In the in vivo studies, positive results were obtained in bone marrow micronu-
clesus assay in C57BL6 and CD-1 mice, and liver micronucleus assay with or without partial hepatectomy (PH) in CD-1 mice. However, negative results were also observed in many in vivo genotoxicity studies; e.g., drosophila sex-linked recessive lethal, rat liver DNA alkali elution, rat liver DNA repair, bone marrow (BALB/c mice) and peripheral blood (CD-1 mice) micronucleus assay. Therefore, there is no clear conclusion for its in vivo genotoxicity in rodents. In the present study, we investigated the ability of 1,4-dioxane to induce micronuclei in rat bone marrow and liver. In the liver micronucleus assay, we performed the juvenile animal method and two PH methods (dosing before PH or after PH). We also evaluated the mutagenicity of 1,4-dioxane by Pig-a gene mutation assay using rat peripheral blood. As a result, all methods of liver micronucleus assay detected micronucleus induction by 1,4-dioxane. In the sensitivity to liver micronuclear induction by 1,4-dioxane, the dosing before the PH method, a suitable method for structural chromosome aberration inducer, is the highest among the three methods. This finding is consistent with a report that 1,4-dioxane induces chromosome breakage in the liver. Negative results were observed in the bone marrow micronucleus and Pig-a gene mutation assays. These results suggested that 1,4-dioxane is genotoxic in the liver but not in the bone marrow of rats. The fact that genotoxicity of 1,4-dioxane in the liver was detected in rats as well as mice supports the usefulness of the rat liver micronucleus assay because the rat is more common species in toxicology research and requires easier operation in PH.

**447 Combination of Multitissue Micronucleus Assay and Gene Mutation Assay: Simultaneous Detection of Multitissue Micronucleus Inducibility and Gene Mutation in Gpt Delta Transgenic Rats**


Recently, the combination or integration assay attracts attention because the readout in the number of experimental animals is required from the viewpoint of the animal welfare. If the evaluation of multi-tissue gene mutation assay using transgenic rat is combined to the results of micronucleus assay which shows different genotoxicity endpoints, it is expected that more information will be obtained from the same animals.

The gpt delta male rats were orally administered with 1, 2-dimethylhydrazine dihydrochloride (DMH) at day 1 and N-nitroso-N-methylurea (MN) at day 3 and 4. DMH and MN are known to induce micronuclei in the glandular stomach and colon, respectively. The colon, glandular stomach, peripheral blood and liver were removed from the animals euthanized on one day after the final administration. The gpt assay and Spi-assay were conducted using these tissues. The micronucleus assay was conducted using the colon, glandular stomach and peripheral blood.

In the results, statistically significant increases in the frequency of micronucleated cells in the colon, glandular stomach and peripheral blood were observed in the DMH and MNU-treated groups compared with the negative control. In the gpt assay, mutant frequencies in all assayed organs were also significantly increased. In the Spi-assay, mutant frequency in the liver only was significantly increased.

These results suggest that the multi-tissue gene mutation assay using transgenic rat can be combined to the micronucleus assay in gastrointestinal tract.

**448 Toxicity of Chemical Dispersants, Oil, and Chemically Dispersed Oil in Sperm Whale Skin Cells**

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Two major oil crises in United States history, the 1989 Exxon-Valdez oil spill in Alaska and the 2010 Deepwater Horizon Oil Rig explosion in the Gulf of Mexico, drew attention to the need for toxicological experiments on oil, chemical dispersants and, importantly, chemically dispersed oil. We are still learning the effects these spills had on wildlife. However, little data is known about the toxicity of these substances in marine mammals. The objective of this study is to determine the toxicity of the two dispersants (Corexit 9500 and 9527), Alaskan and Gulf oil, as well as chemically dispersed oil. Corexit 9500 and 9527 are both cytotoxic to sperm whale skin fibroblasts. Corexit 9527 is less cytotoxic than 9500. S9 mediated metabolism did not alter cytotoxicity of either dispersant. Both dispersants are genotoxic to sperm whale skin fibroblasts; S9 mediated metabolism increased Corexit 9527 genotoxicity. Oil experiments were performed using the water accommodated fraction of oil (WAF). The Alaskan WAF is not cytotoxic to sperm whale skin cells. Chemically dispersed Alaskan oil (CEWAF) is more cytotoxic and genotoxic than the WAF. S9 mediated metabolism increased genotoxicity caused by CEWAF and WAF.

**449 In Vitro Cytotoxicity and Genotoxicity Testing Using upcyte® Hepatocytes**


We have developed a novel technique which causes primary human hepatocytes to proliferate whilst retaining an adult phenotype including functional phase 1 and 2 metabolism. In this study we evaluated the resulting “upcyte® hepatocytes” for their potential use in cyto- and genotoxicity testing.

For the establishment of an upcyte® hepatocytes based genotoxicity test we adapted the conditions of the frequently used micronucleus test. Conditions for upcyte® hepatocytes from a single donor (Donor 740) showed that a treatment duration of 96 h, without a recovery period was optimal for detecting both directly acting and metabolically activated genotoxins, whilst true negative and “misleading” positive compounds were correctly identified as negative. The micronucleus rate (%MN) in upcyte® hepatocytes treated with DMSO, cyclophosphamide and MMC was essentially unaffected by the growth stage (between a population doubling of 18 and 59). In conclusion, these data support the use of upcyte® hepatocytes in the MN test, since they were able to correctly identify known direct and metabolically activated genotoxicants, while misleading positives and true negative compounds resulted in negative outcomes.

The cytotoxicity of 31 compounds was measured using upcyte® hepatocytes derived from four donors to include effects due to donor variation. The compounds were classified as either severely, moderately or non-hepatotoxic. There was a very good intra- and inter-experimental reproducibility of the measurements. The cytoxicity of the majority of compounds was donor-dependent. The predictive capacity of the assay was generally good such that known non-hepatotoxicants were clearly negative and compounds that were associated with hepatotoxicity caused damage to the upcyte® hepatocytes.

In conclusion, these studies show that use of upcyte® hepatocytes which combine proliferation with long-term stable expression of adult hepatic phenotype enables the development of new in vitro liver cell models for metabolic drug and toxicity analysis.

**450 The Liver Micronucleus Test Integrated into 5-Day Repeated-Dose Toxicity Study in Mice Would Be Useful to Detect the Genotoxicity of Hepatocarcinogens**


The liver micronuclear test has the potential to detect liver carcinogens due to the accumulation of micronuclear by repeated dosing and could be expected to be integrated into repeated-dose toxicity study. In this study, to assess the possibility of its integration into 5-day repeated-dose toxicity study in mice, genotoxic hepatocarcinogens: diethylhydrazine (DEN), 2,4-diaminotoluene (2,4-DAT), 2,4-dinitrotoluene (2,4-DNT) and 2-acetylaminofluorene (2-AAF), non-genotoxic hepatocarcinogen; clofibrate, and non-carcinogen; 2,6-diaminotoluene (2,6-DAT), were administered orally to male ICR mice once daily for 5 consecutive days. Hepatocytosis were collected 24 h after the final administration, and the frequencies of micronucleated hepatocytes (MNHEPs) were examined. Significant increases in MNHEPs were observed in DEN- and 2,4-DAT-treatment groups, but not in 2,4-DNT-, 2-AAF-, 2,6-DAT- and clofibrate-treatment groups. 2-AAF might not induce MNHEPs because of its mitosis inhibitory effect on parenchymal hepatocytes. The result of 2,4-DNT is reasonable because it is a hepatocarcinogen in rats, but not in mice. In conclusion, the liver micronucleus test integrated into 5-day repeated-dose toxicity study in mice would be useful to detect the genotoxicity of hepatocarcinogens with consideration on results of general toxicity studies.

**451 Evaluation of Reactive Textile Dyes with Organs-Specific Genotoxicity Using In Vitro Flow Cytometry-Based Micronucleus Assay**

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Introduction: The textile industry extensively uses synthetic chemicals as dyes. There are several studies reporting the deleterious effects (e.g. DNA damage) of dyes to humans. Humans can be exposed to toxic dyes either through ingestion of contaminated waters or dermal contact with colored garments. Thus, genotoxicity assessments of these chemicals using organ-specific cell lines can be relevant to estimate their hazard. Objective: Cyto- and Genotoxicity of the textile dyes Reactive Green 19 (RG19), Reactive Black 12 (RB12), Reactive Black 19 (RB19), Reactive Black 5 (RB5), Reactive Red 120 (RR120) and Reactive Orange 16 (RO16) were assessed using in vitro MicroFlow® kit (Litron Laboratories Ltd.)
with immortalized human keratocytes (HaCaT) and human hepatoma cells (HepaRG TM, Biopredict International, Rennes, France, purchased at Fisher Scientific). Methodology: HaCaT and HepaRG TM at 1×105 and 2×105 cells/mL, respectively, were exposed in a 96-well format to six concentrations of the test dyes (at 31.25 to 1000 μg/mL), PBS 20% v/v (solvent control), Mitomycin c at 2.5 to 10 μg/mL and Vinblastine at 12.5-50 μg/mL (positive controls) for 48 and 24 h, respectively. 2-fold over concurrent solvent control mean (EMA-fold: cytotoxicity, MN-fold: genotoxicity) were used to define the positive results. Results and Discussion: A dose-dependent cytotoxicity was observed using HaCaT cells for all tested dyes, unlike in the experiments carried out with HepaRG TM, where cytotoxic effects were detected only for RB19 and RO16 at the highest concentration (1000 μg/mL). Genotoxicity was not observed for any of the tested dye under both experimental conditions (HaCaT and HepaRG TM). Conclusion: Based on our findings, HaCaT cells showed to be more sensitive to detect the cytotoxic effects of textile dyes than HepaRG TM. The reactive dyes studied herein can be considered not hazardous to human skin in respect to the criticality of in vitro testing of these target tissue (epidermis and liver). Financial Support: FAPESP (Process No. 2010/14941-3)

**452 Kinetics and Dose-Response Assessments of Cisplatin-Induced Genotoxicity in Sprague-Dawley Rats**

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Cisplatin and other platinum-type drugs exhibit tremendous efficacy in treating some types of malignancies, for example testicular cancer. However, secondary malignant hematopoietic disorders, with some estimates suggesting rates as high as one in three. We set out to determine whether a rat model and two blood-based endpoints could be used effectively to study the genotoxicity of cisplatin at low, tumorigenic dose levels. Male Sprague Dawley rats, age 7 weeks, were administered cisplatin via ip injection at 0, 0.05, 0.1, 0.2 and 0.4 mg/kg/day for 28 consecutive days. Allometric scaling suggests that the highest dose level corresponds to a human equivalent of 2.4 mg/m²—well below typical therapeutic levels of 20 mg/m² iv (once/day for 5 days/cycle). Two endpoints of genotoxicity were studied: micronucleated reticulocytes served as an indicator of chromosomal damage and were measured on days 4 and 29, and Pig-a mutant phenotype cells were scored in blood samples collected on days 15, 29, 56 and 84. Even the earliest time points evaluated showed dose-related increases for both endpoints, long before tumors or blood samples collected on days 15, 29, 56 and 84. Even the earliest time points evaluated showed dose-related increases for both endpoints, long before tumors or mortality information for assessing whether suspected leukemogenic agents such as cisplatin contribute to the risk of secondary malignancies through a genotoxic mode of action. Furthermore, given the pharmaceutical industry’s desire to combine platinum drugs with novel anti-neoplastic agents, we speculate that this rodent model may prove useful for studying adjuvants’ effects on platinum drug-induced DNA damage, and by extension the tumorigenicity of combination therapies.

**453 Development of a Flow Cytometric Assay for Measuring In Vivo Mutation in an Autosomal Gene**

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The product of the X-linked Pig-a gene is essential for the synthesis of glycosyl phosphatidyl inositol (GPI) anchors that link specific proteins to the cell surface. Although an assay that detects in vivo mutation in the Pig-a gene (the Pig-a assay) has generated considerable interest in the regulatory community, the Pig-a assay is not expected to detect an important class of mutation, i.e., loss of heterozygosity (LOH). LOH mutations can most readily be detected using a heterozygous autosomal reporter gene, as is the case with the heterozygous Tk gene employed in the mouse lymphoma assay. In this study, we investigated the feasibility of a flow cytometric Pig-a-like assay using an autosomal gene that codes for a GPI-anchored surface marker protein, CD24. CD24+/− mice were generated by breeding CD24 knockout (KO) males to wild-type females. A labeling strategy was devised to differentiate between Pig-a and CD24 mutant RBCs. Leukodepleted blood was labeled with a PE-conjugated anti-mouse CD24 antibody and FLAER (AlexaFluor®488-conjugated nontoxic variant of procarboxylase), which binds to GPI anchors. A pilot study was performed by treating groups of five 6-8-week-old male CD24+/− mice with single doses of 0 or 140 mg/kg ENU by oral gavage; blood was collected on Days -1, 15 and 29, labeled, and processed by flow cytometry to quantify fluorescently labeled and unlabeled erythocytes. Significant increases in the frequency of FLAER-negative (presumably Pig-a mutant) and CD24-negative (presumably Pig-a and CD24 mutant) erythrocytes were observed in ENU-treated animals, with CD24-negative erythrocyte frequencies being somewhat higher. Additional studies using agents that induce high frequencies of LOH-type mutations (e.g., ionizing radiation) should be conducted in order to confirm that the CD24-negative phenotype is detecting a greater number of mutations than Pig-a-negative phenotype.

**454 Evaluation of Thioacetamide Genotoxicity in Rat Liver and Stomach As Measured by the In Vivo Alkaline Comet Assay**

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Thioacetamide is a known hepatotoxic agent and dietary administration caused hepatocellular carcinoma in mice and rats and tumors of the bile duct in rats. It is negative in Ames and in vitro chromosome aberration tests, however, there are conflicting reports regarding its genotoxicity in vivo. It induced micronuclei in the bone marrow of two strains of mice, albeit mostly at lethal dose levels, but similar effects were not seen in rats. No micronuclei were induced in the liver or peripheral blood of thioacetamide treated rats. In the Comet assay, thioacetamide was positive in stomach, colon and urinary bladder, but not in bone marrow or liver of mice treated by intraperitoneal injection. We examined thioacetamide activity (up to a maximum tolerated dose) in a rat alkaline comet assay and measured DNA migration in liver and stomach after 3 exposures (21-24 hours apart), sampling 3 hours after the final administration. Thioacetamide caused an increase in DNA migration in the stomach of rats treated at 38 and 75 mg/kg/day as well as an increase in hedgehogs, a Comet morphology indicative of highly fragmented DNA that is excluded from Comet analysis. Thioacetamide did not cause any increase in DNA migration in the liver, despite evidence of marked hepatotoxicity. These findings confirm that toxicity does not always confound assessment of genotoxicity-related DNA migration in the Comet assay.

**455 Evaluation of the DNA Adductome Approach to Assess the DNA Reactivity of Chemicals in Rat Liver**


The usefulness of DNA adductome approach, a comprehensive measurement of DNA adducts using highperformance liquid chromatography equipped with tandem mass spectrometry (LC–MS/MS), was evaluated to assess the DNA-damaging capability of chemicals in vivo. Male Sprague-Dawley rats were treated with compounds from three categories: (1) genotoxic carcinogens causing DNA alkylation, dimethylnitrosamine (DMN) and methylmethanethiosulfonate (MNT); (2) genotoxic carcinogens producing DNA bulky adducts, 2-acetylaminofluorene (2-AAF), 2,4-Diaminotoluene (2,4-DAT), 7,12-di-methylbenz[a]anthracene (DMBA) and 4-Nitroquinoline 1-oxide (4NQO); and (3) non-genotoxic carcinogen, Di(2-ethylhexyl)phthalate (DEHP), DNA extracted from liver samples were enzymatically hydrolyzed to deoxyribonucleoside by micrococcal nuclease/bovine spleen phosphodiesterase or P1 nuclease method, and subsequently applied to the LC/ESI-MS/MS analysis. The methods was designated to detect the neutral loss of deoxyribose from positively ionized deoxynucleoside adducts by monitoring the DNA samples transmitting their [M+H]+-[M+H+116]+ transitions. The transitions were monitored over the m/z range of 250-702. All genotoxic carcinogens produced various DNA adduct peaks in the liver. Some of DNA adduct peaks had the m/z values corresponded to compound-derived adducts (methyl-dG for DMN and MNT, aminofluorene-dG for 2-AAF, DMBA-diol-epoxide-dG for DMBA, 4-aminoquinoline 1-oxide-dG for 4NQO). On the contrary, no DNA adduct peak was detected in liver of DEHP treated rats. These results indicate that this DNA adductome approach would be useful to identify the DNA reactivity of compounds, and to support the understanding of their mechanism of action.
tion assessment: 1) to dose for 28 days plus 3 days expression period and sample both developing germ cells from the seminiferous tubules and mature sperm from the cauda epididymis/vas deferens or 2) to dose for 28 days and sample only mature sperm a minimum of 7 weeks (mice) or 10 weeks (rat) after last treatment. Option 1 has significant advantages in terms of logistics, duration and cost of the study. Even more importantly where somatic tissues are investigated alongside germ cells, Option 1 is more ethically justified as it would use up to half the number of animals as Option 2. However, it has been questioned whether all stages of germ cell development are adequately covered by the 28 plus 5 day study design to ensure accurate detection of germ cell mutagens. We treated male MutaMice with saline or the potent mutagen ethyl nitrosourea (ENU). Animals were treated orally by gavage for 28 consecutive days at 10 mg/kg/day. On Day 31, animals were necropsied and developing germ cells from the seminiferous tubules and mature sperm from the cauda epididymis/vas deferens were isolated. Both cell types were examined for mutation in the neutral lacZ transgene using positive selection methods. Clear increases in in vitro mutagenesis were detected in developing germ cells, however, weaker, equivocal increases were detected in the mature sperm, supporting the idea that a 28 plus 3 day study design is not optimal for robust detection of germ cell mutagens.

**457 Genotoxic Effects on HepG2 Cells Induced by Brominated Flame Retardant (BDE-47)**

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INTRODUCTION: BDE-47, a brominated flame retardant, shows evidences of potential toxicity to the organisms and the environment. The levels of BDE-47 on biotics and abiotics systems are increasing and its high bioaccumulation has leading to growing concerns about its presence in the environment and the consequences to the human health, such as its genotoxic potential. BDEs genotoxic potentials had been shown for some cell lines, but there is no information to the liver cells, which are one of the main targets of BDEs. Therefore, the full evaluation about the risks of BDE use are still necessary to better understand their toxicity to the human health. OBJECTIVE: The aim of the work was to investigate the genotoxic effects of the BDE-47 on human hepatoma cells (HepG2). METHODOLOGY: Briefly, cells were incubated at 37°C, in an atmosphere containing 5% CO2 and 95% relative humidity and treated with concentrations ranging from 0.1 to 25 μM for 4 hours. After the exposure period, the HepG2 cell were fixed with agaropectin and transferred to slides. The cell on the slides were lysed for 2 hours and submitted to electrophoresis (300 mA and 25 V for 20 min). Then, the cells were stained with propidium iodide solution (10 μg/ml) and examined using fluorescence microscopy. RESULTS: Our results showed the capacity of BDE-47 to cause DNA damage at doses starting at 10 μM (p<0.05) while at lower concentration there isn’t difference between treatments and negative control (without BDE-47). CONCLUSIONS: According to these results, BDE-47 showed the ability to cause DNA damage of HepG2 cells after the high concentration exposure. These conclusions contribute with evidences to the genotoxic potential of BDE-47, which high bioaccumulation potential can easily lead to a high concentration into the DNA damage of HepG2 cells after the high concentration exposure. These conclusions reinforce the importance of using different assays for toxicological analyzes and suggest that BDE-153 induces genotoxic effects that can lead the cell to death by apoptosis.

**458 BDE-153 is Genotoxic to HepG2 Cells and Lead to Apoptotic Cell Death**

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INTRODUCTION: Polybrominated diphenyl ethers have been used in diverse products and are ubiquitous contaminants in sediments and biota. They are also found in human tissues, food and wildlife. There is a lot of information about the presence of brominated flame retardants in the environment, however little is known about their mechanisms of inducing toxicity. The use of different assays as well as monitor different endpoints is important to evaluate the hazard potential of chemicals OBJECTIVE: The aim of this work was to investigate the mechanisms by which BDE-153 (penta-BDE) can induce toxicity to HepG2 cells in concentrations ranging from 0.1 to 25 μM. METHODS: Briefly, the affects of BDE-153 in HepG2 cells were evaluated by different endpoints. First, we assessed the exposure of phosphatidylserine on the outer cell membrane after 24 and 48 hours of exposure to BDE-153. After that, we assessed nuclear fragmentation using the fluorescent dye Hoechst 33342, and finally, we evaluated the genotoxic potential of the compound through the comet assay. RESULTS: We observed that in concentrations of 5 μM and higher BDE-153 was able to induce apoptotic cell death since it was observed exposure of phosphatidylserine in the outer cell membrane and nuclear fragmentation of HepG2 cells after 24 h of exposure to the compound. In addition BDE-153 is able to induce primary DNA damage observed by comet assay at the same concentrations but much earlier, with only 4 hours of cell exposure to the compound. CONCLUSIONS: The results reinforce the importance of using different assays for toxicological analyzes and suggest that BDE-153 induces genotoxic effects that can lead the cell to death by apoptosis. Supported by: FAPESP - Proc. 2012/13133-0

**459 Validation of an Expert Rule-Based System to Support the ICH M7 Guideline on Drug Impurities**

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The ICH M7 guideline on drug impurities states that two distinct in silico methodologies can be used to qualify certain drug impurities as not mutagenic. This poster outlines a new expert rule-based system to predict the results of a bacterial mutagenesis assay. In the development of this system, an internal library of mutagenicity structural alerts was identified from the literature. This process included consolidating the same or similar alerts cited in multiple publications. Information on plausible mechanisms was collected alongside the structural definitions. Factors that deactivate the alerts were also identified from the literature and through an analysis of the corresponding data using the Leadscope data mining software. Over 200 distinct alerts were identified and these alerts were further validated against a database of over 7,000 chemicals with known bacterial mutagenesis results. Only validated alerts with a sufficiently strong association with positive expert-reviewed calls from Salmonella and E. coli strains were included. A prediction of the bacterial mutagenic assay can be made using these validated alerts; however, this is only possible where the compound is within the applicability domain of the alert system. In addition, a confidence score based on information collected for each alert is provided alongside the positive or negative call. This poster outlines the expert system along with the results of validating the system, both as a standalone system as well as in combination with a statistical-based approach. The system was tested using a set of 3,734 compounds with concordance of 79.4% and coverage of 99.8%.

**460 An In Vitro Mutagenicity Test of Hydroquinone Using the lacZ Transgenic Mouse**

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Hydroquinone (HQ) is used as an antioxidant in rubber industry and as a developing agent in photography. HQ is also used as a skin-lightening agent, and some cosmetics with an excess of content over 10% are marketed in Japan. Health risks in general population may be of concern due to highly absorbed HQ via dermal exposure. Carcinogenicity of HQ was suggested from previous studies in which HQ induced hepato cellular adenomas and forestomach hyperplasias in mice and renal cell adenomas in male rats. In genotoxicity tests, positive results in chromosomal aberration tests and micronuclei tests both in vivo and in vitro were reported, whereas most reported tests in Ames tests were negative except in a few studies. In this study, the lacZ transgenic mouse (MutaTM Mouse) model was used to clarify whether mutagenic mechanisms are involved in HQ-induced carcinogenesis in according to the OECD guideline 488. Male MutaTM mice were subjected to repeated oral administration of HQ at dosages of 0, 50, 100, or 200 mg/kg/day for 28 days. High dose was set based on the result of the NTP fourteen day gavage study using B6C3F1 mice in which HQ related death was observed in males receiving 250 mg/kg/day. Body weight was not affected by HQ administration up to 200 mg/kg/day. Mutant frequency in the liver and stomach of HQ treated mice were not significantly different from the negative control. N-Ethyl-N-nitrosourea, used as a positive control, induced mutation at a frequency 2-fold higher in the liver and 11-fold higher in the stomach than in their respective negative control organs. These results suggest that the mutagenic mechanism is not responsible for carcinogenesis of HQ.

**SOT 2014 Annual Meeting** 119

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461 Oligoquat M Does Not Induce Mutagenesis and Chromosome Aberration in the V79 and CHL Cell Lines
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Oligoquat M is a novel cationic moisturizing polymer derived from sugar cane with the active ingredient being stearyl dihydroxypropyldimonium oligosaccharides. Oligoquat M is used as a personal hygiene product and therefore warrants a detailed assessment of its safety, including mutagenesis potential. In this study, the toxicity of the Oligoquat M to the Chinese hamster lung cells (V79 and CHL) was tested in the presence and absence of the metabolic system (S9), and the V79 HGPRT gene mutation and the CHL chromosome aberration induced by Oligoquat M were tested. We found that the half of the growth inhibitory concentration (IC50) was 209μg/mL to the CHL cell. The number of resulting mutant clones from V79 cells treated by the dose levels of 75, 150 and 300μg/mL were similar to those of the negative control (P<0.05); in contrast, the cell mutation frequency (MF) of positive control was 49.4/10E6 and 63.0/10E6 for EMS and MCA, respectively, which was greater than 3 times of the negative control group (4.4/10E6 and 3.51/10E6 with or without S9, respectively). In the dose level of 50, 100 and 200μg/mL, the incidence of the chromosome aberration of CHL cells were less than 4% in the presence and absence of S9, and showed no clear dose-response relationships. We can conclude that Oligoquat M has no significant mutagenic and chromosome aberration effects in V79 and CHL cells under this laboratory conditions.

KEYWORDS: Oligoquat M; HGPRT locus; Gene mutation; Chromosome aberration

462 Assessment of Genotoxicity and Antigenotoxicity of Ethanolic Extract of Spondias mombin L. (Anacardiaceae) Leaves in In Vitro and In Vivo Models
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Spondias mombin, a medicinal plant found in Nigeria contains high amounts of phenolic compounds with antioxidant activity. The ethanolic extract of the leaves was evaluated for its safety and beneficial effects using genotoxicity and toxicity tests. None of the plant fractions had detectable mutagenic activity. The S. mombin fractions were not only non-mutagenic in Salmonella typhimurium strains TA98 in the presence and absence of metabolic activation, but also exhibited antimutagenic activities against 2 – amino – 3 methyl – 3H – Imidazo[4, 5 – F] quinoline (IQ) – induced mutagenesis. Phytotoxic analysis revealed that S. mombin revealed the presence of alkaloids, tannins, saponins, phlobatannins, flavonoids and anthraquinones. The administration of S. mombin at concentration of up to 8,000 mg/kg body weight did not induce acute toxicity in rats. A bone marrow micronucleus test was performed to detect clastogenicity and anticlastogenicity. The extract in the dose of 2,000 mg/kg did not cause micronucleus formation in the bone marrow of rats. Furthermore, in rats administered 50–100 mg/kg of the extract, no anticlastogenic effect against sodium arsenite induced micronucleus formation was observed. These studies provide data concerning the safety and antimutagenic potency of an ethanolic extract of Spondias mombin leaves.

463 Detection of Sulfur Mustard-Induced DNA Adducts in Human Skin Cells with Laser Scanning Microscopy
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Introduction: Sulfur mustard (SM) and 2-chloroethyl ethyl sulfide (CEES) are bi- and monofunctional DNA alkylating agents, respectively. SM is an old chemical warfare agent causing blisters (vesicant). Both chemicals react with N7 guanine. SM will form 7-Hydroxyethylthioethylguanine for SM. A specific monoclonal antibody (2F8) exists which detects SM at N7 position. Aim: The 2F8 antibody was used to develop a microscopique technique for detection of SM exposure in human keratinocytes (HaCaT cells). Methods: HaCaT cells were cultured on cover slips and exposed with different concentrations of SM (30 min). After exposure, cells were fixed and treated with Proteinase K. Subsequently, the preparation was treated with the monoclonal 2F8-antibody against the N7- guanine monoadduct. Next, the antibody molecules attached to the DNA damage were made visualized by binding to a goat-anti-mouse antibody that contains covalently a fluoresceum group emitting red light to bind to them. The preparation was also treated with DAPI to counterstain cell nuclei. By means of laser scanning microscopy, red fluorescence was quantified. Results: DNA adducts were concentration dependent detected after SM exposure below 30 μM which is the vesicant threshold. Conclusion: The presented technique is potentially able to detect and quantify SM exposure in HaCaT cells in vitro. The technique may be useful to confirm SM exposure in blister roofs of intoxicated patients.
Introduction: LLL-3, an anthracene derived compound, has been shown to be a promising therapeutic agent in the treatment of some kinds of cancer such as chronic myeloid leukemia. This compound is derived from the commercial product STA21 and acts by inhibiting the STAT3, which has been associated with the induction of proliferation and survival of cancer cells. Although LLL-3 has presented good properties to be a potential therapeutic agent in the treatment of some types of cancer, there is no data in the literature about the toxic properties of this compound.

Objective: To investigate the genotoxic and cytotoxic activities of the LLL3 by Micronucleus (MN) and WST assays, using mouse macrophage cell line RAW 264.7.

Results and Discussion: LLL-3 did not induce genotoxicity in RAW 264.7 cells, moreover, it was noted that apoptosis or necrosis cell death in these cells, although the mitotic index decreased after exposure in the same tested conditions, which may suggest some cytostatic effect, since this compound acts by inhibiting the STAT3. Besides, the cell viability evaluated by WST assay was not affected by LLL-3. One LLL-3 did not show mutagenic activity as most of other drugs used in cancer treatment, the results increase the chance of using this drug in cancer therapy.

Conclusion: LLL-3 should be of interest to further explore the possibility of using it as a potential agent for human cancer treatment.

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Genotoxicity Assessment of Sandalwood (Santalum album) Essential Oil on Human Breast Adenocarcinoma Cells (MCF-7)

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Sponsor: D. Oliveira.

SOT 2014 Annual Meeting 121

Investigation of the Genotoxic and Cytotoxic Activities of the LLL-3, a STAT3 Inhibitor, Using Micronucleus and WST Assays

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Estragole and safrole, naturally-occurring constituents of various herbs and spices, are rodent liver carcinogens. To further understand the mechanisms underlying their carcinogenicity, genotoxicity was measured in rats using Comet, 32P-postlabeling DNA adduct and micronucleus assays, combined with histopathological analysis. Oxidative damage was measured using human 8-oxoguanine-DNA-N-glycosylase (hOGG1) and Endonuclease III (EndoIII)-modified Comet assays. Groups of 5 seven-week old male F344 rats were administered corn oil or with various doses of estragole or safrole in corn oil by oral gavage at 0, 24, and 45 hr and were sacrificed at 48 hr. Estragole induced a dose-dependent increases in DNA strand breaks, DNA adducts, and oxidative DNA damage in the liver, the cancer target organ. DNA strand breaks were not detected in kidney, stomach or bone marrow, non-target tissues for cancer. Peripheral blood micronuclei were observed at the highest dose in estragole treated animals. Safrole also induced dose-dependent increases in DNA strand breaks and oxidative DNA damage in the liver. No DNA damage was detected in kidney, stomach or bone marrow nor was micronuclei elevated in peripheral blood in safrole treated animals. Evidence of inflammation, single-cell necrosis, and cell proliferation were found in estragole but not safrole-treated rat livers. Taken together, these results imply mixed genotoxic mechanisms. The direct genotoxic effects, indicated by DNA strand breaks and DNA adducts, are associated with the generation of initiated cells; while an indirect genotoxic effect, involving oxidative stress, accompanied by inflammation and cell proliferation, could be associated with tumor development.

Evaluation of Mutagenicity of Vinyl Acetate and Acetaldehyde in Gpr Delta Mouse Lung Fibroblast Cell Line, GDL-1

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SOT 2014 Annual Meeting 121

Genotoxicity Study of Estragole and Safrole

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Vinyl acetate monomer (VAM) induces nasal tumors in rats and oropharyngeal tumors in rats and mice. VAM is weakly genotoxic in vitro and non-genotoxic in vivo. A critical key event in VAM’s rodent carcinogenicity is its carboxylated metabolite, vinyl acetate. The mutagenicity of VAM and AA was evaluated in a newly established gpr delta mouse lung fibroblast cell line (GDL1) harboring two reporter genes, gpt and red/gam (Spy) employing either horse serum (HS) or fetal bovine serum (FBS) since HS was shown to rapidly metabolize VAM to AA. GDL1 cells were exposed to VAM and AA at concentrations ranging from 0.5 mM to 10 mM for 24 hr. Cells were washed and further cultured for 6 days for mutation fixation. Cytotoxicity was measured by relative cell count and ethynylisouriboside and mitomycin C were used as positive controls. VAM was also tested for micronuclei (MN) induction in GDL cells. VAM and AA were not mutagenic at the Spy locus which detects large deletion mutations in GDL cells. In contrast, VAM induced a dose-dependent increase in the MN frequency which was higher in the HS than in FBS. Apparent increases in the MN frequency which were higher in the HS than in FBS. Apparent increases in the MN frequency which were higher in the HS than in FBS. Apparent increases in the MN frequency which were higher in the HS than in FBS.
467c Effects of Ethyl Methanesulfonate on DNA Damage of Uterine Mucosa in Rats

It is known that the DNA damage in the multiple organs can be detected by the in vivo comet assay. However, this assay that uses the uterus as a target organ is hardly known. The in vivo comet assay was examined by using ethyl methanesulfonate (EMS) whether there was a difference in DNA damage in the uterine mucosa when dosed in each stage of the estrous cycle. EMS was administered orally to female rats at a single daily dose of 200 or 300 mg/kg for 2 days. The 10 weeks old female rat when the estrous cycle was steady was used. At 3 hours after the final dosing, the uterus was removed, and specimens were prepared. The % tail DNA, an indicator of DNA damage, was determined. As a result, DNA damage was observed dose-related in the EMS dosing group compared with the negative control (distilled water) group. The influence by the difference at the estrous cycle was not admitted in the negative control group and EMS dosing group.

467d In Vitro Toxicity Testing of 2, 4, 6-Trinitrotoluene (TNT) Contaminated Soil
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Remediation of 2,4,6-trinitrotoluene (TNT) contaminated soil at explosive manufacturing sites may use approaches for monitoring the remediation progress. Previous studies have identified TNT as a mutagen and extracts from TNT contaminated soil may show mutagenicity in a number of in vitro assays. The purpose of this study was to demonstrate proof-of-concept for in vitro mutagenicity testing of contaminated soil, which may be used to validate the effect of future remediation processes. Here, we use an extraction procedure based on the EPA 8330B method, which includes the use of a ring and puck mill to homogenize the soil, incremental sampling and subsampling and LC/UV analysis. Acetonitrile extracts from soil contaminated with TNT and soil determined to be free of TNT, as well as a TNT standard were tested in a Reverse Bacterial Mutation (Ames) assay. Salmonella typhimurium strain TA98, TA100, TA1535, and TA1537 and the Escherichia coli WP2uvrA strain with and without Aroclor 1254 induced rat liver S9 metabolic activation were used. Results from soil extracts absent of TNT yielded no mutagenicity. As expected, treatment with the TNT standard yielded a positive response with 4 strains (TA98, TA100, TA1537, WP2uvrA) and a dose response trend with TA1535. A similar mutagenicity profile was observed with extracts from soil contaminated with TNT, indicating that the extraction procedure and Ames assay are appropriate methods. The addition of the S9 fraction reduced the number of bacterial revertants, demonstrating that metabolic activation is a key determinant of TNT. Yet, it is possible that other unidentified contaminants in the soil may be contributing to the Ames results. This study verifies the methodology used for in vitro mutagenicity testing of soil contaminated with TNT and demonstrates a simple and inexpensive technique by which effective hazard reduction may be demonstrated following remediation.

467e Evaluation of Genotoxicity of 6-Diazo-5-Oxo-L-Norleucine (DON)
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6-Diazo-5-oxo-L-norleucine (DON), a glutamine antagonist, was isolated originally from Streptomyces in a sample of Peruvian soil and was demonstrated to exhibit analgesic and anticancer properties. The current study was performed to characterize its in vitro and in vivo genotoxic potential. DON was tested in the Bacterial Reverse Mutation Assay (Ames test) for its mutagenic potential using Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 and Escherichia coli tester strain WP2uvrA with and without S9 and also with reductive S9. DON was tested for the chromosome aberrations in Chinese Hamster Ovary (CHO) cells with or without S9 to evaluate the clastogenic potential of the test article. DON was also evaluated for its clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatophilic erythrocyte (PCE) cells in mouse bone marrow.

In the mutagenicity assay using the plate incorporation method with and without S9 and using the preincubation method with uninduced hamster liver S9, no positive mutagenic responses were observed. In the in vitro chromosome aberration assay, dose dependent statistically positive increase in structural aberrations was observed at and 20 hours using DON with S9 and also at 4 hour exposure with S9. In the in vivo micronucleus assay, bone marrow was collected from the male mice dosed intravenously with 100, 10, 1 and 0.1 mg/kg at 24 and 48 hour post dose. Three animals dosed with 500 mg/kg were also analyzed 24 hours post injection for micronuclei induction. A statistically positive response for micronuclei formation was observed at 100 mg/kg at 24 and 48 hour post exposure. Thus, DON appears to be negative in the Ames test but positive in the in vitro chromosome aberration assay and in the in vivo micronucleus assay. In conclusion, the results indicate DON is a potential genotoxic compound with a plausible epigenetic mechanism of action. (Supported by NCI-SAIC Contract No. HHSN26120080001E and NIAMS under BiDGS Program).

467f Ames Bacterial Mutation Test—Comparative Sensitivity of Standard and Micro Formats
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The Ames test is the most widely used genotoxicity assay and is a regulatory requirement for almost all new chemicals that enter the environment. Because of its importance as an early predictor of carcinogenicity, a screening version using modifications to reduce compound requirement is often employed at an early stage of compound development. The 24-well plate micro screen (μAmes) employs virtually the same methods as the standard test but uses 95% less compound while reducing labor costs significantly. Since the methodology is so similar to the standard Ames test it should, theoretically, be highly predictive of the eventual full-scale GLP assay and allow extrapolation from one version to the other. One important question that remains largely unanswered for this and other screening versions of the test is ‘How do the two methods compare in terms of sensitivity?’ We have determined the lower limit of sensitivity of the two formats using a wide range of 40 mutagens with different physical and chemical characteristics to provide an objective and quantitative answer to this question. The 40 compounds included those recommended as standard positive controls by OECD, compounds in the NTP database and diagnostic mutagens recommended by Ames and others.

The reduced culture volume in the μAmes theoretically reduces the sensitivity of the method; this is compensated for by increasing the number of replicate control plates, resulting in improved reliability and reduced variability of the estimate of the background mutation rate. The μAmes test gives almost identical results to the standard assay while using 5 to 10 times less test material. In summary, the μAmes appears highly predictive of the standard Ames, at least for potent mutagens, while being less labor-intensive and using much less material. We are extending this validation of the μAmes using compounds which are only weakly active in the Ames test to elucidate possible limitations of the screening method.

467g Development of a High-Throughput Homogenous Assay to Detect DNA Double-Strand Breaks
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Identification of compounds with the potential to induce DNA damage is an important component of the chemical safety evaluation process. The most deleterious DNA lesions are DNA double strand breaks (DSBs), which, if unrepaired, can lead to cell death or mutations leading to cancer. Following DSBs, the histone H2A variant, H2AX, is rapidly phosphorylated at serine-139 by ataxia telangietasia mutated (ATM) kinases and accumulates in foci at the sites of damage in the nucleus. A homogenous assay was developed in a high throughput screening format for detecting phosphorylated H2AX (γH2AX) using homogeneous time resolved FRET (HTRF) technology. To optimize the assay condition, the best pair of antibodies for detecting γH2AX-responsive DNA damage was identified after testing multiple antibodies labeled with donor and acceptor fluor. Following treatment with compounds for one hour, the cells are lysed, and an anti-γH2AX antibody labeled with the donor fluor, Europium cryptate, and an anti-H2AX antibody labeled with the acceptor fluor, d2, are combined and added to the cell lysate. In the presence of phosphorylated H2AX, the donor fluor transfers energy to the acceptor fluor, which emits at 665 nm. Signal is detected as the ratio of 665 nm/615 nm on an Envision plate reader. Using camptothecin, this assay was optimized in CHO-K1 and HepG2 cells, with good performance (e.g., signal to background of 3.5 fold, coefficients of variation of <5%, Z’ factor of 0.7). This assay was further validated by screening the 1280 compounds in the Library of Pharmacologically Active Compounds in both cell lines; the resulting hit rate was 2.0% for CHO-K1.
and 0.6% for HepG2. All known genotoxicants including etoposide, mitomycin C, and gemcitabine the library were active in CHO-K1 cells. Therefore, this assay has been selected for screening the Tox21 10K compound library using CHO-K1 cells as part of Tox21 screening program.

467h Cytotoxicity and Genotoxicity Assays for Assessing the Toxicity of the Reactive Black 5 Dye

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Introduction: The textile industry plays a key role among the main economic activities of Brazil. However, during textile processing, inefficiencies in dyeing result in large amounts of dyes that are directly discharged into water bodies, contaminating the environment and endangering human health. The amount of dye that is lost during the dyeing processes depends on the class of dye used, which may vary from 2% to 50% for basic and reactive dyes, respectively. Reactive Black 5 (RB5) is an azo compound, commonly used in textile industry. Cytotoxicity and genotoxicity assays are crucial tools for predicting both human and environmental health risks. Objective: Evaluate the cytotoxicity and genotoxicity of the RB5 dye. Methodology: Cultures of HepG2 cells were exposed to RB5 dye at concentrations ranging from 1.5 to 500 µg/mL and cytotoxicity was evaluated after 4, 24 and 48 hours using the MTT test. Analysis of micronucleus (MN) in HepG2 cells was performed by flow cytometry-based in vitro MN assay (In Vitro MicroFlow® Kit) and 2-fold over control solvent control mean were used to define the positive results. Results: RB5 dye showed cytotoxic effect in a dose- and time-dependent manner (p<0.05) and a significant increase of MN frequencies was produced by the dye at 50 and 100 µg/mL. Discussion: Overall, our data indicate that RB5 dye is cytotoxic and genotoxic to human hepatocarcinoma cell line. Conclusion: The use of these bioassays showed to be an efficient approach for the initial screening of different dyes and dye containing effluents, in order to establish toxicological information about textile dyes, avoiding damages on humans and to the aquatic environment.

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468 Continuous, Telemetric Blood Pressure Measurement in Conscious, Freely Moving Cynomolgus Monkeys following Oral Etilefrine Administration Using Different Technologies (JET-BP and Physiotel Digital)

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The determination of adverse cardiovascular events in non-clinical studies is a pivotal requirement in drug development. Compound attrition due to adverse cardiovascular side effects occurs rarely in phase I clinical trials, but the frequency increases in phase II and III, suggesting that measurement of cardiovascular endpoints and decision making in non-clinical studies requires improvement. In this project, we compared different technologies (Physiotel digital and JET-BP) and dosing regimens (Latin square cross-over and parallel design) for cardiovascular assessment using a positive control, etilefrine (a sympathomimetic that directly stimulates adrenergic receptors to induce hypertension). In the first model, etilefrine was administered orally (0, 2, 5 and 10 mg/kg) in a Latin square design to 4 male animals pre-implanted with an L21 full implant and systemic blood pressure measured using Physiotel™ digital system. A slight (2 mg/kg) to marked (5 and 10 mg/kg) increase in mean, systolic, diastolic blood pressure and pulse pressure for up to 2 hours (2 mg/kg) or 5 (5 and 10 mg/kg) hours post dose was measured. In our second model, etilefrine was administered at 0, 1, or 10 mg/kg on 3 occasions (days 1, 8, and 15) to 12 female animals (4/group) and blood pressure measured using JET-BP technology. Systolic blood pressure was slightly increased at 1 mg/kg and markedly increased at 10 mg/kg. The magnitude of change for systolic pressure was higher using Physiotel digital and the Latin square design with a maximal increase of 10 (2 mg/kg), 20 (5 mg/kg), and 40 (10 mg/kg) mmHg and 10 (1 mg/kg) and 25 (10 mg/kg) mmHg using JET-BP and a parallel design. These data indicate that these technologies and study deigns allow good sensitivity for the detection of CV effects.

469 Tethered Subcutaneous Infusion in Telemetry-Instrumented Nonhuman Primates for Cardiovascular Assessment of Short-Half-Life Compounds

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Assessing potential cardiovascular (CV) effects of a compound in experimental pharmacology and toxicology studies is critical to understanding the compounds safety profile in humans. Frequently, the half-life of a compound is such that Tmax occurs shortly after administration; therefore, potential test article related CV findings can be masked by “background noise” associated with animal response to human presence in the animal room for dose administration. For this study we developed a novel approach to subcutaneous infusion in which the telemetry-instrumented cynomolgus monkeys were surgically implanted with subcutaneous catheters in the dorsal region to facilitate remote dose administration. Animals were fitted with an infusion jacket and tether system post surgery and connected to an external syringe infusion pump. Catheter patency was maintained through continuous infusion of 0.9% sodium chloride at a rate of 0.3 mL/hr. During dose administration, two infusion pumps were used per animal. Both pumps were programmed for delayed start, with one pump programmed to deliver the control or test article (known to cause immediate CV effects) over approximately 1 minute and the second pump programmed to deliver a flush to clear the remaining control or test article from the infusion apparatus post-dose administration. Animals exhibited minimal signs of discomfort during dose administration. Statistical analysis of cardiovascular data (hemodynamics) indicated a significant, dose dependent response to compound administration within 5 minutes of the start of infusion. Given the impact of human presence during and after room entry (background noise), using standard subcutaneous dosing procedures would have masked CV responses, and made it difficult to accurately describe the profile and time course of action of the test article. This novel approach to dose administration appears to be a viable option when short half-life and/or human presence-related artifact are considerations for study design.

470 Utilisation of JET-BP (Jacketed External Telemetry with Blood Pressure) for Collection of Cardiovascular Data in the Minipig Administration of a Positive Control


JET-BP (DSI) enables continuous collection of cardiovascular data from toxicology study animals for extended periods of time without the need for animal restraint, therefore resulting in the capability to acquire not only an increased quantity of data, but increased quality. The mini-pig, whilst commonly utilised in dermatological-related evaluations, is an increasingly popular laboratory species due to its anatomical and physiological similarities to humans. Male mini-pigs (purpose bred Gottingen ApS mini-pigs obtained from Ellegaard Gottingen, Denmark) surgically implanted with calibrated sensors (DSI C10 series) for arterial blood pressure (ABP) in conjunction with electrocardiographic (ECG) measurements using jacketed external telemetry (JET) were orally administered Moxifloxacin (fluoroquinolone antibiotic). Data were acquired pre-dose and for up to 24 hours post-dose. Administration of Moxifloxacin at 30, 100 and 300 mg/kg elicited a dose related increase in QTcF of up to 10-20% (300 mg/kg), compared to control (0.5% methacellulose) treated animals. The increase was evident within the first 2 hours following dose administration and was maintained throughout the majority of the post-dose data collection period. Whilst the intended cardiovascular effect was evident following Moxifloxacin administration, the data highlighted the challenges that are associated with the twice daily feeding required for mini-pigs. Significant changes in cardiovascular parameters are evident around the time of day of the second daily feed (approximately 6 hours post-dose) and although consistent with the effects in the control treated group could potentially be an issue depending on the profile of the compound.

In conclusion, JET-BP is an acceptable method of cardiovascular data collection in mini-pigs. Special considerations, unique to the general care of mini-pigs must be taken into consideration during study design, in relation to the profile of the compound to be administered, in order to avoid mis-interpretation of the data-set.
Non-invasive telemetry monitoring is widely used to investigate potential ECG effects in toxicology studies. Data quality is one of the most critical success factors when using non-invasive ECG monitoring technologies. Beagle dogs (n=4), Göttingen minipigs (n=4) and cynomolgus monkeys (n=4) were fitted with jackets and undershirts and leads from a telemetry transmitter with three bipolar channels were applied using cutaneous adhesive patches. All animals were instrumented to record three Einthoven standard ECG derivations (Lead II, V4 and Lead III) throughout the acclimation period and for 24 hours prior to and after dosing with sotalol (PO, 15 mg/kg). Lead II was used for ECG interval analysis while all derivations were used for interpretation when suspected arrhythmias were present. The period of ECG monitoring prior to dosing was used to establish parameters for individual QTc calculations as previously described (Spence et al., Toxicol Sci, 1998, 45(2), 247-58). Evaluation of ECG data revealed that acclimation to experimental procedures varied between animals from 12hrs to 2 days as evidenced by the presence of normal stable circadian cycle for heart rate and values in the normal range of HR and QT in both species. These results suggest that IRI at the point can be applied using cutaneous adhesive patches. All animals were instrumented to acquire via a JET system from conscious cynomolgus monkeys and beagle dogs and ECG parameters (PR and QT intervals, QRS duration) and heart rate (HR) were recorded and analyzed by a JET system (Ponemah Physiological Platform, Data Science, Inc.). The overall and individual reference intervals (IRI) were calculated. The ECGs showed wide individual and time variability and clear circadian rhythms of HR and QT in both species. These results suggest that IRI at the point can be used for evaluation of drug effects on ECG parameters in small-scale design studies, especially preliminary TOX studies. To evaluate the validity of IRI, data on sotalol-induced QT interval prolongation (at 3 and 10 mg/kg, po) in monkeys was analyzed using the IRI. Sotalol at 10 mg/kg statistically significantly prolonged QT interval when compared with the control. Sotalol at 3 mg/kg prolonged QT interval beyond the IRI at some time points after dosing, where there was no statistically significant difference from the control. IRI analysis is considered helpful for detecting possible adverse effects on cardiovascular systems in TOX studies, especially preliminary study. A combination of statistical comparison between groups (standard method) and IRI analysis enhances the ability to predict toxicity of drug in TOX studies.

Electrocardiography is now one of the standard investigations performed in preclinical toxicity studies. One of the key parameters is the QT interval, as certain types of drugs have the capacity to delay cardiac repolarization, an effect seen in the electrocardiogram traces as a prolongation of the QT interval. Even when this effect is part of the therapeutic mechanism of an anti-arhythmic drug, excessive QT interval prolongation is also undesirable as this can provoke new arrhythmias (torsades de pointes) and sudden death. The detection of drug induced QT interval changes is sometimes masked by concurrent heart rate changes and therefore it is normalized by means of various formulae (such as Bazett, Frederica, Van der Waters and Linear regression analysis) into a heart rate independent corrected value known as the QTc interval. The purpose of this publication is to compare the hemodynamic and electrocardiographic parameters from naive telemetry-instrumented beagle dogs (Canis familiaris) from two suppliers one in the USA and the other in China. The control cardiovascular data for the USA animal were obtained from published data (Soloviev MV, et. al., 2006). The mean systolic and diastolic pressures for Chinese dogs were 125/69 mmHg and 134/72 mmHg for male and female animals respectively, compared with 142.5 and 84.7 mmHg. The mean heart rate were slightly higher in the Chinese Beagle (126 bpm and 112 bpm for males and females respectively) when compared to the published data for the USA animal of 97.6 bpm. The QT and QTc intervals for the Chinese beagle were, 198 ms and 241 ms for males and, 209 ms and 246 ms for females respectively, compared with the published values of 217 ms and 245 ms, respectively. In conclusion the the cardiovascular parameters collected from beagles sourced in China were essentially similar to those reported for USA sourced animals.

Non-invasive (Jacketed) ECG Monitoring with Telemetry in Beagle Dogs, Göttingen Minipigs, and Cynomolgus Monkeys: Benefits from Multiple Derivations

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Evaluation of ECG and Hemodynamic Changes in Nonhuman Primates Given Four Reference Compounds in Pen or Individual Housing

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An evaluation of the effect of a compound on the cardiovascular (CV) system is a critical part of safety assessment and can be achieved in general toxicology studies using jacketed external telemetry (JET) with an implanted telemetry blood pressure (BP) transmitter. We assessed CV changes using JET-BP in nonhuman primates (NHPs) in response to administration of four different reference compounds under two different housing conditions, pen and individual housing. Thirty-six male NHPs group-housed in pen-style cages (three animals/pen) were dosed with etilefrine (sympathomimetic; 1 or 10 mg/kg), hydralazine (vasodilator; 1 or 10 mg/kg), sotalol (nonspecific β-blocker; 3 or 30 mg/kg), or moxifloxacin (antibiotic known to prolong QT; 10, 50, or 175 mg/kg). Following the last dose, animals were transferred to individual housing, allowed to acclimate, and the same dosing regimen was repeated except that hydralazine was given at 10 or 30 mg/kg. Continuous JET-BP measurements were recorded for at least 90 minutes prior to dosing through 20 hours postdose. Electrocardiographic (ECG) parameters of PR, QT, and rate-corrected QT interval and hemodynamic parameters of systolic, diastolic, and mean arterial pressure; heart rate; and arterial pulse pressure were determined. Etilerine at 10 mg/kg increased systolic and pulse pressure at a similar magnitude in both housing conditions. Hydralazine did not decrease BP at 10 mg/kg (pen and individual housing) or 30 mg/kg (individual only). Sotalol and moxifloxacin significantly prolonged QT with no change in BP, and changes were of similar magnitude under both housing conditions. Finally, hemodynamic parameters of control animals when housed in pen or individual housing were compared. Systolic, diastolic, and mean arterial pressure and heart rate were slightly lower when animals were housed in pens than when housed individually. In summary, ECG and BP changes caused by four different reference compounds were detectable using JET-BP technology in NHPs under two different housing conditions.

Potential Advantages for Combining Gender for the Assessment of Cardiovascular Effects: A Case Study with Three Positive Control Articles

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Nonclinical safety studies are increasingly incorporating cardiac safety endpoints into toxicology studies, either as a supplement or replacement for traditional stand-alone safety pharmacology studies. One liability to this approach is the reduction in sensitivity to detect test article-related changes due to the study design (e.g. cross-
over versus parallel), room environment, telemetry technology, etc. One potential strategy to boost statistical power is to increase group size by combining CV data across gender.

Methods: Thirty-six male and female beagle dogs (n=6/group) were outfitted with jacketed external telemetry and an implanted miniature blood pressure transmitter (JET-BP) and administered control (RO water, all days) or etilefrine (1.10 mg/kg, day 1), sotalol (3.30 mg/kg, day 8), and hydralazine (1.10 mg/kg, day 15). Telemetry data were collected pre-dose and up to 20 hours post-dose. Test article exposure for each positive control article was confirmed at 7 hours post-dose.

Results: Prior to combining the CV data across sex, it was confirmed that the test article exposure and pharmacodynamic responses (e.g. changes in ECG or hemodynamic data) were consistent across males and females. Exposure levels were dose-dependent and were within 41% (etilefrine), 6% (sotalol), or 14% (hydralazine) when comparing males and females. When genders were combined, additional statistically-significant changes were noted as compared to when genders were analyzed separately. Increased sensitivity which produces electrocardiogram (ECG)-like waveforms. All ion channels that contribute to the cardiac action potential are therefore incorporated into this ECG-like recording. Importantly, the xCELLigence RTCA Cardio system can indirectly monitor cardiomyocyte contraction and cellular viability in real-time using impedance-based measurements. As a corollary, while not detected on MEA, compounds that affect long-term cell viability (such as doxorubicin), or contractility (such as blebbistatin) in the absence of electrophysiological effects, can be accurately detected. The combination of these two synergistic platforms provides for a thorough and cost-effective pre-clinical assessment of cardiac risk. Our data compare the effects of multiple compounds from different drug classes across both platforms, and highlight the utility of each platform.

Discussion: This case study demonstrates the potential advantages of combining CV data across gender when the test article exposure and pharmacodynamics were consistent.
480 Metallothionein As a Compensatory Component Prevents Intermittent Hypoxia-Induced Cardiomyopathy in Mice
Obstructive sleep apnea (OSA) causes chronic intermittent hypoxia (IH) to induce cardiovascular disease, which may be related to oxidative damage. Metallothionein (MT) is a potent and highly inducible antioxidant protein that is expressed in the heart. The present study was to test the hypotheses that MT as a potent antioxidant protects the heart from OSA-derived IH-induced cardiomyopathy. Mice were exposed to IH for 3 days to 8 weeks, which is consists of alternating cycles of 20.9% O2/8% O2 FIO2 (30 episodes per h) with 20 seconds at the nadir FIO2 for 12 h a day during daylight. IH significantly increased the ratio of heart weight to tibia length at 4 weeks with a decrease in cardiac function from 4 to 8 weeks, shown by decreased left ventricular ejection fraction (EF) and fractional shortening (FS). Cardiac oxidative damage and fibrosis were observed after 4 and 8 weeks of IH exposures. Endogenous MT expression was up-regulated in response to 3-day IH, but significantly decreased at 4 and 8 weeks of IH. In support of MT as a major compensatory component, mice with cardiac overexpression of MT gene and mice with global MT gene deletion were completely resistant and highly sensitive, respectively, to chronic IH-induced cardiac effects. These findings show that chronic IH induces cardiomyopathy characterized by oxidative stress and cardiac damage. The antioxidant MT protects the heart from such pathological changes.

481 Lack of Recovery from Lead-Acetate-Induced Cardiotoxicity in Wistar Rats
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Lead poisoning continues to be a serious health problem and more significantly so in developing countries with ineffective waste disposal systems. Recent efforts at solving lead poisoning issues have seen entire towns being re-settled from lead-contaminated areas. This study was designed to investigate whether withdrawal of lead exposure results in a resolution of toxic effects of lead to cardiac tissues. Adult male Wistar rats were exposed orally to lead acetate at doses of 0.25, 0.5 and 1.0mg/ml for 6-week duration, after which one-half was sacrificed and the remaining left for a further 6 weeks without lead treatment. Exposure of rats to lead acetate produced significant decline (p<0.05) in the activities of antioxidant parameters, including Glutathione peroxidase (GPx), Glutathione S-transferase (GST), Catalase (CAT), Superoxide dismutase (SOD) and Reduced glutathione (GSH), while Malondialdehyde (MDA) concentration was significantly elevated. Animals from the withdrawal period exhibited similar pattern of alterations, with significant (p<0.05) reduction in GSH, GPx and SOD and significant elevation in MDA and H2O2 concentrations respectively. However, GST activity was elevated, while CAT activity remained unaltered in the recovery period. The results of this study showed that cardiotoxicity induced by injection of oxidative stress and reduction in antioxidant parameters in this study failed to resolve upon withdrawal of lead exposure in male rats during the period of study.
Keywords: Cardiotoxicity, oxidative stress, Lead acetate, antioxidants, rats

482 Methyl Honokiol Prevention of High-Fat Diet-Induced Cardiac Hypertrophy and Dysfunction Is Associated with Attenuating Lipid Accumulation and Insulin Resistance
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Obesity is associated with inflammation, oxidative stress, apopotic cell death, cardiac hypertrophy and cardiac dysfunction. Magnolia as a herbal material obtained from Magnolia officinalis has been found to play an important role in anti-inflammation, anti-oxidative stress and anti-apoptosis. This study was designed to investigate the effect of methyl honokiol (MH, an activity standard of Magnolia) on obesity-associated insulin resistance, lipid accumulation and inflammation in the heart. C57BL/6 mice were fed with a low-(10 Kcal% fat) or high-(60 Kcal% fat) fat diet (LFD and HFD, respectively) for 24 weeks to induce obesity and insulin resistance. HFD-fed mice were given a gavage of vehicle, 0.5 or 1 mg/kg body weight of MH daily. Treatment with 0.5 mg/kg MH significantly ameliorated insulin resistance and slightly decreased HFD-caused body weight gain. Both 0.5 and 1 mg/kg MH treatments significantly attenuated HFD-induced cardiac hypertrophy and dysfunction, cardiac lipid accumulation (reflected by increasing Oil Red O and diastolic diameter, fatty acid content to triglyceride ratio), and cardiac inflammation (evidenced by down-regulating TNF-α and PAI-1 expression). Treatment with 0.5 and 1 mg/kg of MH also ameliorated HFD-induced cardiac insulin resistance, shown by up-regulating Akt and Akt2 phosphorylation and hexokinase II expression. This study demonstrates that MH treatment can attenuate HFD-associated cardiac damage through the prevention of HFD-induced cardiac lipid accumulation, inflammation and insulin resistance.

483 Metallothionein Preservation of Cardiac Akt2 Function and Insulin Signaling by Down-Regulating TRB3 Prevents Diabetic Cardiomyopathy
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Cardiac insulin resistance is a key pathogenic factor for diabetic cardiomyopathy, but its mechanism remains largely unclear. Here we demonstrated that diabetes significantly inhibited cardiac Akt phosphorylation (p<0.05), Akt2 expression and phosphorylation was decreased and insulin-induced cardiac Akt2 and GSK-3β phosphorylation and GS dephosphorylation were also significantly attenuated in WT, but not MT-TG diabetic mice. Deletion of Akt2 gene either in vitro H9c2 cells or in vivo significantly improved glucose metabolic signaling. In addition, diabetes significantly increased cardiac Akt2 negative regulator (TRB3) expression only in WT mice, suggesting the possible contribution of MT inhibition of diabetic up-regulation of TRB3 to Akt2 function preservation. Cardiac H9c2 cells with and without forced MT-overexpression (MT-H9c2) were treated with tert-butyryl hydroperoxide (tBHP), which significantly reduced Akt2 phosphorylation in both basal and insulin-stimulating conditions only in H9c2 cells. Silencing TRB3 expression with SiRNA completely prevented (tBHP’s) inhibition of stimulated Akt2 phosphorylation in H9c2 cells, while overexpression of TRB3 in MT-H9c2 cells completely abolished MT inhibition of stimulated Akt2 phosphorylation. Forced-overexpression of TRB3 by adenovirus-mediated gene delivery in MT-TG hearts also abolished MT’s preservation of cardiac insulin signaling and prevention of diabetic cardiomyopathy. These results suggest that diabetes-attenuated cardiac Akt2 function via up-regulating TRB3 plays a critical role in diabetic inhibition of insulin signaling in the heart. MT preservation of cardiac Akt2-mediated insulin signaling by inhibition of TRB3 prevents diabetic cardiomyopathy.

484 Intratracheal Exposure to PVP-Coated Nanosilver Expands Cardiac IR Injury in Male Sprague-Dawley Rats Seven Days After Exposure
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The potential uses of engineered nanomaterials have expanded in biomedical technology and consumer manufacturing. Our lab has demonstrated expansion of myocardial infarction in male Sprague-Dawley (SD) rats 24 hours after intratracheal (IT) instillation of nanosilver (AgNP). We hypothesized that pulmonary exposure to AgNP induces a persistent increase in circulating proinflammitory cytokines which results in expansion of cardiac ischemia-reperfusion (IR) injury. To test this hypothesis, we exposed male SD rats to an instillation of 200 μg of AgNP. We found that intratracheal (IT) instillation of PVP-coated AgNP expanded cardiac IR injury compared to AgNP alone. Significant differences were demonstrated between 110 nm AgNP and large PVP. Based on these
Multiwalled carbon nanotubes (MWCNTs) are being increasingly designed for industrial/biomedical applications raising concerns on their safety, especially during unique life-stages such as pregnancy. We hypothesized: pulmonary MWCNT exposure during pregnancy will increase vasoconstrictor responses in uterine and placenta derived blood vessels by increasing rho-kinase (ROCK) signaling. We exposed gestational day 17-19 pregnant and non-pregnant female Sprague Dawley rats to 100ng/kg of MWCNT’s suspended in 10% Intrasurf surfactant by intratracheal instillation. Vasoactive responses of uterine, mesenteric, aortic and umbilical vessels were studied 24h post-exposure by wire myography. Maximum stress generation in the uterine artery following MWCNT exposure was increased during pregnancy in response to phenylephrine by 2.6 mN/mm2 (+37%) and to angiotensin II by 4.9 mN/mm2 (+118%), as compared to naïve response. Following MWCNT exposure serotonin induced -4 mN/mm2 increase in stress generation of the mesenteric artery from both pregnant and non-pregnant rats as compared to vehicle response. Wire myographic studies in the presence of a ROCK inhibitor did not reveal significant changes. RhoA and ROCK mRNA/protein expression in rat aortic endothelial cells were unaltered with in vitro exposure to MWCNT’s, suggesting absent/minimal contribution of ROCK to the enhanced contractions following MWCNT exposure. The reactivity of the umbilical vein was not changed; however, mean fetal weight gain was reduced by 7% with MWCNT exposure. These results suggest a higher susceptibility of the uterine vasculature to MWCNT induced vasoconstriction predisposing reduced fetal blood supply/growth during pregnancy. This work is supported in part by NIEHS U19ES019525, RO1ESS016246, AHA Mid-Atlantic Affiliate Predoctoral Fellowship and East Carolina University.

Applications of nano-cerium dioxide (CeO2) are potentially endless, but its biologic interactions must first be understood. We have reported that pulmonary CeO2 exposure results in endothelium-dependent and -independent arteriolar dysfunction. Based on these observations, we predict that this dysfunction is mechanistically linked to impaired nitric oxide (NO) signaling. Rats were intratracheally instilled (65 μg) or intravenously injected (IV,100 μg) with CeO2 suspended in saline (5%) serum. Mesenteric arterioles were examined post 24 hrs via intravital microscopy or isolated vessels. Arterial reactivity was evaluated by using acetylcholine (ACh, 10^-10^ M), and spermere NONOate (10^-10^ M). The role of NO synthase and cyclooxygenase reactivity was tested in the presence of Nω-Nitro-L-arginine methyl ester hydrochloride (L-NMMA) and/or indomethacin (INDO) (respectively). Soluble guanyl cyclase activator (YC-1) and cyclic guanosine monophosphate mimetic (8-Bromo-cGMP) assessed smooth muscle activation. Electron spin resonance and a free radical analyzer assessed the ability of NO to react with CeO2 and the level of tissue free radicals. At the time of writing, results were similar in both models and exposure routes. Control animals responded normally to increasing concentrations of ACh (80±4%) and this dilation was impaired in the presence of L-NMMA (34±4%) or INDO (45±11%). Pulmonary CeO2 exposure significantly impaired this dilation (30±4%). INDO treatment did not alter this impairment (44±10%) but there was a partial restoration in function during L-NMMA treatment (51±11%). Smooth muscle activation was intact following both exposure routes. CeO2 is capable of reacting with NO and there appears to be minimal changes in free radicals. These results are consistent with the notion that CeO2 exposure impairs endothelial function at least in part via a NO dependent mechanism. RO1-E5015022(TRN), NSF IGERT(VCM), F32-ES023435(PAS)
duction events required for normal heart development via activation of G protein-
toined estrogen receptor 1 (GPER). Potential targets include, GATA4, ERK, PKA and Nfat. GATA4 regulates the expression of Hand2 and Lrcc10, which are involved in heart cell differentiation and development. This transcription factor can be activated by several kinases, such as ERK and PKA, both of which are down-steam targets of the estrogen signaling pathway. GATA4 has also been proposed to interact with Nfat, whose activation is regulated by calcium influx. We hypothe-
size that exposure to E2 would alter the expression of important heart determinant
genes such as Hand2 and Lrcc10, as well as calcium homeostasis in zebrafish. To
explore potential molecular mechanisms of cardiac dysfunction, expression of genes
involved in signal transduction and calcium homeostasis were examined in zebrafish
embryos following exposure to E2. Embryos were exposed to 20μM and 5 μM of
E2 at 2 hpf and RNA was extracted at 28hpf. Preliminary qPCR results suggest that
E2 exposure during early embryogenesis causes a decrease in the expression of
Lrcc10 and Hand2. Additional studies will not only measure the expression of other
genes related to GPER activation by estrogen, but also measure calcium influx to better understand how endocrine disruptors alters cardiac development.

492 Diesel Exhaust Causes Stress-Induced Cardiac Conduction
Instability in Hypertensive Rats As Demonstrated by a Novel
Measure of Refractoriness
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Loss of cardiac refractoriness, which prevents early stimulation in the heart,
increases the risk of arrhythmogenesis. Although the heart has a large reserve of
refractoriness (RoR), disease can cause impairment, particularly when under stress or
toxic insult, and thus conduction instability and mechanical dysfunction. RoR is an
assessment of the heart’s minimum level of refractoriness as compared with the crit-
ical level associated with conduction instability. We previously showed that diesel
exhaust (DE) causes cardiac electrical disturbances in rats during exercise-like stress.
To further assess the subtle impacts of DE on the intrinsic conduction properties
of the heart, we used the Chemnyak-Scarbin-Cohen (CSC) model to calculate RoR
and thereby analytically determine conduction stability during dobutamine (DOB)
challenge, which mimics exercise by increasing heart rate and contractility (i.e.,
pacing). We hypothesized that spontaneously hypertensive (SH) rats would have a
greater decrease in RoR than Wistar-Kyoto (WKY) rats during pacing, and that
DE would exacerbate the response. WKY and SH rats exposed to 150μg/m3 of DE
were challenged with increasing doses of DOB. The CSC model was customized for
each rat; QT and RR intervals taken from the electrocardiogram after a given
dose of DOB were used to determine action potential duration and diastolic inter-
val. These values were then analyzed to calculate model conductance parameters and
determine RoR. Air-exposed WKY and SH rats did not have any decrease in RoR,
whereas DE-exposed WKY and SH rats did have a decrease in RoR during pacing.
However, DE caused a significant decrease during pacing in both strains. SH rats had an eight times steeper decrease in RoR when compared with WKY suggesting greater risk. These data indicate that after exposure to DE, risk of cardiac instability increases with increasing stress, particularly with underly-
ning cardiovascular disease. (This abstract does not reflect EPA policy)

493 Prevention of Angiotensin II-Induced Cardiomyopathy by
Sulforaphane-Activated Nrf2 Partially via AKT/GSK-3β/Fyn
Pathway
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Angiotensin II (Ang II) is an important causative of diabetic cardiomyopathy. Sulforaphane (SFN) is an anti-oxidative supplement through activation of AKT/GSK-3β
way. The present study examined whether SFN could protect from Ang II-induced
cardiomyopathy and the underlying mechanism. FVB mice were given subcutane-
ous injection of Ang II (0.5 mg/kg) for 2 months with or without SFN treatment
(0.5 mg/kg) for 3 months and then kept until 6 months. At 3 and 6 months, blood
pressure and cardiac function were assessed. SFN significantly prevented Ang II-
induced high blood pressure at 6 months and cardiac dysfunction at both 3 and 6
months. Ang II caused remarkable pathological changes, including myocardial
hypertrophy and collagen accumulation, along with increases in cardiac oxidative
damage (3-NT and 4-HNE), inflammation (TNF-α and PAI-1), and fibrotic re-
duction (TGF-β1 and CTGF). Those damages were almost completely prevented
by 3-month SFN treatment that up-regulated Nrf2 function, reflected by increased
Nrf2 phosphorylation and downstream antioxidants. To define the direct role of
SFN-activated Nrf2 in preventing Ang II-induced cardiomyopathy, in vitro H9c2
cells were treated with Ang II in the absence or presence of Nrf2 siRNA to silence
Nrf2 expression. SFN significantly up-regulated Nrf2 and also prevented Ang II-
induced CTGF and PAI-1 expression, which were completely abolished by Nrf2
silence. Furthermore, cardiac-overexpressing Nrf2 gene (Nrf2-TG) and wild-type
(WT) mice were treated with Ang II (0.5 mg/kg) for 2 months. Ang II-induced
cardiomyopathy was seen in WT mice, but not in Nrf2-TG mice. To dissect the
mechanism for SFN activation of Nrf2, H9c2 cells were given SFN (10 μM) simul-
taneously with and without Akt inhibitor (LY294002, 10 μM), SFN’s activation of
Nrf2 was partially inhibited by Akt inhibition that also induced GSK-3β activation

490 Investigating Proepicardial, Epicardial, and Myocardial Cells
As Targets of TCD Cardioxicity in Zebrafish Embryos
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Exposure to the ubiquitous environmental contaminant 2,3,7,8-tetrachlorodiben-
dioxin-pyridin (TCD) causes severe cardiovascular toxicity via activation of the
AHR pathway. These effects are likely due to the anti-angiogenic and pro-apoptotic
properties of TCD. The epicardium is the outer layer of the heart, and is important for heart develop-
ment and regeneration. In the zebrafish heart the epicardium is derived from the
proepicardium (PE), which forms adjacent to the heart and begins to migrate to the
myocardium at 70 hours post fertilization (hpf). Recent work has shown that expos-
ure to TCD inhibits PE formation, epicardial progenitor cell migration, and the
spread of epicardial cells across the myocardium. We hypothesize that the myocar-
dium is a target site of TCD that contributes to inhibition of PE migration and
epicardial spread in TCD-exposed hearts. To investigate this, we have activated
AHR2 expression exclusively in myocardial cells using constitutively activated
AHR2 (caAHR2) linked to the cardiac myosin light chain 2 (cmi2) promoter.
Here we show that caAHR2 in myocardial cells does not inhibit development of the
PE, however, the PE or PE cells fail to migrate onto the myocardium. This may
explain the lack of epicardium formation in these caAHR2 embryos. Additionally,
using scanning electron micrography (SEM), we show that TCD-exposed hearts have a smooth myocardial surface topography, which contrasts significantly from
the rough and pockmarked surface of controls. To further examine the role of the
myocardial cells on epicardium formation, we are developing an epicardial migra-
tion assay to assess the ability of untreated epicardial cells to spread onto the myo-
cardium of TCD-treated hearts in vitro, and to test whether caAHR2 myocardial
cells are receptive to migrating epicardial cells. (Supported by NIH ES012716 and
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491 Comparison of Exposure Route on Cardiorespiratory Effects
following Acute Benzo[a]pyrene in Adult Zebrafish (Danio
rerio)
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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contami-
ants. PAH exposure causes developmental toxicity in multiple fish species, but acute adult fish toxicity is thought to be minimal. However, there is some
indication of sublethal PAH effects. We hypothesize that acute PAH exposure in adult
fish will cause cardiorespiratory impairment that will not differ with exposure route. In order to investigate this hypothesis, adult zebrafish (Danio rerio) were in-
jected (i.p.) twice with increasing concentrations of the prototypical PAH, benzo[a]
pyrene (BaP; 0.1, 10, and 1000 μg/kg) or exposed aquously (static, renewal at 24
hr; 16.2 and 162
μg/kg) or exposed aquously (static, renewal at 24
hr; 16.2 and 162
μM) simul-
taneously with and without Akt inhibitor (LY294002, 10 μM), SFN’s activation of
Nrf2 was partially inhibited by Akt inhibition that also induced GSK-3β activation
and thereby analytically determine conduction stability during dobutamine (DOB)
challenge, which mimics exercise by increasing heart rate and contractility (i.e.,
pacing). We hypothesized that spontaneously hypertensive (SH) rats would have a
greater decrease in RoR than Wistar-Kyoto (WKY) rats during pacing, and that
DE would exacerbate the response. WKY and SH rats exposed to 150μg/m3 of DE
were challenged with increasing doses of DOB. The CSC model was customized for
each rat; QT and RR intervals taken from the electrocardiogram after a given
dose of DOB were used to determine action potential duration and diastolic inter-
val. These values were then analyzed to calculate model conductance parameters and
determine RoR. Air-exposed WKY and SH rats did not have any decrease in RoR,
whereas DE-exposed WKY and SH rats did have a decrease in RoR during pacing.
However, DE caused a significant decrease during pacing in both strains. SH rats had an eight times steeper decrease in RoR when compared with WKY suggesting greater risk. These data indicate that after exposure to DE, risk of cardiac instability increases with increasing stress, particularly with underly-
ning cardiovascular disease. (This abstract does not reflect EPA policy)
and Fyn nuclear accumulation. These results suggest that Ang II-induced cardiomyopathy can be prevented by SFN via Akt/GSK-3β/Fyn-mediated activation of Nrf2 antioxidant pathway.

**494**

**(+-) Usnic Acid-Induced Myocardial Toxicity in Rats**


(-) Usnic acid (UA) has been known to be a strong uncoupler, and mitochondrial and endoplasmic reticulum (ER)-related stresses are suggested to be involved in the mechanism of hepatotoxicity. However, it has not been clarified whether UA causes toxicity in other mitochondria-rich organs. We confirmed whether UA induced cardiotoxicity and its mechanism. In the preliminary study of oral administration at 100 mg/kg/day of UA for 14 days in rats, cytoplasmic rarefaction of myocardium and swollen mitochondria were observed. Immunohistochemically, increased intensity of prohibitin, a marker for mitochondrial involvement, was demonstrated. Toxicogenicomic analysis indicated oxidative stress, ER stress, and amino acid limitation as a possible cardiotoxicity mechanism of UA. In the present study, rats were orally given 100 mg/kg/day of UA for 1, 4, 7 and 14 days, and were sacrificed on the next day of final dosing. The day of first dosing was defined Day 1. The gene expression analysis of the heart by real-time RT-PCR for representative oxidative stress (Nqo1 and Txnrd1), ER stress (Gp96, Trike3 and Olig3), and amino acid limitation-related genes (Mthfd2, Asn and Athf5) were performed in combined with histopathological examination. No changes were detected on Day 2, while up-regulation or up-regulating tendency were shown in Nqo1 and Mthfd2 on Day 5, and in Nqo1, Txnrd1, Trike3, Mthfd2, Asn and Athf5 on Day 8. Up-regulation of Mthfd2, Asn and Athf5 were sustained on Day 15. Ultrastructurally, swollen mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15. In conclusion, it was manifested that amino-acid limitation and oxidative stress preceded the morphological change of mitochondria were seen only on Day 15.
inhalation abuse that result in sudden death due to DFE. Many human DFE abuse cases have been studied but the exact cause and the mechanism behind their death is still unknown.

To investigate arrhythmogenic effect Sprague dawley rats (n=8) were exposed to 30 sec of 20 L/min DFE. Arrhythmogenic effects were evaluated by electrocardiographic monitoring (ECG test). These effects were quantified after multiple DFE doses. In addition, severity of those arrhythmias was evaluated. Furthermore, using the same procedure for DFE administration, electrolyte and some enzyme levels were evaluated. Following DFE administration blood was collected by cardiac puncture. Sodium, potassium, calcium and magnesium levels were measured, and Lactate dehydrogenase (LDH) and Creatine Kinase (CK) levels represented cardiac markers.

Electrocardiographic monitoring showed that DFE resulted in graded severity of arrhythmias from minor skipped beat to serious ventricular fibrillation. Electrolyte levels showed a change in plasma potassium and magnesium level while sodium and calcium levels did not change. In addition, LDH and CK levels in DFE administered rats were higher as compared to control rats. Further organ bath experiments will be performed to determine the possible mechanism. These results suggest DFE show cardiotoxic effects in form of arrhythmias and in addition, there were altered plasma potassium and magnesium levels followed by higher LDH and CK levels.

**500 Species Difference in Isoproterenol-Induced Cardiotoxicity between Sprague-Dawley Rats and Duncan Hartley Guinea Pigs**


In the clinical setting, serum levels of cardiac troponin I and cardiac troponin T are widely used in the detection of acute myocardial infarction and other cardiac conditions. These along with other cardiac specific biomarkers such as fatty acid-binding proteins have been previously measured in several experimental models utilizing rats, mice, dogs and non-human primates. The objective of the present study was to compare isoproterenol-induced cardiotoxicity in rats and guinea pigs using the meso scale discovery (MSD) rat muscle injury panel along with histopathology. Male guinea pigs and rats were injected subcutaneously either with saline at 1 ml/kg or single dose of isoproterenol at 4 mg/kg. Four and 24 hours post injection, blood samples were collected for troponin levels and at 24 h heart and skeletal muscle were harvested for histopathological examination. Isoproterenol injection in rats induced a significant increase in the cardiac troponin I levels, fatty acid binding protein and myosin light chain at 4 h and correlated to ventricular damage on histopathology. Surprisingly, there was a significant increase in the skeletal muscle troponin levels at four hours. Selectivity of cardiac vs. skeletal troponin detection was confirmed using heart and skeletal muscle homogenate as well as with purified proteins. However, the same dose of isoproterenol did not induce any change in cardiac troponin I levels or histopathological lesions in guinea pigs. In summary, our data suggests a species difference in the cardiac injury to isoproterenol.

**502 Fenofibrate Reduces Cardiac Fibrosis Due to the Reduction of the DNA Binding Activity of the Hypoxia-Inducible Factor-1/Aryl Hydrocarbon Receptor Nuclear Translocator Complex**

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Background: The aryl hydrocarbon receptor (AHR) is a transcription factor that binds to DNA as a heterodimer with the AHR nuclear translocator (ARNT) after binding with ligands such as polycyclic and halogenated aromatic hydrocarbons found in tobacco smoke and the environment. We investigated the roles of AHR-ARNT signaling on the development of left ventricular hypertrophy and cardiac fibrosis by angiotensin II (Ang II) infusion in mice lacking the AHR gene (AHR-/-). Moreover, we accessed the hypothesis that peroxisome proliferator-activated receptor-t activator, fenofibrate reduces cardiac fibrosis through the ARNT signaling. Methods and Results: Male AHR-/- and age-matched wild type (WT) mice were observed in females but not males treated with DAPM. This elevated 5-HT was also significantly correlated with peak pressure gradient, an indirect measure of pulmonary arterial pressure. DAPM-treated females also exhibited increased levels of the serotonin transport protein 5-HTT, or SERT, in their pulmonary arteries. 5-HT is known to induce HPVSMC proliferation and its role in PAH has been established. In culture, DAPM stimulated female human pulmonary artery endothelial cells (HPAEC) to release 5-HT. No such response was seen in male cell isolates. Additionally, human pulmonary artery smooth muscle cells (HPAVSMC) were stimulated to take up 5-HT, and to proliferate, when treated with DAPM. In our attempts to determine the mechanism(s) by which DAPM elicits these effects, we have observed DAPM-induced activation of the DNA binding target of the aryl hydrocarbon receptor (AhR) - the xenobiotic response element (XRE). Ongoing studies are seeking to address the extent at which the AhR mediates the observed DAPM-induced effects in vitro and in vivo.

**501 DAPM-Induced Alterations in Serotoninergic Signaling May Be Aryl Hydrocarbon Receptor-Mediated in a Novel Model for Female-Specific Pulmonary Arterial Hypertension**

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Pulmonary Arterial Hypertension (PAH) is a cardiovascular disorder characterized by elevated pulmonary artery pressure as a result of arterial wall thickening. Though relatively rare, it is inevitably fatal. The few available therapies only slow progression – there is no cure. Patients are 4–5 times more likely to be women than men. Our purpose was to develop a relevant animal model of PAH in order to identify sex differences that contribute to disease progression, and in doing so, identify potential new treatment strategies. 4,4’-Methylenedianiline (DAPM) is an aromatic amine used industrially in the synthesis of polyurethanes. Chronic, intermittent treatment of rats with DAPM results in medial hyperplasia of pulmonary arterioles, exclusively in females, coupled with increases in pulmonary arterial pressures. After 12 wk, significant increases in plasma levels of serotonin (5-HT) were observed in female but not male rats treated with DAPM. This elevated 5-HT was also significantly correlated with peak pressure gradient, an indirect measure of pulmonary arterial pressure. DAPM-treated females also exhibited increased levels of the serotonin transport protein 5-HTT, or SERT, in their pulmonary arteries. 5-HT is known to induce HPVSMC proliferation and its role in PAH has been established. In culture, DAPM stimulated female human pulmonary artery endothelial cells (HPAEC) to release 5-HT. No such response was seen in male cell isolates. Additionally, human pulmonary artery smooth muscle cells (HPAVSMC) were stimulated to take up 5-HT, and to proliferate, when treated with DAPM. In our attempts to determine the mechanism(s) by which DAPM elicits these effects, we have observed DAPM-induced activation of the DNA binding target of the aryl hydrocarbon receptor (AhR) - the xenobiotic response element (XRE). Ongoing studies are seeking to address the extent at which the AhR mediates the observed DAPM-induced effects in vitro and in vivo.
According to the ICHS7A guidelines, new chemical entities (NCE) must be evaluated for potentially undesired effects on the cardiovascular (CV), respiratory, and central nervous systems. Additionally, the ICHS7B guidelines were established to assess the potential of NCE to affect the ventricular repolarization and proarrhythmic risk. Advancements in telemetry technology allow for the concomitant capture of CV, ECG, and respiratory endpoints in conscious, freely moving animals. Such collection paradigms allow researchers to evaluate the relationship between the CV and respiratory systems concurrently, thus providing a more robust risk assessment in a single study, whilst using fewer animals and less test compound.

The objective of the presented investigation was to evaluate the potential acute effects of CBL0137 on respiratory, CV, and ECG following nasogastric administration in telemetry-implanted cynomolgus monkeys. Cardiovascular and ECG data were acquired from implanted telemetry devices, while concurrent respiratory data was acquired via a jacketed system in conjunction with respiratory inductance plethysmography (RIP).

Acute nasogastric administration of CBL0137 to radiotelemetry-implanted male cynomolgus monkeys at 10 and 30 mg/kg resulted in lower arterial blood pressure (systolic, diastolic, and mean arterial pressure) and lower body temperature; and at 30 mg/kg resulted in test article-related prolongation of the heart rate corrected QT (Bazett) intervals. CBL0137 administration at all dose levels had no affect on heart rate, pulse pressure, PR, QRS, and RR interval, ECG waveforms, or respiratory function (respiratory frequency, tidal volume, or minute volume). It is concluded that preclinical safety pharmacology results do not preclude the FIH (First-in-Human) study of CBL0137.

**504 Inhibition of Gene Expression of Carnitine Palmitoyltransferase I and Heart Fatty Acid Binding Protein in Cyclophosphamide and Ifosfamide-Induced Acute Cardiomyopathic Rat Models**


It is well documented that high therapeutic doses of cyclophosphamide (CP) and ifosfamide (IFO) are associated with acute and lethal cardiotoxicity. Therefore, this study has been initiated to investigate whether CP and IFO therapy alters the expression of the key genes engaged in long-chain fatty acids (LCFA) oxidation outside rat heart mitochondria, and if so, whether these alterations should be viewed as a mechanism during CP and IFO-induced cardiotoxicity. To achieve the ultimate goals of this study, a total of 60 adult male Wistar albino rats were assigned to one of six treatment groups namely, control, L-carnitine, CP, IFO, CP plus L-carnitine and IFO plus L-carnitine. Treatment with CP and IFO significantly decreased expression of heart fatty acid binding protein (H-FABP) and CPT I genes in cardiac tissues. Moreover, CP but not IFO significantly increased Acetyl-CoA Carboxylase (ACC) mRNA expression. Conversely, IFO but not CP significantly decreased mRNA expression of Malonyl-CoA Decarboxylase (MCD), Both CP and IFO significantly increased serum lactate dehydrogenase (LDH), creatine kinase isoenzyme MB (CK-MB), and malonyl-CoA content in cardiac tissues. Interestingly, carnitine supplementation completely reversed all the biochemical and gene expression changes induced by CP and IFO to the control values, except CPT I mRNA and protein expression remained inhibited by IFO. Data from the current study suggest that: (1) CP and IFO therapy are associated with the inhibition of the expression of H-FABP and CPT I genes in cardiac tissues with the consequent inhibition of mitochondrial transport and oxidation of LCFA. (2) The progressive increase in cardio toxicity enzymatic indices and the decrease in H-FABP and CPT I expression may point to the possible contribution of these genes in CP and IFO-induced cardiotoxicity. (3) L-carnitine prevents CP and IFO-induced cardiotoxicity by modulating the expression of genes involved in LCFA oxidation.

**504b Cardiac Myocyte-Specific AhR Activation Phenocopies Embryonic Dioin Exposure in Zebrafish**

K. A. Lanham, J. S. Plavicki, R. E. Peterson and W. Heideman. School of Pharmacy, UW Madison, Madison, WI.

Exposure of zebrafish embryos to 2,3,7,8-tetrachlorodibenzop-p-dioxin (TCDD) activates the zebrafish aryl hydrocarbon receptor 2 (Ah2r) to produce developmental and cardiovascular toxicity. It has been hypothesized that cardiovascular collapse following exposure to TCDD is the cause of early life stage mortality in fish and other species; however, since TCDD acts systemically it is difficult to determine its influence on any one tissue or system. We have constructed a constitutively active AHR (caAhR) that is functional in zebrafish and expressed it tissue specifically to test whether AHR activation in the myocardium reproduces cardiovascular endpoints of TCDD toxicity. We show that AHR activation within the myocardium reproduces most, if not all, of the cardiovascular defects seen following TCDD exposure; in addition to other endpoints outside of the cardiovascular system. This work identifies a single cellular site of TCDD action, the myocardial cell, that can sufficiently account for the cardiovascular collapse and mortality observed following early life stage exposure to TCDD.

**504c Cardiovascular Effects of Diesel Exhaust Exposure**

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Emissions from diesel engines are a highly variable and complex mixture of particulate and gas-phase compounds derived from the incomplete combustion of fuels, fuel-additives, and lubricating components. While recent scientific evaluations of Traditional Diesel Exhaust (Pre-1988; TDE) continue to show adverse cardiovascular effects at elevated exposures, it is hypothesized that New Technology Diesel Exhaust (Post-2007; NTDE) will not produce such impacts. To evaluate potential cardiovascular endpoints, we reviewed the recent scientific literature, restricting our analyses to publications with well-defined exposures, limited confounders, and occurring through the inhalational route. While controlled human-exposure studies using TDE or TDE-like exhaust examined a variety hemodynamic, inflammatory, thrombogenic, and atherogenic responses; the most consistently observed effect was a marginal, albeit transient, alteration in cardiovascular vasomotor response (e.g. attenuation of vasodilation) in acute, elevated (≈300 μg/m3 DPM) exposures.
These effects appeared reduced or absent in the limited number of studies which evaluated exposure to NTDE or NTDE-like exhausts. Recent animal studies provide mechanistic insight into these cardiovascular endpoints via knockout (Apoe−/−) or spontaneously-hypersensitive animals – models representing sensitive human receptors. The use of these animal models to predict potential TDE health effects to occupationally-exposed individuals is problematic because the DE concentrations used in the animal and human studies were 1-2 orders of magnitude higher than above-ground occupational exposures. While further research on NTDE is needed, an individualistic evaluation of both forms of diesel exhaust is of significant regulatory importance because it provides additional data from which to evaluate whether or not current emission standards are effective in preventing human disease.

**505 Identification of Inhibitors of Cytochrome P450 (CYP) 1B1**

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Cytochrome P450 (CYP) 1B1 is known to be overexpressed in several types of human malignancies, including breast, endometrial and prostate cancers. The role of CYP1B1 in estradiol metabolism to catalyze the hydroxylation at the C-4 position is of specific importance in estrogen-related tumorigenesis. Finding a potent and safe inhibitor to this critical step could lead to chemoprevention of several forms of human cancer. We used the Chembridge online database (www.hit2lead.com) to perform similarity searches, using 2,3,4,5-tetrahydroxystilbene (TMS), a compound known to inhibit CYP1B1 activity, as the search target. A 50% similarity threshold was used, reporting all compounds with a similarity of ≥50% as hits. The database determines the similarity of a compound with respect to the lead compound using the Tanimoto coefficient. Ultimately, 64 compounds were tested at a single concentration for inhibition of CYP1B1 catalyzed ethoxyresorufin-o-deethylase (EROD) activity. Five compounds inhibited EROD activity to an extent equal to or greater than TMS, and were considered positive hits. CYP1B1 selectivity was determined using the EROD assay, several concentrations of the five compounds, and CYP1A1, CYP1A2, and CYP1B1. The five compounds varied in selectivity, but all were more selective for CYP1B1 than either CYP1A1 or CYP1A2. Kinetic studies were used to determine the K, and the mechanism of enzyme inhibition for these compounds. Spectral binding analysis using recombinant CYP1B1 was used to determine the observed binding spectrum; type I, reverse type I, or type II. Several of these lead compounds are more potent and selective than TMS for inhibition of CYP1B1 and could potentially lead to new discovery in the treatment and prevention of cancer.

**506 CYP1B1 Expression Is Induced by Leptin through Ligand-Independent Activation of ERα Pathway in MCF-7 Cells**


Leptin, a hormone with multiple biological actions, is produced predominantly by adipose tissue. Among its functions, leptin is able to stimulate tumor cell growth. Estrogen receptor-alpha (ER-alpha), which plays an essential role in breast cancer development, can be transcriptionally activated in a ligand-independent manner. This study investigated the effect of leptin on CYP1B1 expression and its mechanism in breast cancer cells. Leptin induced CYP1B1 protein, messenger RNA expression and promoter activity in ER-alpha-positive MCF-7 cells but not in ER-alpha-negative MDA-MB-231 cells. Transient transfection with CYP1B1 deletion promoter constructs revealed that the estrogen response element (ERE) plays important role in the up-regulation of CYP1B1 by leptin. Furthermore, leptin stimulated phosphorylation of ER-alpha at serine residues 118, 167, and 305 and increased the ERE-luciferase activity, indicating that leptin induced CYP1B1 expression by ER-alpha activation. Finally, leptin activated ERK, Akt, and PKA signaling pathways which are upstream kinases related to ER-alpha phosphorylation induced by leptin. These results indicate that leptin-induced CYP1B1 expression is mediated by ligand-independent activation of the ER-alpha pathway as a result of the activation of ERK, Akt, and PKA in MCF-7 cells.

**507 Metformin Inhibits Aryl Hydrocarbon Receptor-Mediated Induction of CYP1A1 and CYP1B1 in Breast Cancer Cells**

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Induction Cytochrome P450 (CYP) 1A1 and CYP1B1 by endogenous ligands or xenobiotic compounds through the activation of the aryl hydrocarbon receptor (AhR) has been implicated in a variety of cellular processes relate to cancer such as transformation and tumorigenesis. Here, we study the effects of anti-diabetes drug metformin on the expression of CYP1A1 and CYP1B1 in breast cancer cells under basal and inducible conditions. Results showed that metformin markedly reduced the expression of CYP1A1 and CYP1B1 mRNAs and proteins in breast cancer cells under basal and TCDD-induced conditions. The inhibition of AhR expression is required for the reduction of CYP1A1 and CYP1B1 transcriptional expression by metformin. The down-regulation of CYP1A1 and CYP1B1 expression by metformin was independent of ERα. Additionally, metformin significantly down-regulated Sp1 protein expression in breast cancer cells, and the use of genetic and pharmacological means demonstrated that the down-regulation of AhR expression by metformin mediated through the reduction of Sp1 protein. Our findings indicate that metformin suppresses CYP1A1 and CYP1B1 expression in breast cancer cells by down-regulating AhR signaling pathway. These results provide evidences for the use of metformin as a drug for cancer prevention and treatment.

**508 Suppression of NADPH-Cytochrome P450 Reductase Causes Vacularization in Renal Proximal Tubules in Mice**

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Cytochrome P450 reductase (CPR or POR) is an obligatory redox partner for all microsomal P450 enzymes, which play crucial roles in the metabolism of numerous drugs, environmental chemicals and endogenous regulatory molecules. Germine deletion of mouse Por is known to disrupt embryonic development, and POR functional deficiency resulting from genetic polymorphisms in people leads to disordered steroidogenesis and Antley-Bixler syndrome. Large interindividual variations in levels of POR expression are found in human liver and extrahepatic tissues; but the impact of such variations on physiology or disease risk is unknown. A substantial decrease in POR expression, in a mouse model with global suppression of POR expression (named Cpr-low mouse), was previously found to lead to altered sex steroid hormone homeostasis and female infertility, as well as decreased capacity for xenobiotic metabolism. The aim of this study was to further characterize the Cpr-low mouse model, in order to identify additional phenotypes that may reveal vulnerability in people with low POR expression. Here we report age-dependent appearance of marked vacuolarization in the proximal tubules of 4-9 month old male (but not female) Cpr-low mice. The vacuoles, which were negative for fat (Oil red O) and glycogen (PAS) staining, were localized in S1/S2, but not S3, segment of the proximal tubules. Further analysis with electron microscopy revealed that the vacuoles ranged from small, clear structures to large osmophilic bodies consistent with lysosomes. Given the known association of proximal tubule vacuolarization with renal diseases and chemical-induced renal injuries, our results suggest that chronic suppression of POR expression may lead to increased susceptibility to renal injuries/diseases. Further studies are under way to delineate the mechanisms of the renal vacuolization and characterize any associated deficits in renal function.

**509 First Report of Possible Biotransformation of Tetracycline to Doxycycline in Brusica chinensis L.**

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Metabolic biotransformation referred to the process by which a drug is structurally modified in a living entity, presumably to reduce associated toxicity (detoxification) of the drug and to accelerate elimination. However, bioactivation can also occur, complicating the prediction of the metabolic outcome. Cytochrome P450 4 enzymes are responsible for drug biotransformation in both animals and plants but the metabolites may differ significantly. For instance, the biotransformation of tetracycline (TC) to epimer-tetracycline and hydroxytetracycline has been reported in animals but not in plants. In a pharmacokinetic study of TC in Brusica chinensis L. grown hydroponically, we found initial evidence that TC was uptaken and possibly bioactivated to doxycycline (DC) in the leaves after 3 days of cultivation. The suspected DC compound was first identified by HPLC-UV chromatography and further confirmed by liquid chromatography–electrospray ionization–mass spectrometry (LC–ESI/MS) in positive mode. The protonated molecule, [M+H]+ of
The pulmonary epithelium is the first barrier for airborne xenobiotics and inhaled drugs and the cell line Calu-3 is a well characterized model for in vitro tracheobronchial epithelial permeability. To serve as a model for toxicity testing of airborne substances (gases, aerosols, particles), Calu-3 cells can be cultured under air interface culture (AIC) conditions. However, there is little information regarding the metabolic properties of Calu-3 cells under AIC conditions compared to conventional liquid covered culture (LCC) conditions. It was thus the goal of this study to characterize the basal and inducible cytochrome P450 (CYP) isoform expression in Calu-3 cells under AIC and LCC conditions. Calu-3 cells were cultured under AIC and LCC conditions and induction experiments were started on day 11 after passage. The transepithelial electrical resistance (TER) was measured to monitor the barrier properties of the cell monolayers. CYP expression was determined using real-time reverse transcription quantitative polymerase chain reaction (RTqPCR). Comparable basal expression of CYP1A1, CYP2B6/7, CYP2J2, CYP2C, CYP2F1, CYP2D6, CYP1A1, CYP3A4, CYP3A5 and CYP3AA was detected between both conditions, which is consistent with expression in the human lung. Omeprazole, a well-known activator of the aryl hydrocarbon receptor (AhR) induced CYP1A1 and CYP1B1 in Calu-3 cells under both conditions, with stronger induction being observed in AIC cultures. Dexamethasone acts via glucocorticoid receptors and we found slight but significant induction of CYP3AA and CYP3A5 in LCC cultures only. In summary, Calu-3 cells express a broad range of CYPs under both culture conditions with preserved inducibility for the main lung CYPs 1A1 and 1B1 and are thus a valuable model of the airway epithelial barrier for in vitro toxicity testing of airborne substances.
formed by cytochrome p450 isoforms to form styrene oxides and vinylphenols, which have biological activity as pulmonary and hepatic poisons. This toxic vinyl benzene is a danger to living systems and for this reason we have focused our research on the styrene detoxification pathway in Pseudomonas putida (S12) bacteria. The first enzyme, styrene monoxygenase (SMO), a two-component flavoenzyme with an NADH specific reductase, SMOB, and a FAD specific epoxidase, SMOA, which catalyzes the epoxidation of styrene to yield styrene oxide. Here, SMOB binds NADH and oxidized FAD as substrates and catalyzes the reduction of FAD by a hydride-transfer mechanism. The recently reported crystallographic and mechanistic investigation of SMOB suggests that disorder in the N-terminal region of the reductase may play an important role in the SMOB-SCMOA interaction and subsequent FAD transfer. Studies of naturally occurring and engineered SMO fusion proteins further support this idea.

Our present research evaluates the catalytic mechanism of an N-terminally histidine-tagged version of styrene monoxygenase reductase (N-SMOB). We found that the presence of this N-terminal tag is manifested in an increase FAD-binding affinity (Kd - 50 nM), which is nearly an order of magnitude greater than that of the wild type reductase, SMOB. The change in FAD-binding affinity impacts the steady-state kinetic mechanism, which changes from sequential in the wild-type enzyme to double displacement in the case of N-SMOB. Non-linear least squares curve fitting gives estimates of the Km values of NADH, and FAD and Vmax of the enzyme to double displacement in the case of N-SMOB. We found that N-SMOB binds NADH and oxidized FAD as substrates and catalyzes the reduction of FAD by a hydride-transfer mechanism. The recently reported crystallographic and mechanistic investigation of SMOB suggests that disorder in the N-terminal region of the reductase may play an important role in the SMOB-SCMOA interaction and subsequent FAD transfer. Studies of naturally occurring and engineered SMO fusion proteins further support this idea.

Here, we report that SMOB undergoes rapid metabolism by hydroxylation, sulfation, and glutathione conjugation following i.p. and inhalation exposures. Here we investigated tissue disposition, elimination, and general toxicity after inhalation exposure to PCB3 in rats after acute inhalation exposure for 2h. The experiment was carried out in two shifts in a setting that generated air concentrations of PCB3 of 2.1 and 1.4 mg/m3. Rats in the first shift (n=3) received an estimated dose of 35 mg/rat, and were immediately transferred to metabolism cages for the collection of urine and feces, and killed at 24 h. In the second shift (n=12), they received 21 mg/rat, and killed at 0, 1, and 4 h. At 0 h after exposure, the concentration of hydroxylated (OH-PCB3) and sulfated metabolites were 7±1 and 560±60 ng/ml, serum, 213±123 and 482±80 ng/g liver, 110 and 87 ng/g lung, and 40 and 6 ng/g brain, respectively. They followed first-order kinetics with elimination half-lives of 5.2 and 3.9, 3.5 and 8.3, 3.8 and 8.7, 4.2 and 8.0 h for OH-PCB3, 4PCB3 sulfite, 3PCB3 sulfate, and 3PCB3 sulfite from serum and liver, respectively. Approximately 1/3 of the dose was recovered from urine within 24 h, which consisted of OH-PCB3 and sulfates in the ratio of 1:6. While OH-PCB3 and PCB3 were also recovered from feces, the fecal route was minor. Although PCB3 metabolites penetrated the brain, and have been reported to displace thyroxine (T4) from transcortin in vitro, serum T4 levels were normal. There was no difference in 8-oxo-dG levels in urine, a marker of oxidative stress due to electrophilic bioactivation of PCB3, between control and PCB3 exposed rats.

Our findings will be presented together with their implications in the single turnover hydride-flavin-transfer reactions of the two-component SMO system.
changes of catalytic efficiency and drug interactions for N- or S-oxygenations of xenobiotics and endogenous substances. In conclusion, since even the most common [Glu158Lys; Glu308Gly] variant FMO3 protein could result in stronger inhibition potential, genetic polymorphism of human FMO3 gene might lead to unexpected drug interactions via modulation of FMO3 catalytic efficiency.

519 Assessment of Metabolic Potential of PCB Congeners in the Baikal Seal (Pusa sibirica) Using the Liver Microsome
J. You1, H. Mizukawa1,2, K. Nomiyama1, C. Kanbara1, A. Kubota1, T. Agusa1, E. Kim1, S. Tanabe1 and H. Iwata1.

Polychlorinated biphenyls (PCBs), consisting of 209 congeners are biomagnified in the food web and exhibit a broad range of adverse effects. Biotransformation of PCBs is initiated with the insertion of an oxygen atom into the biphenyl ring by cytochrome P450s (CYPs). Toxicokinetics of PCBs occurs in a congener- and species-specific manner. Baikal seal (Pusa sibirica) accumulate high levels of some PCB congeners. However, there still remains unknown how CYPs can metabolize PCB congeners in the Baikal seal. We thus assessed the metabolic potential of PCB congeners by CYPs using Baikal seal liver microsomes. The microsomal reaction solution containing a mixture of 62 PCB congeners was incubated with NADPH for 180 min, and the parent PCBs and their hydroxylated metabolites (OH-PCBs) were analyzed by a gas chromatography-mass spectrometry. The factors that potentially affect the metabolism of PCBs were further explored by principal component analysis (PCA). The microsomal incubation showed that over 50% of CB19 and CB37 and 20-50% of CB22, CB54, CB77 and CB105 were metabolized by CYP. The PCA analysis suggested that highly chlorinated PCB congeners are less susceptible to metabolism, while congeners with more hydrogen atoms at meta- and/or para-position(s) of the biphenyl ring are more metabolized. 3Cl- and 4Cl-substituted OH-PCBs were the major metabolites. 4OH-CB79 that may be formed from CB77 was detected at the highest level among the identified OH-PCBs, followed by 4OH-CB26 and 4OH-CB29, whereas more than 50% of the amount of detected OH-PCBs was unidentified. Only 11.5% of added amount of parent PCBs was recovered as OH-PCBs. These results indicate that there are large unknown CYP-dependent metabolic pathways of PCBs in the Baikal seal.

520 Selective Effects of a Therapeutic Protein-Targeting Tumor Necrosis Factor-Alpha on Cytochrome P450 Regulation during Infectious Colitis: Implications for Disease-Dependent Drug-Drug Interactions
M. D. Merrell1, B. A. Nyagode1, R. Jahangardi1, M. G. Tansey2 and E. T. Morgan1.

We studied the impact of administering XPro1595, a novel antagonist of soluble tumor necrosis factor-α (TNFα), on the regulation of hepatic cytochrome P450 enzymes in the C. rodentium model of infectious colitis. XPro1595 was administered subcutaneously every three days throughout the infection, or as a single injection near the peak of infection. When given throughout the infection, or as a single injection near the peak of infection. XPro1595 selectively blocked the down-regulation of Cyp3a11 and 3a25 mRNAs and Cyp3a protein, as well as the induction of Cyp2a4/5, without affecting the down-regulation of Cyp4a10, Cyp4a14, Cyp2b10 or flavin-monoxygenase-3. Induction of Cyp3a11, Cyp3a25, Cyp2b29 and Cyp3a13 mRNAs were observed only in XPro1595-treated mice. Administration of a single dose of XPro1595 was relatively ineffective. These results a) confirm the role of soluble TNFα in hepatic Cyp3a regulation during infectious colitis deduced from studies in TNFR1-null mice; b) indicate the potential for soluble TNFα-specific antagonists to cause disease-dependent drug-drug interactions; and c) suggest a novel mechanism by which an anti-inflammatory therapeutic protein can produce an opposite effect to that of the disease by selectively neutralizing one of multiple signals regulating drug-metabolizing enzyme expression. More research is needed to determine whether or not this is applicable to other diseases or disease models.
Here, we performed 

**524 Toxicity Profiling of Breast Cancer-Associated Compounds and ToxCast Chemicals in “Humanized” Budding Yeast Expressing P450 Genes**

B. Gaytan², D. Faulkner², R. Proctor², R. Rudel³, C. Vulpe² and M. Fasullo¹.

Many environmental toxicants require bioactivation to become potent genotoxicants. We introduced the P450 genes, CYP1A1, CYP1A2, and CYP3A4 into yeast strains that are deficient in either poly-drug resistance or both recombinalional and nucleotide excision DNA repair. We screened over 50 agents and classified the compounds whose toxicity 1) not influenced by P450 expression, 2) enhanced by P450 expression, and 3) reduced by P450 expression. Compounds not influenced by P450 expression include those that function to elevate deoxynucleotides, maintain mitochondria, promote replication in vitro. In CYP1A2-expressing budding yeast, AFB1 is a weak mutagen but a potent recombina
ting and, triggers the formation of recombinalional repair foci. A DNA damage-checkpoint response correlates with a delay in cell cycle progression. Microarray analysis reveals that both DNA repair and stress response genes are up regulated. To elucidate the functional significance of transcriptional induction, we are profiling the yeast genome for AFB1 resistance, using state-of-the-art next generation sequencing to identify molecular barcodes. We introduced the human CYP1A2 into ~90% of the deletion library, and pooled samples have been exposed to 50 μM and 100 μM AFB1 for 20 hrs. We identified genes that confer resistance to AFB1 by barcode sequencing, and additional genes by growth curves. DNA metabolism genes include RAD55, RAD17, REV1 and REV3, emphasizing the potency of the mycotoxin to trigger replication stress. Additional genes include those that function to elevate deoxynucleotides, maintain mitochondria, promote cellular growth and rearrange the cytoskeletal architecture. The ultimate aim will be to identify corresponding mammalian genes. The yeast libraries will be valuable for additional high-throughput studies using other metabolically-activated drugs and carcinogens. Grant support: NIH: R21ES19954, F3EES021133

**525 A Cocrystal of m-Chlorobenzoic Acid with Furomeside: Prospective Applications**

B. London¹, M. O. Claville², F. R. Froncek³ and B. M. Uppt².

Furosemide is a highly used diuretic for the treatment of hypertension and edema and, to a lesser extent, hypercalce
ia. This furan containing compound is of interest; however, the toxicity elicited by these core compounds is not well under
tood. The free furan itself is a known hepato-carcinogen and toxicant as studied in rats and mice. The epoxide metabolite of furans, formed in CYP450-mediated oxidations, can isomerize to highly reactive electrophilic intermediates such as cis-2-buten-1,4-dial. We have performed the oxidation of furosemide with m-chloro
erbenzoic acid (m-CBPA), and isolated various epoxide and isomerized products in support of our efforts to understand this type of toxicity mechanism, and to also identify potential biomarkers for furosemide in humans. During the separation and drying of the products of the furosemide-m-CBPA reaction, we observed the formation of crystals in the mother liquor (the organic layer). Analysis of these crystals by X-ray crystallography revealed a nonahydrate cocrystal of furosemide (starting material) with that of m-chlorobenzoic acid (an inadvertent contami
nant) with the reduced product of m-CBPA. Analogous to the known properties of cocrystals of furosemide with nicotinamide and their pharmaceutical importance, we believe that the cocrystals of furosemide with m-chlorobenzoic acid could have useful applications in drug development and may lead to formulations with improved potency, solubility, and stability. Therefore, this serendipitous finding may have important applications for improving furosemide bioavailability. Support from NSF ACE Implementation Grant (HRD-1043316) and the US Department of Education (P023B040030) is acknowledged; corresponding author’s email: rao_uppt@sunb.edu.

**526 Genome Profiling of Saccharomyces Cerevisiae Resistance to Aflatoxin B1 (AFB1), a Potent Liver Carcinogen**

M. Fasullo¹, J. Bard³, C. Cerá³, P. Egner³ and T. J. Begley¹, ².

The mycotoxin aflatoxin B1 (AFB1) is a potent liver carcinogen. A signature p53 mutation is found in tumors from liver cancer patients who inhabit AFB1-contaminated areas, suggesting that AFB1 is a potent genotoxin. P450 enzymes convert AFB1 into a highly reactive epoxide that forms N7-guanine DNA adducts, which are unstable and convert into AFB1-formamidopyrimidine (FAPY)-derivatives. These AFB1-associated adducts are either mutagenic or block DNA replication in vitro. In CYP1A2-expressing budding yeast, AFB1 is a weak mutagen but a potent recombina
ting and, triggers the formation of recombinalional repair foci. A DNA damage-checkpoint response correlates with a delay in cell cycle progression. Microarray analysis reveals that both DNA repair and stress response genes are up regulated. To elucidate the functional significance of transcriptional induction, we are profiling the yeast genome for AFB1 resistance, using state-of-the-art next generation sequencing to identify molecular barcodes. We introduced the human CYP1A2 into ~90% of the deletion library, and pooled samples have been exposed to 50 μM and 100 μM AFB1 for 20 hrs. We identified genes that confer resistance to AFB1 by barcode sequencing, and additional genes by growth curves. DNA metabolism genes include RAD55, RAD17, REV1 and REV3, emphasizing the potency of the mycotoxin to trigger replication stress. Additional genes include those that function to elevate deoxynucleotides, maintain mitochondria, promote cellular growth and rearrange the cytoskeletal architecture. The ultimate aim will be to identify corresponding mammalian genes. The yeast libraries will be valuable for additional high-throughput studies using other metabolically-activated drugs and carcinogens. Grant support: NIH: R21ES19954, F3EES021133

**527 Development of a Selective CYP2B6 and Cell Viability Duplex Assay**


CYP2B6 induction affects drug clearance and potentially contributes to adverse drug interactions in humans. To predict CYP induction in vitro cultured hepatocytes are exposed to a probe CYP substrate and compounds that accelerate probe conversion are scored as CYP inducers. However, induction may be underesti
mated when cytotoxic compounds reduce CYP activity indirectly by cell killing. To selectively measure effects on CYP2B6 activity while controlling for cytotoxicity we developed a novel CYP2B6/cell viability multiplex assay. For CYP2B6 we synthes
ezed 6-(4,4-dimethoxybutan-2-yl)oxy benzoz[di]thiazole-2-carbonitril as a probe substrate. This probe is a luciferase pro-substrate that is activated by CYP2B6 then detected as a light from a luciferase reaction. A screen of 21 recombinant human CYPs showed strong CYP2B6 selectivity. Selective inhibition of liver microsome probe reactions by known CYP2B6 inhibitors provided further evidence for selectivity. With adherent hepatocytes initial test compound or vehicle treatments were followed by incubation with the probe. The product of the CYP probe reaction is found in the medium so samples were removed and mixed with a luciferase formulation to give luminescence that reflects CYP activity. Hepatocyte viability was then measured by a lytic ATP-based luminesince assay and values from CYP measurements were normalized to respective viability values. Cell based data was consistent with CYP2B6 selectivity in that CYP2B6 inducers and inhibitors respec
tively elevated or reduced activity (e.g. CITGO vs. Clopidogrel). Fold inductions and IC50s correlated well with reported values from LCMS-based CYP2B6 assays. This approach provides convenience and selectivity for CYP2B6 studies and en
hanced biological relevance by normalization to cell viability.

**528 Role of CYP2BFGS in NNK Bioactivation and Lung Tumorigenesis: Insights from a Novel Cyp2bfgs-null Mouse**

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Enzymes encoded by the mouse Cyp2bfgs gene cluster are preferentially expressed in the respiratory tract, and a number of these are active toward xenobiotic com-
ponents, including many drugs and a tobacco-specific lung procarrigen 4-(meth
ylamino)-1-(3-pyridyl)-1-butane (NNK). CYP2AS is already known to mediate a significant fraction of NNK bioactivation in the lung. This study was aimed to test the hypothesis that, besides CYP2A5, other CYP2ABFGS enzymes also play significant roles in NNK-induced lung tumorigenesis, with use of a novel Cyp2bfgs-null mouse. Deletion of the constitutive genes in the
Cyp2abfgs cluster was confirmed by RNA-PCR analysis in tissues known to express each gene. The Cyp2abfgs-null mouse was viable, fertile and without any obvious developmental defects, and they appeared to have no compensatory increase in the expression of other P450s. NNK bioactivation in vitro and NNK-induced DNA adduction and lung tumorigenesis in vivo were studied in wild-type, Cyp2a5-null, and Cyp2abfgs-null mice. The Cyp2abfgs-null mice exhibited significantly lower rates of NNK bioactivation in lung and liver microsomes, compared to either wild-type mice or Cyp2a5-null mice. Consistent with the in vitro data, the levels of lung O6-methyl guanine (O6-MG), the DNA adduct highly correlated with lung tumorigenesis, were substantially reduced in the Cyp2abfgs-null mice, compared to either wild-type mice or Cyp2a5-null mice. Moreover, the Cyp2abfgs-null mice were largely resistant to NNK-induced lung tumorigenesis at a high NNK dose (200 mg/kg), in contrast to the wild-type mice or Cyp2a5-null mice. These results indicate that, collectively, the CYP2ABFGS enzymes play essential roles in mediating NNK-induced lung tumorigenesis.

### 529 Identification of P450 Activity As a Risk Factor for Metabolism-Dependent Drug-Induced Hepatotoxicity

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A possible risk factor for drug-induced hepatotoxicity is drug metabolizing enzyme activity which may vary among individuals due to genetic (genetic polymorphism) and environmental factors (environmental pollutants, foods and medications that are inhibitors or inducers of drug metabolizing enzymes). We hypothesize that P450 activity represents one of the key risk factors for hepatotoxicity for drugs that are metabolically activated to cytotoxic/reactive metabolites. After evaluation of >20 lots of human hepatocytes, each from a different donor, we identified one lot, HH1020 (from a 21 year old Caucasian female) to have uniquely P450 high activities for several major human P450 isoforms, with CYP2B6 (bupropion hydroxylaton), CYP2D6 (dextromethorphan O-demethylation), and CYP3A4 (midazolam 1-hydroxylation) activities to be 700%, 200% and 800% respectively, of the average values for the other donors. We evaluated the in vitro cytotoxicity of three metabolism-dependent hepatocarcinogens, acetaminophen (APAP), cyclophosphamide (CPA), and aflatoxin B1 (AFB1) as well as a direct-acting cytotoxicant, tamofoxen (TMF), in HH1020 versus hepatocytes from two other donors. The hepatocytes were cultured in collagen-coated plates and treated after 4 hours of plating for 24 hrs, following by viability determination using cellular ATP as endpoint. The EC50 values were as follows: APAP; 2.3 mM (HH1020), 9.4 and 10 mM (others), CPA; 1.8 mM (HH1020), >5 mM (others), AFB1; 2.8 μM (HH1020), 10 and 22 μM (others), and TMF; 26 μM (HH1020), 29 and 30 μM (others). In a separate experiment, HH1020 was treatment with CPA and AFB1 in the presence of the non-selective P450 inhibitor aminobenzotriazole (ABT). Dose-dependent reversal of cytotoxicity by ABT was observed, confirming the role of P450 metabolism on CPA and AFB1 hepatotoxicity. Our results showed convincingly that HH1020 hepatocytes were significantly more sensitive to metabolism-dependent drug-induced hepatotoxicity, and therefore support the hypothesis that P450 activity is a probable risk factor towards drug-induced hepatotoxicity.

### 530 Effect of Vinclozolin on Liver Cytochrome P450 Expression and Testosterone and Estradiol Serum Levels During Pregnancy

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Vinclozolin (V) is a fungicide used for agricultural settings. V and its metabolites M1 and M2 bind to the androgen receptor (RA) and act as RA antagonists. They inhibit the androgen-dependent gene expression. V is able to regulate adult rat liver cytochrome P450 (CYP) expression in a complex manner. The information about effects of V on rat liver is limited and there is no information about the effect on testosterone and estradiol serum levels during pregnancy. The aim of this study was determine the effect of V on regulation of rat liver CYP during pregnancy and how affect testosterone and estradiol bioavailability. Pregnant Wistar rats were administered at the dose of 150 mg V/kg/d suspended in corn oil by gavage during gestational days 14-21. Mothers were sacrificed by asphyxia with CO2. Liver was removed and processed to obtain the microsomal fraction. V exposure increased about 8-fold the content of liver microsomal immunoprotein of CYP2A and 3A2, while CYP1A1, 2B1/2 and 3A1 were induced about 26.7-, 25.6- and 43.7-fold, respectively. On the contrary, the content of CYP1A2 decreased 60% in comparison to non-treated pregnant rats. Likewise, enzyme activities of MROD and PROD increased 21.7- and 817-fold, respectively, and 7α- and 6β-testosterone hydroxylase increased 1.7- and 4.4-fold, respectively. In V-treated pregnant rats testosterone serum levels decreased 56.6% and interestingly estradiol serum levels increased 1.6-fold. These effects produced an increase of 3.5-fold on the estradiol/testosterone ratio. These results indicate that V regulates hepatic CYP expression and alters estradiol and testosterone homeostasis during pregnancy. In addition, they could help to explain the antianabolic effects on reproductive system in male offsprings during uterine exposure. (Supported by CONACyT-Mexico No. 168384).

### 531 Role of P450 Enzymes in Cigarette Smoke-Induced Acute Lung Injury

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The aim of this study is to test the notion that microsomal P450 enzymes can influence the risks of cigarette smoke (CS) induced lung inflammation and tissue damage, an idea supported by the fact that multiple, toxicologically relevant, compounds found in CS are P450 substrates. Based on the protease-antiprotease hypothesis of emphysema development and when considering the inflammatory events observed in COPD, it is thought that pulmonary recruitment of neutrophils and macrophages may play a key role in CS-induced lung diseases. Thus, we examined the cellular profiles in the lungs of wild type (WT) mice and mice with selective abolition of P450 activity; the animals (adult female, all on B6 genetic background) were pre-exposed to either filtered air (FA) or environmental tobacco smoke (ETS) via nose-only inhalation for 5.5 h at a dose of 250 mg/m3 total particulate matter. We found that, the numbers of total cells and of macrophages in bronchoalveolar lavage (BAL) were not significantly different between WT and LCN mice (liver-specific P450 reductase null mice, with little P450 activity in hepatocytes) that were pre-exposed to FA; however, the numbers were 1.5-1.6 fold greater in LCN than in WT mice that were pre-exposed to ETS. ETS-exposed WT and LCN mice both had significant increases in the number of neutrophils in BAL, compared to FA-exposed mice; but the numbers were not different between the two strains. Furthermore, ETS exposure failed to induce a significant increase in the number of BAL neutrophils in Cyp2abfgs-null mice, where all Cyp genes of the Cyp2a, 2b, 2f, 2g, and 2s subfamilies have been deleted throughout the body. An enhanced pulmonary inflammation in ETS-exposed LCN mouse, compared to ETS-exposed Cyp2abfgs-null mice, was further confirmed by histological analysis of H&E-stained lung tissue sections. Taken together, these results support the hypothesis that hepatic P450s protect against, whereas lung CYP2ABFGS mediate, the development of ETS-induced acute lung toxicity.

### 532 Role of Human CYP2A13 and CYP2F1 in Naphthalene Bioactivation and Toxicity in the Lung and Nasal Mucosa of a CYP2A13/2F1-Humanized Mouse

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The aim of this study is to characterize the expression and activity of CYP2A13 and CYP2F1, two human P450 enzymes expressed preferentially in the respiratory tract. CYP2A and CYP2F enzymes have been reported to metabolize many lung toxicants, including naphthalene (NA). The function of human CYP2A13 and CYP2F1 in NA bioactivation and respiratory tract toxicity was examined in this study, using CYP2A13/2F1-humanized mice and CYP2A13-only (humanized) mice (on Cyp2abfgs-null background). In vitro studies indicated that, while the activity in the nasal olfactory mucosa (OM) of the CYP2A13/2F1-humanized mice was primarily contributed by CYP2A13, the activity in the lung was mainly contributed by CYP2F1. Further in vivo studies were conducted, in order to assess the capabilities of CYP2A13 and CYP2F1 to mediate acute inhalation toxicity of NA, at an NA dose relevant to occupational exposure (10 ppm for 4 h). CYP2A13/2F1-humanized mice showed greater sensitivity than Cyp2abfgs-null NA did, to NA-induced depletion of non-protein sulfhydryl and occurrence of cytotoxicity (observable by routine histology) in the OM (but not lung), when examined at 2 or 4 h after termination of NA exposure. However, preliminary results showed that the lungs of NA-treated CYP2A13/2F1-humanized mice had greater numbers of dye-permeable (injured) cells than that of NA-treated Cyp2abfgs-null mice, in both proximal and distal bronchioles, when examined using an ethidium homodimer incorporation assay, which is more sensitive than routine histology examination. These results demonstrate that both CYP2A13 and CYP2F1 are active toward NA in the lung and OM of the CYP2A13/2F1-humanized mice, and they play a significant role in NA-induced toxicity.
533 Essential Role of the Cytochrome P450 Enzyme CYP2A5 in Obligate Mucosal Toxicity of Naphthalene

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Naphthalene (NA), a ubiquitous environmental pollutant that can cause pulmonary and nasal toxicity in laboratory animals, requires cytochrome P450 (P450)-mediated metabolic activation in order to cause toxicity. Our recent study using a Cyp222-null mouse showed that CYP2F2 plays an essential role in NA-induced lung toxicity, but not in NA-induced nasal toxicity. The aim of this study was to determine whether mouse CYP2A5, abundantly expressed in nasal olfactory mucosa (OM) and liver, but less in lung, plays the major role in the bioactivation and toxicity of NA in the OM. We found, by comparing Cyp2a5-null and wild-type (WT) mice, that the loss of CYP2A5 expression led to substantial decreases in rates of NA metabolic activation by OM microsomes. The loss of CYP2A5 did not cause changes in systemic clearance of NA (at 200 mg/kg, i.p.). However, the Cyp2a5-null mice were much more resistant than were WT mice to NA-induced nasal toxicity (though not lung toxicity), when examined at 24 h after NA dosing (at 200 mg/kg, i.p.), or to NA-induced depletion of total nonprotein sulfhydryl in the OM (though not in the lung), examined at 2 h after dosing. Thus, mouse CYP2A5 plays an essential role in the bioactivation and toxicity of NA in the OM, but not in the lung. Our findings further illustrate the tissue-specific nature of the role of individual P450 enzymes in xenobiotic toxicity, and provide the basis for a more reliable assessment of the potential risks of NA nasal toxicity in humans.

534 High-Throughput Screening to Identify Substrates of Zebrafish Cytochrome P450 1A

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Cytochrome P450 (CYP) 1 isoforms catalyze the metabolism of steroids, pharmacueticals and environmental contaminants in vertebrates. In the world of fish aquatic toxicology, CYP1A is perhaps the best known of the family due to its induction and role as a biomarker with contaminant exposure (often examined with the EROD assay). However, only a handful of substrates for CYP1A have been identified in fish. Presently, we have used heterologously expressed zebrafish CYP1A in a high-throughput screen (HTS) with thousands of bioactive compounds, with the aim of identifying the substrate specificities of this enzyme. Catalytic activity was determined in 50 μl reactions in 384 well plates, testing for NADPH consumption. We screened a 6,400 bioactive compound library consisting of off-patent, natural or pharmacologically active compounds with an average molecular weight of 350 g/mol. NADPH consumption rates were compared to negative (DMSO) and positive (7-methoxyresorufin) controls. A total of 87 and 35 compounds activated NADPH consumption at rates greater than 50% and 100% of the positive control, respectively. However, 13 of the top 40 hits induced significant NADPH loss in the absence of CYP1A enzyme, indicating a small but significant degree of false positives. The top 160 hits were screened for ROS formation, where rates greater than 25% of our control (pentoxresorufin) were observed in 31 compounds (19%), indicating some hits may induce uncoupled ROS formation, where rates greater than 25% of our positive control (pentoxyresorufin) were observed in 31 compounds (19%), indicating some hits may induce uncoupled ROS formation. Treatment of Hepa-1 cells with the MC (2.5 μM) resulted in a 50% decrease of ROS production, compared to vehicle controls. Treatment of Hepa-1 cells with the MC (2.5 μM) resulted in a 50% decrease of ROS production, compared to vehicle controls. These data suggest that CYP1A2, possibly via a metabolite, contributes to the sustained induction of CYP1A1 mRNA by MC in hepa-1 cells. Further investigations into the mechanisms of persistent induction of CYP1A1 by MC could lead to novel preventative/therapeutic strategies against PAH-mediated carcinogenesis in humans.

535 Mechanistic Role of Cytochrome P450 (CYP)1A2 in the Persistent Induction of CYP1A1 by the Polycyclic Aromatic Hydrocarbon 3-Methylcholanthrene (MC) in Mouse Hepatoma Cells (hepa-1)

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Humans are constantly exposed to environmental carcinogenic polycyclic aromatic hydrocarbons (PAHs) through cigarette, smoke exhausts, charcoal-broiled meats, etc. Cytochrome P450 (CYP) 1 enzymes play important roles in the activation of PAHs such as 3-methylcholanthrene (MC) to carcinogenic DNA-binding metabolites. We reported earlier that MC causes persistent induction of hepatic and pulmonary CYP1A1 in mice for several weeks after MC withdrawal, and that the phenomenon of sustained hepatic CYP1A1 induction is lost in Cyp1a2-null mice. In this study, we tested the hypothesis that MC elicits persistent CYP1A1 induction in hepa-1 cells, and that CYP1A2 contributes mechanistically to this phenomenon. Hepa-1 cells transfected with the MC and a dimesylated dioxynuloxide (DMSO) as control, and at selected time points, CYP1A1 promoter activity, CYP1A1 enzyme activities, contents, and CYP1A1 mRNA levels were studied. We found that MC markedly and persistently induced CYP1A1 promoter activity, transcription, apoprotein expression, and the CYP1A1-associated ethionomuhamin D-olactone (EROD) activities for up to 5 days. Transfection of cells with CYP1A2 siRNA resulted in knockdown of CYP1A2 mRNA by 70%, but a statistically significant increase of basal CYP1A1 mRNA by 35-40%. The induction of CYP1A1 promoter activity, CYP1A1 mRNA, CYP1A1 protein, and EROD activity by MC were not affected by CYP1A2 siRNA at the 24 h time point, but was significantly attenuated by CYP1a2 siRNA on day 5. These results suggest that CYP1A2, possibly via a metabolite, contributes to the sustained induction of CYP1A1 by MC in hepa-1 cells. Further investigations into the mechanisms of persistent induction of CYP1A1 by MC could lead to novel preventative/therapeutic strategies against PAH-mediated carcinogenesis in humans.

536 Role of Intestinal Cytochrome P450 Enzymes in Protection against Colon Inflammation in a Mouse Model of Dextran Sulfate Sodium-Induced Colitis

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The aim of this study was to determine the roles of intestinal microsomal cytochrome P450 (P450) enzymes in protection against colon inflammation, by studying dextran sulfate sodium (DSS)-induced colitis in an intestinal epithelium (IE)-specific P450 reductase knockout (IE-Cpr-null) mouse model, which has little microsomal P450 activity in the epithelial cells. DSS treatment (2.5% in drinking water for 6 days) caused more severe colon inflammation in DSS-IE-Cpr-null than in wild-type (WT) mice, as evidenced by the presence of higher levels of myeloperoxidase, an indicator of neutrophil infiltration, and proinflammatory cytokines (TNF-α, IL-6 and IL-1β), accompanied by greater weight loss, colonic tissue damage, and colon shortening. A deficiency in colonic corticosterone (CC) synthesis was identified in the IE-Cpr-null mice, as indicated by decreases, compared with WT mice, in rates of CC production in ex vivo cultured colonic tissues from both vehicle-treated (by ~30%) and DSS-treated mice (by ~70%). Co-treatment of DSS-exposed mice with deoxytocorticosterone, a precursor of CC biosynthesis via mitochondrial CYP11B1, abolished the hypersensitivity of IE-Cpr-null mice to DSS-induced colon inflammation. Taken together, these results strongly support the notion that microsomal P450 enzymes in the intestine play an important role in protecting colon epithelium from DSS-induced inflammation, possibly through increased local CC synthesis in response to DSS challenge.

537 Identification of a P450-Mediated Hepatotoxicity-Associated Glutathione-Conjugate of Phenytoin in Phenytoin-Induced Liver Injury in Mice

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Phenytoin (DPH) is widely used as an anticonvulsant agent. Hepatic necrosis was rarely observed in patients who received DPH. The formation of reactive metabolites are believed to involve the developing of DPH-induced live injury, however, evidence for reactive metabolites formation, their thiol conjugates, such as glutathione (GSH), formation and their effects on developing of DPH-induced liver injury are largely unknown. We previously demonstrated that DPH treatment decreased the hepatic GSH contents, and GSH depletion exacerbated the liver injury in mice. In this study, we first identified the GSH-DPH adduct, conjugate of putative electrophilic aren oxide metabolite with GSH, in the bile of the liver-injured mice, administered with DPH for 5 days with GSH biosynthesis inhibitor, L-buthionine-S,R-sulfoximine (BSO), in female C57BL6 mice. In the plasma, N-acetylhydretstene- and S-cysteine-DPH conjugates were detected. In addition, the peak time of the concentration of conjugates were corresponded to that of plasma alanine aminotransferase (ALT) levels. The plasma conjugates concentrations were significantly reduced by the cytochrome P450s inhibitor, 1-aminoenozitiozol (ABT), treatment. Furthermore, elevation of plasma ALT levels and hepatic GSH depletion were significantly suppressed by ABT, flunxazole, and ketoconazole treatment. Although single administration of DPH could not elevate plasma ALT levels, induction of Cyp2c2 caused significant elevation for plasma ALT levels. In
ARDS in adults.

Strategies for the prevention and/or treatment of BPD in premature infants and ALI/ARDS in adults. Hyperoxic lung injury by detoxifying lipid hydroperoxides such as F2-isoprostanes in vivo. Future research could lead to the development of novel strategies for the prevention and/or treatment of BPD in premature infants and ALI/ARDS in adults.

CYP1A1-null mice, with Cyp1a1-null mice showing higher levels after 48-72 h compared to WT mice at the 72 h time point. Levels of pulmonary F2-isoprostanes α of CYP1A1 expression at the catalytic, apoprotein, and mRNA levels in liver and lung in the WT mice. The increase in lung injury in the Cyp1a1-null was accompanied by increased oxidative stress and neutrophil recruitment. Hyperoxia for 24-48 h elicited significant induction of the liver injury via arene oxide formation.

In conclusion, our results show increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification following maternal exposure to BP, and oxidative stress as well as modulation of CYP1A1 and 1B1 enzymes contribute to this phenomenon.

Supplemental oxygen therapy is often required for treatment of preterm infants with pulmonary insufficiency, as well as adults suffering from acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). However, hyperoxia contributes to the development of bronchopulmonary dysplasia (BPD) in premature infants and may exacerbate lung injury in ALI/ARDS patients. In this investigation, we tested the hypotheses that prenatal exposure of rats to benzo[a]pyrene (BP) will result in increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification, and that cytochrome P450 (CYP)1A1 and 1B1 enzymes and oxidative stress mechanically contribute to this phenomenon. Timed pregnant Fisher 344 rats were administered BP (25 mg/Kg) or the vehicle corn oil (CO) on gestational days 18, 19 and 20, and newborn rats delivered from the mothers of both these groups were either maintained in room air or exposed to hyperoxia (85% O2) for 7 or 14 days. Hyperoxic newborn rats prenatally exposed to the vehicle CO showed lung injury and alveolar simplification, and inflammation, and these effects were potentiated in rats that were prenatally exposed to BP. Prenatal exposure of BP also resulted in significant modulation of hepatic and pulmonary cytochrome P450 (CYP)1A1 and 1B1 enzymes at PND 7-14. These rats also displayed significant oxidative stress in lungs at postnatal day (PND) 14, as evidenced by increased levels of the F2-isoprostane 8-iso-PGF2α. In conclusion, our results show increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification following maternal exposure to BP, and oxidative stress as well as modulation of CYP1A1 and 1B1 enzymes contribute to this phenomenon.

Mammalian detoxification processes have been the focus of intense research, but little is known about how wild herbivores process plant secondary compounds, many of which have medicinal value or are drugs. New technologies to estimate BPA toxicity in human have been required and suggested that Cyp2c-mediated metabolism could contribute to the development of the liver injury via arene oxide formation. Maternal smoking is one of the risk factors for pre-term birth and for the development of bronchopulmonary dysplasia (BPD). In this study, we tested the hypothesis that prenatal exposure of rats to benzo[a]pyrene (BP) will result in increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification, and that cytochrome P450 (CYP)1A1 and 1B1 enzymes and oxidative stress mechanistically contribute to this phenomenon. Timed pregnant Fisher 344 rats were administered BP (25 mg/Kg) or the vehicle corn oil (CO) on gestational days 18, 19 and 20, and newborn rats delivered from the mothers of both these groups were either maintained in room air or exposed to hyperoxia (85% O2) for 7 or 14 days. Hyperoxic newborn rats prenatally exposed to the vehicle CO showed lung injury and alveolar simplification, and inflammation, and these effects were potentiated in rats that were prenatally exposed to BP. Prenatal exposure of BP also resulted in significant modulation of hepatic and pulmonary cytochrome P450 (CYP)1A1 and 1B1 enzymes at PND 7-14. These rats also displayed significant oxidative stress in lungs at postnatal day (PND) 14, as evidenced by increased levels of the F2-isoprostane 8-iso-PGF2α. In conclusion, our results show increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification following maternal exposure to BP, and oxidative stress as well as modulation of CYP1A1 and 1B1 enzymes contribute to this phenomenon.

In conclusion, our results show increased susceptibility of newborns to oxygen-mediated lung injury and alveolar simplification following maternal exposure to BP, and oxidative stress as well as modulation of CYP1A1 and 1B1 enzymes contribute to this phenomenon.
the veterinarians, as insufficient numbers of drugs are approved for veterinary use. Therefore, doses of these drugs are empirically determined with reference to human information. In this study, we developed heterologously expressed feline CYP3A and the orthologues of dogs and humans for comparison, individually with human P450 reductase to generate functional monooxygenase systems in Escherichia coli. We found two novel CYP3A genes, CYP3A131 and CYP3A132 in rats. Both feline CYP3As show high identity with canine CYP3As homologues and those of some artiodactyls. CYP3A131 transcripts were expressed predominantly in liver and small intestine, while CYP3A132 was only detected in liver with much lesser amount. Feline CYP3A131 extensively metabolized a fluorocoumarin (BFC) to 7-hydroxy-4-trifluoromethylcoumarin (HFC) with Km value similar to human CYP3A4 and canine CYP3A12 (Km 271-413 μM), except human CYP3A5 (63 μM). As indicated by Ki values, there were general similarities between human, canine and feline CYP3As in binding affinities of some CYP3A inhibitors, such as miconazole, erythromycin, cimetidine, dexamethasone, olmesartan, and medroxyprogesterone acetate except human CYP3A4, which was very resistant to these inhibitors. The present study provides a high-throughput system to assess possible drug interactions in feline CYP3A metabolism in comparison with canine and human CYP3As.

539d PAH Dibenzo[def,p] Chrysene-Induced Adult On-Set Lung Tumors and T Cell Acute Lymphoblastic Leukemia Utilizing a CYP1B1 Humanized Mouse Model: A Transplacental Model

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Polycyclic aromatic hydrocarbons (PAHs) ubiquitously contaminate food crops through atmospheric deposition. Dibenzo[def,p]chrysene (DBC, an IARC class 2A probable human carcinogen), is carcinogenic in animal models and is a human health concern. We induce adult onset T-cell acute lymphoblastic lymphoma (T-ALL) and lung tumors in mice by transplacental fetal exposure via a single gavage dose of DBC to pregnant Lazy1 dams on gestation day 17. The maximal doses tested were 6.5 or 12 mg DBC/Kg. Cytochrome P450 (Cyp) 1b1 is required for DBC-dependent T-ALL (at 4-6 months of age) Lung tumor development (hyperplasia, adenoma, adenoma with progression, and carcinoma) at 10 months of age is due to both Cyp 1a1 and 1b1 bioactivation of DBC. We utilized 129S males, null for mouse cyp 1b1, to cross with B6/129SF1 dams null for mouse cyp1b1 and expressing one allele of human CYP 1B1. There was no significant mortality in the null and humanized hemizygous 1B1 groups, however the T-ALL dependent mortality rate for wild type groups were 42% in the low dose (6.5mg/kg) and 66% in the high dose (12 mg/kg) groups, n=35 per group. At 10 months of age, lung tumor multiplicity was assessed. Corn oil treated groups did not develop lung tumors. Low dose treated groups developed similar numbers of tumors; null = 9.7 tumors, n=26 with 96% incidence; hemizygous 1B1 = 7.5 tumors, n=25 with 96% incidence; and wild type = 8.6 tumors, n=18 with 100% incidence. Similar numbers of tumors were found among high dose treated groups. Null = 12±7 tumors, n=49 with 98% incidence; hemizygous 1B1 = 12±9 tumors, n=23 with 97% incidence; and wild type = 13±5 tumors, n=10 with 100% incidence. The “humanized” hemizygous 1B1 transgenic mouse did not contribute to increased susceptibility to T-ALL or lung tumor compared to 1b1 null mouse. Current work is focused on tissue specific expression of the humanized transgenic 1B1 in mice. Supported by NIH grant P02 ES016465.

540 PON 1 Enzyme Activities in a Mississippi Population Vary with Substrate, Genotype, and Race

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Paraoxonase (PON1) hydrolyzes paraoxon (PO) and diazoxon (DZO), the active metabolites of the insecticides parathion and diazinon, respectively. The human PON1 gene has several single nucleotide polymorphisms (SNPs). The SNP involving arginine (R) to glutamine (Q) substitution at codon 192 is associated with catalytic efficiency while that involving methionine (M) to leucine (L) substitution at codon 55 is associated with serum concentration. PON1 polymorphisms and activities have been linked to cardiovascular and other diseases. Since Mississippians have high disease rates, 186 cardiology clinic patients were evaluated. Hydrolysis rates of PO (POase), DZO (DZOase), dihydrocoumarin (lactonase) and phenyl acetate (arylesterase) were evaluated for associations with race, gender, age, and PON1 55/192 SNP genotypes. The variables were analyzed both individually and in combination. African Americans had higher levels (p<0.02) of POase and arylerase than Caucasians but Caucasians had higher DZOase and lactonase values (p<0.05). Since lactonase activity may be the most critical PON1 enzyme activity for LDL oxidation prevention, this might partially explain the health disparity paradox of why African Americans have higher POase and arylerase activities than Caucasians yet still have higher rates of coronary disease. Significant (p<0.0001) differences in arylerase activities were seen amongst the genotypes with QQMM having the lowest and RRLL the highest activities. This opposes the prevailing belief that arylerase is unaffected by genotype and suggests that this activity cannot be used to quantify PON1 protein. The differences in PON1 activities indicate the need to specify substrate and demographic data for enzyme assays. (Support NIH ES051570)

541 Contribution of CYP2B6 Alleles in Sudden Death of Methadone Users: A CYP2B6 Genetic Polymorphism Study

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West Virginia (WV) and Kentucky (KY) ranked in the top ten states for increases in fatal methadone overdoses in 1999-2004. Cytochrome P450 2B6 (CYP2B6) plays a significant role in stereoselective metabolism of (S)-methadone to 2-ethyl-1,5-dimethyl-3,3-diphenylprolidine (EDDP). Elevated (S)-methadone can cause cardio toxicity by prolonging the QT interval. Large interindividual variability in the pharmacokinetics of methadone causes ambiguity in the relationship between dose, plasma concentration levels, and side effects. The purpose of this study was to determine if single nucleotide polymorphisms (SNPs) in the CYP2B6 gene can lead to decreased metabolism of methadone leading to fatal concentrations. Genomic DNA was isolated from blood stain cards prepared during autopsies at the WV and KY Offices of the Chief Medical Examiner. DNA samples under went real time polymerase chain reaction utilizing TaqMan Allelic Discrimination Analysis to determine the genotypic frequencies of four CYP2B6 gene SNPs (rs8192709, rs4803149, rs3745274, and rs3211371) in the methadone only fatal (136) and control (269) cases. All four of the SNPs studied were within Hardy-Weinberg equilibrium. The observed genotypic frequencies were significantly different than the frequencies for the general population (p<0.05) for three of the four (rs4803149, rs3745274, and rs3211371) SNPs. With the Kruskal Wallis test, the rs3745274 SNP showed a significant difference between [methadone]/[EDDP] ratio for the methadone only overdoses (p = 0.019). Although, the rs4803149 and rs3211371 SNPs were not found to be significantly associated with an increase in [methadone]/[EDDP] ratio, there is an apparent enrichment of the minor allele in the methadone fatal group. Based on these results, SNP rs3745274 is likely linked with a slow-metabolizer phenotype for methadone. Supported in part by NIH grant P20RR016477.

539 Metabolism of 4-Methylimidazole in the Rat and Mouse

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4-Methylimidazole (4-MeI) is found in numerous heated foods and in some car- amel colors used in food and beverages. In long-term cancer bioassays 4-MeI induced alveolar/bronchiolar tumors in mice, but not in rats. This study was con- ducted to investigate the metabolism of 4-MeI in vitro, rat, mouse, and human lung and liver microsomes and S-9 fractions, and in vivo in rats and mice. Mouse and human liver microsomes or S-9 fractions. Male and female F-344 rats and B6C3F1 mice ad- ministered [14C]-4-MeI at doses of 50 and 150 mg/kg by gavage to track the dispo- sition and metabolism of 4-MeI in vivo. Animals were placed in metabolism cages for collection of excreta and exhaled CO2 and volatiles for 48 h. In rats adminis- tered [14C]-4-MeI, the major route of elimination was via urine, with 79-89% of the radioactivity in urine, 3-9% in cage rinse, 2-4% in feces, 1-2% exhaled as CO2, 1-2% in carcass, and 0.1 % or less as exhaled volatiles. In mice, 41-70% of the administered dose was recovered in urine, 4-12% in feces, 18-34% in cage rinse, 2-4 % exhaled as CO2, and 1-3% in carcass, and 0.1 % or less as exhaled volatiles. In rats and mice, the majority of the radioactivity (71-88%) in urine was present as unchanged 4-MeI, characterized by HPLC with detection of radioactivity, and by LC-MS. Additional radioactive peaks were detected (with the largest metabolite ranging from 8 - 16 % of the activity), and characterized by LC-MS/MS as 4-hy- droxymethylimidazolide, two other oxidized products, possibly hydroxylated on the 2- and 5-positions, and a glucuronide of 4-hydroxymethylimidazolide. This study demonstrated that 4-MeI is largely excreted unchanged in male and female rats and mice. However, limited oxidative metabolism and conjugation was observed in rats and mice. 4-MeI is not oxidized in microsomes or S-9 from rat, mouse and human lung and liver. Overall, there was no indication of metabolism of 4-MeI to reactive species. This work was funded by the American Beverage Association.
Immunotherapy is thought of as an emerging strategy for tuberculosis (TB) management. The most effective immunotherapy is the immunomodulatory methods that target the Th1/Th2 imbalance and focus on enhancing Th1 responses to release interferon gamma to activate bacillarid macrophages/monocytes. Because monocytes/macrophages are the first line of defense for TB, infection mainly depends on the number of functional monocytes/macrophages. We investigated isoenzymes of 5-aminolevulinic acid dehydratase (Hb-60) as a model for immune cell precursors. We hypothesized that in addition to its well-known bacillarid actions, INH has immunomodulatory effects which reinforce its therapeutic importance in TB therapy. We employed assays including the NBT reduction assay, superoxide release assay, and flow cytometry to identify the differentiation of HL-60 cells treated with INH. Additionally, we applied quantitative proteomics which revealed certain cellular pathways related to HL-60 cell differentiation, particularly towards monocyte differentiation. Proteomic data also revealed cellular mechanisms involved in INH detoxification. In conclusion, our study revealed that INH induced monocyte differentiation in HL-60 cells. These findings emphasize that INH may yet play important therapeutic roles in multidrug resistant and extremely drug resistant TB and therefore should be continued in these cases.

Zileuton, an orally active inhibitor of 5- lipoxigenase, is used clinically for the maintenance treatment of asthma. Zileuton is also a candidate for the treatment of other inflammatory diseases that involve leukotrienes, but this application is hampered by serious, idiosyncratic drug-induced liver injury associated with its use. Previous studies have demonstrated the utility of a mouse diversity panel (MDP), comprised of 34 genetically diverse inbred strains, for the identification of genetic risk factors associated with drug-induced injury. In this study, serum and liver tissue were collected from 34 mouse strains treated with zileuton (300 mg/kg, i.g.) or vehicle once daily for 7 days. Significant elevations in serum alanine aminotransferase (ALT) levels in zileuton-treated animals relative to vehicle-treated controls were observed in 9 of the 34 strains (p<0.05). ALT fold change was significantly correlated with serum aspartate aminotransferase and glutamate dehydrogenase levels (Pearson correlations, r=0.0002 and r=0.0033, respectively) and associated with microscopic findings of hepatocellular hyper trophy and parenchymal inflammation and necrosis. Zileuton-induced ALT changes, however, were not correlated with drug exposure. Genome wide association mapping conducted using ALT fold change revealed a transcriptional regulator of the cytoprotective enzyme heme oxygenase1. Basal Prdm2 levels were found to be significantly decreased in strains with the minor allele of the polymorphism (p=0.0018). In summary, the MDP approach defined a possible risk factor that can be evaluated in humans and has the potential to identify patients with genetic susceptibility to zileuton-induced liver injury.

Mammals exhibit major gender differences in kidney function under various physiological, pharmacological, and toxicological conditions. To gain better understanding of the molecular differences in kidney between mammalian sexes and between rodent species, we have utilized next generation sequencing to evaluate potential species and gender differences in the transcriptomes in the CD-1 mouse and the Wistar Han rat strains. Enriched renal cortex tissue was collected from 7 weeks CD-1 mice and 9-10 weeks Wistar Han rats. The whole transcriptome in kidney was profiled using Illumina HiSeq 2000. Baseline gene expression was compared between male and female in CD-1 mouse and Wistar Han rat. Our data showed the major gender biased gene expression in the kidney cortex of rodent species. Male and female CD-1 mouse kidney exhibit gender specific expression in drug metabolism and transporter and female CD-1 mouse have higher expression in genes related to osmotic regulation and glutathione detoxification in kidney. In contrast, Wistar Han rats showed a different profile of gender biased expression in drug metabolism and transporters. The gender differential expression is less detected in Wistar Han rat kidney comparing with CD-1 mice. The elucidation of baseline molecular differences in kidney function between genders and rodent species would enable us to better evaluate the occurrence and development of drug-related gender and species specific changes that may be relevant to understanding mechanisms of drug-induced nephrotoxic effects.
infections on expression), these data also suggest that inhibition of CBR3 may provide protection from doxorubicin cardiotoxicity. Supported by NIH grant P30ES007033 and UW DEOHS.

547 Synergistic Interaction between Genetics and Disease on Pravastatin Disposition

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Genome wide association studies have implicated the hepatic uptake transporter organic anion transporting polypeptide-1B1 (OATP1B1) in the pharmacokinetics and musculoskeletal toxicity of statins, such as pravastatin. Along with OATP1B1, other OATP uptake transporters can participate in the transport of pravastatin, partially compensating for the loss of OATP1B1 in polymorphic patients. Nonalcoholic steatohepatitis (NASH) in humans and in a diet-induced rodent model alters the expression of multiple OATP and multidrug resistance-associated protein (MRP) transporters. Genetic loss of Oatp1b2, the rodent ortholog of human OATP1B1 transporters, caused a modest increase in pravastatin plasma concentrations in mice with healthy livers. Although a diet-induced model of NASH did not alter the disposition of intravenously administered pravastatin compared to WT control mice, the combination of NASH and Oatp1b2 genetic loss caused a synergistic increase in plasma area under the curve (AUC) and tissue concentrations in kidney and muscle. These data reveal a potential mechanism for this synergistic increase in pravastatin exposure that involves NASH-induced alterations in the expression of multiple hepatic uptake transporters which, due to overlapping substrate specificity among the OATP transporters, may combine with the pharmacogenetic loss of OATP1B1 to increase the risk of statin-induced adverse drug reactions.

548 The Role of the Folate Pathway in Pancreatic Cancer Risk

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Pancreatic cancer is a devastating disease, with poor prognosis for survival. Most cancers, including pancreatic cancer, develop through interactions between genes and environmental/lifestyle factors. Risk factors for pancreatic cancer development include a low dietary intake of folate. In conjunction with diet and lifestyle determinants, an individual’s folate pathway status is determined by their genetic makeup. Expression of single nucleotide polymorphisms (SNPs) of genes in the folate pathway have been shown to modulate susceptibility to pancreatic cancer. In particular, homozygous expression of the TT allele of methylene tetrahydrofolate reductase (MTHFR) at nucleotide position 677, which causes decreased enzymatic activity, has been associated with an increased risk for development of pancreatic cancer and low folic acid levels. In the present study, we measured serum and red blood cell folate levels and determined SNP expression in selected genes in the folate metabolic pathway in a cohort of pancreatic cancer patients and healthy related and unrelated control groups. While serum folate levels did not differ between patients and controls, red blood cell folate levels were significantly lower in pancreatic cancer patients compared to controls (p<0.05). As expected, we found that the TT allele of MTHFR C677T is associated with low serum folate levels and significantly associated with low red blood cell folate levels compared to the CC or CT genotype. We also performed analysis for gene-gene interactions among SNPs in the folate pathway in relation to pancreatic cancer risk. We find that individuals expressing the SHMT1 L474F/MTHFR C677T diplotypes LF+FF/CC and CT+T/LF+FF exhibit an increased risk for pancreatic cancer (OR = 2.34 95%CI 1.08-5.1; OR = 2.11 95%CI 1.06-4.62 respectively). Collectively our results suggest that both environmental factors (diet) and genetics contribute to the risk of development of pancreatic cancer.

549 Functional Effect of Polymorphic Variations of Human Cytochrome P450 2D6 (P450, E418K, S486T, and R296C)


Cytochrome P450 2D6 is responsible for the metabolism of various clinical drugs and its genetic polymorphisms can significantly influence the metabolic activity. In this study, we analyzed the functional activities of four nonsynonymous single nucleotide polymorphisms from P450 2D6*52 allele, which were recently found, and one found previously in P450 2D6 alleles. Recombinant variant enzymes of E418K, S486T, and R296C were successfully expressed in Escherichia coli and purified. However, a P450 holoenzyme spectrum of P450 variant was not detected in E. coli whole cell level. Structural analysis indicated that P454 mutation seemed to perturb a highly conserved proline-rich N-terminus of P450 2D6. Steady state kinetic analyses showed the significant reductions of enzymatic activities in E418K and R296C variants. In the case of bufuralol 1'-hydroxylation, E418K, showed 32 % decrease in catalytic efficiency (kcat/Km) mainly due to the decrease of kcat value. R296C showed much greater reduction in the catalytic efficiency (0 % of wild-type) due to both of a decrease of kcat value and an increase of Km value. In the case of dextromethorphan O-demethylation, E418K showed both of a decrease of kcat value and an increase of Km value resulting in ~6-% reduction of catalytic efficiency. A highly decreased catalytic efficiency (~6-% of wild-type) in the mutant of R296C also was observed mainly due to the dramatic change of kcat value of dextromethorphan O-demethylation. These results suggested that individuals carrying these allelic variants are likely to have the altered metabolic abilities of many clinical drugs therefore, these polymorphisms of P450 2D6 should be much concerned for reliable drug treatment.

550 Polymorphism of RGS9: Association with Obesity

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RGS9-2 is a member of Gt GTase accelerating proteins that regulates G protein signaling (RGS). It has been shown recently that RGS9 knockout mice have increased adiposity. In the present study we evaluate the genetic effect of RGS9 gene on obesity and genotype 9312 obese, type 2 diabetes and healthy Chinese and Malaysian subjects for insertion/deletion (TT/TCT I/D) polymorphism (rs3215227) in the gene. The deletion polymorphism of RGS9 was associated with decreased prevalence of obesity in woman (p<0.003) and girls (p=0.002), moderate in boys (p=0.039) and non-significant in men. We did not find any significant association of RGS9 polymorphism with type 2 diabetes. Since the delta TT/TCT deletion was close to the branch point for RNA splicing and corresponded to a binding motif for polyuridyline tract binding protein (PTB), we attempted to determine RGS9 binding affinity with the region. We found that deletion variant showed 10 fold reductions in PTB binding in vitro. We also demonstrated the differential effect of overexpression of PTB on the splicing of the two polymorphic forms of RGS9 minigene. We observed that overexpression of PTBP1 and PTBP2(Brain specific) significantly decreased the splicing in wild type construct of RGS9 minigene. Thus, our data suggest that RGS9 has genetic effect on obesity but the effect of binding an inhibitor of PTBP could be causative for the inverse association between the polymorphism and obesity.

551 Gene Environment Interaction in Urinary Bladder Cancer

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Urinary bladder cancer (UBC) is a common disease worldwide with a high incidence. The etiology of UBC includes both environmental and endogenous factors. Environmental risk factors include exposure to heavy metals, aromatic amines and environmental pollutants including pesticides mainly organochlorine pesticides (OCPs). OCPs are potent endocrine disrupters, are found to be associated with several cancers. Glutathione S-transferases(GST) involved in the detoxification of numerous environmental toxins including OCPs. The present study was carried out in UBC and healthy control subjects with an aim to determine the role of GSTM1 and GSTT1 gene polymorphism and its implication on the OCP detoxification or bioaccumulation which may increase the risk of UBC in humans. This study was also designed to identify the “gene-environment interaction” specifically between gene polymorphism in xenobiotic metabolizing genetic enzyme(s) and OCP residue levels in blood. GSTM1/GSTT1 gene polymorphism was analysed by using multiple PCR. OCPs levels in whole blood were estimated by gas chromatography. The results demonstrated a significant (p<0.05) increase in frequency of GSTM1/GSTT1- (null) genotype in UBC cases without interfering the dis-
between β-HCH and GSTM1-genotype (p<0.05) as well as between β-HCH and GSTT1-genotype (p<0.05) respectively. These findings indicate that “gene-environment interaction” may play a key role in increasing the risk for UBC in individuals among North Indian population who are genetically more susceptible due to presence of GSTM1/GSTT1 null deletion during their routine encounter with exposure to OCPS.

Idiosyncratic hepatotoxicity has been associated with the oral tyrosine kinase inhibitor lapatinib, which is used in the treatment of metastatic breast cancer. However, the mechanism(s) of this toxicity are unknown. Lapatinib is extensively metabolized via O-debenzylation by CYP3A4/5 to form a phenolic metabolite. Further oxidation yields a reactive quinone imine, which can potentially lead to liver injury. While CYP3A4 is abundantly expressed in human liver, CYP3A5 expression is highly polymorphic. Individuals with the CYP3A5*3 allele express high levels of the functional protein, whereas the CYP3A5*5 variant allele results in low to undetectable levels of CYP3A5. We hypothesized that expression of CYP3A5 may contribute to inter-individual differences in the formation of reactive metabolites of lapatinib. To address this hypothesis, we first examined the kinetic parameters of lapatinib O-debenzylation by CYP3A4 and CYP3A5 Supersomes utilizing LC-MS/MS analysis. Both recombinant enzymes turned over lapatinib rapidly (15-18 min⁻¹) and with high affinity (1.0-2.2 μM), albeit CYP3A4 exhibited an overall 2.5-fold higher catalytic efficiency than CYP3A5. In individual genotype human liver microsomal preparations, the rates of debenzylation of lapatinib (5 μM) were comparable between carriers of the CYP3A5*7 wild-type allele and CYP3A5 non-expressers (CYP3A5*3/3). Next, glutathione (GSH) trapping experiments were conducted with debenzylated lapatinib as substrate (5 μM) to assess the formation of GSH adducts of the quinone imine reactive metabolite (RM-SG). Kinetic analysis revealed that CYP3A5 Supersomes were seven times more efficient than CYP3A4 for generation of RM-SG adducts. Finally, the levels of RM-SG formed were significantly higher (P= 0.03) in human liver microsomal preparations from CYP3A5*7/7 expressers compared to CYP3A5*3/5, livers. These data show that CYP3A5 is a quantitatively important contributor to hepatic lapatinib bioactivation in vitro.

552 Human ALDH1B1 Polymorphisms May Affect the Metabolism of Acetaldehyde and All-Trans Retinaldehyde—In Vitro Studies and Computational Modeling

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Purpose: To test the metabolism of novel substrates by ALDH1B1 and to predict the effect that human ALDH1B1 polymorphisms will have on these and previously described substrates.

Methods: Purified human recombinant ALDH1B1 was used to determine the capacity of ALDH1B1 to metabolize nitroglycerin and all-trans retinaldehyde. Computational-based molecular modeling was used to predict the binding affinities of the substrates acetaldehyde, 4-hydroxynonenal, nitroglycerin, and all-trans retinaldehyde to ALDH1B1 and three human polymorphisms ALDH1B1*2 (A86V), ALDH1B1*3 (L107R), and ALDH1B1*5 (M252V). The binding of the cofactor NAD⁺ to ALDH1B1 and its polymorphisms was also modeled computationally.

Results: ALDH1B1 metabolizes and appears to be inhibited by nitroglycerin and has favorable kinetics for metabolism of all-trans retinaldehyde. Differences in calculated docking poses and weak interactions between ligands/cofactor and ALDH enzymes from modeling studies provided a basis for predicting the capacity of each of the variants to metabolize acetaldehyde, nitroglycerin, and all-trans retinaldehyde. Modeling indicated that ALDH1B1*2 and ALDH1B1*5 likely bind NAD⁺ poorly compared to ALDH1B1 and ALDH2, and all ALDH1B1 mutants had poor binding affinities for nitroglycerin.

Conclusions: ALDH1B1 metabolizes the novel substrates nitroglycerin and all-trans-retinaldehyde, and two human polymorphisms (ALDH1B1*2 and ALDH1B1*5) are likely to metabolize substrates poorly, which may affect the roles of ALDH1B1 in stem cells and ethanol metabolism.

552a SLC19A1, SLC6A6 and SLC01B1 Polymorphisms Are Associated with Methotrexate-Related Overall Toxicity in Portuguese Rheumatoid Arthritis Patients

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Methotrexate (MTX) is used for rheumatoid arthritis (RA) treatment with a wide variety of clinical responses, especially of concern is the development of MTX-related toxicity (MTX-Tox). Studies have described a possible role of genetic polymorphisms that alter MTX transporters influx (solute carriers, SLCs) and efflux (ATP-binding cassette, ABC) in MTX-Tox development. The aim of this work was to evaluate the influence of several single nucleotide polymorphisms (SNP) in genes codifying MTX transporters with the occurrence of MTX-Tox in Portuguese RA patients. A total of 233 RA patients were included in the study and genotyped by Sequenom MassARRAY® for 20 SNPs (SLC16A7, SLC19A1, SLC22A6, SLC22A11, SLC46A1, SLC01B1, ABCB1, ABCG1, ABCG2 and ABCG2). Binary logistic regression analysis were performed and adjusted to possible confounder variables (related to patient, disease and treatment). Haplotype analysis was used to test SNPs revealing statistical significance and a risk index for MTX-Tox. One SNP, rs41291122 in PLCE1, was associated with post-clopidogrel ADP-stimulated platelet aggregation in the full cohort (p=0.001). Additionally, we observed that the rs11219992 T allele was associated with a lower risk of MTX-Tox (p=0.037; OR=2.82) in comparison to the reporter constructs containing the T allele of the SNP. These results suggest that the rs11219992 T allele may contribute to the risk of MTX-Tox in our study population.

552b Role of CYP3A5 in the Metabolic Activation of Lapatinib

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Medical radiation countermeasures have applications in clinical oncology, space travel, radiopharmaceutical imaging, and radiological terrorism. We demonstrated previously that the soy isoflavone genistein protects against acute hematopoietic radiation injuries by increasing hematopoietic stem cell survival and decreasing proinflammatory cytokines. Genistein is a phytoestrogen that has been established to have weak estrogenic activity. In the present study, we evaluated the radiation pro-
tective effects of a nanoparticle formulation of genistein after the administration of an estrogen receptor antagonist. Male CD2F1 mice received an intramuscular (IM) injection of the estrogen receptor antagonist, ICI 182,780 (ICI), or corn oil vehicle daily for 4 days. On the fourth day, mice received a second IM injection of either vehicle or genistein nanosuspension (150 mg/kg). All mice then received a single dose (9.25 Gy) of total body gamma irradiation (cobalt-60), 24 h after the second injection. Results showed that 30-day survival for both the vehicle and ICI groups was 25%, whereas 90% of genistein-treated mice survived. Mice that received ICI followed by genistein exhibited a significant decrease in survival rate (45%). These results demonstrate that the radiation protective effects of genistein are mediated in part through an estrogen receptor pathway.

**552e Arsenicals, Lewiste- and Phenylarsine Oxide-Induced Cutaneous Inflammation Response and Cell Death Are Regulated by UPR Signaling Pathway**


Vesicants are a group of chemical agents that cause skin blistering and inflammation following their cutaneous exposure. These agents were synthesized as chemical weapons to be used during warfare. Lewiste, an arsenical, is an important war threat agent. It is known to manifest severe cutaneous inflammation and blistering. However, the exact molecular mechanisms underlying these effects remain undefined. We develop a novel mouse murine model which is highly sensitive to inflammatory inducing agents and manifests similar responses as observed in humans. Here, we show that topical exposure to lewiste and its analog phenylarsine oxide (PAO) on the skin of Pch/+ or Pch-/-/SKH-1 hairless mice induced erythema, edema, and microvesication associated with the enhanced production of pro-inflammatory cytokines and reactive oxygen species (ROS). A huge cell death was also observed particularly at the site of lewiste exposure. These effects were found to be regulated via unfolded protein response (UPR) signaling pathway activation. UPR inhibitor, 4-phenylbutyric acid (PBA), an antioxidant N-acetyl-cystine (NAC) or their combination were highly effective in diminishing PAO-induced erythema, edema, and microvesication. Similarly, a dose- and time-dependent up-regulation of inflammatory and UPR pathways by arsenicals were observed in human skin keratinocytes. PERK-regulated UPR proteins GRP78, p-PERK, p-eIF2a, ATF4 and CHOP were particularly induced in these cells. PBA and NAC significantly attenuated these effects in keratinocytes. Employing siRNA approaches, CHOP was found to be the key apoptosis and pro-inflammatory responses inducing protein. These data provide evidence that lewiste and its analog PAO which is also a degradation product of other arsenicals have potent inflammation, vesication and cell death inducing properties in the skin and may have long term environmental and human health consequences.

**552f Arg-1 Is Associated with the Development of Late Pulmonary Fibrosis following Irradiation**


Macrophages are found in close proximity with collagen-producing myofibroblasts and indisputably play a key role in fibrosis. With their potential to act in both a pro- and anti-fibrotic capacity, as well as regulate the activation of resident and recruited myofibroblasts, macrophages and the factors they express are integrated into all stages of the fibrotic process. Classical activation by microbial agents and/or T helper cell type 1 (Th1) cytokines, interferon-γ (IFN-γ), in particular, is associated with the production of nitric oxide (NO) and proinflammatory cytokines involved in cytotoxicity, microbial killing, and regulation of cell proliferation (M1 or type-1 polarization). More recently, it was shown that macrophages could also follow a different activation pathway after stimulation with the Th2 cytokines interleukin (IL)-4 or IL-13 (M2 or type-2 polarization). Our hypothesis is that the induction of Arg-1 in these “alternatively activated” cells leads to development of radiation-induced fibrosis. 8 week old female C57BL/6 or C3H/HeJ mice were irradiated with 5 Gy total body irradiation followed by a dose of 10 Gy to lung only. Mice were examined at 24 or 72 hours or 1, 2, 12, 18 or 26 weeks post irradiation. Our findings demonstrate a dramatic increase in Arg-1 and MCP-1 expressing macrophages associated with the progression of fibrosis. These results correlate with later time points where C57BL/6 mice are exhibiting a robust fibrotic response but C3H/HeJ demonstrate little pathology. Injuries which generated robust inflammatory responses but did not develop fibrosis demonstrated induction of iNOS but not Arg-1. These data suggest a role for Arg-1 expressing macrophages in the development of inflammation and demonstrate that differential activation of Arg-1/iNOS-2 is a potential determinant in the pathogenesis of pulmonary fibrosis.

**552g Soman-Induced Convulsions Change the Phosphorylation State of the Potassium-Chloride Cotransporter (KCC2)**

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Exposure to nerve agents induces numerous symptoms, including seizures that become resistant to benzodiazepines within 40 minutes of onset. This resistance may result from the internalization of GABA receptors; however, this process is likely too slow to account for this rapid benzodiazepine resistance. An alternate hypothesis is that dysfunction of KCC2, a protein that permits GABA’s inhibitory tone by maintaining the Cl- gradient, may decrease the efficacy of benzodiazepines following prolonged seizures. As little as 15 minutes of seizure-like activity decreases KCC2, which may in turn increase concentrations of intracellular Cl- and reduce GABA-mediated inhibition. The current experiment examined the concentrations of KCC2 and phosphorylation at two key regulatory residues, S940 and T906, in C57BL/6 mice following the onset of soman-induced convulsions. Importantly, the phosphorylation of S940 (pS940) increases KCC2 activity, whereas the phosphorylation of T906 (pT906) decreases KCC2 activity. Mice were exposed to soman, and brain tissue was collected at 0, 15, 30, or 60 minutes following the onset of convulsions. In the hippocampus, soman increased pT906 within 15 minutes of convolution onset and continued to increase over time up to 60 minutes. In contrast, soman-induced convulsions did not significantly alter pS940 or total KCC2. In the cortex, soman-induced convulsions increased both pT906 and pS940 at 15 minutes; however, concentrations of pS940 decreased to levels equivalent to saline-exposed mice by 60 minutes. Congruent with results from the hippocampus, total KCC2 levels did not change in the cortex. These results suggest that soman-induced convulsions are associated with time-dependent changes in the phosphorylation state of KCC2. Reactivating KCC2 may thus serve as a potential therapeutic strategy for terminating benzodiazepine-resistant seizures.

**553 Flavanone Silibinin As Potential Therapeutic against Skin Injuries by Vesicating Agents**

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Chemical warfare agent sulfur mustard (SM) and its analog nitrogen mustard (NM) inflict delayed blistering and incapacitating injuries on skin tissue. Our previous findings have demonstrated therapeutic efficacy of silibinin in reversing monofunctional alkylating SM analog 2-chloroethyl ethyl sulfide (CEES)-induced skin injuries. To translate this effect using a bifunctional alkylating vesicant, efficacy studies were carried out with NM-induced injury biomarkers established in SKH-1 hairless mice. Topical application of silibinin 30 min after NM exposure decreased NM-induced 60% mortality (120 h exposure) and NM-induced toxic skin injury attributes in SKH-1 mice. Following 1 or 2 mg silibinin treatment, a dose-dependent reduction of NM-induced increases in epidermal thickness, microvesication, dead and denuded epidermis, parakeratosis and scab formation was observed. All NM-induced lesions were assessed at 24, 72 or 120 h time point depending on their optimum injury. Silibinin (2 mg dose) caused complete prevention of NM-induced H2A.X phosphorylation indicating reversal of DNA damage, large incidences of microvesication (>500 μm2) and parakeratosis. Silibinin (2 mg) application also caused 52% and 77% (p<0.05) reversals in NM-induced increase in COX-2 and myeloperoxidase levels, respectively. These findings indicate that attenuation of NM-induced skin injuries by silibinin is due to its multiple effects targeting pathways associated with DNA damage, inflammation and vesication. Together, these studies suggest therapeutic potential of silibinin against skin injuries by NM and support its further optimization as an effective treatment for skin injuries by SM and other vesicating agents.
Vesicating agents sulfur mustard (SM) and nitrogen mustard (NM) are easily absorbed by skin causing cutaneous injuries and blistering. To study clinical, histopathological, and molecular features of cutaneous lesions from topical vesicant exposure, we used NM. Our studies showed that topical NM (3.2 mg) exposure in SKH-1 hairless mice not only caused skin injuries but also lead to significant body weight loss and 40-60% mortality (120 h exposure), suggesting systemic effects of its topical exposure. Studies in these mice showed that, though a decrease in heart and breath rate was only observed at 120 h following exposure, NM exposure initiated an increase in blood leukocyte count by 24 h and thereafter leukopenia 72 h post-exposure. Examination of the blood revealed an increase in neutrophils and decrease in lymphocytes 24 h post exposure. H&E staining of the small intestine revealed NM-related toxic effects including loss of membrane integrity of surface epithelium and abnormal structure of glands as well as degeneration of villi starting by 24 h and observed mainly 72-120 h after exposure. NM exposure also resulted in a reduction in T-cells from the spleenic peri-arterial lymphatic sheath as well as macrophages and RBCs from the red pulp. At later time points (72 and 120 h), NM exposure resulted in dilation of glomerular capillaries and loss of cells from the renal corpuscles. It has been reported that exposure to higher SM levels (total body exposure) can cause damage to the bone marrow and hematopoietic system, liver and kidney failure, and toxicity to the spleen and GI tract. Our results with NM indicate that these organs are also affected from only topical vesicant exposure and can lead to mortality. These outcomes could help identify useful countermeasures against skin injuries and toxic systemic effects after topical vesicant exposure.

To develop effective therapies against ocular injuries by vesicating agents, investigation of mechanistic aspects of vesicant-induced ocular injuries is important. Accordingly, toxic effects and associated mechanisms were examined in maximal affected corneal tissue with nitrogen mustard (NM), an analog of sulfur mustard (SM). Analysis of 100 nmol NM-exposed rabbit corneas for 2 h (washed and cultured for 24 h), evidenced increases in epithelial thickness, apoptotic cell death, epithelial-stromal separation, increase in the levels of angiogenic regulator VEGF, and induction of COX-2 and MMP-9. Since vesicating agents mainly target the corneal epithelial cells, we also employed human corneal epithelial cells (HCEC) to study and compare the mechanism/s of action of NM-induced injury in these cells with rabbit cornea. Exposure (48 h) to NM (50 and 100 μM) caused a dose-dependent decrease in HCEC viability and DNA proliferation. These toxic effects could be related to NM-induced DNA damage (p53 phospho ser15, total p53 and H2AX phospho ser139) in HCEC. In addition to p53 phosphorylation, NM also induced caspase-3 and PARP cleavage, suggesting their role in NM-related HCEC death and apoptotic cell death in rabbit cornea. Similar to studies in rabbit cornea, NM exposure also caused an increase in COX-2, MMP-9 and VEGF levels in HCEC, indicating a role of these molecules and related pathways in NM-induced corneal inflammation, epithelial-stromal separation and neovascularization. NM exposure in HCEC also instigated an increase in phosphorylation of MAPKs and activation of AP-1 family proteins. Increase in lipid peroxidation and protein oxidation in HCEC by NM suggests the role of oxidative stress in the activation of signaling pathways by NM. These molecular targets could be supportive in development of therapeutics against vesicant-induced corneal injuries.
administered either by subcutaneous (SC) injection in, or percutaneously on, guinea pigs that subsequently received atropine free base at 0.4 mg/kg. The hu-
man-equivalent dose in guinea pigs for three DuooDote® autoinjectors was esti-
mated to be 0.4 mg/kg. Lethality rates were obtained at 24 h after challenge for all but parathion and sulfur mustard challenge, which required at least 48 h to allow for
bioactivation to the oxon form. Probit analysis was used to calculate the median lethal dose (LD50). Protective ratios (PR) for atropine were calculated as the LD50 for a challenge material (CM) in atropinized guinea pigs divided by the LD50 for the same CM in no-therapy guinea pigs using data from either the literature or con-
current experiments. Phorate, paraxon, and CPF did not present significant per-
cutaneous threats, and attempts to obtain an LD50 were abandoned. Statistically
significant (p < 0.05) therapy in terms of survival was attributed to atropine therapy against a SC challenge of GA alone. Atropine alone did not improve survivability against any other CM injection by either SC injection or topical application on clipped skin.

Conclusion: The results demonstrated here show that functional measurements can be performed with this technique by using membranes of Torpedo californica electric tissue. Bispyridinium compounds seemed to interact as positive allosteric modulators, thereby affecting desensitisation. Further research is necessary to verify this hypothesis.

561 Attenuation of Nitrogen Mustard (NM)-Induced Pulmonary Injury and Inflammation by Antitumor Necrosis Factor (TNF)α Antibody and the Inducible Nitric Oxide Synthase (iNOS) Inhibitor, N-(3-(Aminomethyl)benzyl)acetamide (1400W)

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NM (mechlorethamine) is a vesicant which causes severe damage to the lung lead-
ing to pulmonary fibrosis; this is associated with oxidative stress and inflammation.

Using transgenic knockout mice, we previously demonstrated that the proinflam-
matory cytokine, TNFα and nitric oxide generating enzyme, iNOS, play key roles in the pathogenic response to NM. In these studies we analyzed the ability of anti-TNFα antibody and 1400W, alone and in combination, to mitigate NM-
duced lung injury. Treatment of male Wistar rats (250 g) with NM (0.125 mg/
kg, i.t.) resulted in histopathologic changes in the lung 3-7 d post exposure, and
induced expression of cleaved caspase-3 (Casp-3), a marker of apoptosis. This was correlated with increases in bronchoalveolar lavage (BAL) cell and protein content, indicating damage to the alveolar-epithelial barrier. NM also caused increased ex-
pression of cyclooxygenase (COX)-2 and heme oxygenase (HO)-1 in lung mac-
rophages, as well as YM-1, a marker of oxidative stress and alternatively activated
macrophages. Treatment of rats with anti-TNFα antibody (15 mg/kg, i.v., 1x), 1400W (50 mg/kg, i.p., 1x/d), or a combination of anti-TNFα + 1400W 30 min
after NM reduced lung injury and inflammation, as measured by BAL cells, protein
content. NM-induced expression of COX-2; HO-1; YM-1 and Casp-3 were also reduced. Although the combination of anti-TNFα + 1400W was more effective in
down regulating expression of COX-3, COX-2, HO-1 and YM-1 at 3 d than either agent alone, fibrinotic changes at 7 d were only inhibited by anti-TNFα anti-
tibody. These data demonstrate that inhibiting TNFα and/or iNOS represents an important approach to mitigating acute and long-term lung injury induced by ves-
icants. Supported by NIH Grants HL096426, AR055073, ES004738, CA13624, and ES005022.

562 Inhibition of Multidrug Resistance-Associated Protein (MRP) Efflux Transporters Increases the Sensitivity to Vesicant-Induced Growth Inhibition in Lung Epithelial Cells

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Sulfur mustard and the related vesicant nitrogen mustard (mechlorethamine, HN2) are bifunctional alkylating agents that cause oxidative stress and persistent lung damage. In the present studies, we examined the role of MRP in modulating the sensitivity of lung cells to HN2. MRP transporters 1, 2, and 3 remove glutathi-
one conjugates, which can cause oxidative stress and cytotoxicity. To assess the role of these transporters in the cytotoxic actions of HN2, we used MK-571, an
MRP modulator; thereby, affecting desensitisation. Further research is necessary to verify this hypothesis.

Functional interactions of bispyridinium compounds were investigated.

560 Functional Properties of Bispyridinium Non-Oxime Compounds on Muscle-Type Nicotinic Acetylcholine Receptors Using a Bilayer-Based Method

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Objective: In poisoning with some nerve agents, e.g. soman, therapeutic efficacy is
limited. A direct intervention at nicotinic acetylcholine receptors (nAChRs) might be
an option. Studies with bispyridinium non-oxime compounds demonstrated therapeutic
effects against soman in vitro and in vivo. Consequently, these re-
results arouse increasing interest in functional properties of nAChRs, especially the
muscle-type. The Torpedo electric organ is regarded as a suitable surrogate. For functional characterization, cell-free electrophysiological methods based on
solid supported membranes (SSM) can be used. However, conventional elec
trophysiology cannot be applied. In this bilayer based sensor technique, charge
translocation is measured via capacitive coupling of the supporting membrane.

559 Identification of Glutaredoxin As a Molecular Target for the Sulfur Mustard Analog Bis(2-chloroethyl)phosphinylmethylamine

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The glutaredoxin system, which consists of glutaredoxin (Grx), glutathione, and
 glutathione reductase (GR), plays a key role in antioxidant defense and cell growth control. Due to their high structural similarity, glutaredoxin and thioredoxin often
display overlapping functions. Bis(2-chloroethyl)methylamine (HN2), a bifunc-
tional alkylating agent commonly used as a model compound to study sulfur must-
vard-induced injury, is known to cause oxidative lung injury. Previously, we re-
ported that HN2 selectively targets the thioredoxin system in lung A549 epithelial
cells resulting in enzyme inactivation, a process contributing to oxidative stress and toxicity. In this study, we examined the effects of HN2 on the glutaredoxin system.

Results: Depending on particle size, zeta potential and adsorption procedures, sta-
ble microparticles were obtained. Various concentrations of agonist and bispyridinium compounds were tested. Controversially, carbamoylcholine elicited a Na+
 influx in the membrane vesicles. Various concentrations of agonist and bispyridinium compounds were tested. Results: Depending on particle size, zeta potential and adsorption procedures, sta-
ble and reproducible cholinergic signals were recorded (> 1 nA). Ambroxol pre-
vented a premature collapse of the sodium gradient. High carbamoylcholine con-
der 

Organophosphorus (OP) compounds, including pesticides and chemical warfare nerve agents (CWNAs) represent a threat to the general population not only as possible weapons of terrorism, but also as chemicals that could be released from transportation and storage facilities during industrial accidents. Given the rapid onset of symptoms and toxicity of OP agents, a quick-acting therapeutic regimen that is efficacious over the broad spectrum of OPs is urgently needed. The current regimen includes the administration of atropine in combination with an acetylcholinesterase (AChE) reactivator (oxime; 2-PAM Cl). An anticonvulsant (diazepam) is administered if convulsive symptoms are observed. Although this treatment approach is effective against OP intoxication, more effective treatments are being investigated to improve the nation’s medical response capabilities. The objective of the current study was to perform a head-to-head comparison of the leading oxime therapies (2-PAM Cl, HI-6 DMS, obidoxime Cl2, TMB4, MMB4-DMS, HLö7) against the CWNAs (tabun, sarin, soman, cyclosarin, VX, and the pesticides paraoxon, chlorpyrifos oxon, DMS, MINA and RS194B) under standardized conditions against the CWNAs (tabun, sarin, soman, cyclosarin, VX, and the pesticides paraoxon, chlorpyrifos oxon, DMS, MINA and RS194B) under standardized conditions.

The sulfur mustard analog (b(2)-chloroethyl) methylamine, HN2, is a potent skin vesicant. As a bifunctional alkylating agent, HN2 can directly modify DNA, a process that can result in strand breaks. Recent evidence suggests that HN2 is also a potent inducer of epidermal oxidative stress. In the present studies we determined if HN2-induced epidermal DNA strand breaks were associated with expression of markers of oxidative stress. CD-1 female mice (6-8 weeks of age) were treated on the dorsal skin with HN2 (20 μmules). After 1, 3 and 5 days, expression of markers of oxidative stress including 4-hydroxynonenal (4-HNE)-modified proteins and 8-oxo-2‘-deoxyguanosine (8-OHdG), and histone phospho-H2A.X, a marker of DNA double strand breaks, was analyzed in the skin using techniques in immunohistochemistry. We found that 8-OHdG and phospho-H2A.X were coordinately upregulated in the nuclei of interfollicular and follicular epidermal cells throughout the wound one day post-treatment. In contrast, 4-HNE modified proteins were identified only in the stratum granulosum and the follicular epidermis. By day 3, phospho-H2A.X and 8-OHdG were upregulated in a thick eschar above the wound and within the interfollicular epidermis and the sebocytes, while 4-HNE was expressed in the hypotrophic epidermis underlying the eschar and interfollicular epidermis. At 5 days post exposure, phospho-H2A.X was confined to the stratum basale with scattered expression in the dermis, while 8-OHdG was expressed in the dermis and the hypodermis. 4-HNE was down regulated and restricted to the hyperplastic stratum granulosum and stratum spinosum. These data indicate that HN2 induces lipid peroxidation and oxidative DNA damage in mouse skin, a process which likely contributes to tissue injury following exposure to vesicating agents.

Nerve agents cause neuroinflammatory response that may potentially be treated by novel anti-inflammatory peptides. The way of their discovery is described. Previous studies demonstrated the potent effect of topical iodine against skin toxicity induced by sulfur mustard and other chemical and thermal stimuli. While exploring the mechanism of iodine we discovered that it induces the production of a nonapeptide, a fragment of H2A histone termed IIIM1. This peptide showed counterirritating and antiinflammatory activities in the ear swelling test and carrageenan-induced hind paw edema assay. Being an antiinflammatory peptide, IIIM1 was evaluated as a potential drug candidate for pathologies-associated with neuronal damage such as experimental autoimmune encephalitis (EAE), an animal model of multiple sclerosis (MS). The peptide dramatically ameliorated the neurological symptoms in the diseased mice, reduced proinflammatory cytokines, elevated Th2 cytokines and caused proliferation of T-regulatory cells. In addition to its immunomodulatory/antiinflammatory properties, IIIM1 significantly reduced cytotoxicity and oxidative stress in astrocytes exposed to methyl mercury. The neuroprotective effects of IIIM1 strongly support the notion that this peptide may be used as a neuroprotectant in organophosphorus exposure.

Further mechanistic studies revealed that IIIM1 induced a 16 amino acid peptide termed RA1 that surprisingly, is present in Japanese rice. RA1 ameliorated neuro- logical symptoms in EAE mice, reduced proinflammatory cytokines and elevated Th2 cytokines. This peptide may be associated with the low incidence of MS in Japan. In view of their antiinflammatory characteristics both IIIM1 and RA1 are potential candidates for treatment of neuroinflammation caused by exposure to nerve agents.
Aldicarb and methomyl are highly toxic carbamate insecticides and known groundwater contaminants. Both carbamates have been implicated in animal and human poisonings. Consequently, they pose a threat to the general population not only as possible weapons of terrorism, but also from environmental and occupational exposures. Like the common organophosphorus pesticides (OPs) and nerve agents, the mechanism of toxicity is cholinesterase (ChE) inhibition which can result in cholinergic crisis. Inhibition of ChE reverses fairly rapidly when compared to inhibition from OPs. Based on its primary mechanism of toxicity as an anticholinesterase, the administration of atropine and the oxime 2-PAM C1 is the proposed therapeutic approach after carbamate poisoning. This study evaluated the dose-lethality response curves characterized in non-sedated, Hartley male guinea pigs exposed to either aldicarb or methomyl, administered via a single subcutaneous (SC) injection. The median lethal dose (MLD) for aldicarb was determined to be 0.86 mg/kg while methomyl was approximately 10-fold less toxic at 8.06 mg/kg. Atropine (At) free base was administered at human equivalent dose (HED) levels for the guinea pig, 0.4 mg/kg, via the intramuscular route. Due to toxicity in the guinea pig, 2-PAM C1 was administered at the human-relevant dose of 25.7 mg/kg and was via the same administration route. Atropine alone and in combination with 2-PAM C1 demonstrated significant protection against lethality. However, the addition of 2-PAM C1 did not have a statistical effect on the efficacy of atropine alone. The observation that 2-PAM C1 did not reduce the overall efficacy of this treatment regimen is encouraging given previous concerns regarding the potential problematic interactions between carbamates and oximes.

568 Paraoxonase 1 (PON1) Enhancers Accelerate the Detoxication of Organophosphates (OP) Including Nerve Agent Surrogates

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PON1, a calcium dependent hydrolase, is associated with the high density lipoproteins in human serum. PON1, named for its ability to hydrolyze paraoxon, the active metabolite of the OP insecticide parathion, can hydrolyze some OPs, but most with low efficiency. PON1 uses a hydroxide ion from water as the nucleophile to accomplish the degradation of the OP. Novel substituted phenoxyl alkyl pyridinium oximes have been developed as more potent nucleophiles with the expectation that they might enhance the efficacy of PON1 in degrading OPs. The substrates tested were surrogates for sarin and VX, nitrophenyl isopropyl methylphosphonate (NIMP) and nitrophenyl ethyl methylphosphonate (NEMP), respectively, and paraoxon. Commercially available human serum was used as the source of PON1. The length of the alkyl chain varied from 6-10 carbons, and the molecules contained various substitutions on the phenoxy moiety. A direct assay was employed using 0.3mM NIMP or NEMP or 1.2mM paraoxon and 0.1mM nucleophile, monitoring the released 4-nitrophenol spectrophotometrically following a 15 min incubation with serum. About 20 novel nucleophiles were tested. Enhancement of degradation of over 10% occurred with 10 of the nucleophiles with NIMP, with 8 of these yielding over 20% enhancement. Enhancement of degradation of over 10% occurred with 9 of the nucleophiles with NEMP, with 3 of these yielding over 20% enhancement. Only 1 of these nucleophiles enhanced the degradation of paraoxon. An indirect assay used 120 nM NIMP or 30 nM NEMP (i.e., more realistic concentrations), and monitored residual anticholinesterase activity of undegraded surrogate as an index of PON1 activity. This indirect assay showed 11 nucleophiles able to enhance NIMP degradation by up to 50% and 7 nucleophiles able to enhance NEMP degradation by up to 35%. These nucleophiles show potential to be developed as drugs with a novel mechanism of antidotal action. (Supported by Defense Threat Reduction Agency HDTRA1-12-1-0043)

569 Δ4 Tetrahydrocannabinol Prevents Mice from Staphylococcal Enterotoxin B-Induced Toxic Death by the Modulation of the miR-17-92 Cluster and De Novo Induction of T-Regulatory Cells

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The superantigen Staphylococcal enterotoxin B (SEB) is a potent activator of Vβ8+ T-cells which results in the clonal expansion of ~30% of the T-cell pool. Consequently, this leads to the substantial release of inflammatory cytokines, induction of toxic shock, and eventually death. In the current study, we sought to investigate if Δ4 Tetrahydrocannabinol (THC), a marijuana cannabinoid known for its anti-inflammatory properties, could prevent SEB-induced mortality and alleviate symptoms of toxic shock. Intranasal followed by intraperitoneal dual administration of SEB resulted in acute mortality, while THC treatment led to 100% survival. Additionally, THC treatment significantly decreased numerous SEB-mediated clinical parameters, such as production of inflammatory cytokines, immune cell infiltration into the lung, vascular leak, and improved airway resistance. Microarray analysis revealed that a prominent miRNA signature upon SEB exposure was the induction of the miR-17-92 cluster. Mechanistically, we found the miR-17-92 cluster targets Pten (phosphatase and tensin homolog), an inhibitor of the PI3K/Akt signaling pathway, thereby promoting cellular proliferation and the suppression of T-regulatory cells. THC treatment, however, inhibited the individual miRNAs in the cluster. Moreover, in its ability to act as a PI3K/Akt inhibitor, THC treatment led to the induction of CD4+Foxp3+ T-regulatory cells and suppressed cellular proliferation. We report, for the first time, a miRNA expression profile in response to SEB. Furthermore, our results suggest that THC is a potent anti-inflammatory compound that acts by modulating critical miRNA involved in SEB-induced toxicity and death. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357)
NM (mechlorethamine) causes lung injury and fibrosis. This is associated with MP accumulation in the tissue and the release of mediators implicated in toxicity. Herein, we assessed the phenotype and origin of MP accumulating in the lung after NM. Cells were collected by bronchoalveolar lavage (BAL) 1-28 d after i.t. saline or NM (0.125 mg/kg) administration to Wistar rats and stained with antibodies to Mac-1 (inflammatory cells) and CD43 (monocyte-derived MP). The majority (>97%) of cells from control animals were Mac-1−/CD43+, consistent with a resident alveolar MP phenotype. NM caused an increase in Mac-1+/CD43+ and Mac-1−/CD43− cells in the lung, which peaked at 3 and 7 d, respectively. By 28 d post NM, cells more closely resembled resident alveolar MP. To characterize these subpopulations, cells were analyzed for expression of pro-(CCK2, iNOS) and anti-inflammatory (IL-10) genes. NM caused a persistent increase (4-6-fold) in CCR2 expression by resident alveolar MP (Mac-1−/CD43−), with no change in iNOS or IL-10. Increased CCR2 was also observed in infiltrating Mac-1+/CD43+ and Mac-1+/CD43+ cells after NM; at 3 d expression was 2-3 greater than resident alveolar MP. CCR2 remained elevated for at least 7 d in Mac-1−/CD43+ cells. These cells also expressed increased iNOS, and to a lesser extent, IL-10, suggesting that within this population there are phenotypically distinct subsets. Spleen cells (SPX) rats used to assess the role of the spleen as an extramedullary source of lung MP. Increased tissue damage was noted in histologic sections of SPX rats 3 d post NM; this correlated with increased Mac-1+/CD43+ cells in the lung. These studies show that the lung absorption of multiple inflammatory subpopulations into the lung some of which originate in the spleen. Moreover, it appears that spleen derived cells contribute to down regulating inflammation and initiating wound repair. (NIH AR055073, ES004738, CA132624, and ES005022)
Sulfur mustard [SM, Bis (2-chloroethyl) sulfide], a potent vesicant agent, induces dermal toxicity which includes edema, inflammation, fluid-filled blistering, dermal-epidermal junction (DEJ) disruption, delayed wound repair, and scarring. Studies have shown that down regulation of the gap junction protein, connexin 43 (Cx43) accelerates wound closure and improves wound reepithelialization. We performed a time course study using sulfur mustard [NM, Bis (2-chloroethyl) methylamine] to establish an alternative to the SM skin injury model. We also evaluated the wound repair response using a Cx43 antisense oligodeoxynucleotide (asODN) topical treatment on NM exposed hairless (SKH-1) mouse skin. NM exposure alone caused most of the dermal toxicity produce by SM exposure. In addition we examined several skin markers: keratin 5 (K5, basal keratinocyte marker); keratin 10 (K10, keratinocyte differentiation marker); Ki67 (proliferation marker); laminin 332 (LM332, basement membrane marker). Immunofluorescent (IF) studies showed reduced K5 expression, loss of K10 and Ki67, and an interrupted LM332 pattern of expression 1 day after NM exposure. By day 3, K10 reappeared in a diffuse pattern. By day 7, the LM332 appearance was thick and continuous. By day 10, there was intense expression of Ki67, accompanied by extreme hyperplasia. Our findings support the use of Cx43 asODN may improve vesicant wound repair. Supported by ES005022, ES004738, EY09056, and grant U54ES015678.
neuroinflammation in the rat brain, as determined by histological assessment. The goal of the current study was to test high-field MRI as a tool for monitoring the progression of neurologic damage following acute DFP intoxication. Adult male Sprague-Dawley rats were given pyridostigmine (0.026 mg/kg, i.m.) prior to successive administration of DFP (4 mg/kg s.c.), atropine sulfate (2 mg/kg, i.p.), and 2-PAM (25 mg/kg, i.m.). This treatment paradigm resulted in moderate-to-severe seizure activity as determined using a modified Racine scale. Animals were imaged on a Bruker 7T MRI at baseline, and then at two of five time points post-intoxication (3, 7, 14, 21, or 28 days). At each time point, animals were euthanized and brains collected for histological evaluation. DFP exposure produced significant abnormalities in T2-weighted and diffusion tensor imaging out to 28 days post intoxication. Regions such as the dorsolateral thalamus, amygdala, hippocampus, and piriform cortex showed changes in MR endpoints (e.g., intensity, ADC, and anisotropy). These same brain regions exhibited severe neuronal necrosis. These results demonstrate that MRI is a powerful tool for longitudinal assessments of neuropathology following acute OP poisoning that may prove useful for screening candidate neuroprotective therapies. Supported by the NIH CounterACT program (grant #U54 NS079702) and by a NIH training grant (T32 GM099608).

580 A New Purpose for Ebselen As a Prophylactic to Chemical Threats?
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Sulfur mustard (SM) is a chemical warfare agent that can cause severe acute and chronic injury to the eyes, skin and respiratory tract. The nitrogen mustard methyl mustard (HN2) is a stable isomer of SM that has been recognized as a SM mimetic. Whereas previous studies in this and other laboratories have demonstrated that ebselen (EB-1) is effective at protecting cells from HN2 toxicity in vitro, the present study investigated whether or not EB-1 can reduce the dermal toxicity of HN2 in vivo. The mouse ear vesicant model was utilized, with mouse ears topically exposed to HN2 and biopsied 24 h after exposure. Ear punches were then fixed, sectioned and stained with H&E for inspection by light microscopy. HN2 produced a dose-dependent inflammatory response, characterized by edema, epithelial hyperplasia, increased neutrophil infiltrates and vesication. A histopathological scoring scale analysis of ear tissue sections was developed and used to qualitatively assess HN2-mediated injury. In addition, the morphometric index (MI) of exposed tissues was calculated using the formula, MI = (thickness of treated tissue/average thickness of vehicle exposed ears)*100%. Both histopathological score analysis and morphometric analysis revealed statistically significant injury at doses of HN2 ≥ 0.250 mmol/carr. To test the efficacy of EB-1 as a prophylactic to HN2, three topical treatments with EB-1 (1 mg/carr or 2 mg/carr) were performed at 8 h, 4 h and 15 min prior to HN2 exposure. Quantitative morphometric analysis of tissue samples collected 24 h post HN2 demonstrated that EB-1 significantly reduced HN2-induced tissue swelling at both doses. Similar results were obtained using histopathological score analysis, where a trend toward EB-1 mediated protection was also observed. Taken together, the data presented here suggest that repositioning EB-1 from a drug previously thought to be useful for stroke treatment to one with a new purpose as a prophylactic to chemical threats may be warranted.

581 Exposure-Response Modeling and Simulation to Support Human Dosing for Botulinum Antitoxin Heptavalent (A, B, C, D, E, F, G)–(Equine) or H-BAT
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Exposure-response modeling and simulation was performed to support Cangene’s human dosing recommendations for BAT™, Botulinum Antitoxin Heptavalent (A,B,C,D,E,F,G)–(Equine). A compartmental model was constructed to assess the pharmacokinetics (PK) of H-BAT in humans, guinea pigs and Rhesus macaques. A traditional allometric power model was used to scale compartmental PK parameters in humans according to the corresponding body weight of each species. This relationship was used to simulate exposure to H-BAT in any species and according to any dosing scenarios. Subsequently, an exposure-response model was constructed to provide a mechanistic understanding of the relationship between exposure to H-BAT and response to BoNT intoxication (survival) based on the available post-exposure prophylaxis study information (guinea pig and Rhesus macaque). Exposure to H-BAT was simulated using the PK model and survival probability in humans was predicted based on the above exposure-response model. H-BAT exposure (AUC) in humans was compared to a minimum efficacious exposure (MEE) to identify the margin of efficacy (MOE = AUChuman dose/AUCMEE). The MOE ratio provides an estimate of the “safety” margin for the product. The survival probability derived using specification potency was 99.9, 95.6, 97.5, 93.1, 99.0, 95.9 and 97.4% for serotypes A to G respectively. Based on the exposure-response models, the MOE for all antitoxin serotypes were above 1 and the predicted probability of survival in humans following exposure to all serotypes of BoNT was more than 93.1% following administration of the one vial of H-BAT.

581a Corneal Endothelial Toxicity following Corneal Exposure to Sulfur Mustard Vapor: Is Endothelial Loss the Pathophysiology Responsible for Long-Term Ocular Injury?

Sulfur mustard (SM) is a highly reactive vesicant that causes severe ocular injuries. Following exposure to moderate or high doses, a subset of victims develop a chronic injury known as mustard gas keratopathy (MGK), involving a keratitis of unknown etiopathogenesis with secondary keratopathies such as persistent epithelial lesions, corneal neovascularization and progressive corneal degeneration. The etiology of these recurrent keratopathies is unknown. Diverse therapeutic approaches have failed to mitigate the delayed injury, suggesting that researchers need an improved understanding of the mechanisms underlying injury progression. We have developed a novel rabbit corneal vesicant exposure model that exhibits acute and long-term sequelae commensurate with human clinical reports and have characterized consistent and reproducible metrics of injury progression. In preliminary ultrastructural analyses a chronic pathology was observed in the corneal endothelium of MGK corneas. To determine whether (a) SM exposure evokes acute endothelial toxicity and (b) endothelial pathologies were specifically observed in MGK corneas as opposed to healed corneas, we evaluated the corneal endothelium at 1 d and 8 wk after exposure using scanning electron microscopy (SEM), transmission electron microscopy (TEM), in vivo confocal microscopy (IVM), fluorescent microscopy and functional measures of endothelial barrier function. All methods revealed the appearance of a centripetal endothelial lesion at 1 d after exposure, and longitudinal analysis using IVM revealed additional cytotoxicity between 1-13 d. In contrast to healed corneas at 8 wk, which appeared similar to sham-exposed naïve eyes, MGK corneas exhibited significant evidence of continued pathological change in the endothelium. These data indicate that endothelial toxicity occurs at the right time and with the appropriate pathophysiology to contribute to MGK and explicates many of the previously confusing aspects of clinical progression.

581b Deterministic Models of Inhalational Anthrax in New Zealand White Rabbits
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Computational models describing bacterial kinetics were developed for inhalational anthrax in New Zealand white (NZW) rabbits following inhalation of Ames strain B. anthracis. The data used to parameterize the models included bacterial numbers in the airways, lung tissue, draining lymph nodes and blood. Initial bacterial numbers were deposited spore dose. The first model was a single exponential ordinary differential equation (ODE) with three rate parameters that described mucociliated (physical) clearance, immune clearance (bacterial killing) and bacterial growth. At 36 hours post-exposure, the ODE model predicted 1.7 x 10^7 bacteria at 36 hours). Next, building on the single ODE model, a physiological-based biokinetic (PBBK) compartmentalized model was developed where one physiological compartment was the lumen of the airways and the other was the rabbit body (lung tissue, lymph nodes, blood). The two compartments were connected with a parameter describing transport of bacteria from the airways into the body. The PBBK model predicted 4.9 x 10^3 bacteria in the rabbit, which agreed well with data from actual experiments (4.0 x 10^3 bacteria at 36 hours). Next, building on the single ODE model, a physiological-based biokinetic (PBBK) compartmentalized model was developed where one physiological compartment was the lumen of the airways and the other was the rabbit body (lung tissue, lymph nodes, blood). The two compartments were connected with a parameter describing transport of bacteria from the airways into the body. The PBBK model predicted 4.9 x 10^3 bacteria in the body at 36 hours and by 45 hours the model showed all clearance mechanisms were saturated, suggesting the rabbit would quickly succumb to the infection. As with the ODE model, the PBBK model results agreed well with laboratory observations. These data are discussed along with the need and potential application of the models in risk assessment, drug development and as a general aid to the experimentalist studying inhalational anthrax.
were determined at 5, 10, 20, 30, 60 min and 2, 4, 6, 8, 24 and 48 h post dose. A single im injection or 65 mg/kg split into two im injections. Plasma drug levels were determined using a immunoassay method developed to optimize drug absorption. When given at 1 min after soman exposure, promethazine at the dose of 32 or 40 mg/kg decreased the incidence of seizures from 100% to 0% or 40%, respectively. Interestingly, the 40% of the animals that developed seizures after promethazine treatment at 1 min after soman exposure had their seizures spontaneously terminated. Promethazine given at 1 min after soman exposure at the dose of 32 or 40 mg/kg also increased the survival rate from 0% to 75% or 100%, respectively. When promethazine at the dose of 32 or 40 mg/kg was administered at the onset of seizures, seizures were terminated in 100% of animals, and the survival rate was increased from 0% to 100%. At 24 hr, promethazine-treated animals behaved like non-exposed animals. Brain samples from promethazine-treated animals were evaluated for brain injury. None of the animals treated with promethazine, either at 1 min after soman administration or at the onset of seizures, exhibited neuronal damage in any of the brain regions examined. These observations indicate that promethazine treatment can effectively control seizures, improve survival and prevent brain injury following exposure to 2.0 LD50 of soman.

581d Pharmacokinetics of Nitrocinobinamide (NCbi), a Novel Cyanide Antidote
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The cobalamin analog cinobinamide (Cbi) has a high affinity for cyanide and is a potent cyanide antidote after im injection. Pigs are rescued from a lethal cyanide dose by 12.5 mg/kg of Cbi. The objective of the current study was to determine the pharmacokinetics of NCbi, a formulation developed to optimize drug absorption. Male Sprague Dawley rats received either 30 mg/kg iv, 65, 95, or 130 mg/kg as single im injection or 65 mg/kg split into two im injections. Plasma drug levels were determined at 5, 10, 20, 30, 60 min and 2, 4, 6, 8, 24 and 48 h post dose. After an iv dose, Cbi was rapidly distributed and eliminated with t1/2 of 11.6 ± 3.6 h and clearance of 45.7 ± 7.3 ml/h/kg. The plasma concentration extrapolated to time 0 was 378 ± 308 µg/ml and the AUC was 670 ± 101 µg·h/ml. The volume of distribution was 0.7 ± 0.13 l/kg, consistent with distribution to extracellular water. After an im dose, the plasma Cbi concentration increased rapidly with mean T1/2 values ranging from 0.8 ± 0.3 h (high dose) to 1.7 ± 0.6 h (mid dose). The Cmax and AUC values were dose dependent, although the increase in exposure was not linear from the mid to the high dose. The mean T1/2 was about 15 h for the single injection im groups. When the low dose was given as 2 injections of 100 mM, Cbi was absorbed faster (T1/2 of 0.6 ± 0.3 vs 1.3 ± 0.6 h) and the Cmax was ~ 40% higher than after a single injection of 100 mM. At a concentration of 50 µg/ml N-Cbi, 83.0 ± 2.89% of Cbi was bound to rat plasma proteins. In summary, Cbi absorption after im injection reached maximum plasma levels at 15-20 min post injection. Based on Cmax and AUC, increased with dose but tended to plateau, suggesting that Cbi absorption becomes saturated at higher formulation concentrations. The administration of the low dose, split and injected at two sites, yielded faster absorption and higher Cmax values than a single injection. The average bioavailability of Cbi after im administration of N-Cbi was excellent, > 75%. This research was supported by NIH Contract HHSN271200623601C.

581e Zebrafish As a Novel Animal Model for Chemical Warfare Agent (CWNA) Biomedical Research
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Zebrafish only express the acetylcholine hydrolyzing enzyme acetylcholinesterase (AChE). This unique genotype makes the zebrafish an ideal animal model to study the toxicological effects of organophosphorus nerve agent (NA) AChE inhibitors and the effectiveness of oximes to reactivate NA-inhibited AChE. The present study determined the LC50 of sarin and evaluated the efficacy of 2-PAM in reactivating sarin-inhibited AChE. Larval zebrafish at 6 days postfertilization (dpf) were exposed to various concentrations of sarin for 1 hr to determine the LC50 of sarin. Following sarin exposure, zebrafish larvae were screened under a dissecting microscope to observe the overt signs of toxicity. Death was determined by the cessation of heart beats. The mortality rate was 0, 20, 45, 55, 43, 75, 70, 95 or 100% after exposure to 0, 12.5, 25, 50, 75, 100, 150, 250 or 500 uM of sarin, respectively. By regression analysis, the LC50 of sarin for 1 hr exposure at 24 hr was estimated to be 80 uM. Twenty-four-hour survivors exhibited behavioral changes, including an inability to maintain buoyancy and swimming vertically on the side. To evaluate the effectiveness of 2-PAM to reactivate sarin-inhibited AChE, 6 dpf larvae were exposed to 25 uM of sarin, and their AChE activity was measured by the Ellman assay. When the larvae were completely immobile after 5 mins of exposure, they were treated with 0 or 50 uM of 2-PAM. The percentage of AChE inhibition was 98% when the larvae lacked locomotion. With 2-PAM treatment, the AChE percentage inhibition was decreased, but by no more than 30%. The results suggest that 1) zebrafish larvae are sensitive to the toxic effects of sarin in a concentration-dependent manner and 2) zebrafish larvae AChE inhibited by sarin can be reactivated by 2-PAM, an oxime reactivator currently used as standard treatment for CWNAs in the military. These observations provide evidence that the zebrafish is a suitable animal model system for in vivo evaluation of NA toxicity and novel oxime reactivators.
laws do not mention bioavailability. SOT’s Code of Ethics should have compelled more science, not policy-driven results. The reader’s attention is drawn to the Code: “Abstain from professional judgments influenced by undisclosed conflict of interest, disclose any material conflicts of interest, and avoid situations that imply a conflict of interest. The authors’ functions were resisted to the DVA for creation of negative responses to concerns raised by exposed veterans. No disclosure was offered or mention made of challenges raised by other toxicologists not employed by the DVA, which was not in keeping with the Code.

581h Blood Pressure, Heart Rate, Temperature, and Central Nervous System Evaluation of Cyanide Intoxication in Juvenile and Adult Mice

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Cyanide (CN) affects organ systems with the highest demand for energy such as the central nervous, cardiovascular (CV), and pulmonary systems. A mouse model of oral KCN intoxication is needed for screening and testing of CN countermeasures. Adult and juvenile CD-1 mice were dosed orally with a single dose of water or 8 mg/kg KCN, a toxic but generally non-lethal dose. Sensory, motor, cognitive, and behavioral changes were measured using a functional observation battery (FOB) and Tier II CNS testing up to 42 days post-dosing. Histopathology was conducted on select tissues and telemetry was used for CV evaluations and body temperature. KCN reduced sensorimotor responsivity or spontaneous locomotor activity (FOB and Tier II testing) in juvenile and adult mice at 30 min post-dose. Core body temperature decreased at 30 min post-challenge consistent with the general malaise observed. No FOB differences were observed on Day 2 (24 hr post-dose), but decreases in spontaneous locomotor activity and/or rectal temperature were observed on Days 7, 14, 28, and 42. Tier II tests demonstrated no significant differences between KCN-treated and control groups on Days 2, 7, 14, 28, or 42. KCN caused immediate effects related to bradycardia, hypotension, and hypothermia with recovery 1 to 3 hours post-dosing. Twenty percent of mice in the KCN group experienced ECG events within the first 5 min post-dosing that ranged from bradycardia, to left ventricular premature contractions (VPCs), and a large Ta wave. At 30-min post-dosing, ECG included PR prolongation, alternans, and Ta prolongation. The bradycardia and VPCs as well as the PR and Ta prolongation appear to be effects of KCN in a small percentage of mice, most likely due to effects on the SA node. There were no KCN related histological findings through Study Day 42. The effect of treatments on KCN-induced changes in CV and CNS parameters can be used in subsequent studies to determine the efficacy of countermeasures.

581i Younger Rats Are More Susceptible to the Lethal Effects of Sarin Than Adult Rats: 24 h LC50 for Whole-Body (60 Min) Exposure

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Chemical warfare nerve agents (CWNA) are among the most lethal chemicals known to man, and children are a particularly vulnerable subpopulation because of their smaller body masses, smaller airway diameters, higher respiratory rates and immature central nervous systems. While there have been a handful of studies on the effects of CWNA in younger animals, exposure routes relevant to battlefield or terrorist situations (inhalation for G-agents and dermal for V-agents) were not used. Thus, we determined the 24 h LC50 for whole-body (60 min) exposure to sarin using a stagewise, adaptive dose design. Briefly, both male and female Sprague-Dawley rats were exposed to a range of sarin concentrations at five different time points during their development (postnatal day [PND] 7, 14, 21, 42 and 70). For both male and female rats, the lowest LC50 value was observed on PND 14 and the highest LC50 value on PND 42. Females had slightly smaller LC50 values than males at all five time points, and the LC50 values were significantly smaller on PND 21 and 70. Thus, younger rats are more susceptible to the lethal effects of whole-body exposure to sarin than older rats, and females are more susceptible than males. The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committees at USAMRICD and ECBC, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by interagency agreement between the Biomedical Advanced Research and Development Authority and USAMRICD.

581j Natural History Study of Cyanide Intoxication in Adult and Juvenile Mice

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Cyanide (CN) is an inhibitor of cytochrome C oxidase causing rapid death due to hypoxia. CN is of concern due to its use as a warfare agent, industrially, exposure from burning plastics, and possible terrorist use. A well-characterized model of oral KCN intoxication is needed to test new therapeutics for KCN intoxication under the FDA Animal Rule. The objective was to describe CN intoxication after a single lethal and sub-lethal acute oral KCN administration to juvenile and adult mice. The oral LD50 of KCN was 11.5 ± 0.4 mg/kg KCN in adult mice and 10.4 ± 0.3 mg/kg KCN in juveniles. The oral KCN LD50 value for male mice was about 0.9 mg/kg greater than female mice, for either age. Death following KCN challenge occurred within 30 min post-dose in adult and juvenile mice at doses above 10 mg/kg. Juveniles tended to have a shorter time to death with a rapid seizure response. CN and its major metabolite, thiocyanate, were quickly metabolized (Tmax of 0.5 hr and 2-4 hr, respectively) and excreted. A survey of biomarkers indicated that blood pH and lactate were rapid and quantitative, although non-specific, markers of CN intoxication. Serum FABP3 increased with KCN dose in adult, but not juvenile, mice. No microscopic pathological findings were observed 1 day post-dose. The model description indicates that the effects of KCN are rapid and, at doses up to a least the LD50, completely reversible in surviving mice. The toxicokinetics of CN in mice is consistent with the rapid clinical signs and recovery. Based on the rapid toxicity observed, treatment would have to be initiated quickly following exposures for optimal results. Rapid analysis of blood pH and/or lactate using a point of care device, such as the l-Stat, could be used as a trigger for treatment in efficacy studies. The results provide a well characterized model of acute oral KCN intoxication of adult and juvenile mice that may be used to screen or conduct pre-clinical efficacy studies of potential countermeasures.

581k Treatment of Lung Injury from Phosgene Chemical Inhalation with NOS-2 Inhibition

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Acute lung injury (ALI) induced by the chemical agent phosgene (CCl2O) is characterized by increased lung expression of inducible nitric oxide synthase (iNOS, NO2S) and production of reactive nitrogen species including peroxynitrite, which triggers epithelial and endothelial cell injury, surfactant dysfunction, and loss of alveolar epithelial barrier integrity. Our research demonstrates that phosgene-induced lung injury also leads to significant reduction in the tight junction protein zonula occludens (ZO)-1, resulting in exaggerated alveolar epithelial permeability in vivo under acute phosgene exposure. We have previously demonstrated that NO2S expression in lung epithelial cells plays a key role in surfactant dysfunction and is critical for the development of endotoxin-induced ALI. Furthermore, we have identified that NO2S-derived NO from lung epithelial cells downregulates surfactant protein B (SP-B) expression at the transcriptional level. We therefore hypothesized that NO2S-generated NO contributes to phosgene-induced ALI and that selective inhibition of NO2S in the lung epithelium will attenuate surfactant dysfunction, disruption of tight junction proteins, and vascular leak induced by phosgene. Our research demonstrates that administration of a NO2S-specific inhibitor 1400W delivered via inhalation, but not systemically, dramatically attenuates phosgene-induced lung injury. Aerosol delivery of 1400W augmented SP-B expression and restored ZO-1 expression in the lung leading to attenuation of histologic lung injury and physiologic dysfunction in a murine phosgene ALI model. Consistent with our previous studies and work by other investigators, these findings support a role for inhibition of localized NO2S production within the lung in attenuating chemical agent-induced lung injury. We will present on this research, and consider the current proposed standard of care for phosgene induced ALI against this potential therapy in terms of feasibility and efficacy.

582 Modulation of Inflammasome Activation by Carbon Nanotubes in Asthma

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Introduction: Multi-walled carbon nanotubes (MWCNT) represent a potential health hazard for human lung disease based on studies in mice which show lung inflammation after exposure. A possible mechanism of MWCNT-induced inflam-
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ulate MWCNT-induced inflammasome activation and how MWCNT exacerbate inflammation. Further study will address the mechanism through which Th2 cytokines modulate MWCNT-induced inflammasome activation and how MWCNT exacerbate allergic airway inflammation in asthma.

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583 Atomic Layer Deposition Coating of Multiwalled Carbon Nanotubes with Aluminum Oxide Alters Innate Immune Responses In Vitro and In Vivo

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Multi-walled carbon nanotubes (MWCNTs) pose a possible human health risk for lung disease as a result of occupational or environmental exposure. Macrophages are the first line of defense that engulf and remove inhaled MWCNTs from the lungs. MWCNTs stimulate macrophage inflammasome activation and interleukin (IL)-1β release, key cellular components of the innate immune response that mediate the initiation and resolution of acute inflammation. Alternatively, macrophages are a source of secreted osteopontin (OPN), which promotes tissue matrix remodeling and fibrosis. Atomic layer deposition (ALD) is a novel process used to enhance surface and functional properties of MWCNTs. The purpose of this study was to determine whether ALD coating with Al2O3 would alter toxicity, phagocytosis, or the production of soluble mediators (IL-1β or OPN) in human macrophages or monocytes exposed to MWCNTs as well as in mice in vivo. IL-1β and OPN protein secretion was measured by ELISA at 24h post nanotube exposure. ALD surface coating of MWCNTs with Al2O3 enhanced IL-1β secretion, yet inhibited OPN production by THP-1 cells. Experiments conducted with primary peripheral blood monocytes obtained from normal human donors also showed that the ALD-coating on MWCNTs enhanced IL-1β secretion and yet suppressed OPN production. C57BL6 mice exposed to uncoated MWCNTs for 1 day had increased levels of IL-1β and OPN in the bronchoalveolar lavage fluid. ALD coated MWCNTs increased IL-1β in the BAL fluid as well, yet had a suppressive effect on OPN levels. These findings indicate that thin film surface coating of MWCNTs with Al2O3 by ALD enhances the innate immune response of mononuclear phagocytes but decreases pro-fibrogenic activity in vitro and in vivo.

584 Activation and Interplay of the Tumor Suppressor Genes p53 and STAT-1 in Response to Multiwalled Carbon Nanotubes

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Multi-walled carbon nanotubes (MWCNTs) are engineered nanomaterials that possess unique physical properties, making them ideal candidates for use in electronics, engineering and medicine. As they are used in increasing quantities, the potential for occupational and/or environmental exposures is inevitable. There is increasing evidence that MWCNTs may produce toxic effects both in cultured cells and in the lungs of rodents. Studies in human epithelial cells and rats in vivo have shown MWCNTs to cause DNA damage. The tumor suppressor gene p53 is a DNA damage inducible transcription factor involved in cell cycle arrest and apoptosis. Likewise, the transcription factor, STAT-1 serves many of the same functions as p53 and STAT-1 has been found to interact with p53 in mouse embryonic fibroblasts and human fibrosarcoma cells in vitro. We explored a possible relationship between p53 and STAT-1 in human mesothelial cells, the target cells of mesothelioma, as well as in the mouse lung in vivo, following exposure to MWCNTs. Human mesothelial cells (Met5A cells) were dosed with 10 µg/cm2 MWCNTs, and using western blot analysis, we observed that the phosphorylated or "active" forms of both p53 and STAT-1 were increased 4 hours post-exposure. To determine whether the induction in p-p53 was affected by the presence of STAT-1, we utilized both STAT-1 siRNA to knockdown STAT-1 message in the Met5A cells, as well as a STAT-1 knockout mouse model. In both models, decreased or absent STAT-1 protein, resulted in decreased p53 protein levels. Our results suggest that both p-p53 and p-STAT-1 can be induced as a result of MWCNT exposure and that the induction in p-p53 is dependent on the presence of STAT-1. The findings presented here are relevant to our understanding of the potential for carbon nanotubes to cause pleural disease after inhalation exposure.

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585 Different Functionalizations of MWCNT Influence Transformation Potential in Primary Human Lung Epithelial Cells

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MWCNT have received increased scrutiny for potential human health impacts based on their fibrogenicity and promotion of pulmonary carcinogenesis. Functionalized CNT (fCNT) development has intensified to improve surface activity in technological applications, and potentially reduce toxicity. Subchronic in vitro CNT exposure causes neoplastic-like transformation; however, tumorigenic risk associated with fMWCNT exposure in human lung epithelium is presently unknown. To identify early steps in fMWCNT-induced cell transformation, this study hypothesized that different functional groups of MWCNT determine their neoplastic transformation potential in primary human small airway epithelial cells (SAECs). Cells were continuously exposed (0.06 µg/cm²) to dispersed pure (pMWCNT), carboxylated (cMWCNT), and aminated MWCNT (nMWCNT) for 8 and 12 weeks. Dispersed ultratine carbon black (UFBCB) and crocidolite asbestos served as controls. Exposed cells were assessed for several established cancer hallmarks and morphological transformation. UFBCB and pMWCNT cells at 48h and all MWCNT cells at 6d post-treatment exhibited significant increased proliferation compared to controls. UFBCB exposure stimulated significant invasion and migration behavior while pMWCNT and cMWCNT showed moderate significant increases; however, these trends disappeared at 6d post-exposure. Conversely, nMWCNT displayed the largest significant increase in colony formation potential while UFBCB showed a moderate significant increase. All other treatments did not differ from controls. UFBCB and nMWCNT cells exhibited increased foci frequency, indicative of neoplastic transformation, compared to all other treatments. In summary, surface charge characteristics of carbon nanoparticles can impact transient, early neoplastic transformation events in vitro following occupationally relevant exposures.

Disclaimer: Present findings and conclusions are the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

586 Lactoperoxidase-Mediated Degradation of Oxidized Single-Walled Carbon Nanotubes and Its Modulatory Effects on Airway Antibacterial Activity

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Studies in recent years have shown that carbon nanotubes (CNTs) may elicit inflammatory responses. On the other hand, enzymatic biodegradation of CNTs by inflammatory cells has also been reported thus illustrating the reciprocal interactions between CNTs and the immune system. Here we analyzed the biodegradation of oxidized SWCNTs by recombinant lactoperoxidase (LPO), in the presence or absence of lung surfactant (Curosurf®). UV/Vis-NIR and Raman spectroscopy techniques were employed to monitor the biodegradation. There was effective degradation of SWCNTs by LPO and no interference by Curosurf®. AFM and
SEM analyses suggested the attachment of LPO on the carboxylated side-walls of the bundles of SWCNTs. We also confirmed the ex vivo biodegradation of SWCNTs in murine bronchoalveolar lavage (mBALF) shown to express peroxidase activity. Lastly, we addressed whether the pre-occupancy of LPO with SWCNTs would interfere with its antibacterial properties. To this end, experiments with Pseudomonas aeruginosa, an opportunistic human pathogen, were conducted and we noted that the pre-occupancy of LPO with SWCNTs severely impaired the antibacterial activity of LPO. Our study provides evidence for the degradation of carboxylated SWCNTs by LPO, a secreted peroxidase that is present in the airways. However, the antibacterial activity of LPO is impaired as a result of the pre-occupancy of LPO with SWCNTs. This latter finding implies that airborne defenses may be compromised in individuals exposed to SWCNTs, possibly leading to more persistent infections.

587 Effects of MWCNT and Nitrogen-Doped MWCNT in Lung Epithelial Cells
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The growing use of multi-walled carbon nanotubes (MWCNT) and their derivatives in academic and industrial settings has raised the need to efficiently and accurately determine their potential toxicity in the workplace. Nitrogen-doped multi-walled carbon nanotubes (ND-MWCNT) are modified MWCNT that have enhanced electrical conductivity and are increasingly used in a variety of aerospace and fuel cell applications. Although similar in structure to MWCNT, limited toxicological data is available, and the biocompatibility and mechanism of action of ND-MWCNT have yet to be elucidated. Recent in vivo data showed that ND-MWCNT induced inflammation and fibrosis in mouse lungs. In this study, we assess uptake of ND-MWCNT into small airway epithelial cells (SAEC), which may induce molecular toxicological effects. We showed that ND-MWCNT induced higher levels of reactive oxygen species (ROS) in SAEC when compared to their parent MWCNT in short-term in vitro exposure. Treatment of SAEC with low doses (1.25 µg/mL) of ND-MWCNT and MWCNT indicated that both induced a significant increase in cell proliferation in a time-dependent manner. Furthermore, significant alterations to the cell cycle were observed in cells treated with both ND-MWCNT and MWCNT in a time- and dose-dependent manner, as shown by an increased percentage of cells in the S and G2 phases of the cell cycle. Confocal images showed alterations in acetylated and total tubulin levels in both ND-MWCNT and MWCNT treated cells as well. Since increases in ROS production and cellular proliferation are associated with multiple pathological conditions, ND-MWCNT exposure may play a role in the initiation of these diseases.

588 Autophagy and Extracellular HMGB1 Are Mediators of Inflammammasome Activity in Response to MWCNT Exposure
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RATIONALE: Pulmonary exposure to multi-walled Carbon Nanotubes (MWCNT) has been shown to induce chronic inflammation in animal models, and therefore may pose a health risk to humans. Pathology induced by MWCNT exposure is dependent upon NLRP3 Inflammasome activity, specifically IL-1β signaling. Mechanisms regulating Inflammasome activity are not clear. In this study, we establish critical roles for High Mobility Group Box 1 (HMGB1) and autophagy in regulating Inflammasome activity. METHODS: Secretion of HMGB1 was assessed in vitro from primary Alveolar Macrophages (AM) and in vivo in C57Bl/6 mice (24 and 72 hours) after MWCNT exposure. The contribution of extracellular HMGB1 to Inflammasome activity was delineated in vitro via HMGB1 elimination by immunoprecipitation techniques and in vivo using neutralizing antibodies. To assess the contribution of autophagy in mediating inflammasome activity, primary AM exposed to MWCNT were treated with molecular inhibitors (LY294002, Bafilomycin, Imipramine) targeting different sites along the autophagic flux, and IL-1β production assessed after 24 hours. RESULTS: HMGB1 secretion and autophagic flux are increased following MWCNT exposure. HMGB1 removal by immunoprecipitation techniques or neutralization reduces IL-1β production in primary AM in vitro and in vivo, respectively. Additionally, targeting autophagy by stabilizing the lysosome also decreases IL-1β production after MWCNT exposure. However, inhibition of PI3 kinase activity, responsible for autophagosome formation, enhances IL-1β secretion. CONCLUSIONS: HMGB1 signaling and autophagy play critical roles in the acute Inflammasome response to MWCNT exposure, and may be potential therapeutic targets in particle induced inflammatory diseases.

589 Effects of SWCNT Fiber Length and Functionalization on ROS and Collagen Production
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Single-walled carbon nanotubes (SWCNTs) present a significantly higher chance of occupational and environmental exposure owing to their widespread use in technology and commercial products. Currently, there is a lack of consensus on the various CNT characteristics that induce pulmonary toxicity. Therefore, our aim was to study the effect of SWCNT length and chemical functionalization on ROS production and collagen synthesis in human lung fibroblasts. SWCNTs with varying functionalization including carboxylic acid, amine and hydroxyl, and varying lengths, i.e. long 5-30 nm and short 1-2 nm, obtained from Cheap Tubes Inc. were used. SWCNT dimension and elemental composition characterization was conducted using atomic force microscopy and energy dispersive X-ray spectroscopy respectively. SWCNT toxicity in normal human lung fibroblast were first determined using WST-1 assay and sub-toxic concentrations of SWCNTs were selected for further experiments. Cellular ROS production was determined fluorometrically using DCF-DA as fluorescent probes for peroxide measurement. Type I Collagen expression was determined by western blotting in the presence and absence of ROS inhibitors. Cellular collagen content was determined by SiriusRed® assay. Our results showed that: 1) Pristine > COOH > NH2 > OH SWCNT in ROS and collagen production in a dose- and time-dependent manner; 2) longer length SWCNTs induced higher ROS generation and collagen I expression than those of short length; and 3) SWCNT-induced collagen expression was significantly blocked by various ROS inhibitors revealing a role for oxidative stress in SWCNT-induced fibrogenesis. These results suggest that SWCNT length and functionalization impact lung fibroblast ROS and collagen synthesis, which contribute to SWCNT-induced pulmonary fibrosis. Disclaimer: Research findings and conclusions are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

590 Differential Gene Expression in SAEC and HMVEC Grown in Monoculture or Coculture and Exposed to MWCNT: Correlation with In Vivo Studies
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In vitro coculture systems are at the forefront of molecular research due to their increased ability for cell-cell communication. In this study, small airway epithelial cells (SAEC) and human microvascular endothelial cells (HMVEC) were grown separately in monocyte or together in an alveolar-capillary coculture and exposed to either dispersion media control or multi-walled carbon nanotubes (MWCNT) for 6 or 24 h. Global mRNA profiling determined genes that were differentially expressed in coculture as compared to monoculture, and Ingenuity Pathway Analysis determined the biological functions and related pathways of these genes. A total of 1505 SAEC and 54 HMVEC genes, commonly involved in the cell cycle and cell proliferation, were differentially expressed in control coculture experiments as compared to monoculture (SAM 1%, FC >1.5). A total of 1601 SAEC and 2016 HMVEC genes, commonly involved in cell movement and survival, were differentially expressed in coculture as compared to monoculture following MWCNT exposure (SAM 1%, FC >1.5). A correlation study of gene expression between monocyte, coculture, and in vivo gene expression from mice lungs exposed to MWCNT determined that coculture gene expression had a better correlation with in vivo gene expression than monoculture. In this study, we determined that gene expression in cells from coculture models is different from expression in the corresponding cells in monoculture. As coculture gene expression better correlates with gene expression seen in vivo, we hypothesize that coculture may offer an enhanced in vitro model for nanoparticle risk assessment.
Determination of Stoichiometric ROS Degeneration and Relationship between Redox Potential and Bioavailability to Design Safe CNTs

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An important goal is to design safe carbon nanotubes (CNTs) by controlling their properties. Biological evaluations have been conducted with individual CNTs, and such results have been used for risk assessment (NIOSH CIB 65, 2013). However, information is lacking to define the relationships between the chemical properties of CNTs and their bioavailability. The present work aimed to obtain a stoichiometric expression of ROS degeneration by CNTs, which predicted ROS scavenging results with CNTs. ROS degeneration rate was measured with an ESR-DMPO method. The Fenton reaction was used to generate OH radicals. To evaluate CNT surface reactions, surfactant concentration was minimized to eliminate its influence, and reactivity of CNTs was determined. Results indicate that this allows kinetic quantification of the relationship between degeneration reactions and CNT morphology. Since the degeneration of ROS is attributed to a redox reaction according to the hypothesis in reaction kinetics on CNT surface, CNT bioactivity is defined as a redox potential that can be used to elucidate the relationship between bioactivity and CNT physicochemical properties. In conclusion, redox potential will be used to predict CNT bioactivity using its surface morphology and to design safer CNTs. (This work was supported by the Exotic Nanotube Project, Japan Regional Innovation Strategy Program by the Excellence, JST(Japan Science and Technology Agency)(ST, KK, YU, ME)).

Determination of Stoichiometric ROS Degeneration and Relationship between Redox Potential and Bioavailability to Design Safe CNTs

Acute Inhalation Toxicity of Graphene Oxide and 5-Day Repeated Inhalation Toxicity of Graphene

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Hazard of graphene exposure to human health, however, has not been known well. Acute pulmonary toxic effect of graphene oxide was evaluated via nose-only inhalation for 6 hours to experimental animals. Male Sprague-Dawley rats were divided into 3 groups (4 rats in each group), the fresh air control (0 mg/m3), low (0.46 ± 0.06 mg/m3), and high (3.76 ± 0.24 mg/m3) concentration group, respectively. Lactate dehydrogenase (LDH), microprotein (mP), and microalbumin (mALB) levels were evaluated from the bronchoalveolar lavage (BAL) fluid on day 1, 7, and 14 after 6 hours exposure to graphene oxide. In addition, 5-day repeated inhalation toxicity of graphene was conducted to rats by assigning 3 groups (20 rats in each group); the control (0 mg/m3), low dose (0.68 ± 0.14 mg/m3) and high dose (3.86 ± 0.94 mg/m3). The rats were exposed to graphene for 6 hr/day for 5 day. The exposed rats were allowed to recover for 14 to 28 days to evaluate the biopersistent effect of graphene oxide and graphene on the lungs after the acute and 5-day repeated exposure. The ingestion of graphene oxide and graphene in the alveolar macrophages was also evaluated. There was no statistical significant difference in average concentrations of LDH, mP, and mALB in the exposed groups when comparing with the unexposed control in all post-exposure groups (1, 7, and 14 days). The ingestion of graphene oxide in the alveolar macrophages was observed in all post exposure groups of the high dose group, but it was difficult to observe the graphene oxide in the control and low concentration group. Graphene oxide did not induce acute pulmonary toxic effect in the lungs of experimental animals. Graphene also did not induce any significant body weight, organ weight and lung weight changes after 5 day exposure and during 28 days of recovery.

Development of Determination Method of Single-Walled Carbon Nanotubes with Distinct Chiral Enrichment

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The rapid expansion of nanomaterial use in industrial, consumer and medical sectors has raised concern regarding associated health effects from unintended exposure. There is exceptional interest in single-walled carbon nanotubes (SWNT), as they are used in many common applications. As SWNT share a resemblance to asbestos, there is concern regarding inhalation exposure and associated lung diseases. Although consequences of SWNT exposure in the lung include pulmonary injury and fibrosis, few studies have focused on the ability of these particles to modulate infections by pathogenic agents. Recognition of invading pathogens occurs through interaction with toll-like receptors (TLRs) that stimulate the immune system through activation of nuclear factor kappa beta (NF-κB). Based on this knowledge, we hypothesized that SWNT interacted with TLR2 or 3 activities. Overall, these data suggest that chiral, aggregate size and stability of SWNTs may influence the activity of TLRs and have implications for interferring with normal pathogen defense systems.
Correlation of Toxicity and Material Properties: Oral and Inhalation Exposure of 16 Surface-Functionalized Nanomaterials

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16 nanomaterials were tested by short-term inhalation studies (R. Landsiedel and al. Advanced Materials 22.24.10: 2651–2667) and oral gavage (28-day OECD TG407). The results were correlated to the complete physical-chemical properties (according to the REACH R.7.1 nano-specific guidance) and to the in-situ corona structures (in lung surfactant, pure lipids, serum-containing media) The surface of suspended nanoparticles with sizes of 10 nm (ZnO2), 15 nm (SiO2) and 50 nm or 200 nm (Ag) received acid, amino, -PEG, and -steric functionalities. The organic molecules were bound to the surface of the particles to improve properties such as processability, solubility or stability of the products. All results were benchmarked against OECD reference materials (TiO2, ZnO, BaSO4). None of the materials elicited an adverse effect in oral testing (see separate poster). For inhalation at up to 50 mg/m3, inflammations of the lung were observed with most of the nanomaterials. In general, functionalizations reduced the acute inflammatory response, especially for negative charges and for sterically stabilized materials. The correlation of inflammatory potency (e.g. monitored by PMN) to in-situ corona parameters is vanishing. A weak correlation was found between the particle’s affinity to phospholipids and the deposition rate, but not further correlated to clearance. In contrast, correlations are strong with the chemical composition of the core particle, suggesting grouping and prioritization for in-vivo testing by surface charge and by core substances. The project NanoGEM (Nanostructured materials – Health, Exposure and Material Properties, 2010 – 2013) involved 19 research institutions and companies, supported by the Federal Ministry of Education and Research (BMBF).

Determination of Nanomaterial Single Nanoparticle and Nanoparticle Aggregation in Complex Biological Environments

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The plasmonic nanomaterials are attractive for a variety of biomedical applications such as drug delivery and bio-imaging agents. Many of these nanomaterials were introduce to human or animals through intravenous injection and work most effectively following cellular uptake. However, aggregation/agglomeration of nanoparticles will affect their biological behavior, distribution and toxicity. Rapid 'in situ' characterizations are urgently needed to monitor the physical nature of the nanoparticles as they were introduced into blood and intracellular compartment representing extremely complex environments. We proposed that the confocal Raman microscopy (cRM) and hyperspectral dark field microscopy (hpDFM) are able to discriminate the status of the gold nanoparticles (AuNPs) and aggregates in biological fluid blood and cultured macrophage. Citric acid coated Au nanoparticle (50 nm) and nanoparticle aggregates were characterized using transmission electron microscopy (TEM), Zetaizer, Nanoparticle Track Analysis (NTA), UV/Vis spectroscopy and induced-coupled Plasma microsopy (ICP-MS). A series of complementary methods are used to characterize the single Au NPs and status of particle aggregates following incubation in rat blood and cultured macrophage. hpDFM was demonstrated to monitor Au NPs and aggregates in blood in situ, and the spectra were shifted from 540 nm with single nanoparticles to 600-700 nm for the nanoparticle aggregates. In contrast, Au NPs aggregates were detected in the cytosol of macrophages after exposure to Au nanoparticles. Additionally, cRM was demonstrated as complementary to detect Au NPs aggregation state. This detection method was further confirmed by single particle ICP-MS analysis. The study suggested that the hpDFM imaging system is likely robust and can be used to assess physiological status of new plasmonic material in biological system.

CCEF-LRI N1 Project: Oral Toxicity of a Synthetic Amorphous Silica (SAS) in Rats


The test item was a synthetic amorphous silica (SAS) coded NM-200 and available at the EU JRC nanomaterials repository. It is a nanostructured, precipitated SAS. As NM-200 is used as an ingredient in food it was the objective of this study to...
evaluate oral toxicity. The toxicity of NM-200 after daily oral gavage in male rats for 28 days with an additional 14-day recovery period (OECD TG 407; in addition: Si ion and Si particle analysis in selected organs) was tested. Dose levels of 100, 300 and 1000 mg/kg bw (vehicle: 0.5% methylhydroxypropylcellulose) were used. Each group (control, recovery high dose recovery) was kept for the 14 days of post-observation. Body weights, food consumption, the functional observation battery (FOB) and hematology and clinical chemistry did not reveal any treatment-related effects. Also organ weights, necropsy, and histopathological examination did not show any substance-related findings in the high dose group, as compared to controls. Chemical analysis of silicon ions in blood, kidney and liver tissues did not reveal differences between the control and the high dose group. As a result, NM-200 did not cause any substance-related effects at doses up to 1000 mg/kg bw. After oral exposure for 28 days in male Wistar (WU) rats. Therefore, the highest dose tested (1000 mg/kg bw.) was determined to represent the NOAEL in this study. By exemplary TEM analysis no particles of NM-200 could be detected in selected tissues.

602 Modulation of Macrophage Phenotype and Function by Engineered Nanoparticles

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Epidemiological studies have established a correlation between exposure to ambient air pollution particulates and the incidence of pneumonia and lung infections. Using an in vitro assay of macrophage phagocytic function, we investigated the effects of an array of 18 different ENP types on the ability to macrophages to recognize and phagocytize S. pneumoniae. We identified three major groups of ENPs based on their effect on macrophage function. ENPs which most potently inhibited macrophage phagocytosis included metals and metal oxides which are expected to undergo significant dissolution and have direct cytokotoxic effects. An additional group of materials, including iron oxide and MWCNTs, were found to cause dose-dependent inhibition of phagocytic function, yet had no direct cytokotoxic or proinflammatory effects of their own. The third group of benign ENPs had no effect on direct cytokotoxicity or phagocytic capacity. Oropharyngeal instillation of superparamagnetic iron oxide (SPIO) an ENP from group 2 in C57BL/6 mice confirmed the in vitro results, demonstrating SPIO exposure decreased S. pneumoniae lung clearance in vivo. Macrophage transcriptional response to two ENPs silica and SPIO from class 2 and class 3 before and after bacterial lipopolysaccharide (LPS) challenge showed that while SPIO treatment modulated nearby 500 genes, silica pretreatment regulated only 67 genes. Macrophages exposed to SPIO displayed a phenotype suggesting an impaired ability to transition from an M1 to M2-like activation state, characterized by suppressed IL-10 induction, enhanced TNFα production, and diminished expression of complement and phagocytic receptors. Our results illustrate adverse biological consequences of nanomaterials may not always be directly mediated, but may be manifested by altering susceptibility to other common environmental exposures. Supported by NIEHS Grant U19-ES019544

603 Amphiphilic Polymer-Coated CdSe/ZnS Quantum Dot-Induced Hemolysis and Glutathione Depletion Are Enhanced in Gclm Null Mouse Erythrocytes

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Quantum dot (QD) nanoparticles have unique fluorescent properties, making them ideal for biomedical imaging. However, QDs contain heavy metals such as cadmium (Cd) and selenium (Se) and have been demonstrated to generate reactive oxygen intermediates, elevate oxidative stress biomarkers such as heme oxygenase-1 (Hmox1) and to cause pulmonary inflammation in vivo. Glutamate cysteine ligase (GCL), the rate-limiting enzyme in glutathione (GSH) synthesis, is important for antioxidant defense. Absence of GCLM (GCL modifier subunit) results in greatly diminished GSH. GCLM polymorphisms exist in humans some of which have been linked to increased risk of cardiovascular disease. We tested the effects of QDs on cultured erythrocytes obtained from Gclm wild-type (+/+) and heterozygous (+/-) and null (-/-) mice. Gclm+/+ and Gclm+/- erythrocytes had diminished GSH as compared to Gclm++/+ erythrocytes (51% and 6%, respectively). Erythrocytes from Gclm++/+ and Gclm+/+ mice greatly resisted QD-induced hemolysis until 48 hours at which point a mild dose-response increase in hemolysis was observed. However, Gclm--/- erythrocytes experienced increased hemolysis as early as 12 hours with all QD treatments yielding equally high levels of hemolysis at 48 hours. Interestingly, after 24 hours of exposure to 40 nM QDs, erythrocytes from Gclm++/+ and Gclm+/+ mice (but not Gclm--/- mice) had higher total GSH levels. This may be due to enhanced formation of GCL holoenzyme in these erythrocytes, thus increasing GSH synthesis. These findings suggest that QDs are capable of producing oxidative stress-mediated hemolysis and raise concerns regarding the effects of prolonged exposure to QDs or other engineered nanomaterials that cause oxidative stress, especially in individuals having genetic or epigenetic modifications to DNA resulting in low expression of GCLM. Supported by NIH Grants U19ES01945, P30ES007053, T32ES007052 and UW DEOHS.
Dermal exposure to metals may result in irritant contact dermatitis. The objective of this study was to examine the in vitro potential of metal-containing nanoparticles to elicit irritant dermatitis in human-derived epidermal keratinocytes (HDEK). Dermal exposure to metals may result in irritant contact dermatitis. The objective of this study was to examine the in vitro potential of metal-containing nanoparticles to elicit irritant dermatitis in human-derived epidermal keratinocytes (HDEK). The cells examined were non-malignant, human epidermal keratinocytes (HDEK) derived from fetal foreskins. The HDEK were cultured as monolayer cultures in 24-well plates. The HDEK were incubated with metal nanoparticles at concentrations ranging from 100 to 1000 μg/mL for 24 hours. The HDEK were then examined for cytotoxicity using a number of different assays, including the MTT assay, the LDH assay, and the Annexin V/7-AAD assay. The MTT assay was used to measure cell viability, the LDH assay was used to measure cytotoxicity, and the Annexin V/7-AAD assay was used to measure apoptosis. The results of these assays were then compared to the results obtained from the control groups, which were cultured in media alone. The results of these assays showed that the metal nanoparticles had a significant effect on cell viability and cytotoxicity. The MTT assay showed that the HDEK treated with metal nanoparticles had significantly lower cell viability compared to the control groups. The LDH assay showed that the HDEK treated with metal nanoparticles had significantly higher cytotoxicity compared to the control groups. The Annexin V/7-AAD assay showed that the HDEK treated with metal nanoparticles had a significantly higher percentage of apoptotic cells compared to the control groups. These results suggest that metal nanoparticles have the potential to elicit irritant contact dermatitis in human skin.
The aim of the study was to assess potential fibrogenic risk upon real occupational exposure to multi-walled carbon nanotubes (MWCNT) using biomarkers tested in preceding animal experiment. The study was conducted at 2 MWCNT-producing enterprises with the same reactor type. 11 workers who had more than 1 year contact with MWCNT aerosol composed the exposure group, the control group consisted of 14 people. Elemental carbon was evaluated in air samples and the CNT presence was confirmed by TEM-analysis. Blood and induced sputum samples were obtained from workers, TGF-β1, KL-6 and osteopontin levels evaluated. To assess the relationship between MWCNT exposure and biomarker levels (age, gender, smoking have been chosen as cofounders) general linear models including main effects and interactions in-pairs were created. The regression coefficients confidence intervals were refined by bootstrap analysis. TWA respirable MWCNT fraction was up to 6.11 mg/m³. TEM has shown the presence of MWCNT agglomerates sized 0.5-10 μm in all air samples. It was found that exposure to MWCNT aerosol at workplaces may alter the fibrosis biomarkers in blood serum and induced sputum. The levels of TGF-β1 in serum were dependent on exposure to MWCNTs (β=10.5, 95%BCa=1.2-51.8), the KL-6 levels in induced sputum were significantly higher in exposure group (β=235.9, 95%BCa=21.2-482). Osteopontin proved to be as an uninformative indicator. Data suggest that MWCNT exposure may lead to the changes in serum and induced sputum samples specific fibrogenic biomarkers content in workers. MWCNT-producing companies have to introduce control measures, as well as provide the adequate medical services organization.
In this project, the synthesis, characterization and antimicrobial activity of calcium alginate nanocapsules containing active ingredients of Matricaria chamomilla and Catharanthus roseus were evaluated. Plants were collected from Puebla, Mexico, and stem, flowers and leaves were used to obtain hexane, chloroform and methanol extracts of both plants, and extracts were dissolved in glycerol and/or water. Synthesis of calcium alginate nanocapsules was developed by microemulsion method, employing the dissolved extracts during the process. The nanocapsules obtained were characterized by Low Voltage Electron Microscopy (LVEM) at 45 kV, Dynamic Light Scattering (DLS) and DRIZZLE method. Antimicrobial activity of extracts and nanocapsules was evaluated employing antibiograms containing 10 μL of suspensions containing 10, 1, 0.1 and 0.01 mg/mL of extracts, upon Staphylococcus aureus and Escherichia coli as Gram positive and Gram negative models, growth in Mueller-Hinton agar and adjusting the inoculum with the standard 0.5 McFarland. Antimicrobial activity of capsules was evaluated employing the same protocol, however their quantity was determined by DLS. Results obtained by LVEM showed that nanocapsules size range was between 35-100 nm, with morphology previously reported for soft spheres (1). DLS demonstrated a hydrodynamic ratio between 13-100 nm, and large aggregates on 1 nm. DRIZZLE analysis showed that aggregates were conformed by nanocapsules in a size range of 50-100 nm. Antimicrobial activity showed that nanocapsules containing chloroform extracts of flower and leaves in M. chamomilla, and nanocapsules containing hexane extracts of C. roseus inhibited the bacterial growth.

(1) J.P. Blitz and V.M. Gun’ko (Eds.), Surface Chemistry in Biomedical and Cancer Research, Frederick, MD.
605 Estimating an Acceptable Daily Intake of Anthraquinone (AQ) in Paper-Packaged Food

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AQ is used in the pulping process in making paper and may be present in finished paper products used for food packaging. IARC recently concluded that AQ was carcinogenic based on NTP chronic dietary AQ bioassay results for B6C3F1 mice and F344/N rats, possibly with a genotoxic mode of action (MOA). This conclusion raised questions about AQ in food packaging. Exponent performed a margin of exposure (MOE) evaluation for AQ to estimate derived maximum exposure levels (DMELs) assuming that AQ is a genotoxic carcinogen, and alternatively assuming a scientifically supported nongenotoxic MOA. AQ is not genotoxic as questionable because: 1) tests directly comparing purified vs. commercial sources of AQ found genotoxicity was induced only with AQ containing detectable levels of mutagenic contaminants; 2) NCI found no evidence that dietary AQ exposure of male or female B6C3F1 or B6AKF1 mice increased tumor incidence, and the NCI vs. NTP data for B6C3F1 mice are significantly inconsistent; 3) hydroxy-AQ derivatives have long been hypothesized and 1-hydroxy-AQ is known to promote tumorigenesis by PGE2-mediated target-tissue inflammation (a nonlinear MOA). In view of the contradictory NCI and NTP results for mice, a reference point of 14 mg/kg/day was identified for increased tumor risk based on benchmark dose analysis of NTP rat tumor data. Applying EU guidelines for MOE assessments, and assuming AQ is a genotoxic carcinogen with an MOE of 10,000, yields a DMEL of 98 ug/day. Assuming instead, based on the overall weight of evidence indicating that contaminant-free AQ and its in vivo metabolites are probably nongenotoxic at relevant doses, a DMEL value of 9,800 ug/day is derived using an effective MOE value of 100, the safety factor typically applied for a classical toxicity endpoint. More conservatively under this interpretation, a DMEL value of 980 ug/kg/day was derived by applying an effective MOE value of 1,000 to address uncertainty concerning any form of genotoxicity that might dominate an otherwise predominant inflammation-mediated MOA expected for AQ and its metabolites.

606 Updated Dose-Response Assessment and Derivation of Acceptable Drinking Water Levels for O-Phenylphenol


The general population may be orally exposed to O-phenylphenol (OPP) or its water-soluble sodium salt (SOPP) through industrial applications that result in food and drinking water contact. Both an oral RfD and a cancer slope factor have previously been derived for OPP-induced bladder tumors in male rats, the most sensitive tested species and sex. Lacking from these analyses is a dose-response assessment for bladder hyperplaasia, a precursor key event, for consideration as a point-of-departure for a threshold mode of action. In contrast to previous assessments, the key study in the present evaluation was considered the Nho et al. (2002) chronic oral study with SOPP in F344 rats, since it included additional and lower dose levels than other studies in F344 or SD rats that examined bladder lesions, thus potentially increasing confidence in quantitative dose-response modeling. Use of data for the more potent SOPP is supported by limited data suggesting SD rats may be more susceptible to bladder toxicity than F344 rats, the only strain tested chronically. The relationship of the necessary non-cancer precursor key events (cytotoxicity and regenerative hyperplasia) and the lack of DNA-OPP adducts in target tissue in vivo support the most plausible mode of action for OPP or SOPP being the ability of the reactive metabolite, phenylbenzoxquinone, to react with the urothelium, possibly forming urothelial protein adducts if detoxification pathways become saturated. Benchmark dose modeling suggests only a marginal (1.5x) increased potency of SOPP compared to OPP in F344 rats when high doses associated with sodium ion-induced bladder calculi are excluded. The oral RfD for OPP of 1 mg/kg/day based on a human equivalent benchmark dose (BMDL10) of 33 mg/kg-day for bladder hyperplasia corresponds to a total allowable concentration of 7 mg/L in drinking water. A short-term exposure level (STEL) of 20 mg/L was derived based on maternal toxicity in rats, since the weight-of-evidence does not indicate any specific effects on reproduction or development.

607 Mode-of-Action Evaluation and Oral Risk Assessment of Hydroquinone


The general population may be exposed to hydroquinone through dietary sources, pharmaceuticals, drinking water, cigarette smoke and metabolism of benzene and phenol. Exposure to hydroquinone may also occur by ingestion of arbutin (hydroquinone-1-O-β-D-glucopyranoside), a naturally-occurring conjugate of hydroquinone present in plant-derived foods. A recent oral risk level incorporating current risk assessment methodology and new data was not identified for hydroquinone and thus was the purpose of the present assessment. Due to insufficient quantitative data in humans, hazard identification relies on laboratory animal data. In chronic bioassays, hydroquinone induced atypical tubule hyperplasia and benign neoplasms in the kidneys of male F344/N rats dosed orally either by gavage or diet, and in kidneys of male B6C3F1 mice dosed via the diet. The renal lesions in male F344/N rats occurred only in areas of the kidney with severe or end-stage chronic progressive nephropathy. In female F344/N rats, a dose-related increase in mononuclear cell cellularity was observed; however, the incidences in the exposure groups were within the historical control range. Findings of hepatic adenomas in mice were not consistent between two chronic bioassays; hydroquinone exposure was associated with hepatocellular adenomas in female B6C3F1 mice in one study and in male B6C3F1 mice in another study. No increases in hepatocellular carcinomas were observed in either chronic bioassay. The combined incidence of renal tubule adenoma and atypical tubule hyperplasia observed in the male F344/N rats was selected as the critical effect with a resulting Human Equivalent BMDL10 of 3.1 mg/kg-day. The evidence for a prooxidant, cytotoxic mode of action potentiated in male F344 rats by chronic progressive nephropathy supports a threshold. Recognizing the F344 rat as being a multiple-site and species carcinogen, a hydroquinone nephrotoxicity relative to other animal models, an interspecies uncertainty factor (UF) was not applied. Using a 10x UF to account for possible human variability, an oral RfD of 0.3 mg/kg-day was determined for hydroquinone.

608 Derivation of Acceptable Drinking Water Levels for N-Nitrosomorpholine That Accommodate Susceptibility during Early Life Stages


N-Nitrosomorpholine (NMOR) is a drinking water contaminant that may be present in source water and as a byproduct of disinfection. Successful methods of remediation have not been identified. The general population can also be exposed to NMOR through tobacco, air, food and consumer and industrial products. The purpose of this assessment was to establish acceptable lifetime drinking water levels for NMOR that account for potential increased susceptibility during early life stages (i.e. birth to 16 years of age). No epidemiology studies that specifically evaluated NMOR were identified, but rubber industry exposure to elevated combined NMOR/N-nitrosodimethylamine concentrations in the workplace was associated with increased relative risks for cancer of the esophagus, oral cavity, and pharynx. In laboratory animals NMOR is a multiple-site and species carcinogen. Target tissues following oral exposure include the liver, esophagus, nasal cavity, lung, and kidneys. Hepatocellular tumors were seen in both sexes of NMRI mice, rats of several strains, and Syrian golden hamsters. Dose-response assessment identified the liver in rats as the most sensitive target tissue and species. The weight-of-evidence for a mutagenic mode of action included mutagenicity, clastogenicity, and oxidative DNA damage in vitro, in vivo evidence for genotoxicity in the liver included the induction of micronuclei, DNA adducts, and unscheduled DNA synthesis. A 103 cancer risk level was estimated from the human equivalent benchmark dose (BMDL10) of 250 μg/kg-day based on the incidence of hepatocellular carcinomas in female F344 rats from a chronic drinking water study in which the exposure commenced at eight weeks of age. After adjusting for potential increased susceptibility by post-natal life stage, the drinking water unit risk was 2.9 x 10-5/L-1 corresponding to a total allowable concentration for NMOR of 40 ng/L in drinking water. Contemporary methods permit quantification of nitrosamine in water at less than part-per-trillion levels.

SOT 2014 ANNUAL MEETING 162
Arsenic, boron, nickel, and vanadium are four elements for which data suggest beneficial roles in physiological processes in some species; however, the data are incomplete in humans and nutritional levels such as Recommended Dietary Allowances (RDAs) have not been set for any of these elements. Key information for these elements has been evaluated, including their potential essentiality and toxicity, current areas of research, and the basis for the following human health benchmarks: Institute of Medicine’s Tolerable Upper Intake Level; EPA’s Reference Dose, Maximum Contaminant Level, or lifetime Health Advisory; Agency for Toxic Substances and Disease Registry’s Minimal Risk Level; California EPA’s Public Health Goal; International Programme for Chemical Safety’s Tolerable Daily or Weekly Intake; and other benchmarks developed by States or private organizations. An analysis of the human health benchmarks shows that the range of human health benchmarks for boron and vanadium are within a 3-fold difference between the highest and lowest benchmarks for boron and within a 6-fold difference for vanadium, while there is a large range between the human health benchmarks for arsenic and nickel (10,000-fold difference for arsenic and 254-fold for nickel). A comparison between the human health benchmarks and the average adult intake of the elements in the U.S. revealed that for boron, vanadium, and nickel, all of the benchmarks exceed the mean adult intake by 4- to 150-fold, while for arsenic, the mean adult intake exceeds two of the human health benchmarks by 100- to 1000-fold. An analysis of the reasons for the large spread between the human health benchmarks for arsenic is also presented in this work.

The Texas Commission on Environmental Quality (TCEQ) has developed a conservative 24-hour AMCV based on Glaser et al. (1990) study in comparison studies available in the CrVI toxicological database. Consequently, the TCEQ has only evaluated 24-hour data in terms of comparability of the resulting annual averages to chemical-specific chronic Air Monitoring Comparison Values (AMCVs) to evaluate the potential for chronic health and/or neurological effects in workers. Overall, through integration of epidemiology, monkey, and toxicokinetic data, our preliminary WoE analysis suggests that predicted brain concentrations in welders at Mn exposure concentrations of 100-200 μg/m³ are not likely to lead to more than a very small (10%) increased risk of slight to mild (subclinical) neuromotor effects; therefore, a Mn occupational exposure level of 100-200 μg/m³ is protective for welding workers. Our results are in contrast to the recently adopted American Conference of Industrial Hygienists (ACGIH) 8-hour time-weighted average threshold limit value (TLV-TWA) for respirable particulate Mn of 20 μg/m³. A more statistically robust approach for derivation of the TLV (i.e., benchmark dose) would be more consistent with the current standard of risk assessment practice, and would result in a TLV approximately 5- to 10-fold higher.

The European Chemical Agency (ECHA) previously released manufacturer/importer REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) chemical registration files, making available the Derived No Effect Levels (DNELs) for hundreds of substances. The DNEL is defined as “the level of exposure above which humans should not be exposed” and needs to reflect the likely route(s), duration and frequency of exposure. Specifically, worker DNELs are levels intended to protect workers during manufacture. Typically, DNELs are developed by industry using ECHA’s guidance; the registrant has the final decision on the selection of the key studies, endpoints of concern, and the assessment factors to account for sources of uncertainty. By comparison, currently accepted occupational exposure limits (OELs), such as permissible exposure limits (PELs) or threshold limit values (TLVs) developed by the Occupational Safety and Health Administration (OSHA) and the American Conference of Industrial Hygienists (ACGIH), respectively, may have been derived using different methods, some of which incorporate economic and technical feasibility concerns and/or health-based assessment. In addition, these other occupational values may have been based on different toxicological data and endpoints. Because the worker DNELs may potentially be used as guideline levels for worker exposures in Europe and have the potential to become de facto OELs in other regions due to the global extent of REACH, a comparison analysis between the long-term inhalation DNELs for the worker population and OELs developed within Europe and by OSHA and ACGIH was completed for eighteen common inorganic and insoluble metals (in powder form). This analysis showed that in most cases the long-term inhalation worker DNELs for metals that were used for comparison were equivalent to or lower than the accepted OELs.

Although there are thousands of chemicals in use in the US, there are only several hundred Occupational Exposure Limits (OELs) for these chemicals. Many chemicals have sufficient toxicological data to generate OELs. However, there is still a limited pool of experts to evaluate the data and create a consensus-based OEL. One such mechanism is the approach used by the Workplace Environmental Exposure Level (WEELOEL committee under the Occupational Alliance for Risk Science (OARS), a collaborative initiative managed by the not-for-profit Toxicology Excellence for Risk Assessment (TERA). The goal of this arrangement is to promote worker health through increased access to high quality OELs, enhancement in methods for establishing worker-health exposure guidelines, and education and training in occupational risk assessment methods. The OARS-WEELOEL Committee is...
a volunteer group of toxicologists and industrial hygienists from academic, governmental, corporate, and consulting backgrounds, who create health-based guideline values for chemical agents, called WEELs. WEELs are limits that represent “safe” or “relatively safe” air concentrations that will protect most workers from adverse health effects related to occupational chemical exposures over a lifetime. They can be used across industries as a central tool for worker health protection. OARS promotes the development of WEELs by encouraging stakeholder engagement. This is achieved through open and transparent processes, invitation of stakeholders to attend science deliberations, free online access to WEELs and associated documentation, and active outreach, while using a scientifically-based methodological approach to evaluate the toxicological data including the application of appropriate safety & uncertainty factors. The WEEL development process will be explained using a selected WEEL as an example.

614 Derivation of a Human Equivalent Concentration for Diacetyl for Hyperplasia of the Bronchiolar Epithelium

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Severe respiratory disorders, including bronchiolitis obliterans (BO), have been alleged in some cases in which workers and consumers were exposed to diacetyl in microwave popcorn and other products. Recently, results have become available from an unpublished study conducted by the National Toxicology Program in which rodents were exposed to diacetyl for 6 hours per day, 5 days per week for 13 to 14 weeks. Using these data, we performed a BMC analysis to estimate the diacetyl concentration associated with a 10% excess risk (BMCL10) of hyperplasia of the bronchiolar epithelium and a 95% lower confidence bound concentration estimate (BMCL5%) of the endpoint of interest in the upper airways (minimal to mild peribronchial lymphocytic inflammation). Our range of HECs is based on a sensitive endpoint and new-toxicity factors. The estimated BMCL10 values were adjusted to human equivalent concentrations (HECs) using the following procedure: 1) the computed BMCL10 were converted to an equivalent exposure of 8 hours per day; 2) a scrubbing factor was applied using the computational fluid dynamics model developed for diacetyl for rodents and humans; and 3) a regional gas dose ratio (RGRD) was calculated and applied using EPA methods. Based on our analysis, the BMCL10 ranged from 39.7 ppm to 92.9 ppm, and the BMCL5% ranged from 31.9 ppm to 60.4 ppm over all models; the HECs were estimated to range from 15.9 ppm to 30.1 ppm. The results of our analysis were consistent with those reported by Maier et al. (2010), which calculated an HEC of 1.8 ppm for an endpoint of interest in the upper airways (minimal to mild peribronchial lymphocytic inflammation). The developmental effects of NMP are well studied in rats following oral, inhalation, and dermal exposure routes. A hazard identification and dose-response assessment was conducted. Based on consideration of the dose-response range covered, on the inclusion of late-gestational exposures, and on consistency with current test guidelines, the oral and inhalation studies were considered to be more robust than the dermal study. Fetal body weight changes in rats are considered to be the most sensitive endpoint for NMP. However, this effect appears to be reversible, and because it reflects a cumulative response, is not expected to result from acute (1-day) exposures. Because other developmental endpoints (e.g., delayed ossification) were highly variable and occurred only at higher internal doses, these effects were considered secondary to fetal body weight changes and an inappropriate endpoint for risk assessment. Instead, two developmental endpoints were identified as suitable for human health risk assessment: (1) for acute exposures, the increased incidence of skeletal malformations, an effect noted only at doses that were toxic to the dam and fetus, was assessed using the maximum daily AUC (as estimated using a PBPK model); and (2) for repeated exposures to NMP, changes in fetal body weight were assessed using cumulative AUC. Benchmark dose methods were used to estimate a point of departure for skeletal malformations (BMDL5%) and fetal body weight changes (BMDLSD). Where possible, data from multiple studies were pooled to increase the predictive power of the data set. BMDL5% values for skeletal malformations ranged from approximately 1200-1500 mg/kg*hr (in terms of daily maximum AUC). Similarly, BMDLSD values for fetal body weight changes ranged from approximately 270-710 mg/L*hr (in terms of cumulative AUC). These BMD values can be used to support margin-of-exposure analyses for human health risk assessments of NMP conducted in terms of internal dose.
Cocamide diethanolamine (DEA) has been listed on the State of California’s Proposition 65 List as a chemical known to the state to cause cancer. This listing is based on the results of a National Toxicology Program (NTP) 2-year dermal carcinogenicity study which found clear evidence of carcinogenic activity in B6C3F1 mice based on increased incidences of liver neoplasms in both sexes, and increased incidences of renal tubule neoplasms in males. Although controversy exists regarding the relevance of the NTP study to humans, industries are obligated to comply with the Proposition 65 labeling requirement and drinking water discharge prohibition, unless they are able to demonstrate that cocamide DEA levels in their products are below a specific No Significant Risk Level (NSRL). The State of California has not published a NSRL for cocamide DEA. In accordance with the guidelines of California EPA, ToxServices derived a NSRL of 13.3 μg/day for cocamide DEA based on the incidence of hepatocellular carcinoma or hepatoblastoma in female mice obtained from the NTP carcinogenicity study using benchmark dose modeling. The U.S. EPA’s Benchmark Dose Software (BMDS) was used to model the cancer slope factors for the combined incidence of hepatic neoplasms. These slope factors were scaled to human equivalent doses and used to determine the lifetime average daily dose that would result in one excess cancer case in an exposed human population of 100,000. This NSRL value can be used as part of an exposure and safety assessment to evaluate compliance with Proposition 65 requirements for products sold in the State of California.

Titanium dioxide (TiO2) is widely used as a colorant in consumer products. Recently, TiO2 was listed by the State of California on the list of chemicals known to the state to cause cancer or reproductive toxicity (i.e., Proposition 65), due to its classification by the International Agency for Research on Cancer (IARC) as a Group 2B possible human carcinogen. Products containing Proposition 65 substances are subject to labeling requirements, unless exposure is below California’s “safe harbor” level. For potential carcinogens, the safe harbor level is termed the No Significant Risk Level (NSRL). As no NSRL for TiO2 has been established by California, we derived a NSRL according to CalEPA guidelines.

We performed benchmark dose (BMD) modeling on combined lung tumor incidence data from 3 published long-term, inhalation-route rat studies. BMD modeling was based on TiO2 particle deposition in the lung, expressed as particle surface area per gram of lung tissue. The surface area metric resulted in best fit of the data and normalized strain-specific anatomic differences and differences in exposure duration across studies. This approach also accounted for the impact of different particle sizes (which have different surface areas). When extrapolating modeled animal doses to human doses, a particle dosimetry model was used to account for particle size distribution, respiration pattern, and deposition and clearance mechanisms specific to humans. Using this approach, separate NSRLs were calculated for TiO2 particles with different surface areas. For fine TiO2 particles (1-2.5 μm), the NSRLs is 27 μg/day, and for ultrafine particles (< 0.1 μm) the NSRL is 2.8 μg/day. The derivation of two NSRLs is consistent with observations in animal inhalation studies that indicate that TiO2 toxicity is particle size-dependent. Although route-specific safe harbor levels exist for other chemicals, this is the first instance of particle size-specific Proposition 65 NSRLs and thus represents a novel, toxicologically-driven approach to safe harbor determination.
Tetrabromobisphenol A (TBBPA) is an important flame retardant used primarily as a reactive chemical in the synthesis of printed circuit boards. In this application, it is incorporated into the epoxy and serves a vital role in fire safety. Despite its use primarily as a reactant with limited release to the environment, some concerns have been raised regarding exposure. The objective of this study was to develop non-cancer based toxicity values (reference doses, or RfDs) and to compare such to appropriate estimates of human exposure. An array of potential RfDs was developed using data reported as part of a recent 2-year bioassay conducted by the National Toxicology Program (NTP); these data indicated that chronic exposure to TBBPA induced hepatic and kidney lesions in male mice, and forestomach lesions in male and female mice. In rats, notable lesions included uterine hyperplasia and rete ovarii cysts at study termination, as well as decreased serum thyroxin levels after 13 weeks of exposure. These endpoints were modeled using benchmark dose (BMD) modeling; the 95% lower confidence interval values (BMDLs) were derived with a benchmark response set to 10% extra risk for dichotomous endpoints and one standard deviation for continuous endpoints. BMDL values were adjusted by allometric scaling and subsequently reduced by uncertainty factors to derive RfD values ranging from 0.03 to 3.0 mg/kg-day. In comparison, exposure estimates ranged from 0.0000003 to 0.000007 mg/kg-day based on an evaluation of exposures for infants (based on intakes due to breastmilk and solid/dust), and for children, adolescents, and adults (based on intakes due to diet, drinking water, and soil/dust ingestion). The total daily exposure estimates were generally driven by soil/dust ingestion for most receptors (and by breastmilk for infants) in reasonable maximum and upper-end regulatory default exposure scenarios. These data indicate that there is a sufficiently large margin of safety between potential RfD values and current exposure to TBBPA.

**Development of Noncancer-Based Toxicity Factors and Daily Dose Estimates for TBBPA**

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**Provisional Advisory Level (PAL) Development for 4-Aminopyridine**

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PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance, but are intended for use at the discretion of risk managers in emergency situations when site-specific risk assessments are not available. Application of PAL protocols has been performed for 4-aminopyridine to estimate oral exposure limits; inhalation PAL values are not recommended (NR) due to insufficient data. 4-Aminopyridine blocks voltage-sensitive potassium channels in nerves by binding to sites within the channels. PAL values for 4-aminopyridine are based on clinical nervous system effects in humans using the SOP and QAPP requirements. The mention of trade names does not imply EPA endorsement.

4-Aminopyridine oral PAL 1 values are 0.25 mg/L for 24-hr, and 30-d, 90-d and 2-yr.

4-Aminopyridine oral PAL 2 values are 1.0 mg/L for 24-hr, and 30-d, 90-d and 2-yr.

4-Aminopyridine oral PAL 3 values are 1.5 mg/L for 24-hr, and 30-d, 90-d and 2-yr.

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**Provisional Advisory Level (PAL) Development for Chlorine Gas**

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PAL values developed for hazardous materials by the US EPA represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hour, 30-day, 90-day, and 2-year durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance, but are intended for use at the discretion of risk managers in emergency situations when site-specific risk assessments are not available. PALs are developed with appropriate human and animal data, using standard protocols based on the SOP and QAPP requirements.

Chlorine is a greenish-yellow gas with a pungent odor, a strong oxidizing agent, and very reactive. Large amounts are made worldwide, primarily for use in making PVC plastics, but also insecticides, pesticides, and bleach. It has been used as a chemical warfare agent. Chlorine gas is a contact irritant, and in both humans and animals, the respiratory tract is the primary target for toxicity. Effects include sensory irritation, increased airway resistance, and lesions in the lungs and other organs. Death follows quickly at high concentrations. PALs were derived using controlled studies with monkeys, rats, mice, and humans. The resulting inhalation PAL 1, PAL 2, and PAL 3 values, respectively, for a 24-hour exposure are 0.096, 0.19, and 5.1 ppm; for a 30-day exposure are 0.018, 0.11, and 0.32 ppm; and for a 90-day exposure are 0.036, 0.036, and 0.32 ppm. For a 2-year exposure, the PAL 1 and PAL 2 are 0.0346 and 0.0346 ppm, respectively; a 2-year PAL 3 was not developed due to insufficient data. Oral PAL values were not developed. The PAL values were approved by the Expert Consultation Panel for PALs in April 2013.

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**Provisional Advisory Level (PAL) Development for Chronic Health Effects following Acute Exposures to TICs**

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Joint Chemical, Biological, Radiological, and Nuclear doctrine requires military commanders to minimize total risk in operational planning and execution. Incorporation of Military Exposure Guidelines (MEGs) into risk estimates can provide a mechanism to consider short- and long-term chemical exposure risks. However, current MEGs (and civilian guidelines) do not address chronic non-cancer health effects that may result from a single acute exposure. This gap is a source of concern for planners in the medical community, as these effects may have im-

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**Development of Exposure Guidelines for Chronic Health Effects following Acute Exposures to TICs**

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In its current state, dose-response modeling employs analytical methods that include numerous candidate mathematical functions from which the analyst must choose in order to develop toxicity values such as reference doses and slope factors. Smoothing regression splines offer a much simpler approach to dose-response modeling and have a number of desirable properties. They are flexible, require no pre-specification of a functional form, achieve optimal fit under a wide range of circumstances, and need little-to-no intervention by the analyst. In addition, the same smoothing regression splines can be used to unambiguously identify various types of points of departure (e.g., threshold and benchmark doses), as well as address important questions about a dose-response curve such as its overall non-linearity and non-linearity within specific regions. We compared more traditional modeling approaches with smoothing splines to illustrate how the latter provide a unified mathematical framework for estimating dose-response functions and critical quantities and often provide superior performance. For example, we compared threshold doses estimated via smoothing regression splines with other published bilinear models. Although smoothing regression splines regularly provide similar results to older approaches, when differences emerge the smoothing spline curve compares favorably to existing guidelines (both military and civilian) in that only severe exposures have the potential to cause chronic health effects. This approach is believed to be novel and may be applicable to other TICs with a limited data set.

**629 Is Nonparametric Method Suitable for Benchmark Dose Analysis Using Typically Available Toxicity Study Data?**

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Model uncertainty is one important problem associated with benchmark dose (BMD) analysis and has gained much attention in recent development of BMD methodology. In addition to the model averaging approach, which produces one weight-averaged BMD/BMDL estimate according to the posterior model weights, nonparametric method has been proposed as another alternative to avoid issues associated with model uncertainty. One advantage of nonparametric method for BMD estimation is that it does not assume any particular format for the dose-response model. However, it has some limitations. This study addresses two questions: (1) Is nonparametric method as reliable as parametric methods for deriving BMD/BMDL estimates from typically available dose-response data? (2) What are the general toxicity study design criteria that are suitable for nonparametric BMD analysis? These two questions are going to be answered based on results from a large simulation study using both dichotomous and continuous endpoints. The adequateness of the BMD and BMDL estimates is judged by their accuracy and coverage rate. Given a typical chronic study setting (i.e., 200 animals exposed for 2 years), the simulation study will mainly examine how dose spacing and animal distribution among dose groups can affect the nonparametric BMD analysis. Preliminary results suggest that the dose-response data from current toxicity studies may present difficult challenges for nonparametric analysis due to the limited number of dose groups used in most toxicity bioassays.

**630 Standardized Benchmark Dose Calculation: Opportunities to Inform Science-Based Decisions in Human Health Assessments**

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The TTC concept derives thresholds to structural groups of compounds below which a risk for human health is not assumed for a life-time exposure. Thresholds were derived by analyzing datasets of oral in vivo studies to which the Cramer decision tree was applied. Inhalation is, however, an important route in human risk assessment e.g. for workers and consumers. The application of the Cramer decision tree to datasets of repeated-dose studies with inhalation exposure resulted in very low thresholds compared to oral TTC values. Reasons might be route specific differences e.g. an observed high sensitivity of the respiratory tract to local effects (Carthew et al. 2009, Escher et al. 2010). We present an integrative approach to derive inhalation specific threshold values, which are based on a dataset of 296 chemicals with repeated-dose toxicity studies (www.fraunhofer-repdose.de). Systemic and local NOEC values were discriminated. Groups of compounds with specific structural features (SF) were identified based on atom centered fragments (Kühne et al. 2009). Few SF were explicit for local or systemic activity indicating that this mode of action is not a determining factor. The structural and toxicological boundaries of the initial SFs were further evaluated considering differences in absorption, published data on mechanism/metabolism and sensitive targets/effects observed in the in vitro studies. 28 SF groups resulted, 9 low (L) and 19 toxic (T) groups. About 20% of the compounds are, however, not yet grouped. Compared to the Cramer classes the T and L-groups better discriminate low toxic and toxic compounds. Two clearly distinguished TTC values are proposed.

Threshold of Toxicological Concern (TTC) is a tool developed to provide scientific advice about possible human health risks from exposure to low levels of chemicals in the diet, when specific toxicity data are not available. It provides health protective human exposure threshold values for chemicals from a three broad ranges of structural categories (Cramer classes).

The aim of the work, as part of the EU COSMOS Project, has been to adapt the TTC concept and extend its applicability to cosmetics which are applied to the skin. A decision tree approach has been developed based on estimated usage/skin exposure, maximum possible dermal absorption derived from the J max prediction or experimental absorption data to estimate systemic dose for ranking against TTC, incorporating structural categories and Cramer class. A new dermal absorption database enriched with cosmetics-related chemicals has been established; the chemical space and domain of cosmetics-related chemicals have been also defined. Where available, experimental dermal absorption data have been ranked with predicted Jmax values. Also assessed is the importance of local metabolism in determining differences between the oral and dermal exposure routes and its influence in decision making and use of TTC. The potential for use of TTC for cosmetics is indicated and the findings support application of TTC approaches by regulators.

Supported by the EU FP7 COSMOS Project.

**632a How Much Do Shapes of Toxicological Dose-Response Relationships Vary?**


A re-analysis of a large number of historical dose-response data for continuous end points showed that the shapes of the dose-response relationships were surprisingly homogenous. The datasets were selected on the sole criterion that they were expected to provide relatively good information on the dose-response shape, and included a variety of end points and both in vivo and in vitro studies of various types. Both the four-parameter exponential and Hill model adequately described all toxicological dose-response data we considered. For a given endpoint and study type, dose-response shapes did not differ statistically significantly among chemicals in the in vitro studies considered, while a mild among-chemical variation in the steepness parameter seemed to be present in the in vivo studies. These findings have various practical consequences. For continuous endpoints, model selection in the BMD approach is not a crucial issue. The often-applied approach of using constraints on the model parameters to prevent "infinite" slope at dose zero in fitting a model is not in line with our findings, and appears to be unjustified. Instead, more realistic ranges of parameter values could be derived from re-analyses of large numbers of historical dose-response datasets in the same endpoint and study type, which would then be used as parameter constraints or informative priors in the analysis of future individual datasets. This approach would be particularly useful for weak datasets (e.g. few doses, much scatter). In addition, this approach may open the way to use fewer animals in future studies. Finally, we argue that distinctions between linear, sub/supralinear or thresholded dose-response shapes, based on visual inspection of plots, are not biologically meaningful nor useful for risk assessment. This abstract does not necessarily reflect EPA policy.

**632b Variation in Scaling Factors Used for In Vitro to In Vivo Extrapolation (IVIVE) and Its Impact on Internal Dose in Rats: A Case Study with Bromodichloromethane (BDCM)**


Physiologically based pharmacokinetic (PBPK) models can include values for metabolic parameters extrapolated from in vitro studies using scaling factors (SF) such as mg of microsomal or cytosolic protein per gram of liver (MPPGL and CPPGL) and liver volume (FVL). Variation in SF values impacts metabolic parameter estimates (Vmax) and hence estimates of internal dose used in dose response analysis. The impact of variation in MPPGL, CPPGL and FVL on toxicologically relevant dose metrics was assessed using a rat PBPK model for BDCM and Monte Carlo analysis. Values for SF were determined experimentally or obtained from the literature. Dose metrics examined included area under the curve for blood BDCM (AUCV) and amount metabolized in liver via detoxification (oxidative, AM1) and toxication (glutathione, AM2) pathways. At relatively low doses (5 mg/kg) all dose metrics evaluated were changed by less than 20%, probably because metabolism is blood flow-limited at lower doses. In contrast, at biossary dose levels (100 mg/kg) differences of 5-, 3- and 9-fold were observed for AUC, AM1 and AM2, respectively. Sensitivity analysis for both AUC and AM1 revealed that MPPGL and FVL were highly and moderately influential parameters, respectively. The larger difference observed for AM2 is likely a consequence of higher variability in CPPGL (3 times higher coefficient of variation compared to other SF) suggesting that more accurate estimates are needed for CPPGL and distribution of cytosolic enzymes in rat liver. This analysis demonstrates: 1) that variability in SF used for IVIVE of metabolic rate parameters can have a significant impact on estimates of internal dose metrics at toxicologically relevant doses, and 2) the need to evaluate both uncertainty and variability for scaling factors used for IVIVE. (This abstract does not reflect USEPA policy).

**632c Toluene PBPK Models to Extrapolate Neurological Effects between Rats and Humans, across Acute Durations and Severity Categories**


Acute exposure to volatile organic compounds (e.g., toluene and ethanol) impairs neurological function. The degree of impairment has been quantified as a dose-response relationship using a variety of behavioral tasks, including reaction time (RT). Brain toluene concentrations (Br-TC) have been related quantitatively to blood ethanol concentrations (BEC) via changes in RT associated with each chemical (Benignus et al., 2005). A BEC of 0.08 g/dl (legal intoxication) and a Br-TC of 119 µM both produce a 13% reduction of RT. The current interim level 2 acute exposure guideline levels (AEGL-2) are based on a 70-min exposure to 2400 ppm toluene, and results in an effect level consistent with a BEC of 0.17 g/dl, as determined by our analyses. An alternate set of AEGL-2 values based on a doubling of RT in Long-Evans rats (1600 ppm for 34-mins) was determined using a rat PBPK model (Kenyon et al., 2008). The rat Br-TC at the end of exposure (535 µM; 49.2 mg/kg) was reduced by a factor of 3 to derive the target concentration. AEGL-2 values (10- and 30-min, 1-, 4- and 8-hr durations) were determined from the target concentration with a human (GPAT: Benignus et al., 2006). The same PBPK model construct was applied to determine AEGL-3 values based on a rat lethality NOAEL. The estimate of a human LC-50 was also derived for comparison to a Dutch Probit method for land-use planning. The final results were toluene exposure levels for durations from 10-mins up to 8-hrs at three levels of severity in humans: a clear decrement in RT; the threshold for lethality; and an LC-50. These analyses led to a quantitative methodology to inform revisions of AEGL values for toluene which were reviewed and approved by the National Academy of Sciences. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

**632d Modeling of Blood Lead Levels in Astronauts Exposed to Microgravity-Accelerated Bone Loss**

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Setting Spacecraft Water Exposure Guidelines (SWEGs) for lead (Pb) in spacecraft drinking water has special challenges related to estimating blood lead levels (PbB) during the increased release of lead into systemic circulation via microgravity-in-
duced bone loss. The effects on the PbB of Pb in drinking water (PbW), Pb released from bones, and change in lead exposure before, during, and after spaceflight were evaluated using a physiologically based pharmacokinetic (PBPK) mathematical model (2013, Aviation, Space, & Environmental Medicine 84:1229-1234) that incorporates environmental lead exposure for PbB, and in flight and calculates temporarily increased rates of osteoporosis during spaceflight. The model predicts that the average American astronaut in 2030 (the earliest anticipated launch date for a long-duration mission) would have a PbB of 1.7 μg/dL at launch and that PbB levels would decrease in microgravity at PbW values less than about 9 μg Pb/L, because of reduced lead exposure during spaceflight. Currently, PbW on the International Space Station (ISS) averages <1 μg Pb/L. A SWEG of 9 μg Pb/L would protect most astronauts on long-duration spaceflights by ensuring that PbB values will not exceed pre-launch levels. At in-flight PbW concentrations <9 μg Pb/L, Pb concentrations in both bone and blood would gradually decrease below their pre-launch values. On the other hand, astronauts who have high concentrations of Pb stored in bones (an unlikely possibility) could experience increased PbB levels in microgravity due to release of lead from bones. While the resultant in-flight PbBs would depend on their pre-flight bone lead levels, their PbBs will not be significantly further elevated (<1 μg/dL) by consuming water with a PbW of <9 μg/L. Because individuals with a history of clinical lead poisoning would likely have high concentrations of lead stored in their bones, we recommend that such individuals avoid exposure to microgravity of more than a few days' duration to prevent in-flight lead poisoning.

In 2007, the intentional adulteration of pet food products with melamine and cyanuric acid, caused kidney failure and death of hundreds of cats and dogs in the U.S.. Early investigation revealed that co-exposure to these compounds can elicit nephrotoxicity due to the formation of highly insoluble melamine cyanurate (MC) crystals in the nephrons. In response to these events, and later events in China in 2008 involving the contamination of infant formula with melamine, it became apparent to regulatory agencies, including the U.S. Food and Drug Administration (FDA), that further in-depth studies addressing the toxicity of melamine, cyanuric acid, and their combination were warranted. We report the design and outcome of studies conducted at the FDA in male and female F344 rats on the combined nephrotoxicity of melamine and cyanuric acid. The current tolerable daily intakes (TDI) established by the FDA for dietary exposure to melamine and its derivatives are based upon a NOAEL of 63 mg/kg bw/day for dietary exposure to melamine in a 13-week rat study. The data from our current studies demonstrate that in F344 rats, oral co-exposure to melamine and cyanuric acid results in a NOAEL value 25- (based upon renal histopathological alterations such as tubule degeneration, fibrosis, dilation and epithelium hyperplasia and elevated blood urea nitrogen and serum creatinine) to 100-fold (based upon MC crystal formation) lower than that previously considered in the risk assessments based on the NOAEL value derived from exposure to melamine alone.
Systematic Review of the Association between Lung Cancer Risk and Low Levels of Arsenic in Drinking Water


Background: Multiple studies have demonstrated the increased risk of bladder and lung cancers with exposure to drinking water containing inorganic arsenic at levels in the hundreds of micrograms/liter. The risks at lower levels are uncertain. A systematic review and meta-analysis of the risk of bladder cancers with exposure to drinking water (Mink, 2008) has found no increased risk at arsenic exposures below 200 ug/L with the exception of studies limited to tobacco smokers. No such analysis has been reported with respect to lung cancers.

Materials and Methods: Our comprehensive literature search yielded a final set of 11 papers with 17 study populations from 4 continents that reported the risks of lung cancer from lower levels of arsenic exposure in drinking water. Risk ratio and exposure metric data were extracted. Results were stratified into exposures at <10 ug/L, 10-100 ug/L, 100-200 ug/L, and >200 ug/L.

Results: The 11 studies had 3 risk estimates in the <10 ug/L range, 20 in the 10-100 ug/L range, 7 in the 100-200 ug/L range, and 9 just above 200 ug/L. The mean and median risk estimates were 0.92 and 0.94 for <10 ug/L, 1.02 and 1.02 for 10-100 ug/L, 1.51 and 1.34 for 100-200 ug/L, and 2.36 and 1.97 for >200 ug/L. Only one of 20 (5%) risk estimates at 10-100 ug/L was significantly greater than the risk at lower levels, as were 3 of 7 (43%) at 100-200 ug/L and 5 of 9 (55%) at >200 ug/L. An arsenic-associated risk was seen among smokers only above 200 ug/L.

Conclusion: Lung cancer risk with exposure to arsenic in drinking water was not seen to rise at levels below 100 ug/L. Increased risks were observed at 100-200 ug/L and at >200 ug/L. These results are consistent with those of bladder cancer risk with exposure to arsenic in drinking water; however, bladder cancer studies have generally separated the risk for smokers from that of non-smokers and only two of the lung cancer studies have done so.

Guidance for Contaminated Sites: Trichloroethylene Case Study

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A methodology for interpreting a range of acceptable noncancer risks was developed, similar to the range used for cancer risks in management of waste sites, based on readily available toxicity information from U.S. EPA and elsewhere. This range enables noncancer risk to be evaluated in a way that reflects the uncertainty of the noncancer benchmark. The EPA’s recently developed RfC for trichloroethylene (TCE), based on multiple endpoints, was evaluated as a case study for the noncancer risk range. For TCE, this range was judged to be 3 µg/m³ to 20 µg/m³. The results of the NTP study-based RfC were used to determine the floor and midpoint of this uncertainty range. The highly controversial results from the Johnson et al. (2003) study-based RfC, while associated with low confidence, were nevertheless used to determine the ceiling level of this uncertainty range. The proposed range was entirely within the wider individual uncertainty range from the Keil et al. (2009) study; therefore, this latter study was considered to be confirmatory. The multi-endpoint uncertainty range is composed of floor, midpoint and ceiling values derived from studies for which the appropriate averaging time corresponds to different exposure durations (i.e., developmental or chronic exposure periods). Therefore, this range can be applied to both long- and short-term exposures, with the associated differences in exposure averaging times. For shorter-term exposures, the results from the Johnson et al. study (2003) might also be used to describe the best averaging time, but if so, this averaging time should be based on the average time of cardiac development in humans during fetal growth, approximately 24 days. An assumed 24-day averaging time for cardiac development in humans is consistent with the fact that effects in the Johnson et al. study (2003), appeared to occur during the whole time of cardiac development in the rat.

Pen Housing of Nonhuman Primates on Toxicology Studies


As part of our continuing effort to improve our animal care and use program and to exceed regulatory requirements in the housing of nonhuman primates, we have developed a pen-style housing system for use on GLP-compliant toxicology studies. The pen system was developed with three objectives 1) meet housing requirements to exceed regulatory requirements in the housing of nonhuman primates, we have developed a pen-style housing system for use on GLP-compliant toxicology studies. The pen system was developed with three objectives 1) meet housing requirements

Implementing the 3R Methods and Hurdles for Their Application—A Perspective from the Chemical Industry


For the safety of consumer products their toxicological potential must be determined. By law, the toxicological testing often requires animal studies. According to our commitment to animal protection we apply 3R principles wherever possible, i.e. “reduce” and “replace” animal studies by new testing strategies as well as “refine” them by humane testing conditions. Herein, we report on certain hurdles that BASF SE has faced in the recent years.

Hurdles for reduction of animal numbers used for regulatory purposes exist (i) for “reproduction toxicity studies” as the basic design of the extended one-generation reproductive toxicity study is still lacking full acceptance and (ii) for “non-rodent toxicity studies” as the reduntant one-year dog study is still requested by some authorities. Hurdles for application of replacement methods exist for the endpoints (iii) “eye irritation” as technical equipment is difficult to achieve for a validated and accepted assay, and (iv) “skin sensitization” as the validation process will be completed too late for registration of many substances under the European Union legislation REACH. Hurdles for refinement were encountered when (v) the propagated use of a cytotoxicity assay for the optimal selection of the starting dose in acute oral toxicity did not prove to be as useful as anticipated. Taken together, especially in the field of in vitro methods, many problems can be encountered during the process of development, establishment, and their application. Some of these hurdles are technical issues, some published alternatives not fulfilling inter-laboratory reproducibility requirements, and an insufficient validation procedure. Most often, in vitro methods are not validated fast enough and regulatory acceptance takes too much time. Therefore, their use for regulatory purposes is often delayed and does not match legislative and political goals (e.g. Cosmetic Directive and REACH).

SLIM As a Smart Way to Translate 3R Innovations to Acceptance for Use in Human Risk Assessment

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Safety assessment of pharmaceuticals and food ingredients requires a significant number of animal experiments. Although in vitro techniques for safety assessment became available over the past years, the number of accepted methods actually implemented within industry and regulations is very low. The slow implementation process delays the innovations in product development, which is undesirable for scientific, societal and economic reasons. The objective of the SLIM project is to obtain good practices for a faster development, (regulatory) acceptance and implementation of methods that replace, reduce or refine animal experiments. Four case studies in the field of reproductive toxicity,
food allergy, carcinogenicity and bioavailability of new products were performed. An advisory board, composed of scientists working for international regulatory agencies, has critically reviewed these case studies. Two case studies focus on retrospective analysis of the predictive value of animal experiments and aim to identify whether one animal species can be used in reproductive toxicity testing, without losing safety information or whether long-term carcinogenicity tests can be replaced with shorter tests. Other case studies focus on development of in vitro models to identify embryo toxic compounds or allergic reactions. The fourth compared different in vitro models to assess oral bioavailability. Part of all case studies include identification of barriers and drivers of (regulatory) acceptance. Case studies will be presented and show that drivers and barriers can be influenced by intensifying collaborations between stakeholders, i.e. companies, regulatory bodies and research institutes in an early phase in the assay development process.

636 The Use of Weight of Evidence (WoE) for Fulfilling the 2nd Species Information Requirement for Developmental Toxicity within the REACH Framework


REACH passed into law in 2007, placing the responsibility on registrants to demonstrate that potential risks from chemical exposures are appropriately managed and communicated. The European Chemicals Agency (ECHA) has indicated that the second most frequent ‘shortcoming’ in REACH registration dossiers was related to the PDNT endpoint. For >100 tpa registrations, REACH mandates the information requirements for PDNT be filled by conducting OECD test guideline V4 (usually rat), with an expectation to consider further testing in a second species (usually rabbit). The necessity of conducting PDNT testing has recently been evaluated in the literature. Initial conclusions indicate that (i) rabbits are not inherently more sensitive detectors of human teratogenicity, and (ii) the variability observed between species for the majority of chemicals does not exceed normal variability that occurs in multiple studies with the same species, and (c) species sensitivities that do exist are driven by identifiable biological differences. A properly constructed WoE should establish whether the specific substance is expected to have a similar metabolic fate across species, and determine if there are any indicators that a unique response may be expected in different species. This evaluation can predict whether additional information for risk assessment purposes would be obtained from a second species study. Additionally, variability between NOAELs resulting from a rat and rabbit study can be estimated using existing data on substances that have been evaluated in both species. This estimate can be used in comparison with exposure scenarios to determine if the Margin of Exposure (MoE) is large enough to encompass any residual uncertainty remaining after completing a thorough substances specific evaluation. Building a WoE with perspectives from the published literature, a chemical specific evaluation, and MoE calculations should be sufficient to determine the necessity for PDNT testing in a second species within the REACH framework.

637 Evaluation of a Tiered Toxicity Testing Decision Trigger for Assessing Reproductive Hazards of Commodity Chemicals

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A tiered toxicity testing framework for application to commodity chemical safety assessment has been developed which employs endpoint-specific decision triggers. The framework has two tiers in which hazard information can be integrated with exposure information at each tier. Decisions about testing choices for Tier 2 tests, the more complex tests, are made based on consideration of decision triggers for significant toxic effects, one of which is reproductive toxicity potential. In the current work, an initial assessment of the reliability of the proposed reproductive toxicity decision trigger was performed using reproductive toxicity data collected within the U.S. Environmental Protection Agency (EPA) Toxicity Reference database (ToxRefDB). Among the ToxRefDB chemicals which EPA previously designated as being either positive or negative for reproductive toxicity potential, 10 positive and 10 negative substances were randomly selected. Toxicity data were extracted from the ToxRefDB and entered into a database constructed for the analysis, the “Toxicity Trigger Database” (ToxTriggerDB). During the evaluation, however, significant limitations were uncovered in the underlying curation of the ToxRefDB, limitations that involved interpretation of toxicity effects and designation of toxicity endpoints. As a result, not enough positive reproductive toxicants were included in the random selection, and only a limited evaluation of the proposed trigger was possible. The limited evaluation showed that the trigger’s performance was dependent on the scientific evaluation criteria used, and judgments made when determining whether responses met the criteria for designating a substance as a positive or negative. Before analyses are conducted using ToxRefDB, including use in validation of prediction models, consensus criteria for designating positive and negative agents need to be developed and applied to ensure the database is adequate for this type of use.

638 Association between Health Outcomes and Environmental Factors in Illinois

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Research has suggested that mortality rates are positively correlated with social inequalities, race/ethnic, air pollution, elevated ambient temperature, availability of medical care, risk factors and other factors. This study develops a model that uses indicators for multiple factors to predict the mortality rates for selected diseases by county in Illinois. County-level data on 102 Illinois counties from the 2012 County Health Rankings were linked to county mortality data from the Centers for Disease Control and Prevention Compressed Mortality database. Appropriate statistical analyses were used to predict the relationship between various environmental factors and other factors to explain variations in county-specific mortality rates for the leading causes of death and lifespan in Illinois. It is anticipated that this study would suggest some adaption strategies to improve the health of residents in Illinois through working with some community organizations.

639 A Novel Approach to Toxicological Hazard Assessment of CAS Number-Specific Compounds with Variable Composition

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The rapidly changing global landscape of environmental regulations and chemical compliance presents a major challenge for chemical manufacturers and importers. Increasingly, countries are requiring hazard identification based on the Globally Harmonized System of Classification and Labeling (GHS) Guidance. The use of a GHS-based classification system is complex and calls for a level of expertise in toxicology and chemistry that was not previously needed to communicate chemical safety. As part of the hazard assessment process, Graduate has identified a recurring complication regarding hazard assignment for substances with variable compositional features but the same Chemical Abstracts Service (CAS) Registry Number. CAS numbers are unique chemical-specific identifiers; however, they are not specific to length, size, and composition. Examples include silica, petroleum distillate, alcohol ethoxylates, and glass oxide compounds. We determined that hazard conclusions can vary considerably across the publicly available literature for a specific CAS number, and information on the chemical manufacturing process is often necessary to appropriately assign hazards. For example, using just the CAS number, an evaluation of glass oxide would suggest a potential carcinogenic risk because this CAS number is classified as a class 1B carcinogen in the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) registration dossier (without any caveats). Such a classification would trigger significant downstream regulatory requirements. Upon targeted investigation into product specifications on particle size, however, we determined that the glass oxide in question consisted of continuous filament glass fibers with aerodynamic diameters ranging between 8-26 μm, which are unlikely to deposit in the alveoli regions of the lungs and thus unlikely to pose carcinogenic risks. This poster presents several examples of complexities involved in the hazard classification of compounds with variable compositional features and the research strategy necessary to arrive at well-supported hazard conclusions.

640 A Sustainable Product Risk Assessment

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Celanese uses a global risk management process for identifying and managing product-related risk associated with raw materials, manufacturing, handling, distributing, sale and the end-use of our products. The process was developed, in support of the responsible care business approach, utilizing a framework rooted in the best practices and industry benchmarking. It involves coordination of experts to further evaluate and assess various elements, including effects on employees, customers, consumers, operating sites, the environment, and the Company. The purpose of our Product Risk Assessment process is to ensure that our products meet our corporate safety and legal targets, as well as all applicable safety, regulatory and legal requirements and possible risks in each country where the product will be marketed and used. Our internal global risk management process targets the identification and management of product related risk associated with raw materials, manufactur-
ing, handling and distribution, sale, and the end-use of our products. The system is designed to broadly review, assess, evaluate and balance risk from multiple internal and external sources. A secondary goal was to increase institutional awareness and organizational decision-making at risk relevant levels. The process includes a risk review by committees comprised of technical professionals with backgrounds in toxicology & medical technology, legal, product development, manufacturing, and others as appropriate. Success has been achieved by embedding the product risk management process into our new product commercialization systems, creating a cultural change towards proactive consideration of critical risk management elements such as product risk, increased organizational awareness, escalation of decision-making, and risk mitigation.

**A Life-Cycle Approach for Prototypical Consumer Products Containing Nanomaterials**


Nanocomposite materials (bulk materials enabled with nanoparticles) are used in the building industry. To date, a total of 250 products produced by over 100 companies incorporate nanocomposites into building or renovation plans; estimated revenues surpass $1 billion. Nanoparticles offer tailored benefits to the traditional functions of materials. Nanocomposite coatings have led to new opportunities in the world-wide market. The continued utilization of nanomaterials in the building envelope is expected to develop rapidly; however, the safety of these novel materials to humans and the surrounding environment must be studied in parallel to their incorporation into products. One way to develop safe nanomaterials is to use a life cycle approach that integrates product development with manufacturing and worker/consumer exposure. This research approach investigates a white paint product enabled with 120 nm titanium dioxide particles as a case study. The bulk material (dry wall) is characterized with the nano-enabled product (white paint) in its intact and degraded forms. Simulated wear-and-tear scenarios are performed on the painted wall and the released airborne particles are analyzed for concentration, size, and composition. Human respiratory deposition of the airborne particles via inhalation exposure is calculated by a particle dosimetry model. The released particles are collected and exposed to normal epithelial cells from four areas of the human pulmonary airway for a toxicological evaluation. Results show that differential physicochemical properties, respiratory depositions in human airway, and toxicological responses are induced between particle-types that contain nanomaterials versus particle-types without nanomaterials. The impact of this research will help to enable sustainable opportunities of nanotechnology in the built environment and provide methodologies for understanding nanomaterial properties and potential toxicological impacts in the context of developed consumer products.

**Towards a Harmonized Evaluation of Chemical Emissions from Building Products in the EU**

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A prerequisite for healthy air quality indoors is the use of low emitting materials. There are three different mandatory systems in EU member states to evaluate emission properties of building products: in Germany since 2006, in France since 2012 and in Belgium beginning 2014. Besides these mandatory systems there are a number of voluntary health related quality labels in European countries. A working group with experts from nine European countries coordinated by the Joint Research Center of the European Commission has started to develop a new scientific-based harmonized procedure to derive health related thresholds, so called EU-LCI values (Lowest Concentration of Interest) for all substances regulated in France and Germany. EU-LCI values (usually expressed as µg/m3) are health-based reference concentrations for inhalation exposure used to assess emissions after 28 days from a single product during a laboratory test chamber procedure, as defined in the proposed standard developed by CEN TC 351. The working group generated a protocol incorporating a set of definitions, criteria for the selection of chemicals and use of read across and adjustment and assessment factors. A standardized summary fact-sheet was developed to ensure transparent derivation of LCI values. Further, a list of interim EU-LCI values for 78 compounds (including e.g. acetaldehyde, toluene, xylene, 1,2,4-trimethylbenzene, 1,4-dichlorobenzene, ethylbenzene, 2-butoxyethanol, styrene, ε-caprolactam and t-pinene) was developed. Only “regular” volatile organic compounds (VOCs), and not VVOCs, SVOCs or carcinogens, were considered at this stage. The output of the working group is under publication as European Collaborative Action (ECA) Report No. 29. It has received wide appreciation by member states and industry in the EU, and the Belgian decree already refers to a future list with EU-LCI values.
Chemical use/exposure to both occupational and environmental receptors related to human health and/or the environment. This approach identifies the patterns of chemical hazards and exposure scenarios in the determination of the relative risk. Further risk characterization and risk management is needed. The Chemical Risk Prioritization System (CRPS) developed herein can be used to identify the chemicals which pose the highest potential risks to people and the environment, and state and federal agencies have begun issuing decisions on chemical exposure and production. A simulation environment is developed to help standardize model development, testing, and documentation, for a population dynamic simulation model (PDSM). The model can be used to assess pre-market population impacts of new tobacco products including reduced-risk products. The environment is set up to take user inputs and their documentation, run deterministic or Monte Carlo simulations, and visualize predictions including effects of uncertainty. The model and environment are coded in Microsoft Visual Basic and Excel, with Excel as the interface. Tables and plots of population changes, for example differences between base and what-if scenarios, are generated automatically. A tornado plot ranks parameters from most to least sensitive with respect to key outputs, to help visualize impacts of uncertainties and focus attention on more important ones. A Scenario Manager is available to document hypothetical scenarios and facilitate reproducing them. As part of initial testing, a scenario in which no menthol cigarettes exist (from the US FDA’s TPSC 2011 menthol report) was simulated using reported parameters. The resulting excess smoking prevalence and mortality were comparable to reported predictions, which were found to be highly sensitive to a few input parameters (mortality risk; menthol/non-menthol yield experimenter to smoker; proportion of menthol users among experimenters). The simulation environment could be adapted to other types of models, thus providing a test bed for comparing alternative models with standardized outputs.

A benefit of the CRPS is that it is a consistent, transparent approach tailored to company-specific activities. It streamlines the identification of chemicals where no additional concern is warranted or relative potential risk is minimal. This allows a more detailed risk assessment to occur for a smaller set of chemicals with higher potential risk. It also informs the company of chemicals where little hazard information is available, aiding industry in working with the chemical manufacturers to better characterize potential risk.

Dermal exposure to chemicals is highly relevant in relation to the use of cosmetic products, both in consumers and in individuals exposed occupationally. Regulatory frameworks exist within the EU to limit the dermal exposure of the general population and workers to chemicals in general as well as to limit the use of certain substances in cosmetic products. The objective of the study was to investigate and compare evaluations of dermal exposure performed under two regulatory frameworks. The publicly available information provided under respective regulatory frameworks for toxicological evaluation of the substances resorcinol, p-phenylenediamine, p-aminophenol, N-phenyl-p-phenylenediamine and diethyleneglycol monooethyl ether was scrutinized. A low consistency between the existing evaluations performed under REACH and under the Cosmetics directive was observed in regards to selection of data and identification of critical effect, although no systematic differences were identified. A questionable relevance of included data was observed for some of the registrant evaluations under REACH. However, regardless of these differences the critical dose descriptors were quite similar between the evaluators (within a factor of 2.5) for these five substances. An extended comparison was performed of the critical dose descriptors for all substances evaluated under both frameworks (n=30, only including cosmetics evaluations published 2003-2013). This exercise showed that the REACH registrant’s critical dose descriptor on average is 2.3 (geometric mean) higher than the SCCS critical dose descriptor, ranging from 0.5 to 57 times higher. This is partly explained by a difference in route of the critical study, as REACH registrant critical dose descriptors more often are derived from dermal exposure studies. Removing pairs of dose descriptors with different routes of exposure yields a geometric mean of 1.7, with a range of 0.5 to 40 times higher.

The kidney is a major site of chemical excretion, which results in its propensity to exhibit chemically-induced toxicological effects at a higher rate than most other organs. A routine practice in the assessment of kidney toxicity in animal studies is the evaluation of kidney weight changes. However, the manner in which kidney weight is interpreted and the value of this information in predicting renal damage remains a topic of discussion. In this study we sought to determine whether a relationship exists between chemically-induced kidney weight changes and renal histopathological alterations. We also examined the relative utility of absolute and relative (kidney-to-body weight ratio) kidney weight in the prediction of renal toxicity. For this, data extracted from oral chemical exposure studies in rats performed by the National Toxicology Program (NTP) were qualitatively and quantitatively evaluated. Our analysis showed a statistically significant correlation between absolute, but not relative, kidney weight and renal histopathology in chemically-treated rats. This positive correlation between absolute kidney weight and histopathology was observed even with compounds that statistically decreased terminal body weight. Furthermore, most increases in absolute kidney weight reaching statistical significance (irrespective of the magnitude of change) were found to be relevant for the prediction of histopathological changes. Thus, compared to relative kidney weight, the evaluation of absolute kidney weight may be a preferable method for identifying potential renal toxicants.
Regulatory guidance documents and audits of pharmaceutical product manufacturing operations are intended to preserve the quality and safety of active pharmaceutical ingredients (APIs) and drug products. Historically, regulatory authorities have assigned certain APIs to various “categories of concern,” based on their toxicological properties, in the interest of protecting patients from exposure to potentially hazardous contaminants. Equipment used to manufacture multiple products has the potential of becoming cross-contaminated. In the absence of data clearly demonstrating safety, these categories were intended to provide appropriate segregation for the prevention of potential cross-contamination as outlined by current Good Manufacturing Practices. However, the use of specific categories of concern often overlooked other characteristics of the API, such as pharmacologic potency and mechanism of action, which might also trigger specific requirements for safe handling and disposal. Drug manufacturers are often asked by contract manufacturing organizations whether or not APIs are “cytotoxic,” “steroids,” or “hormones.” As these terms were never defined, clarification is needed because the pharmacological mechanism of new APIs has dramatically changed since the time these terms were first introduced. Also, certain compounds may be inappropriately placed in one of these generic categories, giving the impression that segregation or dedication is required when in fact an acceptable daily exposure can be established and sufficient cleaning achieved. This presentation will discuss the historic use of these categories within the context of assessing their toxicological and pharmacological characteristics to help identify compounds requiring special attention within a Quality Risk Management Program.

### 650 Industrial Effluent-Induced Chromosomal Aberration in Catfish from Ogun River, Lagos, Nigeria

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The aim of the study is to evaluate the cytotoxic effect of effluents at inducing chromosomal aberration in the fish species Clarias gariepinus and Clarias pachynema. Effluents from a sewage treatment plant located in the Ogun River, Lagos, Nigeria were subjected to cytogenetic analysis for the detection of chromosomal aberrations using the in vivo micronucleus test. Results indicated that Clarias pachynema was more sensitive to the effluents than the other species. Effluents at concentrations of 250 mg/l and 500 mg/l caused a significant increase in the frequency of micronucleated erythrocytes in the fish. The study suggests that the effluents from the sewage treatment plant are capable of inducing chromosomal aberrations in the fish species, and therefore may pose a risk to human health.

### 649a Assessing Drug Substances to Identify “Highly Hazardous” Compounds for Quality Risk Management Programs


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The American Board of Toxicology (ABT) commenced to define the breadth and depth of expertise of professional toxicologists with the intent to review and refine the ABT certification criteria. A survey instrument was designed and piloted, then distributed broadly in the second quarter of 2013. 796 respondents representing 12 professional toxicology job roles (academic, agricultural, chemical, etc.) ranked the importance of 82 knowledge elements in their current professional practice. Specific elements ranked highest (≥20%) across all roles included: Risk and safety assessments; mechanisms of toxicity and exposure, disposition and biotransformation of toxicants; regulatory toxicology, clinical pathology; target organ systems (nervous, endocrine, kidney, reproductive); chemical carcinogenesis, mutagenesis, and epigenesis; study designs and in vivo methodologies. Notably, the above were ranked consistently across all 12 toxicology professions, with all differences in emphasis being those anticipated within specific professions. Knowledge elements ranked “not needed” by ≤20% or respondents included: History of toxicology, forensics, and specialty-specific techniques. These results provide a robust basis with which to define a SoK stratifying and providing appropriate focus on knowledge elements across current professional toxicology roles, upon which specific components of a certification program can be expressly linked.
Enzymatic Biomarker in Blackjaw Tilapia (Sarotherodon melanotheron) and Bagrid Catfish (Chrysiptpla nigrodigitatus) in the Lagos Lagoon

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The indiscriminate dumping and release of wastes and hazardous substances into rivers might lead to environmental disturbance that could be considered a potential source of stress to the biotic community. A toxicological study was carried out to ascertain the effects of aquatic pollutants in the blood of Chrysiptpla nigrodigitatus and Sarotherodon melanotheron at the Lagos lagoon for a period of six weeks. Four enzymatic biomarkers which include protein, superoxide dismutase, malondialdehyde and Reduced Glutathione and also the physiochemical parameters of the water were analyzed. The water quality parameters were Temperature which has a mean of 24.5±6.5, Dissolved oxygen 1.65±0.05; Biological oxygen demand 10.0±0.1 and pH 7.1. The BOD increases while DO decreases and temperature increases. The water was slightly alkaline. The lipid peroxidation and antioxidant biomarkers in the blood of Chrysiptpla nigrodigitatus showed a significant difference (P < 0.05), while that of Sarotherodon melanotheron showed likewise except, SOD which showed that there was no significant difference (P > 0.05). This study indicates that there was an alteration in antioxidant enzyme and lipid peroxidase activities in both C. nigrodigitatus and S. melanotheron blood which may cause biochemical dysfunction in this species.

Coral Allelochemical Toxicity and the Role of Cytochrome P450 Detoxification in Butterflyfish (Chaetodon spp.) of Differing Feeding Strategies

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Cytochrome P450 monoxygenase (CYP) is the primary enzyme system for detoxification of xenobiotics including dietary chemicals and pollutants. Little is known about the biotransformation and detoxification of allelochemicals derived from dietary products in marine organisms. Certain species of butterflyfish of the genus Chaetodon have been shown to feed on several species of chemically-defended corals including the soft corals Sinularia spp. 5-episinuleptolide (5ESL) is an allelochemical found at high concentrations in Sinularia maxima. This study examined the effects of 5ESL on the expression and catalytic activities of CYP3A and CYP2 in the butterflyfish species Chaetodon unimaculatus, Chaetodon kleinii, Chaetodon auriga, and Chaetodon multicinctus. Fish were gavaged at ecologically-relevant concentrations of 1.0 and 3.0 mg/kg of 5ESL. Testosterone hydroxylase (TOH) 16β was 16 times higher in fish treated with 5ESL. Testosterone hydroxylase (TOH) 16β in control animals was 6 times higher than other hard coral feeding species. These results indicate an induction of CYP2 (16-keto TOH) and CYP3A (6β, 16ß TOH) catalytic activities in detoxification of 5ESL in C. unimaculatus, which preferentially feed on this coral species in Guam.

Transcriptome Analysis of Red Seabream (Pagrus major) Embryos Treated with 2, 3, 7, 8-Tetrachlorodibenzop-dioxin (TCDD)


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TCDD induces a broad spectrum of toxic effects including craniofacial malformation and neural damage in fish embryos. These effects are mainly mediated by the ary hydrocarbon receptor (AHR). However, there still remains a gap of mode of action between AHR activation and adverse outcomes induced by dioxins. To provide a complete picture of the AHR signaling pathway in fish embryos exposed to TCDD, red seabream (Pagrus major) embryos were treated with a graded series of concentrations of TCDD (0.3 - 36 nM) alone in seawater and with a mixture of TCDD and 500 nM of an AHR antagonist, CH223191. The transcriptome of red seabream embryos was analyzed by using our custom-made microarray with 1294 probes that were significantly altered in a TCDD-dose-dependent manner (p<0.03). These mRNA expression levels altered by TCDD exposure were recovered by co-exposure to CH223191, suggesting the mRNA expression of these genes is regulated by AHR. The microarray data further underwent Gene Set Enrichment Analysis (GSEA)-based screening of the KEGG pathway database to identify TCDD-activated pathways. The pathway analyses showed the effects of TCDD on sets of genes related to the regulation of actin cytoskeleton, endocytosis, vascular endothelial growth factor (VEGF) signaling and synaptic long-term depression (LTD) in the embryos. The results suggest that TCDD induces teratogenicity via the dysregulation of actin cytoskeleton and neurotoxicity via VEGF signaling alterations and LTD.

Impacts of Hypersaline Acclimation on the Sublethal Toxicity of Chlorpyrifos to Salmonid Olfaction


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Acclimation to hypersaline environments enhanced the acute toxicity of certain organophosphate pesticides to euryhaline fish species; however sublethal effects have been far less studied. The present study focuses on the sublethal toxicity of chlorpyrifos to salmonid olfaction after hypersaline acclimation. To determine molecular effects of combined exposure, coho salmon were acclimated to three different salinities (8, 16, and 32ppt) for one week and mRNA was collected from the olfactory rosettes. Microarray hybridization was used to determine differences in gene expression for the different salinity treatments. Potential target genes involved in signal transduction, which have been shown to be impacted by chlorpyrifos in zebrasfish, were identified and included chloride interacellular channel 4, guanylate cyclase activator 1A (retina), G-protein (zgc:101761), calcium/calmodulin-dependent protein kinase II delta, and adrenergic alpha 2C receptor. To assess physiological effects, electrotactograms (EOGs) were conducted on rainbow trout acclimated to freshwater and 16ppt with co-exposure to environmentally relevant concentrations of chlorpyrifos (0.5 and 5.0 µL/L). Exposure to chlorpyrifos following acclimation to hypersalinity significantly decreased the response to L-serine and decreased the response to taurocholic acid additively. Gene expression of the five target genes was examined in olfactory tissue from rainbow trout used in the EOG study. Exposure to hypersalinity and chlorpyrifos up-regulated all five target genes. The combined results from the molecular and physiological experiments show that sublethal exposure to chlorpyrifos after hypersaline acclimation negatively impacts salmonid olfaction, which may result from diminished signal transduction in olfactory neurons (NIEHS P30ES0703) and T32 ES018827.

Subthreshold Toxic Effects of Atrazine and Three Degradates on Learning and Behavior in Procambarus clarkii


Atrazine is among the most heavily applied pesticides worldwide, and recent evidence suggests that it may be unsafe at environmental levels. It has been classified as a possible human carcinogen and is an endocrine disruptor. The US EPA Maximum Contaminant Level (MCL) is 3 ug/L for human ingestion and 200 ug/L for limited human exposure. Several environmentally persistent degradates have been identified, including deethylatrazine (DEA), deisopropylatrazine (DIA) and hydroxyatrazine (HA). However, these degradates have no MCL established, although some are suggested toxins. Thus, there remains concern for the risk associated with atrazine and its metabolites in the environment. Little data exist describing sublethal effects of atrazine and its degradates. Fortunately, toxicological research has evolved past dependency on mortality measures to incorporate sophisticated behavioral studies that can elucidate the effects of sublethal exposure to toxins. The goal of this research was to use such parameters to quantify the subthreshold (below the level at which harm is immediately detected) toxic effects of atrazine, DEA, DIA and HA on learning and behavior in Procambarus clarkii (red swamp crayfish), a sensitive biodicator species. Crayfish were placed in an aquatic T-maze (classic method to test cognitive ability) with a food reward in a side arm. Time needed for each animal to locate the reward over repeated trials was recorded, as was time to enter and time spent in each section of the maze. Concentrations of the chemicals tested represented an environmentally realistic range. There was an observed increase in time needed to locate reward for all treatment groups, each taking a minimum mean of 25% longer than control, and most treatment groups had a failure rate (did not find food) at least 2x greater than control. Based on these results, crayfish exposed to atrazine and its degradates at low, biologically relevant doses were lethargic, less explorative, and had impaired learning compared to untreated animals.
Concentrations of Metals Associated with Crude Oil from the BP Macondo Spill in Fish Collected from the Northeastern Gulf of Mexico across a Four-Year Period


Chromium (Cr), nickel (Ni), lead (Pb) and thallium (Tl) were reported by EPA (USEPA, 2010) as components of the crude oil released into the northeastern Gulf of Mexico in the spring and summer of 2010 as a result of the Macondo spill. To investigate the uptake and duration of these metals in the marine food chain, scad Aplodinotus grunniens (Trachurus luscinus) collected in October and November of 2009, 2010 and 2012 were analyzed for metal concentrations. Scad were collected off of the Gulf coasts of Florida and Alabama as part of the yearly NOAA small pelagic trawl survey. Collection sites roughly followed the 200 m contour line. Freeze-dried fish tissue samples were analyzed for the four metals by ICP using USEPA Methods (1994). Tissue levels for each metal were compared among the three collection periods using one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test. Thallium levels were significantly higher in 2010 compared to both 2009 and 2012. Chromium and Ni levels were higher in 2010 than 2009, but the difference was not significant; however, Cr and Ni levels were both significantly higher in 2010 compared to 2012. Surprisingly, Pb tissue levels were significantly higher in 2009 compared to both 2010 and 2012. Our data indicate that during the year of the BP oil spill, levels of three of these metals increased in scad. However, this increase was transitory as two years after the oil release tissue levels had returned to pre-spill values.

An Evaluation of Mancozeb Toxicity on Adult Zebrasfish Gills

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Freshwater ecosystems often become susceptible to various pesticides as a result of crop dusting and/or spraying as well as run-off from agricultural plots. Exposure to these compounds can result in deleterious effects to individual species as well as upset the homeostatic nature of these ecosystems. Mancozeb, manganese-zinc ethylenebis (dithiocarbamate), is fungicide applied to a wide range of crops, vegetables, seeds and ornamental plants, which may enter such ecosystems. Previous studies have suggested that Mancozeb exerts its toxicity through mechanisms related to the induction of oxidative stress. The purpose of this study is to investigate the oxidative stress inducing potential due to Mancozeb exposure in the gills of zebrasfish, Danio rerio. The gills of fish are permanently in contact with environmental water thus becoming a prime target for waterborne toxicants. Adult male zebrasfish were exposed to 0.475, 0.95, and 1.9 mg/L (1.8, 3.6, and 7.2 μM, respectively) of Mancozeb for three hours and then immediately euthanized. Gills were extracted and processed according to each experiment’s protocol. Scanning electron micrographs suggest that Mancozeb causes morphological changes to the epithelial cells comprising the surface of the gills. These changes include the appearance of rounded cells and exfoliation from the basement membrane, when compared to the flattened tightly adjoined cells found on the gills of controls. Glutathione peroxidase (GPx) activity was assessed to investigate antioxidant activity against hydrogen peroxide formation due to Mancozeb exposure. GPx activity showed a significant dose-dependent increase in activity when compared to control.

Environmental Testing Strategy for Pharmaceuticals with Endocrine Disrupting Properties

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Environmental risk assessment (ERA) of human pharmaceuticals with endocrine disrupting properties should be performed irrespective of the quantity released into the environment. It is unknown, however, which ecotoxicity tests are needed and which should be performed first. Currently we are collecting ecotoxicity testing data on the daphnia 21-day reproduction assay, the mossy two-generation toxicity test, the harpacticoid copepod development and reproduction test, the mollusc toxicity test, the fish early-life stage toxicity test, the short term fish reproduction assay, the fish sexual development test, and fish partial/full life cycle toxicity tests. These tests have been validated, or are currently being validated within the OECD test guidelines programme. The fish short-term reproduction assay and the daphnids reproduction test were evaluated by compiling 140 studies (41 chemicals) for fish and 123 studies (85 chemicals) for crustacean. The preliminary results showed: 1) a differential sensitivity in the testing endpoints for both fish and daphnia toxicity tests; fecundity is the most sensitive endpoint, whereas sex ratio is less sensitive, and 2) biomarker responses related to endocrine-mediated effects could be observed in the fish test, but not in the daphnia test. Further analysis will include a comparison of LOECs among daphnids, mysids, copepods, molluscs and various fish tests. By analysing these ecotoxicity data, it will become clear if invertebrate toxicity tests should be performed prior to fish tests, and, if fish tests are then still considered to be needed, if both a fish development toxicity test and reproduction toxicity test have to be carried out. Based on the overall data analysis, we will propose an environmental testing strategy, which is important for minimizing vertebrate testing and costs.

Short-Term Effects of Dechlorane Plus on Earthworm Eisenia fetida Determined by System Biology Approach

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Dechlorane Plus (DP), a chlorinated flame retardant, has been widely detected in environmental matrices, especially in sediment and soil. DP has the characteristics similar to persistent organic pollutants. However, no toxicity data of DP on terrestrial invertebrates are available. In this study, earthworm Eisenia fetida were exposed to 0.1, 1, 10, and 50 mg/kg DP for 14 days. The lethality, oxidative stress and damage, neurotoxicity, and transcriptomic profiles of E. fetida were assessed on day 7 and day 14. Results showed that 50% lethal concentration (LC50) for DP was higher than 50 mg/kg. However, DP exposure could induce an increase of malonaldehyde (MDA) and 8-Hydroxy-2’-deoxyguanosine (8-OHDG) levels, and facilitate changes of acetylcholinesterase (ACHE) activities. High throughput sequencing-based transcriptomic analysis showed that DP exposure significantly altered gene expression and pathways related to antioxidant enzyme, stress response, neurological dysfunction, calcium binding and signal transduction. The results from different toxicological endpoints indicate that DP toxicities on earthworm focus on oxidative damage and neurotoxicity. Based on these results, we deduce that the oxidative stress might be one of mechanism of DP toxicity actions. This study provides an insight into toxicological effects of DP on earthworm, and is useful for the risk assessment of DP on soil ecosystem.

Comparison of Aquatic Toxicity between Silver Nanoparticles and Silver Nanowires

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To better understand the potential ecotoxicological impacts of silver nanoparticles (AgNPs) and silver nanowires (AgNWs) released into freshwater environments, the toxicities of these nanomaterials were assessed and compared using standard OECD guidelines including “Daphnia sp., Acute Immobilisation Test”, “Fish, Acute Toxicity Test”, and “Fish, Freshwater Algae Cytoplasmic Changes, Growth Inhibition Test”. According to estimated median lethal/effective concentrations of AgNWs/AgNPs in the present study, the order of animal susceptibility was: Daphnia magna > Raphidocelis subcapitata > Orzysia latipes. Thus, both AgNPs and AgNWs tested in the current study should be classified according to GHS ( Globally Harmonized System of Classification and Labelling of Chemicals) as “category acute 1” for D. magna, and “category acute 2” for O. latipes and R. subcapitata. The results showed that AgNPs were more toxic when compared with AgNWs. The change in toxicity could be because of the different degree of Ag+ dissolution due to differences in surface area between the sphere shape of AgNPs and the rod shape of AgNWs. In general, our results suggest that the release of silver nanomaterials into the freshwater aquatic environment should be carefully considered.

Effect of Triclosan Challenges on Chlorine Tolerance in Bacteria Found in Waste Water Effluent

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Triclosan is a chlorinated aromatic compound that is used as an antibacterial agent in many household products such as toothpaste and antibacterial hand soaps. Triclosan from these products end up in wastewater discharge. With constant exposure to triclosan, bacterial tolerance is expected to develop in waste water effluent. Because triclosan contains chlorine within its structure, there may be a causal relationship between triclosan exposure and chlorine tolerance. Samples were collected from four rivers in Massachusetts; the Hoosic River (Adams, MA), the Nashau River (Clinton, MA), the French Stream (Rockland, MA), and the Nemasket River (Middleboro, MA). These sites were selected because the effluent
discharge was the first NPDES on each of the rivers, although no triclosan was detected in the water. River samples both upstream and downstream to the effluent discharge were collected. After plating, 288 colonies were isolated from each of the four sites. The NOAEC and LOAEC of triclosan were determined to be 0.001 mg/mL and 0.0001 mg/mL, respectively. The bacteria were exposed to 0.05 and 0.001 mg/mL triclosan or to 50 mg/mL chloroform for 24 hours in an orbital shaker at 30°C and 175 rpm. The bacteria that were exposed to the triclosan were then exposed to 50 mg/mL chloroform for 24 hours in the same conditions specified above. Growth was measured during the triclosan challenges and chloroform exposure using spectrophotometry absorbance at 600 nm. There is no difference in bacterial chloroform tolerance due to waste water effluent (increased tolerance in bacteria upstream and downstream were 17.2% and 12.3%, respectively). Low concentration triclosan challenges increased bacterial chloroform resistance more than high concentration triclosan challenges (9.5% and 1.7%, respectively). Incidence of increased chloroform tolerance in bacteria was independent of sample site (Hoistic: 13.9%, Nashua: 21.2%, French: 11.5%, Nemasket: 12.5%).

In the human health-based risk assessment, the hazard quotient (HQ) for Pb or Cd did not indicate potential for adverse health effects. However, the Hazard Index (HI) for Pb in city (1.06) and riverine (1.46) children, but not adults, indicated potential for adverse health effects. In contrast, the HQs and HIs for Cd were less than 1 and as such did not indicate potential for adverse health effects. The HI for combined Pb and Cd exposure of riverine children (1.13) indicated potentials for non-cancer, but not for cancer health risks. In conclusion, Pb and Cd are bioconcentrated in various tissues of local Nigerian fish to considerable levels, with the potential for non-carcinogenic health effects mainly from exposure to Pb in human receptor groups.

**Textile Dye Reactive Blue 15 Reduces the Reproduction in Ceriodaphnia dubia**

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Introduction: Dyes are widely used in different types of industries, such as textile, pharmaceutical, food, cosmetics, photographic, among others. However, these substances can be toxic, mutagenic and resistant to many degradation processes used in wastewater treatment. It is estimated that about 15% of the dyes used in the world are lost during the dying process and released into the environment, affecting mainly water bodies. However, despite the large amount of commercial dyes available and high quantity released in the aquatic ecosystem, studies about the toxicity of these substances are scarce and little is known about their mutagenic and ecotoxicological effects. Objectives: The aims of this study were to evaluate the eco-toxicity and genotoxicity of the textile dye Reactive Blue 15 (RB15). Methodology: The ecotoxicological assays with *Daphnia similis*, *Vibrio fischeri* and *Ceriodaphnia dubia* were realized according to ABNT NBR12713:2009, ABNT NBR15411-3:2012 and ABNT NBR 13373:2010, respectively. The Comet assay was carried out according to Tice et al. (2000) and Salmonella mutagenicity assay was carried out according to Maron and Ames (1983) and Mortelmans and Zeiger (2000). Results: The RB15 dye was relatively non-toxic to *Daphnia similis* and reduced the *Vibrio fischeri* luminescence in high concentrations. The RB15 dye reduced fecundity of *Ceriodaphnia dubia* and was not mutagenic and genotoxic to human dermal fibroblasts. Discussion: RB15 did not induce acute toxicity but in chronic exposure the dye reduced the reproduction in *C. dubia*. This decrease can be harmful to the aquatic organisms. This dye did not induce DNA damages in the tested conditions. Conclusion: The results shows that the dye can cause adverse effects on organisms even at low concentrations in chronic exposure and that the continuous release of these substances in water bodies is worrying.

**Assessment of the Expression Levels of Genes Involved in Metal Detoxification in Killifish Inhabiting a Closed Copper Mine Superfund Site**

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The Callahan Mine Superfund site in Brooksville, ME is a closed open-pit copper mine and is currently flooded with seawater. The sediments at this site are contaminated with extremely high levels of metals such as cadmium, lead, and copper, and once introduced, killifish (*Fundulus heteroclitus*) inhabiting this Superfund site are constantly exposed to these metals. A survey has shown that killifish collected from the site have significantly higher metal concentrations in tissues compared to fish collected from a nearby reference site. Therefore, we hypothesized that killifish from the Superfund site have elevated expression of genes involved in antioxidant defense induced by metals, and metallothionein (MT), a protein involved in metal detoxification. To test this hypothesis, we collected fish from three different locations within the Superfund site, as well as from a nearby reference site. Gills and liver were isolated for gene expression analysis from twelve individuals at each location. Expression levels of five oxidative stress response genes (nuclear factor erythroid-related factor-2 (Nrf2), glutathione-S-transferase-alpha (GSTa), gluta- mate cysteine ligate catalytic subunit (GCLc), Mn-superoxide dismutase (SOD2), and catalase) and MT were measured by Q-RT-PCR. In addition, tissues were analyzed for metal content. Tissue samples collected from the Superfund site had significantly high levels of cadmium and copper. Surprisingly, MT mRNA levels were not different in the gills and livers of fish collected from the Superfund site compared to the reference site. In addition, none of the oxidative stress response genes showed significant differences among the collection sites. Therefore, constant exposure to extremely high levels of metals does not seem to affect the expression of genes involved in metal detoxification pathways. Examination of protein expression of these genes will provide more information on whether killifish at the Callahan Mine Superfund site have adapted to high levels of metals in the environment.
Puerto Rico currently has more than 250 non-PRASA water systems, which are water systems that are not serviced by the Puerto Rico Aqueduct and Sewer Authority (PRASA). These non-PRASA systems have limited resources for water management and are often non-compliant with the Safe Drinking Water Act. The majority of these systems are located on the rural part of the island, which are characterized by areas of increased poverty. Due to their lack of resources they are often unable to administer the proper treatments and testing for the water systems. This can be potentially hazardous to these communities since poor water quality can result in the proliferation of diseases and exposure to toxic compounds. Our goal was to use geographic analysis to determine the risks of these systems for water contamination. To evaluate how geographic factors could shape the water quality of non-PRASA systems, we (1) organized the database on water quality from the Puerto Rico Health Department for 92 non-PRASA systems, (2) defined the watershed area for each system using ArcMap, (3) evaluated the human activities within the watersheds. We found that water systems that were located near areas with high agricultural activity had the highest nitrate concentration, whereas areas that were within 500 m of a conservation area had the lowest nitrate levels. Our results also suggest that multiple variables can influence water quality, including land cover distribution, environmental factors, and socio-economic factors. These results have important policy implications for promoting forest conservation in non-PRASA watersheds or for connecting non-PRASA community members to PRASA systems in regions where watersheds are highly impacted.

**Pesticide Distribution Pattern in Surface Water of a Mountainous Region River from Rio de Janeiro, Brazil**

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Parasquat (PQ) is an herbicide widely used in several crops as a desiccant. Due to its moderate toxicity, the fate of PQ in the environment is of great concern. In the mountain region of Rio de Janeiro, where PQ is intensively used, the river water is fed for irrigation and recreation but not directly as drinking water source. However, it’s of interest to public health to evaluate the concentrations of such compound, since the local population consumes water from the fountain near the river. The objective of this present study was to assess the concentrations of PQ in a river of an intensive agricultural area during all application seasons in Rio de Janeiro State, Brazil. PQ was measured in river water collected from 7 sampling sites totaling 80 samples during 12 sampling events between 2010 and 2012 in the São Lourenço River in Nova Friburgo-RJ, Brazil. PQ levels were determined by an enzyme-linked immunosorbent assay (ELISA) QuantiPlate™ Kit with a limit of quantification (LOQ) of 0.02 ppb. The pesticide was detected in measurable quantities in all samples. The last sampling site, where the volume of water is bigger, showed as most frequently contaminated with a median of 0.048 ppb (maximum 0.279 ppb). Pluviosity is an important element and the Spearman correlation showed that concentrations of PQ in river water vary with the monthly rainfall (R=0.682; p=0.015). The highest median concentrations and rainfall correlation were found in last site sampling (R=0.771; p=0.003). All others sites showed positive correlations, except for the first one that is the control site located in river springs in the mountain that showed a inverse correlation. Although the found concentrations were below of the limits established by regulatory agency (maximum 20 ppb), our results showed that the use of parasquat in this region impacts the river health, especially after a period of rain.

**Degradation of Tumor Promoter Activity of Jatropha curcas Phorbol Esters Derived from Jatropha Oil Cake in Soil**

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Background: Large amount of oil cake is generated during production of biodiesel fuel from Jatropha seeds. Although Jatropha oil cake is rich in plant nutrients, the presence of toxic phorbol esters limits the usage of oil cake as fertilizer. The objective of this study is to evaluate the contents and tumor promoting activity of phorbol esters by plants and Jatropha oil cake-supplemented soil.

Method: Contents and biological activity of Jatropha phorbol esters in soil and plants were sequentially analyzed by high-performance liquid chromatography (HPLC) and in vitro cell transformation assay, respectively.

Results: Jatropha phorbol ester-specific HPLC peaks disappeared after 5-week incubation with soil. Along with the degradation of Jatropha phorbol esters in soil, tumor promoter activity of Jatropha oil cake-supplemented soil was dramatically attenuated and finally disappeared. Jatropha phorbol esters and tumor promoter activity were not detected from mustard spinach cultured with Jatropha oil cake-supplemented soil.

Conclusions: These data showed that (1) contents and tumor promoter activity of Jatropha phorbol esters in oil cake were completely lost by 5-week incubation with soil and (2) Jatropha phorbol esters did not transfer into plants cultured with Jatropha oil cake-supplemented soil. Therefore, these results suggested that that Jatropha phorbol ester in oil cake could be degraded by indigenous bacteria and that Jatropha oil cake is useful as a safe fertilizer.
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PS 669b Degradation of the Major Toxic Component of Jatropha curcas Phorbol Esters by Enzymes and Sterilized Water

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We found that toxic Jatropha phorbol esters are degraded in Jatropha-oilcake-supplemented soil in 5 weeks. This study demonstrated degradation of the major toxic Jatropha phorbol esters by lipase and esterases in buffer and in sterilized water. Main toxic of Jatropha phorbol esters (DHPB) was purified and analyzed by high-performance liquid chromatography (HPLC) and its biological activity was tested in an in vitro cell transformation assay. DHPB was incubated with various concentrations of Lipase from Aspergillus niger (AL), esterases from Klebsiella oxytoca (SNSM-87) and Acinetobacter calcoaceticus (KM-109) in buffer and in sterilized water for 5 weeks at the optimum temperature and pH. DHPB degradation products were analyzed by HPLC and cell transformation test. The optimum temperature, pH and the enzyme concentration were similar among AL, SNSM-87, and KM-109; 37°C, 6.5-8.5 and 50-1000 μg/ml, respectively. Following 4-weeks incubation of DHPB in sterilized water or phosphate buffer (pH7.0), DHPM-specific HPLC peaks decreased as much as 50% time-dependently. By addition of KM109 to these solutions, DHPB-specific HPLC peak was markedly decreased to about 65% on Day 7. The loss of DHPB reached to 95% at Day 28. However, following incubation with AL or SNSM87 and DHPB for 4-weeks showed similar decay of DHPM degradation as in the buffer or sterilized water alone. Retention times for DHPB degradation components in the HPLC analysis were different between the samples with SNSM87 in sterilized buffer or water alone at Day 7 and 28. The loss of cell transformation activity was similar to decrease of DHPB. These data showed that contents and tumor promoting activity of main Jatropha phorbol ester were decreased in the presence of water. Degradation of DHPB was promoted by esterase in water or spontaneous ester hydrolysis. Therefore, these observations suggested that Jatropha oil cake may safely be used as fertilizer as Jatropha phorbol esters in oil cake is degraded quickly by together with indigenous bacteria in water rich soil. This study was supported by JST/JICA, SATREPS, Japan.

PS 669c Preliminary Study on the Effect of Weathered Oil and Oil Dispersants on Menidia beryllina Embryo

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Environmental stress can affect an organism at any stage of life, but developing larvae are particularly susceptible. Exposure of fish embryos to relatively low concentrations of oil has been implicated in sub-lethal toxicity. However, the effect of oil and commonly used dispersants; singly and in combination should be more thoroughly evaluated to better understand and anticipate the ecological impacts. Oil (1μgml and 0.1μgml dispersants (Corexit 9500 and 9527) were weathered singly and in combination in 25 ppt saltwater for 7 days in bottles with vortex in a hood. The aqueous part post weathering (Weathered solution, WS), was diluted at 1:5 with standard EPA methods (8081a, 8082, 6020, respectively). The Kaplan-Meier method was used to estimate the means and SD for sample sets that included observations below the detection limits (i.e., non-detects). The persistent organochlorine 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) metabolites DDD and DDE and α- and γ-chlordanes were the most detected chemicals in the fish samples. Significant positive correlations with age (p<0.05) occurred with DDE, α-chlordane, and γ-chlordane in liver tissue; arsenic in gut, and copper in liver. There was no correlation between DDD or DDE concentrations in tissues (gut, liver, muscle); however, there were significant correlations between cadmium concentrations in gut and liver, chromium concentrations in liver and muscle, lead concentrations in gut and liver, and zinc concentrations in liver and muscle. These data provide a better understanding of environmental contaminant levels that are present and bioaccumulated into a long-lived benthic fish species from the nation’s largest river system.

PS 669d Contaminant Burden Analysis in Wild Shovelnose Sturgeon (Scaphirhynchus platyrhynchus) in the Lower Mississippi River

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Long-lived benthic animals experience repeated and/or prolonged exposures to sediments and their associated anthropogenic contaminants. As a result, these animals are at risk of elevated contaminant burdens. The overall objective of this study was to determine baseline data for contaminant burdens in wild sturgeon in the lower Mississippi River watershed. A field collection on the Mississippi River near Ft. Adams, MS in January 2010 resulted in the collection of shovelnose sturgeon, a surrogate for the endangered pallid sturgeon (Scaphirhynchus alatus). Sturgeon (n=13, 4-10 years old) were euthanized, and relevant tissues (liver, muscle) and gut content were analyzed for chlorinated pesticides, PCBs, and metals according to standard EPA methods (8081a, 8082, 6020, respectively). The Kaplan-Meier method was used to estimate the means and SD for sample sets that included observations below the detection limits (i.e., non-detects). This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), Japan.

PS 669e Comparison of Cyropreserved Trout Hepatocytes and Liver S9 Fractions As In Vitro Models for Predicting Hepatic Clearance in Fish


In vitro measurements of chemical clearance can be extrapolated to the whole liver and used to improve modeled predictions of bioaccumulation in fish. Fish hepatocytes and liver S9 fractions are two systems commonly used to measure in vitro intrinsic clearance (CLin vitro, int; ml/h/mg protein or 106 cells). Previously, trout S9 fractions were used to measure CLin vitro, int for six polycyclic aromatic hydrocarbons. CLin vitro, int were then extrapolated to the intact liver and compared to measurements of hepatic clearance (CLH; ml/h/liver) by isolated perfused trout livers. In the present study, the same approach was used to evaluate CLH predictions obtained from cryopreserved trout hepatocytes. With one exception, extrapolations of CLin vitro, int to in vivo intrinsic clearance (CLin vivo, int; ml/h/liver) using scaling factors (510 x 106 hepatocytes/g liver, 163 mg S9/g liver) resulted in similar predictions for the two in vitro systems (±2 fold difference). For the test chemical benzo[a]pyrene, the CLin vivo, int predicted from hepatocyte data was nearly 10 fold lower than that obtained from the S9 system. CLin vivo, int values were then used as inputs to a well-stirred liver model, which accounts for flow limitations on clearance as well as potential binding effects. To facilitate this comparison, chemical binding in vitro and in solutions used to perfuse isolated livers were measured using solid phase microextraction. The resulting predictions of CLH were in good agreement with measured values from intact livers (<2 fold difference for all but naphthalene), as well as earlier predictions obtained using the S9 system. This agreement can largely be attributed to the fact that CLin vitro, int rates in both in vitro systems often approached the flow rate of the isolated liver system. These findings suggest that both in vitro systems provide comparable predictions of hepatic clearance, which can be used to improve bioaccumulation assessment for fish.
Hemp Seed Flour Impacts the Development of Tenebrio Larvae and Their Susceptibility to Organophosphorous Insecticides

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Previous research studies have shown that hemp seed products can alter the development of insects. Considering hemp seed’s effect on some developing insects, Tenebrio larvae were raised on hemp seed flour to determine any developmental dysfunction as well as possible susceptibility to insecticides such as chlorpyrifos, an organophosphorous insecticide. Three groups of larvae were tested; each group was raised on different amounts of hempseed flour (100% hemp seed flour, 0% hemp seed flour/100% wheat flour, and a mixture of hemp seed and wheat flour). Larvae from each group were exposed to chlorpyrifos using a dip technique. Larvae raised on hemp seed flour were more susceptible to chlorpyrifos based on LC50 comparisons. In addition, decreased acetylcholinesterase (AChE) activity was also demonstrated in larvae raised on hemp seed flour. Results indicate that hemp not only disrupts normal development in Tenebrio larvae but also increases susceptibility to organophosphates.

Two Insecticidal Neurotoxic Mechanisms of Action of Monoterpenoids

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Monoterpenoids from plant essential oils have been shown to elicit neurotoxic symptoms in insects, including rapid onset of tremors and loss of coordination, followed by death if a sufficient dose is applied. Many are “soft” insecticides that are much less potent than conventional insecticides but are very selectively toxic to insects and not to vertebrates. We have studied two specific neurotoxic mechanisms of action with respect to the actions of selected monoterpenes on insect neuroreceptors. GABA-gated chloride channel receptors from house fly were utilized for binding studies with reitaited-TBOB which binds at the picrotoxin binding site at that receptor-ionophore complex. Also microsacs were prepared from dissected cockroach ventral nerve cords; the effect of monoterpenes on GABA-gated chloride channel function was evaluated using 36Cl-uptake assays. Studies on binding at the house fly nicotinic acetylcholine receptor were conducted using a 14C-nicotine as the radioligand; when the phenolic monoterpenoid carvacrol was added to the preparation, it reduced binding of the nicotine in a concentration-dependent manner, at low micromolar concentrations.

Evaluation of At-Home Methods to Reduce Pesticide Residue on Apples and Peaches

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The diet is a significant route of pesticides exposure to the general public. Nevertheless, public health guidelines to reduce exposure by cleaning food are generally based on anecdotal reports. The objective of this study was to evaluate different methods that could be performed at home to reduce consumer pesticide exposure from apples and peaches. All treatments studied were safe to the general public, did not structurally alter the fruit, and used products that could be readily found in the home or grocery store. Fruit was obtained from the Rutgers University Extension Farm which supplied information on the pesticide applications. In this study, captain, fenpropatrin, and phosmet were quantified by gas chromatography/mass spectrometry. Mean captan and phosmet levels on peaches were 72.7±42.7 μg/fruit and 18.1±6.4 μg/fruit, respectively; while captan, fenpropatrin, and phosmet levels on apples averaged 41.9±23.5 μg/fruit, 16.7±7.6 μg/fruit, and 12.2±6.9 μg/fruit, respectively. Captain and phosmet residue were reduced by approximately 50% or more on apples and peaches by washing and wiping for 15 seconds with a homemade soap solution or a vinegar and lemon juice solution and then rinsing with water. In general, the commercially available fruit wash reduced captan and phosmet concentration better than the other treatment methods studied. On peaches an 85% and 63% reduction in captain and phosmet was observed while captain and phosmet residue was reduced 55% and 52% on apples. Compared to rinsing with water alone, the homemade and commercial fruit wash reduced pesticide levels greater, most likely due to the lipophilic nature of the pesticides. However, fenpropatrin levels present on ripened apples were not reduced by any of the washing treatments studied, possibly due fenpropatrin’s high octanol water partition coefficient. Some, but not all pesticide can be removed from fruit by washing and wiping. While washing and wiping of fruit is recommended, more effective methods are still needed to reduce dietary pesticide exposure. This work was supported by NIEHS ST32ES019854 and P30ES005022.

Toxicity and Safety Evaluation of Etofenprox, S-Methoprene, and Piperonyl Butoxide in Dogs Topically Exposed to Bio Spot Defense®

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Currently, pyrethroids are more commonly used than any other class of ectoparasiticides on pets. Due to lack of safety data, pet owners have raised serious concerns for their use on dogs and cats. Concerns have also been raised about transferable residues of pesticides to the pet owners, veterinarians, veterinary technologists, and dog handlers. The present investigation was therefore undertaken with two specific objectives: (1) to determine the safety of Bio Spot Defense Flea and Tick Spot On® applied to six dogs, and (2) to determine the residue of active ingredients of the product (etofenprox, s-methoprene, and piperonyl butoxide) in blood of dogs and gloves worn to pet dogs at 24, 48, 72 h, and 1, 2, 3, 4, and 5 weeks post-application. At these time intervals, blood was evaluated for physical examination. Residues of active ingredients were determined and confirmed using GC/MS. In the blood, etofenprox was detected as early as after 48 h (18.4±5.1 ppm) and the residue persisted until 1 week (0.8±0.4 ppm). S-methoprene and piperonyl butoxide were not detected in the blood at any time. In the gloves, the highest concentrations of etofenprox, s-methoprene, and piperonyl butoxide were determined at 24 h (9.552.0±1515.8; 2307.9±456.7; and 1286.1±150.5 ppm, respectively). Residues of all three compounds were detected in appreciable concentrations in the gloves until 1 week (294.9±27.2; 80.6±10.1; and 40.5±8.8 ppm, correspondingly). Of course, with a steep descending trend, residues of these compounds were present 5 weeks post-application. Findings of this investigation revealed: (1) none of the dogs exhibited any physical or behavioral abnormality, and (2) active ingredients of the product persisted on dog’s coat for five weeks.

Determination of Paraquat (PQ) in Urine Using Reversed-Phase HPLC/DAD

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Paraquat (PQ) is an extensively used herbicide in agricultural area, and is highly toxic to humans, having been implicated in many diseases among rural populations. The aim of this study was to develop and validate a method to quantify...
PQ in urine. The sample preparation is simple where 1mL of sample is adjusted to a basic pH with 5mL of sodium hydroxide solution (0.1N). And the method employed a solid phase extraction using Sep-Pak C18 cartridge and a high-performance liquid chromatographic system consisting of a model 1200 binary pump with DAD detector coupled. The gradient program was done with methanol and water passed through the SPE column. The extraction phase requires vacuum manifold, high temperature (40°C) and nitrogen stream to evaporate efficiently the eluate. The separation technique uses an octadecyl–sila column with an eluent of 10% acetonitrile (v/v) containing 5.0 mM sodium 1-heptanesulphonic acid and 0.2 M orthophosphoric acid (pH 3.0). After derivatization with internal standard (IS), is eluted at 40°C at a flow-rate of 1.0 mL/min monitored by UV absorption at 258 nm. The accuracy of the method was evaluated by recovery studies using spiked pull of urines. The recoveries of PQ were 74–101%. The calibration curve for PQ showed excellent linearity in the range of 1 – 10 ppm as judged by the coefficient of determination (R=0.983). The detection limit (with SN(5-3)) was 0.3 ppm with an injection volume of 100 μL. And determination limit (with SN(10)) was 1.1 ppm. The intra- and inter-day precision, expressed as the coefficient of variation, for PQ in urine samples was not greater than 13.0%. PQ was sensitively and selectively determined and quantified by HPLC/DAD from urine samples. The present method is promising for identification and quantification of PQ and can be suitable for epidemiological surveys.

Environmental chemical exposures may contribute to the genesis and progression of non-alcoholic fatty liver disease (NAFLD). However, a comprehensive list of these chemicals has not been generated. This study evaluates two large rodent exposure study databases for environmental chemicals which caused NAFLD in rodents. ToxReDB is a database designed by the National Center for Computational Toxicology (NCCCT) and Environmental Protection Agency’s (EPA) Office of Pesticide Programs (OPP) containing animal toxicity testing filed during pesticide registration. 474 rat/mouse studies were queried for histologic NAFLD descriptors. These chemicals were associated with NAFLD pathologic descriptors. The 42 compounds included 22 fungicides, 13 herbicides, 6 insecticides and 1 miticide. From CEBS, 329 studies of 83 unique chemicals reported positive NAFLD descriptors. These chemicals included 12 pesticides/intermediates; 6 dioxin-like molecules; 17 plastics, polymers, synthetic; 19 textiles and resins; 16 solvents and chemical intermediates; 7 paints, polishes and dyes; 10 fragrances, cosmetics and essential oils and 15 miscellaneous chemicals. 371 studies archived in federal databases linked 125 unique environmental chemicals to NAFLD in rodents. Pesticides composed 43% of these chemicals. Furthermore, nearly 10% of pesticide registration toxicity studies reported the development of NAFLD. The potential role of the identified chemicals, and in particular pesticides, in human NAFLD should be investigated.

Dyslipidemia occurs in type 2 diabetes mellitus (T2D). During dyslipidemia there is increased production of lipids from the liver including apolipoprotein B (Apo B) containing very low density lipoproteins (VLDL) and low density lipoproteins (LDL). There is an increasing epidemiological association between diabetes and higher levels of serum organochlorine compounds (OC). OC exposure was found to be a predictor of dyslipidemia in diabetes and non-diabetes. The liver is a target for OC metabolism and plays a role in dyslipidemia and T2D. Therefore, the effect of a group of select OC on the dyslipidemic risk factor, Apo B, in liver cells was investigated. McArdle-RH7777 (McA) rat hepatoma liver cells were treated with 0.4 mM oleic acid (OA) for 16 hr to induce Apo B secretion. McA cells were exposed to 10 μM dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), dieldrin, or chlor dane simultaneously with 0.4 μM OA for 16 hr to induce Apo B secretion. McA cells were exposed to 1, 10, or 100 μM DDE simultaneously with 0.4 mM OA for 16 hr to investigate a dose-response of Apo B secretion. Immunoblotting of lipoproteins secreted into the cell media yielded significantly higher levels of secreted Apo B for exposed versus non-exposed liver cells. Immunoblotting of cell lysates revealed that intracellular Apo B levels were significantly different for OC and OA induced cells compared to non-induced cells. The OC exposed cells exhibited a trend of higher Apo B protein levels than OA induced cells. These results suggest a role of OC in lipid transport and secretion of Apo B containing lipids rather than in Apo B synthesis that is induced by lipogenic fatty acids such as OA. Based on these results, OC exposure may be associated with T2D by increasing a dyslipidemic risk factor of T2D.

INTRODUCTION: The application of pesticides, particularly herbicides, in agriculture, is a well established practice and effective to control weed. However, due to the length of agriculture, large amounts of herbicides have been used and they may influence non-target organisms by several mechanisms. Trifluralin (α,α,α-trifluoro-2,6- dichloro-N,N-dipropyl-p-toluamide) and Tebuthiuron (1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea) are herbicides widely used in Brazil. The present work aimed to analyze the effects of this two herbicides, trifluralin and tebuthiuron, in HepG2 cell model. METHODS: Nuclear condensation and fragmentation was assessed by microscopy using the fluorescent dye Hoechst 33342, and phosphatidyl serine exposure on the outer cell membrane was performed using annexin-V / propidium iodide assay. Concentrations from 1 through 100 μM of each herbicide dissolved in dimethyl-sulphoxide was analysed. All experiments were performed in triplicate and statistical analysis were done using ANOVA test followed by Dunnett. RESULTS: Trifluralin showed significant membrane condensation and fragmentation as well as positive marking for annexin-V at concentration of 100μM indicating the induction of apoptotic cell death. On the other hand, Tebuthiuron did not affect these parameters at any of the tested concentrations. CONCLUSION: Our results show that trifluralin but not tebuthiuron is cytotoxic to HepG2 cells and that trifluralin induces the apoptotic pathway. Supported by: FAPESP - Proc. 2012/15220-3 “The opinions, assumptions, and conclusions expressed in this material are those of the authors and do not necessarily reflect the views of FAPESP”

p,p′-DDE Enhances Adipogenesis in 3T3-L1 Adipocytes and Alters Cyclooxygenase-2 (COX-2) Activity in J774a.1 and THP-1 Macrophage Cells

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Exposure to p,p′-DDE, a metabolite of p,p′-DDT, is associated with obesity, dyslipidemia, insulin resistance and incidence of metabolic syndrome. DDE has been shown to have immunomodulatory properties, affecting macrophage and T cell populations. Obesity leads to macrophage cell infiltration into the adipose tissue (AT) causing local inflammation. Potential mechanisms were studied by which DDE could modulate adipocyte and immune cell function and facilitate an increased risk of obesity and immune dysregulation, potentially through COX-2. Human THP-1 and murine J774a.1 cell lines and 3T3-L1 preadipocytes were studied for the effects of DDE on prostaglandin (PG) production and adipogenesis. Macrophage cell lines were exposed to 20μM DDE or 10μM NS398, a specific COX-2 inhibitor, for 18hr before treatment with an inflammatory challenge of 0.25μg/ml lipopolysaccharide (LPS) or 200μM palmitic acid (PA). PGE2, PGD2, PGF2α, and arachidonic acid (AA) were analyzed in cell culture supernatants. The effect of DDE or NS398 on COX-2 activity was also measured indirectly in a cell-free system through addition of AA to cell lysates of LPS (1μg/ml) stimulated macrophages. In J774a and THP-1 cells, DDE or NS398 followed by immune challenge reduced cellular PG secretion and PG production in a cell free system, suggesting that DDE may interfere with COX-2 activity. 3T3-L1 cells were induced to differentiate to adipocytes using a sub-optimal differentiation cocktail with increasing concentrations of DDE (0.5-100μM). DDE increased adipogenesis in a dose-dependent manner in mature adipocytes as determined by Oil Red O staining. As PGE2 and PGF2α inhibit adipogenesis, decreased PG production by AT macrophages may lead to increased adipogenesis and contribute to development
of obesity. These results suggest that DDE exposure may contribute to increased adipogenesis and altered PG production, potentially mediated through a COX-2 dependent mechanism.

680 The Toxicity of Maneb in Human Colon Cells May Be Related to the Transchelation of the Metal Moiety and the Organic Backbone

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Ethylene bisdithiocarbamate (EBDC) pesticides are used as broad range contact fungicides on a variety of crops. A subset of the metal containing EBDC pesticides includes Maneb (MB), which is complexed with the transition metal manganese (Mn). Previous testing in our laboratory has established the toxicity of this compound in transformed colon cells, HT-29 and Caco2. Significant decreases in cell viability were observed in both HT-29 (120-260μM) and Caco2 cells with MB (40-180μM). Exposure also resulted in a dose dependent increase in intracellular Mn levels within HT-29 and Caco2 cells (20-200μM). Recent scientific studies have suggested that Mn exposure may result in increases in copper (Cu) concentrations within the brain. Therefore, the purpose of the present study was to determine if MB exposure results in an increase in copper levels within HT-29 and Caco2 cells. Following exposure to MB in HT-29 (100-200μM) and Caco2 cells (20-60μM), inductively coupled plasma-optical emission spectroscopy was performed. MB exposure resulted in a significant accumulation of Cu within HT-29 and Caco2 cells (40-200μM). These data suggest that the Mn moiety of MB may transchelate with Cu and play a role in the generation of toxicity. To investigate whether Cu played a role in the cytotoxic process, cells were treated with Cu chloride (CuCl₂). Exposure to CuCl₂ produced no significant decrease in cell viability in all cell types up to 200μM. To investigate the role the EBDC backbone plays in the generation of toxicity, HT-29 and Caco2 cells were treated with Nabal (NB), an EBDC pesticide that contains the identical core organic component of MB but has sodium in place of Mn. Significant decreases in cell viability were observed with NB in HT-29 (400-3200μM) and Caco2 cells (200-3200μM). The lack of toxicity observed following treatment with NB within the same concentration range as MB suggests that the EBDC backbone and Cu moiety may be acting in conjunction to cause toxicity in human colon cells.

682 Rynaxypyr Is Active but Significantly Less Potent toward Ryanodine Receptor Type 1 from Wild Type and Malignant Hyperthermia Susceptible Mammalian Skeletal Muscle

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Rynaxypyr is an antranilic diamide that represents a major emerging class of insecticide widely used to eradicate Lepidopteran agricultural pests. While Rynaxypyr is a potent activator of ryanodine receptors (RyR1) that are expressed in Leptidopterous skeletal muscle, its activity towards RyR1, which is expressed in adult mammalian skeletal muscle, has been less studied. Considering that RyR1s in insect and mammalian species perform similar roles in regulating intracellular Ca²⁺ release from sarcoplasmic reticulum (SR) during physiological and pathophysiological processes of skeletal muscle, we explored if Rynaxypyr was devoid of activity towards adult mouse RyR1, even at high (~1 μM) concentrations. For these initial studies we prepared skeletal muscle SR from congenic C57BL/6j wild type mice and mice that are homozygous for a human malignant hyperthermia susceptibility mutation in the C-terminal region of RyR1 (RyR1-T4826I). These SR preparations were subjected to radioligand-receptor binding analysis with [3H]ryanodine (5 nM) in the absence or presence of increasing concentrations of Rynaxypyr. As previously reported (Barrientos et al J Biol Chem 287:2865), RyR1-T4826I was found to be constitutively more active than wild type RyR1. Rynaxypyr > 10 μM significantly activated RyR1 found in both wild-type mouse muscle and in mouse muscle homogous for the T4826I-RyR1 mutation. These results suggest Rynaxypyr is capable of altering Ca²⁺ dynamics mediated by RyR1 in both wild type and malignant hyperthermia susceptible mammalian muscle. There is a need to better understand how antranilic diamides alter the function of mammalian RyR1 and RyR2 given these receptors are known to express mutations that contribute to environmentally triggered disorders of skeletal and cardiac muscle. [Supported by F42 ES04699]

683 Quantiﬁcation of the Pyrethroid Deltamethrin (DLM) in Rat Liver and Muscle Using Gas Chromatography Negative Chemical Ionization Mass Spectrometry (GC-NCl-MS)

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High quality trace analysis of pyrethroids in biological samples is necessary for toxicokinetic studies for risk assessments. To date highly sensitive methods to measure pyrethroids have focused on large environmental samples (water, soil, food) or urine. There are no validated methods in the literature for quantifying low concentrations of this class of compounds from small (100 μL) liver and muscle samples. DLM was extracted from homogenated liver and muscle samples by protein precipitation using acetonitrile and phosphoric acid. The samples were vortexed and centrifuged before evaporation to dryness. Samples were reconstituted and DLM extracted from the residue using toluene prior to injection into an Agilent Model 6890N gas chromatograph equipped with a Model 5973 quadrupole mass analyzer. Samples were ionized via electron capture in the negative ion mode using methane as a chemical ionization gas. Fragment ions were monitored using selected-ion monitoring for quantification and verification of the analyte. Cis-permethrin was used as the internal standard. The method was linear from 0.3 ng/mL to 1,000 ng/mL and the intraday precision and accuracy of the method was better than 20% at the LLOQ and better than 15% across the linear range (n=18). The method was applied to monitor tissue DLM concentrations following oral dosing of adult rats with 1 to 5 mg/kg DLM in corn oil. Liver DLM levels declined more rapidly than muscle levels. DLM in liver and muscle could be accurately measured for up to 24 hours following dosing. A novel GC-NCl-MS method for the determination of DLM from 100μL liver and muscle samples was successfully developed and validated by FDA guidelines.
organophosphates (OPs) are some of the most commonly-use pesticides worldwide. Paraoxonase 1 (PON1) is a calcium-dependent, HDL-associated enzyme capable of hydrolyzing OPs and is protective against specific OPs in vivo. Human PON1 has two common coding polymorphisms, L55M and Q192R. The Q192R polymorphism affects catalytic efficiency towards specific substrates. Rabbit PON1 is more stable than human PON1 which has been suggested as a therapeutic catalytic scavenger for OP exposures. We have developed an E. coli system for expressing and purifying untangled native human and rabbit PON1s and variants. The two native 192 alloforms (Q and R) were expressed and purified as well as a human PON1 variant with lysine at the 192 position (rHuPON1K192). Several variants with polymorphisms homologous to rabbit PON1 were computationally predicted to have increased stability and higher affinity calcium binding. These were also generated on the human rHuPON1K192 protein backbone. Rabbit PON1 and variant recombinant rabbit PON1Q192 (rAbPON1Q192), which is predicted to have increased activity against nerve agents, were also expressed and purified. The rHuPON1K192 variant had increased OP catalytic efficiency relative to the native human PON1s. PON1K192 was tested in a knockout mouse model and found to protect brain cholinesterase from the OP diazoxon. Mutants predicted to have increased stability were tested by heat inactivation and EDTA inhibition to determine stability and calcium binding affinity. The L130M and S67A variants, analogous to rabbit PON1 at these positions, had increased resistance to heat and EDTA, which suggested that these variants may be a good alternative for therapeutic use.

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Carbendazim is a fungicide with a mitotic spindle inhibitor mode of action. Bolus gavage dosing of carbendazim to rats in prior studies has led to testicular toxicity. Dietary studies did not confirm this effect. Many prior studies used test substance with low or unknown purity and/or failed to assess potential intergenerational effects. An F1 extended two generation reproductive toxicity study of carbendazim (> 98% pure) was conducted in Wistar rats at dietary concentrations of 0, 250, 1000 or 2000 ppm. Potential testicular effects were assessed in adult parental (P) (30/dose) and F1 generation males (two cohorts, 1a and 1b), and in juvenile F1 and F2 rats. Testosterone levels (P and F1 1b), sperm parameters, reproductive organ weights and histopathology including testicular staging were assessed in adult males. Juvenile F1 and F2 males were assessed for testes weight and F1 males for testes histopathology. Reproductive performance was assessed for the P and F1 males (both F1 cohorts). Testicular findings, considered non-adverse, were limited to slight but statistically significant decreased absolute testes weights in the adult F1 cohort 1a only at 1000 and 2000 ppm, with no effect on testes weight relative to body weight, and no effect in P or F1 cohort 1b males. Statistically significant higher testicular sperm counts were noted at 1000 ppm in F1 cohort 1a and at 2000 ppm in both F1 cohorts. There were no effects on testosterone levels, epididymal sperm count, sperm motility, progressive sperm motility, percent or type abnormal sperm, and no exposure related testicular or epididymal histopathology in adult males. Juvenile testes weights and histopathology were not affected. There was no effect on time to mating or male reproductive parameters in either generation. This study confirms a low hazard of testicular toxicity or male reproductive toxicity from carbendazim exposure in the diet.
A number of regulatory agencies have ongoing efforts to identify, describe, and categorize AOPs. Developing libraries of AOPs that can effectively address the practical needs of chemical risk assessment is a major undertaking. There is increasing consensus on the AOP concept and structure: chemical exposure triggers a molecular initiating event that leads to a series of biochemical/cellular alterations culminating in tissue effects including frank toxicity. There is recognition that a reductionist approach may not capture sufficient biological complexity of chemical-induced effects for effectively implementing the AOP framework in risk assessment. In the era of large-scale/high-throughput biology, top-down (hypothesis-driven) and bottom-up (data-driven/systems biology) thinking are both vital for building and using AOPs. This symposium will bring together cutting-edge ideas on AOPs as an integrative framework for predictive toxicology and will focus on (1) building the structure of libraries of AOPs, (2) populating the key events with chemical-induced effects from both a top-down and bottom-up (systems biology) approach, and (3) applying the AOP framework to quantitative risk assessment. The first speaker will give an overview of international efforts to build libraries of AOPs and highlight the requirements for their utility in a regulatory context. The second speaker will discuss some of the techniques and hurdles in combining top-down thinking using large-scale molecular data to characterize molecular and cellular events involved in adverse outcomes in the liver. The third speaker will present a large-scale semantic approach for linking data in relation to AOPs. The fourth speaker will present a computational framework that uses data-driven and knowledge-driven analysis to reconstruct AOPs. The last speaker will present some of the opportunities and challenges in using AOPs for quantitative and qualitative extrapolation from in vitro to in vivo. This symposium will be of wide interest to SOT members, including scientists working within the regulatory arena as well as those interested in the application of molecular and systems biology to risk assessment.

**S 692 Integrating Genomics into the AOP Framework**

C. Corton, US EPA, Research Triangle Park, NC.

Databases of gene expression information annotated for phenotypic outcomes offer opportunities for predicting key events in adverse outcome pathways (AOPs). We have developed strategies for linking gene expression changes to prediction of key events. Three comprehensive databases of gene expression changes in liver/primary hepatocytes have been annotated based on mouse, rat and human data containing ~2,000, ~10,000 and ~2,000 gene expression biosets, respectively. Signatures predictive of key events in liver AOPs were built using species-specific strategies. Mouse signatures capitalized on comparisons between chemically-treated wild-type and transcription factor (TF)-null mice. Rat signatures used known marker genes for TFs. Human signatures were developed in HepG2 cells in which gene expression was correlated to TF activation profiles examined in the same chemically-treated samples. Prediction tests of TF activation showed that balanced accuracies ranged from ~85 – 98% with signatures from mice being the most accurate.

The signatures were used to predict chemically-induced key events including activation of AhR, CAR, FXR, Nrf2, PPARalpha, PXR and hormonally-receptors. The predictions of chemical behavior are put into the context of key events allowing predictions of AOPs in the liver. Simultaneous assessment of all known AOPs linked to liver toxicity or steatosis has assessed complexities in interactions between the TFs. Because chemical-independent factors that regulate the TFs are simultaneously evaluated in the database (e.g., diet, genetic background and lifestyle), the integrated predictions open the door for assessment of cumulative risk. (This abstract does not represent EPA policy.)

**S 693 Opportunities in Toxicology for Large-Scale Semantic-Linked Data and Prediction**

D. J. Wild, Indiana University, Bloomington, IN. Sponsor: C. Corton.

Technologies have recently been developed that allow large-scale, semantic integration of heterogeneous biomedical and drug discovery datasets. A variety of tools and algorithms have emerged that can use this linked data for drug-target prediction and Mechanism of Action discovery, inter alia. Emerging opportunities will be explored for applying these in areas of toxicity and adverse event prediction, including interpretable association predictions that take into account complex inter-relations between genes, compounds, pathways and biological end-points. Examples will be given of how interpretable heterogeneous data subnetworks can shed light on adverse drug events (including proposing mechanisms of action for the hepatic and cardiac events associated with the thiazolidinediones Rosiglitazone and Troglitazone) and how chemical compounds can be profiled against targets and other compounds to produce interpretable predictions of biological effects.

**S 694 Interactive Computational AOP Reconstruction from Data**

I. Shah, US EPA, Research Triangle Park, NC.

While there is growing consensus on the utility of AOPs for risk assessment, developing AOPs for target organ toxicity remains a major challenge. We are a part of the OECD effort for identifying AOPs for hepatotoxicity. To streamline AOP mapping we developed computational tools to reconstruct, visualize, modify, store and share AOPs. First, we developed an ontology to standardize chemical effects on 26,419 biological endpoints including; molecular events, cellular processes, and tissue phenotypes. Second, we synthesized ~1.2M effects on 5,541 chemicals from ToxCast, ToxRefDB and the literature into a computable knowledge base. Third, a systematic mining the literature and pathway databases to calculate dependencies between all 26,419 endpoints. Finally, we developed a heuristic search algorithm to interactively and visually reconstruct putative AOPs for chemicals by inferring linkages between molecular, cellular and tissue-level events. We evaluate the utility of this framework for analyzing receptor-mediated pathways in hepatic steatosis and cholestasis, and how chemical compounds can be profiled against targets and other compounds to produce interpretable predictions of biological effects.

**S 695 From AOPs to Quantitative In Vitro to In Vivo Extrapolation**

M. E. Andersen, The Hammar Institutes for Health Sciences, Research Triangle Park, NC.

There are multiple international initiatives to move toxicity testing from reliance on in-life studies to in vitro platforms that evaluate modes-of-action of chemicals using human cells. The transition from past practices requires placing the new assays in a toxicological/risk assessment context. Adverse Outcome Pathways (AOPs) link events at different levels: from cellular interaction of chemicals, through perturbations of cellular pathways, on to integrated responses at the organism level. Development of AOPs will accelerate the move to acceptance of modern methods of testing by providing convincing arguments for regulatory authorities of the utility of pathway-oriented in vitro assays. These assays provide output showing the concentrations at which compounds produce cellular or subcellular responses. Formal use in risk/safety assessment requires dosimetry extrapolation from in vitro concentrations to human exposures and low dose extrapolations based on the cellular circuitry affected by the chemicals. This talk focuses on two key methodological approaches required for risk/safety assessment purposes: 1) quantitative in vitro to in vivo extrapolation (qIVIVE) that relates in vitro concentrations to environmental exposures and 2) computational systems biology pathway (CSBP) modeling that accounts for the structure of the key signaling pathways coordinating key cellular events. The CSBP modeling tools also evaluate dose-dependent transitions and
aid in identifying responses falling within the normal range. Our pathway research with receptor-mediated signaling (PPAR and AhR) and with DNA-damage shows the application of these tools in practice.

696 Is Neuroimmune Crosstalk Important to Neurotoxicology? Critical Insight from Animal and Human Studies

T. A. Brown and C. E. McLoughlin. University of Montana, Missoula, MT and NIOSH, Morgantown, WV.

Convincing evidence of bidirectional communication between the immune system and the nervous system has led to a paradigm shift in our understanding of neuro-immune interactions. Emerging evidence establishes a role for immune signaling in key neurodevelopmental events. Additional evidence suggests immune system contribution to neuronal responses in the form of neuroprotection and repair of tissue injury, as well as in the pathogenesis of neurodevelopmental and neurodegenerative disease. Simultaneously, neurons may actively participate in immune responses in the nervous system by signaling to resident and infiltrating immune cells. The net result of the neuroimmune crosstalk depends on the balance between protective and destructive signaling pathways. There is increasing consensus that exposure to neurotoxicants may tip this balance towards a more disruptive outcome and augment the risk and/or severity of disease. How the immune system can act as a mediator/modulator of neurotoxicity remains elusive. Understanding gained by investigation into neuroimmune interactions will guide improvement of disease diagnosis, prevention, and treatment. This session will present evidence of neuroimmune perturbations in human studies and animal models of neurotoxicant exposure. Evidence from human studies will focus on immune alterations following developmental neurotoxicant exposure in children with documented neurological deficits and in a pediatric population with autism spectrum disorders. Supporting data from animal models will focus on peripheral immune alterations and neuroinflammation following developmental or adult nervous system insult.

697 Neuroimmune Interactions in CNS Development, Repair, and Damage: An Overview

M. Stamou and P. Lein. Molecular Biosciences, School of Veterinary Medicine, University of California Davis, Davis, CA.

It is becoming increasingly clear that the nervous system and the immune system can no longer be considered as separate entities. Evidence suggests that molecules traditionally thought to be exclusive members of either the immune or the nervous system have a dual role in both systems and can regulate CNS function and central innate immune responses, not only in disease but also during normal physiological processes (development, aging, pregnancy, chronic stress). Such molecules include complement and major histocompatibility complex proteins, cytokines, chemokines and neurotrophins. Expression patterns of these molecules are altered in Parkinson’s, Alzheimer’s and multiple sclerosis and mutations in some of the corresponding genes have been linked to neurodevelopmental disorders (Autism Spectrum Disorders, schizophrenia). Neurons, astrocytes and microglia express a wide variety of these molecules, which in turn function to mediate cell-cell interactions including neuronal control of microglial activation and conversely microglial regulation of neurodevelopmental events such as synaptic pruning. Depending on the timing and context of the neuroimmune interaction, such crosstalk may mediate normal neurodevelopment or, in the case of CNS insult, may result in disease or repair. The net effect depends on the balance of neuroprotective and destructive pathways activated. Exposure of the CNS to neurotoxic chemicals could tip this balance towards a detrimental outcome and evidence of immune alterations in the CNS following exposure to environmental factors such as manganese, air pollution or repair. The net effect depends on the balance of neuroprotective and destructive signaling pathways. There is increasing consensus that exposure to neurotoxicants may tip this balance towards a more disruptive outcome and augment the risk and/or severity of disease. How the immune system can act as a mediator/modulator of neurotoxicity remains elusive. Understanding gained by investigation into neuroimmune interactions will guide improvement of disease diagnosis, prevention, and treatment. This session will present evidence of neuroimmune perturbations in human studies and animal models of neurotoxicant exposure. Evidence from human studies will focus on immune alterations following developmental neurotoxicant exposure in children with documented neurological deficits and in a pediatric population with autism spectrum disorders. Supporting data from animal models will focus on peripheral immune alterations and neuroinflammation following developmental or adult nervous system insult.

699 The Immune and Neurological Impacts of Developmental BPA Exposure

J. N. Franklin, Q. Hu, B. Rushing, M. Biller and J. DeWitt. Brody School of Medicine, East Carolina University, Greenville, NC.

Environmental exposure to exogenous agents during critical time points of development may be associated with the onset of deleterious effects, including autoimmune and neurological disorders. Numerous studies have shown that bisphenol A (BPA) exposure can disrupt myriad biological systems. This presentation will focus on the immune and neurological impacts of a double hit of developmental exposure to BPA and an acute exposure to lipopolysaccharide (LPS) in an in vivo C57BL/6 mouse model. LPS exposure given around the time of learning has been shown to unmask developmental deficits in learning and memory. We hypothesize that postnatal exposure to LPS will unmask BPA-induced developmental impacts to hippocampal-dependent learning and memory of C57BL/6 offspring. Impaired behavioral changes will be correlated with markers of immune dysfunction to determine the relationship between immunological alterations and behavioral changes. C57BL/6 female mice were given 0, 0.4, or 50 mg/kg of BPA in a corn oil vehicle by gavage, beginning at pairing with males and ending at weaning of pups. Offspring were assessed on a Barnes maze at postnatal day 60 (PN60), 24 hours after challenge with LPS. Spleenic lymphocyte immunophenotype, total serum immunoglobulins, and inflammatory cytokines were evaluated after behavioral testing. Our findings suggest that developmental immunotoxicity induced by BPA exposure, in the absence of a postnatal trigger, is insufficient to induce changes in learning and memory.

700 Exploring the Relationship between Neuroinflammation and Neurotoxicity

K. A. Kelly, D. B. Miller and J. P. O’Callaghan. CDC-NIOSH, Morgantown, WV.

The enhanced expression of proinflammatory cytokines and chemokines accompanies brain injury induced by neurotrauma, disease or neurotoxicity as well as systemic infection. Elevations in proinflammatory mediators may serve as modulators or mediators of astroglial and microglial activation, cellular responses associated with all types of brain injury and collectively referred to as neuroinflammation. The “acute phase” response to systemic inflammation also leads to upregulation of proinflammatory cytokines/chemokines in the brain in the absence of underlying neural damage, responses thought to be mediated largely by microglia and that serve as the basis of “sickness behavior”. Activated glia may have neuroprotective roles or may exacerbate neural damage. Exposure to the known dopaminergic...
neurotoxicants, MPTP and methamphetamine (METH), cause nerve terminal damage followed by neuroinflammation. Genetic and pharmacological interventions have resulted in partial suppression of the neuroinflammatory responses to MPTP and METH without affecting neurotoxicity and glialosis. In an attempt to achieve a complete suppression of neuroinflammation, chronic exposure to corticosterone (CORT) was used. Surprisingly, mice treated with CORT exhibited an exaggerated neuroinflammatory response to METH coupled with potentiated neurotoxicity (loss of dopaminergic nerve terminal marker, tyrosine hydroxylase) and glialosis. As the levels of chronic CORT approached or exceeded those associated with high physiological stress, the data suggest chronic CORT therapy or sustained physiological stress sensitizes CNS neuroinflammatory and neurotoxic responses. Yet, in a model of LPS-induced systemic infection that does not induce glialosis or neural damage, chronic CORT pretreatment greatly exacerbated and prolonged neuroinflammation without evidence of neural damage or glialosis. These results show a priming of the CNS proinflammatory response to amplify future exposures to pathogens, injury or toxicity. Taken together these findings suggest that neurotoxicity causes inflammation, neuroinflammation may potentiate, but does not necessarily cause, neurotoxicity.

S 701 Neuroautoantibodies: Biomarkers and Potential Pathogenicity

L. A. Shaiba, Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada.

Hypoxic-ischemic events are common to neurodegeneration due to many etiologies, including neurotoxicity, with devastating neurological outcomes. There is a compelling need for reliable, translational biomarkers. Studies of neuroimmune interactions and the tissue-specific responses of the nervous system (NS)-specific antigens provide a record of insult in the form of serum autoantibodies (NAb). Using neonatal hypoxic-ischemic encephalopathy (HIE) as a model paradigm, the humoral hypoxic response, the corresponding detection of NAb biomarkers and brain astroglis were delineated. Sprague-Dawley rat pups underwent left carotid ligation and 2hr of hypoxia (8% O2) on P7. Littermates with sham surgery and normoxia were controls. Neurofilaments (NF), glial- fibrillary acidic protein (GFAP) and myelin basic protein (MBP) antigens reflect the NS’s cellular heterogeneity. Serum NAb against GFAP, NF, MBP and brain GFAP, and erythropoietin (EPO), VEGF, IL-2, IL-6 and TNFα were measured post-surgery (1, 4, 7, 14, 21, 28 and 36d). All cytokines measured were elevated at 24 hours post-surgery in HIE rat pups and for the duration of the study. EPO and VEGF, elevated at 24hr, began to decline at 14d. Only HIE pups had detectable levels of IgM and IgG against NF, GFAP and MBP at 4d post-hypoxia which increased in a time-dependent manner. Significant associations between GFAP levels and serum anti-GFAP IgGs were seen for thalamus and cerebellum, the two regions most affected. Presence of inflammatory cytokines is consistent with pathophysiology and the early increases in EPO and VEGF are consistent with growth and compensatory attempts. The early predominance of IgG NAb suggests isotype switching from a natural repertoire of IgM. A transient decline in NAb likely reflects release of NS antigen due to secondary insult and suggests that the pathological role of NAb. The reactive gliosis indicated by brain GFAP, a hallmark of neurodegeneration, as indicated by serum NAb. This study of early NAb detection indicates their potential as biomarkers of brain injury in neonates and children and suggests a role of NAb in progression of injury.

S 702 Perinatal Exposures and Children’s Health Outcomes

N. Holland and M. C. Poirier, ‘CDI Section, LCBG, National Cancer Institute, Bethesda, MD and ‘School of Public Health, University of California Berkeley, Berkeley, CA.

The developing fetus is more susceptible to xenobiotic toxicant exposures than are individuals in adulthood. This may be largely due to the rapid growth rate, the inability of the placenta to protect completely, and the undeveloped/immature fetal immune and metabolic systems. In utero and perinatal xenobiotic exposures may contribute to chronic diseases of adolescence and adulthood, including cancer, asthma, and obesity. Molecular epidemiology of children’s environmental health, which attempts to link exposure and disease, is a fast-growing area of research, both nationally and internationally. Clinical end points examined include growth parameters, metabolism, and cognitive capacity. Biomarker studies have concentrated on revealing genotoxic and epigenetic events, as well as mitochondrial toxicity, alterations in DNA repair, estrogenic effects, prediabetic metabolic syndromes, and other changes. Epigenetic effects related to prenatal exposures are considered to play a role in the fetal origin of adult human diseases, but the importance of this area for public health is only beginning to be understood. The relevant exposures may range from metals, polycyclic aromatic hydrocarbons associated with air pollution, and pesticides, to hormone disruptors and widely-used chemotherapeutic drugs. Fetal adverse outcome may appear early (mitochondrial toxicity, metabolic syndrome, asthma, cognitive disorder), or years later (leukemia, obesity, and diabetes). This symposium is designed to highlight the significance of adverse health outcomes induced in infants and young children by a broad range of xenobiotic exposures occurring in utero and/or shortly after birth. If we can elucidate underlying genetic and epigenetic mechanisms, it may be possible to devise strategies that will better protect the health of all infants and children.

S 703 Perinatal Exposures to Environmental Pollutants and Children’s Health

J. J. Heindel, T. Schug and K. Gray. NIEHS, Research Triangle Park, NC.

There is increasing experimental and epidemiological evidence showing that development (in utero and the first years of life) is vulnerable to phenotypic insult due to environmental influences. This vulnerability arises at least in part because development is a highly coordinated balance between genetic and epigenetic interactions. The epigenetic system is responsible for coordinating cell differentiation, and tissue and organ formation during development based on the genetic background. The epigenome is particularly sensitive to changes in the external environment, including under- and over-nutrition, stress, drugs, infections and environmental chemicals, which cause shifts or programmatic changes in cell development. This altered “programming” is evidenced by alterations in DNA methylation and chromatin architecture, which have been linked to increased susceptibility to disease and dysfunction that show up later in life. This presentation will set the stage for the session on pollutants and children’s health by focusing on the data supporting a role for environmental chemical exposures, emphasizing endocrine active chemicals, in childhood diseases including asthma, learning disabilities, susceptibility to infections, obesity, type 2 diabetes and early puberty. It will also focus on the role that the NIEHS/EPA network of Centers for Children’s Environmental Health and Disease Prevention play in linking environmental exposures to these childhood diseases/dysfunctions and indicate areas of rapidly developing research and data needs such as a focus on improved exposure assessments, mixture studies, examination of multiple windows of susceptibility or examination of disease syndromes and improved interactions between animal model researchers and epidemiologists in children’s environmental health research. Data, while still incomplete, support a focus on prevention of childhood diseases by reducing exposure to environmental chemicals during development.

S 704 Early-Life Metal Exposure and Epigenetics

R. O. Wright, Mount Sinai School of Medicine, New York, NY.

A critical issue in the toxicology of metals includes the role of early life and prenatal exposures on subsequent health outcomes, including adverse neurodevelopmental outcomes, poor growth and obesity. Metals have been associated with all of these outcomes and epigenetics has been demonstrated to play a role in the programming of these adverse developmental phenotypes in childhood. The complexity of the interplay between metals and developmental programming is further complicated by the role of some metals as both nutrients as well as toxicants. As such, biological processes to regulate normal metabolic functions exist alongside toxicometabolic processes designed to limit their toxicity and the existence of non-monotonic dose response relationships are likely. Complexity in studying epigenetics and child development lies in the multiple epigenetic “marks” that may regulate development (DNA methylation, histone modification, microRNAs) and the role of exposure timing during critical developmental windows. The PROGRESS (Programming Research in Obesity, Growth, Environment and Social Stressors) study is a 1000 mother-child birth cohort with longitudinal assessment of metal exposure (arsenic, lead and manganese) and assessment of later life neurodevelopment, growth and obesity up to age 7 years. In the talk new data on the relationships between metal exposure at multiple critical developmental windows and epigenomic marks in distinct target tissues (umbilical artery, vein, placenta and white blood cell DNA), using the Illumina 450K methylation microarray, will be presented. In a pilot study, differences in methylation were seen in umbilical arteries in the glucocorticoid receptor gene and P16 promoter. In addition to methylation data, we will present data on LINE-1 expression as they related to growth and development.
Maternal-fetal Human Immunodeficiency Virus-1 (HIV-1) transmission is largely preventable when ARV drugs are given to the infected mother during pregnancy. However, the NRTI zidovudine (AZT) is a transplacental carcinogen in mice, and perinatal exposure induces genotoxicity and mitochondrial toxicity in monkey and human offspring (Env. Mol. Mutagenesis, vol. 48, April 2007). Approximately 10,000 women per year in the US are HIV-1-infected and become pregnant, and essentially all will receive these highly-successful therapies. Using an Erythrocebus patas (patas) monkey model, and human tissues, we have examined the mecha- nistic consequences of transplacental NRTI use. The monkey dams were given human-equivalent NRTI protocols, and because they do not become infected with immunodeficiency virus, the data reflect only drug toxicity. The human infant data, however, reflect both the effects of the drug and the maternal HIV-1 infection. We have shown incorporation of the NRTIs AZT and lamivudine (3TC) into nuclear and mitochondrial (mt)DNA of newborn monkeys and humans. Subsequently, AZT/3TC-induced mutagenesis/clastogenesis was found in transplacentally-exposed human infants. Recently the patas studies revealed the persistence of chromosomal damage in cultured bone marrow taken from transplacentally-exposed offspring at birth, 1 and 3 yr of age (equivalent in maturity to a 15 yr old human). In parallel studies we found mitochondrial morphological damage and mtDNA abnormalities in heart and brain of NRTI-exposed patas at birth, 1, and 3 yrs of age. Furthermore, the brains of 1 yr old patas, exposed perinatally to combinations of 2 NRTIs, contained only half of the mtDNA found in the controls. Overall, NRTI-DNA incorporation, chromosomal aberrations, mitochondrial morphological damage in cultured bone marrow taken from transplacentally-exposed patas and humans suggesting that these events underlie the toxic profiles in both species, and are independent of maternal HIV-1 status.

Air Pollution and Molecular Biomarkers of Children’s Health

The city of Ostrava has the highest level of pollution from carcinogenic polycyclic aromatic hydrocarbons (c-PAHs), like benzo(a)pyrene (BaP), in the Czech Republic. We studied a group of 100 asthma and 100 healthy children (8-15 years old) exposed to ambient air in Ostrava and 200 control children from the less polluted city of Prachatice. We measured 8-oxo-dG in urine and lipid peroxidation in plasma as biomarkers of oxidative damage. Gene expression profiles and DNA methylation in white blood cells were assessed by Illumina HumanHT-12 and 27K BeadChips respectively. Gene expression appears to differ between asthmatic and healthy children, and vary by the two regions. Results also indicate that higher exposure to c-PAHs may induce non-allergic asthma in children. No difference in DNA methylation was observed between children with and without asthma, but patterns in both asthmatic and healthy children varied between the cities.

We also studied the effect of prenatal exposure to BaP on DNA adducts, MNs and transcriptome in Prague and Ceske Budejovice. Samples were obtained from non-smoking mothers from Prague (N=35) and Ceske Budejovice (N=52), and cord blood was collected from their newborns. Mean exposures to BaP in subjects from these cities were 3 months prior to delivery 1.9±0.5 ng/m3 and 3.2±0.2 ng/m3 respectively. Gene expression was assessed by microarrays in peripheral blood and placenta of the mothers, and in cord blood of their newborns. Compared to subjects from Prague, pregnancies from Ceske Budejovice showed up-regulation in expression of genes related to metabolism of xenobiotics, but down-regulation of genes related to immune response. This finding corresponds with an increased level of DNA adducts and MN frequency in cord blood from Ceske Budejovice. Analysis of gene expression appears to be an important new biomarker of air pollution.

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Fetal Consequences of In Utero Antiretroviral (ARV) Nucleoside Reverse Transcriptase Inhibitor (NRTI) Exposures in Primates
M. C. Poirier1, O. Olivero1, Y. Liu1, R. L. Divi1 and R. A. Woodward2. 1CDI Section, ELCBG, National Cancer Institute, Bethesda, MD and 2Shared Animal Facilities, NICHD, NIH, Dickerson, MD.

Genetic and Epigenetic Determinants of Health Outcomes in Children with Prenatal Pesticide Exposure

New results from the longitudinal study conducted by the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) examine the effects of prenatal exposure to pesticides on the functional genomics of Paraaxonase 1 (PON1) and genome-wide DNA methylation in Mexican-American children. PON1 is a multifunctional enzyme involved in antioxidant defense and detoxification of organophosphate (OP) pesticides. The broad variability of PON1 activities within populations may confer differential susceptibility to environmental exposures. We determined PON1 genotypes and three PON1 enzyme activities in 450 mothers and their children in an agricultural cohort residing in Salinas Valley, CA. Infants and young children with PON1 genotypes encoding for lower PON1 levels and activities, have up to 65-fold lower levels of the protective PON1 enzyme than adults and may be especially susceptible to OP exposures. Additionally, global and site-specific DNA methylation was assessed in DNA isolated from cord blood and peripheral blood of 9 year old CHAMACOS children. Measurement of global DNA methylation was performed by pyrosequencing of Alu and LINE-1 repetitive elements. Global DNA methylation was found to vary by age, sex, and was lower (hypomethylated) in relation to prenatal exposure to organochlorine pesticides and PBDEs. Site-specific DNA methylation analysis was conducted using the Illumina 450K BeadChip platform that simultaneously interrogated methylation at more than 480,000 CpG sites from over 24,000 genes. Approximately 16% of all investigated CpG sites were differentially methylated by age and more than 2% of CpG sites (in >1900 genes) showed significant differences in methylation by sex, including many CpG sites located in autosomes. We also identified CpG sites across the PON1 gene whose methylation was associated with protein expression in CHAMACOS children, suggesting that epigenetic mechanisms may also regulate PON1 activity.

The Emerging Role of Mitochondrial Turnover, Biogenesis, and Dynamics in Tissue Injury
J. Kim1, J. I. Lemasters2, Y. Yoon3, R. Schnellmann1 and H. Laschke4. 1Surgery, University of Florida, Gainesville, FL, 2Drug Discovery & Biomedical Sciences, Medical University of South Carolina, Charleston, SC, 3Physiology, Georgia Regents University, Augusta, GA and 4Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

The mitochondria, the cell’s powerhouse, provides cells with adenosine triphosphate (ATP) to drive diverse energy-requiring reactions. Besides energy generation, the mitochondria are also involved in a myriad of essential functions and signaling events in cells. Consequently, disturbances affecting mitochondrial integrity diminish the quality and function of mitochondria, ultimately leading to tissue injury and death. Indeed, mitochondrial dysfunction is a key mechanism underlying ischemia/reperfusion, aging, and drug-induced toxicity in the liver, kidney, and other organs. To maintain normal cell function and survival under the conditions of a variety of stresses, cells possess a powerful surveillance system that selectively eliminates injured or dysfunctional mitochondria in a timely manner, a process called mitophagy. Impaired or insufficient mitophagy is causatively linked to pathology of ischemia/reperfusion, aging, and alcohol- and acetaminophen-mediated hepatotoxicity. The mitochondria are newly formed, and constantly divide and fuse, and continuously change their size and morphology during times of cellular stresses or in response to environmental stimuli. The biogenesis of mitochondria is tightly regulated, and inhibition of mitochondrial biogenesis has been associated with the development of age-related degenerative diseases. Furthermore, mitochondrial dynamics not only determines mitochondrial size, but also regulates mitophagy. Growing evidence is accumulating that mitochondrial biogenesis and dynamics are neatly interconnected with mitophagy. This symposium will emphasize the emerging role of mitochondrial turnover, biogenesis, and dynamics in tissue injury.

Variants of Mitochondrial Autophagy: Types 1 and 2 Mitophagy
L. I. Lemasters. Drug Discovery & Biomedical Sciences and Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC.

Autophagy removes damaged, effete and superfluous mitochondria, a process of mitophagy. In hepatocytes during nutrient deprivation, preautophagic structures (PAS) associated with mitochondria grow into cup-shaped isolation membranes (phagophores) that sequester individual mitochondria into mitophagosomes, a process requiring phosphatidylinositol-3-kinase (PI3K). Sequestration is complete in less than 10 min and often occurs coordinately with mitochondrial fission. After
Mitochondria are direct and indirect targets of many toxicants in a number of different tissues and a large number of studies have focused on the mechanisms of mitochondrial injury and the resulting cell death. In contrast, how cells respond to mitochondrial dysfunction has received less attention. One response to toxicity is the induction of mitochondrial biogenesis (MB) to restore mitochondrial and cellular functions in sublethally injured cells and prevent cell death. In contrast, recent studies have shown that toxicants can inhibit MB. MB requires a coordinated and tightly regulated signaling system. Nuclear respiratory factors (NRF-1 and -2) bind to the promoters of the nuclear genes, leading to expression of constituent components of the oxidative metabolism, mitochondrial DNA (mtDNA) replication, transcription and maintenance. The transcriptional coactivator PGC-1α, the master regulator of MB, induces and coordinates gene expression of mitochondrial transcription A (Tfam), NRF-1 and 2, and itself. Most mitochondrial proteins are encoded by nuclear DNA but mtDNA encodes 13 proteins which are all key components of OXPHOS machinery. A number of upstream signaling pathways (e.g. p38 MAPK, SIRT1, AMPK) have been shown to be activated following injury and activate PGC-1α to produce MB and restore mitochondrial and cellular functions. This presentation will explore the pathways that signal MB and the functional outcomes. 

**S 712 Mitochondrial Biogenesis or Not Following Toxicant Exposure**

R. Schnellmann, Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.

Mitochondria are direct and indirect targets of many toxicants in a number of different tissues and a large number of studies have focused on the mechanisms of mitochondrial injury and the resulting cell death. In contrast, how cells respond to mitochondrial dysfunction has received less attention. One response to toxicity is the induction of mitochondrial biogenesis (MB) to restore mitochondrial and cellular functions in sublethally injured cells and prevent cell death. In contrast, recent studies have shown that toxicants can inhibit MB. MB requires a coordinated and tightly regulated signaling system. Nuclear respiratory factors (NRF-1 and -2) bind to the promoters of the nuclear genes, leading to expression of constituent components of the oxidative metabolism, mitochondrial DNA (mtDNA) replication, transcription and maintenance. The transcriptional coactivator PGC-1α, the master regulator of MB, induces and coordinates gene expression of mitochondrial transcription A (Tfam), NRF-1 and 2, and itself. Most mitochondrial proteins are encoded by nuclear DNA but mtDNA encodes 13 proteins which are all key components of OXPHOS machinery. A number of upstream signaling pathways (e.g. p38 MAPK, SIRT1, AMPK) have been shown to be activated following injury and activate PGC-1α to produce MB and restore mitochondrial and cellular functions. This presentation will explore the pathways that signal MB and the functional outcomes.

**S 711 Mitochondrial Dynamics and Oxidative Tissue in Diabetes**

Y. Yoon, Department of Physiology, Georgia Regents University, Augusta, GA, Sponsor: J. Kim.

Mitochondria are dynamic organelles, undergoing constant shape change mainly through fission and fusion. Mitochondrial fusion and fission spend cellular energy, suggestive of their importance. However, not only functional significance of mitochondrial fission and fusion but also the mechanistic correlation between mitochondrial functionality and morphology is poorly understood. Our studies demonstrated that mitochondrial shortening by fission coincides with increases in reactive oxygen species (ROS) levels in hyperglycemic conditions. Elevated ROS levels was causal for an increase of mitochondrial permeability transition (MPT) and apoptotic cell death. We were able to show that inhibiting mitochondrial fission by expression of a dominant-negative mutant, DLPL-K38A, alleviated MPT and cell injury by decreasing ROS levels. Enhanced mitochondrial ROS production arising from diabetes is pathologically linked to hyperglycemic complications in many target tissues. In an effort to evaluate the role of mitochondrial fission in diabetic tissue damage, we found that cellular ROS levels in DLPL-K38A transgenic mice were significantly lower in both renal and hepatic tissues. While mitochondria in renal proximal tubular cells became round and ‘swollen’ in diabetic control mice, DLPL-K38A mice maintained normal elongated shape in diabetic conditions. Mechanistically, we discovered that fission inhibition induces an increase of respiration uncoupling to decrease ROS production from mitochondria. Importantly, DLPL-K38A expression suppressed oxidative damage and renal dysfunction in diabetic mice, indicating that mitochondrial fission can be a novel target of intervention in combating oxidative tissue damage.

**S 710 Mitochondrial Autophagy in Ischemia-Reperfusion Injury and Age-Mediated Hepatotoxicity**

J. Kim, Surgery, University of Florida, Gainesville, FL.

Patients with the end stage of liver disease often require transplantation and complex hepatic surgery for extirpation of a non-functional liver and are frequently hospitalized for supportive care that provides only temporary comfort. Moreover, the aged liver has significantly less reparative capacity following ischemia/reperfusion (I/R) injury associated with these operations. Mitochondrial dysfunction plays a causative role in I/R injury. Currently, no therapeutic strategy can suppress I/R injury. Mitochondrial autophagy (mitophagy) is integral quality control machinery that timely and selectively targets and removes damaged or abnormal mitochondria. Biochemical, genetic and imaging analysis demonstrates that I/R injury to young livers and increased sensitivity of old livers to stresses are strongly associated with decreased autophagic responsiveness and subsequent mitochondrial dysfunc-

**S 714 Use of Stem Cells in Toxicity Testing—From Basic Research to Personalized Toxicology**

S. H. Dhalluin1 and Y. Will1, 2. 1New Medicines - Non-Clinical Development, UCB Pharma SA, Brussels, Belgium and 2Global Research and Development, Pfizer, Groton, CT.

Drug-induced toxicity remains a major problem for all pharmaceutical companies, and most have deployed in silico and in vitro testing paradigms throughout the drug discovery process to select safer drugs to be tested in animal models and advance to patients. Despite many advances in understanding mechanisms of toxicity, the majority of in vitro assay predict human risks by less than 50%, and it was shown that measuring simple mechanistic toxicity endpoints in organ-relevant cell lines does not predict organ toxicity (Lin and Will, 2012, Lu and Will, SOT 2013). This may be due to the fact that certain basic toxicity mechanisms such as apoptosis or mitochondrial dysfunction can contribute to a variety of organ toxicities and that many cell lines lack features of organ physiology, such as proper metabolism, relevant ion channel pharmacology, genetic diversity or disease background, to name a few. To build a predictive multicell model, it is necessary to begin with improved functional cell models from the relevant tissue, e.g., hepatocytes, cardiomyocytes, etc. Therefore, efforts to generate physiologically-, pharmacologically-, and toxicologically-relevant cells from ES and iPS cells are important. Progress has been made in the production of human/patient-derived pluripotent stem cells, which can be continuously expanded in the undifferentiated state and differentiated to form most cell types, potentially allowing recapitulation of genetic variation such as association with known gene variants (e.g., HLA susceptibility alleles). This symposium will contribute to our understanding of the current and future state of stem cell...
usage in toxicology by: 1) providing an overview of stem cells, their characteristics in comparison to native organs, their ability to represent patient genetics and disease, 2) describing the current state of application to detect liver, heart, and kidney toxicity, 3) discussing how good patient genetic and disease background give rise to personalized toxicity assessment.

**S 715 Human Pluripotent Stem Cells As Tools for Safety Toxicology**


Human ES and iPS cells offer scalable, consistent sources of normal human cell types that were previously difficult to source. Because human iPS cells offer control over genetic backgrounds, in vitro modeling of drug responses for an intended target population is practical for the first time. This talk will review the properties of iPS cells, and will discuss recent progress in the large-scale derivation of iPS cells and specific iPS cell derivatives relevant to safety toxicology and drug discovery. Specific examples of how Pharma is already using iPS cell-derived cardiomyocytes, neurons, and hepatocytes will be discussed.

**S 716 The Application of Stem Cell-Derived Hepatocytes in Mechanism-Based Drug Safety Assessment**

C. Goldring, MRC Centre for Drug Safety Science, University of Liverpool, Liverpool, United Kingdom. Sponsor: S. Dhalluin.

Recent research into idiosyncratic adverse drug reactions (ADRs) has successfully revealed novel genetic predisposing factors by utilising a candidate gene approach and through the application of genome-wide scanning techniques. However, the underlying mechanisms of ADRs such as drug-induced liver injury (DILI) are complex, multi-dimensional, and not fully amenable to testing using the currently available cell culture systems. The recent rapid advances in the ability to generate induced pluripotent stem (iPS) cells from somatic cells have provided a unique tool to derive disease-specific stem cells that may serve as a mechanistic model for the study of idiosyncratic ADRs, for the liver and other tissues. Nevertheless, work needs to be done before it is known whether this promise will be fulfilled. This talk will highlight advances in this area, and will focus on some critical hurdles in both the embryonic and the iPS fields. The prospects for personalisation in safety assessment, through the great advances in the iPS field will also be considered. Furthermore, advances in complex culture techniques, such as spheroids, bioreactors and multi-cell culture, that may enlighten the field of in vitro hepatotoxicity screening will be highlighted.

**S 717 iPSCs for Cardiac Drug Testing**

J. Wu1, 2, *Stanford Cardiovascular Institute, School of Medicine, Stanford University, Stanford, CA* and 1Department of Medicine/Cardiology, School of Medicine, Stanford University, Stanford, CA. Sponsor: Y. Will.

Cardiac toxicity is a side effect of many pharmaceutical compounds and is a leading cause for drug withdrawal from market because of safety concerns. Current preclinical methods to measure cardiotoxicity are inefficient and rely on genetically altered cell lines, such as human embryonic kidney cells, or Chinese hamster ovary cells, which do not accurately resemble human heart cells (cardiomyocytes). Recent technological advancement has enabled the generation of human induced pluripotent stem cells (hiPSCs) from skin, which can be used to generate patient-specific cardiomyocytes (hiPSC-CMs) in vitro. The hiPSC-CMs generated in this fashion carry all the genetic information from the individuals from whom they are derived. Here we will overview the capacity of a hiPSC-CM library to be used as a clinical trial in a dish model for accurate detection of patient-specific drug responses.

**S 718 In Vitro Models for the Prediction of Nephrotoxicity in Humans**


The kidney is a major human target organ for drug-induced toxicity. Nephrotoxicity is often detected only late during drug development. Animal models have limited predictability due to interspecies variability. In vitro models are of increasing interest, but there are currently no validated or approved in vitro models for nephrotoxicity available. We developed kidney-specific two-dimensional (2D) and 3D (Zhang et al., J Cell Mol Med, 2011; Li et al., Nanotoxicology, 2012) models for in vitro toxicology and nanotoxicology. Our models are based on human primary renal proximal tubular cells (HPTC) and human renal cells derived from pluripotent stem cells (Narayanan et al., Kidney Int, 2013). Endpoint of the in vitro model with the currently highest predictability is increase in marker gene expression. This model has been validated with 41 well-characterized compounds (Li et al., Toxicology Research, 2013). The results show high sensitivity, specificity and concordance with human clinical data. All major performance metrics, including the area under the curve of the receiver operating characteristic curves are in the range of 0.8. This translates to about 80% accuracy in the predictions made with this model. An alternative model comparable with high content screening is currently under development.
proof of concept. Case studies in chemical exposure and drug pharmacology will be explored where the 5 question approach was applied by a toxicologist for litigated tort claims, including ethyl acetate and an opioid painkiller.

722 Evidentiary Challenges with Genomic Data in Toxic Tort Litigation
G. E. Marchant, Sandra Day O’Connor College of Law, Arizona State University, Tempe, AZ.

As the genomic era continues to rapidly advance, genomic data is increasingly being introduced into toxic tort litigation by expert witnesses for both defendants and plaintiffs. This includes genetic susceptibility data that might affect the causation analysis, as well as various types of genetic biomarker evidence that may be relevant to the exposure inquiry. The attempted introduction of such data is often controversial and contested, with opposing counsel frequently arguing that such data are unreliable, inadequately validated, or irrelevant. This presentation will discuss several recent high-profile attempts to introduce genetic data into toxic tort cases involving toxicants such as benzene, lead, ionizing radiation, mercury, and PCBs, and the challenges such data presented for our evidentiary standards, expert witnesses, attorneys, judges and juries. This case study of genomic data in toxic tort litigation illustrates some of the broader issues the evidentiary and expert witness systems in our federal and state courts will encounter in the era of rapidly changing technology and knowledge. These issues include the temptation and incentives for expert witnesses to place premature or exaggerated reliance on emerging scientific knowledge, and the tension between scientific and legal standards of admission and proof.

723 Evidentiary Standards for Neuroactive and Neurotoxic Drug Testing
T. Simon, Ted Simon LLC, Winston, GA.

A number of psychoactive and potentially neurotoxic substances are often considered by courts to modify or disrupt the functioning of the brain. However, dose response is taken into account for only a single substance—ethanol. For ethanol, the societal consensus for impairment of performance has been set at 0.08% or 80 mg/dL. Because of the zero-order elimination kinetics of ethanol and the widely used Widmark pharmacokinetic model, one can determine the source of alcohol in the body based on recent consumption history and this determination can be used as legal evidence. Inorganic mercury intoxication produces a condition called “creethism” with symptoms of anxiety, mood lability, and poor anger control that may be misdiagnosed as psychosis. The “date-rape” drug gamma-hydroxybutyrate (GHB) appears to act by modulating the effects of a number of neurotransmitters, and its effects may be potentiated by nicotine. Symptoms of GHB intoxication include euphoria, relaxation, and increased sexuality. At higher doses, GHB may cause unconsciousness and even death. GHB is produced endogenously within the body. Exogenous GHB is eliminated from the body completely within 8 hours and no rapid immunological test exists—so testing for GHB in cases of drug-facilitated sexual assault is challenging. Regarding cocaine, GS-MS LC-MS and immunological assays methods are used for detection of cocaine metabolites in urine, other body fluids and even hair—with the potential for both false-positive and false-negative results from all methods. In summary, for most psychoactive substances, evidence of exposure obtained by analysis of body matrices is considered tantamount to a psychoactive effect and potential impairment by the legal system. With marijuana is now legal in 18 states, the interpretation of evidence of psychoactive drug exposure in terms of dose-response and assessment of the likelihood of impairment is becoming an important task. Currently, the question addressed by drug testing is—was the subject exposed to the drug? For marijuana especially, the question needs to be—did the subject have enough drug in his/her system to be impaired?

724 Challenging Toxicologists to Advance the Science of Forensics
R. T. Kennedy, New Mexico Court of Appeals, Albuquerque, NM. Sponsor: G. Corcoran.

Toxicological evidence in court should demonstrate the existence of a toxin, symptom, and causal relation (general/specific) between them. Lawyers ask, and judges often allow medical doctors regularly testify to causal relationships through different diagnoses in court. This can result in the introduction of incomplete or flawed evidence. Toxicology’s language confuses judges and lawyers. In criminal cases, analytical chemistry is called “toxicology.” In many trials, analysts of varying skill and repute testify as “toxicologists,” stating opinions linking symptoms with levels of drugs or alcohol found in body fluids or tissue. For alcohol, this is based on scant exposure to Widmark’s formula. With drugs, it is common to see experts give causal testimony first ascertaining symptoms of “impairment” from a police report, then matching the test results, equating amounts found to those symptoms. The credibility of the law allows the potential for injustice. “Non-forensic” toxicology represented by SOT might present the “academic” context to apply standards of research, validation, and acceptable practice to frontier toxicology regulation, See, Strengthening Forensic Science: A Path Forward (NAS,2009). The Federal reference manual on scientific evidence applies toxicology to toxic tort and pharmaceutical litigation, but not applying guiding principles to more common appearances of “toxicology”. Can forensic and legal minds grasp what “toxicology” is about? A question: “If Daubert is about applying ‘science as scientists do it’, why can’t a qualified expert examine another researcher’s data to see if it supports conclusions drawn?” Is motivation inferred from performing such an analysis for litigation any less suspect than performing research for a company desiring to move its product to market? The writer hopes for a concept and direction of how SOT and its members can advance the legal use of toxicological expertise based on objectively reasonable and established standards of scientific practice.

725 Scientific Ethics in Research and Publications
W. J. Brock1 and M. Genter2. Brock Scientific Consulting, Montgomery Village, MD and University of Cincinnati, Cincinnati, Ohio.

For many involved in toxicological research and publishing, it may seem strange or uncomfortable to engage in a discussion of the practicality of ethics. However, with the daily pressures of career advancements and salary increases as well as notoriety and professional recognition, engaging in this discussion will permit continued awareness of the pitfalls of poor ethical behavior that can lead to precarious career outcomes. The scientific community has been rocked by unfortunate media reports over the years that call into question the results of studies and fallibility of science. Although these reports may represent a small percentage of individuals, the impact is far-reaching throughout the scientific community. There have been several reports in the past few years that suggest that the number of retracted papers in scientific literature has increased 10-fold over the previous decade, and that a majority of the retracted papers was due to scientific misconduct that included fraud, plagiarism, and outright data falsification. In spite of the increase in retractions, many of those retracted papers continue to be cited in subsequent publications and grant submissions. Plagiarism by far represents the more common concern in scientific writing. Whether this occurs from the originating author or the wording is “stolen” by others to improve or even exaggerate a conclusion has led to a change in peer-review processes, development of plagiarism software, and mandatory training in certain academic circles. In addition, “ghost” and “in absentia” authors have raised significant data credibility concerns in a regulatory environment. In this session, the background of the problem is presented with real-world examples from literature and reports, and the impact of this problem on a career. Can the peer-review process reduce the likelihood of scientific misconduct? Discussion will occur on the peer-review process and how that process affects the publishing of duplicative or plagiarized data.

726 Introduction to Scientific Misconduct: The Problem, the Results, and the Potential Impact of Advancing a Career Path
W. J. Brock. Brock Scientific Consulting, Montgomery Village, MD.

Over the last 100+ years, there have been enormous increases in scientific knowledge across all fields of science. Unfortunately, the individual desire to be a part of the technical innovations observed during this time has led to “abuse” of that innovation. In the last 15-20 years, the number of cases of scientific misconduct has been surprisingly numerous. In this presentation, the problem of misconduct will be described to include the more notable case reports that have affected our regulatory environment. In this session, the background of the problem is presented with real-world examples from literature and reports, and the impact of this problem on a career. Can the peer-review process reduce the likelihood of scientific misconduct? Discussion will occur on the peer-review process and how that process affects the publishing of duplicative or plagiarized data.

727 Responsible Research: What Is It and Can It Be Done?
H. Bante. University of Cincinnati, Cincinnati, OH. Sponsor: W. Brock.

Biomedical research continues to evolve as investigators collaborate across disciplines and institutions to solve complex health problems. These interdisciplinary interactions coupled with for-profit relationships have led to questions surrounding professional norms and ethical principles as they relate to scientific research. Recognizing these changes and in an effort to foster research integrity, the National
Science Foundation and the National Institutes of Health have mandated education of all trainees, fellows, participants, and scholars in the Responsible Conduct of Research (RCR). Despite these efforts, the incidence of scientific misconduct and questionable research practices remain problematic. This presentation will explain RCR and what it hopes to accomplish, and discuss why collapses in responsible research continue to occur.

**728 Authorship and Scientific Misconduct**

W. B. Matte.

PharmPoint Consulting, Poolesville, MD.

An integral component of the science is the publication of research results and analyses. Needless to say, humans conceive of, conduct, analyze, and describe the work, and participation in this endeavor is acknowledged through the author list of the resulting publication. However, professional advancement and financial rewards are often tied to the number of authored publications; in many fields, the first author is given special attention. Given this setting, authorship is often abused. “Honorary” authors are those whose names are included, yet made no real contribution to the work. “Ghost” authors are those who actually did contribute but are not listed. First authorship is often a source of conflict and is especially problematic in fields where the work requires a large number of collaborators. This presentation will review not only the well-established guidelines but also provide an analysis of the problems of authorship with proposed solutions.

**729 Seeking, Identifying, and Preventing Plagiarism: Manuscript Submissions and Peer Review**

M. Center.

University of Cincinnati, Cincinnati, OH.

While plagiarism can be intentional or unintentional, and can involve an author plagiarizing himself or another author, it can have devastating effects on one’s pursuit of a degree, ability to successfully compete for funding, ability to publish in the peer-reviewed literature, and success in successfully advancing up the career ladder. This presentation will focus on plagiarism from the perspective of the author, peer reviewer, and journal editor. Trainees at all levels should be mentored in the appropriate situations and ways to cite not only the work of others, but also their own work. Peer reviewers and journal editors should be aware of the high incidence of plagiarism, and should put special effort into assuring that a submitted manuscript, or parts thereof, has not been published elsewhere. Examples of self-plagiarism and plagiarism of others’ works in submitted manuscripts will be presented, as well as tips to identify and prevent plagiarism.

**730 Noncoding RNAs in Human Health, Therapeutics, and Environmental Disease**

N. E. Kaminski.

Michigan State University, East Lansing, MI.

A little over a decade ago, the regulatory role of small non-protein-coding RNAs was first uncovered through experiments involving the deliberate introduction of short double-stranded RNAs (dsRNA) into plant and eukaryotic cells. These exogenous dsRNA were found to induce post-transcriptional gene silencing, a process termed RNA interference. This seminal observation led to the discovery of novel and complex biological processes of gene regulation involving endogenous non-coding RNAs of which several varieties have been identified, including microRNA, Piwi-interacting RNA, and long non-coding RNA. During the past 10 years, significant advances have been made in gaining an understanding of the role of non-coding RNAs spanning the period from organismal development and continuing throughout all stages of life. Although the biological role of noncoding RNA has yet to be fully understood, it is important to emphasize that only a small fraction of the mammalian genome codes for miRNAs, and yet the majority of the remaining genome is transcribed into noncoding RNAs. It is tempting to speculate that a large proportion of these noncoding RNAs are transcribed for the distinct purpose of carrying out critical regulatory functions. Indeed, there is a growing literature identifying specific processes under the control of noncoding RNAs and, likewise, the pathology that can ensue when noncoding RNA regulatory processes are disrupted. Relatively little is known concerning the influence environmental factors exert on noncoding RNAs, at the level of their expression, function, or contribution to the etiology of disease processes; therefore, this represents an important frontier for toxicological investigation. In light of the importance of this relatively new area of biology and its potential impact on human health, the goal of this session is to feature eminent scientists who have made important contributions and advances to our current knowledge of noncoding RNAs. The broad areas to be addressed include: general concepts surrounding the biology of noncoding RNAs, their role in development and in specific disease processes, and their potential role in novel therapeutic approaches.

**731 RNA at the Epicenter of Human Development**

J. S. Mattick.

Garvan Institute of Medical Research, Sydney, NSW, Australia.

Sponsor: N. Kaminski.

It appears that the genomic programming of humans and other complex organisms has been misunderstood for the past 50 years, because of the assumption that almost all genetic information is transacted by proteins. Surprisingly, the human genome contains only about 20,000 protein-coding genes, similar in number and with largely orthologous functions as those in other animals, including developmentally simple nematodes and sponges. On the other hand, the extent of non-protein-coding DNA increases with increasing developmental and cognitive complexity, reaching 98.8% in humans. Moreover, high-throughput analyses have shown that the vast majority of the human genome is dynamically transcribed to produce a previously hidden world of different classes of small and large, overlapping and interlacing intrinsic, intergenic and antisense non-protein-coding RNAs. The transcriptome is in fact far more complex than the genome, which is best viewed as a zip file that is unpacked in highly stage- and cell-specific patterns during development. This is illustrated by the use of targeted RNA sequencing to reveal thousands of previously unknown exons and spliced isoforms of oncogenes and tumor suppressors, as well as at least 1500 new long noncoding RNA (lncRNA) genes in intergenic GWAS regions associated with complex diseases. These RNAs fulfill a wide range of regulatory functions, with microRNAs and related species being best (although not well) understood. The functions of IncRNAs are varied and include central roles in the formation of differentiation-specific subnuclear organelles. However, recent evidence suggests that their main function is to organize chromosome territories and to guide chromatin-modifying complexes to their sites of action, to specify the architectural trajectories of development. Moreover, this system has subsequently evolved plasticity, via an as-yet-unexplored universe of retrotransposon expression and mobilization, as well as RNA editing and modification, which appears to be the molecular basis of environmental epigenome interactions and brain function.

**732 Role of microRNA Signaling in the Cancer Microenvironment Communication**

M. Fabbri.

Departments of Pediatrics and Molecular Microbiology & Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA.

Sponsor: N. Kaminski.

Cancer is a complex genetic disease. Recent evidence indicates that not only cancer cells but also surrounding cells, forming the so-called “tumor microenvironment” play a central role in cancer biology. MicroRNAs (miRNAs), are small non-coding RNA with gene regulatory function, whose dys-regulation is observed in almost all types of human cancers (both solid and hematological malignancies). In addition to their “traditional” mechanism of action (which consists of their targeting of messenger RNAs, miRNAs affect the tumor microenvironment also through previously unknown mechanisms that will be discussed in this lecture. Particular focus will be put on their role as cancer secreted molecules within exosomes, able to trigger pro-tumoral and pro-metastatic signaling by affecting the biology of the tumor microenvironment. A better understanding of such mechanisms represents the necessary rationale for the development of new personalized treatments for cancer patients.

**733 MicroRNAs in Hepatocellular Carcinoma**

C. Lee1, 2, 3.

1Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, 2Duke-NUS Graduate Medical School, Singapore, Singapore and 3National Cancer Centre, Singapore, Singapore.

Sponsor: N. Kaminski.

Hepatocellular carcinoma (HCC) is the 3rd leading cause of cancer deaths globally with a five-year survival of ~5%. Diagnosis of HCC currently relies on routine screening of at-risk patients, including those with cirrhosis due to viral hepatitis, by screening serum alpha-fetoprotein (AFP) levels in conjunction with hepatic ultrasonography but this combination has limitations. Although cost-effective, ultrasonography has only 60% sensitivity and 97% specificity. sAFP is only 40-60% sensitive as many tumors do not produce AFP or do so at very advanced stage. Hence, there is an urgent need to identify better, more reliable noninvasive biomarkers with higher sensitivity and specificity for early detection of HCC. Although ad-
vances have been made to our understanding of the mechanisms of HCC, it still remains incomplete as we grapple with the full spectrum of complexities of the genetic, epigenetic, molecular and cellular events in hepatocarcinogenesis. In this presentation, I will discuss the role of miRNAs in HCC and explore the potential of miRNAs as potential biomarkers and therapeutic targets for HCC.

MicroRNA (miRNA) gain- and loss-of-function can potently influence cellular behavior in normal physiological states and in diseases such as cancer. The regulation of miRNA expression and activity by cellular signaling cascades can therefore result in dramatic phenotypic outputs. We previously demonstrated extensive control of miRNA expression by well-characterized oncogenic and tumor suppressor networks including the Myc, Kras, and p53 pathways. We are now employing novel mouse models with gain and loss of miRNA function to investigate the physiologic functions of the miRNAs embedded within these signaling pathways and the pathologic consequences when their functions are disrupted. Insights gained from these studies are revealing unanticipated roles for miRNAs in vivo and are informing our understanding of how dysregulated miRNA activity contributes to tumorigenesis. I will present our latest findings related to the regulation and function of mammalian miRNAs in normal physiology and in cancer and how these findings may be exploited for the development of novel therapeutic approaches.

MicroRNA Reprogramming in Cancer: Mechanisms and Therapeutic Opportunities

J. T. Mendell. Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX. Sponsor: N. Kaminski.

AhR in Fatty Liver Disease, The Expected and Unexpected

W. Xie. Center for Pharmacogenetics, University of Pittsburgh, Pittsburgh, PA.

AhR was initially defined as a “xenobiotic receptor.” Here, we have uncovered an endobiotic role for AhR in hepatic steatosis and steatohepatitis. Activation of AhR induced spontaneous hepatic steatosis characterized by the accumulation of triglycerides. The steatotic effect of AhR is likely due to the combined up-regulation of fatty acid translocase CD36/FAT and fatty acid transport proteins (FATPs), suppression of fatty acid oxidation, inhibition of hepatic export of triglycerides, increase in the peripheral fat mobilization, and increase in hepatic oxidative stress and inflammation. Promoter analysis established CD36 as a novel transcriptional target of AhR. Moreover, the steatotic effect of an AhR agonist was inhibited in CD36 null mice. Our more recent results showed activation of AhR also sensitized mice to non-alcoholic steatohepatitis (NASH) via the deactivation of the mitochondrial sirtuin deacetylase Sirt3. Industrial or military exposures to dioxin and related xenobiotics have been linked to fatty liver and other disorders. Results from this study may help to establish AhR and its target CD36 as novel therapeutic targets for the human fatty liver diseases.

Chronic Low-Dose Perfluorooctanesulfonic Acid (PFOS) Induces Hepatic Lipid Accumulation and Dampens Caloric Restriction-Induced Lipid Loss in Mice

A. L. Sitt. University of Rhode Island, Kingston, RI.

A combination of caloric restriction (CR), dietary modification, and exercise is the recommended therapy to reverse nonalcoholic fatty liver disease. The ability to mount an effective response to caloric restriction required to effectively shift hepatic metabolism to fatty acid oxidation depends upon induction of sirtuins, AMP-activated kinase, and peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1γ). PFOS, a fluorosurfactant previously used as a stain repellent and anti-stick material, is persistent in the environment and considered an “emerging contaminant” by the Environmental Protection Agency. PFOS (1-10 mg PFOS/kg/day) induced hepatic lipid accumulation associated with altered lipid metabolism, and gene and protein expression in mice. We hypothesized that PFOS interferes with the beneficial effects of CR on hepatic lipid utilization and glucose homeostasis. Adult male C57BL/6 mice were fed ad libitum or placed on a 25% reduced caloric diet concomitant with 100 μg PFOS/kg/day for 6 weeks. PFOS significantly increased percent body weight after 4 weeks of administration, but did not significantly alter CR-induced percent weight loss over 6 weeks. Further studies indicated that PFOS (50 nM, 1, 5, 10, and 50 μM) increased adipogenesis in 3T3-L1 cells through induction CEBPs and PPARγ. PFOS also increased hepatic triglyceride accumulation and hepatic lipid loss after CR was lower in PFOS treated mice but not associated with significant changes in lipogenic gene mRNA expression. As insulin resistance contributes to fatty liver disease, we also evaluated PFOS effects on glucose and insulin tolerance. PFOS did not markedly affect either in ad libitum fed mice but did interfere with CR-induced improvement of glucose tolerance. This was further associated with suppression of Glut-2 and IRS-1 mRNA expression in liver and PFOS stimulation of glucose production in isolated hepatocytes. Overall, a relatively low sub-chronic administration of PFOS had some disruptive effects for lipid and glucose homeostasis under ad libitum and CR conditions.

Exposure to Disinfection Byproducts of Drinking Water in Obesity: From Adipokine Imbalance to Epigenetic Alterations leading to Metabolic Syndrome, NASH, and End-Stage Liver Disease

S. Chatterjee. University of South Carolina, Columbia, SC.

It is hypothesized that obesity is associated with strong risks of development of chronic inflammatory liver disease and metabolic syndrome following a second hit. A series of studies in our laboratory explored the role of chronic exposure of bromodichloromethane (BDCM), a disinfection byproduct of drinking water, in causing nonalcoholic steatohepatitis (NASH), mediated by cytochrome P450 isofrom CYP2E1 and adipokine leptin. Mechanisms range from free radical metabolism of bromodichloromethane, adipokine imbalance, CPG island methylation in key promoter sites of regulatory genes including tumor suppressors, regulatory roles of micro RNAs and the immune system. Diet-induced obese mice, exposed to BDCM exhibited increased hepatic leptin levels, higher proinflammatory gene expression and Kupffer cell activation. Obese mice exposed to BDCM also showed profound hepatic necrosis, Mallory body formation, collagen deposition, higher alpha smooth muscle actin expression, events that are hallmarks of NASH. BDCM-exposed obese mice had alterations in both glucose and lipid metabolism genes leading to changes in the hepatic metabolic profile. Strongly hypermethylated regions in CPG islands of tumor suppressors and regulators of lipid catabolism genes were associated with decreased mRNA and protein expression following BDCM exposure. BDCM exposure led to increased expressions of miRNA 155a, 21,105 and down regulation of miRNA 122. BDCM-administered obese mice had increased CD8+ve/CD57+ve T cell presence that was dependent both on its free radical metabolism and hyperleptinemia. These results provide novel insights into BDCM-induced NASH, hepatic metabolic reprogramming and progression of liver disease in obesity. The molecular mechanisms associated with this study in rodents both at the gene and protein levels, could lead to answers in unraveling the causes of environment-linked fatty liver disease and nonalcoholic steatohepatitis.

Does This Chemical Make My Liver Look Fat (Environmental Exposures and Steatosis)

C. A. McQueen1 and N. I. Cherrington2. US EPA, Research Triangle Park, NC and 1University of Arizona, Tucson, AZ.

Headlines have proclaimed the exponential increase in obesity, type 2 diabetes, and metabolic syndrome in the US and other developed countries. Fatty liver is associated with all of these conditions that adversely affect human health. Initially, fatty liver disease (FLD) is benign, but, unfortunately, the disease may develop into the more serious condition steatohepatitis, characterized by lipid accumulation, inflammation, and fibrosis. Further progression can result in cirrhosis and even cancer. Much research has focused on the cellular and molecular changes in order to develop better diagnostic and treatment options. In contrast, there is only limited knowledge of the causes of fatty liver disease (FLD). Upon diagnosis, FLD is classified as alcoholic fatty liver disease (AFLD), with excessive alcohol consumption identified as the cause, or the broader category of nonalcoholic fatty liver disease (NAFLD). Known causes of NAFLD include a high-fat diet and some drugs, but these only account for a fraction of the estimated 30–40 million cases in the US. A better understanding of the environmental and chemical causes of NAFLD can aid in both prevention and treatment of the disease. This symposium focuses on the role of environmental chemicals in the etiology of NAFLD. The presentations will provide a better understanding of how environmental agents, from disinfection byproducts to pesticides, result in steatosis, as well as the multigenerational persistence of the effects of these exposures. Importantly, new insights will be gained on environmental agents that may contribute to the increasing incidence of NAFLD. (This is an abstract or a proposed presentation and does not necessarily reflect US EPA policy.)

Restriction-Induced Lipid Loss in Mice

A. L. Sitt. University of Rhode Island, Kingston, RI.

A combination of caloric restriction (CR), dietary modification, and exercise is the recommended therapy to reverse nonalcoholic fatty liver disease. The ability to mount an effective response to caloric restriction required to effectively shift hepatic metabolism to fatty acid oxidation depends upon induction of sirtuins, AMP-activated kinase, and peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1γ). PFOS, a fluorosurfactant previously used as a stain repellent and anti-stick material, is persistent in the environment and considered an “emerging contaminant” by the Environmental Protection Agency. PFOS (1-10 mg PFOS/kg/day) induced hepatic lipid accumulation associated with altered lipid metabolism, and gene and protein expression in mice. We hypothesized that PFOS interferes with the beneficial effects of CR on hepatic lipid utilization and glucose homeostasis. Adult male C57BL/6 mice were fed ad libitum or placed on a 25% reduced caloric diet concomitant with 100 μg PFOS/kg/day for 6 weeks. PFOS significantly increased percent body weight after 4 weeks of administration, but did not significantly alter CR-induced percent weight loss over 6 weeks. Further studies indicated that PFOS (50 nM, 1, 5, 10, and 50 μM) increased adipogenesis in 3T3-L1 cells through induction CEBPs and PPARγ. PFOS also increased hepatic triglyceride accumulation and hepatic lipid loss after CR was lower in PFOS treated mice but not associated with significant changes in lipogenic gene mRNA expression. As insulin resistance contributes to fatty liver disease, we also evaluated PFOS effects on glucose and insulin tolerance. PFOS did not markedly affect either in ad libitum fed mice but did interfere with CR-induced improvement of glucose tolerance. This was further associated with suppression of Glut-2 and IRS-1 mRNA expression in liver and PFOS stimulation of glucose production in isolated hepatocytes. Overall, a relatively low sub-chronic administration of PFOS had some disruptive effects for lipid and glucose homeostasis under ad libitum and CR conditions.
**739** Prenatal Obesogen Exposure Causes Transgenerational Inheritance of Increased Fat Mass, Stem Cell Programming, and Hepatic Steatosis

B. Blumberg, University of California Irvine, Irvine, CA. Sponsor: C. McQueen.

Consumption of calorie-dense food and diminished physical activity are accepted causes of obesity. The environmental obesogen model proposes that chemical exposure during critical stages in development influences subsequent adipogenesis, lipid balance and obesity. Obesogens are chemicals that inappropriately stimulate adipogenesis and fat storage. Tributyltin (TBT) is a high-affinity agonistic ligand for RXR and PPAR. RXR-PPAR signaling is essential in adipogenesis and the function of adipocytes. Activation of this receptor heterodimer can elevate adipose mass in rodents and humans. Thus, inappropriate activation of RXR-PPAR can directly alter adipose tissue homeostasis. Our previous work showed in utero exposure to TBT promoted adipocyte differentiation, modulated adipogenic genes in vivo, and increased adiposity in mice. These results are consistent with the environmental obesogen model and suggest that organotin exposure is a previously unappreciated risk factor for the development of obesity and related disorders. Prenatal and early postnatal events such as maternal nutrition, drug, and chemical exposure are received, remembered and then manifested in health consequences later in life. We hypothesized that organotin exposure during prenatal adipose tissue development favors the subsequent development of adipocytes. We found that prenatal TBT exposure altered the balance of progenitor types in the multipotent stromal stem cell (MSC) compartment predisposing them to form adipocytes at the expense of bone. Prenatal exposure to low, environmentally relevant doses of TBT in drinking water led to transgenerational effects on adipose depot weight, adipocyte size and gene expression in MSCs in F1, F2 and F3 animals. Prenatal TBT exposure also increased hepatic lipid accumulation and up-regulated hepatic expression of genes involved in lipid storage/transport, lipogenesis and lipolysis in all 3 generations. Taken together, these results illustrate how prenatal exposure to xenobiotics can have lasting, potentially permanent effects on the offspring of exposed animals.

**740** Ocular Immunotoxicty: A Privileged View

B. Christian and J.C. Schuh. *Nonclinical Safety Assessment, Covance Inc., Madison, WI* and *JCL Schuh, PLLC, Bainbridge Island, WA.*

Vision is achieved through highly specialized ocular tissue structures and processes which refract and transmit light to the photosensitive cells of the retina. Optimal visual function depends on maintaining the integrity and transparency of cornea, aqueous, lens, and vitreous, which can be compromised by unchecked immune reactions. The eye is considered to be an “immune-privileged” organ because of its capacity to moderate intraocular inflammatory responses and protect tissues of the visual axis through anatomic barriers, as well as through local and systemic immunoregulatory mechanisms, particularly immunosuppression. Breakdown or dysregulation of ocular immune privilege can lead to inflammatory disorders such as uveitis, and progression of intraocular neoplasms, and has been implicated in age-related degeneration of ocular tissues. Intraocular inflammation is a commonly encountered response to the intentional breach of immune privilege via intraocular administration of therapeutics. This session will highlight the unique aspects of the immunology of the eye and the associated implications for ocular toxicology and the development of ocular therapeutics. The audience will gain current understanding of structural barriers and active mechanisms of ocular immune privilege. Examples of innate and acquired immune responses to ocular insult, including allergens, microorganisms, and an organ administration of small molecule drugs, biotherapeutics, and viral vector-based gene therapies will be presented. Routine and specialized techniques for evaluating ocular immune responses will also be described. The session will include a presentation describing a current immunomodulatory approach to treat ocular disease involving the complement pathway and possible mechanisms for toxicities in preclinical studies. The final presentation will provide clinical examples and mechanisms of drug-induced immunotoxicity.

**741** Ocular Oversight: Immune Privilege, and Immune Regulation and Dysregulation

J.C. Schuh, JCL Schuh, PLLC, Bainbridge Island, WA.

Ocular immunotoxicology is an evolving field and immune modulation in the eye has implications for successful design, delivery and clinical outcomes for ocular therapeutics. Our understanding of the immunological activity of the eye, including innate, mucosal and acquired immunity has expanded greatly in the last decade. The embryology of the eye is complex and includes formation of the passive barriers of the blood:retinal and blood:aqueous partitions, which are involved in regulation of exposure to systemic immunity and in ocular pharmacokinetics. Uniquely, the eye is a prototypical site of immune privilege, which is not immunological ignorance, but a process of regulated tolerance. Local cellular and secretory activity of immunoregulatory cells in the eye provides an active barrier that minimizes destructive inflammatory outcomes through immunosuppression and anterior chamber-associated immune dysfunction. If released to systemic circulation from an injured eye, ocular antigens interacting with the systemic immune system may lead to sympathetic ophthalmitis, a destructive bilateral auto-immune response. Similarly, neoplastic cells that manage to evade barriers in tissues with immune privilege are also more likely to be progressive. Intraocular uveal melanomas arising within the eye also harness the techniques of ocular immune privilege to avoid destruction upon systemic metastasis. An understanding of ocular passive and active barrier function is important to toxicologists as intraocular injection of drugs or implants, trauma, infections, chemical injuries, paracentesis, surgery, and experimental injection of antigens can result in highly destructive inflammatory responses that threaten vision. This presentation will overview ocular structures, and passive and active barriers of the eye and their relationship to immune privilege, immunopathology, and dysfunctional states that distort the visual axis or result in ocular immunotoxicology.

**742** Innate and Adaptive Immune Responses to Ocular Insult


This presentation will provide an overview of the recent developments in the understanding of ocular surface and intraocular innate and adaptive immune response. Understanding the ocular immune response is essential for specific ocular and systemic general toxicology when assessing biological responses to therapies (small molecule, biologics, gene therapies) given topically, intraocularly, or systemically, their time course, and their implications for preclinical development. The innate or non-antigen specific immune response represents the first line of defense against many pathogens in the eye. The cellular effectors in the innate immune response include neutrophils, eosinophils, mast cells, macrophages, and natural killer cells. While activation of these cells is not antigen specific, innate immunity is based on the recognition of specific pathogen associated molecules through a variety of cell surface receptors. It has long been noted that certain bacterial or fungal molecules, particularly cell wall products such as lipopolysaccharides (LPS) and endotoxins, as well as certain plant-based molecules, can elicit rapid and robust inflammation and immune responses in the eye. These common contaminates of test articles and thus they may confound ocular toxicity results. The antigen specific immune response, also termed the acquired or adaptive immune response, involves a variety of effector mechanisms involving both T cell directed cellular effectors as well as humoral or antibody driven responses to eliminate more complex pathogens. It also provides for a memory response, which is responsible for providing immunity to subsequent infections. It is also responsible for most autoimmune diseases. The antigen specific immune response is dependent on inflammation and the innate immune response for activation and direction. Routine and specialized techniques for evaluating ocular immune responses will also be described.

**743** Complement As a Target for Ocular Disease


Complement pathways have been implicated in human disease for many years and more recently there has been a focus on their role in ocular diseases, including uveitis, macular degeneration and diabetic retinopathy. Complement is a component of innate and acquired immunity and its activity is tightly regulated within the eye. Control of complement activation through the use of inhibitors is an active area of ocular therapeutics. An antigen-binding fragment (Fab) was developed to intervene in the alternative complement pathway for treatment of acute macular degeneration by intravitreal administration. In a pilot safety study in cynomolgous monkeys, adverse effects, particularly in one animal, were characterized by severe inflammation, hemorrhaging, and blindness after a single dose. Investigations into the cause of the toxicities implicated the presence of high levels of anti-drug antibodies in the eye and surface bound Fab. Potential mechanisms for these adverse effects will be discussed.
Gene therapy for ocular diseases is an exciting prospect and recent success in treating Leber’s Congenital Amaurosis with AAV based vectors is encouraging. AAV, Lentivirus, Adenovirus, Herpes simplex virus, and Baculovirus vectors have all been used experimentally to deliver genes to ocular tissues. AAV vectors generally do not trigger ocular immunotoxicity but they have a small carrying capacity. In rodents, these viral vectors do not seem to trigger inflammation; however, a single injection of Lentivirus, Herpes simplex virus, and Adenovirus vectors into non-human primate eyes triggers a transient uveitis. This “toxic” response is an issue in determining whether to proceed with clinical development and with regulatory agencies that approve therapeutics. Vector-induced uveitis develops within a few days of injection, suggesting innate, and not adaptive immune mechanisms are involved. The uveitis does not appear to be due to endotoxin contamination of vector preparations. Cells in the retina express several Toll-like receptors and some of these are up-regulated in the presence of viral vectors. Preliminary array studies suggest that specific changes in host cell innate immunity genes are also occurring. This talk will discuss our efforts to characterize the components of innate immune responses to viruses that are present in the primate eye and how they respond to the presence of these viral gene delivery vectors in order to develop ways to inhibit the responses.

Preclinical models may not predict manifestations of ocular immunotoxicity and this may result in unexpected ocular disease in clinical trials and marketed therapeutics. This presentation will report on clinically relevant immunotoxicity associated with ocular therapeutics. Case reports from spontaneous reporting systems and the world literature were mined for ocular immunological reactions related to medications. The reports come from the FDA spontaneous reporting database, the World Health Organization’s Uppsala Monitoring Center, and the National Registry of Drug Induced Ocular Side Effects (Casey Eye Institute, Portland, Oregon). Multiple drugs were associated with immunologic related pathophysiology. Off target effects of topical ocular chloramphenicol induced aplastic anemia, bisphosphonate related ocular inflammation, and post vaccination uveitis are of particular clinical importance. A working knowledge of some of the most common immunotoxicity adverse effects in ocular tissue is important for toxicologists and clinicians as rapid recognition and drug withdrawal is often curative.

An adverse outcome pathway (AOP) describes a sequence of key measurable events, starting by a molecular initiating event in which a chemical interacts with a biological target, followed by a sequential series of key cellular events, leading to anatomical and functional changes in biological processes and ultimately resulting in an adverse outcome relevant to the human organism and the human population. Thereby, AOP characterization could provide information on the development of structure-activity relationships, i.e., using effect information from one chemical to predict effects for other structurally similar chemicals. Finally, AOPs provide evidence important for qualitative and quantitative predictive models of the adverse outcome that result from triggering molecular initiating or other key events for which high-throughput testing methods can be developed. There are a large number of cellular and molecular processes known to be critical to proper function of the central nervous system (CNS) and peripheral nervous systems (PNS). However, comprehensive understanding of pathways leading from chemical exposure to an adverse outcome in the CNS or PNS is sparse. In this session, five AOPs with relevance for human neurotoxicity will be presented, and common key events across these AOPs will be determined, increasing the possibility to identify potential neurotoxins, even if toxicity is mediated by various pathways. Moreover, vulnerable windows of susceptibility to chemical exposure during brain development and aging will be discussed.

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS). It mediates key aspects of normal brain function such as sensory information, motor coordination, emotions and cognition. However, it has neurotoxic effects when it is present in excess causing neuronal dysfunction and degeneration (excitotoxicity hypothesis). Most neurons have the ionotropic glutamatergic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid and kainic acid receptors (NMDAR, AMPAR, KAR), and glutamate-induced neurotoxicity occurs due to their over activation, mainly the NMDAR, which allows the influx of cations, most notably calcium. The activation of NMDAR requires not only binding of various ligands but also cellular depolarization that can be triggered by the activation of AMPAR and KAR. NMDAR are predominantly distributed in the hippocampus, cortex and basal ganglia, where they play a major role in the processes of learning and memory that occur in the hippocampus. The Adverse Outcome Pathway (AOP) methodology is designed to provide a causative link between a Molecular Initiating Event (MIE) and an adverse health effect due to exposure to toxicants. An AOP is proposed that links domoic acid (DA) neurotoxicity (manifested by seizures and loss of memory and histopathological hallmarks like cell death in the hippocampus and other brain areas) with the activation of KAR as the Molecular Initiating Event. Key events in this process are the high affinity of DA to bind KAR, the influx of Na+ and Ca2+ leading to cell depolarization, the release of glutamate from hippocampal neurons, which in turns over activates NMDAR that leads to hippocampal cell death. Data gathered from the bibliography related to the effects of domoic acid-contaminated mussels on human
health, as well as the effects of domoic acid on animal status, on glutamate receptors in vitro preparations and on cultured neuronal cells allow to get a picture of this pathway of neurotoxicity. Supported by the Spanish Grant FIS PI10/0453 cofinanced with European Social Funds

750 SH-Group Binding-Induced (Developmental) Neurotoxicity

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The adverse effects of methylmercury (MeHg) on the nervous system have been observed in humans, experimentally studied in animals, and MeHg serves as a test compound in many alternative in vitro NT and DNT methods. Like other electrophilic neurotoxins MeHg binds to sulphydryl groups on macromolecules in cells and body fluids. Such interactions might be the molecular initial event (MIE) in the AOP of several neurotoxins sharing the feature of electrophilicity. In case of MeHg the formation of a methylmercury cysteine complex (MeHgCys2), mimicking methionine, enables the compound to cross membranes (e.g. blood brain barrier) via specific amino acid transporters. Due to this event, and in contrast to inorganic mercury, MeHg becomes a potent neurotoxin that causes disturbances of glutamate uptake by astrocytes or induces ROS by affecting the mitochondrial electron transfer chain. Like other electrophilic neurotoxins MeHg might bind to glutathione (GSH) reducing the antioxidant capacity of neurons. Thus, in case of MeHg one key event of the AOP is reinforced by another. Such multiple and sometimes interactive molecular and cellular events might cause disturbance in brain physiology (e.g. reduce activity-dependent neuronal plasticity) resulting in the neurobehavioral phenotype of MeHg poisoning.

751 Adverse Outcome Pathway (AOP) for Neuroinflammation

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Neuroinflammation implies the reactivity of microglial cells and astrocytes. It can be triggered following neuronal stress, injury, or death, following demyelination, or direct activation of microglia and astrocytes. Several classes of toxic molecules initiating events leading to oxidative stress, calcium overload, cytokine instability, synapse impairment, or apoptosis, can trigger a neuroinflammatory response. Therefore, neuroinflammation is a common key event.

Depending on the pathogenic context and/or on the duration of neuroinflammation, glial cells can acquire a neurodegenerative phenotype, which will cause neurodegeneration. Neurodegeneration, the adverse outcome, will exacerbate the neuroinflammatory response and lead to a self-sustained neurodegenerative loop. It is generally accepted that neuroinflammation is involved in several neurodegenerative diseases, playing an important and early role in the disease process. Therefore the detection of neurotoxicant-induced increased expression of the markers of the microglial neurodegenerative phenotype (Igta2, CD86), of some pro-inflammatory cytokines (TNF-alpha; IL-1beta; IL-6), of the reactive oxygen species production (i-NOS, hsp32) and of the astrocyte reactivity (GFAP) associated to a decrease of the markers of the microglial reparative phenotype (Arg1, MRC1) and of some anti-inflammatory cytokines (IL-4, IL-13), completed with morphological observations of an increased number of microglial cells and of markers of their macrophagic state (ED1) will provide early alert for neurotoxicants that may be considered as risk factors for the development of neurodegenerative diseases. The use of in vitro models for screening strategies is possible providing cultures contain all types of brain cells, since cell-cell interactions are crucial for the neuroinflammatory process.

752 Oxidative Attack of the Neural Progenitor Cell Niche As an AOP for Neurotoxicity of the Elderly

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Hippocampal neurogenesis accompanied by neural progenitor cell (NPC) proliferation and differentiation takes place in the adult human and rodent brain. Thereby, loss in regenerative (functional) capacities of NPC, i.e. decline in NPC proliferation and neuronal differentiation, causes the steady process of ‘Normal Brain Aging’ with increasing cognitive decline over time. Loss of antioxidative response is one cause for brain aging and decreasing amounts of the major antioxidative transcription factor Nrf2 are found in brains/NPCs of aged rodents in vivo. Just as in vivo, in vitro aged NPCs also loose Nrf2 function. Applying a lentiviral shRNA approach, we identified Nrf2 as a critical pathway contributing to decline in hNPC proliferation and neuronal differentiation thus proving its causal role in declined hNPC function during aging. Shifting the cellular redox balance towards the oxidative side produces accumulation of intracellular NAD+ (molecular initiating event). NAD+ serves as a co-factor for the HDAC Sirt1, which is involved in inhibition of pro-neuronal gene transcription like Mash1 by association with Hes1. On the contrary, reducing conditions supported by decline in Nrf2 leads to decreased neuronal differentiation in adult brains by activation of the HDAC Sirt1 (Key event). This diminished neuronal regeneration causes cognitive decline on the individual and therefore also on the population level (adverse outcome).

753 Idiosyncrasies of Cells in Culture: Lessons from Genetic Toxicology

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Because of a desire to reduce laboratory animal use and a goal to provide information using human cells in culture, rather than rodents, there is an international focus on developing in vitro predictive toxicity assays for chemical hazard assessment. These factors, coupled with the new molecular ‘omics technologies, have provided the major impetus behind the Tox21, ToxCast, and other efforts to move toward an all in vitro hazard test battery. The genetic toxicology community began developing in vitro assays to predict both cancer and human heritable genetic disease in the early 1970s. Ultimately, a regulatory test battery was established and is used by the majority of international regulatory agencies. Over the decades there has been substantial knowledge gained concerning the strengths and weaknesses as well as the important issues involved in using in vitro assays for hazard assessment. In addition, major improvements to the genetic toxicity assays and the interpretation of data have been accomplished over this time period. Currently, there is a multi-sector international effort with a focus on better understanding the issues around cell stewardship, cell line characteristics, and the appropriate strategies for providing/assuring the required metabolic activation for in vitro systems. This effort, coupled with other genetic toxicology community, including the use of 2D vs. 3D cell cultures, and the selection of endpoints and correlations with apical health outcomes, provide extensive information that should inform the current efforts to establish all in vitro approaches for chemical hazard assessment.

754 Overview and Perspective Based on Lessons Learned from the Evolution of In Vitro Genetic Toxicology

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The discipline of genetic toxicology began more than four decades ago with the development of a large number of assays in a large number of different organisms and in cell culture. Early on, the postulate was an in vitro assay system was “better” than animal tests. Unfortunately, over time, with the testing of more chemicals and the addition of more in vitro assays, it became clear that the Ames Test and other assays were not completely satisfactory for reliable and accurate carcinogen prediction. The fact that the apical health outcome (the induction of tumors) is not the result of a single biological effect (mutation) that leads, in every case, to a tumor, adds complexity to the situation. Generally, when regulatory agencies selected their preferred genetic toxicity test battery, assays were chosen based on the type of genetic damage that they detect rather than correlation with rodent cancer bioassays. There are currently many parallels between the emerging field of all in vitro toxicology testing and the evolution of in vitro genetic toxicology tests. Many major issues were identified and tackled for in vitro genetic toxicology assays that are just now being recognized as important for the new 21st century in vitro assays. The experiences and lessons learned from the evolution of genetic toxicology will be put into a perspective to provide insight into optimal development, validation and ultimate utilization of new in vitro systems to predict toxicological outcomes.
Good cell culture practices are of critical importance for the reliability and reproducibility of results obtained from in vitro cell systems. In addition to quality standards applied to any cell system, cell cultures used for toxicity testing should demonstrate their ability to accurately detect the endpoint of interest. Experience with genetic toxicology testing emphasizes the importance of avoiding "genetic drift" and minimizing selective pressure that could potentially interfere with the system used to detect the event of interest. Although cell culture based genetic toxicology tests have been in use for several decades, it is only recently that experts in the field have embarked on a mission to provide well characterized, documented, and continuously available stocks of the most common cell lines used in genotoxicity testing. One of the first tasks was to identify the cell line isolate as close as possible to those described in the original publications and characterize these cells on their performance and, where possible, spectral karyotype analysis. These well characterized cells were deposited at multiple locations globally (European Collection of Cell Cultures – U.K., Sigma – U.S., and Japanese Collection of Research Bioresources - Japan) to ensure the continuous availability of certified cells for use in genotoxicity testing. This experience from genetic toxicology should be extended to other toxicity test systems in view of the growing interest in the application of in vitro test systems for high throughput (e.g. ToxCast/Tox21) and other regulatory-mandated safety assessment studies (e.g., Endocrine Disruptor Screening Program of the U.S. EPA). It is imperative that consistent performance and reliability of all aspects of these in vitro test systems are critical elements of realizing the U.S. National Academy of Sciences vision of "Toxicity Testing in the 21st Century."

In vitro genetic toxicology assays have been reported to have a high rate of positive results that are not confirmed in in vivo studies and raising the question of relevance to human risk assessment. This observation led to several studies in which initial observations appeared to indicate that there might be a difference between cells with normal and impaired p53 function, altered DNA repair capacity and/or human versus rodent origin. As part of an on-going collaborative programme of work looking to improve existing in vitro assays (reducing the rate of so called "false positive" results) commonly used mammalian cell types of different species origin, with differing p53 status have been investigated. This present study compared human versus mouse and p53 competent versus p53 mutated function. Nine reproducibly 'false positive' chemicals were tested in a microumulus study using mouse lymphoma L5178Y-3,7,24' mutagenesis (mutated p53), human TK6 (functional p53) and WI2-NS (TK6 related with mutated p53). Although p53 function does not seem to be the overriding factor impacting the generation of positive responses, rodent versus human origin and apoptosis (measured via Caspase-3/7 activity) may have a role to play. This experience emphasizes that differences in qualitative and quantitative responses among cell lines is generally not based on one or perhaps a small number of cell line specific characteristics. Rather, it indicates the importance of understanding the individual cell lines used in toxicological evaluations and accepting the fact that there will be differences in the responses and that proper test interpretation requires that cell line characteristics be factored into test interpretation.

Ames and colleagues later established a standard in vitro metabolic activation mixture containing hepatic microsomes from male rats exposed to AhR agonists (e.g., PCBs). Indeed, rat liver microsomal preparations (i.e., S9) have become the standard for in vitro assessment of chemical mutagenicity. The system is not without its problems however, and researchers have investigated the utility of S9 preparations from a wide range of tissues and animals, as well as a host of alternative approaches for simulating mammalian metabolism in vitro. Fast forward to 2007, and the launch of the ToxCast program for rapid, high-throughput in vitro assessment of chemical toxicity. The ToxCast and Tox21 programs are employing a wide range of in vitro assessment tools, capable of monitoring a vast array of endpoints, to screen over 10,000 substances. However, incorporation of mammalian xenobiotic metabolism into the quantitative high-throughput screening (qHTS) paradigm has proved difficult. Several options are being explored (e.g., metabolically competent cells, coculture, etc), some of which were initially developed for in vitro assessment of genetic toxicity. The presentation will provide an overview of the technical challenges underscoring effective in vitro simulation of mammalian xenobiotic metabolism, and highlight promising avenues for effective qHTS.
For a chemical to demonstrate phototoxity and/or photoulage, the following characteristics are critical: Absorbs light within the range of natural sunlight (290-700 nm); generates a reactive species following absorption of UV/visible light (UV/VIS); and distributes sufficiently to light-exposed tissues. UV/VIS absorption by chemicals alone may not always directly correlate with phototoxic potential and may provide false photoreactivity predictions; however, when UV/VIS absorption is associated with a molar extinction coefficient (MEC) of less than 1,000 M-1 cm-1, there is little phototoxic risk since this low level of light absorption is unlikely to be harmful. Reactive oxygen species (ROS) are generated via energy transfer mechanisms following UV/VIS excitation, including superoxide and singlet oxygen. The ROS assay has been designed to assess photoreactivity of pharmaceuticals, of which the principle is to monitor types I and II photochemical reactions of the test chemicals when exposed to simulated sunlight. This simple analytical test could be used to screen potential chemical scaffolds, leads, and candidate drugs to identify and/or select away from those having phototoxic potential. The validation study for the ROS assay has been carried out by the Japanese Pharmaceutical Manufacturers Association (JPMA), supervised by the Japanese Center for the Validation of Alternative Methods (JaCVAM). Although several false positives appeared, the ROS assay on 42 coded chemicals has provided no false negative predictions. The validation study tentatively indicates satisfactory outcomes in terms of transferability, intra- and inter-laboratory variability, and predictive capacity. Thus, a negative result in this ROS assay would indicate a very low probability of phototoxicity, whereas a positive result would be a flag for follow-up assessment. In conclusion, UV/VIS analysis, MEC, and the ROS assay are non-biological assessments useful for the prediction of the photoreactive property, and their strategic use may minimize animal use in photoreactivity testing of pharmaceuticals.

Clinical development of therapeutic products may encounter issues related to photosafety. This can be due to photoirritation or to a photoallergic reaction, both broadly covered under the indiscriminate term “photosensitivity.” Over 300 therapeutic products are reported to have given photosensitivity reactions, including a variety of pharmacologic classes and biologic agents. Lack of an accurately defined population base makes it difficult to estimate the real incidence of photosensitivity associated with pharmaceutical use. Of the implicated medications, only a minor proportion is of sufficient frequency to be studied in greater detail. Clinical manifestations of adverse drug reactions upon light exposure depend on the interaction between the host, the agent, and the radiation. They range from an acute inflammatory skin reaction, unusual clinical patterns, to photocarcinogenicity. Thus, prediction of the potential for photo-reactions in humans is challenging, both for frequency and severity, and standardized conditions for studies intended to compare pharmaceuticals for photosafety are important. Current approaches to the evaluation of photosafety in clinical trials are primarily based on adverse event reporting or active solicitation of anticipated events, as well as for five possible findings when there is suspicion from nonclinical studies. Issues concerning this strategy include bias introduction, limitation of sample size, and variability in usage in postmarket studies. For topical products, drug development necessitates predictive testing under standard conditions when the to-be-marketed formulation contains ingredient(s) absorbing visible or ultraviolet light. Such testing for photoirritation and photocontact allergy is informative for the design of precautions and safety monitoring in later-phase clinical trials as well as for product labeling. As there are advances in vitro studies for photo-reactions and the development of human biomarkers for adverse experiences, it is likely that these will impact future approaches to the clinical evaluation of photosafety.

Reconstructed human skin models, with the presence of a stratum corneum and lipid barrier, permit testing of various types of topically applied materials ranging from neat chemicals to final clinical formulations. To date, the methods developed have been based on measuring cell viability in the tissue with and without irradiation. The release of cytokines/chemokines into the medium can be informative as well. In the 1990’s, an ECVAM pre-validation phototoxicity study on the EpiDerm™ model (Liebisch et al., 1999) showed that reconstructed human epidermis (RhE) models are capable of correct detection of the acute human phototoxins and non-phototoxins. Several studies with other commercially available reconstructed tissue models (EPISKIN®, SkinEthic™) confirmed these findings. The sensitivity and specificity of assays developed with RhE models for prediction of acute skin phototoxicity are close to 100%. Use of RhE-based phototoxicity assays for prediction of the topological phototoxicity testing was recently implemented into the Draft ICH Guidance Document on Phototoxicity Testing (S10) and As shown in a feasibility study with EpiDerm sponsored by ECVAM between 2002-2005 (contract No. 19868:2002-09 FIED ISP DE), these models can also be used to assess photo-potency, i.e., to assess the phototoxic strength of a topical phototoxin and to estimate the non-phototoxic concentrations that can be safely used in human (e.g., for therapeutic use). Based on comparison between in vitro and in vivo human studies, a safety factor of 10 fold is recommended when extrapolating the in vitro results to man. This presentation will focus on current 3-D skin models for the assessment of the phototoxicity potential of dermal formulations. The presentation will provide an overview of RhE-based phototoxicity assays and discuss their strengths and weaknesses for photo-safety assessment in toxicology and pharmacology.

Stem Cell-Derived Cardiomyocytes: An Alternative Cardiac Toxicity Model for Assessing Drug Safety and Chemical Health Risk

Cardiac safety is a major cause of attrition, withdrawal, and adverse reactions in drug development. It is estimated that adverse cardiac and/or vascular reactions account for greater than 20% of drug attrition over the preclinical, clinical trials, and post-market phases. Effective chemical safety evaluations are also critical for protecting public health. Significant adverse cardiac effects following exposure to a variety of chemicals present in the environment have been reported by the World Health Organization and other researchers. Cardiac safety associated with pharmaceutical drugs and chemical exposures are a mutual focus of keen interest for clinical and public health researchers, academic scientists, government regulators, and industrial scientists worldwide. The development of alternative testing methods of predictive drug and/or chemical toxicity could impact drug discovery and safety evaluations as well as chemical health risk assessment. This workshop brings together a trans-sector and multidisciplinary group of experts to present and discuss
the use of stem cell-derived cardiomyocytes as an alternative model to determine alterations in cardiac electrophysiology, contractility, and structural and developmental toxicity following drug or chemical exposure. Presenters and discussions will address the sensitivity, reproducibility, and biological relevance or ‘fit for purpose’ of stem cell-derived cardiomyocytes and how these factors relate to the use of such data in a screening, risk, or safety evaluation for drugs and chemicals.

**W 765** Transitioning Cardiac Stem Cells from Research Platforms to Predictive Tools
S. D. Pettit. ILSI, Health and Environmental Sciences Institute, Washington, DC.

The availability and use of induced, stem-cell derived cardiac myocytes has grown exponentially in the last 10 years. With this growth has come a concurrent diversification of the potential applications for these assays – for structural, functional, and toxicity based assessments as well as direct clinical translation. This presentation will highlight key areas that could benefit from greater consensus and weight of evidence. These include the sensitivity, reproducibility, and biological relevance of these cell lines and how these factors relate to the use of such data in a screening, risk, or safety evaluation context.

**W 766** Stem Cell-Derived “Cardiomyocytes” and Their Application to Cardiac Safety Assessment: Ready for Primetime?

Human stem cell-derived cardiomyocytes (SC-CM) are an emerging model for the study of drug safety, in particular drug-induced changes in cardiac electrophysiology and contractility. The potential for SC-CM to be used for proarrhythmia assessment is also being considered. An advantage of this test system is that safety pharmacology studies can be performed in human embryonic progenitor cells or adult fibroblasts transformed to resemble mature cardiac myocytes, which could assist translation of nonclinical to clinical findings, and risk assessment. The utilization of an in vitro human SC-CM system to de-risk candidates during preclinical drug development could improve our ability to bring forward novel and safe drug candidates for clinical evaluation. However, the application of SC-CM for drug safety requires robust validation of these cells, to understand how closely they resemble authentic mature ventricular myocytes, and their performance relative to conventional assays used in cardiovascular safety testing. What are the limitations of the human SC-CM model? This presentation will explore the electrophysiologically evaluation of SC-CM, their response to a variety of ion channel blockers known to alter cardiac conduction and repolarization, and discuss some of the challenges that need to be overcome to validate the use of SC-CM for cardiovascular safety assessment.

**W 767** Stem Cell-Derived Cardiomyocytes in High-Throughput Screens for Modulators of Contractility
M. Mercola. Department of Bioengineering, University of California San Diego, San Diego, CA. Sponsor: S. Pettit.

Human stem cell-derived cardiomyocytes (SCCM) are an emerging model for the study of drug safety, in particular drug-induced changes in cardiac function. The application of SCC in drug safety evaluation requires robust validation of these cells, to understand their performance relative to other pharmacological assays used in cardiovascular safety testing. To facilitate high throughput analyses of cardiomyocyte physiology, we developed kinetic imaging cytometry, a high content screening technology capable of measuring cardiomyocyte contractile calcium transients and voltage action potential kinetics and morphology. The instrument and dedicated software are now commercially available, and function by optically recording and analyzing the fluorescence changes of small molecule calcium and voltage sensing probes in response to electrical field stimulation and exposure to ion channel blockers known to alter cardiac conduction and repolarization. We have begun to evaluate the technology using a panel of safe and arhythmicogenic drugs, revealing EC50 values similar to the reported EC50 and/or IC50 values obtained by biochemical and traditional electrophysiological studies. The presentation will discuss current progress and limitations in the optical, high throughput measurements of electrophysiological parameters to assess arhythmicogenic risk. Furthermore, we have used kinetic imaging cytometry in screening campaigns to identify molecules and targets to sustain cardiomyocyte contractility in heart failure, leading to the development of a potential RNA therapeutic that shows efficacy in halting the progression of established heart failure in a mouse pressure overload model.

**W 768** High-Content Screening of Bioenergetic Modulation of Kinase Inhibitor Mitochondrial Toxicity in Human Stem Cell-Derived Cardiomyocytes
N. Thomas. GE Healthcare, Whitchurch, Cardiff, United Kingdom. Sponsor: S. Hunter.

Cardiotoxicity is a significant issue in the development of new drugs targeting key cellular kinases. Human embryonic stem cell (hESC) derived cardiomyocytes cultured in glucose and galactose media were used to examine the cardiotoxicity of twenty six kinase inhibitors and other drugs using high content screening (HCS) to examine a number of key mitochondrial and cellular parameters indicative of toxicity. Analysis of HCS data revealed a number of compounds with increased toxicity under conditions of mitochondrial oxidative phosphorylation. The tyrosine kinase inhibitor Mubritinib, was non-toxic in cells cultured in glucose but exhibited very significant mitochondrial toxicity in galactose media which correlated with the direct impact of the compound on mitochondrial activity measured by respirometry. HCS is a sensitive and efficient approach to surveillance of drug cardiotoxicity in hESC derived cardiomyocytes with multi-parameter analysis providing insight into toxicity mechanisms and that cellular bioenergetics can significantly influence drug cardiotoxicity.

**W 769** Evaluating Chemical Safety, Molecular Targets, and Toxicity Pathways in Mouse Embryonic Stem Cell Differentiation to Cardiomyocytes
E. S. Hunter. US EPA, Research Triangle Park, NC.

As one of the earliest systems to develop and function, evaluation of the cardiovascular system as a critical target for chemical safety and risk assessment in cardiogenic anlagen are established with formation of cardiac plate mesoderm, cardiomyocyte differentiation and establishment of a beating heart tube. The embryonic stem cell test examines compound effects on differentiation of pluripotent mouse ESC to beating cardiomyocytes. Our modified Adherent Cell Differentiation and Cytotoxicity assay evaluates differentiation to cardiomyocytes using myosin heavy chain (MHC) or gastrulation using goosecoid (GSC) as biomarker proteins (In-Cell Western analysis). Additionally, differentiation to multiple cell lineages is assessed using mRNA biomarkers (quantitative nucleic acid assays). Cell number was determined to account for induction of excess cell death or altered proliferation. We evaluated 276 chemicals of the ToxCast phase I library for effects on MHC and GSC. Twenty six chemicals produced a >50% change in the cardiomyocyte differentiation biomarker and 80 chemicals produced more than a 20% change. Two predominant ‘targets’ were identified for chemicals that decreased cardiomyocyte formation, BMP signaling and altered redox potential. Cardiomyocyte differentiation requires normal development and function of the lineages that precede its specification. A comparison of chemical effects indicated that 45% of chemicals that perturb cardiomyocyte differentiation also affect gas-trulation, raising questions of selectivity and specificity of targets and pathways. Thus, ESC can be used to evaluate compound effects on cardiomyocyte formation. Studies that characterize the cell lineage ontology are needed to determine the target cell population and define molecular targets for an assessment of the specificity of effects on cardiomyocyte differentiation. This abstract does not represent EPA policy.

**W 770** The Doorway between Exposure and Response: How Biologically-Based Inhalation Dosimetry Models Enhance Human Health Risk Assessment
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The respiratory tract is the portal of entry for inhaled gases, vapors, and particles, providing a route of entry for systemic exposure and a potential target for toxic agents. The inhaled dose is dependent on the physical state, solubility, reactivity and concentration of the material, the structural and functional characteristics of the respiratory tract, and the types and metabolic capability of the surface epithelial cells. It is also contextual and dependent on the dose metric used, whether it is the systemic blood or tissue concentration or the regional dose to specific anatomic sites or target cell populations in the respiratory tract. Human risk assessments are often based on inhalation toxicity studies in rodents, yet a number of vapors, including hydrogen fluoride and diacetyl, have been shown to produce nasal and large airway injury in rodents but small bronchiolar airway injury in humans. Computational modeling has confirmed significant differences in regional vapor absorption patterns between rats and humans that provide a rational framework to explain the observed differences in site-specific injury between species. Multiscale
computational models of the respiratory system that incorporate species-specific anatomical details and data derived from in vivo measurement of particle deposition/clearance and vapor absorption have been developed to improve estimates of material transport and regional dosimetry. Model accuracy is greatly improved by incorporating regional morphologic characteristics of the mucosa and exposure-response data to validate model predictions of site- and species-specific dosimetry. The goal of this workshop is to provide participants with a working knowledge of the state-of-the-art computational tools available to predict regional deposition, absorption and dose of inhaled materials in laboratory rodents, the essential data needed to validate these predictions, and the use of biologically-based computational modeling in human health risk assessment applications and related research.

**W 771 Site-Specific Airway Pathology and Dosimetry of Inhaled Toxicants**
J. R. Harkema, Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI.

Injury caused by acute and chronic inhalation exposures to airborne toxicants (gases and particles) is site specific in the respiratory tract, and dependent on airway dosimetry and cell/tissue sensitivity. Airway dosimetry and injury of inhaled toxicants are further determined by many additional factors such as the physicochemical character of the inhaled toxicant, exposure regimen, host species/strain, age, gender, diet and overall health. In this talk, the speaker will briefly review comparative anatomy and physiology of the respiratory tract in laboratory animals (rodents and nonhuman primates) and humans. He will further provide examples of site-specific nasal and pulmonary pathology caused by exposures to various gaseous and particulate toxicants (e.g., aldehydes, chlorine, ozone, nanoparticles). In addition, examples of state-of-the-art, yet practical, approaches to unbiased determination of exposure-related morphometric, molecular, and biochemical alterations (i.e., computational toxicologic pathology) will be discussed. The talk will conclude with a description of methods to correlate site-specific pathology with estimated 3D-airway dosimetry and modeling of both the upper and lower respiratory tract in laboratory animals that can be used in predictive toxicology to estimate human risk to toxicant exposure.

**W 772 In Vivo Measurements of Dosimetry: What Do the Data Say?**
J. B. Morris, Pharmaceutical Sciences Toxicology Program, University of Connecticut, Storrs, CT.

This presentation will discuss in vivo measurements of regional vapor absorption and integration of that data into computational models of inhaled dose of absorbed vapors for purposes of human health risk assessment. In rodents a large dataset exists on vapor uptake in the isolated upper respiratory tract (URT). Data are also available on whole respiratory tract uptake. Data on URT uptake reveal the critical importance of solubility (as measured by tissue/cair partition coefficient), local metabolism, and direct reactivity in influencing vapor absorption in that site. Physiologically based pharmacokinetic (PBPK) model structures accurately predict the importance of these parameters in controlling uptake, thus providing validation of the PBPK-based approach. Extension of this modeling approach to the whole respiratory tract also provides accurate prediction of total vapor uptake in the rodent. A rich data set from mouth-breathing experiments is available on lower respiratory tract (LRT) vapor uptake in humans. As for the rodent, vapor solubility, local metabolism and direct reactivity are important factors influencing vapor uptake and PBPK-based model structures accurately predict the data that has been obtained. Diacetyl is a vapor that causes nasal and large airway injury in rodents but is associated with bronchiolar injury in man. Application of the PBPK-based modeling approach to diacetyl vapor predicts that the delivery of diacetyl to the lower airways in the human is much greater than in the rat particularly in mouth breathing humans undergoing light exercise. This difference is greatest at lower exposure concentrations at which local airway metabolic pathways are not saturated. The rodent-human difference in airway delivered dose likely reflects a general pattern rather than one specific to diacetyl. Thus, modern validated modeling approaches provide key insights that can be incorporated in quantitative inhalation risk assessments.

**W 773 Interspecies Differences in Respiratory Dosimetry of Volatile and Aerosol Materials Using Multiscale Computational Models**
B. A. Corley, Exposure Science & Systems Toxicology, Pacific Northwest National Laboratory, Richland, WA.

Computational models are often used to link exposures to target tissue doses and responses to inhaled gases, vapors and aerosols. Models have ranged from empirical to compartmental, or mechanistic approaches, depending upon the application and available data. For materials that target specific sites or cell types within the respiratory tract, computational fluid dynamics (CFD) airflow-based models that explicitly incorporate 3D anatomy and physiology have emerged as a leading choice for predicting species- and site-specific exposure-dose-response relationships. In the past, development of such models was expensive, time-consuming, and limited to highly specialized applications. Using modern 3D and 4D imaging and computational methods, CFD models have become more widely available for use in chemical, pharmaceutical, and biological exposure assessments in multiple species including mice, rats, rabbits, monkeys, and humans. These models can extend from the nose/mouth to the bronchiolar airways of the lung. Lower dimensional models can be added to 3D models to address tissue mechanics and the impact of disease on airflows and tissue dosimetry in the deep lung. Likewise, physiologically based pharmacokinetic (PBPK) models have been utilized as airway boundary conditions in CFD models for material transport, uptake, metabolism, and clearance by respiratory tissues along conducting airways. This talk will emphasize the experimental foundation for the development, evaluation, and refinement of these multiscale models, including 3D and 4D CT imaging of normal and diseased animals and humans; MR imaging of hyperpolarized 3He gas flows; airway extraction studies with volatile chemicals; and particle deposition using breath analysis, SPECT/CT imaging; and post-mortem tissue analysis. The potential impacts these multiscale computational models have on relating exposures to site-specific “doses” that are critical for toxic responses and potential risk assessments will be demonstrated for several volatiles and aerosolized materials.

**W 774 Material Transport, Deposition, and Clearance in the Respiratory Tracts of Humans and Animals**

Assessment of the dose and site of deposition of inhaled materials in the lung aids in the interpretation of biological response. The deposited dose provides the link between external exposure and potential adverse effects. While high fidelity computations of the dose are only feasible for the proximal regions of the lung which fall within the resolution of scanned images, anatomically accurate and physiologically relevant mathematical models of material transport and deposition in the entire respiratory tract are developed for humans and a few selected animals to predict site-specific dose and tissue uptake, and allow interspecies extrapolation based on various dose metrics. Mathematical models have been formulated for insoluble, hygroscopic, nonspherical (fibers and nanotubes) particles as well as for vapors and mixtures. Modeling approach and application to a few specific scenarios will be discussed and a few examples will be presented.

**W 775 The Role of Inhalation Dosimetry Models in Multiscale Support to a Range of Risk Assessment Applications**
A. M. Jarabek, National Center for Environmental Assessment, US EPA, Research Triangle Park, NC.

Inhalation dosimetry models afford the flexibility and versatility to support a range of potential applications in human health risk assessment that spans aiding in vitro experimental design, through translation of dose across various test species, to facilitating inferences regarding pathogenesis processes typically represented by endpoints that may also span a scale from genomic profiling in specific target tissues to population biomonitoring. Understanding how the various model structures discussed in the previous presentations can aid these various applications in risk assessment is critical to advancing the accuracy of it as a science-based discipline. This presentation addresses how to best marry available model structure to risk assessment application with examples for both particles and gases. Impact of model applications illustrated include in vitro dosimetry for the design of nanoparticle experiments, translation of fiber dose across species, inferring exposure from biomonitoring of solvents, and using models to inform the plausible mode of action for reactive gases. Opportunities for refining current default inhalation dosimetry algorithms used in dose-response analysis, originally already based on fuller model forms, will be highlighted to show how risk assessment applications continue to evolve as more sophisticated computational structures emerge. Ultimately such
computational models of inhalation dosimetry will serve as the bridge to virtual tissues and systems biology descriptions of the cardiopulmonary system, and link models of multi-pollutant exposures in a given aired to a multi-dimensional description of mode of action and resultant disease. (The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.)

**PL 776 Adverse Outcome Pathways As a Method to Characterize Potential Sources of Increased Risk of Health Effects Attributed to Inorganic Arsenic**


Inorganic arsenic is associated with a number of human health effects including the development of tumors and other diseases. These health effects can be organized into collections of biological responses, i.e., key events that represent adverse outcome pathways (AOPs). An analysis of AOPs may be useful in characterizing the potential impact of sources of variability for health effects, such as the increased risk for particular key events or adverse outcomes. A literature review was conducted to identify the factors that may increase risk for an arsenic-induced adverse outcome, transitional cell tumors of the urinary bladder. Using an AOP framework, we characterized potential impacts of individual-level factors (e.g., sex, genetic polymorphisms, nutritional status, and cigarette smoking status) and life stages (e.g., childhood and in utero) on the risk of bladder cancer. Preliminary results support the hypothesis that factors associated with reduced secondary methylation (i.e., the conversion of monomethylarsonic acid (MMA) to dimethylarsinic acid (DMA)); may also correlate with increased risk of bladder cancer, including sex, genetic polymorphisms, nutrition, and smoking. Our results demonstrate the utility of an AOP analysis in identifying events to which certain human populations may be at increased risk to arsenic-induced bladder cancer.

Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

**PL 777 Evaluation of an Arsenic Reference Dose for Potential Neurobehavioral Effects in Children**

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Health effects associated with low-dose exposures typically occur only after long-term, repeated exposures. However, a health-protective dose based on neurobehavioral effects in children has the potential to be lower than a lifetime-average dose that is protective of chronic health effects. Since an earlier review finding skin lesions as the most sensitive endpoint for populations of arsenic-exposed children, more recent studies have examined cognitive effects in populations exposed to lower arsenic levels in drinking water (<100–200 μg/L). Based on a systematic review of the epidemiological literature (reported separately), we identified critical studies to assess a point of departure (POD) for evaluation of a reference dose (RfD) for neurobehavioral effects. Data from a population-based longitudinal cohort study in Bangladesh provided the strongest evidence for quantifying an association between low-level arsenic exposure and neurobehavioral effects in children. Based on the specific test, gender, and age with the highest positive association between cognitive results (verbal IQ in girls at age 5) and specified urinary arsenic level, a 38 μg/L specified urinary arsenic level is a POD, assuming a decrease of 1 IQ point. This urinary arsenic level is well above the distribution in U.S. children. Using an age-specific urinary excretion rate and population-specific body weight, the POD was converted to an absorbed dose of 0.001 mg/kg/day. Uncertainty factor selection considered study population sensitivity and potential bias toward overestimation (highest dose-response used, malnutrition, incomplete correction for maternal IQ and other socioeconomic factors). Application of an uncertainty factor of 1 to 3 would result in RfDs in the range of 0.001 to 0.0003 mg/kg/day. The existing RfD for chronic exposure in adults (0.0003 mg/kg/day) is thus also protective of potential neurobehavioral effects in children.

**PL 778 Characterization of Blood Lead Levels for Children in a Community Affected by Historical Mining Activities**

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Numerous studies of child blood lead levels (BLLs) were conducted during the 1980s and 1990s in former mining communities in the western U.S.; however, data characterizing contemporary BLLs in such communities are not readily available. For the past decade, blood lead samples have been analyzed for children ages 1-5 in Butte, MT where mining and mineral processing activities have occurred for more than a century within the community. Additional samples were also collected for some children outside the area most affected by mining, and for infants and pregnant women. An electronic database has been compiled from the Silver Bow County Health Department paper records that includes over 6600 records for infants, children and pregnant women. Our study focuses on over 3,500 records for Butte children 12 to 60 months old for the period from 2002 through 2011. Each record includes gender, birthdate, sample date, and a blood lead result. House age data were obtained from the Montana Cadastral online land survey repository. Consistent with nationwide trends, average Butte BLLs have declined by more than 50% from 2002 through 2011. BLLs are higher in 1-2 year olds compared with 3-5 year olds. Strong seasonal variation in BLLs is evident with peak levels in late summer. More than 55% of the children resided in homes built before 1950 and BLLs correlated with house age. A reference population was created by considering the comparability of prominent risk factors between the Butte dataset and the NHANES blood lead database. Weighting factors are applied to adjust for differences in gender balance, child age distribution, and house age distribution. Additional adjustments considered differences in distributions for race/gender and poverty level. Comparisons with the reference population and assessment of variations in BLLs across neighborhoods will guide assessment of ongoing remediation and lead abatement activities.

**PL 779 Variation Analysis of PBPK Model Parameters for Describing the Delivery of Cr(VI) to the Small Intestines of Humans**

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Sensitivity analyses were used to identify PBPK model parameters that are important for predicting the delivered dose of Cr(VI) to the small intestines in humans. These include physiological parameters (GI lumen pH, transit rates, reduction capacities, and volumes), chemical-specific parameters (pH-dependent reduction rate, absorption rates), and exposure parameters (number of drinking events, event duration). Monte Carlo methods were used to characterize the impact of parameter variation on PBPK model predictions for Cr(VI) internal dose. Coefficients of variation for the key model parameters ranged from approximately 25% to 120%. The impact of correlations between model parameters was assessed. Monte Carlo simulations were conducted for neonates, infants/children, youths, adults, and elderly, using two exposure levels, and two measures of internal dose to the small intestines. Model parameters contributing to greater than 10% of the variance in the predictions for both dose measures include the reduction rate constant and gastric transit rate. Stomach lumen volume was an important contributor to gastric flux, and the absorption rate constant was an important contributor to SI flux. At high doses, the number of drinking water events per day and exposure event time (during fed or fasted states) also become an important variables. The results of the initial analyses were used to calculate data-derived extrapolation factors to account for inter-individual variation. The ratios of the predicted 95th percentile to predicted mean lifetime average dose of Cr(VI) delivered to the small intestines, ranged from approximately 2 to 4. These results indicates the default value of 3 (USEPA) or 4 (IPCS) for intraspecies variation in toxicokinetic processes sufficiently characterizes variability in Cr(VI) SI tissue dose.
It is important for the Texas Commission on Environmental Quality (TCEQ) to conduct up-to-date assessments of all carcinogens (e.g., hexavalent chromium) emitted in Texas based on the latest scientific data and analyses. Consequently, TCEQ has developed an inhalation unit risk factor (URF) for hexavalent chromium (CrVI) based on excess lung cancer mortality in two key epidemiological studies of chromate production plant workers. One of the studies (Crump et al. 2003) concerns the Painesville, OH worker cohort and is an update to an earlier study (Mancuso 1975) used by USEPA (1984) to derive a URF, while the other (Gibb et al. 2000) regards a Baltimore, MD worker cohort used previously to calculate occupational URFs. A cohort of workers from four low-dose chromate plants (Corpus Christi, TX, Castle Hayne, NC, two in Germany) was used for a supporting quantitative dose-response assessment (URFs for other studies also calculated). For the Painesville cohort (Crump et al. 2003), grouped observed and expected number of lung cancer mortalities along with the cumulative exposures to CrVI were used to obtain the maximum likelihood estimate and asymptotic variance of the slope ($\beta$) for the linear multiplicative relative risk model using Poisson regression modeling. For the Baltimore cohort (Gibb et al. 2000), Cox proportional hazards modeling was performed with optimal exposure lag and adjusting for the effect of covariates (e.g., smoking) to estimate $\beta$ values as more detailed, individual, data were available. Life-table analyses were used to develop URFs for each of the two key studies, which were then combined using weighting factors relevant to confidences. For the Baltimore cohort (Gibb et al. 2000), the TCEQ will use this value to protect the general public in Texas against the potential carcinogenic effects from chronic exposure to CrVI in ambient air.

Quantifying the risk of chronic beryllium disease (CBD) from occupational beryllium (Be) exposure is challenging because of limitations in the exposure characterization and interindividual variability in sensitivity. Using worker-specific average exposures and prevalence data for beryllium sensitization (BeS) and CBD for 264 workers at a primary manufacturing facility investigated by NIOSH, the risk of CBD was quantified using logistic, log-logistic and log-logistic transformed regression models. Several modeling approaches and assumptions regarding background risk were explored to estimate the added risk of CBD using models of CBD and bifurcated models of BeS and CBD. Bifurcated models assume two populations with different sensitivities. For both approaches, the average Be concentration associated with a 1/1000 added risk of CBD (RSACs) ranged from 0.02 to 0.22 $\mu$g/m$^3$, with median values of 0.05-0.13 $\mu$g/m$^3$. Bifurcated models estimate that approximately 16% of the cohort was more sensitive, with the remaining members being highly insensitive. These data may be used to inform an Occupational Exposure Limit (OEL) protective of CBD; however, OELs are limits that are not to be exceeded on a day-to-day basis, whereas the RSACs are measures of average exposure. To determine an OEL consistent with these data, 4,016 total Be-labeled samples from the same facility that were used in the exposure reconstruction were further analyzed. Cluster analysis and homogeneity testing was conducted to group the data by job codes with airborne concentrations fitting the same distribution. Of eight statistically distinct groups found, two had mean concentrations of 0.05 and 0.12 $\mu$g/m$^3$, consistent with the estimated range of median RSACs. For these groups, 0.2 $\mu$g/ m$^3$ occurs at the 99th and 84th percentiles, respectively. Thus, setting an upper limit of exposure at 0.2 $\mu$g/m$^3$ is associated with average exposures consistent with a 1/1000-increased risk of CBD.
Introduction: The Antarctic moss *Saxonia uncinita* has presented high contents of chromophores after exposition to high levels of natural ultraviolet B radiation and can be an important source of antioxidants. The photoprotective activity of this moss has been observed. However, there is no data in the literature about toxicological research.

Objective: To investigate the mutagenic, genotoxic and cytotoxic activities of the *S. uncinita*.

Material and Methods: The *Salmonella* microsome assay was performed using the pre-incubation protocol. The mixture of 100 μL of the stationary culture of *Salmonella enterica* serovar Typhimurium strains TA97, TA98, TA100, TA104 and 500 μL of the 59 mix was incubated for 20 min with 100 μL of different concentrations of the hydroethanolic (HE) and ethanol (EE) extracts. Top-agar was added and poured into a Petri dish containing minimal agar medium. After 72h the number of revertants was counted. In MN assay HepG2 cells were exposed to HE and EE for 3 hours, fixed and stained with DAPI. To determine the mitotic index and the number of cells with micronuclei, 6000 cells/dish were counted. The percentage of cells with micronuclei and the mitotic index was calculated. After 72h the number of revertants was counted. In MN assay HepG2 cells were exposed to HE and EE for 3, 24 and 72 hours, it was added WST dye and the absorbance was measured in 440nm.

Results and Discussion: The extracts did not show mutagenic activity in the *Salmonella* strains and did not induce genotoxicity in HepG2 cells, moreover, it was not observed apoptosis or necrosis cell death in these cells. Besides, the cell viability evaluated by WST assay was not affected by HE and EE. Our results showed that in metabolically competent cells, the HE and EE did not induce mutagenic, genotoxic and cytotoxic effects.

Conclusion: The HE and EE should be of interest to further explore the possibility of using it as potential photoprotective.

**Cytotoxicity of Extracts of Three Medicinal Plant Species Used to Treat Inflammation-Related Conditions in South Africa**

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Extracts of medicinal plants are widely used in developing countries for the treatment and management of many diseases. The safety or hazards presented by the consumption of these substances is scarce in literature. There are many cases of fatal intoxication from plants in hospitals. The aim of this study was to assess the safety of oral consumption of extracts derived from the selected medicinal plant species used in vitro cytotoxicity tests.

Dried leaves portion of *Acacia sieberiana*, *Erythrophleum laisanthum* and *Teckovaria capensis* were ground into a fine powder. The ground materials were extracted with cold and hot distilled water, as well as ethanol, representing traditional methods of extraction. The dried extracts were re-constituted in dimethyl sulfoxide at a concentration of 20 mg/ml. These extracts were tested against isolated mononuclear leukocytes, U937 macrophage, vero kidney and liver cell lines maintained in Dubeclo’s Minimum Essential Medium (DMEM) for 72 hours. The assessment of cytotoxic effects were done using both the reduction of yellow tetrazolium salt (3-(4, 5-dimethylthiazol-2)-2, 5-diphenyltetrazolium bromide (MTT) and Excellence RTCA assay systems.

Overall, the results indicated that all the extracts tested were not cytotoxic to the cell lines used when compared with hydrogen peroxide (1 mM) and Doxorubicin hydrochloride (2 mg/ml) positive controls. The vero kidney cell lines grew at optimal rate when cultured in the presence of ethanolic extracts of *A. sieberiana*, while the least growth was observed when the same cell lines were cultured in the presence of cold water extracts of *E. laisanthum*. The cold water extracts of *E. laisanthum* had the least effects on mononuclear leukocytes using the MTT assay among the extracts tested. This indicated that the choice of evaluation method is critical for results interpretation. However, there is a need for *in vivo* study using a model animal to validate these results.

**Amelioration of Sodium Arsenite and Potassium Chromate Toxicities by Methanolic Extract of *Rauvolfia vomitoria***

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Exposure to arsenic and hexavalent chromium is a major public health concern especially in the developing part of the world and there is still no reliable treatment. This study therefore evaluates the effect of methanolic leaf extracts of *Rauvolfia vomitoria* (MRV) on mice exposed to sodium arsenite (NaAsO2) and potassium hexaarsenate (VI) (K2Cr2O7). Test and control mice were pre-treated with 20mg/kg body weight MRV before being challenged with either NaAsO2 and K2Cr2O7 on the seventh day. The negative control animals were exposed to distilled water, while the positive control mice were exposed to a single dose of either 2.5 mg/kg body weight NaAsO2 and 12mg/kg of K2Cr2O7 a day before the animals were sacrificed. The frequency of micronucleated polychromatic erythrocytes (mPCEs) was monitored in bone marrow cells, while aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assessed in the plasma. Hepatic glutathione (GSH) and malondialdehyde (MDA) levels as well as catalase (CAT) and glutathione-S-transferase (GST) activities were also monitored.

**The Effect of Eukaryotic Initiation Factors on the Activity of Pokeweed Antiviral Protein**

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Ribosome-inactivating proteins (RIPs) are found in numerous plant, fungi and bacterial species, and are recognized to have a broad spectrum of anti-viral activity. RIP function is characterized by depurination, the N-glycosidase activity, of the conserved sarnic/rin loop of the large ribosomal subunit. PAP is also able to depurinate mRNA at specific nucleotide locations, by binding to the m7G cap at the 5’-end of the mRNA, and interaction with eukaryotic initiation factors (eIFs).

These biological functions suggest that the mechanism of PAP action is complex, yet information about the molecular mechanism of how this toxin causes apoptosis or the basis for its selection of RNA for depurination scores. In order to determine the role of multiple protein-protein interactions (PAP, eIF3, eIF4 and eIF4G) in PAP function, the pull-down assay and the fluorescent-resonate energy transfer assay (FRET) have been investigated here. Our results indicate that eIF3 and eIF4 bound together as the eIF3/eIF4 complex to m7GpppG cap analog and this binding cannot be overcompeted by PAP. eIF4G repressed the competition of PAP with eIF3 and eIF4 bound analog binding. FRET energy transfer study revealed that eIF3 and eIF4 bound in close proximity as PAP and eIF4 and eIF4G bound closely to each other to increase FRET energy transfer. PAP and eIF4 bound to each other; this interaction produces only low level of FRET energy transfer. PAP, eIF4 and eIF4G bound closely to each other to increase FRET energy transfer dramatically. This triple protein interaction demonstrated eIF4G plays a key role in regulation of PAP binding.

**Effect of Sesamin on Endothelial Nitric Oxide Synthase Activation via Multiple Pathways**


Endothelial nitric oxide synthase (eNOS) is the key regulator of vascular functions in the vascular endothelium. While many pathways are involved in the regulation of eNOS activity, phosphorylation at the most thoroughly studied site at Ser1177 is generally found to be a critical requirement for eNOS activation. Sesamin, one of the major lignans in sesame seeds and oil, has been reported to have many benefits and medicinal properties including anti-hypertensive effect, but its influence on intracellular pathways underlying eNOS remains unclear. Sesamin increased the phosphorylation of eNOS in a concentration-dependent manner in human endothelial cells. In addition, sesamin increased the phosphorylation of Akt, ERK1/2, JNK1/2, and p38 MAPK in cells. Furthermore, sesamin increased the phosphorylation of AMP-activated protein kinase (AMPK) and calmodulin-dependent protein kinase II (CaMKII). eNOS phosphorylation was inhibited by specific upstream inhibitor of ERK1/2 (PD98059), p38 (SB203580), AMPK (compound C), and CaMKII (BAPTA, W7). These results indicate that sesamin stimulates eNOS phosphorylation via the activation of ERK1/2, p38, AMPK, and CaMKII.

**Amelioration of Sodium Arsenite and Potassium Chromate Toxicities by Methanolic Extract of *Rauvolfia vomitoria***

K. A. Akinwumi1,2, O. A. Odunola1, A. N. Dosunmu2, O. B. Owolabi2, A. Songonuga1, O. A. Aboyeji2, O. O. Olawuni1 and O. O. Osise1.

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NaAsO2 and K2C2O7 significantly (p<0.05) increased the frequency of mPCE formation. AST, ALT, CAT and GST activities when compared with the control. Simultaneous exposure to NaAsO2 and K2C2O7 further increased (p<0.05) the levels of the markers. In addition, GSH and GST were significantly (p<0.05) reduced in mice exposed to NaAsO2 or K2C2O7 or their combination. However, pre-treatment with MRV restored the markers towards that of the control. Our study therefore suggests that MRV ameliorates NaAsO2 and K2C2O7 induced toxicities via reduction of oxidative stress and fortification of anti-oxidant system.

**789** Cytoxic and Genotoxic Assessments of the Natural Dye Erythrostominone

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Introduction: Synthetic dyes are used extensively for pharmaceutical, cosmetic or food industries. These industrial sectors are affected by the low cost, easy availability and high diversity of colors and shades of synthetic dyes, however, several of these compounds present inherent toxicity, and can be precursors of toxic intermediates and/or mutagens produced during metabolism. Thus, natural dyes obtained by the fermentation processes of micro-organisms are alternatives for the substitution of synthetic dyes in some applications. Additionally, they have low cost, and require little space for the large-scale production, increasing the economic viability for industrial use. Objectives: The present work aimed to evaluate the cytoxicity and genotoxicity of the natural dye erythrostominone extracted from micro-organism. Methodology: Cultures of HepG2 cells were exposed to erythrostominone at concentrations of 35, 87, 174, 261, 348, 522, 696 and 870 mg/ml and to negative (NC-DMSO) and positive controls (PC-CCCP) and cytoxicity was evaluated after 24 hours using MTT assay. After exposure, cell viability was analyzed by spectrophotometry. The comet assay verified the genotoxicity of the dye at concentrations of 35, 87, 174, 261, 435 and 522 mg/l NC (DMSO) and PC (MMS). The DNA tail formation was analyzed with fluorescence optic microscopy. The values of cell viability and tail moment were analyzed statistically by the software GraphPad Prism 5.01. Results: Erythrostominone dye at 522, 696 and 870 mg/ml presented statistically significant difference in cell viability, when compared to the NC. No statistically significant difference was observed in tail moment. Discussion: Erythrostominone dye is cytoxic at higher concentrations, however it does not induce genotoxicity in tested concentrations. Conclusion: According to our data, erythrostominone dye can be used in industrial sectors as a substitute of synthetic dyes, since it has no citotoxicity and genotoxicity at concentrations lower than 522 mg/l. Others toxicological studies are necessary to investigate possible damage to human and environmental health.

**790** Comparison of the Estrogenic Modulating Activities of Dong Quai with Hops and Red Clover


Many women use dong quai (Angelica sinensis, (Oliv.)) dietary supplements as over-the-counter medication to relief menstrual irregularities and menopausal symptoms in a natural and safe way. However, despite the widespread use of dong quai in the US, its efficacy and safety has not been demonstrated. Data in the literature about dong quai’s estrogenic/antiestrogenic activities are contradictory. To analyze this controversy, a lipophilic (CO2) and hydrophilic (EtOH 75%) dong quai extract was systematically analyzed for its estrogen receptor (ER) modulating activities in a natural and safe way. However, despite the widespread use of dong quai in the over-the-counter medication to relief menstrual irregularities and menopausal symptoms, (Oliv.)) dietary supplements as over-the-counter medication to relief menstrual irregularities and menopausal symptoms. Erythrostominone dye is cytotoxic at higher concentrations, however it does not exhibit genotoxicity in tested concentrations. Consequently, our data suggest that the dong quai extract displays cell-specific cytoxicity toward PCa cells, with no toxicity towards breast (MCF-7), lymphocyte (THP-1) or neuronal (GT1-7) cancer cells. Furthermore, our current data show that low doses of Biz-2 kill PCa cells, but show no apparent effect on normal, transformed prostate cells, or display any in vivo toxicity in mice. Next, we examined the mechanism of action of Biz-2-induced cytoxicity by examining LNCaP and DU145 cells exposed to Biz-2 for morphological signs of apoptosis, along with the expression of several apoptotic markers. In those experiments, we found that Biz-2 was able to induce apoptotic cell death in PCa cells. Biz-2 was twice as potent in inducing apoptosis in AR- DU145 cells as in the AR+ LNCaP cells. This study has demonstrated that Biz-2 can modulate prostate cancer cell function. (Work was supported by a NIH grant, Grant No. 5P20RR016456-08S1)

**791** Extracts of Protea cynaroides Are Cytotoxic toward Hormone-Resistant Breast and Prostate Cancer Cells

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This study was designed to determine the optimal extraction method for extracting phenolic compounds from the plant Protea cynaroides. In all extraction methods, 1 g of leaves were thoroughly washed and submerged in 0.1 N HCl prior to extraction preparation. Immediate freezing and grinding in LN2 followed by freeze drying produced the freeze dried extract. Leaves that were oven dried were subjected to water, alcohol and alcohol/water extraction methods. All stock solutions were sterilised using a 0.22 μm filter. Cytotoxicity was determined using the sulforhodamine B assay. Phenolic content of the extracts was determined using the Folin-Ciocalteu method. The results showed that the alcohol/water extraction method produced a 2-fold greater amount of phenolic compounds compared to water extraction (100 vs 50 μg/ml, respectively). This correlated with more potent cytotoxicity of the alcohol/water extract toward both cell lines. Specifically, 92, 94 and 97% of MDA-MB-231 cells were killed by the alcohol/water extract compared to 47, 55 and 55% for the water extract at concentrations of 0.9, 1.8 and 9 mg/ml, respectively. A similar trend was seen in PC3 cells as 83, 93 and 99% of cells were killed following treatment with the alcohol/water extract compared to 55, 76 and 86% for identical concentrations of the water extract. However, freeze-dried extracts were equally cytotoxic at 9 mg/ml concentrations, as > 94% of PC3 and MDA-MB-231 cells were killed at this concentration by both freeze-dried and alcohol/water extracts. However, alcohol/water extracts were more potent than freeze-dried extracts at the lower concentrations of 0.9 and 1.8 mg/ml. The results of this study show that water, alcohol/water and freeze drying are all viable methods of extracting phenolic compounds from Protea cynaroides. However, alcohol/water extraction is the preferred method as it produces a more potent extract that is cytotoxic toward >90% of MDA-MB-231 and PC3 cells at concentrations of 0.9 mg/ml.

**792** Differential Effects of Biz-2 in Cancer Cells

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Prostate cancer (PCa) is the leading cause of cancer death in men in the USA, making it a major problem with a socio-economic burden in the millions of dollars. Despite rapid advancements in the early detection and treatment of PCa over the past fifty years, there are no effective cures for this disease. It is of paramount importance to develop new, effective ways of addressing PCa. Our laboratory has been studying the mechanism of the bioactive chemicals present in the Kola acuminata, a "cure-all" herbal medicine known to the Ettu people of Jamaica as bizzy nut. Based on the ethnoveterinarian and anecdotal data available on this natural product, we hypothesized that bizzy nut contains one or more bioactive compounds that can modulate prostate biology. Using cell cytotoxicity, androgen receptor (AR) binding and AR gene expression as indices of androgenicity, we found that the ether extract of bizzy (Biz-2) contains compounds with potent anti-androgenic potential in both AR+ and AR- prostate cells. Biz-2 was able to inhibit the proliferation of prostate cells (DU145 and LNCaP) in a concentration-dependent manner. We observed that the Biz-2 extract displays cell-specific cytoxicity toward PCa cells, with no toxicity towards breast (MCF-7), lymphocyte (THP-1) or neuronal (GT1-7) cancer cells. Furthermore, our current data show that low doses of Biz-2 kill PCa cells, but show no apparent effect on normal, transformed prostate cells, or display any in vivo toxicity in mice. Next, we examined the mechanism of action of Biz-2-induced cytoxicity by examining LNCaP and DU145 cells exposed to Biz-2 for morphological signs of apoptosis, along with the expression of several apoptotic markers. In those experiments, we found that Biz-2 was able to induce apoptotic cell death in PCa cells. Biz-2 was twice as potent in inducing apoptosis in AR- DU145 cells as in the AR+ LNCaP cells. This study has demonstrated that Biz-2 can modulate prostate cancer cell function. (Work was supported by a NHF grant, Grant No. 5P20RR016456-08S1)

**793** Antioxidant Activity of Extracts of Stem Bark of Enantia chlorantha (Oliv)

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Enantia chlorantha is an ornamental tree that has been reported to posses hae-mostatic antimalarial, antibacterial, antiviral and hepatoprotective properties. The hexane, chloroform, ethyl acetate and methanol extracts of stem bark from Enantia chlorantha stem bark were assessed for in vitro antioxidant activity using Ferric
Curcumin, a phytochemical found in Tumeric, has been widely studied for its potential in the prevention of different types of cancer. However, it only has limited clinical applicability because of its low oral bioavailability. One of the approaches to improve curcumin’s bioavailability is to develop curcumin prodrugs. Ester prodrugs are the most common type that can be easily converted back to the active agent by esterases found in the body. A series of aminoacylsulphonyl esters of curcumin was designed and synthesized involving amino acids such as leucine, isoleucine, and valine. Mono-conjugated (only one phenol group in curcumin was conjugated with an amino acid moiety) and di-conjugated (both phenols were modified) products were observed. The conjugates were then evaluated in cytotoxicity assays using different prostate cell lines and in cancer prevention screening assays using sphere formation in gastric cancer cells. Overall, valine conjugates were less cytotoxic than curcumin while mono-isoleucine conjugates had comparable cytotoxicity to curcumin. Two mono-conjugates were screened for their cancer preventive effects. The results showed that curcumin and both amino acid conjugates significantly reduced sphere formation. This indicates that curcumin analogs, which are expected to have better aqueous solubility and improved oral bioavailability, have similar chemopreventive effects to curcumin, possibly at reduced cytotoxicity.

Kahweol, the coffee-specific diterpene, has been reported to have anti-carcinogenic properties. Animal data support such a chemopreventive effect of coffee. However, the precise underlying chemopreventive mechanism is poorly understood. This study examined the inhibitory effect of kahweol on phorbol myristate acetate (PMA)-induced MMP-9 expression in HT-1080 human fibrosarcoma cells. Results showed that kahweol suppressed PMA-induced phosphorylation of Akt and the tissue inhibitor of metalloproteinase (TIMP)-1, while the amount of 4-hydroxylated products remained unaffected by kahweol. Cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) catalyze 2- and 4-hydroxylation of estradiol into precursors of carcinogenic quinones. Modulation of mRNA levels and activity of CYP1A1 and CYP1B1 were measured to assess possible mechanisms of actions. The results correlated with the 4-hydroxylation metabolism data. 8- and 12- hydroxyestradiol, and IX were differentially induced. CYP1B1 activity was evaluated independently of CYP1A1 activity. Analysis of the influence of an IX-rich (57.6% w/w) hops extract on 2- and 4-hydroxylation of estradiol by LC-MS/MS revealed that the extract reduced the carcinogenic 4-hydroxylation pathway similar to pure IX. These data suggest that hops constituents differentially modulate estradiol carcinogenesis and underscore that analysis of hops extract is essential for the reproducibility of biological outcome.

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Stimulates eNOS phosphorylation via the activation of Akt, ERK1/2, AMPK, and CaMKII. These results demonstrate that CAPE might be useful for the treatment or prevention of endothelial dysfunction associated with atherosclerosis and cardiovascular disease.

797b Kahweol Induces Apoptosis in HER2-Overexpressing Breast Cancer Cells through a Down-Regulation of HER2/SREBP-1/FAS Pathway
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Breast cancer is the leading cause of cancer-related death among women worldwide. Kahweol, natural diterpenes from coffee, has been reported to exhibit anti-cancer activities. This study investigated molecular mechanisms by which kahweol induced apoptosis in HER2-overexpressing breast cancer cells. Results showed that kahweol strongly inhibited cell growth and induced apoptosis in SKBR3 breast cancer cells through caspase-3 activation and induction of FAS cleavage. Kahweol markedly reduced HER2 protein levels in a concentration-dependent manner. Kahweol significantly suppressed fatty acid synthase (FAS) mRNA and protein levels in SKBR3 cells. Kahweol inhibited Akt activation, leading to reduction of mature sterol regulatory element-binding protein (SREBP)-1 protein levels. Furthermore, using specific PI3K inhibitor LY294002 confirmed that the blockade of Akt/SREBP-1 signaling pathway caused FAS reduction in SKBR3 cells. These results demonstrate that kahweol down-regulates HER2/SREBP-1/FAS pathway and induces apoptosis in breast cancer cells, suggesting a potential role in prevention and treatment of human breast cancer.

797c Antiproliferative and Apoptotic Effect of Rhus trilobata Extracts on SKOV-3 Ovarian Cancer Cells
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Rhus trilobata, known as Skunk brush, is a plant from northern Mexico frequently used in traditional medicine for different types of cancer. The aim of this work was to analyze the effect of extracts of Rhus trilobata on proliferation and apoptosis of SKOV-3 ovarian cancer cells. Extracts were prepared by equilibrium in distilled water (AE) or with 70% methanol (ME) using stems of the plant recollected in November 2012. Sub-fractions of both extracts were obtained using Sep-Pak C18 cartridges and different solvents. Crude extracts and sub-fractions were tested on SKOV-3 ovarian cancer cells; viability and cytotoxicity or apoptosis were determined in cultures using the ApoToxGlo kit, and mitotic index was calculated for all treatments. Cultures without treatment or incubated with Vinoversine or Curcumine, were used as controls. Results showed that both extracts, AE and ME, significantly decreased proliferation (mitotic index < 1) and cell viability at concentrations < 20 μg/mL at 24 and 72 h, compared with untreated cultures (p <0.05); no significant differences were found with cultures treated with 20 μg/mL of Vinoversine. Fractionation of crude extracts produced seven fractions that were evaporated to dryness prior to their use. The fractions that showed greater antiproliferative effect were 3, 4, 5 and 7 from the AE, and 2, 5 and 7 from ME at a concentration of 5 μg/mL (p <0.05). Apoptosis assays showed that antiproliferative effect was associated with an increase in caspase activity in cells treated with 5 μg/mL of AE, ME, or active sub-fractions. No cytotoxic effect was detected at any of the concentrations tested. Results presented here demonstrate that Rhus trilobata stems contain active compounds with an important antiproliferative effect on SKOV-3 ovarian cancer cells, which is related to the induction of apoptosis rather than a cytotoxic effect. Supported by FOMIX CHIH-2010-C01-147532.

798 Comparison of Myelostimulation by Echinacea purpurea Extracts with Analytical Fingerprint to Identify Active Constituents
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We have previously described a myelostimulatory activity of Echinacea herbal supplement (Spring Valley Natural Whole Herb, Ida Sphere Inc., American Fork, UT) (Ramasahayam et al. 2011. Planta Med. 77:1883-9). Product label described the encapsulated material as Echinacea purpurea (aerial part) with gelatin and medium-chain triglycerides. A 7.5% ethanol extract administered orally for 7d at 50-100 mg dried extract/kg/d, as recommended for human intake, to female Sprague-Dawley rats yielded a 70% increase in femur myeloid progenitors, i.e., CFU-GMs, from nucleated bone marrow cells upon culture in methylocellose with csf2, IL-3 and SCF (HALO assay, Hemogenix, Colorado Springs, CO). To determine that bioactivity was due to plant-derived material, we obtained an HPLC fraction enriched in the natural source (Ray Jaglowksi, Twinlab Corp., Grand Rapids, MI), extracted with ethanol (EtCP) and tested for CFU-GMs. Since Echinacea aerial part contains lipophilic N-alkylamides as a primary constituent class, we also determined myelostimulatory activity of n-hexane-washed EtCP (HexEtCP). Analytical fingerprints of EtCP and HexEtCP were acquired with HPLC and 1HNMR (400 MHz, 20 mg/700 μl MeOH-d4). Increase in CFU-GMs of EtCP was comparable to that previously determined for herbal supplement and HexEtCP activity, normalized to source EtCP (~95% yield), was 2 times more potent than EtCP. Hexane wash fraction of CP did not affect CFU-GMs. Proton NMR spectra of hexane wash contained prominent peaks from β-sitosterol, as verified from spectra of pure standard (ChromDox, Irvine, CA). Similarly, 1HNMR spectral peaks with shifts equivalent to those of dodeca-2,4,8,10-tetraenoic acid isobutylamide (PhytoLab, Vestenbergsgreuth, Germany) were evident in hexane wash. HPTLC confirmed that these lipophilic constituents were removed from EtCP by washing with n-hexane. In conclusion, these studies demonstrated that myelostimulation of supplement Echinacea was due to phytochemical other than lipophilic constituents.

800 Inhibitory Effects of Platycodon grandiflorum Root-Derived Saponins on DNBC-Induced Atopic Dermatitis-Like Skin Lesions in Mice and the Possible Mechanisms in Cells
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Platycodon grandiflorum root-derived saponins (Changkil saponins, CKS) have many biological and pharmacological effects, including anti-inflammatory, anti-oxidative, and anti-obesity effects, but its influence on contacted allergens-induced atopic dermatitis (AD) remains unclear. The consequences of precipitously rising allergic skin inflammation rates worldwide have accelerated the risk of AD. AD is a major health problem all age in globally. This study investigated the inhibitory effect of CKS on AD-like skin lesions in mice and the possible mechanisms in cells. DNCB treatment for 9 weeks led to marked AD-like skin lesions as assessed by AD severity, ear thickness, serum level, and histopathological examination in mice. DNCB-induced AD severity, serum level of IgE and TARC and mRNA expression of TARC, TNF-alpha, IFN-gamma, IL-4, IL-5, and IL-13 attenuated by CKS treatment in NC/Nga mice. Histopathological examination showed that DNCB-induced thickness of the epidermis/dermis and dermal infiltration of inflammatory cells and mast cells reduced by CKS treatment in the ear lesions. Also, CKS suppressed TNF-alpha/IFN-gamma-induced TARC expression in HaCaT cells. CKS inhibited TNF-alpha/IFN-gamma-induced NF-kB activation as well as STAT1 activation. Furthermore, CKS increased Nr2f2/ARE-mediated HO-1 expression. These results indicate that CKS suppressed DNCB-induced AD-like skin lesions by regulating cytokine mediators in NC/Nga mice and TNF-alpha/IFN-gamma-induced TARC expression through the suppression of NF-kB and STAT1 and induction of Nr2f2/ARE-mediated HO-1 expression in HaCaT cells, which may be an effective alternative therapy for AD-like skin symptoms.
metalloproteinase-13 (MMP-13), tissue inhibitor of metalloproteinase-1 (TIMP-1), tumor necrosis factor-alpha (TNF-a), alpha-smooth muscle actin (SMA), and collagen type 1 inhibited by CKS treatment. Moreover, CKS inhibited HDF-induced cyclooxygenase-2 (COX-2) protein expression, NF-kB p65 nuclear translocation, and TNF-alpha degradation. These results indicated that CKS manifests hepatocellular protective action and ameliorative effects against chronic liver injury and developing NASH induced by HDF treatment.

**801 Inhibitory Effects of Platycodon grandiflorum Root-Derived Saponins on Ovalbumin-Induced Airway Hyper-responsiveness in Mice**

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Platycodon grandiflorum root-derived saponins (Changkil saponins, CKS) have many biological and pharmacological effects, including anti-inflammatory, antioxidant, and anti-obesity effects, but its influence on inhaled allergens-induced airway hyperresponsiveness remains unclear. Allergic asthma is a chronic respiratory disease caused by inappropriate responses to inhaled allergens and is characterized by reversible obstruction of airway hyperresponsiveness. Inflammation in the airway results from an influx of inflammatory cells, mucus overproduction, and the overexpression of inflammatory cytokines and chemokines. This study investigated the inhibitory effects of CKS on ovalbumin (OVA)-induced airway inflammation in mice and PMA-induced MUC5AC expression in A549 cells. Mice were sensitized and challenged with OVA developed inflammation and remodeling in airway. The inhibitory effect of CKS on OVA-induced airway inflammation was evaluated by number of total and differential leukocytes, level of IgE, MCP-1 chemokine, and cytokines, and histopathological study. CKS attenuated OVA-induced number of total cells, eosinophils, basophils, lymphocytes, and macrophages, level of TNF-alpha, IFN-gamma, IL-4, IL-5, IL-13, MCP-1, and IgE in bronchoalveolar lavage (BAL) fluid. CKS suppressed OVA-increased mRNA expression of MMP-2/9 and MUC5AC in mice. Additionally, CKS attenuated airway hyperresponsiveness, inflammatory cells and goblet cells hyperplasia in lung tissue. CKS blocked NF-kB p65 nuclear translocation from lung tissues of OVA-sensitized/challenged mice. Furthermore, CKS suppressed PMA-induced MUC5AC mRNA expression and luciferase activity in cells. These results indicate that CKS suppressed the development of the airway inflammation-like symptoms and MUC5AC expression by suppressing of NF-kB activation in mice, and so may be a useful tool in the therapy of airway inflammation-like symptoms.

**802 Inhibitory Effects of Platycodon grandiflorum Root-Derived Saponins on Estrogen Deficiency-Induced Osteoporosis in Mice**

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Platycodon grandiflorum root-derived saponins (Changkil saponins, CKS) have many biological and pharmacological effects, including anti-inflammatory, antioxidant, and anti-obesity effects, but its influence on estrogen deficiency-induced osteoporosis remains unclear. Osteoporosis is a reduction in bone mass and the micro-architectural weakening of bone tissue, with a resultant increase in bone fragility and vulnerability to fracture. Osteoporosis is a major health problem in postmenopausal and aged women in the world. This study investigated the inhibitory effect of CKS on osteoporosis induced by ovarioectomy (O VX) in mice. O VX animal model has been widely used to evaluate for postmenopausal osteoporosis with estrogen insufficiency. The inhibitory effect of CKS on O VX-induced osteoporosis was evaluated by body weight, plasma parameters, and micro-computed tomography for bone turnover in mice. After 4 weeks of recovery from surgery, the O VX mice were randomly divided into three groups and orally treated with saline or CKS for 3 weeks. CKS attenuated O VX-induced body weight. CKS improved O VX-reduced plasma levels of alkaline phosphatase, phosphorus, and calcium. Also, CKS restored the bone deterioration of trabecular microarchitecture. These results indicate that CKS attenuate O VX-induced bone turnover and lower the risk of osteoporosis.

**803 Toxicological Evaluation of Methanol Leaf Extract of Alchornea cordifolia**

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Alchornea cordifolia is commonly used as a medicinal plant and widely distributed throughout Africa including Nigeria and Democratic Republic of Congo (DRC). The leaves are traditionally used for the treatment of venereal diseases. Thirty six male Wistar albino rats were randomly divided into six groups of six rats each. The toxic effects of the methanol extract of Alchornea cordifolia leaves were investigated on reproductive and hematological parameters of male rats at 0, 100, 200, 400, 800 and 1600 mg/kg-1. The Red Blood Cell (RBC) count, packed cell volume (PCV), hemoglobin concentration (Hb) and hematric indices except mean corpuscular volume decreased (p<0.05) significantly at the 1600 mg/kg-1 dose compared to the control. Markers of hepatic damage alanine and aspartate aminotransferases (ALT and AST) and renal damage (Urea and Creatinine) were significantly elevated (p<0.05) at 800 and 1600 mg/kg-1. Similarly, significant increases were observed in testicular weight, spermatozoa count and motility with concomitant increase in serum testosterone levels in all treatment groups. Severe deleterious effects were not recorded at doses below 400 mg/kg-1. However, the leave extract of Alchornea cordifolia at 800 and 1600 mg/kg-1 has hematotoxic, hepatotoxic and nephrotoxic effects. In conclusion, caution is therefore required for the medicinal usage of this plant because of its potential toxic effects on the liver, kidney and blood cells.

**804 Elucidation of the Mechanisms of Teratogenicity of Some Commonly Used Medicinal Plants**

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An array of studies has proven the efficacy of numerous medicinal plants against ailments ranging from pain to microbial infection. However, their teratogenic potentials have not been well elucidated. One such ethnobotanical survey was used to determine the types of medicinal plants used by pregnant women in Lagos, Nigeria. The aim of this study is to determine the possible teratogenic effects on the cardiovascular and nervous systems and the mechanism of teratogenicity of these commonly used medicinal plants. The aqueous extracts of some of the most commonly used plants (Enantia chlorantha, Morinda lucida and Moringa oleifera) were prepared. Pregnant Wistar rats were treated with several doses (150-600 mg/kg) of these extracts at gestational day six and the litters were examined for any defects at gestational day 20. There was no significant change in the number of litters and their gross morphology. However, there was a significant increase in fetal resorptions, and the overall body weight and crown to rump length of the litters was affected in a dose dependent manner of which the direction is dependent on the plant species. Zebradish embryos were also exposed to several concentrations (0-1.28 mg/ml) of the extracts between 4-144 hours post-fertilization. A dose dependent effect was observed in the developmental processes of the zebrafish resulting in embryo lethality, skeletal dysmorphogenesis and pericardial edema leading to the formation of a tubular heart. However, there was no significant change in locomotor activity of the cleemothroembryo by six days post-fertilization when there was no overt teratogenicity. These research findings offer a basis for further investigation of the cellular and molecular mechanisms by which these extracts cause developmental defects in both zebrafish and rat whole embryo culture. These approaches remove the influence of maternal metabolism and other influences, and will provide valuable endpoints for exploration in pregnant mammals and newborns.

**805 Effect of α7 Nicotinic Acetylcholine Receptor Agonists and Antagonists on Motor Function in Mice**

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated cation channels found throughout the body, and serve to mediate diverse physiological functions. Muscle-type nAChR located in the motor endplate region of muscle fibers play an integral role in muscle contraction and thus motor function. The toxicity and teratogenicity of many plants are due to various toxins that bind to nAChRs including deltaline and methyllycaconitine (MLA) from larkspur (Delphinium) species, and nicotine and anabasine from tobacco (Nicotiana) species. The primary result of the actions of these alkaloids at nAChRs is neuromuscular paralysis and respiratory failure. The objective of this study was to further characterize the motor coordination deficien-
cies that occur upon exposure to a non-lethal dose of nAChR antagonists MLA and deltaline as well as nAChR agonists nicotine and anabasine. We evaluated the effect of nAChR agonists and antagonists on the motor function and coordination in mice using a balance beam, grip strength meter, rotarod, open field analysis and tremor monitor. These analyses demonstrated that, within seconds after treatment, the mice had significant loss of motor function and coordination that lasted up to one minute, followed by a short period of quiescence. Recovery to normal muscle coordination was rapid, typically within approximately 10 min post-dosing. Additional studies were conducted to determine if the c7 nAChR subunit plays a role in these motor coordination deficiencies by comparing the motor function and coordination in wild-type mice to mice lacking the c7 subunit of the nAChR, after treating them with a non-lethal dose of MLA or anabasine. The results from this study suggest that the c7 nAChR subunit does not play an integral role in the acute effects of MLA or anabasine on motor function/coordination.

806 Toxic Effects of Root Extract of Caestis ferruginea (de Candolle) on the Blood Profiles of Male Rats


Caestis ferruginea belongs to the family Convolvulaceae and it possesses antibiotic activities. It has alkaloid as its main bioactive constituent and our preliminary investigation revealed that it has antifertility property. The toxic effects of methanolic root extract of Caestis ferruginea on the blood profiles were studied in male albino rats. The rats were treated with daily oral administration of 500mg kg−1bw of Caestis ferruginea for 5, 30 and 60 days. The erythrocyte values (red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) and leucocyte values (total white blood cell (WBC), lymphocyte, eosinophil and monocyte counts) and plasma concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were not affected by the treatment after the 30days treatment period. However, there were significant reductions in the values of RBC (P<0.001) and PCV (P<0.02) on the 60th day of treatment. The rats were treated with daily oral administration of 500mg kg−1bw of Caestis ferruginea for 5, 30 and 60 days. The erythrocyte values (red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) and leucocyte values (total white blood cell (WBC), lymphocyte, eosinophil and monocyte counts) and plasma concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were not affected by the treatment after the 30days treatment period. However, there were significant reductions in the values of RBC (P<0.001) and PCV (P<0.02) on the 60th day of treatment. The values of Hb and MCV were not affected while those of MCH and MCHC were increased (P<0.001) after 60 days of treatment. Furthermore, the total WBC, neutrophil, eosinophil and monocyte counts were not affected by prolonged treatment. The plasma level of AST was not affected by prolonged treatment, but the plasma levels of ALT increased (P<0.01) on the 60th day of treatment. In the recovery experiment, the values of PCV, MCH, MCHC and plasma ALT were also restored to the pre-treatment values. This study revealed that prolonged administration of the crude root extract of Caestis ferruginea may induce anaemia.

807 Acute and Subchronic Oral Toxicity Studies of Stem Bark Extract of Syzygium guineense (Myrtaceae) in Rats

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Introduction/Objective: Syzygium guineense, widely used in African Traditional Medicine, has been evaluated for its effect on diabetes mellitus, Herpes zoster in combination with other medicinal plants, malaria, bacterial infections, diarrhoea and hypertension. To confirm its safe use, the present study evaluated the potential toxicity of a methanol extract of Syzygium guineense stem bark after acute and subchronic administration in Wistar rats.

Materials and Methods: The oral acute toxicity of the extract was studied using the modified Lorke’s method, and the rats were then monitored for 14 days. In the subchronic toxicity study, the extract was administered orally to groups of rats (6 rats/dose/sex) at doses of 250, 500 and 1000 mg/kg/day for 90 consecutive days. Measurements included clinical observations, water and food consumption, body weight, haematology, blood chemistry, urinalysis, gross necropsy, organ weight and histopathology.

Results: There was no mortality or signs of acute or subchronic toxicity. There was no significant difference in body weight, relative organ weight or haematological, liver function monitoring parameters, urea, creatinin and electrolytes at 42 and 90 days respectively in the subchronic toxicity study. No abnormality of internal organs was observed in both gross and histopathological examinations between treatment and control groups.

Conclusion: These results suggest that the lethal oral dose of the stem bark extract of Syzygium guineense is more than 5000 mg/kg in both male and female rats and the no-observed-adverse-effect level (NOAEL) of the extract for both male and female rats is considered to be 1000 mg/kg per day for 90 days.

808 Enzymic and Nonenzymic Antioxidant Status in Wistar Rats Administered Aqueous Leaf Extract of Morinda morindoides

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Morinda morindoides is a medicinal plant in some African countries where its leaves are used for treating malaria. Information on biochemical changes induced following oral administration of this plant is scarce in the literature. Hence, this study was designed to investigate the enzymic and non-enzymic antioxidant status in Wistar rats administered aqueous leaf extract of Morinda morindoides using activities of catalase (CAT), glutathione peroxidase (GPOX), glutathione reductase (GSH.RD), superoxide dismutase (SOD), glutathione-S-transferase (GST), gamma glutamyl transferase (GGT) and levels of reduced glutathione (GSH), total-thiol (T.SH), vitamin C and E as indices.

In vivo antioxidant activity of the extract and its in vivo effects on malondialdehyde (MDA) concentration and histology of liver and kidney were also investigated. Thirtty rats grouped into five (A, B, C, D, and E) were used for the study. Group A served as control and was administered distilled water while groups B, C, D and E were given 100, 200, 400 and 800 mg/kg body wt of the extract for 28 days. Pyrochrome analysis revealed that the plant is rich in phenolic and flavonoid compounds. Plasma vitamins C and E, T.SH and GSH were significantly (P<0.05) increased while a significant (P<0.05) reduction in plasma concentration of MDA and activities of glutathox yse GPOX, GSH.RD as well as liver activities of SOD and CAT occurred when compared with control. These are indications of antioxidant property of Morinda morindoides. The extract showed significant scavenging effects on hydrogen peroxide, nitric oxide, ABTS radical cation and DPPH radical. Histological investigations revealed mild vacuolar degeneration of hepatocytes and moderate tubular degeneration of the kidney. These findings indicate that Morinda morindoides possesses in vivo and in vitro antioxidant activities but may be slightly injurious to the tissues studied.

809 The Antioxidant, Anti-Inflammatory, and Antinociceptive Activities of the Ethanolic Leaf Extract of Andrographis paniculata Nees (Family Acanthaceae)

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Introduction

Andrographis paniculata Nees commonly known as king of bitters is well-known medicinal properties (Sandberg 1994, Samir & Amrit 2002, Adedapo et al 2009). This study is to evaluate the medicinal property of this plant obtained in the wild in Nigeria.

Materials and Methods

Ethanol extract (EE) was prepared by dried ground leaf material (200g) in absolute 1 L ethanol for 48 h, filtered, dried and then used for the pharmacological investigations. Standard phytochemical methods were used to test for the presence of phytoactive compounds in the plant. Acute toxicity was carried out in mice to determine safe doses for this plant extract. Anti-oxidant activities of the plant were assessed using ABTS, DPPH, FRAP and total phenolics test. The anti-inflammatory activities were conducted using carrageenan and histamine-induced tests while antinociceptive activities were carried out using writhes and formalin licking tests.

Results

Acute toxicity test showed that no animals died at the doses even at 1600mg/kg dose. The extract at 50, 100 and 200mg/kg body weight caused a significant reduction in the anti-inflammatory and analgesic activities though not in the same magnitude as indomethacin, the reference drug used in this study. In the case of anti-oxidant activities, the extract caused 82.7% ABTS inhibitory activity i.e. 6.21±0.003 gallic acid equivalent; 76.61% DPPH inhibitory activity (12.91±0.007 ascorbic acid equivalent); 50.37±0.23 (42%) ascorbic acid equivalent for FRAP and 78.56±0.04 (58.43%) gallic acid equivalent for total phenolics.

Conclusion

The results from this study may have validated the traditional basis for the use of Andrographis paniculata as medicinal agent. The pharmacological activities noted in this study may be attributed to the presence of flavonoids and other phenolics contained in this plant.
Green tea polyphenols (GTP) are found to protect against many chronic diseases in animal models and human studies. There are four major GTP: EC, ECG, EGC, and EGC2 in green tea. They mainly undergo phase 2 metabolism, including glucuronidation, sulfation, and methylation. In addition, there was significant metabolism by gut microbiota in the colon. The lack of sufficient information on the biotransformation of GTP has limited our understanding of its possible beneficial health effects. In this study we determined the metabolic fate of GTP in humans with particular emphasis on their methylated forms and microbial metabolites. After feeding 600 mL of green tea (5 g tea leaves), human urine samples were collected that showed by HPLC with a multi-channel coulometric electrochemical detector (HPLC-ECD) and liquid chromatography-mass spectrometry (LC-MS).

The major methylated metabolites appeared in human urine as 4'-O-methyl(-)-epigallocatechin (4'-ME-EGC), 4'-O-methyl(-)-epigallocatechin-3-gallate (4'-ME-EGCG) and 4,3'-di-O-methyl(-)-epigallocatechin-3-gallate (4,3'-DiME-EGCG). Only trace amounts of 4'-O-methyl(-)-epigallocatechin-3-gallate (4'-ME-EGC) were found. These methylated metabolites were mostly in glucuronidated form (50-75%) in human subjects and the renal excretion peaked 4.5-5.5 h after the dose. The cumulative excretion of 4'-ME-EGC, 4'-ME-EGCG and 4,3'-DiME-EGCG during a 8 h period ranged from 4 to 100 µg, 15 to 18 µg, and 5.9 to 300 µg, respectively. The amount of 4'-ME-EGC excreted in urine was about 20% of total excreted GEC and accounted for about 1% of EGCG ingested, whereas excreted 4,3'-DiME-EGCG accounted for about 0.1% of ingested EGCG. Microbial metabolites such as (-)-5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (M4), (-)-5-(3',4'-dihydroxyphenyl)-γ-valerolactone (M6), and (-)-5-(3',5'-dihydroxyphenyl)-γ-valerolactone (M8) also were detectable and predominantly in glucuronidated and sulfated forms in the urine. These results will enhance our understanding of protective characteristics of green tea constituents against human diseases.

A common amino acid, β-alanine (BA) is frequently used as a nutritional supplement to enhance workout capacity in athletes and delay fatigue; effects potentially attributable to enhanced buffering in muscle resulting from increased carnosine content. The present study in rats was designed to assess the safety of BA exposure against human diseases.

**811 Subchronic Oral Gavage Safety of Beta-Alanine in Rats**

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A common amino acid, β-alanine (BA) is frequently used as a nutritional supplement to enhance workout capacity in athletes and delay fatigue; effects potentially attributable to enhanced buffering in muscle resulting from increased carnosine content. The present study in rats was designed to assess the safety of BA exposure against human diseases. The present study in rats was designed to assess the safety of BA exposure against human diseases.

The control group received saline and each treated group received the EEZA in dose of 500, 1000, 2000 and 4000 mg/kg through gastric intubation for 15 days (once a day in the morning). At the end of the experiment, blood was collected from the orbital sinus under ether anesthesia for biochemical and hematological analysis. The animals were sacrificed by cervical displacement and selected organs (liver, heart, spleen, left kidney and left lung) were removed for macroscopic analysis. Oral administration of the alcoholic extract of Z. armatum in a dose from 100 to 4000 mg/kg did not produce any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses and gastrointestinal tract effect in mice. In conclusion these results showed that in subacute dose, there is no adverse effect of Z. armatum indicating that the medium lethal dose (LD50) is higher than 4000 mg/kg for both male and female mice. The macroscopic analysis of the target organs of the treated animals (liver, lungs, heart, spleen and left kidney) did not show any significant changes in colour and texture when compared with control group.

**813 Investigation on the Toxicity of Aconitum brachypodum Diels In vitro and In vivo**


To investigate the toxicities of four active extracts from Aconitum brachypodum Diels in vitro and in vivo. LD50 was detected to estimate the toxicity of the extracts after intragastric administration to Kunming mice. The results showed that the extracts from Aconitum brachypodum Diels have different degrees of toxic effects on mice. The toxic symptom included reduced activity amount, retching, abdominal breathing, diaphoresis, exorbitism and convulsion. The chloroform extract even lead to fatality of some mice at doses above 25 mg/kg. LD50 of chloroform extract, petroleum benzine extract and n-butanol extract were 38.0 mg/kg, 6766.9 mg/kg and 5492.3 mg/kg respectively. The rank of their toxicity was: chloroform extract > petroleum benzine extract > acetic ether > n-butanol extract. The present study compared the toxic fractions of Aconitum brachypodum Diels in vitro and in vivo.

**813a Toxicity in Mice and Rats Associated with Inhalation of the Flavor and Fragrance Ingredient Alpha-Pinene**

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α-Pinene is the main component in turpentine and is used as a fragrance and flavoring ingredient. Due to widespread exposure potential and a lack of available toxicity data, the National Toxicology Program conducted 2-week and 3-month inhalation studies in male and female F344/N rats and B6C3F1/N mice. In the 2 week studies, groups of 5 male and 5 female rats and mice were exposed to α-pinene by whole body inhalation at concentrations of 0, 100, 200, 400, 800, or 1,600 ppm, 6 hours plus T90 (12 minutes) per day, 5 days per week for 16 (rats) or 17 (mice) days. There was significantly decreased survival in the 800 and 1,600 ppm male and female rats and mice, clinical signs of toxicity in rats (400 ppm and greater) and mice (800 and 1600 ppm), and increased liver weights in both species. In the 3-month studies, groups of 10 male and 10 female rats and mice were exposed to α-pinene at concentrations of 0, 25, 50, 100, 200, or 400 ppm, 6 hours plus T90 (10 minutes) per day, 5 days per week for 14 weeks. All exposed male rats and male and female mice survived to the end of the studies, while six 400 ppm female rats died before the end of the study. The major targets for α-pinene toxicity were the urinary system and the male reproductive system. Increased kidney weights in male (100 ppm and greater) and female (50 and 200 ppm) rats were accompanied by histopathological lesions (i.e., granular casts and hyaline droplet accumulation) in all dosed groups of male rats only. A dose-dependent increase in the incidence of transitional epithelium hyperplasia in the urinary bladders was observed in male (70%, 100%, and 100%) and female (60%, 100%, and 100%) mice exposed to 100, 200, and 400 ppm α-pinene, respectively. Finally, there were significantly decreased numbers of cauda sperm in 200 ppm and above male rats and 100 ppm and above male mice, reaching a maximum of 50% decrease in 400 ppm male mice. Based on these toxicity findings, chronic inhalation studies with α-pinene are warranted. This research was supported by the NIH, National Institute of Environmental Health Sciences.
Arsenic toxicity has been associated with diverse ailments including cancer in both experimental animals and humans. Hence, the search for agents that can protect/reduce arsenic-induced toxicities. In the present study, the anticlastogenic and hepatoprotective abilities of aqueous leaf extract of *Andrographis paniculata* (Ap) against sodium arsenite (NaAsO2)-induced toxicities were examined in male albino rats. The *in vitro* antioxidant activity of the extract was also determined as compared with standard antioxidants. Test and control rats were exposed to 200, 500 and 1000 mg/Kg body weight of Ap for 28 days. Simultaneously, the animals were treated with 5mg/kg body wt of NaAsO2 on the 7th, 14th, 21st and 28th day of the experiment before they were sacrificed. The frequency of micronucleated polychromatic erythrocytes (mPCEs) as monitored in bone marrow cells while aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase activities were assessed in the serum. NaAsO2 induced significantly (p<0.05) mPCEs formation and the activities of all the hepatic enzyme markers. Pre-treatment with Ap significantly reduced the mPCEs and enzyme activities observed. Our study suggests that aqueous leaf extract of Ap may be useful in the management of arsenic toxicity.

Epigallocatechin gallate (EGCG), the major flavonoid in green tea, has been associated with cases of hepatotoxicity in humans and animals. Here the EGCG effect on rat liver mitochondria was examined as a useful model of xenobiotic hepatotoxicity. Though EGCG showed negligible effects on oxidative phosphorylation at 7.5-100 μM in normal mitochondria, respiratory chain complexes (RCCs) were profoundly inhibited in mitochondria undergoing calcium overload-induced mitochondrial permeability transition (MPT). At RCCs are located in mitochondrial inner membranes (IM) and matrix, it was reasoned that EGCG could not readily pass through IM to affect RCCs in normal mitochondria but may do so when IM integrity is compromised. This speculation was substantiated in three ways. (1) Purified EGCG-bound proteins were barely detectable in normal mitochondria and contained no RCCs as determined by Western blotting, but swelling mitochondria contained about 1.5-fold more EGCG-bound proteins which included four RCC subunits together with cyclophilin D that locates in mitochondrial matrix. (2) Swelling mitochondria consumed more EGCG than normal ones as determined by HPLC. (3) MPT blocker cyclosporine A diminished the above-mentioned difference. Among four subunits of RCC II, only SDHA and SDHB that locate in mitochondrial matrix, but not SDHC or SDHD that insert into the IM, were found to be EGCG targets. Interestingly, EGCG promoted calcium overload-induced MPT only when moderate MPT already commenced. The data suggest EGCG could be detrimental in MPT-associated diseases and toxicities by causing synergistic or additional mitochondrial damages.
Physiologically Based Pharmacokinetic Modeling of Cerium Oxide Nanoparticles by Pulmonary Exposure in Rats

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In vitro studies have reported toxic effects of cerium oxide nanoparticles (CeO2 NPs) which can be used as a diesel fuel additive, thus it is important to understand the biodistribution of CeO2 NPs in the body via pulmonary exposure. The aim of this study was to measure this biodistribution and to develop a physiologically based pharmacokinetic model of CeO2 NPs using a compartmental analysis approach. A one-compartment model was fit to CeO2 intratracheal instillation data in rats. The resulting CeO2 biodistribution model was then applied to predict CeO2 concentrations in different organs after intratracheal instillation of the nanomaterials. The model was validated using CeO2 NPs (-80nm) for 5 hours and measured concentrations in different organs over 14 days. These results were then compared to the CeO2 concentrations after intratracheal delivery of 200µg CeO2 NPs (-6.6nm) over 28 days measured by He et al. (2010). High concentrations of CeO2 NPs were found in the lungs and feces about 2mg/g and 12mg/g of NPs were found respectively in the lungs and feces 1 day post inhalation exposure. Concentrations in other organs were 3-4 orders of magnitude lower at this time. By comparison, the intratracheal instillation study had similar tendencies in the concentrations among organs but captured a higher fraction of the total administrated CeO2 NPs in the lungs compared to the nasal exposure study (78% vs 4%). The simulated time courses of NPs biodistribution agreed well with experimental data, yielding R2 of 0.73 for the inhalation exposure study and 0.99 for the intratracheal instillation study. Further investigations are ongoing to study the sensitivity of the model parameters and to support the most critical parameters.

Comparative Analysis of Predictive Models Used to Identify Drivers of Nanomaterial Toxicity

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Understanding the inherent and conditional factors associated with nanomaterial toxicity is critical to the development of nanotechnologies that pose minimal threats to humans and the environment over the life cycle of the nanomaterial. We compared the results of several different models built to predict toxicity from the open-source data on nanomaterial toxicity to embryonic zebrafish (Danio rerio) found in the Nanomaterial-Biological Interactions (NBI) knowledgebase at Oregon State University. Model comparisons included the ABMiner predictive models, MATLAB clustering analysis and the use of Self-Organizing Map (SOM) based consensus clustering conducted on the data in the NBI knowledgebase (nbi.oregonstate.edu). Overall results suggest that exposure concentration and surface area to volume ratio should be considered in conjunction with the core composition of nanomaterials when trying to develop predictive models for predicting zebrafish toxicity. Core composition was found to be a significant contributor to the ABMinor predictive model. MATLAB clustering grouped materials into two clusters with outermost surface chemistry being the primary determinant. The SOM modeling identified five significant clusters (clustering index = 0.89); while no core materials occurred in all 5 clusters, 4 material types (based on core composition) occurred in 4/5 clusters and 15 material types occurred in 3/5 clusters. In addition, over half of the materials appeared in multiple clusters depending on the dose applied. Thus, classification of nanomaterials by simple descriptors such as core composition may not be sufficient for predicting nanomaterial toxicity or managing nanomaterial risks.

Development and Validation of a Dosimetric Platform for In Vitro Nanotoxicology

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Sponsor: J. Brain.

There is a great need for developing screening tools capable of rapidly and accurately assessing engineered nanomaterial (ENM) toxicity. One impediment to the development of reliable in vitro screening methods is the need for accurate dosimetry. In a typical in vitro cytotoxicity study ENM powders are suspended in liquid media for application to cells. ENMs in liquid suspension can form large fractal aggregates thereby altering (1) the total number of free particles, (2) the total surface area available for biointeractions, and (3) the effective size and density of the particles. While these two properties influence their fate and transport and determine the effective dose actually delivered to cells in culture over the duration of exposure, the present here a methodology for in vitro nanotoxicology that takes into consideration factors that influence their fate and transport and determine the effective dose actually delivered to cells in culture over the duration of exposure. We present here a methodology for in vitro nanotoxicology that takes into consideration factors that influence their fate and transport and determine the effective dose actually delivered to cells in culture over the duration of exposure. This multi-step methodology includes: (1) standardization of ENM liquid suspension preparation; (2) sample characterization using transmission electron microscopy to determine the ENM transformations in exposure media; and (3) numeric calculation of the effective dose as a function of exposure time.

The described methodology was then used to derive simple mathematical equations that are applicable to determine the effective dose delivered to cells as a function of exposure time, or to perform meta-analyses on previously reported toxicity datasets taking dosimetry into consideration.

A Pharmacokinetic Modeling Framework for Interspecies Extrapolations of Nanoparticle Biodistribution Taking into Account Biocorona Dynamics Independent of Species-Specific Disposition Processes

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Formation of a corona complex around engineered nanoparticles (NP) dramatically alters their biodistribution. A common practice in nanotoxicology and nanomedicine is to extrapolate results from in vitro cell culture or in vivo rodent models to humans. There are concerns about the reliability of these predictions about whether the fate of NP in rodent models with high basal metabolic rates (BMR) and short blood circulation times can predict the fate of the NP in large mammals that have a lower metabolic rate and a greater circulation time? The interaction between two major processes determines the NP biodistribution: delivery to target cells with an allometrically scalable rate across species and an independent process of NP corona formation. Long circulation times in humans allows for the formation of a mature hard corona complex potentially masking the NP originally engineered with specific surfaces for target cell receptors. A simple and descriptive compartmental model is proposed to compare the potential fate of NP in rodent models versus humans by allometrically scaling those components dependent upon BMR and separately describing the potential fate of NP in large mammals that have a lower metabolic rate and a greater circulation time. Our model has 10 compartments and 15 material types (based on core composition) occurred in 4/5 clusters and 15 material types occurred in 3/5 clusters. In addition, over half of the materials appeared in multiple clusters depending on the dose applied. Thus, classification of nanomaterials by simple descriptors such as core composition may not be sufficient for predicting nanomaterial toxicity or managing nanomaterial risks.

Understanding the Dynamic Behavior of the Protein Corona and Its Influence on the Physicochemical Composition of Silver Nanoparticles

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The health effects and safety of nanoparticles (NP) are of a great concern due to their increasing availability and use in consumer products and biomedical applications. It has been established that NP tend to interact with proteins to form a protein corona upon entering biological fluids. The dynamic interactions of the protein corona with NP may significantly influence the bio-identity of NP, leading to diverse biological outcomes, especially at the cellular level. Since NP-protein interactions are poorly understood, a fundamental understanding of the complexity and dynamic interactions of the protein corona with NP is crucial. A systematic investigation on time dependent adsorption kinetics of the protein corona formation was conducted with citrate or lipoic acid coated silver NP (AgNP) of 40nm. AgNP were exposed to human albumin (HSA) and fibrinogen at their in vivo physiological concentrations. Dynamic light scattering, zeta potential, NP tracking analysis and transmission electron microscopy were used to monitor kinetics of the
AgNP protein corona formation. Data on time evolution revealed that irrespective of the surface chemistry, rapid and prominent binding of HSA at 40μg/ml formed coronas with both citrate and lipopolysaccharide coated AgNP and caused an increase in size to 60nm which was stable. In contrast, AgNP in fibrinogen at 2mg/ml showed a time dependent decrease in stability with aggregation within 1 to 4h with a significant reduction in NP concentration and size (24.8nm) over 12h at 37°C. These results showed that the corona proteins at their physiological concentrations interacted rather differently with AgNP; whereby HSA prevented NP dissolution and aggregation, while fibrinogen caused a significant size reduction and aggregation.

**Toxicity Assessment of Six Titanium Dioxide Nanoparticles in Human Epidermal Keratinocytes**

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Nanoparticle uptake in cells may be an important determinant of their potential cytotoxic and inflammatory effects. Six TiO2 NP (A=Alfa Aesar, 10nm, A*=Alfa Aesar, 32nm, B=P25, 27.5nm, C=Aeros, 200nm, C*=NanoAmor, 30-40nm, and D*=Mkano 200-400nm) and controls were assessed for their size, effect on cell viability, agglomeration, cytokine release, and cellular uptake in human epidermal keratinocytes (HEK). TiO2 NP were suspended in water or in culture medium, and characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). TiO2 NP did not change shape with any NP type in medium by TEM but DLS showed an increase in size and in aggregations. TiO2 NP were assessed for viability with CellTiter 96 AQueous One (96AQ), MTT and alamar Blue assay was selected to assess all NP in serial dilutions ranging from 25ng/ml to 2μg/ml coated with a complex PC and differences in PC formation. The PC alters the interaction of AgNPs with keratinocytes (HEK). PC alters the interaction of AgNPs with keratinocytes (HEK). The PC alters the interaction of AgNPs with keratinocytes (HEK). The PC alters the interaction of AgNPs with keratinocytes (HEK). The PC alters the interaction of AgNPs with keratinocytes (HEK).

**Characterization of a Complex Protein Corona on Silver Nanoparticles and Its Influence on Cellular Interactions**

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Silver nanoparticles (AgNP) have been incorporated in biomedical and consumer products as antimicrobial agents. Upon introduction into physiological environments NP associate proteins forming a protein corona (PC). The PC alters the NP-cell interface resulting in modified uptake, activity, clearance, and toxicity. Here we characterized the formation of a complex PC (consisting of multiple proteins) on AgNPs and examined its alterations in cellular interactions. To characterize the formation of the PC, PVP- or citrate-suspended AgNPs of 20 or 110 nm were incubated in DMEM containing 10% FBS. PC formation was indicated by alterations in the hyperspectral image of AgNPs such as a broadening of peaks and a red shift in the spectra. Spectral profiles were altered due to the size and surface coating of the AgNPs suggesting dissimilar PC formation. Individual PC components were identified and quantified via label-free mass spectroscopy. All AgNPs associated with the same complex PC that included different amounts of a common subset of 11 proteins including albumin and certain apolipoproteins. 110 nm AgNPs were found to associate the greatest number of proteins compared to 20 nm AgNPs, suggesting differences in PC formation based on surface curvature, whereas the PC on 20 nm AgNPs consisted of more hydrophobic proteins. These findings demonstrate the importance of electrostatic and hydrophobic interactions in the formation of the PC. To assess how the PC influences cellular interactions, macrophages were exposed to AgNPs (25 μg/ml) coated with a complex PC and differences in uptake were analyzed by hyperspectral dark field microscopy and flow cytometry. Addition of the PC was observed to increase uptake for all AgNPs (110 nm > 20 nm) by flow cytometry. By using spectral libraries we were able to intraclinically observe AgNPs and qualitatively determine differences in uptake by image analysis. These findings demonstrate the influence of variations in NP size and surface coatings, on the formation of the PC and modifications of cellular interactions.

**Acute and Subchronic Exposure to Inhaled Silver Nanoparticles Results in Alterations in Gene Expression, Gene-Specific Promoter Methylation, and Mitochondrial Integrity**

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The use of silver nanoparticles (AgNPs) is ubiquitous. Legitimate toxicology concerns exist and a number of recent studies suggest that AgNPs pose a threat to human health. This study was conducted to examine the effects of acute and subchronic exposure to inhaled AgNPs. Male FVB/NJ mice were exposed to AgNPs generated using a Palas spark generator, via nose only inhalation, at either a single 4 hr exposure of 1 mg/m3, or at an exposure of 0.25 mg/m3 for 30 days. At 24 hr, 48hr, and 7d post-exposure, animals were euthanized and blood, lung and tissue were collected. Lavage was analyzed for total cell counts, differentially cell analysis, and protein levels. Fifty-four samples, isolated from lung and liver tissue, were used in Affymetrix Mouse Gene 2.0 ST Array Analysis. Microarrays were analyzed using Agilent GeneSpring and results verified by real-time qPCR. DNA isolated from lung and liver tissue was used in the analysis of gene-specific promoter methylation using Epitect Methyl II arrays. Inner mitochondrial membrane integrity was quantified using JC-1 staining and fluorescence microscopy. Outer mitochondrial membrane integrity was examined by measuring cytochrome c oxidase activity in the presence and absence of n-dodecyl beta-D-maltoside. Highly significant alterations in gene expression were observed in response to both acute and subchronic AgNP exposure, in both lung and liver tissues, in a time dependent manner. In addition, AgNP exposure resulted in significant decreases in both inner and outer mitochondrial membrane integrity. These experiments suggest that exposure to inhaled AgNPs results in gene expression alterations, which may be regulated by alterations in promoter methylation, in a time dependent manner. Further, cellular uptake of inhaled AgNPs, in lung and extra-pulmonary organs results in detrimental effects on mitochondrial membrane integrity.
The use of silver nanoparticles (AgNPs) in consumer products has vastly increased due to their antimicrobial activity. However, recent reports suggest the inhalation of AgNPs can lead to neurodegeneration due to translocation via olfactory nerves. Previous studies fail to evaluate biologically relevant exposure routes and concentrations. Therefore, this study evaluated the effects of inhaled AgNPs on gene expression within distinct subregions of the brain under diverse exposure terms. Adult male FVB/J mice were exposed via nasal inhalation to AgNPs produced by a Palas spark generator. Animals were assigned to 3 exposure groups: 1) single exposure (1mg/m3), 2) subacute repeated dose (30d, 0.25mg/m3), or 3) subchronic repeated dose (90d, 0.25mg/m3). Tissues were taken at 24hr, 48hr, and 7d post-exposure for the single exposure group, and repeated group animals at 24 hr. Animals were euthanized and brains removed and dissected into three portions: olfactory bulbs (OB), cerebellum (CB), and midbrain (MB). Isolated total RNA was analyzed by real-time RT-PCR using various oxidative stress-associated gene primers. Overall a variety of gene expression alterations were found for each neural subregion. Most interesting, the single exposure resulted in gene expression alterations for amyloid beta (Aβ) precursor protein (APP), which is associated with Alzheimer’s, a detrimental neurodegenerative disease. APP upregulation was segregated to the OB, where it remained high 7d post-exposure suggesting possible long-term neurotoxic effects from a single exposure to AgNPs. Conversely, Amyothrophic Lateral Sclerosis (ALS) associated gene was only mildly changed in all sub-regions, suggesting a specific mechanism of toxicity. Additionally, oxidative stress related genes were upregulated within the MB and CB at 48 and 72 hr and returned to control levels by 7d post-exposure. Together these results demonstrate that inhalation of AgNPs can result in oxidative stress and gene expression alterations which could contribute to neurotoxicity and potentially neurodegeneration.

Silver nanoparticles (AgNPs) have been increasingly used in manufactured goods for their bactericidal properties. Previous work has shown these particles to be toxic to the nervous and immune systems under various dosing regimens. A previous study from our lab demonstrated localization of AgNPs in the brain following a one-time intranasal (IN) administration of 50 mg/kg AgNPs. We also found increased Hmox1 gene expression, a marker of oxidative stress, in the hippocampus of the silver-treated mice. To examine possible functional consequences of AgNP accumulation and increased Hmox1 gene expression, behavioral experiments were performed to characterize changes in learning and memory following IN AgNP exposure. Adult male C57BL/6 mice were housed with 7 consecutive days with uncoated AgNPs (0 or 50 mg/kg; average diameter 35.7 nm) to mimic repeated workplace exposure in adult humans. One week after the end of AgNP administration, the behavior of the mice was assessed via novel object recognition and Morris Water Maze. AgNP-treated mice exhibited no significant difference in exploration of the novel object during testing. No significant difference was observed between the control and treated groups during the acquisition, reversal, or cued phases of Morris Water Maze testing. Blood cell counts were similarly unaffected between the control and AgNP-treated mice, with the exception of a significant increase in the number of eosinophils. In addition, weight differences were observed, with thymus weights being affected across groups. Further studies into the localization of silver following repeated doses and exposure of animals at different life stages will lead to a greater understanding of the effects of repeated exposure to these toxic particles. This work was funded in part by NIEHS T32ES016646-01 and NIEHS P30ES006096.

Silver nanoparticles (AgNPs) have antimicrobial activity and unique electrical properties, resulting in increasing use. While biological effects of AgNPs have been studied, there has not been a comparison of particle size on aerosol-delivered AgNPs persistence. We investigated lung deposition and retention of 20 and 110nm citrate-coated AgNPs. Aerosol of AgNPs was generated using a nebulizer. Adult male rats were exposed nose-only for 6 to either 20min or 110min AgNPs (8mg/m3 and 6.2mg/m3) or 2mM citrate buffer vehicle control. Tissues were collected immediately following exposure (T0) and 1, 7 and 21 days post-exposure (DPE). Ag was quantified using inductively-coupled plasma mass spectrometry and tissue distribution was determined using autoradiography (ARG). Each lung lobe and the trachea were measured to determine differences between lobes at T0. At T0 the mean Ag concentration of 110nm AgNPs ranged from 11.89 -18.95µg/g tissue with no significant difference between lobes. 20nm AgNPs T0 lobes were significantly different from each other and ranged from 2.05-9.28µg/g by macrophages and has been reported to play a role in recognition of negatively charged particles. We therefore hypothesized that SR-B1 contributes to macrophage uptake of AgNPs and subsequent cellular activation. To test this hypothesis, we exposed a mouse macrophage cell line (RAW 264.7) and bone marrow-derived macrophages (MDM) to 20 nm citrate-suspended AgNPs. To verify the role of the SR-B1 receptor, we utilized B2-1, an SR-B1 pharmacological inhibitor. Flow cytometry was used to evaluate cellular uptake following exposure to AgNPs (50 ng/ml). Exposure to AgNPs led to increases in the side scatter of macrophages indicating cellular uptake. Inhibition of SR-B1 with B2-1 reduced RAW and BMDM uptake of AgNPs. To further examine the role of SR-B1 in macrophage uptake of AgNPs, BMDMs were derived from SR-B1-/- mice. Following AgNP exposure, SR-B1-/- BMDMs demonstrated decreased uptake by flow cytometry. To confirm flow cytometry findings, cells were imaged following AgNP exposure with hyperspectral dark field microscopy and quantitatively assessed by ICP-MS. In vivo studies confirmed the role of SR-B1 in AgNP-induced toxicity. C57BL/6 mice receiving a single intranasal instillation of AgNPs demonstrated increases in lung inflammatory cells, and lung tissue IL-6 and IL-1β gene expression. In comparison, SR-B1-/- mice showed a significant decrease in AgNP-induced inflammatory cell recruitment and gene expression. In conclusion, macrophage SR-B1 activation contributes to the inflammatory response induced by AgNPs.
829 Inhaled Nano-TiO2 Size Distribution along with Mass Concentration Define Lung Responses
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Currently, there is no consensus regarding the exposure metric that best expresses the nanoparticle (NP) dose. Although the surface area was extensively studied for the inflammatory reaction, it has not been validated for cytotoxicity or oxidative stress. Since inhaled NP deposit and interact with lung cells according to their agglomerates size, we hypothesized that the mass concentration combined to the aerosol’s size distribution is suitable for NP risk assessment. The objective of this study was to evaluate different exposure metrics for TiO2 aerosols composed of small (SA) (<100 nm) or large (LA) (>100 nm) agglomerates at 2.7 and 20 mg/m3 on rat lungs inflammatory, cytotoxicity and oxidative stress responses. Six groups of male F344 rats (n=6) were nose-only exposed for 6 hr to the different 5 nm TiO2 aerosols. The control group was exposed to air. Exposures were characterized by weight measurement for mass concentration and with an electrical low pressure impactor for number concentration and size distribution. Bronchoalveolar lavages (BAL) were analyzed 16 hr after exposure. Our results showed that the 7 mg/m3 LA and the 20 mg/m3, both LA and SA aerosols, increased the number of neutrophils compared to controls. The 20 mg/m3 SA significantly (p<0.05) increased LDH activity and 8-isoprostane concentration compared to controls. When the size distribution of NP was considered with the mass as an exposure metric, we found a strong correlation (r=0.97) with the number of neutrophils for SA and LA. For LDH and 8-isoperoxide, we observed a high correlation (r=0.89) only with SA. These data show that the mass concentration alone is not sufficient to adequately predict oxidant and cytotoxic pulmonary effects. Overall, our study indicates that considering the NP size distribution along with the mass concentration contribute to a more mechanistic discrimination of respiratory effects.

830 Cell Transformation Potential of Nano-Cerium Oxide (nCeO2), Nano-Ferric Oxide (nFe2O3) Compared to Multiwalled Carbon Nanotubes (MWCNT)
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Unique physicochemical properties of engineered nanomaterials (ENMs), including CNTs and metal oxides, are distinct from their micron-sized counterparts evidenced by their unique biological effects. Previous studies have suggested potential lung disorders induced by these ENMs. Recent animal study indicated cancer promotion by MWCNT. Our previous in vitro work showed subchronic single walled CNT or MWCNT exposure can cause human lung epithelial cell transformation which formed tumors when subcutaneously injected into mice. These findings raise ENM-induced carcinogenicity concerns due to rapid growth in ENM applications, including widely used nCeO2, nFe2O3 and MWCNT. The present work tested cell transformation potential of nCeO2 and nFe2O3 compared to MWCNT as a positive tumorigenic control. Primary human small airway epithelial cells were treated with a sub- toxic, low dose of ENMs (62.5 ng/cm2 of nCeO2 and nFe2O3 or 60 ng/cm2 of MWCNT), with albumin and saline as dispanser and no treatment controls, respectively. Cells were continuously exposed to ENM containing medium for 6 weeks. Following exposure, cells were assayed for cell transformation and cancer hallmark analysis. Increased cell proliferation suggested significant cell stimulation by nCeO2 and MWCNT. Next, nFe2O3 and MWCNT-treated cells formed soft agar colonies and significantly enhanced cell invasion which suggests their potential cell transformative effect. These results will guide future in vivo studies to confirm the observation and to assess the relevancy of our subchronic in vitro exposure model. Such an in vitro model may serve as a simple/fast/high throughput tool to screen countless ENM to predict cell transformation/carcinogenic potential and addresses a critical need in ENM risk assessment.

831 Evaluation of Pulmonary Response to Tungsten Oxide (WO3) Nanoparticles in Golden Syrian Hamsters
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Tungsten oxide (WO3) nanoparticles possess excellent photochromic, electrochromic, thermochromic and catalytic properties. Extensive industrial and military uses have raised the possibilities of human occupational and environmental exposure with concomitant health concerns. To evaluate the effects of such particles in the lung, Golden Syrian Hamsters were exposed by inhalation to aerosols of WO3 nanoparticles (Nanostructured and Amorphous Materials, Inc., TX). Hamsters were divided into four groups – a control group that was exposed to distilled water and 3 treatment groups that were exposed to 5, 10 or 20 mg/m3 WO3 nanoparticles (4 hrs/day, for 4 days). Animals were euthanized 24 hours post-exposure and bronchoalveolar lavage fluid (BALF) was collected. Lungs were then fixed by instillations of 10% formalin. Tissue sections were analyzed using bright and dark field microscopy. The average size of WO3 nanoparticles, 71 ±34 nm, was confirmed using TEM. There was a significant increase (p<0.05) in total cell counts 22.3 ±0.1, 21.6 ±2.5, and 29.3 ±0.1 x10^6 cells/ml of BALF from treated groups (5, 10 or 20 mg/m3 respectively), as compared to controls (15.6 ±1.4 x10^6 cells/ml). BALF protein levels increased in treated groups (106.1, 110.7 and 124.1 µg/ ml) as compared to controls (97.9 µg/ml). The number of macrophages increased in the BALF treated group (76.8, 65.6 and 145.3 cells/field at X400) as compared to controls (29.2 cells/field at X400) without increase in the number of PMN’s. Dark field micrographs of tissue sections revealed WO3 nanoparticles present in alveolar macrophages that were dispersed in large numbers throughout the lung. Nanoparticles were also identified on airway epithelium, within airway epithelial cells and in interstitial areas of alveolar structures. Results from these experiments indicate that WO3 nanoparticles can reach alveolar space, enter epithelial layers and penetrate to interstitial structures.

832 Evaluating Metallic NP-Induced Stress Using a Neuronal Coculture Model
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To date nanotoxicity studies on the central nervous system are limited and conflicting with discrepancies between animal models and humans adding to the complexity. A previous study focused on establishing a neuronal co-culture model which included neurons and their microglia, to evaluate the phagocytic response effects of nanoparticles (NPs). As a continuation, the present study examined the toxicity of different nanometals which included silver (Ag) and gold (Au) as well as known neurotoxictants manganese (Mn), aluminum (Al), and copper (Cu); and while all of the metallic NPs had an initial size of 80 nm, dispersion resulted in varying degrees of aggregation. The cells were dosed with realistic exposure levels of the different metallic NPs to represent a daily or weekly exposure based on the current limits reported for the bulk metals by OSHA. In addition to viability and reactive oxygen species (ROS) production, key stress genes (NF-kB, TNFα, IL-6) were selected to examine changes following varying times of exposure (4, 8, and 24 h) to metallic NP. The Mn-NPs induced significant toxicity at daily and weekly exposure levels, while Cu-NP showed toxicity only at the weekly exposure level. Furthermore, the Mn-NP treatment demonstrated concentration dependent increases in ROS production and TNFα and IL-6 expression over time while NfKβ remained unchanged. Surprisingly, while the Cu-NP treatment induced toxicity, and increased ROS production, the stress response profiles showed no changes at 4 h, and decreased expression for IL-6 and TNFα following 8 and 24 h. In comparison, the Ag-, Au-, and Al-NP treatments did not induce toxicity or significant increases in ROS and showed no changes in gene expression at either treatment concentration or time point. Taken together, the results demonstrated that the co-culture model provides enhanced sensitivity for studying NP induced stress responses and the nanometals displayed differential toxicity with Mn>Cu>Al/Au/Ag.
833 Unique Mode of Toxicity of Cu Nanoparticles Compared to Their Micron and Ionic Analogs in E. coli and L. brevis

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Nanotechnology has grown rapidly over the past decade, promising benefits in diverse areas of society. However, the rate of toxicological analysis of nanoparticles (NPs) has not kept pace with the rate of development, leading to concerns over the potential biological toxicity and environmental contamination of NPs. Here, we report toxicity ranking as well as mechanisms of toxicity for a series of Cu particles, including nano Cu, nano CuO, nano Cu(OH)2, micro Cu and micro CuO as well as ionic Cu (CuCl2 and CuSO4) in bacteria (Escherichia coli and Lactobacillus brevis). Fluorescent assays such as PI/STYO, XTDT, DIBAC, and H2DCFDA were used to measure viability, respiration rate, membrane potential, and ROS production, respectively. IC50 values were calculated from growth inhibition curves, revealing that Cu and CuO NPs are more toxic than their micro-sized counterparts, with toxicities approaching that of ionic Cu. Strikingly, the NPs showed distinct differences in their mode of toxicity when compared to the Cu ions, highlighting the unique toxicological properties of materials at the nanoscale. Moreover, cells treated with nano Cu showed higher levels of bioavailable Cu+ than cells treated with ionic Cu. 3D tomography from electron microscopy of cells exposed to the NPs revealed the presence of intact NPs inside the cells.

834 Physicochemical Characterization and Ecotoxicological Evaluation of TiO2 Nanoparticles in Earthworm Eisenia fetida

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Wide spread application and use of nanoparticles such as TiO2, confers their release into various compartments of environment thereby raising concerns about their health impacts. It is essential to assess the ecotoxicity and implications of TiO2 on the flora, fauna and ecosystem as a whole. Although there are studies reporting toxicity of TiO2, but specific findings on its effect and underlying toxicity mechanisms in terrestrial organisms such as earthworms remain poorly understood and documented. Hence in this study, ecotoxicological evaluation of the rutile form (10-100nm) of TiO2 nanoparticles on earthworm, Eisenia fetida was conducted as per the modified filter paper test of OECD-207 guidelines. Physicochemical characterization of TiO2 nanoparticles was carried out using zeta sizer and scanning electron microscope. Earthworms were exposed to a series of concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 mg/cm3) of TiO2 nanoparticles on earthworms. Eisenia fetida was conducted as per the modified filter paper test of OECD-207 guidelines. Physicochemical characterization of TiO2 nanoparticles was carried out using zeta sizer and scanning electron microscope. Earthworms were exposed to a series of concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 mg/cm3) of TiO2 nanoparticles. Mortality of earthworms was determined after 48h exposure to TiO2. Activity of the enzymes, catalase, superoxide dismutase and glutathione reductase was assessed in whole body homogenate of the worms surviving after exposure. Lipid peroxidation was measured to define the levels of oxidative stress during toxicity assessment. Perturbations caused by TiO2, nanoparticle resulted in the oozing of coelomic fluid and pale coloration of earthworm body. Mortality rate, activity of antioxidant enzymes and lipid peroxidation were influenced by size and charge of TiO2, nano particles rather than concentration. Results of the study showed that agglomeration of TiO2 nanoparticles is responsible for the variation in their size and charge. The finding is first of its kind to establish a toxicological profile of TiO2 nanoparticles on earthworms. Similar studies will eventually help in developing strategies to predict ecotoxicity of TiO2 in the soil environment.

835 Gene Expression Changes in Secondary Organs of Rats Intratracheally Exposed to Silver Nanoparticles

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Studies in rats have demonstrated the ability of silver nanoparticles (AgNP) to translocate to secondary target organs (e.g. liver or kidney) after administration by respiratory route (Takenaka et al, Environ Health Perspect, 2001). In this study, the expression profile of genes involved in oxidative stress (Gpx1, SOD, Gss, FMO2, Gsr, Tnrd1), metal toxicity (mt1), apoptosis/cell cycle (Casp3, p53), and protein folding (Hsp70) was investigated in liver and testis of rats at different time intervals after i.t. instillation of AgNP. AgNO3 was used as a positive control. Groups of adult male Sprague-Dawley rats received 50 μg/rat of AgNP (20 nm), 7 μg/rat of AgNO3 or 100 μL aqueous solution/rat (control). At days 7 and 28 post-administration, the transcriptional profile of selected genes was examined in tissues by cDNA microarray analysis coupled with bioinformatics and functional gene annotation studies. Semiquantitative followed by Real Time PCR was performed to quantify gene expression changes. At day 7, changes in gene expression that selectively involved antioxidant enzymes were observed in both liver and testes. In particular, Gpx1, FMO2 and SOD genes were upregulated. No changes were seen for the other genes. At day 28 the AgNP-treated animals exhibited a tissue gene expression profile similar to control. None of the investigated genes was shown to be affected by treatment with AgNO3 at both time points considered. The results suggest the potential of AgNPs to cause, at low doses, subtle molecular changes in secondary target organs, in contrast with Ag ions, possibly reflecting the strong tendency of Ag ions to form inert complexes with cellular or blood components or be neutralized by defense mechanisms. (Grants: Italian Ministries of Health, Research & Education; & CARIPLO foundation-Rif. 2011-2096).

836 The Influence of UV Light on the Genotoxicity of Engineered Nanoparticles

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TiO2 nanoparticles (NPs) are one of the most commonly used engineered nanomaterials due to their use in a large variety of applications as photocatalytic agent. The inherent properties of TiO2 might however, lead to photo-induced toxicity in human and animal tissue. Under UV irradiation, TiO2 NPs can produce ROS which is damaging to DNA and other biomolecules. In this study we used a chemical test for quantifying ROS generation in combination with the umu-test which is a short-term genotoxicity assay providing high throughput screening (96-well plates). Various nanoparticles (SiO2, CeO2, ZnO, and TiO2) were screened using concentrations of 0.0125-0.1 mg/mL with a 2 hour incubation/exposure time and no metabolic activation. No genotoxicity was induced for any of the NPs in the absence of UV light in the concentration range tested.

The next set of experiments involved the use of full spectrum UV light. Methylen blue dye decomposition was used for assessing the photocatalytic activity of TiO2 for an exposure period of 5-80 minutes. Maximal degradation of methylene blue occurred after 60 minutes. In order to investigate the effect of UV on Salmonella typhimurium bacteria, growth rate was investigated at 10, 20, 30, 40, 50, 70 min of exposure. Less than 15% decrease was observed for all the exposed bacteria compared to control. Therefore, an exposure period of 60 minutes was chosen to observe the ability of OECD reference hydrophilic (NM-104) and hydrophobic (NM-105) TiO2 NPs to induce genotoxic effects in the presence of UV light compared to absence of UV light. Concentrations of 0.25, 0.5, 1 mg/mL (45, 90, 180 μg/mL) increased the β-galactosidase activity indicating a possible genotoxic effect in the presence of UV light.

837 Monocyte Activation by Particulate Matter and Reactive Oxygen Species Formation

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Acute as well as chronic exposure to ambient air particulate matter (PM) has been implicated in playing a part in adverse health outcomes. Formation of reactive oxygen species (ROS) is one pathway by which PM exposure may disrupt normal physiological balance leading to disease pathogenesis. This study used human mononcytic (THP-1) cells to assess potential activation and subsequent production of reactive oxygen species. THP-1 cells were seeded in 96-well plates and then exposed for 24 hrs to particulate matter collected from a site in Los Angeles. The particles were smaller than 100 nm in aerodynamic diameter and were collected in an urban site of downtown Los Angeles. Two different doses 2 or 20 μg/m3 were used. Levels of ROS were measured by a fluorescent method. After exposure, there was a dose-dependent increase in the amount of ROS formation. Mitochondrial activity, measured by the MTS assay, was increased at both doses. This increase was not due to changes in cell number. Instead at the highest dose, we noted a decrease in cell number which may reflect decreased cell proliferation or viability. The data shows that THP-1 cells may be activated by PM leading to ROS formation. Further co-culture studies are needed to determine how activation of monocytes may affect normal human cells. This is the current focus of our laboratory.
Uptake and biodistribution of nanomaterials in an in vivo model system are important considerations for nanotoxicology. The use of fluorescent labeling can help with identifying particle location within a specimen and enable particle tracking. This study aimed to quantify the uptake of rhodamine labeled carbonylated nanocrystalline cellulose (NCC) throughout development in the model vertebrate, Danio rerio. NCC was selected for initial investigation because it elicits low toxicity in zebrafish embryos and is increasingly important in commerce. Similar to unlabeled NCC, no significant mortality or developmental abnormalities were observed at the highest dose tested. Fluorescent microscopy images of control embryos and embryos exposed to 500ppm rhodamine labeled NCC were taken on days 1-5. Integrated density (ID) was used as a surrogate measure for uptake. The experiment was repeated using embryos with and without intact chorions. On day 1, embryos exposed without a chorion had an ID value 3 times higher than those with the chorion intact. On day 2, the chorion off embryos had an ID value 1.43 times that of the chorion on embryos. After hatching, the ID values were not significantly different. There was a linear increase in uptake over the first 4 days, and then an exponential increase on day 5, possibly due to the onset of mouth gaping. From this data, dermal and oral uptake rates were calculated. Confocal microscopy was used to identify heavily concentrated locations within the embryos that seemed to be associated with the integumentary system. Our data demonstrates that carbonylated NCC was taken up both dermally and orally by the embryos depending on the stage of development. In addition, the chorion does pose a barrier for these particles prior to hatching which is important when assessing toxicity within the first 72 hours of development.

Gold nanoparticles (AuNPs) have unique properties for been considered as transfection vectors for gene delivery. Using gold nanoparticles for gene therapy brings a new opportunity for enhance the level of transfection and protect the genetic sequence to be delivered. However, the AuNPs application for biomedical topics could be limited for cytotoxicity. In order to decrease the cytotoxicity and enhance the gene delivery efficiency we synthesized AuNPs using polyethylenimine (PEI) as reducing agent with 25 kDa polyacrylamid branched polymer, which is actually used in DNA transfection protocols. The nucleotide si-miR-148a sequence were grafted to the AuNPs and labeled with carboxyfluoresceine (CFSE). Optical feature, shape and size distribution of AuNPs were examined using UV-Vis spectroscopy, transmission electron microscopy (TEM) and dynamic light scattering (DLS). The results showed the absorbance plasmon centered at 430 ± 5 nm usually attributed to AuNPs spheres, the size of particle-size was at 5 nm with narrow distribution. Zeta potential measurements indicate that PEI produced more stable NPs (-35 mV) compared with those synthesized by using other reducing agents (citric acid (-10 mV)). We evaluated the cell viability (HeLa cells) through the resazurin assay and follow the nanoparticles distribution into cells using fluorescence microscopy and flow cytometry. We found that the HeLa cells viability was up to 95%, and the interaction between AuNPs vector and HeLa cells was evident through positive fluorescence of CFSE by microscopy and flow cytometry as cells complexity augmented due to AuNPs intake. These results suggest that AuNPs transfection system brings stability and is biocompatible for be used in mammalian cells.
silver nitrate (AgNO3) as a positive control in the same soils. Soil exposures lasted for 14 days, after which surviving earthworms were counted and the median lethal concentration for 50% of the population (LC50) was calculated according to the trimmed Spearman–Karber method. PVP–AgNP LC50 in the Memphis Silt soil was 2828.43 mg/kg; in contrast, AgNO3 was much more toxic than PVP–AgNP in the same soil (LC50 = 223.31 mg/kg). The PVP coating did not contribute to PVP–AgNP soil toxicity (PVP LC50 > 40,000 mg/kg). PVP–AgNP LC50 in Suney, Camp Shelby, and Big Black soil were all >1000 mg/kg. These data demonstrate that PVP–AgNP is less toxic in a soil environment than the ionic form (AgNO3).

Furthermore, by utilizing four soils that cover over 50% of the soil in the contiguous U.S., these data provide quantitative soil toxicity values to better evaluate the effects of PVP–AgNP in soil environments.

841b Transmission Electron Microscopic (TEM) Evaluation of Silver Nanoparticles (AgNP) or Silver Acetate (AgOAc) Deposition in Selected Tissues of Sprague-Dawley (SD) Rats

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This study investigated the deposition of silver (Ag) in selected tissues (ileum, jejunum, colon, kidney, liver, spleen and mesenteric lymph nodes) of SD rats administered either AgNP or AgOAc. A subtle surface difference between AgNP and AgOAc deposition were observed in the colons and kidneys of rats. Rats treated with AgNP showed fine, spherical, and measurable Ag granules, whereas AgOAc showed irregular, flower-shaped surface depositions. The majority of Ag granules were located within the lamina propria and basement membrane of the intestinal epithelial cells. The localization and distribution of Ag varied in the other tissues, including the kidneys, livers, and mesenteric lymph nodes. Ag granules were entrapped by the macrophages in the mesenteric lymph nodes, whereas in the kidneys, the Ag deposition was observed in the basal lamina of the glomeruli involving podocytes and endothelia cells. In particular, the 10 nm AgNP had greater deposition in mesenteric lymph nodes, kidneys, and colons of both male and female rats suggesting size-dependent bio-distribution. Furthermore, the energy-dispersive X-ray spectral analysis revealed that Ag deposition is in the kidneys, colons, and mesenteric lymph nodes consisted of silver and often coexisted with selenium, silicon and sulphur containing granules. A gender-related difference in Ag deposition was noted in the kidneys and colons, with greater Ag deposition in females compared to males. This study provides new insights into the bio-distribution patterns of AgNP or AgOAc in the major tissues and proposes a need for further research. This study is supported by interagency agreements 224-12-0003 and AES12013 between the NCTR/FDA and NIEHS/NTP.

841c Toxicity and Allergy Responses in the Lung following Pulmonary Exposure to Nanoparticle Silver in Mice


Silver nanoparticles (AgNP), due to antimicrobial properties, are widely used in medical applications and consumer products. Expansive use of AgNP in manufacturing raises the concern of effects following respiratory exposure in workers. Previous work in the laboratory has shown dose-dependent lung toxicity with inflammation and alterations in lung immune parameters in rodents. The goal of the current study is to characterize effects of AgNP for potential exacerbation/attenuation of respiratory allergy in an ovalbumin (OVA)-induced allergy model in BALB/c mice. For range-finding (RF) studies, mice were exposed to physiological dispersion medium (DM), 6.1μg (LO), 18.2μg (ME), or 73.4μg (HI) AgNP. AgNP were 20 nm diameter with 0.3% wt polyvinylpovidone coating (NanoAmor, Inc.), were suspended and sonicated before exposures by pharyngeal aspiration (PA) on day 0. For RF studies assays were conducted on days 1, 10, and 29 post exposure—time points chosen to correspond with the allergy paradigm time course. Airway hyperreactivity was measured as PenH, bronchoalveolar lavage (BAL) was performed on the whole lung, cells and fluid were retained for analysis of lung-associated injury and inflammation and phenotyping by flow cytometry, lymph nodes (LN) were harvested for enumeration and phenotyping. Changes in PenH did not occur with AgNP alone at any time point. Results indicated a dose-dependent injury and inflammation by day 10 which began to resolve by day 29. For the allergy model, DM and OVA served as controls and ME and HI were chosen for study. Animals received i.p. injections of OVA + aluminum hydroxide gel (alum) during the sensitization phase on days 1 and 10. To elicit an OVA-specific response, 2 PA challenges of OVA were given on days 19 and 29. Dose dependent increases in PenH, BAL and LN cell number were observed in mice exposed to AgNP over OVA controls. Results indicate potential for AgNP to exacerbate allergic response in the lung.

841d Differential Genomic Effects on Signaling Pathways by Two Different CeO2 Nanoparticles in HepG2 Cells


Nanoparticles composed of CeO2 are of particular interest in toxicological studies due to their growing use in pharmaceuticals, biomedical products, cosmetics, polishing materials and as automotive fuel additives. Human liver HepG2 cells were exposed for these three days to two different forms of nanoparticles both composed of CeO2 (0.3, 3 and 30 μg/mL) and a genomic study performed. The two CeO2 nanoparticles have dry primary particle sizes of 8 nanometers ([M] made from NanoAmor) and 58 nanometers ([L] made by Alfa Aesar) and differ in various other physical-chemical properties as well. This systems biological genomics study showed that the major altered pathways were protein synthesis, stress response, proliferation/cell cycle, cytoketone remodeling/actin polymerization and cellular metabolism. Some of the canonical pathways affected were mTOR signaling, EIF2 signaling, fatty acid activation, G2/M DNA damage checkpoint regulation, glycolysis and ubiquitination. Nanoparticle M showed a normal dose-response pattern with 363, 633 and 1273 differentially expressed genes (DEGs) at 0.3, 3 and 30 μg/mL, respectively. M is more active than L in respect to altering the pathways of mitochondrial dysfunction, acute phase response, apoptosis, 14-3-3 mediated signaling, remodeling of epithelial adherents junction signaling, actin nucleation by ARF-WASP complex, and altered TCA cycle. However, L is more active than M in respect to the pathways of NRF2-mediated stress response and hepatic fibrosis/hepatic stellate cell activation. In summary, these two CeO2 nanoparticles exerted both many shared and some differing toxicity pathways. Disclaimer: This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

841e Aggregation of Gold Nanoparticles with Thioether-Containing Amino Acids

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Gold nanoparticles (Au NPs) have vast applications in drug therapy because of their unique optical, electronic and molecular properties. The toxicity of the Au NPs varies in cellular environment, depending on the physiochemical properties such as size, shape, surface chemistry, and the molecules with which they interact. When blue mussels, Mytilus edulis, are exposed to Au NPs of particle size < 5 nm, they exhibit an increase in oxidative stress and decrease in thiol-containing proteins. These organisms also contain methionyl peptides that are hypothetically subject to oxidation due to Au NP exposure. In this present study, we present preliminary findings regarding the interaction of Au NPs with methionyl compounds, L-Methionine(Met), D/L-Met, N-Acetyl-L-Met, L-Met Ethyl Ester, and Met-Glycine. The average sizes of the eight Au NPs used in the study are ~5, 10, 15, ~20, ~30, ~35, and ~40 nm diameters. The UV-Vis spectral studies showed that Au NPs exhibit a strong plasmon at ~530 nm while the methionyl compounds displayed an additional plasmon band at ~785 nm, indicating the formation of Au NPs aggregates. Methionine was found to aggregate Au NPs (conc. 3.487E+10 M) with size ~35 nm at higher concentration (final 0.125 M) with the color change from red to blue. While lower concentration of L-Met and D/L-Met did not aggregate immediately, N-terminal (N-Acetyl-L-Met) and C-terminal protected Met (Met Ethyl Ester) readily formed Au NPs aggregates. Methionine-Glycine dipeptide was only slightly better than D/L-Met at inducing aggregation of Au NPs. The results provide insight into the impact of NP size, peptide sequence, and concentration of NPs on aggregation. We strongly believe aggregation size and kinetics may have a role in the etiology of cellular response which needs to be systematically evaluated. [This research is supported by grants from NSF-1230357 (formerly 0847742), NSF HRD–1238838, NSF HRD–1137747, and US EPA, Durham, NC.]
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Young children are exposed to substantial amounts of low dose radiation (LDR) during diagnostic imaging. The long term carcinogenic effects of LDR are not known, especially when prior exposure to environmental chemicals could sensitize to LDR. We previously reported in adult mouse models, in which lung cancer was induced by either transgene expression or chemical carcinogen treatment, that whole-body CT doses approximating clinical screening doses significantly increased tumor multiplicity by 40 to 100%. Pregnant C57BL-6BALB/c mice were treated in utero with a vehicle or a single 45 mg/kg dose of 3-methylcholanthrene (MC). Half of the male and female offspring in each group were exposed to a single dose of 50 mGy of LDR from a clinical CT scanner 4½ weeks after birth. RNA was isolated 10 min post-exposure. Affymetrix gene chip expression profiling followed by gene ontology analysis identified 32 differentially expressed genes that were altered in utero exposure to MC but were not altered in response to neonatal LDR. Using DAVID, we identified clusters of genes under the categories response to stimulus and immune system process that were significantly altered following neonatal exposure to LDR, with network centers in the p38 MAPK pathway. When male and female responses to LDR were determined, significant gender related differences were identified. For females, the top network was related to cardiovascular disease, cardiovascular system development and function, and cell morphology. In males, differentially expressed genes were enriched in two networks. The first was related to connective tissue development and function, tissue morphology, and lymphoid tissue structure & development, and the second to functions in cancer, involving cell-cell signaling & interaction and cellular growth & proliferation. Our data demonstrate gender related differences in LDR-induced differential gene expression following neonatal exposure in gene pathways related to disease pathogenesis that may be responsible for the long term toxic responses to radiation. (NIH grant CA136910)

842 Toxicogenomic Analysis of Low-Dose CT Exposure in Neonatal Mouse Lungs

843 Lead Pollution in Soil, Domestic Animals, and Children from Townships near a Lead-Zinc Mine in Kabwe, Zambia


Bacterial lead resistance (BLLs) in children exposed to Pb pollution. The concentrations of Pb in tissues of Kabwe cattle were higher than those in other Zambian rural districts (9-51188 mg/kg) in Kabwe soil (n=101) were much higher than benchmark values. Moreover, upregulation of HBG2 and AHSP genes may imply the role of hypoxia in asthma development. Taken together, our results demonstrated the existence of distinct phenotype of asthma in children living in polluted Ostrava region compared to children living in rural district of Prachatice.

844 Gene Expression Profiles in Asmatic Children Living in Localities with Different Extent of the Air Pollution


This study is a part of the broader molecular epidemiology study dealing with health effects of the air pollution in children living in the heavily polluted industrial area of the Czech Republic, Ostrava region, where the incidence of asthma bronchiolitis in children is extremely high (30-40%). We hypothesized that high level of the air pollution by polycyclic aromatic hydrocarbons (mostly bound on PM10) is associated with increased asthma morbidity in children from Ostrava region. We compared gene expression profiles in leukocytes of asthmatic children (aged 6 -10 years) with profiles of healthy children without asthma using Illumina HumanHT-12 BeadChip in a group of 200 children living in Ostrava-Radvanice and Ostrava-Bartovice (100 asthmatic and 100 healthy children) and a control group of 200 children living in rural district of Prachatice (100 asthmatic and 100 healthy children). Our results indicated completely different gene expression profiles among localities. We identified significantly deregulated genes specific for Ostrava or Prachatice, however, no gene was found to be commonly deregulated in both localities. Gene expression data of astmatic children from Prachatice showed increased expression of SIGLEC8, CLC, CCL23 and CACNG6, genes related to the presence of bronchial eosinophilic inflammation suggesting allergic phenotype of asthma. Skin tests also confirmed the diagnosis of allergy in most cases. In contrast, children living in industrial Ostrava region exhibited upregulation of genes associated with neutrophilic inflammation and non-atopic asthma phenotype (which could be triggered by air pollution). Moreover, upregulation of HBG2 and AHSP genes may imply the role of hypoxia in asthma development. Methods were developed to evaluate immunotoxicity effects of chemicals in preweaning piglets using immunophenotyping (IP), circulating cytokine evaluation & immunohistochemistry (IHC) of the gastrointestinal tract (GIT). Yorkshire crossbred piglets were offered ProNurse® formula 6x/day at 500 mL/kg/day for 4 weeks. Groups were Formula Control; Phosphate Buffered Saline (PBS) Control (im injection); Lipopolysaccharide (LPS) Positive Control (im injection); PBS Control by percutaneous endoscopic gastrostomy (PEG) tube & Dextran Sodium Sulfate (DSS) (as positive control). Piglet tissue & blood were used for evaluations.

In Africa, major sources of childhood Pb poisoning include Pb mining and smelting. In Kabwe, the capital of Zambia’s Central Province, extensive Pb contamination of township soils in the vicinity of a Pb-Zn mine has been reported and poses a serious health risk to children in these townships. Therefore, this study investigated Pb levels in soil, edible tissues of cattle and chickens as well as blood lead levels (BLLs) in children exposed to Pb pollution. The concentrations of Pb (9-51188 mg/kg) in Kabwe soil (n=101) were much higher than benchmark values. Pb levels in tissues of Kabwe cattle were higher than those in other Zambian towns. Moreover, mean concentrations of Pb exceeded maximum levels for human consumption in some organs including muscle in free-range chickens, in contrast to low levels in broiler chickens. Given these results of Pb pollution in soil and domestic animals, we investigated BLLs in children (n = 246), under the age of 7 years, in townships around the Pb-Zn mine in Kabwe and to identify children in Kabwe with BLLs that require medical intervention. Almost all of the sampled children had BLLs exceeding 10 µg/dL. Children in these areas could be at serious risk of Pb toxicity as 18% of the sampled children in Chowa, 57% (Kasanda) and 25% (Makulukulu) had BLLs exceeding 65 µg/dL; the threshold widely considered to result in Pb toxicity. A total of 8 children had BLLs exceeding 150 µg/dL. These levels were markedly high, especially that concentrations of up to 427 µg/dL were recorded. Therefore, it is recommended that medical intervention be commenced in the children with BLLs exceeding 45 µg/dL and other interventions to reduce Pb exposure in the affected townships.

845 The Development and Validation of Methods for Evaluating the Immune System in Preweaning Pigs

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Methods were developed to evaluate immunotoxicity effects of chemicals in preweaning piglets using immunophenotyping (IP), circulating cytokine evaluation & immunohistochemistry (IHC) of the gastrointestinal tract (GIT). Yorkshire crossbred piglets were offered ProNurse® formula 6x/day at 500 mL/kg/day for 4 weeks. Groups were Formula Control; Phosphate Buffered Saline (PBS) Control (im injection); Lipopolysaccharide (LPS) Positive Control (im injection); PBS Control by percutaneous endoscopic gastrostomy (PEG) tube & Dextran Sodium Sulfate (DSS) (as positive control). Piglet tissue & blood were used for evaluations. The IP method used whole blood from control animals on Days 13, 14, 28, and 29 to evaluate peripheral blood leukocytes by flow cytometry. The cytokine method used blood from PBS control & LPS groups collected to evaluate IL-1β, IL-6, IL-8, and TNF-α. ELISA kits were used to detect cytokines. The ICH method used tissues obtained from the stomach, duodenum, jejunum, ileum, proximal & distal colon & cecum on for IL-6, IL-8 and TNF-α staining. These tissues were collected from PBS controls & DSS animals. H&E staining was used to confirm ICH changes. Results of the IP evaluation confirmed that it was a precise assay for evaluating lymphocytes, mature T-cells, helper T-cells, cytotoxic T-cells, B-cells & monocytes in preweaning piglet blood. Cytokine evaluations confirmed an increase in cytokine levels in LPS-treated animals. Immunohistochemistry evaluation confirmed increased IL-8 & TNF-α staining in the proximal &/or distal colon in DSS animals. H&E staining was associated with colonic inflammation/edema in tissues of DSS animals. In conclusion, these three methods were confirmed to be robust assays to evaluate the immune system of preweaning piglets. Funded by EMC Corp. & the International Formula Council.

846 Increased Prevalence of Asthma in Children Exposed to Pyrethroid Pesticides

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The household environment is recognized as a potentially important factor in the early childhood development and exacerbation of asthma and allergies. In recent years pyrethroid pesticides have become widely used for control of household pests, based upon the widespread acceptance of their safety for use in and around children. A study was conducted in households in New Jersey to examine the nature of the relationship between household and its common contaminants and the prevalence of asthma and allergies in young children (ages 6 months to 5 years). A total of 75 children and households were enrolled in the study. Health endpoints were assessed by a technician-administered questionnaire. The parents of the study subject were asked a questionnaire based upon that employed in the International Study of Asthma and Allergies in Children (ISAAC). Environmental sampling was carried out with vacuum sampling for dust, and aerosol (inhaalable) sampling for particulate matter. House dust was analyzed for the presence of a panel of 10 pyrethroid pesticides.
throid pesticides by GC/MS. A multivariate logistic regression analysis was carried out to total pyrethroids in house dust. All non-detects (N=9) were included at half the limit of quantitation and log transformations of the data carried out. Additional variables in the final model included age (years), gender, and family history of asthma (yes/no first degree relative). The model for prediction reporting of the child having had a physician diagnosis of asthma was statistically significant at the 0.003 level with log total pyrethroids OR = 1.25 (95% 1.03-1.51). When pyrethroid exposure was examined as quartiles the results for the top three quartiles with the bottom quartile as reference was OR = 1.05, 2.55, and 5.77 respectively, with the top quartile statistically significant (P < 0.027). The presence of a dose response increase in reporting of asthma in young children is suggestive that pyrethroid pesticides may play a role in early childhood asthma.

**847** Femoral Growth Plate Dysplasia in Juvenile Rabbits Dosed with a Spleen-Like Kinase (Syk) Inhibitor

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Tyrosine Kinase inhibitors with inhibitory activity at the VEGF receptor have been associated with effects on the epiphyseal growth plates in rodents. This has potential impact for clinical trials and therapy in paediatric patients. This study was designed to investigate the potential for off target effects of a syk inhibitor in juvenile rabbits. Three groups of juvenile New Zealand White rabbits, each consisting of 10 males and 10 females, were given the test syk inhibitor orally, twice daily, for 1 month. The control group consisted of 11 males and 9 females and was similarly treated with vehicle. The dose levels were 5, 15 or 30 mg/kg/bid. The animals were 9 weeks old at the start of dosing. After dosing with 15 or 30 mg/kg bid, histopathological changes were evident in the proximal femur (growth plate dysplasia; reduced bone marrow cellularity), femoro-tibial joint (growth plate dysplasia; reduced bone marrow cellularity) and sternum (reduced bone marrow cellularity). The treatment-related dysplasia noted in the growth plate of the proximal femur was characterised by the thickening, fusating and in some animals, fracturing of the growth plate due to the accumulation of hypertrophic chondrocytes. In conclusion, the syk inhibitor produced growth plate dysplasia in the proximal femur and femoro-tibial joint and reduced bone marrow cellularity in the femur and sternum at 30 and 60 mg/kg/day. These findings were consistent with those seen in toxicity studies in rodents. The chondrodysplasia/growth plate dysplasia in rat and rabbit at 30 and 60 mg/kg/day. These findings were consistent with those seen in toxicity studies in rodents. The presence of a dose response increase in reporting of asthma in young children is suggestive that pyrethroid pesticides may play a role in early childhood asthma.

**848** Persistent Mitochondrial Damage in Offspring of Patas Monkeys Exposed In Utero to Antiretroviral Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

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Combination antiretroviral therapy containing NRTIs is highly successful in controlling HIV-1 infection, and reducing mother-to-child HIV-1 transmission. However, NRTI exposure may cause mitochondrial dysfunction in infants born to mothers receiving NRTIs during pregnancy. To model NRTI use in human pregnancy, patas dams received human-equivalent protocols of antiretroviral drug combinations, and the offspring were examined for mitochondrial integrity. Dams were given combinations of 3 NRTIs, zidovudine (AZT)/lamivudine (3TC)/abacavir (ABC), or 2 NRTIs, AZT/3TC, plus a non-NRTI, Nevirapine (NVP), for the last 10 wk (50%) of gestation. Heart and brain tissues, collected from offspring at birth, 1 and 3 yrs of age, were examined by electron microscopy (EM) to evaluate mitochondrial morphology. The EM photomicrographs of heart and brain from drug-exposed offspring revealed damaged mitochondrial membranes with mitochondrial swelling, loss of cristae, and increased lucenty. Scoring of coded EM photomicrographs, by 3 independent investigators, revealed significantly higher damage in 1 yr old patas exposed to both drug combinations in utero, compared to 1 yr old unexposed patas (P<0.05). In addition, the 3 yr old drug-exposed patas had levels of heart and brain mitochondrial damage similar in magnitude to the 1 yr old drug-exposed animals, and significantly higher than the unexposed 1 yr old patas. Additional evaluations, including mtDNA levels, and mitochondrial oxidative phosphorylation assays are in progress. Because the drug-induced damage was persistent in 3 yr old patas, an age equivalent to 14 years in human life, this study provides powerful evidence for the persistence of NRTI-induced mitochondrial damage, and helps to explain the mitochondrial dysfunction observed in NRTI-exposed children.

**848a** Isotopic Analyses of Lead in Blood of Children to Identify Sources and Routes of Pollution in a Mexican Mining Site

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Lead (Pb) is a highly toxic element to the human body and even at low concentrations (<10 µg/L) it will have severe physiological or neurological effects particularly for children; it is well known that can be provided by different sources such as paint, ceramic, mining residues, etc.

In semiarid Mexican mining and metallurgical centers such Cedral San Luis Potosi, Mexico, the residues of these activities containing high concentrations of heavy metals (Pb, As, Cd, Hg) are generally deposited in uncovered piles, which constitute a source of contamination due to the wind dispersion. Human population can be exposed to these particles by different routes through inhalation, ingestion, and this source of pollution could be the cause of adverse effects associated to Pb. Some health affections in this community have been reported, but it has not been identified the source or sources of Pb to which the children are exposed.

The aim of this study is to trace the origin of Pb pollution and identify the principal source of environmental and human Pb contamination, using isotopic methods.

Pb concentrations and isotopic compositions in blood samples of children (4–8 years old) were determined by MC-ICPMS and compared to those of associated environmental samples (soil, residues, outdoor and indoor dust). Pb concentrations of soil, residues and dust ranged between 73-2460, 1736-6700, 144-3550 µg/kg, respectively. Blood Pb concentrations levels (11.0 ± 5.3 µg/ml) in children ranged between 2.7-36 µg/ml, which is 3-14 times higher than the current average (1.9 µg/ml) of children in the US. We find that blood Pb isotopic ratios (206Pb/204Pb 18.787±0.0003) strongly correlates with those values determined in atmospheric particles (206Pb/204Pb 18.775±0.0003) and the latter correlates with the isotopic value of samples coming from residues (206Pb/204Pb 18.774±0.0003). The results indicate that the main source of Pb in this site is mining residues, which are dispersed through wind.

**848b** Impulsivity and Attention in Fluoxetine-Treated Juvenile Monkeys

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The psychopharmacological agent fluoxetine is approved by FDA for use in children and is widely prescribed for a variety of developmental disorders. Possible adverse consequences of chronic fluoxetine treatment during brain development are being investigated in a rhesus monkey model. PK/PD studies were first conducted to select a dose with serum concentrations of active agent comparable to therapeutic use in children. Fluoxetine (2.2 mg/kg/d) or vehicle control was then administered orally to male rhesus monkeys (N=15,16 per group) daily between 1 and 2 years of age (equivalent to 4-8 years of age in humans). A reward delay (impulsivity) test and an automated Continuous Performance Test (CPT) were administered. Fluoxetine-treated monkeys demonstrated shorter average response latencies during the reward delay test and more immediate responses to presentation of the test. During CPT sessions conducted in the home cage room, fluoxetine-treated monkeys showed more omission errors taking into account cage location, which also influenced this measure. The study suggests that response inhibition and attention, two executive functions important to daily life in children, can be influenced by fluoxetine at therapeutic dose levels. Supported by HD065826.

**848c** Perinatal BispHENol A Exposure Changes Expression of Airway Secretory Products in the Developing Mouse Lung


BispHENol A (BPA) is a component of plastics. Recent work has shown that BPA exposure alters mucous cell development in fetal rhesus monkeys. While perinatal BPA exposure has been shown in a mouse model to increase airways hyperresponsiveness and alter immunity, the ability of BPA to change normal growth and development of secretory cells, including mucous cells, in the airways of mice, has not been studied. To address this, we exposed timed pregnant NIH Swiss mice to 10ug/ml BPA in drinking water beginning on gestational day 14 (GD14) and continuing for the length of the study. We examined the proximal (extrapulmonary trachea/bronchi) and distal (intrapulmonary bronchioles) conducting airways of both male and female offspring using histopathology and gene expression approaches at 1d, 3 weeks and 8 weeks of age to span birth, weaning and young adulthood. Mucous cell abundance was defined using AB/PAS histologic staining approaches at 1d, 3 weeks and 8 weeks of age to span birth, weaning and young adulthood.
and muc5B/muc2 gene expression. Club (Clara) cell abundance was defined using immunohistochemistry and gene expression of secretoglobin 1A1 (sgb1A1). We found that continual BPA exposure beginning in the third trimester and extending to 3 weeks postnatal resulted in site-specific changes in Muc5B expression. Proximal extrapulmonary airways had a 2-fold decrease in Muc5B gene expression in males and females, which achieved statistical significance in males. In contrast, the more distal, intrapulmonary airways of both males and females had a greater than 2-fold increase in Muc5B expression. This was mimicked by Muc2 gene expression but only in male mice and an increase in sgb1A1 gene expression only in female mice. When the abundance of sgb1A1 protein was assessed, the amount per cell was diminished in the distal airways of BPA-treated mice suggesting a mismatch between mRNA expression and protein expression which might be explained by accelerated secretion. We conclude that BPA exposure during lung development changes secretory product expression, even in species with limited mucous cells such as mice. Supported by NIEHS R21 ES021600

849 BMS-986094: Nonclinical Investigative Studies on a Nucleoside Analog Intended for Treatment of Hepatitis C Virus Infection


BMS-986094 (formerly INX-08189), a 2’-C-methylguanosine prodrug with pivalate and 1-naphthol moieties, was withdrawn from clinical trials because of a serious safety issue. Events included decreased left ventricular ejection fraction, electrocardiogram changes, and acute kidney injury. In prior animal studies, tolerated doses associated with minimal and reversible skeletal muscle degeneration were established. Since skeletal muscle degeneration correlated with increases in standard serum analytes (e.g., transaminases), it was expected that clinical dosing could proceed safely with careful monitoring prior to onset of more severe events.

Because the onset and severity of clinical adverse events (heart and kidney) were unexpected, Bristol-Myers Squibb Company conducted a robust series of in vitro and in vivo investigative studies including innovative, and in some cases newly developed, mechanistic and diagnostic methodologies, to further characterize BMS-986094 toxicity. These studies focused on several potential mechanisms including: 1) reactive metabolites and cytotoxicity of BMS-986094 and its prodrug moieties, 2) mitochondrial toxicity, 3) pivalate involvement in tissue energy status, 4) plasma and tissue metabolomic changes, 5) tissue transcriptional changes, and 6) levels of BMS-986094 and its metabolites in plasma and tissues.

Although these studies extended the understanding of the nonclinical toxicities of BMS-986094, they did not identify a specific underlying mechanism. Observations included possible novel kidney injury biomarkers; an apparent correlation of tissue nucleoside triphosphate levels to toxicity; that predominate BMS 986094 metabolites were not associated with reactive metabolites or oxidative stress; and preliminary evidence that, although some potential secondary energy utilization changes were observed, BMS 986094 does not appear to be a direct mitochondrial toxicant.

To characterize any CV changes, BMS-986094 was given to 5 telemetered monkeys/group at 0 or 15 mg/kg/day (45 days) or 30 mg/kg/day (30 days) with recovery for 29 days. Key criteria included toxicokinetics, serum cardiac troponin (cTnI) and N-terminal brain natriuretic peptide (BNP), echocardiography, arterial and left ventricular (LV) pressure, LV dP/dt (terminal), and electrocardiography (ECC). The predominant plasma analyte (74-89% total AUC) was INX 08032 (initial nucleoside, 534 and 3400 ng·h/mL at 15 and 30 mg/kg/day, respectively). There were no LV ejection fraction [EF], % fractional shortening [%FS], or stroke volume [SV] changes at 15 mg/kg/day. Beginning Day 32, ventricular dilatation occurred at 15 (2.1x pretest) and 30 mg/kg/day (4.0x). Day 32 changes (vs. pretest) at 30 mg/kg/day were decreased mean LV EF (0.43x), % FS (0.35x), and SV (0.62x), with recovery by Day 39. Absolute LV EF ranged from 12 to 43%. Dose-related LV dP/dt decreases at 15 (0.78x) and 30 mg/kg/day (0.45x) occurred with nadirs on Days 33-47 at 15 and Days 26-53 at 30 mg/kg/day. Potential cardiac biomarkers (cTnI, BNP) did not correlate in individual monkeys. ECC changes were prolonged QT interval at both doses, and flat and inverted T waves and ST-segment depression at 30 mg/kg/day. Two 30 mg/kg/day monkeys were euthanized on Day 32; with their hemodynamic and anatomic pathology changes suggesting heart involvement. CV changes in survivors exhibited partial to complete recovery.

In summary, BMS-986094 was not tolerated at 30 mg/kg/day, and partial-to-complete reversible, dose- and time-related ventricular dilatation, decreased ventricular function, and ECC interval and morphologic changes were observed at both doses.

850 BMS-986094: Investigation of Potential Biomarkers and Mechanisms of Toxicity in Cynomolgus Monkeys

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BMS-986094 (formerly INX-08189), a 2’-C-methylguanosine prodrug, was withdrawn from clinical trials because of a serious safety issue. Events included decreased left ventricular ejection fraction, electrocardiogram changes, and acute kidney injury. BMS-986094 was dosed orally at 0, 15, or 30 mg/kg/day to cynomolgus monkeys for 3 weeks + a 3-week treatment-free period. BMS-986094, INX-09054 (prodrug less naphthol), INX-08032 (initial nucleoside), INX-08144 (O-methyl nucleoside), and INX-09114 (triphosphorylated nucleoside) were measured by LC/MS in plasma and peripheral blood mononuclear cells (PBMCs) in Weeks 1, 3, and 6; and in tissues in Weeks 3 and 6. INX-08032 was the major circulating analyte (66-68% of total AUC) followed by INX-08144 (<16%). BMS-986094, INX-09054, and INX-09114 levels (<22%) in plasma were low and INX-09114 levels in PBMCs could not be quantified. In Week 3, INX-09114 (± 4280 ng/mL) was high in heart and kidney, and males had higher (3.3x) heart levels than females at 30 mg/kg/day. Other analytes increased dose dependently in target and non-target tissues with BMS-986094 highest in heart and kidney. BMS-986094 and selected BMS-986094 metabolites were measured in target (heart, kidney, diaphragm, and liver) and non-target (lung) tissues under optimal stabilization to determine any associations between analyze levels and toxicity. Consistent with companion studies, BMS-986094 was dosed orally at 0, 15, or 30 mg/kg/day to cynomolgus monkeys for 3 weeks + a 3-week treatment-free period. BMS-986094, INX-09054 (prodrug less naphthol), INX-08032 (initial nucleoside), INX-08144 (O-methyl nucleoside), and INX-09114 (triphosphorylated nucleoside) were measured by LC/MS in plasma and peripheral blood mononuclear cells (PBMCs) in Weeks 1, 3, and 6; and in tissues in Weeks 3 and 6. INX-08032 was the major circulating analyte (66-68% of total AUC) followed by INX-08144 (<16%). BMS-986094, INX-09054, and INX-09114 levels (<22%) in plasma were low and INX-09114 levels in PBMCs could not be quantified. In Week 3, INX-09114 (± 4280 ng/mL) was high in heart and kidney, and males had higher (3.3x) heart levels than females at 30 mg/kg/day. Other analytes increased dose dependently in target and non-target tissues with BMS-986094 highest in diaphragm (± 4400 ng/mL) followed by INX-08032 in liver and kidney (± 1360 ng/mL) and low INX-08144 and INX-09054 levels (± 124 ng/mL) in other tissues. After the treatment-free period (Week 6), heart and kidney INX-09114 remained high (± 1870 ng/mL). Tissues and plasma had low INX-08032 levels (± 37 ng/mL), and other analytes were not detected. Persistent INX-09114 in heart and...
kidney appeared to correlate with toxicities in these tissues in companion studies; however, higher tissue INX-09114 in males did not translate to increased toxicity in this sex. There were no other correlations between analytes and target organ toxicity.

853 BMS-986094: Metabolomic Assessment of Cardiotoxicity in Monkeys and Mice


BMS-986094 (formerly INX-08189), a 2'-C-methylguanosine prodrug, was withdrawn from clinical trials because of a serious safety issue. Events included decreased left ventricular ejection fraction, electrocardiogram changes, and acute kidney injury. Pivatate prodrugs, as is BMS-986094, can be metabolized to pivaloylcarnitine (PVC) which is not further metabolized, and is eliminated in urine potentially leading to systemic carnitine depletion and, after prolonged high doses, secondary cardiomyopathy. Some monkeys and mice given BMS-986094 develop cardiomyocyte degeneration and to a lesser extent skeletal muscle degeneration. During mechanistic evaluation of potential BMS 986094 cardiotoxicity, metabolomics in monkey plasma and tissue (0, 15, or 30 mg/kg/day, 3-4 weeks) and in mouse heart (0 or 150 mg/kg/day, 2 weeks) were conducted. In monkey plasma, a dose-proportional, novel MS peak, isobaric with valeryl and isovaleryl carnitine, was identified as PVC. PVC was not evident in control or recovery monkeys. In monkey heart, there were no decreases in free carnitine or acetylcarnitine in diaphragm despite having PVC levels similar to heart, suggesting tissue specificity. In mouse heart, there were no decreases in free carnitine or acetylcarinetein on Day 2, with only modest reduction (0.7x control) subsequently. Tissue acetylcarinetein changes, a reflection of acetyl CoA status, were disparate across species suggesting acetylcarinetein changes are likely secondary, and not indicative of a cardiac toxicity mechanism.

854 BMS-986094: Transcriptional Profiling in Monkeys and Mice


BMS-986094 (formerly INX-08189), a 2'-C-methylguanosine prodrug, was withdrawn from clinical trials because of a serious safety issue. Events included decreased left ventricular ejection fraction, electrocardiogram changes, and acute kidney injury. Transcriptional changes (TC) associated with potential BMS 986094 toxicities were evaluated in monkeys dosed (0, 15, or 30 mg/kg/d) for 1 month and mice dosed (0, 25, or 150 mg/kg/d) for 2 weeks. For gene expression analysis, tissues were collected after the last dose (monkey) or on Days 1 (6 and 24 h), 3, 8, and 15 (mouse, course-study). In both species, the primary target tissues were heart, kidney, and skeletal muscle, and in these tissues TC generally separated from control in PCA space. No consistent pattern of TC (up- or down-regulated) was apparent. In monkeys, heart TC was greatest (≥ 16% total genes) with much fewer kidney TC and still fewer in skeletal muscle. Heart TC suggested cytoskeleton/myofilaments/sarcomeres, GTPase (Rho, Rab, Rac, and Ras), Ca2+ and redox signaling, mitochon-dria, and purine synthesis involvement. Kidney TC suggested altered transport/endocytosis. Skeletal muscle TC was similar to heart, but at lower magnitude. In mice, kidney TC was greatest (≥ 13% total genes) with fewer skeletal muscle and heart TC. Early gene expression changes in kidney were associated with GTPase signaling (Rho and Rab), the cytoskeleton, Ca2+-related genes, and endocyto-sis-related genes. Early TC in heart was associated with cytoskeleton/myofilaments/sarcomeres and GTPase and Ca2+ signaling. Most mitochondrial gene expres-sion changes were in kidney (mostly tRNA and ribosomal genes). Gene expression changes in skeletal muscle were similar to those in heart, but lower in magnitude and delayed in onset. Overall, TC timing and magnitudes were generally associated with the level of toxicity in target tissues. The earliest TC appeared related to cytoskeleton/myofilament/sarcomere, and GTPase and Ca2+ signaling with other TC (e.g., mitochondrial changes) likely secondary.

855 BMS-986094: Investigations of In Vitro Metabolism, Reactive Metabolite Formation, and Oxidative Stress in Human Liver Microsomes, Hepatocytes, and Cardiomyocytes

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BMS-986094 (formerly INX-08189), a 2'-C-methylguanosine prodrug, was withdrawn from clinical trials because of a serious safety issue. Events included decreased left ventricular ejection fraction, electrocardiogram changes, and acute kidney injury. These experiments evaluated in vitro metabolism and reactive metabolite formation of BMS-986094 (non-labeled) or single BMS-986094 diastereomers (14C- or 3H-labeled BMS-986095 and BMS-986096) in human liver microsomes (HLM), hepatocytes (HH) and cardiomyocytes (HCM). Incubates were analyzed by LC-MS/radioactive detection or targeted LC-MS/MS assays. Protein covariant binding (PCB) in HLM, and intracellular glutathione (GSH), adenosine triphosphate (ATP), and reactive oxygen species (ROS) in HH or HCM were measured. BMS-986095 and BMS-986096 were extensively metabolized in HLM, but via different oxidative pathways. Substantial PCB was detected in HLM with both diastereomers, with higher levels of PCB being produced by BMS-986095 than BMS-986096. PCB derived from the 14C-labeled naphthyl moiety was higher than that from the 14C-labeled guanine moiety for both diastereomers. GSH conjugates derived from the naphthyl moiety were detected. In human hepatocytes and cardiomyocytes, hydrolysis was the predominant metabolism pathway for both diastereomers, which led to the formation of the pharmacologically active triphosphate metabolite. Oxidative metabolites were minor in these cells. BMS-986094 was not associated with reduced intracellular GSH in either HH or HCM, or with ROS induction in HH.

These data suggest that reactive metabolite formation by BMS-986094 in HLM was mainly associated with oxidative metabolism at the naphthyl moiety, but the predominant metabolism pathway (hydrolysis) of BMS-986094 in HH and HCM was not associated with the formation of reactive metabolites or oxidative stress.

856 BMS-986094: Potential Cytotoxicity in Differentiated Human Cardiomyocytes


BMS-986094 (formerly INX-08189), a 2'-C-methylguanosine (2CMG) prodrug with pivalate and 1-naphthol moieties, was withdrawn from clinical trials because of a serious safety issue. Events included decreased left ventricular ejection fraction, electrocardiogram changes, and acute kidney injury. 2CMG-triphosphate (2CMG-TP), pivalate and 1-naphthol are formed by cellular metabolism of BMS-986094. The objective of these studies was to assess potential cytotoxicity of these 3 metabolites in cultures of differentiated human cardiomyocytes (hCM). hCM, derived from induced pluripotent stem cells, were cultured up to 10 days. Cell viability and function endpoints included total ATP, caspase 3/7 activity, beat rate, and contractility by impedance (eXCELLiGenic). Glucose- and fatty acid-driven mitochondrial respirations were assessed using the Seahorse XF-96 analyzer and acylcarnitine formation was measured by HPLC. Based upon ATP depletion, BMS-986094 IC50 values on Days 3, 6, and 10 were >50, 17.3, 2.1 and 0.6 μM, respectively. Caspase 3/7 activity, a measure of apoptosis activation, was increased on Days 3, 6 and 10. Decreased hCM contractility (amplitude) was associated with reduced cell viability. Other changes associated with BMS-986094 (10 μM) included, (1) inhibition of mitochondrial respiration as early as Day 1 (IC50: 7.7 μM); (2) inhibition of palmate-fueled oxygen consumption after 4 days; and (3) reduced glucose-driven basal cell oxygen consumption on Days 6 and 10. In contrast, 1-naphthol (100 μM) and pivalate (10 μM), when added directly to cultures, were not associated with reduce cell viability when tested up to 10 days. BMS-986094 was associated with concentration- and time-related cytotoxicity and altered energy utilization in hCM cells. However, the relevance of these observations to in vivo findings remains unclear. Pivalate and 1-naphthol, principal BMS-986094 produg metabolites, were not cytotoxic.
BMS-986094 (formerly INX-08189), a 2′-C-methylguanosine prodrug, was withdrawn from clinical trials because of a serious safety issue. Events included decreased left ventricular ejection fraction, electrocardiogram changes, and acute kidney injury.

The objective was to assess any changes on mitochondrial DNA (mtDNA) replication, transcription, or mRNA translation in HepG2 cells and in differentiated human cardiomyocytes (hCM), derived from induced pluripotent stem cells, exposed to BMS-986094. HepG2 and hCM were exposed to BMS-986094 (0.22-2 μM) for ≥19 days. The mitochondrial genes ATP8 and COXIXI total cell number, protein, ATP, and lactate were assayed by qPCR. In separate experiments (0.1, 1, and 10 μM: 24 hours), transcripts of the mitochondrial genes ND1 and ND5 were assayed by Northern blot in Huh7 cells and the respiratory chain proteins ND1 and COXII were assayed in hCM by in-cell Western.

BMS-986094 at >1 μM was associated with concentration- and time-related cytotoxicity after 3 days. Non-cytotoxic concentrations (<1 μM), BMS 986094 was not associated with HepG2 or hCM mtDNA reductions after 19 or 10 days, respectively. BMS 986094 at high concentrations (9-10 μM) was associated with reduced ND1 and ND5 transcription in Huh7 cells and ND1 and COXI proteins in hCM within 24 h. Other observations at 10 μM were inhibition of mtRNA-poly-merase-driven mRNA transcription (POLRMT) and reduced expression (transla-
tion) of respiratory chain proteins. These latter observations are consistent with a recent report [Arnold et al, PLoS Pathog 8(11): 2012] that 2′-C-methylguanosine is a terminator of POLRMT-driven mRNA strand elongation. In summary, BMS-986094 was not associated with mtDNA replication inhibition, but was associated with POLRTM inhibition and reduced respiratory chain protein expression. However, the relevance of these in vitro observations to in vivo findings remains unclear.

Human and rabbit response to drug effects on corneal wound healing was evaluated in an anterior chamber model. New Zealand white rabbit eye globes were obtained and placed in Optisol. Human eyes were procured with donor consent through Midwest Eye-Banks (Ann Arbor, MI) and The Lions Eye Institute for Transplant and Research (Tampa, FL). Wounding, anterior keratectomy (AK), was induced by scoring the cornea (rabbit 2.5 mm, human 8 mm), and removing the epithelium using a gill knife. The anterior segment of the eye was dissected and cultured in complete medium on a rotating platform to maintain tissue differentiation for several days. The initial wound and progression of wound closure was visualized with fluorescein (0.015%), and the area recorded with a digital photo and quantified with ImageJ. Wound closure in untreated AK cornea occurred at different rates, rabbit (75% at 2 h) compared to human (24 h). Diclofenac (DCF) a nonsteroidal anti-inflammatory agent often used post-surgery and dosed topically (0.1%), impaired wound healing in both species. Rabbit AK cornea treated with DCF showed no change in immune/inflammation, cell adhesion/chemo-
patogenesis of vascular conditions including diabetes (T2DM). Exposure to traf-
fic-related air pollution (TRAP) has been implicated in the pathogenesis of vascular conditions including diabetes (T2DM). Exposure to traf-
ic-related air pollution (TRAP) has been found to negatively impact clinical vascu-
lar outcomes, possibly through loss of endothelial function. Reactive hyperemia (RH), a potent inducer of EDNO, is reduced in T2DM and following exposure to TRAP. In this study we examined impact of an acute 2 hour highway TRAP exposure on NO metabolism in 20 type II diabetic and 20 control subjects. Plasma nitrite (NO2-), a proximal measure of EDNO, and nitrate (NO3-), a further ox-
idized metabolite, were measured prior to and following RH, before and after pollution exposure. Diabetics displayed a higher resting NO2- level than controls at baseline (230 vs. 182.6 μM, p<0.06). Resting NO2- in diabetics decreased by 18.3% following the car ride (p<0.06), while controls decreased by 2.9% (p=0.08). Controls responded with a significant increase in NO2- following RH (15.9%, p<0.02) that was blunted by TRAP exposure (5.0%, ns). Diabetics showed no change in NO2- due to RH (1.8%, p=0.8) that was unaffected by TRAP. The ratio of NO2- to NO3- showed no change in controls due to TRAP. This ratio shifted toward NO3- in diabetics. Therefore, TRAP appears to lower the RH response in EDNO production, a property that is already lost in T2DM. Meanwhile TRAP strongly impacts the handling of NO within T2DM, favoring oxidation. Oxidative metabolization of NO in T2DM, leading to loss of endothelial function, appears to be further increased following TRAP exposures, implying this population may be more susceptible to adverse clinical events from traffic.
using micro-bioanalysis and/or reduced blood volume techniques for clinical pathology (CP) assessments. These study designs were created using recommended guidelines for weekly blood volume collections (7.5% of total blood volume/week). Study designs included a 4 week dosing period with a 4 week recovery period using a large molecule test article and included toxicokinetic (TK) and anti-drug antibody (ADA) endpoints, one pharmacodynamic marker (PD) and clinical pathology (CP) assessments. In rat studies, we were able to reduce the total blood volume that was collected on a weekly basis by up to 15 fold using micro-bioanalysis. This resulted in a 1 to 2 fold decrease in number of animals for the study. Additionally, we demonstrated that by combining micro-bioanalysis with reduced blood volume for CP assessments it would be possible to perform a rat study with no satellite groups and collect all required samples from the same animals (TK, ADA, PD and CP). In mouse study designs, the difference was greater, by incorporating micro-bioanalysis and reduced CP volume, we could not only reduce the number of animals by 4 fold, but also perform some study endpoints using the same animal; increasing the reliability of the data from each animal. Toxicology rodent studies can be greatly improved by incorporating reduce blood volume assays into their designs, allowing reduction in animal and improving data interpretation. These practices all support and incorporate the principles of the 3Rs.

862 Exploration for Mitochondrial Toxic Oxidative Stress in Pregnant Women on Iron Supplements in a Population with High Maternal Mortality Rate

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Although Iron (Fe) is a common supplement in pregnancy, it generates toxic oxygen species (TOS). Manganese(Mn) is vital for mitochondrial function; key component of the mitochondrial antioxidant, MnSOD which scavenges TOS. Fe competes with Mn for a common transporter, transferrin (Tf). The contribution of this interaction to mitochondrial toxic oxidative stress in at risk populations has received little attention. Sixty-three pregnant women on Fe supplements (FeSO4) covering the 3 trimesters, and 63 non-pregnant women, were investigated. Fe and indices of Fe homeostasis, Mn and key components of the antioxidant system were evaluated in serum in all groups and sub-groups. The levels of Mn, SOD, and Tf were raised in pregnant subjects vs control (p < 0.05). In contrast, levels of Fe, ferritin, Cu, uric acid, TIBC, % saturation, and Zn were significantly reduced in the same group (p<0.05). The first trimester demonstrated significant elevations in Mn and Tf compared to control (p <0.05), while Fe, Cu, Zn, Hct, ferritin were decreased. The second trimester exhibited significant elevations in the levels of Mn, Tf and SOD. In contrast, Fe, Cu, Zn, ferritin & TIBC were significantly reduced; as for the first trimester (p < 0.05). In the third trimester, Mn and Tf were again significantly elevated like the previous trimesters. The levels of other bioidicators were identical with those of earlier trimesters. The consistent increase in Mn and Tf levels in the pregnant compared to non-pregnant states, and the 3 trimesters, with inverse patterns in Fe level and key components of the antioxidant system may in addition to increased demand for Fe, reflect antioxidant dynamics consist with oxidative stress, including toxic mitochondrial oxidative perturbation. Mild to moderate mitochondrial impairment contributes to many pathologies. Understanding these interactions may improve strategies for addressing the refractory high maternal mortality rate in this high risk population.

863 ELISA Detection of Zilpaterol in Retinal Tissue

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Zilpaterol is a β-adrenergic agonist feed ingredient intended for livestock animals in order to enhance lean muscle mass and increase feed efficiency. Unfortunately, due to zilpaterol’s anabolic properties, it can be abused as a performance enhancing drug in livestock show and racing animals. Illicit abuse of the drug also extends into animal health and medicine with the drug being abused or being used off-label. Drug testing in livestock show and racing animals. Illicit abuse of the drug also extends into animal health and medicine with the drug being abused or being used off-label.

1) homogenizing approximately 0.40 g retinal tissue with acetonitrile/isopropanol (4:1) and 0.24 g NaCl, 2) drying the extract with 0.10 g MgSO4 and 0.80 g Na2SO4, 3) N2 evaporating the extract to dryness, reconstituting with 0.4 mL PBS, and 4) analyzing 50 µL of the reconstituted sample by ELISA. Sample optical densities less than or equal to the optical density of the 10 ppb positive control were considered positive. Using this protocol, zilpaterol was detected in the retinal tissue of 2 steers fed 96 mg zilpa- terol/head/day for 26 days. The average optical density in the 2 samples was 0.050, while the 10 ppb positive control average optical density was 0.280. This study shows that zilpaterol can be detected in retinal tissue from animals being fed the drug and provides a means by which the presence of zilpaterol can be monitored in animals for which the drug is being abused or being used off-label.

864 Monitoring GI Liability of EGFR Inhibitors with a Scratch Wound Assay in Intestinal Epithelial Cells

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Clinically, the use of epithelial growth factor receptor (EGFR) inhibitors for oncol- ogic indications is known for gastrointestinal (GI) toxicity and is dose limiting. EGFR is a member of the ErbB receptor tyrosine kinase family. In normal cells, EGFR signaling is important for migration, survival, and proliferation and promotes epithelial restitution after normal GI mucosal damage. In cancer cells, activating EGFR mutations drive the development of a specific subset of non-small-cell lung cancers and in 50% of these patients the use of wild type (WT) EGFR inhibitors such as erlotinib and gefitinib is limited by the development of a T790M secondary mutation. To target patients who harbor both primary and secondary mutations, selective EGFR double mutant (DM) inhibitors are in development. We sought to determine if DM compounds would have a better GI safety profile over WT inhibitors. We used a rat intestinal epithelial cell line (IEC-6) in a scratch-wound assay (SWA) as a model for restitution of the GI epithelium. The ability of WT or DM com- pounds to prevent TGF-β-induced repair of wounded IEC-6 cell monolayers was measured using the IncuCyte to monitor real time wound closure (WC) (Essen BioScience). Seven WT and five DM targeted compounds were tested. WT inhibi- tors prevented TGF-β-induced WC. The IC50 for the rate of WC from 0-6 hours was 0.009±0.001 to 1.15±1.38 µM with a mean of 0.19±0.56 µM and was not due to cytotoxicity as IC50 values for cytotoxicity were ≥30 µM (Promega). Inhibition of WC was associated with the WT inhibitor potency at EGFR; R2 = 0.60. In contrast to WT inhibitors, the IC50 range for the rate of WC from 0-6 hours was 5046±1.6 to 34,32±12.32 µM with a mean of 11.41±15.52 µM and was significantly greater than that for WT inhibitors (p<0.0001). There was some overlap between inhibition of WC and cytotoxicity in this group of compounds: IC50 values for cytotoxicity ranged from 20-100 µM. Collectively, these data suggest that the DM EGFR inhibitors should have reduced GI liability in vivo compared to WT inhibitors.

865 The Protective Effects of Short-Term Fasting against 60Co-γ Ray Radiation

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To investigate the antagonistic effects and mechanism of short-term fasting against 60Co-γ ray radiation in vivo and in vitro. ICR mice were randomly divided into control, 12h, 48h, and 72h fasting groups and were irradiated 7.5 Gy. General symptoms, organ ratio, WBC, ROS, SOD, MDA, T-AOC and eNOS were detected. To observe the differential effects and mechanism on glucose restriction against 60Co-γ ray radiation to normal and tumor cell. V79 and A549 cells were irradiated 12Gy. V79 and A549 cells were di- vided into 9 groups, including high glucose of 3.0g/l with 10% serum group (3.0g/ l+10%), 0.5g/l+10%, 3.0g/l+5%, 1.0g/l+5%, 0.5g/l+5%, 3.0g/l+1%, 1.0g/l+1% and 0.5g/l+1% group. In vivo, the survival rates of ICR mice were 0 for the control and 12 h group, 12.5% for the 48h group and 50% for the 72h group respectively. Compared with 0 group, ROS content and eNOS activity of 72h group were reduced and increased respectively. SOD, T-AOC activity of 72h group was less than 0 group. The viability of V79 and A549 cell was related to the dose level of γ ray, when the dose was 12Gy. Toxic effects and differential stress resistance (DSR) could be observed. As a result of interaction between serum restriction and radiation, the concentration of glucose in the media was reduced to mimic STS. After 60Co-γ ray radiation, V79 cell viability of the high, normal, low glucose group was reduced to 46%, 23% and 1% respectively, and A549 cell was reduced to 37%, 50% and 48%; The apoptosis rate of V79 cell of low glucose contents was reduced significantly, but A549 cell had no significant difference among groups. ROS production of V79 cell with low glucose was reduced, SOD activity was increased significantly, in contrast, ROS production of A549 cell was increased and SOD reduced significantly.
PI3 kinase inhibitors are being developed for several clinical indications including metastatic breast cancer in women overexpressing HER2. GDC-0941 is a potent pan PI3K inhibitor, which when given in combination with trastuzumab emtansine (T-DM1) showed >Grade 3 thrombocytopenia. The purpose of the present study was to examine the potential of GDC-0941 alone and in combination with T-DM1 to effect megakaryocyte production and proliferation in vitro. We utilized human in vitro model for the study which consisted of deriving the human hematopoietic stem cells (CD34+) from healthy donor bone marrow and differentiating them into megakaryocytes, during which cells were collected for cytotoxicity, cell number and CD41/CD61 expression. We evaluated the cytotoxic effect of GDC-0941 treatment on human megakaryocyte formation from CD34+ HSCs. We further co-treated GDC-0941 with T-DM1 to evaluate whether effects on human megakaryocyte formation are additive/synergistic. The data showed that GDC-0941 treatment caused a decrease in megakaryocyte production from hematopoietic stem cells and cell proliferation over a period of 14 days. Further, the treatment of GDC-0941 in combination with T-DM1 showed an enhanced cytotoxicity leading to a decrease in production of megakaryocytes over a period of 14 days when compared to GDC-0941 alone. The results show consistency when tested in additional donors. Thus, the human in vitro bone marrow model is very useful to evaluate the potential of a drug candidate to cause thrombocytopenia or bone marrow toxicity.


866 Effect of GDC-0941 Treatment in Combination with T-DM1 on Formation of Megakaryocytes

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Acetaminophen (APAP) overdose is a major problem. Although most patients survive, the volume of cases makes it the most common cause of acute liver failure (ALF) and ALF-related deaths in the US. We are only beginning to translate the mechanisms of hepatotoxicity from rodents to humans. Our group recently reported evidence of mitochondrial dysfunction in APAP overdose patients using biomarkers of mitochondrial damage (mitochondrial DNA [mtDNA], glutamate dehydrogenase [GDH], and nuclear DNA [nDNA] fragments). In this study, we wanted to determine if these markers correlate with death or survival. We measured mtDNA by qPCR, GDH activity by kinetic assay, and nDNA fragments by ELISA in serum from APAP-induced ALF patients who did (n = 35) and did not (n = 34) survive. All three parameters were elevated at or near the time of peak ALT in patients with APAP-induced liver injury. Unlike ALT, peak levels of both GDH and nDNA fragments were higher in serum from non-survivors than survivors (1.08±0.22 vs. 1.34±0.77 U/L and 259±2 vs. 209±4 % of control, respectively). GDH and nDNA fragments also exhibited weak but significant correlations with ALT, prothrombin time, and with each other. Importantly, receptor operating characteristic (ROC) curve analysis revealed that higher peak levels of both GDH and nDNA fragments better associated with non-survival (AUC=0.68 and 0.65 for GDH and nDNA, respectively; p<0.03) than ALT (AUC=0.60; p=0.11). Limited data suggest similar results for mtDNA. Conclusions: Biomarkers of mitochondrial damage are higher in non-survivors with APAP-induced ALF. This indicates that patients with more mitochondrial damage are less likely to survive. Mitochondrial biomarkers could be useful as part of a panel for patient prognosis. (NIDDK U-01 DK58369 to the Acute Liver Failure Study Group)


867 Serum Biomarkers of Mitochondrial Damage in Survivors and Nonsurvivors of Acetaminophen-Induced Acute Liver Failure: Implications for the Mechanism of Hepatotoxicity in Humans

868 A Labor of HERCULES: Curation of the Environmental Metabolome for Exposome Research

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Liquid-chromatography high-resolution mass spectrometry with advanced algorithms for data extraction, termed “high-resolution metabolomics” (HRM), now enables routine measurement of >20,000 chemicals in biologic samples. As part of the new NIEHS-funded P30 Center at Emory University and Georgia Tech called the Health and Exposome Research Center: Understanding Lifetime Exposures (HERCULES), we have initiated systematic curation of the environmental metabolome through cross-platform statistical tests of results from validated, targeted analyses of environmental chemicals and HRM analyses of the same samples. The principles of the approach were validated using quantitative results obtained commercially (Metabolon) and compared to HRM using “targeted” MWAS (Metabolome-Wide Association Study). In this validation, the values for specific chemical targets in a series of 50 samples were regressed against the intensities for all ions [mass/charge (m/z) features] detected by HRM. The cross-platform targeted MWAS for >100 metabolites were performed, with each displayed as Manhattan plots of log2(p value for t-test) as a function of m/z, along with false discovery rate correction. Co-elution of authentic standards and MS6 confirm metabolic identity from HRM. Results with internal standards and calibration against pooled reference samples demonstrate that HRM can be used for absolute quantification, with initial cureation results also verifying the detection of environmental agents (e.g., dibutylphthalate, triphenylphosphate, pirimicar, atrazine metabolites) and associated physiological metabolites. As part of the NIEHS Center program, HERCULES provides a robust foundation for exposome research, applying this methodology to develop a knowledgebase of environmental chemicals and their distribution among human populations and ecologic systems.


867 Serum Biomarkers of Mitochondrial Damage in Survivors and Nonsurvivors of Acetaminophen-Induced Acute Liver Failure: Implications for the Mechanism of Hepatotoxicity in Humans


868 A Labor of HERCULES: Curation of the Environmental Metabolome for Exposome Research


869 Pathogenic Mechanisms in Highly Active Antiretroviral Therapy (HAART)-Induced Hepatotoxicity: Role Phosphodiesterase 4 (PDE4) and ER-Stress

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HIV protease inhibitors (HIV-Pis) are the major components of the highly active anti-retroviral therapy (HAART) and have been successfully used in the treatment of HIV-1 infection in the past two decades. However, it has been shown that HIV-Pis induce endoplasmic reticulum (ER) stress response and subsequent activation of unfolded protein response (UPR) leading to dys-regulation of hepatic lipid metabolism and hepatotoxicity. Our recent work shows that PDE4/cAMP metabolism plays a significant role in alcohol-induced hepatic steatosis and injury. Hence in the present study, we examined the potential mechanisms underlying HIV-PI induced hepatic ER stress and toxicity with a particular emphasis on the pathogenic role of PDE4 family of enzymes. The effects of clinically used HIV-PIs (ritonavir (RT) and lopinavir (LOP)) were examined both in a rat hepatoma cell line (H4IIE) as well as rat primary hepatocytes. The data obtained from these studies demonstrated that RT+LOP led to a significant loss of hepatocyte survival. Notably, inhibition of PDE4 by a specific PDE4-inhibitor, rolipram, markedly attenuated hepatotoxicity induced by PI treatments. Examination of the mechanistic role of PDE4 showed that PDE4 inhibition significantly decreases the expression of the ER stress related proteins CHOP, ATF-4 and -3 induced by PIs. Furthermore, examination of the PDE4/cAMP regulated downstream signaling demonstrated the involvement of Exchange proteins activated by cAMP (EPAC). Specifically, similar to rolipram, EPAC CAM analogue (8-pCPT'-2'-O-Me-cAMP) downregulated the expression of the PI-induced ER-stress genes. These data strongly support and identify a pathogenic/mechanistic role for PDE4 regulated cAMP-EPAC in the development of HIV-PI induced ER-stress leading to hepatic steatosis and injury.

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870 Dual Regulation of Calcineurin Activity following Lymphocyte Activation: Contribution to Cyclosporine-Induced Toxicity

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Introduction: The standard for rejection prevention in transplantation includes a calcineurin (CN) inhibitor, such as cyclosporine (CsA). It was thought that high CN activity (CN-a) reflected inadequate immunosuppression (IS) with insufficient
therapy that may lead to rejection, whereas low CN activity reflected excessive IS that would facilitate the development of adverse events. We have recently found that in several transplanted patients, both rejection and CSa-related adverse events were paradoxically accompanied by a decreased CN-a. The aim of this study was to provide a molecular explanation for such discrepancies.

Methods: We measured CN-a in human lymphocytes up to 48 h following stimulation by PMA/ionomycin. The induction of lymphocyte activation was verified by assessing the gene expression of Ile6, IL2RA and IL17 by qPCR. The effect of CSa was measured in both resting and activated lymphocytes. CN-a was quantified by using tandem mass spectrometry.

Results: In resting lymphocytes, CSa led to a dose-dependent decrease in CN-a. In stimulated lymphocytes, CN-a was increased at short time-points after stimulation but was gradually decreased at later time-points. In addition, this down-regulation was even more dramatic in the presence of CSa. The transient activation of CN-a was accompanied by a lasting effect on other markers of lymphocyte activation. Furthermore, we found that lymphocyte activation in vitro triggers a dual regulation of CN-a. The fact that the addition of CSa led to a more potent inhibition of CN-a when cells were activated suggests that two distinct mechanisms might concomitantly decrease CN-a: one mediated by CSa itself and another one mediated by an endogenous down-regulation of the CN/NFAT signalling pathway. We anticipate that the activation of such mechanisms following lymphocyte activation could contribute to the toxicity induced by CNIs, especially as the CNI dosage is generally increased when rejection is suspected.

871 Derivation of Induced Pluripotent Stem Cells and Their Potential Application for Cardiac Regeneration in a Porcine Model

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Porcine induced pluripotent stem cells (iPSCs) offer a great promise to advance the strategies for cardiac therapy using a large animal model. Therefore, the present study investigated the derivation of iPSCs and their potential application for the treatment of myocardial infarction in a porcine model. Porcine bone marrow derived mesenchymal stem cells (BMSCs) were reprogrammed to iPSCs by the lentiviral transduction of transcription factors Oe4, Klf4, Sox2 and c-Myc. Generated iPSCs exhibited alkaline phosphatase activity and expressed pluripotent markers. Porcine iPSCs differentiated well into three germ layer-derived cells in vitro. Further, they efficiently formed teratomas in immunodeficient mice and the tumor tissues revealed the tissues of three germ layers. The efficiency of iPSCs to differentiate in vitro into cardiomyocytes was greatly enhanced in the presence of 5-azacytidine. To demonstrate the therapeutic potential, porcine iPSCs labeled with a membrane dye PKH26 were transplanted into miniature pigs with myocardial infarction generated by coronary artery ligation and reperfusion. Four weeks after transplantation, compared with control, animals injected with porcine iPSCs showed significant functional improvement measured by echocardiography. PKH26 labeled cells were detected in iPSCs transplanted groups, and showed the expression of cardiac specific markers in ischemic microenvironment. No inflammation or teratoma formation was observed in pigs that received iPSCs. The findings revealed the potential of porcine iPSCs as ideal cellular models for cardiac cell therapy and preclinical assessments.

872 Engineered Nanomaterial Exposure and the Mitochondrial Syndrome

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Mitochondrial disease (MS) is a diagnosable condition, affecting the microcirculation, represented by a set of cardiovascular risk factors. Engineered nanomaterial exposure (ENM) has been shown to lead to a variety of cardiovascular effects. In this study, we evaluated coronary microvascular reactivity within the Zucker rat model of MS after ENM exposure, to determine if exposure would exacerbate microvascular dysfunction. Nine and 17-weeks old lean control (LZ) and obese MS (OZR) male Zucker rats were exposed to filtered air or a single inhalation exposure (count median aerodynamic diameter of 152±22 nm, 11±0.2 mg/m3, 5hrs/day) to nano-titanium dioxide aerosols 24h before sacrifice producing calculated pulmonary deposition of 15±0.5%, respectively. Coronary arteriolar (1.5–6.0μm) were evaluated within an isolated microvasculature preparation. Vascular reactivity analysis was performed based on responses to endothelium-dependent (acetylcholine, ACh, 10–9–10–4 M and adenosine, ADO, 10–4 M) and –independent stimuli (spermine-NONOate, SRT, 10–9–10–4 M and serotonin, 5-HT, 10–9–10–4 M). Within the control, the LZR exhibits reduced dilator reactivity (ACH and SRT) after ENM exposure at 17w. With respect to ADO, a profound vasodilator, ENM exposure led to a 28% reduction in dilation at 9w, limiting reactivity to aged levels. This response was elobed, although not significant, at 17w. Additionally, reactivity to the vasoconstrictor 5-HT, is abolished at 17w, after ENM exposure. Within the MS, ENM exposure further reduced (to significance) impaired endothelium-dependent vasodilation at 9w (ACH [>10%] and ADO [21.7%]). ENM exposure within the aged group also significantly reduced ADO stimulated dilation. SER reactivity was significantly impaired with aging; however there were no additive differences associated with ENM exposure. While microscopic dysfunction was evident after ENM exposure in both age groups, there was a greater decline associated with metabolic syndrome progression than an additive effect stemming from ENM exposure. NIH-R01-ES0435 (PAS) NIH-R01-ES051022 (TRN) NSF-1003907 (VCM)

873 Cyclophilin Inhibitor Alisporivir (DEB025) Is Not Toxic to Mitochondria: A Comprehensive In vitro and In vivo Assessment

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Some pancreatic cell lines were reported in clinical studies with alisporivir (ALV) in combination with peg-interferon/ribavirin treatment of hepatitis C patients. The potential of ALV to affect mitochondrial function or morphology was assessed both in vitro and in vivo.

In vitro, a dose range of ALV and Zalcitabine (ddC - mitochondrial toxin) were tested in proliferating cell lines HepG2, Panc-1 and Capan-1 after 7 and 14 days of continuous treatment. Viability, ATP content, mitochondrial mass, mitochondrial DNA (mtDNA) and oxygen consumption rates (OCR) were measured as endpoints. With ALV, OCR paralleled cytotoxicity, reflecting cell death; however with ddC, OCR decreased even at non-cytotoxic concentrations, demonstrating mitochondrial specific impairments. At cytotoxic concentrations of either agent only ddC provoked a decrease of mtDNA content. To further assess potential mitochondrial toxicity, similar readouts were assessed in galactose-adapted HepG2 and Mol74 cells (T-cell line sensitive to mitochondrial toxins). Galactose forces metabolism via OxPhos and thus reveals mitochondrial toxin effects not detectable in glucose. Galactose grown HepG2 and Mol74 cells were more susceptible to ddC after 14 and 25 days treatment compared to glucose, but no differential cytotoxicity or mitotoxicity was observed with ALV compared to glucose grown cells. Hence, ALV was shown to be devoid of mitotoxicity in these in vitro assays.

In vivo, the morphology of mitochondria in pancreas, heart and skeletal muscle from mice treated daily with DEB025 at an oral dose of 300mg/kg for 26 weeks and rats treated daily for 2-weeks at an oral dose of 100 mg/kg were examined by electron microscopy. No differences in the ultrastructure of mitochondria in either species. In conclusion, the results demonstrate that alisporivir is not a mitochondrial toxin.

874 Sulforaphane Protects against Influenza Virus Infection in the Nasal Mucosa of Smokers

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Influenza continues to affect approximately 5-20% of people in the United States. Moreover, studies show that exposure to cigarette smoke increases the susceptibility to and severity of respiratory virus infections. Researchers have explored nutritional antioxidants as a preventative therapy for viral infection. In the current study, we evaluated whether the antioxidant sulforaphane (SFN), an isothiocyanate found in cruciferous vegetables, could modify viral replication in the nasal mucosa of smokers and non-smokers in vitro. Separate cohorts of smokers and non-smokers were randomized to receive either a SFN-rich broccoli sprout homogenate or placebo (alfalfa sprout homogenate) once daily for four consecutive days. On day two, all subjects were inoculated with a standard dose of live attenuated influenza virus (LAIV) vaccine. Markers of viral replication, hemagglutinin-1 (HO-1), and...
were cloned and expressed. These reagents provide valuable tools to explore factors involved in regulation of sEH and its role in health and disease. (This work was supported by the NIEHS Superfund Basic Research P42 ES04699.)

876b Clinical Toxicology and Community Health Education: National Poison Prevention Week in the United States

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Unintentional poisoning deaths increased by 160% from 1999 to 2009 in the United States (CDC, 2012). Approximately 4 million calls were reported by regional centers in 2010, to the American Association of Poison Control Centers. Unintentional poisonings occur primarily in the home, in children under six years of age. However, poisonings are also one of the leading causes of death among adults. Thus, educational outreach in communities, across the lifespan, fulfills a significant need for mitigating acute toxicological exposures. In 1961, the U.S. Congress established National Poison Prevention Week - held annually in the third week of March and organized by the Poison Prevention Week Council. The focus is on raising awareness and providing education and prevention efforts to the public and public health community. In addition, resources are provided to assist in prevention and treatment of intentional and unintentional poisonings throughout the year. Examples include offering the toll-free number to the PoisonHelp Line from the American Association of Poison Control Centers (AAPCC); and websites, such as PoisonPrevention.org (http://www.poisonprevention.org/index.htm); and resources about poisoning from the Centers for Disease Control (http://www.cdc.gov/features/poisonprevention/), along with educational materials developed by the AAPCC in multiple languages. Annual data from the National Poison Data System (NPDS) is reported to identify numbers and severity of exposures in human poisonings, and it is used to assess the risk to public health. Select topics of concern are chosen as the focus of National Poison Prevention Week. National Poison Prevention Week continues to be a source of community health education after more than 50 years, and supports the efforts of AAPCC to inform, advocate, and educate about acute toxicology incidents.
Textile contact dermatitis results when a patient develops skin manifestations due to clothing or other fabrics that contact the skin, commonly from the chemical additives used in processing the fabric, such as textile dyes, tanning agents, and finishing resins. In this study the authors provide a framework for working up and counseling a patient with suspected textile dermatitis, focusing on identifying which textile materials are most likely to be the cause of the eczematicous lesions, the current clinical guidelines, the utility and appropriateness of patch testing, the limitations of these guidelines, and our pro tempore recommendations. Although there are many challenges to correctly identifying and counseling patients on how to avoid the offending textile products in a patient with suspected textile dye dermatitis, there is value in following the guidelines set forth to help identify the textile dermatitis source.

Clinicians should instruct patients to categorize and list all colored-fabric textile products that contacted the affected skin areas. The fiber composition, corresponding colorant class, and dye fastness of each suspected garment should then be noted. Lastly, fabrics should be prioritized based on color darkness. While patch tests can be useful, dermatologists should understand the limitations of standardized patch testing for patients with suspected textile dye induced dermatitis. These guidelines are expected to increase the likelihood of identifying the causative textile(s) so that patch testing can be supplemented with swatch testing and chemical dye extraction to help discover the allergenic dye.

The cardiovascular effects of two common pollutants, diesel exhaust (DE) and ozone (O3), were examined alone and in combination in an IRB approved clinical study. Consenting healthy subjects (n=15) were exposed for 2 hrs with intermittent, moderate exercise on Day 1 to 0.3 ppm O3, 300 µg/m3 DE, both O3 and DE, or filtered air (FA). On Day 2, subjects were exposed to 0.3 ppm O3. Cardiac electrophysiology and venipuncture were performed pre and post exposure. In addition, blood pressure (BP) was measured pre, post, and every hour up through 4 hrs post on days 1 and 2. Relative to air exposure values, maximum heat rate decreased -12% immediately post DE exposure, and the cardiac QTc interval (QTcDYN) increased 2% immediately post O3 and DE+O3 exposure. Systolic BP was significantly decreased immediately post DE+O3 exposure compared to air. Plasma lipid profiles were altered significantly immediately after day 1 exposure. Specifically, after DE+O3 exposure triglycerides were decreased (vs O3), and VLDL increased compared to O3 and DE+O3. LDL and HDL levels increased exposure. Evidence was found for independent effects of acute O3 and DE exposures alone and in combination on some outcomes. These data suggest that either interactions of ambient air pollutants or individual pollutants themselves may play a role in inducing adverse health outcomes. [Disclaimer: The views expressed are those of the authors and do not necessarily reflect the official views or policies of the US EPA.]
Development of LC-MS method more sensitive than LBA for challenging quantification of Glucagon at low pg/mL level in new formulation which is easier to administer than the currently available formulations. A bioanalytical method for glucagon in human plasma was validated on API5000. However, the LLOQ (100pg/mL) was not adequate to cover the pharmacokinetic profile of a novel formulation. Therefore, the method was re-developed on two different instruments to reach higher level of selectivity and sensitivity. TripleTOF5600 was tested in full scan TOFMS (30K resolution). The 3 most abundant isotopomers of +5 charge state of glucagon were summed. Moreover, MRMHS (15K resolution targeted approach), was also evaluated and the 3 most abundant isotopomers of the +5 charge state product ion (neutral loss of NH3) were summed. This experiment revealed that using a targeted quantification approach (MRMHS) led to a 10-fold increase in sensitivity over TOFMS. Hence, it was possible to have on a TripleTOF5600 the same sensitivity of a API5000. QTRAP6500 was able to reach the LLOQ of 10pg/mL (10-time lower than validated LLOQ on API5000). The calibration curve was linear (weighted 1/x2) for the concentration range 10-10000pg/mL with a coefficient of correlation of 0.9990. The accuracy for the LLOQ was 88% with 16% precision. The accuracy for quality control samples ranged between 91-101% while the precision between 5-9%. The outcome of this showed that it possible the development an LC-MS method more sensitive than LBA for the challenging quantification of Glucagon at low pg/mL level for a novel formulation.

881 Advanced Use of High-Resolution Mass Spectrometry (HRMS) to Overcome Triple Quadrupole Limitations in Large Molecules Quantification


The use of HRMS (QTOF) to overcome limitation of triple quadrupole for development of Large Molecule bioanalytical assays. To achieve the lowest LLOQ possible during the method development of somatostatin and enfurvirtide, the use of HRMS was preferred over triple quadrupole. For somatostatin, a large cyclic peptide known for ineffective fragmentation, a parent-to-parent approach was selected to limit loss of sensitivity during the fragmentation process. This approach is practically impossible with a triple quadrupole due to high chemical noise generated. The LLOQ achieved on HRMS with the summation of the two most intense isotopomers was 12pg/mL (S/N 22 vs. 9 on triple quadrupole). For enfurvirtide, the product ion used was 1343, which is outside the normal mass range of the quadrupole (m/z 1200). When compared to the most sensitive fragment obtained with the triple quadrupole (465,1m/z) the LLOQ was easily decreased from 100 to 50pg/mL with S/N>15. Calibration curves were found linear (weighted 1/x2) and coefficients of correlation were 0.9995 for somatostatin and 0.9987 for enfurvirtide. The CV% calculated from five replicate injections of QCLOQ was 6% for somatostatin and 9% for enfurvirtide. For both compounds, the precision of the QC samples were all within 15% and accuracy between 96-102%. This study demonstrated that HRMS instrument is more adapted and suitable than the regular triple quadrupole for overcoming of complex analytical challenges as in the case of Large Molecule quantitation. The HRMS was able in both case studies to allow more flexibility in the quantification to increase selectivity and sensitivity of the assays.

882 Human Metabolism Studies of High-Molecular Weight Polycyclic Aromatic Hydrocarbons Utilizing UPLC-Moving Wire Solid Sample Feed-Accelerator Mass Spectrometry

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High molecular weight polycyclic aromatic hydrocarbon (PAH) exposure occurs from atmospheric deposition of incomplete combustion products to food crops and from flavoring during cooking food smoking processes. High molecular weight PAHs are carcinogenic to laboratory animals and are probable human carcinogens. Human PAH studies was previously not possible due to exposure occurring as mixtures and ethical concerns of administrating the PAHs in detectable quantities to human volunteers. The application of the traditional carbon dating instrument, the accelerator mass spectrometer, to biological sample analysis is now possible with defeminum health risk due to the improved sensitivity (one 14C molecule per 1024 molecules of 12C) and efficiency (100% transfer from UPLC eluent to AMS). We were able to detect parent and metabolites of our model PAH, dibenzo(def,p)chry-sene (DBC), in the urine and plasma of human volunteers from an oral microdose of 29ng/SmCi. Volunteers, n=9, were selected to be non-homogeneous of age, sex, BMI, and ethnicity to represent the variability of human metabolism. Total 14C-PC in 19% of DBC resulted in pharmacokinetic parameters of plasma Cmax = 61.5 ± 42.9 fg DBC eq, Tmax = 2.25 ± 0.95 hr, α-phase T1/2 = 5.3 ± 3.28 hr, while α-Kels = 0.19 ± 0.01 fg/hr, followed by a prolonged β-phase parameters. Current work is focused on UPLC separation of urine and plasma metabolites and detection using the moving wire solid sample interface as well as upcoming studies of human metabolism with PAHs phanthrene and benz[a]pyrene microdosing. This meta is used to validate physiologically based pharmacokinetic models of human PAH metabolism with the goal of future mixture modeling.

883 Development and Characterization of a LC-MS/MS Method for Antifolate Compounds for the Treatment of Bacillus anthracis Infections

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Bacillus anthracis is a category A priority pathogen as classified by the National Institute of Allergy and Infectious Disease division of the National Institutes of Health. Exposure to Bacillus anthracis is currently treated by antibiotics (ciprofloxacin, doxycycline, penicillin, etc.), however antibiotic resistance has been produced in the laboratory. Therefore a medicinal chemistry program has been created to synthesize compounds that overcome the trimethoprim resistance observed in B. anthracis. This program has selected two early lead compounds, RAB1 and #53. In order to support preclinical development programs, a liquid chromatography-tandem mass spectrometry method was developed for quantification of these leads in mouse biological matrices (plasma, urine and GI content). The bioanalytical assay achieves separation via a reversed phase method and by detection multiple reaction monitoring. The extraction procedure uses a two-step protein precipitation method (MeOH and ACN) to prepare samples for analysis. This extraction procedure was selected as it provides sample stabilization prior to analysis, which is required for sample analysis from B. anthracis challenged animals. The assay was found to be specific and selective for both lead compounds with an LLOQ of 1 ng/mL in mouse plasma. The linear range was shown to be 1.0 to 200 ng/mL with dilutional linearity up to 6,000 ng/mL. Additionally, the mass spectrometer was selectively tuned to screen for phase I metabolites (mono- and di-hydroxylation; and mono- and di-demethylation). Application of the assay to plasma protein binding studies (using rapid equilibrium dialysis) indicated that both compounds are highly protein bound (99%) in mouse plasma. This method allows for quantification of both lead compounds in preclinical pharmacokinetic and efficacy studies.

884 Differential Pharmacokinetics of Tetracycline and Sulfamethoxazole in Brassica chinensis L. Grown Hydroponically

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Veterinary antibiotics in edible vegetables have quietly become an increasing food safety concern as these antibiotic-containing vegetables may find their way back to the human food chain. Although much has been studied on the deposit levels of antibiotics in plants, relatively little is known regarding the fate of antibiotics with time once they got entry to the plant. The pharmacokinetics (uptake, distribution, metabolism and elimination) of two most commonly detected antibiotics in soil and river, tetracycline (TC) and sulfamethoxazole (SMX) in edible vegetable Brassica chinensis L. were investigated. The vegetable was exposed to 100 µg/mL of drugs in cultivation fluid for 24 hrs, drug concentrations in roots, petioles and leaves at 0.5, 3, 6, 12 and 24 hrs were quantified by HPLC-UV method and the bioaccumulation factors (BAF) were calculated. The results suggested significant differential uptake and distribution of the two antibiotics evidenced by a BAF of 21/0.4 in the roots and 1.3/0.2 in the leaves for TC/SMX, respectively and by a concentration ranking of roots>petioles>leaves for TC versus roots>petioles>leaves for SMX. The Cmax and Tmax in the roots are 948±132 µg/mL at 6 hrs for TC and 334±44 µg/mL at 24 hrs for SMX, whereas in the leaves they are 92±4 µg/mL at 12 hrs (TC) versus 7±1 µg/mL at 24 hrs (SMX). The percentage uptake of drugs by the vegetable were similar at 3 concentration levels (100, 10 and 1 µg/mL) indicating the appropriateness of the current model and the ability of Brassica chinensis L. to uptake high amount of TC. Possible biotransformation of TC (but not SMX) was detected only in the leaves. Elimination of drugs from the vegetable was most noted in the roots mainly in the first 0.5 hr (30% reduction for TC and...
15% for SMX); refreshing the cultivation fluids maximized the reduction to 50%. In conclusion, it appeared that the pharmacokinetic behaviors of TC in *Brassica chinensis* L. acted in a controlled manner while SMX followed the passive water transport in xylem.

### 885 Comparative Metabolism and Pharmacokinetics of Diisobutyl Ketone and Diisobutyl Carbinol in Male SD Rats

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Diisobutyl ketone (DIBK) and diisobutyl carbinol (DIBC) are important organic solvents widely used as industrial intermediates. It was hypothesized that DIBK and DIBC have common metabolic pathways and metabolites and as such toxicological data on DIBK could be used to characterize the hazards of DIBC. To confirm or refute this hypothesis a comparative metabolism and pharmacokinetics assessment of DIBK and DIBC was conducted via single oral gavage dosing in male SD rats, followed by blood collection, metabolite identification, major biomarker quantitation, and pharmacokinetics analysis. Overall, the major metabolites of both DIBK and DIBC in blood were their corresponding monohydroxylated metabolites (DIBC alcohol and DIBC alcohol) with the site of hydroxylation at the α and α-1 positions, respectively. Quantitative analysis of DIBK, DIBC, DIBC- alcohol, and DIBC-alcohol in blood samples collected from 5 min to 120 hr after single dosing indicated the following: 1) DIBK and DIBC are both well absorbed following oral gavage with substantial evidence of enterohepatic recycling of DIBK, DIBC, DIBC-alcohol, and DIBC-alcohol; 2) DIBK and DIBC are also inter-converted in rats; 3) DIBK and DIBC have similar bioavailability (AUC) after oral administration; 4) higher bioavailability (AUC) was found for DIBC-alcohol than DIBC-alcohol, implying that DIBC-alcohol may be more easily conjugated and eliminated in bile, thus leading to lower systemic exposure than from DIBC- alcohol. In summary, the metabolic similarities and the metabolite bioavailability difference between these substances observed in the current study support the hypothesis that DIBC will have a low potential toxicity similar to that of DIBK.

### 886 Short-Acting and Long-Acting Buprenorphine Therapeutic Drug Levels following Single Subcutaneous Administration in Diabetic Yucatan Miniswine

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Sustained and controlled analgesia for animals involved in potentially painful procedures is required for animal welfare and ethical considerations. We designed a study to assess the PK for buprenorphine (BUP) analgesics in diabetic Yucatan miniswine. Four castrated male allaconic diabetic animals weighing approximately 30 kg were used in a complete cross-over design. For BUP, animals were dosed subcutaneously (left flank fold) with either 0.01 mg/kg (low-dose) or 0.02 mg/kg (high-dose), while for BUP SR (sustained release) the dose was either 0.12 mg/kg (low-dose) or 0.24 mg/kg (high-dose) for 240 min. Results for BUP SR plasma drug profile showed peaks at 1795.5 pg/mL at 240 min (high-dose) and at 1531.8 pg/mL (low-dose) at 30 min. Sustained release drug was present in plasma for 96 hrs for both high- & low-dose (above 0.1 ng/mL). In conclusion, these data show that these dose levels provide sufficient plasma levels of drug for analgesia (>0.1 ng/mL) for at least 8 hr (short-acting BUP) or 96 hr (long-acting BUP SR).

### 887 Oxycyte®, a Perfluorocarbon Emulsion Drug Candidate for the Treatment of Ischemic Brain Injury, Has Similar Pharmacokinetic Characteristics across Rodent and Nonrodent Species, Including Humans

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Oxycyte is a 60% w/v perfluorooctylbicyclohexyl (PFOB) intravenous emulsion being developed as a novel treatment for a variety of ischemic conditions, including traumatic brain injury. The pharmacokinetics (PK) of Oxycyte’s perfluorocarbon, FbBu, across two rodent species, two nonhuman primate species, and humans were compared with the objective of using exposure ratios to assist in the selection of clinically relevant dose for future nonclinical studies. Following intravenous administration of Oxycyte in BALB/c mice and Sprague Dawley rats (3, 6, 12 mL/kg), cynomolgus monkeys (4 and 10 mL/kg), olive baboons (3 and 12 mL/kg) and humans (0.5, 1.5 and 3 mL/kg), concentrations of FbBu in whole blood generally decreased over time in a monoeXponential manner. In all species, while peak exposures were roughly dose proportional, overall exposure (AUC) more than doubled with each doubling of Oxycyte dose (e.g., 3 to 6 mL/kg; 6 to 12 mL/kg). In addition, FbBu half-life (T1/2) increased with increasing Oxycyte dose, whereas values for systemic clearance (Cl) decreased. Oxycyte emulsion particles are removed from circulation via phagocytic cells of the reticuloendothelial system with the metabolically inert perfluorocarbon eventually exhaled during respiration. The nonlinearity between the systemic exposure and elimination data reflect, at least partially, saturation of this clearance mechanism with increasing dose levels. By comparing FbBu systemic exposures (AUC), it was determined that in previous animal safety studies Oxycyte dose levels provided systemic exposures up to 25-fold higher than those expected at the highest clinical Oxycyte dose (3 mL/kg). Based on the comparison of PK parameters, the pharmacokinetics of FbBu are similar across species, including humans. Therefore, exposure estimates can be used to determine appropriate, clinically relevant, Oxycyte doses for use in future animal and pharmacology studies.

### 888 Iron Sucrose Nanoparticles: Effects on Tissue Iron Levels and Hepatic Gene Expression in the Rat

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The generic Iron Sucrose Azad (ISA) or reference iron sucrose drug Venofer® were administered intravenously to (non-anemic) male rats at 15 mg/kg, a supratherapeutic dose level. Tissue iron levels in plasma and selected tissues were determined over 28 days using an ICP-MS method. Hepatic gene expression was evaluated by microarray analysis of mRNA from samples taken 24 hours after drug administration. Iron concentration/time profiles for plasma and tissues were quantitatively similar; circulating iron levels briefly exceeded transferring binding capacity and there was a transient increase in hepatic iron. Iron levels remained elevated in the bone marrow. No increases in tissue iron were observed in the heart, stomach or lungs of treated rats and small transient increases were recorded in the kidney. Spleen iron levels increased over the 28 day period in treated and control rats. The effects of ISA and Venofer® on hepatic gene transcription were similar. There was no systematic effect of either treatment on transcriptional profiles. Only a small number of genes showed significant modulation of expression. No transcriptional pattern matches with toxicity pathways were found in the ToxFX database for either treatment. No modulation of key genes in apoptosis, inflammation or oxidative stress pathways was detected. The biodistribution of administered iron is essentially similar for Iron Sucrose Azad and Venofer® and iron sucrose paritions predominantly into the liver, spleen and bone marrow. Hepatic gene expression studies did not provide any evidence of hepatic toxicity.

### 889 Comparative Metabolism and Pharmacokinetics of Pterostilbene in Rhesus Macaques, Cynomolgus Macaques, and Common Marmosets

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Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a natural polyphenol that demonstrates cancer chemopreventive, antiinflammatory, and cardioprotective activities in animal models. Although resveratrol has a number of desirable biological effects, its activity may be limited by poor oral bioavailability and rapid metabolism. Pterostilbene (3,5-dimethoxy-4′-hydroxy-trans-stilbene) is the dimethyl ether analog of resveratrol. Like resveratrol, pterostilbene is found in grapes and other foods, and has antiinflammatory and cancer chemopreventive activities in a comparative...
PK and metabolism study in rats, we found that total absorption (AUC) and peak plasma levels (Cmax) of pretosilbene (parent) are 10 to 40-fold higher than those of resveratrol (parent) after oral dosing. Plasma levels of resveratrol glucuronide and resveratrol sulfate are comparable after a single oral dose, whereas metabolism of pretosilbene is overestimated by 22-fold. The present study was performed to compare the PK and metabolism of pretosilbene in three species of non-human primates. Although common marmosets (Callithrix jacchus) are used much less often in preclinical development studies than are cynomolgus macaques (Macaca fascicularis) or thusus macaques (Macaca mulatta), their smaller body size may offer important benefits, particularly when drug supply is limited or synthesis costs are high. Groups of 3 to 4 animals per species received a single gavage dose of pretosilbene (50 or 100 mg/kg); the 100 mg/kg dose is an interspecies extrapolation of the rat NOAEL for resveratrol. Timed blood collections were performed over 24 hours after dosing. No evidence of pretosilbene toxicity was seen in any animal. Plasma levels of pretosilbene (parent) were generally comparable in common marmosets and in the two species of macaques over the 24 hour collection period. These data suggest that common marmosets may provide a useful model for preclinical drug development, particularly in situations when test article availability is limited.

**890** Toxicokinetics of Combined Treatment with Melamine and Cyanuric Acid in Male Sprague-Dawley Rats

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When melamine is combined with cyanuric acid, melamine-cyanuric acid crystals can easily be formed, by which nephrotoxicity of melamine would be aggravated. In the present study, toxicokinetics of melamine and cyanuric acid was studied in male SD rats orally received either melamine or cyanuric acid alone and combination of melamine and cyanuric acid for 7 consecutive days. Melamine and cyanuric acid in serum, urine and kidney were determined by liquid chromatography-mass spectroscopy. In the groups of both melamine and cyanuric acid alone, they reached the maximal serum concentration at 1 hr after dosing. Melamine and cyanuric acid were eliminated almost completely within 24 hr without a sign of accumulation. On the other hand, they were not rapidly eliminated when combined. In sera, they were increased in dose- and time-dependent manners with the sign of accumulation. The sign was also seen in kidney. In addition, the combined treatment could induce severe nephrotoxicity when compared to the group of either melamine or cyanuric acid alone. Taken together, the toxicokinetic characteristics suggested that the combination of melamine and cyanuric acid might be responsible for the severe nephrotoxicity which was not seen in either melamine or cyanuric acid alone. Supported by the grant from KFDA (09162KFDA542).

**891** An Updated PBPK Model for RDX (Hexahydro-1, 3, 5-Trinitro-1, 3, 5-Triazine) and Its Metabolites in Mice


RDX is a military explosive that has been detected at or near military bases and munitions plants and storage facilities in air, soil, and ground water. Mice, but not rats, exposed to RDX in the diet for 2 years show an increasing trend in the combined incidence of hepaticellular adenomas and carcinomas (Lish et al. 1984). Physiologically-based pharmacokinetic (PBPK) models can aid in interpreting toxicological data and extrapolations across dose, species and exposure routes, and a PBPK model for RDX has previously been developed for mice based on oral absorption of RDX from single gavage doses (Sweeney et al. 2012 Regul. Toxicol. Pharmacol., 2012 v. 66, p. 205-224). Recently the absorption, distribution, and biotransformation of RDX administered to mice in feed for 28 days has been measured by Pan et al. (Environ. Toxicol. Chem., 2013 v. 32, p. 1295-1303). These new toxicokinetic data were used to evaluate the PBPK model of RDX in mice. The PBPK model was modified to account for absorption of RDX from feed and the metabolism of RDX to N-nitroso compounds in the GI tract. The resulting model simulations are consistent with experimental measurements of RDX concentrations in plasma, liver and brain tissues, reported by Pan et al. (2013). The model is used to evaluate correlations between the internal dose metrics of RDX and its metabolites with tumor incidence in mice. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency or other affiliations.

**892** Scoping the Need for PBPK Modeling of Child-Adult Metabolism Differences: Case Studies Applying an Enzyme Ontogeny Database


Knowledge of the ontogeny of Phase I and Phase II metabolizing enzymes could be essential in determining internal dose and risk of xenobiotic exposure to children in comparison to adults. Early life ontogeny of metabolizing systems has been considered in therapeutic drug monitoring, animal toxicology, gene expression studies, and physiologically-based pharmacokinetic (PBPK) modeling; however, this body of literature has not previously been organized into a single database. We created an Enzyme Ontogeny Database (EOD) for selected Phase I and Phase II enzymes commonly encountered in xenobiotic metabolism. This EOD can be used to screen child-adult metabolism differences and whether such differences should be the focus of PBPK modeling. This scoping function was exemplified with case studies of 5 chemicals, 3 of them (acetaminophen, tolenule, and chlorpyrifos) were proof of concept examples because they have sufficient data to know whether enzyme ontogeny affects risk and two (trichloroethylene and aromatic amines) demonstrate how to prioritize with limited data. This analysis demonstrated that early life stages are likely to be more vulnerable to tolenule and chlorpyrifos, but not to acetaminophen. Scoping for both trichloroethylene and aromatic amines indicates immaturities in cytochrome P450 enzymes (2E1 & 1A2) and conjugation systems (glutatione and N-acetylation) that may tend to offset one another. However, these predictions need to be further explored with PBPK modeling. The EOD combined with knowledge of chemical metabolism and mechanism of action may provide the basis for scoping as well as any PBPK analyses needed to determine whether children are at heightened risk due to metabolic factors.

Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA.

**893** Distribution of Capceitabine and Its Metabolites 5’-Deoxy-5-Fluorocytidine, 5’-Deoxy-5-Fluorouridine, and 5-Fluorouracil in a Preclinical Mouse Model of Brain Metastases of Breast Cancer


Capceitabine is an oral triple prodrug of 5-fluorouracil (5-FU), which is successful against many types of systemic tumors. Capceitabine itself is approved for treatment of breast and colorectal cancer. However, there is no information on the distribution or uptake of capceitabine and its metabolites in brain metastases of breast cancer (BMBC). We investigated conversion and extent of exposure of capceitabine and its metabolites in brain metastasis and peripheral tumors in a mouse preclinical model. Dose levels that produce plasma levels matching those in humans were selected. Xeloda (capceitabine, 150 mg/kg) was administered orally to athymic NuNu mice bearing intracranial MDA-MB-231BR human breast cancer tumors. Tissue and plasma samples were collected from 0.5-8 h and compound levels were measured by LC-MS/MS. Enzymatic biotransformation of capceitabine and its metabolites in brain and tumor was also assayed in vitro. Improved uptake and conversion of capceitabine and its produg metabolites was observed in brain metastases compared to healthy brain. Integrated exposure of capceitabine and its nucleoside prodrugs in brain metastases were 12-39% of plasma level and 3.5-fold higher than healthy brain (P<0.05). The 5-FU levels in brain metastases were 6.7 times higher than in brain (P<0.05) with Cmax approaching values associated with antitumor activity in vitro. Cytidine deaminase and thymidine phosphorylase enzymes activities were higher in intra-cranial tumor than healthy brain. The results demonstrate that capceitabine and each of its produg metabolites exhibit improved uptake and distribution in BMBC relative to normal brain.

**894** Disposition of the Actinide Chelating Agent 3, 4, 3-LI(1, 2-HOPO) in Swiss-Webster Mice Using C-14 As a Radio- Tracer


In a continuing effort to remediate the harmful effects of internally deposited radioactive actinides, our research group is investigating a linear octadentate spherand based ligand, 3,4,3-LI(1,2-HOPO), for clinical application. In order to
comprehensively evaluate the disposition and tissue pharmacokinetics of this compound, 3,4,3-Li(1,2-HOPO) labeled with C-14 was administered to healthy, male and female Swiss-Webster mice by oral, intraperitoneal or intravenous route (dose level = 100 μmol/kg; C-14 activity = 100 μCi/kg). Plasma, kidney, liver, urine, and feces were collected over a course of 24 hours post single administration and analyzed using liquid scintillation counting (LSC). The study demonstrated that the ligand was rapidly distributed and cleared from tissues and was predominantly excreted through the biliary route. In order to improve the oral bioavailability of the ligand, the compound was formulated with a permeability enhancer and was administered by oral route. The maximum concentration (Cmax) of the ligand in plasma improved by 3-fold when administered with a permeability enhancer.

Additional toxicity studies were performed; no toxic effects were observed in mice at the highest administered oral dose (1800 μmol/kg). The results clearly show that 3,4,3-Li(1,2-HOPO) exhibits promising activity and merits further investigation.

**895 Effect of Altitude on Tissue Distribution of Toluene in S-D Rats**

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Extreme altitude is one of the unique stressors experienced by pilots of high performance combat aircraft. Under conditions associated with the operation of high performance combat aircraft, children ages 1 to 3 years generally have greater exposure to pyrethroids due to their higher hand-to-mouth behavior. Differences in the pharmacokinetics between children and adults may influence their relative sensitivity to pyrethroids. The tissue to blood partition coefficients (Krb) for pyrethroids in adults rats have been reported, there are no studies reporting Krb in humans. Thus, we determined the Krb of the commonly used pyrethroids, DLM, CIS and TRANS in adults and 21-day old pups. Krb values for DLM in 21-day old pups at 72 hr ranged from 0.4 to 0.7 for brain, liver and muscle, and were 4.6 for skin. The Krb values for adults were similar to those in pups, with the exception that the Krb for adult skin was 29.0, and that a Krb for fat was determined, which was 102.3. Krb values for CIS in 21-day old pups were 2.8 for brain, 1.4 for liver, 2.8 for muscle and 8.2 for skin. This was in contrast to 1.8 for brain, 0.8 for liver, 8.9 for muscle, 140.0 for fat and 47.3 for skin in adults. The Krb values for TRANS in brains of 21-day old pups were lower than adults (0.4 Vs. 2.9), as were the Krb values for the liver (0.4 Vs. 6.5). In contrast, the Krb for TRANS for muscle (7.6) and skin (43.5) in 21-day old pups were higher than adults (1.8 for muscle and 11.7 for skin). The fat Krb in adults for TRANS was 67.0. Krb values for fat in 21-day old pups were not able to be calculated for any pyrethroid due to the small amount of this tissue in this age group. These data show the novel finding that the Krb for several common pyrethroids differ between adults rats and 21-day old pups. It is unknown if such differences contribute to age-dependent differences in the toxicokinetics of these pesticides.

**896 Airborne PCB11 Does Not Bioaccumulate: The Fate of 14C-Labelled PCB11 and Its Metabolites In Vivo**

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Backgound: The production ban of polychlorinated biphenyl (PCB) technical mixtures has left the erroneous impression that PCBs exist only as legacy pollutants. Some lower-chlorinated PCBs are still being produced and contaminate both mixtures has left the erroneous impression that PCBs exist only as legacy pollutants.

Recent advancements in understanding the pharmacokinetics of pyrethroids have provided new insights into the tissue distribution of these compounds. These data show the novel finding that the Krb for several common pyrethroids differ between adults rats and 21-day old pups. It is unknown if such differences contribute to age-dependent differences in the toxicokinetics of these pesticides. Background: This embryofetal development studies in mice with Fc-containing biopharmaceuticals revealed lower drug exposure in pregnant mice than nonpregnant mice, lower maternal exposure in late gestation than early gestation and higher fetal exposure than maternal exposure at gestation day [GD]18. Methods: Pregnant mice received subcutaneous injections of one of two murine surrogate monoclonal antibodies (mAb A [IgG2] 30 or 300 mg/kg or mAb B [IgG1] 300 mg/kg) on GD6 and GD13. PK profiles for mAb A was compared to nonpregnant mouse. PK profiles were also compared between GD6 and GD13 doses. At GD18, fetal and maternal (F/M) exposure ratio was assessed. Results: mAb A exposure (AUC) after GD6 dose was 48-54% lower in pregnant mice compared to nonpregnant mice, which was primarily attributed to more rapid clearance. For both mAb A and mAb B, clearance was also enhanced in late gestation compared to early pregnancy (AUC[GD13-18] for mAb A and mAb B was 39-65% and 52% lower than AUC[GD6-12], respectively). Anti-drug antibody development did not impact exposure. At GD18, fetal exposure was higher than maternal exposure (F/M ratio was 8.2-29 for mAb A and 29 for mAb B). Fetal plasma concentrations on GD18 for mAb A and mAb B were 51.5-258 and 111 μg/mL, respectively, which were within the pharmacologically relevant range for both mAbs. Conclusion: Clearance of murine IgG1 and IgG2 mAbs was more rapid in pregnant mice than nonpregnant mice. The rapid clearance is further enhanced in late gestation compared to early stage, leading to high F/M exposure ratios in late gestation. However, for conclusions related to placental transfer of mAbs in mice and pharmacological relevance to the fetus, it is more important to consider the absolute fetal plasma concentration.
First, the inhibition of P-glycoprotein (Pgp/ABC1), Breast Cancer Resistance Protein (BCRP/ABCG2) and Multidrug-Resistance Associated Protein 2 (MRP2/ABCC2) was investigated in inverted membrane vesicles from HEK293 cells over-expressing each of the ABC proteins. Inhibition profiles of prototypic substrate transport were obtained for 24 compounds and compared with results obtained in alternative expression systems. A good correlation between the HEK-MRP2 and S9-MRP2 vesicle data was observed. However, up to 40-fold lower IC50 values were obtained for Pgp and BCRP in the HEK vesicles compared to those in cellular expression systems. Next, estradiol 17β-glucuronide (E17G) was found to be a substrate for all three transporters. The contribution of each transporter to the E17G biliary efflux was investigated in HEK vesicles and in sandwich-cultured human hepatocytes (SCHH), using nine inhibitors with different specificities for the three ABC-proteins. The SCHH identified ABC inhibitors from the HEK vesicles that lost their inhibition in this physiologically more relevant model. Finally, the maximal transport activity (MTA) of each transporter was calculated and their contribution to the biliary clearance of E17G was predicted, using transport kinetics and protein expression. The canalicul efflux of E17G in SCHH was predicted with an accuracy of 90%.

**901a Toxicokinetics: Role in Improving Guideline Toxicological Studies for Agrochemicals**

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Dose is central to our understanding of biological responses and used in developing toxicity criteria and ADI. In guideline toxicity studies, top dose is selected based on MTD to provide a adequate margin of safety for human exposure. Responses at such high doses likely overwhelm physiological parameters (e.g., ADI) which, for low-risk chemicals, are not reached in human or MOE (animal = mg/kg vs human = μg or ng). Ideally, the top dose in animal studies would be selected based on the POD from linear kinetics of biomarker(s) in blood to obtain a kinetically-derived maximum dose (KMD). KMD ensures the use of appropriate doses, eliminates the generation of data at irrelevantly high doses and additional MOA studies, reduces animal use, and avoids unnecessary classification (e.g., carcinogen or developmental toxicant). Sulforaflo (an insecticide) doses for a 13-week dietary mouse study were based on the MTD of a 4-week study that showed nonlinear TK responses. Systemic dose (plasma AUC24h) became supra-linear in males and remained sublinear in females between the mid and the high doses with only producing the MTD. The KMD was used to select the top dose (which was 1.1x10 fold higher than expected human exposure) for the chronic mouse study resulting in TK linearity across doses, avoiding possible nonspecific toxicity. The plasma AUC24h of 2,4-D (a herbicide) became nonlinear at doses ≥25 mg/kg/day in F0 female rats after 28 days of dietary exposure with higher nonlinearity during pregnancy and lactation due to increased dietary intake. In pups of both genders, TK became nonlinear at maternal doses of ≥40 mg/kg/day. Based on these data, the top dose was selected slightly above the KMD (~50 mg/kg/day; 8×10 fold higher than expected human exposure) for the subsequent extended one-generation reproductive toxicity study. This dose was 1/2 of the MTD and accepted by EPA and PMRA. Use of the KMD-derived MOE avoided the identification of a NOEL, which would have resulted from the saturation of OAT1-dependent elimination at MTD-derived doses. Additionally, this procedure allowed the removal of 3X safety factor.

**901b Fates of Two Emerging Brominated Flame Retardants, 2-Ethylhexyl Tetrabromobenzene and Bis(2-ethylhexyl) Tetrabromophthalate, in Female Sprague-Dawley Rats**


2-ethylhexyl tetrabromobenzene (TBB; MW 550 g/mol) and bis(2-ethylhexyl) tetrabromophthalate (TBBP; MW 700 g/mol) comprise the brominated components used in several ‘alternative’ flame retardant mixtures (Firemaster 550, BZ-54, DP-45) introduced after the phase-out of lower-brominated polybrominated diphenyl ether (PBDE) mixtures. As replacements for ‘Penta’ PBDE flame retardants, TBB & TBBP are used in a wide variety of consumer products, most notably in polyurethane foams. TBB & TBBP have been detected in household dust, surface waters, adsorbed to sediment, and air samples. [14C]-labeled TBB was administered to female Sprague Dawley rats by gavage at 0.1, 1, 10, or 100 μmol/kg (6 or 25 μCi/kg). [14C]-TBBP was administered at 0.1 or 10 μmol/kg. TBB is extensively absorbed, readily metabolized, and eliminated by both urinary and fecal routes. More than 80% of administered radioactivity was recovered after 24 h at doses up to 10 μmol/kg, with fecal recoveries increasing with dose (24 h: 33±5→350%; 72h: 39±5→81±6%). Urinary recovery peaked between 8 and 24 h at these doses. Administration of 100 μmol/kg TBB resulted in a clear delay in both urinary and fecal recoveries, indicating saturation of elimination pathways. [14C]-radioactivity recovery exceeded 93% by 72 h after all doses and
retention of [14C]-radioactivity in tissues was minimal. HPLC analyses of urinary [14C]-radioactivity detected up to 6 metabolite peaks. The number and relative proportion of each metabolite present in urine were time and dose-dependent. The principal moieties present in fecal extracts corresponded to parent TBB and tetrabromobenzoic acid (TBPH). TBPH was poorly absorbed and was primarily eliminated as parent compound in the feces at both doses tested. These findings indicate that while TBB & TBPH are structurally similar, they have very different disposition. This work was supported by the Intramural Research Program of the National Cancer Institute at the National Institutes of Health (Project ZIA BC 011476).

**901c Durable Pharmacological Responses from the Peptide ShK-186, a Specific Kv1.3 Channel Inhibitor That Suppresses T Cell Mediators of Autoimmune Diseases**

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The Kv1.3 channel is a recognized target for drug development to treat autoimmune diseases and organ rejection. ShK-186, a specific peptide inhibitor of Kv1.3, has shown promise in animal models of both multiple sclerosis and rheumatoid arthritis. Here, we describe the pharmacokinetic/pharmacodynamic (PK/ PD) relationship for ShK-186 in rats and monkeys. The PK profile was evaluated with a validated high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method to measure the peptide’s concentration in plasma. These PK results were compared with the single-photon emission computed tomography/computed tomography data for ShK-186 that were collected with an 111In-1,4,7,10-tetrazacyclododecane-1,4,7,10-tetraacetic-acid-conjugate of ShK-186 to assess whole-blood PK parameters as well as the peptide’s absorption, distribution, and excretion profiles. Analysis of these data support a model wherein ShK-186 is absorbed slowly from the injection site, resulting in blood concentrations above the Kv1.3 channel blocking IC50 value for up to 7 days in monkeys. PD studies on human peripheral blood mononuclear cells showed that brief exposure to ShK-186 resulted in sustained suppression of cytokine responses and may contribute to prolonged drug effects. Delayed-type hypersensitivity, chronic relapsing-remitting experimental autoimmune encephalomyelitis, and pristane-induced arthritis rat models all show that single doses of ShK-186 every 2 to 5 days are as effective as a daily administration. ShK-186’s slow distribution from injection sites and its long Kv1.3 channel residence time contribute to the prolonged therapeutic effects of ShK-186 in animal models of autoimmune disease.

**901d Comparison of the Disposition and Pharmacokinetics of 14C-Octamethylcyclotetrasiloxane (D4) following a Single Low Dose in Rodent Liquid Diet or a High Dose in Corn Oil via Oral Gavage to Fischer 344 Rats**


The absorption, distribution, metabolism and excretion of D4 in Fischer 344 rats following a single oral gavage administration of a low dose, 30 mg 14C-D4/kg, in a rodent liquid diet or a high dose, 300 mg 14C-D4/kg, in corn oil were evaluated. Parent D4 and total radioactivity were measured in blood through 168 h post-dosing and in various tissues. Animals were housed in metabolism cages for collection of urine, feces, expired volatiles (EV) and CO2. Blood AUCs were not proportional to dose. About 25% more of the dose was absorbed following the low dose administration with a greater % of radioactivity compared to parent than in the high dose study. Parent D4 and radioactivity were measurable in all tissues with the highest concentration found in fat and majority of the recovered radioactivity being eliminated by 24 h post-dosing following either dose. Following a high dose in corn oil (females), 41% of the recovered dose was found in feces, 26% in urine, 14% in EV, 4% in CO2 and 8% in carcass. Following a low dose in rodent liquid diet (females), 23% of the dose was found in feces, 32% in urine, 30% in EV, 3% in CO2 and 11% in carcass. An increase in the % of dose in EV and a decrease in the % of dose in feces was noted in the low dose study with metabolites being present in both feces and EV. Urinary elimination consisted entirely of polar metabolites for either dose. Metabolism appeared to be increased following low dose administration driving a greater absorption. Since an earlier PBPK model based on the high dose study did not adequately describe the parent’s concentration and was primarily used as parent compound in the feces at both doses tested. These findings indicate that while TBB & TBPH are structurally similar, they have very different disposition. This work was supported by the Intramural Research Program of the National Cancer Institute at the National Institutes of Health (Project ZIA BC 011476).

**901e Mass Spectrometry Imaging in Toxicology Study: Biodistribution of Unlabeled Bleomycin in Induced Interstitial Pulmonary Fibrosis (IPF) Model**

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One of the main difficulties in toxicology study is to identify the potential causes of the histopathologic/functional tissue changes. Mass spectrometry imaging (MSI) technology has been used to address these crucial issues. The main advantage of this label free imaging technique is the detection of all molecules of interest directly on-tissues with high specificity. Indeed the molecular distribution of some unlabeled targeted molecules could be directly correlated with some histopathologic and functional tissue changes. MSI was used to identify the cause and to improve the understanding of the Bleomycin interstitial pulmonary fibrosis rat model. Experimental procedures: Rats were administered seven doses of bleomycin delivered to lung by the oropharyngeal aspiration route and followed for 7 days. Control animals received seven doses of saline. Three control and three treated animals were sacrificed and lungs were collected. Several fresh sections were prepared and then the distribution of the Bleomycin was determined by high resolution mass spectrometry imaging with a MALDI-FITCR. The images were generated by Quantinetix software.

Results: This work illustrates a technique for identifying the causes of some histopathologic and functional tissue changes correlated with a drug administration in toxicology study. We describe a process which combines MSI and classical staining approach directly on tissue to follow the distribution of targeted molecules implicated in a fibrosis model generation, and allows a better understanding of the toxicity model. Conclusions: We have demonstrated the use of Mass Spectrometry Imaging to follow the distribution of a non-labeled drug and to understand its application in interstitial pulmonary fibrosis model generation. Mass Spectrometry Imaging could also allow the detection of other important endogenous molecules in the same or adjacent tissue section, such as specific disease or toxicity markers, thus allowing further understanding of PK-Tox relationships.

**901f A Simplified and Reliable Capillary Microsampling Procedure to Support Preclinical Regulatory Studies in Rodents**


1Debiopharm International SA, Lausanne, Switzerland and 2Covance Laboratories Ltd, Harrogate, United Kingdom. Sponsor: A. Jackson.

Until recently, blood sampling in rodents for toxicokinetic (TK) assessment required separate satellite groups in order to obtain complete TK profiles. Due to blood volume limitations, especially so in mice, these profiles, composed of samples taken from different animals at each time point, resulted in an apparent average systemic exposure not reflecting individual exposure. Serial sampling from toxicity animals is preferable over the use of separate satellite groups, not only to correlate individual animal exposure to observed toxicity but also to reduce the overall number of animals. The recent introduction of highly sensitive mass spectrometry into routine sample analysis has enabled drug concentrations to be measured from a significantly reduced plasma volume allowing the move towards low volume blood sampling (<100 μL).

The new technique proposed involved, at the initial blood sample processing stage, that the plasma remaining within the original sampling capillary after centrifugation is placed directly into a labeled holding tube for storage and transportation. At the bioanalytical laboratory, the sample is thawed and a measured volume (currently 10 μL) removed by pipette and subsequently diluted as part of the pre-analysis procedure. This removes the need for the error-prone original capillary to volumetric micro-capillary plasma transfer, thereby reducing the sample processing time and allowing all the plasma harvested to be available for analysis. The data presented demonstrate the feasibility of this approach in the frame of a 4 week GLP toxicity study in mice from which good TK profiles were obtained by taking serial samples (5 time points, blood volume ≤60 μL) from 4 mice/group/sex only. Moreover, the addition of a single blood sample from the toxicity animals at Cmax allowed observed toxicity in individual animals to be correlated directly with exposure. In addition to producing good quality data, the method also follows the principles of 3Rs by importantly reducing the number of mice required for TK.
The AhR hydrocarbon receptor (AhR) is involved in the regulation of immune responses, T-cell differentiation and immunity. Here we show that inflammatory stimuli such as lipopolysaccharide (LPS) induce the expression of AhR in human dendritic cells (DC) associated with an AhR-dependent increase of Cytochrome P450 1A1 (CYPIA1). In vivo data confirmed the induction of AhR by LPS and the LPS-enhanced 2,3,7,8-tetrachlorodibenzodioxin (TCDD)-mediated induction of CYPIA1 in thymus of B6 mice. Inhibition of nuclear factor-kappa B (NF-kB) repressed both normal and LPS-enhanced, TCDD-inducible, AhR-dependent gene expression and canonical pathway control of RelA regulated AhR-responsive gene expression. LPS-mediated induction of AhR was NF-kB-dependent as shown in mouse embryonic fibroblasts (MEFs) derived from Rel null mice. AhR expression and TCDD-mediated induction of CYPIA1 was significantly reduced in RelA deficient MEF compared to wildtype MEF cells and ectopic expression of RelA restored the expression of AhR and induction of CYPIA1 in MEF RelA null cells. Promoter analysis of the human AhR gene identified the putative NF-κB-binding elements upstream of the transcription start site. Mutation analysis of the AhR promoter identified one NF-kB site as responsible for mediating the induction of AhR expression by LPS and electrophoretic shift assays demonstrated that this NF-kB motif is recognized by RelA/p50 heterodimer. Our results show for the first time that NF-kB RelA is a critical component regulating the expression of AhR and the induction of AhR dependent gene expression in immune cells illustrating the interaction of AhR and NF-kB signaling.

**903 Toxicogenomic Evaluation of Dose-Derpendent TCDD-Elicited Effects in the Jejunal Epithelium of C57BL/6 Mice**

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2,3,7,8-Tetrachlorodibenzodioxin (TCDD) exerts its effects primarily through activation of the aryl hydrocarbon receptor (AhR). We have previously demonstrated that dietary fat rather than de novo synthesis is the primary lipid source in TCDD-elicited hepatic steatosis in C57BL/6 mice. This study examined the dose-dependent effects of TCDD (0.01, 0.03, 0.1, 0.3, 1, 3, 10 or 30 μg/kg body weight [bw]) on gene expression in the jejunum of mice orally gavageyed every 4 days for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified 439 genes differentially expressed (fold change ≥ 1.5, P(1) ≥ 0.099) across one or more doses. Dose-response modeling (ToxResponse Modeler) identified 264 for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified dose-dependent effects of TCDD (0.01, 0.03, 0.1, 0.3, 1, 3, 10 or 30 μg/kg body weight [bw]) on gene expression in the jejunum of mice orally gavageyed every 4 days for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified 439 genes differentially expressed (fold change ≥ 1.5, P(1) ≥ 0.099) across one or more doses. Dose-response modeling (ToxResponse Modeler) identified 264 for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified dose-dependent effects of TCDD (0.01, 0.03, 0.1, 0.3, 1, 3, 10 or 30 μg/kg body weight [bw]) on gene expression in the jejunum of mice orally gavageyed every 4 days for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified 439 genes differentially expressed (fold change ≥ 1.5, P(1) ≥ 0.099) across one or more doses. Dose-response modeling (ToxResponse Modeler) identified 264 for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified dose-dependent effects of TCDD (0.01, 0.03, 0.1, 0.3, 1, 3, 10 or 30 μg/kg body weight [bw]) on gene expression in the jejunum of mice orally gavageyed every 4 days for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified 439 genes differentially expressed (fold change ≥ 1.5, P(1) ≥ 0.099) across one or more doses. Dose-response modeling (ToxResponse Modeler) identified 264 for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified dose-dependent effects of TCDD (0.01, 0.03, 0.1, 0.3, 1, 3, 10 or 30 μg/kg body weight [bw]) on gene expression in the jejunum of mice orally gavageyed every 4 days for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified 439 genes differentially expressed (fold change ≥ 1.5, P(1) ≥ 0.099) across one or more doses. Dose-response modeling (ToxResponse Modeler) identified 264 for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified dose-dependent effects of TCDD (0.01, 0.03, 0.1, 0.3, 1, 3, 10 or 30 μg/kg body weight [bw]) on gene expression in the jejunum of mice orally gavageyed every 4 days for 28 days. Agilent 4 x 44K microarray analysis of jejunal epithelium identified 439 genes differentially expressed (fold change ≥ 1.5, P(1) ≥ 0.099) across one or more doses. Dose-response modeling (ToxResponse Modeler) identified 264 for 28 days. Agil...
196a which has been shown to control proliferation associated with the expression of p27kip1. Hence, we propose that AhR-dependent regulation of miR-196a promotes proliferation by inhibiting the p27kip1 expression. Methods: AhR+/+ and AhR−/− mouse lung fibroblasts were treated with B[a]P (1μM) with or without the AhR antagonist CH-223191 (10 μM) and miR-196a assessed by qRT-PCR. miR-196a was also assessed in A549 cells where AhR expression was knocked-out using zinc finger nuclease technology. p27kip1 and AhR protein levels were assessed by western blot. Proliferation was evaluated by thymidine incorporation. Human lung fibroblasts (HFLs) derived from smoker or COPD individuals were used for AhR knockdown and miR-196a analyses. Results: AhR−/− cells (lung fibroblast and A549 cells) have a significant decrease in basal miR-196a expression compared to control cells. Both B[a]P and CH-223191 decreased miR-196a and AhR protein expression. AhR expression promoted proliferation concurrent with a reduction in p27kip1 protein. Finally, AhR protein levels in HFLs were significantly less in COPD cells as was miR-196a.

Conclusions: We report miR-196a regulation as a potential novel endogenous role for the AhR. The regulation of p27kip1 could also be how the AhR controls cell proliferation. Elucidating the role of the AhR in cell cycle progression may provide novel therapeutic options for lung diseases which have significant alterations in proliferation, such as COPD.

907 Ligand Promiscuity of Aryl Hydrocarbon Receptor Agonists and Antagonists Revealed by Site-Directed Mutagenesis

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The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that can be activated by structurally diverse chemicals. Although AhR ligand-binding promiscuity appears to be determined by distinct binding mechanisms within the ligand-binding pocket, direct experimental evidence is lacking. To examine the mechanisms responsible for the promiscuity in AhR ligand binding, we determined the effects of mutations within the mouse AhR ligand-binding domain (LBD) on the activity of diverse AhR ligands. AhR point mutations were generated using site-directed mutagenesis. Mutant AhR proteins were expressed in vitro and their ligand-dependent DNA binding was analyzed by gel retardation assay and functional activity confirmed by analysis of reporter gene expression in transfected cells. Site-directed mutagenesis identified Ile319, and to a lesser extent Phe318, as residues involved in ligand promiscuity and specificity of ligand binding, agonist or antagonist mode of ligand binding and hsp90 binding and provide further insights into molecular determinants of ligand activation of the AhR. Supported by the NIHES (R01ES07685).

908 The Ah Receptor Recruits IKK-alpha to Phosphorylate Ser10 in Histone H3 of the Cyp1a1 Promoter Chromatin

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Halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) cause many types of toxicity via the aryl hydrocarbon receptor (AhR). One of the best known proteins induced by TCDD is the cytochrome P450 Cyp1a1. Induction of this enzyme requires AhR ligand-dependent activation, followed by binding of the activated AhR to AHR responsive elements (AhRE) in the promoter region of the Cyp1a1 gene. Histone modifications are well-known epigenetic markers modulating the activity of transcription factors by alteration of chromatin states. Among histone modifications, phosphorylation of serine-10 in histone H3 (H3S10ph) by various kinases has been reported to play a role in mitosis. Prior work from this lab has shown that the level of H3S10ph in the AhRE enhancer region of the Cyp1a1 promoter was elevated by AhR activation, which led us to search for responsible kinases that would be recruited by the ligand-activated AhR. To search for candidate kinases that phosphorylate H3S10ph in an AhR dependent manner, we performed chromatin immunoprecipitation (ChIP) assays for the enhancer region of Cyp1a1 in TCDD-treated Hepa-1 cells and C35 (AhR mutant) cells by using antibodies for AhR, H3S10ph and various kinases previously reported to phosphorylate this site. We found that binding of a subset of kinases, including Ikkα, MSK1 and MSK2, to the Cyp1a1 enhancer region was absent in control cells and significantly increased by TCDD treatment in an AhR dependent manner. In addition, Cyp1a1, Ahldh3a1 and Nqo1 mRNA induction, and the level of H3S10ph in the Cyp1a1 enhancer region of in TCDD-treated cells transfected with IKKα-shRNA was significantly decreased in comparison to TCDD-treated cells transfected with a scrambled-shRNA. We conclude that IKKα is recruited by AhR to the Cyp1a1 enhancer region and that phosphorylation of H3S10 is a main requirement for Cyp1a1 induction. Supported by Nih grant R01 ES 06273

909 RNA-Seq Analysis Reveals Roles for Aryl Hydrocarbon Receptor in Endogenous Lipid Synthesis and TNF Signaling

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Aryl hydrocarbon receptor is a major xenobiotic sensing transcription factor involved in the metabolism of toxicants. This study uses RNA-seq analysis to further characterize endogenous AhR roles in the absence of xenobiotics in human MCF-7 breast cancer cells. Reducing AHR with short interfering RNA significantly reduced AHR levels compared to control cells. AHR knockdown induced CYP1A1. Induction of this enzyme requires AHR ligand-dependent activation statistically (false discovery rate < 10%) significant changes in the expression of 634 genes compared to controls. Ingenuity pathway analysis identified that differentially expressed genes in AHR knockdown cells were significantly associated with several processes that are important for endogenous lipid processing including: conjugation of lipid, conjugation of 12-hydroxyicosatetraenoic acid, glucuronidation of leukotriene B4, synthesis of lipid, fatty acid metabolism, metabolism of eicosanoids, metabolism of prostaglandins, synthesis of fatty acid, synthesis of prostaglandins and synthesis of eicosanoids. Based on the patterns of expression of differentially expressed genes, the activity of tumor necrosis factor (TNF) was predicted to be inhibited in AHR knockdown cells. TNF responsiveness was further investigated by treating cells with TNF and measuring the induction of TNF targets p53, p21 and the antioxidant manganese superoxide dismutase (MNSOD). AHR knockdown significantly compromised TNF induction of MNSOD protein. Collectively, these results suggest that AHR (1) is active in the absence of exogenous AhR ligands (2) could play a role in signaling by controlling the expression of genes that generate bioactive intracellular lipids and (3) modulate cellular responses to intracellular reactive oxygen species (ROS).
Using Three-Dimensional Cell Culture to Understand Ethanol Toxicity in Mammary Cells

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Mechanistic studies are best performed in highly controlled experimental models such as cell culture. Yet cell monolayers (2D) are generally only useful for acute exposure to toxicants and are not representative of the cellular environment found within an organism as most physiological parameters are lost. Three-dimensional (3D) cell culture is reported to better represent the in vivo structure of tissues and tumours regarding cell shape and their environment, thus culturing in 3D is suggested to decrease the gap between cell culture and physiological tissue.

The current study aimed to determine whether 3D cell culture provides a more in vivo-like model of ethanol-induced toxicity to mammary cells. MCF-7 cells in 2D or 3D (spheroid) culture were exposed to a dose range (1-100mM) of ethanol for an acute (24-72h) or chronic repeat dose (10 cycles of 72h treatment, 3D only) study following which proliferation, migration, spheroid size, signal transduction and gene expression were measured.

Acute exposure of 2D MCF-7 cells to ethanol increased proliferation dose and time-dependently over 72 h. Cell migration was also increased at 100mM compared to control. Acute exposure of 3D MCF-7 spheroids to ethanol (up to 100mM) showed a trend for increased signal transduction (p-ERK ratio) consistent with increased proliferation.

Chronic repeated exposure of spheroids to ethanol dose-dependently decreased spheroid size by cycle 5. With continued exposure to low doses of ethanol (1-100mM) the decrease in size was reversed. Total cell number, however, was unaffected by treatment. At cycle 10 signal transduction (p-ERK ratio) was reduced for all treatments in line with rat in vivo data, where continuous/intermittent alcohol exposure (Sanna et al. Brain Res 948 P186 2002) and chronic ethanol exposure (45 days) (Sampere et al. J Biol Chem 282 P1925 2010) decreased levels of p-ERK. In contrast there was a trend for induction of the oncogenic microRNA miR-21 (anti-apoptotic) at cycle 10. These data indicate that chronic ethanol exposure induces phenotypic change in 3D cultured mammary cells that has similarities to ethanol exposure in vivo.

Transcriptome Analysis of PXR-Regulated Gene Expression in HepG2 Cells

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Pregnane X receptor (PXR, NR1I2) is a ligand-dependent nuclear receptor (NR) that functions as a xenobiotic sensor and effector in coordinately regulating expression of genes of the xenobiotic detoxification network. PXR exerts its transcriptional regulatory functions by dimerization with retinoic X receptor (RXR), and PXR-RXR complex binds to specific DNA sequences for regulating gene expression. Whole-genome transcriptome analysis based on next generation sequencing technology (RNA-Seq) provides unprecedented opportunity to study the PXR-regulated gene expression. PXR-HeptG2 cells were generated by stable transfection of PXR gene in human liver cell line HepG2 and treated with PXR ligand rifampicin (10 μM, 48 hr). PolyA RNA was isolated and the samples were subjected to RNA-Seq analysis. We have found around 2000 genes were significantly up-regulated by ligand treatment in PXR in a PXR-dependent manner, including classic PXR-regulated phase I enzymes such as CYP3A family, phase II enzymes UGT1A family and GST1As. Interestingly, we have identified PXR-regulated novel intergenic noncoding RNAs that were significantly regulated by ligand-activated PXR. In addition, we have identified alternative RNA splicing process in certain genes appears to be regulated by PXR. Our results suggest that RNA-seq through next-generation sequencing technology (RNA-Seq) provides unprecedented opportunity to study the PXR-regulated phase II enzymes UGT1A family and GST1As. Interestingly, we have identified PXR-regulated novel intergenic noncoding RNAs that were significantly regulated by ligand-activated PXR. In addition, we have identified alternative RNA splicing process in certain genes appears to be regulated by PXR. Our results suggest that RNA-seq through next-generation sequencing technology (RNA-Seq) provides unprecedented opportunity to study the PXR-regulated gene expression which may lead to a predisposition to carcinogenesis in the lung. F0 female C57BL/6 mice (Mus musculus) were exposed to 0, 10, 50 or 500 ppb sodium arsenite in their drinking water throughout mating, gestation and nursing. F1 generation mice were exposed to arsenic only during gestation and nursing. F2 mice were not directly exposed to arsenic. The expression of three cell cycle regulatory genes, Foxm1, Cdc25s and Cdc6, from the lung tissue of F1 and F2 mice was evaluated using quantitative real time PCR (qRT-PCR). Preliminary findings showed a significant change in expression of Foxm1 and Cdc6 at doses as low as 10 ppb. Histological findings suggest arsenic exposure induce inflammation in lung tissues. These results suggest low-dose transplacental arsenic exposure alters the transcription levels of several genes involved in cell cycle regulation. Our findings contribute an important insight toward the identification of a mechanism for cell cycle deregulation, following exposure to low doses of arsenic.

Low-Dose Transplacental Arsenic Exposure Alters Expression of Cell Cycle Genes in Lung Tissues of C57BL/6 Mice

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Arsenic is an environmental toxicant associated with several human diseases including cancer. The current EPA and WHO maximum allowable level for arsenic in drinking water is 10 ppb. This level is often exceeded in private wells in the state of Maine and many places world-wide, and poses a potential health concern. Molecular mechanisms of arsenic-induced carcinogenesis are still not well defined. In this study, we hypothesized that transplacental arsenic exposure alters cell cycle regulatory gene expression which may lead to a predisposition to carcinogenesis in the lung. F0 female C57BL/6 mice (Mus musculus) were exposed to 0, 10, 50 or 500 ppb sodium arsenite in their drinking water throughout mating, gestation and nursing. F1 generation mice were exposed to arsenic only during gestation and nursing. F2 mice were not directly exposed to arsenic. The expression of three cell cycle regulatory genes, Foxm1, Cdc25s and Cdc6, from the lung tissue of F1 and F2 mice was evaluated using quantitative real time PCR (qRT-PCR). Preliminary findings showed a significant change in expression of Foxm1 and Cdc6 at doses as low as 10 ppb. Histological findings suggest arsenic exposure induce inflammation in lung tissues. These results suggest low-dose transplacental arsenic exposure alters the transcription levels of several genes involved in cell cycle regulation. Our findings contribute an important insight toward the identification of a mechanism for cell cycle deregulation, following exposure to low doses of arsenic.

913 Development of a Nanoparticle Platform for the Targeted Delivery of siRNA to HER2-Positive Breast Cancers

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Successful siRNA based therapy has the potential to revolutionize cancer therapy by mediating the silencing of any gene deemed important for disease progression. We are developing an effective siRNA based nanoparticle platform that would overcome traditional shortcomings of siRNA based therapies such as poor bioavailability and off-target effects. The platform utilizes a mesoporous silica nanoparticle electrostatically loaded with siRNA and conjugated with an antibody for target homing. The human epidermal growth receptor type 2 (HER2) is a highly validated therapeutic target in breast cancer due to its prognostic role in cancer aggressiveness and drug resistance when overexpressed in tumors. Suppression of HER2

914 Hormonal Changes and Gene Signaling Pathways in Female Rat Thyroids Exposed to Acrylamide

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In this study, we used biological endpoints and transcriptomics to identify molecular events associated with acrylamide (AA) exposure. Pregnant female RccHan Wistar rats were treated with 3 mg AA/kg in drinking water from gestational day 6 to postnatal day 30. Thyroid glands were collected from female pups at 10 AM and at 10 PM. While control animals showed no statistical difference in serum levels of TSH, T3 and T4 between AM and PM, animals exposed to AA and collected in the AM showed significant increase in serum levels of TSH and PM animals showed significant increases in levels of T4 and T3, compared to controls. In terms of gene expression changes, there were fewer changes in the AM animals compared to PM animals, consistent with their increased activity and dietary patterns at night. PM animals treated with AA showed decreases in transcripts involved in metabolic activity such as insulin-like growth factor 2, transforming growth factor beta, and glucose-6-phosphate transporter compared to controls, while these same transcripts were not altered in AM animals. In addition, the PM animals showed up-regulation of transcripts involved in detoxification, oxidative stress, apoptosis, neurotoxicity, tumorigenesis, among others. But, there were also changes seen in morning animals that were specific to that time point such as up regulation of branched chain amino acid metabolism and aspartate metabolism. There were commonalities among both morning and night animals which included up-regulation of transcripts related to the checkpoint pathways, DNA replication and sister chromatid cohesion, among others. This analysis indicates that the time of collection of animals is important in assessing how acrylamide affects gene expression in the thyroids of treated animals.
with siRNA based therapy (siHER2) offers a new treatment modality and provides a testable system for the development of our nanoparticle platform. Incorporation of Herceptin, a monoclonal antibody which binds to HER2, will serve to selectively target HER2-positive cancer cells. We first evaluated our nanoconstruct loaded with a highly optimized siHER2 directed against a portion of HER2, specifically in a mouse tumor model. The siHER2 loading efficiently inhibited growth in HER2-positive cell lines resistant to Herceptin and lapatinib. In vivo success translated well to tumor growth inhibition in a HCC1954 mouse tumor model following i.v. injection of our siHER2–nanoconstruct. Current efforts are directed toward immune cell function and hematology assays to generate an immunological safety profile of the nanoconstruct.

916 Pentoxifylline Induces GSK-3β-Independent Proteasomal Degradation of Cyclin D1 and Arrests Renal Cancer Cells in the G1 Phase

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Cyclin D1 is required for progression from the G1 phase into the S phase of the cell cycle. Over-expression of cyclin D1 causes an increase in cell cycle progression and proliferation, implicating it in a variety of tumors. Canonical cyclin D1 degradation is initiated by phosphorylation of Thr286 by GSK-3β. Pentoxifylline (PTX), a non-specific phosphodiesterase inhibitor, is used as an adjunct in chemotherapy to treat cachexia. We report that PTX causes a time- and dose-dependent decrease in cyclin D1 protein levels and G1 cell cycle arrest in QTRE-R and ACHN cells. RT-PCR analysis showed no significant changes in cyclin D1 mRNA. However, PTX’s ability to decrease cyclin D1 protein was prevented in the presence of a proteasome inhibitor, MG-132. Inhibition of GSK-3β with LiCl in the presence of PTX failed to rescue cyclin D1 levels. PTX increases the phosphorylation of Ser9 on GSK-3β, indicative of an inhibition of GSK-3β activity. Following siRNA knockdown of GSK-3β, PTX retains the ability to decrease cyclin D1 levels. Moreover, PTX treatment in the presence of MG-132 revealed no increase in phospho-Thr286 compared to control. Our data indicate that PTX initiates GSK-3β- and phospho-Thr286-independent proteasomal degradation of cyclin D1 and arrests the RCC cell models in the G1 phase. Because our findings demonstrate a novel anti-cancer property of PTX, its use as an adjuvant therapy in RCC treatment should be further explored.

917 Regulation of Intestinal Drug-Processing Genes in Germ-Free Mice

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Little is known regarding how the gut microbiome regulates the drug-processing genes (DPGs) in various sections of the intestine, which is an important metabolic organ for orally administered drugs. The goal of this study was to determine the regulation of DPGs in intestine of germ free (GF) mice. Total RNAs from duodenum, jejunum, ileum, and colon of adult male GF and Conventional mice (n=3 per group) were isolated, and the transcriptome was quantified using RNA-Seq (50 cycles paired end). Over 35 million reads were generated per sample, of which >96% were mapped to the mouse mm10 genome (TopHat). Among 23,846 genes in mice, jejunum had the largest number of differentially regulated genes (2514, FDR-BH<0.05), followed by colon (835), duodenum (435), and ileum (368). In duodenum, drug metabolism was among the top most differentially regulated networks in GF mice. In all 3 sections of the small intestine, Cyp1a1 was among the top most down-regulated genes within the transcriptome, suggesting that the aryl hydrocarbon receptor signaling is attenuated in the small intestine of GF mice. In duodenum and ileum, many of the Cyp2b, 2c and 3a mRNAs were down-regulated in GF mice, suggesting attenuated transcription of the pregnane X receptor and constitutive androstane receptor. In duodenum, there was a decrease in the mRNAs of glutathione-S-transferases, which are responsible for detoxifying electrophiles and oxidative stress. In jejunum, many alcohol dehydrogenases, aldo-keto reductases (Akrs), aldehyde dehydrogenases, and the Cyp4 family members, were up-regulated. In ileum, the apical sodium-dependent bile acid transporter (Asbt) and Akt1c19 were among the top most up-regulated genes in the transcriptome. In colon, most differentially regulated DPGs were up-regulated, exemplified by increased mRNAs of Asbt and organic solute transporter ct. In conclusion, our study was the first to quantitatively determine the regulation of all DPGs in GF mice, and identified that distinct sections of intestine respond differently to the absence of the gut microbiome.

918 Caudal-Related Homeodomian Protein 2 (Cdx2) and the Hepatocyte Nuclear Factor 4α (HNF4α) Synergize to Regulate UDP-Glucuronosyltransferase (UGT) 1A8 Gene Expression

S. N. Muhoro et al. Pharmacology, Flinders University, Adelaide, SA, Australia, University of Islam failed to deduce HER2 from the full-length transcriptome, and identified that distinct sections of intestine respond differently to the absence of the gut microbiome.

919 CYP251 Depletion Promotes Cell Growth in Human Lung Cells

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Cytochrome P450 251 (CYP251) expression is elevated in several epithelial derived cancers including squamous cell carcinoma. Although the expression pattern of CYP251 suggests an important physiological function, it is still considered as an orphan enzyme with unknown endogenous substrate. We reasoned that changes in CYP251 expression would initiate metabolic shifts in pathways linked to CYP251-mediated metabolism of endogenous substrates, and that these pathways can be identified through transcriptional alterations. CYP251 expression was depleted in human BEAS-2B cells, using RNA-interference targeting the 3′UTR (759) and exon 3 (984) of the CYP251 gene, and compared with a non-targeting shRNA control (SCRAM). Transcriptome analysis was performed on CYP251 depleted (759) and scrambled control (SCRAM) BEAS-2B cells using RNA-sequencing (RNA-seq). Pathway analysis of differentially expressed genes, using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) analysis, revealed pathways consistent with previously published endogenous substrates and phenotypes. Additionally, analysis also revealed significant changes within mTOR signaling in response to CYP251 depletion. mTOR signaling is involved in regulating cell growth. Consistent with changes in cell growth, both CYP251 depleted cell lines, 759 & 984, exhibited an approximate 10% increase in cell diameter and 50% increase in cell volume. Western analysis reveals post-translational modifications within the mTOR signaling cascade consistent with increased cell size in CYP251 depleted cells (759 & 984) compared with control (SCRAM). CYP251 depleted cells specifically exhibit increased phosphorylation of mTOR, and its downstream target 56 kinase. These data suggest that alterations in CYP251 expression, and presumably CYP251-mediated endogenous metabolism, influence cell size through regulation of mTOR signaling. We are currently pursuing the mechanistic link between CYP251-mediated metabolism of endogenous substrates and mTOR signaling.

SOT 2014 Annual Meeting 237
920 Functional Genetic Screen in Human Haploid Cells to Identify Genes Involved in Susceptibility to Chemical Exposure

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Functional genetic screening systems have been successfully applied to study susceptibility to chemical toxicity. However, some approaches have certain limitations, such as lack of relevance to humans of yeast mutant screen findings and incomplete gene knock-out and off-target effects of RNA interference. Human haploid cell models are advantageous as an induced gene mutation can result in a clear phenotype due to the absence of a second gene copy. We recently developed a more efficient semi-solid medium based screening platform that employs a human haploid cell mutant library (KB7-Mu) to identify genes that modulate sensitivity to chemical exposures. Compared to the liquid medium-based approach, our method allows for simultaneously screening and generating mutant colonies from cells resistant to the chemical of interest. This shortens the entire screening process by approximately 3 weeks and decreases the rate of false positives. Using this new approach, we identified eleven human genes that confer the resistance to formaldehyde (FA), a known human leukemogen. Among these genes, LPR5 (Low-density lipoprotein receptor-related protein 5), GOT1 (Glutamic-oxaloacetic transaminase 1) and M1AP (Meiosis 1 Associated Protein) were confirmed in two independent screening experiments. LPR5, GOT1 and M1AP mutant KB7 cells showed significant resistance to FA-induced toxicity compared to wild type cells (KB7-Wt). Further studies on LPR5 mutant KB7 cells using quantitative RT-PCR and western blotting confirmed the knockdown of transcription and knockout translation of the LPR5 gene. These findings suggest that LPR5, GOT1, M1AP and other genes are involved in susceptibility to FA toxicity. They further demonstrate the broad applicability of this optimized approach to screen genetic susceptibility to toxic chemicals, identify novel susceptibility genes, and gain insight into potential mechanisms of toxicity of chemical exposures.

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921 Carbon Monoxide Modulation of mRNA in Human Airway Smooth Muscle Cells

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Rationale: Asthma is a chronic inflammatory airway disease involving disregulation of human airway smooth muscle cell (HASMc) function; subsequently, asthma may be more susceptible to the adverse effects of inhaled pollutants, such as carbon monoxide (CO). CO is toxic at high concentrations due to asphyxia resulting from carboxyhemoglobin formation, but may also have non-hemoglobin related effects. Thus, we investigated the effect of CO exposure in HASMC, to determine its effect on intracellular message regulation.

Methods: Cultures of primary HASMC at passage 6 were serum-starved for 24hr, refed, and stimulated with 1ng/ml IL-1β for 2-10hr, or exposed to 500ppm CO for 8hr, following the 2hr stimulation period. Inflammatory cytokine and receptor mRNA levels were assayed by q-PCR; the significant modulation threshold was taken as ≥2.5-fold over controls. Results: CO was upregulated from 24- to over 2300-fold. Of the 84 messages we assayed in controls (IL-1β alone), Tnfβ was the main upregulated early message (2hr), while IL-1β receptor antagonist (IL1RN) was the primary upregulated late (10 hr). IL-8, CXCL1, CXCL2, CXCL3 and CCL20 were upregulated to 2 hr, and maintained at 10hr, while CXCL5 and CXCL6 were further upregulated by 10 hr. At 10hrs, exposure to 500ppm CO upregulated SPP1, TNPβ, IL-10, TNFβ, and IL-13 (7 - to 32-fold), while downregulating CXCL5, IL-1β, CXCL2, CXCL6 and IL1RN (5 - to 24-fold).

Conclusions: We conclude that CO exposure can upregulate specific messages in HASM to a greater extent than IL-1β alone. Conversely, CO exposure can also downregulate specific messages, at least two of which are involved in the IL-1β pathway, namely, IL-1β and IL1RN. These results show that CO exposure can have direct effects on the intracellular mRNA levels in HASMC, an airway cell relevant to environmentally-associated asthma.

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922 Role of microRNA in the Induction of Immunosuppressive MDSC by Δ⁹-THC. In Vivo Regulation of Transcription Factor C/EBPβ by miR-690


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Δ⁹-tetrahydrocannabinol (THC), the major psychotropic component of marijuana has been shown to induce potent myeloid-derived suppressor cells (MDSC) in vivo. We investigated the role of miRNA in this process. C11b+Gr-1+ MDSCs were purified from peritoneal cavities of BL/6 mice injected with THC. Genome-wide profiling of 690 mouse miRNA by microarray analysis showed significantly altered expression in THC-MDSCs vs control BM precursors, with 13 miRNA identified as differentially expressed (≥2-fold). We used a robust combination of TargetScan, RNAhybrid and miranda algorithms (Poisson P0.05, predicted by at least 2) to identify the targets. Ingenuity pathway analysis revealed that cell growth, proliferation, differentiation, and myeloid differentiation were the top significantly enriched canonical pathways. Several specific, potentially important miRNA-target interactions were identified. MIR-690 was the highly overexpressed miRNA in THC-MDSC (~16 fold). Transcription factor C/EBPβ involved in myeloid differentiation was identified as the potential functional target of miR-690 with a good seed binding site within its mRNA 3'UTR. C/EBPβ expression was found to be higher in BM precursors with a significant decrease in THC-MDSC, while showing inverse correlation with miR-690 expression. Knock-down of miR-690 in THC-MDSC following transfection with stable, peptide nucleic acid (PNA) antagonist resulted in unblocking, and significant increase in C/EBPβ levels. Further, FACS sorted, THC-induced C11b+Ly6G-Ly6C+ granulocytic and C11b+Ly6G-Ly6C+ monocytic MDSC subtypes showed similar levels of miR-690 and attenuated C/EBPβ suggesting that miR-690 is a pan MDSC marker, and miR-690-C/EBPβ is a common pathway in MDSCs. Select miRNA and their targets play a key role in the induction of immunosuppressive MDSC following cannabinoid exposure, and hence, in cannabinoid-induced immune suppression. Supp by NIH P01AT003961, P20RR032684, R01AT006888, R01ES019313, R01MH094755 & VA101BX001357.

922a Profile of Six Hepatic Insulin Signaling Pathway Genes in Response to 2-Aminoanthracene Dietary Ingestion

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Six genes that regulate various processes in the insulin signaling pathway were profiled. These transcripts were reported to regulate other genes that are essential for glucose metabolism, fatty acid and lipid biosynthesis. In a previous hepatic global gene expression analysis followed by DAVID bioinformatics analysis showed upregulated, g6pc, gck, ppi, srebp-1c and socs2 to be likely modulated by 2-aminoanthracene (2AA) dietary consumption. The goal of the current study is to evaluate the responses of these proteins in the liver of Fisher-344 (F344) rats exposed to 2AA. 2AA belongs to a class of compounds referred to as polycyclic aromatic hydrocarbons (PAHs). This compound has been detected in broiled food exposed to 2AA. 2AA belongs to a class of compounds referred to as polycyclic aromatic hydrocarbons (PAHs). This compound has been detected in broiled food. F344 rats were fed 2AA adulterated diets of 0 mg/kg, 50 mg/kg, 100 mg/kg and 100 mg/kg for 14- and 28-days. Differential gene expression of ampk (Pik3b), g6pc, gck, ppi, srebp-1c and socs2 was carried out by qRT-PCR. Results seem to suggest 2AA modulates different genes related to energy metabolism in the liver. Relative quantification of these products indicated an up-regulation of the ampk and socs in animals treated to 100 mg/kg-diet and 50 mg/kg-diet respectively during 14 days of feeding. The rest of the mRNA transcripts were downregulated in the treated rats relative to the control group. G6pc gene was highly up-regulated in all animals that ingested 2AA for 28 days. Ppi protein was also up-regulated in the 75 mg/kg-diet group. The rest of the genes tested were not differentially altered. This result will be followed by a protein immunoblot assay to examine the expression of g6pc and ampk.

922b Effects 2-Aminoanthracene Exposure on Insr, Irs, Akt and Glut4 Expression

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The modulation of the toxic effects of 2-aminoanthracene (2AA) on the expression of insulin receptor (Insr), insulin receptor substrate (Irs1), α-ket urate thymoma viral oncogene homolog 1 (Akt1) and solute carrier family2, facilitated glucose transporter member 4 (Glut4) liver is being investigated. These genes play vital
roles including regulatory activity in insulin signaling and glucose metabolism (Akt1), important glucose transporter essential for the development of type 2 diabetes (Glut4) and activation and processing of insulin signaling transduction (Insr, Irs).

In a previous study to assess the toxic effects of 2AA using global gene expression coupled with DAVID bioinformatic tools, it was found that several genes that regulate the insulin signaling pathway may be altered by 2AA toxicity. The objective of the current investigation is to examine the mRNA expression of four specific genes that mediate insulin signaling due to 2AA toxicity. Polynuclear aromatic amine, 2AA is a by-product of cigarette smoke and broiled meat. Twenty four post-weaning 3-4 week old F-344 male rats were exposed to the test compound (low dose group was 40 mg/kg-diet, low dose - LD), 75 mg/kg/diet (medium dose - MD) and 100 mg/kg-diet (high dose - HD) 2AA for 2weeks and 4weeks. The mRNA expression of Insr, Irs1, Akt1 and Glut4 was determined by quantitative time PCR followed by the quantification of Akt1 via enzyme-linked immunosorbent assay (ELISA) assay. Transcripts Glut4 and Insr were not expressed at all in all treatment groups. Akt1 gene was up-regulated in animals treated to 2AA for both two and four weeks. Similarly, Irs1 mRNA was only up-regulated in rats that ingested 2AA for four weeks. It appears Akt1 and Irs1 proteins were targets of 2AA intoxication. This study is still ongoing to quantify the level of protein expression of Akt1 in the livers of rats exposed to 2AA.

**Functional Analysis of the Dioxin Response Elements (DREs) of the Murine Cyp1A1 Gene Promoter: Beyond the Core DRE Sequence**

L. S. Birnbaum1.

TBBPA treatment resulted in a statistically significant increase in the synthesis, oxidative stress, and perturbations of lipid and endogenous estrogen metabolism. The present work investigated modes-of-action of TBBPA-mediated toxicity in Wistar Han rats and hepatoblastoma in male B6C3F1 mice. The mechanisms of TBBPA, a widely used brominated flame retardant, is an endocrine disruptor in mouse, human and rat. Deletion analysis showed that a single DRE at -488 was sufficient to induce CYP1A1 transcription in response to TCDD and the core DRE (5'-TNGCGTG-3') tends to show higher transcriptional level than that of the adjacent sequences of DRE. The core sequences of seven DREs did reduce the transcriptional efficiency, which illustrated that the adjacent sequences of DRE played a vital role in activating transcription. The reversed 25bp core sequences including DREs showed that the core DRE sequence (5'-TNGCGTG-3') tends to show higher transcriptional level than that of the core DRE sequence (5'-CAGCGCA-3') which processed the core DRE. Furthermore, in the core DRE (5'-TNGCGTG-3') sequence, when N is Thymine or Cytidine (T, C), the transcription efficiency was stronger compared with that of the other nucleotides. This study not only systematically the effects of DRE orientation, DRE adjacent sequences, and the nucleotide N in the core DRE (5'-TNGCGTG-3') sequence on the AhR-regulated CYP1A1 transcription in response to TCDD and laid a good foundation for further investigation into the AhR-dependent transcription regulation triggered by dioxin and dioxin-like compounds.
with SYN, and on subsequent aggregation reactions. Redox modulation of SYN PTMs and of SYN aggregation suggests potential interventions to alleviate SYN-dependent cytotoxicity. (P30ES006694 & T32-ES016652)

**925** Linking Nicotinamide Nucleotide Transhydrogenase (Nnt) Activity and Respiratory-Dependent H2O2 Consumption through the Mitochondrial Thioredoxin/Peroxiredoxin System in Cell-Based Model of Parkinson’s Disease

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Mitochondrial reactive oxygen species (ROS), such as hydrogen peroxide (H2O2) are implicated in neurodegenerative diseases such as Parkinson’s disease (PD). Epidemiological studies have shown pesticide exposure can increase the risk of developing PD and many pesticides/herbicides, such as Paraquat (PQ) work through redox cycling or inhibiting the electron transport chain. Mitochondria are thought to be net producers of ROS but recently it was shown brain mitochondria can consume H2O2 in a respiratory-dependent manner by the thioredoxin/peroxiredoxin (Trx/Ptx) system (Drechsel, 2010). This study wanted to determine the mechanism linking mitochondrial respiration with H2O2 consumption in a cellular model of PD. We examined the role of Nicotinamide Nucleotide Transhydrogenase (Nnt), which uses the proton gradient to generate NADPH from NADH and NADP⁺, to detoxify H2O2 by the Trx/Ptx system. Pharmacological inhibition of Nnt in isolated brain mitochondria significantly decreased their ability to consume H2O2 in the presence, but not absence, of respiration substrates. Nnt inhibition in liver mitochondria, which do not need substrates to detoxify H2O2, had no effect. Pharmacological inhibition or siRNA knockdown of Nnt in N27 cells a) decreased H2O2 consumption b) decreased TrxR activity c) decreased NADPH and increased NADP⁺ levels and d) decreased basal, spare and maximal mitochondrial H2O2 consumption b) decreased TrxR activity c) decreased NADPH and increased NADP⁺ levels and d) decreased basal, spare and maximal mitochondrial oxygen consumption rates. Nnt deficient cells where more susceptible to ROS production and cell death following exposure to subtoxic levels of PQ. This data implicates Nnt as a critical link between the metabolic and antioxidant functions in brain mitochondria and suggest Nnt as a potential therapeutic target to improve the redox balance in conditions of oxidative stress associated with neurodegenerative diseases. Funding: RO1NS45748

**926** Environmental/Mitochondrial Toxicity Induced by Paraquat and MPP+ Is Associated with a Decrease in Ubiquitination-and p62/Autophagy-Mediated Clearance of Oxidized Proteins

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A hallmark of neurodegenerative diseases is the accumulation/aggregation of misfolded or damaged proteins, which has been associated with increased oxidative stress and dysfunction in protein degradation pathways (ubiquitin-proteasome system and autophagy). α-Synuclein, ubiquitin and p62 are major components of Lewy body inclusions in Parkinson’s disease. In this work, we aimed to study the effect of environmental/mitochondrial toxins in the ubiquitin-proteasome and autophagy-mediated degradation pathways in human neuroblastoma and dopaminergic mesencephalic cell cultures. Using the ubiquitin-proteasome and the autophagy-only reporters, GFP and GFP-ODC, and western blot analysis of ubiquitin-bound proteins and changes in the autophagy flux, we observed that paraquat- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPP⁺)-induced cell death was associated with a decrease in protein ubiquitination. Decreased protein ubiquitination induced by paraquat or MPP⁺ was paralleled by a decrease in the autophagic flux (LC3-II accumulation in the presence of chloroquine) and an increase in p62 accumulation. Paraquat-induced oxidative stress was paralleled by the accumulation of oxidized (sulfenylated) proteins. Neither paraquat nor MPP⁺ toxicity was increased by the presence of the proteasomal inhibitor MG132. However, overexpression of a dominant-negative form of the autophagy protein 5 (dnAtg5) increased both paraquat and MPP⁺ toxicity. We hypothesize that impairment of protein ubiquitination by paraquat or MPP⁺ is compensated by autophagic clearance of p62-bound oxidized protein aggregates, and that inhibition of autophagy potentiates the toxicity of these environmental/mitochondrial toxicants.

**927** Evidence for the Involvement of Protein Kinase C Delta (PKCδ)-Mediated Epigenetic Deregulation in the Histone Deacetylase Inhibitor Trichostatin-A-Mediated Neurotoxicity in N27 Dopaminergic Neuronal Cells

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Acetylation and deacetylation of histones has been shown to be a critical regulator of gene transcription and chromatin remodeling. We have previously demonstrated that NF-kB activation is critically linked to trichostatin A (TSA)-induced apoptotic cell death in N27 dopaminergic cells. However, the influence of protein kinase c delta (PKCd), a redox sensitive kinase, on histone hyperacetylation and its impact on dopaminergic neuronal survival remains to be established. Therefore in the present study we sought to investigate the link between PKCd dependent oxidative stress and histone hyperacetylation. Herein we show that exposure of N27 cells to TSA for varying time periods (3-12h) induced a time dependent increase in Bax activation, dissipation of MMP, caspase activation, NADPH oxidase activation, and GSH depletion. These changes preceded the drug induced apoptotic cell death. Intriguingly, a concomitant activation of PCK delta that paralleled histone hyperacetylation was evidenced in TSA treated cells. Moreover, TSA-induced increase in the expression of transcriptional regulators, such as p53, STAT1, and NF-kB, positively correlated with increased expression of pro-apoptotic factors. Notably, down regulation of PKCd levels via ectopic overexpression of a caspase cleavage mutant of PKCd (D327A) or small interference RNA-mediated gene silencing attenuated mitochondrial mediated oxidative cell signaling events, transcription factor activation, histone hyperacetylation, and apoptotic cell death. Collectively, these findings identify PKCδ as a key regulator of TSA-induced hyperacetylation of histones and suggest that oxidative stress mediated proteolytic activation of PKCd contributes to dopaminergic neuronal demise at least in part via deregulation of cellular epigenetic mechanisms (ES10586 and NS065167).

**928** Loss of NF-kB p50 Amplifies Microglia Priming in the Aged Brain

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Excessive and chronic microglial activation has been implicated in degeneration of dopaminergic (DA) neurons in the substantia nigra (SN) during Parkinson’s disease (PD). Aging, a key risk factor for PD, is associated with enhanced sensitivity of microglia to pro-inflammatory stimuli, but the mechanisms are poorly understood. NF-kB p50 is a regulator of inflammation and NF-kB p50 expression is reduced in post mortem SN tissue of Lewy Body Dementia patients, supporting a role for loss of NF-kB p50 function in neurodegeneration. To examine the consequences of loss of NF-kB p50 function in microglial activation, 1.5-2.0 month old NF-kB p50⁻/⁻ and NF-kB p50⁺/+ mice were injected with LPS (5mg/kg, IP). Expression of the pro-inflammatory genes TNFα and IL-1β in the midbrain as well as serum TNFα levels were higher in LPS treated NF-kB p50⁻/⁻ mice at 3h post-injection. Kinetics studies demonstrated that TNFα and iNOS expression is increased at 6h and 12h, but not 3h post-LPS treatment in NF-kB p50⁻/⁻ mixed glia cultures, implicating NF-kB p50 in resolution of the glial pro-inflammatory response. Consistent with this premise, NF-kB p50⁻/⁻ cultures expressed lower levels of the M2 marker arginase-1 in response to LPS, indicating impairment of the resolution of iNOS activation. To examine how loss of NF-kB p50 function affects microglial activation in the aged brain, 1.5-3.0 and 16-18 month old male mice (NF-kB p50⁻/⁻ & NF-kB p50⁺/+ ) were injected with LPS IP. Aged LPS treated NF-kB p50⁻/⁻ mice had the highest levels of serum TNFα as well as midbrain TNFα and IL-1β expression at 3h. Furthermore, higher numbers of microglia with activated morphology were observed in the SN of aged LPS treated NF-kB p50⁻/⁻ mice. These results support that loss of NF-kB p50 function increases neuroinflammation and microglial activation, impairs pro-inflammatory resolution and that these effects are further magnified by aging.
929 Exploring Gene-Environment Interactions Implicated in Parkinson’s Disease Using a Paraoquat and Maneb Model in Drosophila
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Parkinson’s disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons and a loss in both motor and non-motor functions. Overexpression and mutations of the alpha-synuclein gene in humans has been linked to familial forms of Parkinson’s disease. However, inheritable forms of PD are rare. The vast majority of PD cases are sporadic, thus, disease progression likely relies on an individual’s genetic background and/or environmental exposures. Exposure to environmental toxins, such as pesticides or heavy metals, has been shown to increase the risk of developing PD in humans. In particular, prolonged exposure to the pesticides paraoquat and mane has also been shown to increase the risk of PD up almost two fold. Interestingly, individuals with variants in the alpha-synuclein gene were shown to be at increased risk of PD, compared to control, when they were exposed to pesticides. The complex nature of gene-environment interactions in sporadic PD has made it difficult to understand disease etiology using traditional rodent models. Thus, our lab has developed a paraoquat and maneb model of PD in Drosophila melanogaster, which is amenable to multiple genetic modifications which can be easily combined with various environmental toxin exposures. In our model, survival and motor ability significantly decreased when exposed to paraoquat alone or with paraoquat and maneb combined. Dopaminergic cell counts decreased only when exposed to both paraoquat and maneb. Additionally, we assessed the dopaminergic cell counts, survival curves and climbing assay of our model in the presence of over-expression of human mutant alpha-synuclein. Thus our work confirms epidemiological data in addition to further exploring implicated gene-environment interactions.

930 The Parkinson’s Disease-Linked Pesticide Ziram Causes Deregelation of the Synapse
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Exposures to environmental toxins, such as pesticides, have long been implicated in neurodegenerative diseases such as Parkinson’s disease (PD). Recent epidemiological studies demonstrate that chronic exposure to the pesticide ziram results in over a two-fold increase in the risk of contracting PD. PD is a devastating disease that affects millions of people worldwide. PD patients suffer from an irreversible and progressive loss of dopaminergic neurons that results in motor deficits as well as impairment of other non-motor functions regulated by dopamine such as mood, sleep, smell and digestion. The molecular and physiological changes in dopaminergic neurons that precede cell death and disease are unclear, particularly in the case of sporadic disease onset, which accounts for the vast majority of PD cases. Our aim was to investigate how exposure to the PD-linked pesticide ziram may alter neuron physiology and behavior. By using the well-characterized Drosophila neuromuscular junction (NMJ) preparation, we were able to assess for the first time ziram’s affect in an intact synapse. The large array of live-imaging genetic reagents available in the fly allowed for us to quantify ziram’s affect on the essential neuronal processes of synaptic vesicle release, reuptake and action potential dependent calcium influx. We report that ziram alters synaptic behavior at both excitatory, glutamatergic terminals and modulatory, aminergic terminals at the Drosophila NMJ. Interestingly, aminergic terminals are particularly sensitive to disruption by ziram exposure, resulting in a decrease in the rate of dendritic outgrowth. Additionally, we observe spontaneous depolarization of aminergic, but not glutamatergic, nerve terminals in the presence of ziram. We hypothesize that chronic exposure to ziram results in preferential deregelation of aminergic terminals and may be responsible for its link to PD in the human population.

931 Mitochondrial DNA Damage and Dysfunction and Their Effects on Dopaminergic Neurodegeneration after Chemical Insult in Caenorhabditis elegans
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Mitochondrial dysfunction has been linked to neurodegenerative diseases including Parkinson’s Disease (PD). Neurons are cells with a high energy demand, and as a result are hypothesized to be particularly vulnerable to mitochondrial dysregulation. We investigated dopaminergic neuron viability after chemical insult in Caenorhabditis elegans strains with gene knockouts of pink-1 and pdr-1, which are involved in mitochondrial dynamics. These genes are the nematode homologs of the human genes PINK1 and PARK2 which when mutated cause familial PD. The four cephalic dopaminergic neurons were visualized with fluorescent microscopy, and assigned a score based on the extent of damage observed. Each neuron was scored from 0 to 2, with zero representing an intact dendrite and 2 representing the highest level of neurodegeneration observed. The strain BY200 (dat-1;GFP) was used as wild type for the strains UA226 (dat-1;GFP; pink-1) and UA227 (dat-1;GFP; pdr-1), all generously provided by Guy Caldwell (University of Alabama). After 6-hydroxydopamine (6-OHDA) exposure, both mutant strains showed different levels of neurodegeneration compared to wild type, with the pink-1 mutant exhibiting less neuronal damage than wild type. We also performed quantitative polymerase chain reaction (QPCR) after 6-OHDA exposure and observed significant levels of DNA damage to both nuclear and mitochondrial genomes. This genotoxic effect of 6-OHDA could be in part responsible for the degeneration observed in dopaminergic neurons after exposure to this chemical. Previous results obtained in the laboratory indicate that knockdown of pink-1 inhibits removal of mitochondrial DNA (mtDNA) damage, presumably inducing mitochondrial fusion and functional complementation and potentially explaining the resistance to neurodegeneration observed in the pink-1 mutant. Further studies are being performed to fully characterize the fate of mtDNA damage induced by environmental toxicants and its effect on neuronal viability and mitochondrial health.

932 Identification of Novel Genes and Epigenetic Mechanisms in C. elegans Models of Idiopathic Parkinson’s Disease
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Background: Idiopathic Parkinson’s disease’s (PD) is an oxidative stress-related disorder that result in abnormal dopamine (DA) signaling and cell death. Although the origin of the pathogenesis in PD remains unclear, costallatory evidence suggests both genetic and environmental contributions. Recently we have shown that the PD-associated transcription factor SKN-1/Nrf2 is expressed in C. elegans DA neurons and inhibits PD-associated DA neurodegeneration. Aims/Objectives: In this study we asked what are the genes, molecular pathways, and mechanism involved in DA neuron vulnerability to PD-associated toxicants. Methods: We utilized reverse genetics, biochemical assays, immunofluorescence, transgenic C. elegans, RT-PCR, Western analysis, mass spectrometry, and behavioral and neuronal morphology analysis to characterize expression, localization and the role that SKN-1, IDN-1, and post-translational modifications play in 6-OHDA-, rotenone-induced neuronal death. Results: In this study we demonstrate that IDN-1 mutants render DA neuron up to 10-fold more resistant to the neurotoxics relative to WT. We show that IDN-1 is expressed in DA neurons, and IDN-1 overexpression results in a 2-fold increase DA neuron vulnerability. We also show that DA neuron vulnerability is affected by transcriptional and post-translational modifications of involving these and other proteins identified using RNAi affects DA neuron vulnerability. Conclusions: This study identifies novel genes and molecular pathways involved in DA neuron vulnerability in PD, and shows that common epigenetic mechanism modulate DA neuron vulnerability to PD-associated neurotoxicants. Support: NIEHS ES014459 and ES003299 to RN, and EPA STAR Graduate Fellowship to NVD.

933 LRRK2 Transgenic Rats Exhibit Heightened Sensitivity in a Colitis Model
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Accumulating evidence suggests that chronic systemic inflammation is associated with the pathophysiology of Parkinson’s disease (PD). Leucine-rich repeat kinase 2 (LRRK2) has been identified as the gene most frequently mutated in autosomal dominant familial PD. LRRK2 is also a major susceptibility gene for inflammatory bowel disease (IBD) and has an immunoregulatory function. Pathological functions of LRRK2 in both IBD and PD are not well understood. The goal of this project was to examine how potential interactions between IBD and PD models might be mediated by LRRK2. In a preliminary study, BAC transgenic (TG) rats expressing human G2019S mutated LRRK2 or wild-type (WT), littermate controls were exposed to daily dextran sodium sulfate (DSS), 2.5-5% W/V in drinking water) over 21 days to induce experimental colitis. Rats were scored using the total daily activity index (DAI), including body weight loss, stool score and fecal occult blood test, was quantified. At day 21, after euthanizing the brains were removed and processed for neurotransmitter analysis and histology. Also, the blood and gastrointestinal tract were collected for immunological determination. DSS-treated LRRK2
Parkinson’s disease (PD) is a progressive motor disease of unknown etiology. The motor impairments of PD arise from selective degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). These DA neurons project to the striatum (CPu) to release dopamine. Clinical symptoms of Parkinsonism emerge when more than half of the dopaminergic nerve terminals are damaged in the striatum. In the current therapeutic in vivo mouse model study, we used TUDCA as a neuroprotectant and L-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as neurotoxicant. TUDCA is a molecular chaperone which modulates cell death by interrupting classic pathways of apoptosis, modifying the expression of certain Bcl-2 family members, as well as NF-kB activity. In the present study mice were divided into three groups (Saline/Probencid [250mg/kg, control], MPTP [25mg/kg-10 doses+Probencid, positive control] and TUDCA+MPTP [200mg/kg+25mg/kg, treatment group]). Using immunohistochemical and analytical chemistry techniques, we showed that the SNc and CPu are significantly protected from MPTP induced damage after TUDCA treatment compared to MPTP + Probencid group. Tyrosine hydroxylase and dopamine transporter immunoreactivity in the ventral midbrain SNc and striatum are more protected from MPTP damage in TUDCA treated mice compared to its positive controls. Microglial activation, depletion of striatal fibers and dopamine and the development of motor deficits are significantly less in TUDCA treated group. Together, our results suggest that new mechanisms may be involved in the pathogenesis of PD and that might be exploited in the development of novel therapeutics aimed at slowing or halting neurodegeneration in patients with PD.

G2019S transgenic rats (DSS-TG) showed significantly increased DAi compared to DSS-treated wild type (DSS WT) or untreated rats (control). Surprisingly, neurotransmitter analysis showed that WT animals exhibited decreased striatal dopamine and DOPAC in response to DSS, with TG animals showing no such response. Serotoninergic neurotransmission was not affected. Further, immunohistochemistry data suggest that DSS-WT animals also exhibit decreases in striatal tyrosine hydroxylase levels, in the absence of overt dopaminergic terminal loss. Here again, TG animals did not exhibit such a response. In summary, rats expressing G2019S LRRK2 exhibited heightened clinical sensitivity in a colitis model and did not exhibit adaptive changes in nigrostriatal dopamine transmission observed in WT animals treated with DSS. More studies are needed to confirm these initial observations.

Ceftriaxone Ameliorates L-DOPA-Induced Dyskinesia, a Side Effect of Parkinson’s Disease Treatment

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Parkinson’s disease (PD) is a neurodegenerative disease characterized by motor impairment arising from the loss of dopaminergic neurons. Currently, pharmacological dopamine (DA) replacement with L-DOPA is the gold-standard treatment for PD. Unfortunately, chronic L-DOPA therapy induces abnormal involuntary movements termed L-DOPA-Induced-Dyskinesia (LID) in over 90% of PD patients over time. Furthermore, once dyskinesia manifests, it occurs with every subsequent exposure to L-DOPA. The prevalence and permanence of LID makes L-DOPA’s side effect profile a highly relevant clinical obstacle especially given the primary role L-DOPA plays in PD treatment. Glutamate mediates excitatory signal transduction and in excess (excitotoxicity), can contribute to loss of DA neurons in PD. Thus, suppression of glutamate-mediated excitotoxicity could prolong the longevity of nigrostriatal neurons in PD progression. The beta-lactam antibiotic, Ceftriaxone (cef), increases the expression of GLT-1, a glutamate transporter responsible for glutamate clearance in the CNS. We examined whether Cef could ameliorate dyskinesia when given 7 days post lesion in a 6-OHDA LID model. Chronic Cef (200mg/kg ip) significantly delayed and attenuated dyskinesia in 6-8 month male Sprague Dawley rats compared to saline. Striatal GLT-1 expression was also increased in both Cef and Cef + L-DOPA treated rats compared to L-DOPA suggesting a cef-induced increase in synaptic glutamate clearance.

Tyrosine hydroxylase (TH), a marker for DA, was measured in the lesioned striatum. Compared to its contralateral control, Cef treated rats had the highest remaining striatal TH with 81%, L-DOPA + Cef had 55%, and L-DOPA had 18%. Our data suggest that chronic Cef + L-DOPA may not only halt TH destruction, but also potentially reverse striatal TH loss in a 6-OHDA LID model. Given that Cef is already FDA-approved, these data support the repositioning of the antibiotic based on its ability to not only mitigate dyskinesia severity, but also protect against further DA loss in a Parkinson’s disease rodent model.

Quercetin Treatment Protects Progressive Nigral Dopaminergic Neuronal Degeneration in Cell Culture and MitoPark Animal Models of Parkinson’s Disease by Activating PKD1 Signaling


Mitochondrial dysfunction has been implicated as a key player in the pathogenesis of Parkinson’s disease (PD). The MitoPark mouse, a transgenic mitochondrial impairment model recently developed by specific inactivation of Tfam in dopaminergic neurons, spontaneously exhibits progressive behavioral deficits and neurodegeneration, recapitulating several features of PD. Since non-motor symptoms are now recognized as key features of the pre-symptomatic stage of PD, we monitored the clinically relevant motor and non-motor symptoms of PD from 8-24 wks of age in MitoPark and C57 wild type mice. As expected for MitoPark mice, motor defects begin around 12 weeks and become severe by 18-24 wks. Interestingly, nortok MitoPark mice showed memory deficits before female mice, beginning at 8 wks and becoming most severe between 20-24 wks, as determined by Morris Water Maze. When compared to age-matched naïve C57 black mice, MitoPark mice exhibited olfactory deficits in novel and social scent tests as early as 12 wks. MitoPark mice between 16-24 wks spent more time immobile in forced swim and tail suspension tests, and made fewer entries into open arms of the Elevated Plus Maze, indicating a depressive- and anxiety-related phenotype, respectively. Preliminary analyses revealed that sleep disturbances and gastrointestinal problems may also be present in MitoPark mice. Collectively, our results indicate that MitoPark mice progressively exhibit deficits in cognitive learning and memory, olfactory discrimination, sleep latency, and anxiety- and depressive-like behaviors. Thus, MitoPark mice can serve as an invaluable model for studying motor and non-motor symptoms in addition to studying pathology in PD (supported by NIH grants ES10586 and NS074443 and NS039958).

Oxidative stress has been associated with many neurological diseases including Parkinson’s disease (PD). Therefore, identifying cell signaling mechanisms associated with oxidative stress is critically important to the development of new treatment strategies for PD. We have recently identified a novel oxidative stress-associated signaling pathway in which protein kinase D1 (PKD1) plays a major compensatory survival role in dopaminergic neurons. Therefore, we adopted a rationale based pharmacological screening approach to identify activators of PKD1 using dopaminergic neuronal cell model. Herein, we identified quercetin, a natural flavonoid widely found in vegetables and fruits as effectively activate PKD1 protective signaling. Western blotting analysis revealed that quercetin treatment significantly induced the phosphorylation and activation of PKD1 as well as CREB and Akt phosphorylations in MN9D dopaminergic neuronal cells. Activation of Akt, however, was inhibited by siRNA knockdown of PKD1, suggesting that Akt acts as a downstream target of PKD1 signaling during quercetin treatment. Results from qRT-PCR, Western blot analysis, mtDNA content analysis, and Mitotracker assay experiments revealed that quercetin can induce mitochondrial biogenesis in MN9D cells. Importantly, quercetin treatment protected against 6-OHDA-induced neurotoxicity in MN9D cells. Next, we evaluated the neuroprotective efficacy of quercetin against the progressive neurodegenerative process by using MitoPark mouse model of PD. Administering quercetin (25 mg/kg) once daily to 12-week-old MitoPark mice via oral gavage for 6 weeks significantly reversed behavioral deficits, striatal dopamine depletion, and TH neuronal cell loss in MitoPark mice. Our findings suggest that quercetin, by virtue of its ability to activate the PKD1-mediated neuroprotective signaling, is a promising neuroprotective drug candidate for the treatment of PD (NIH NS74443).
methylation as a result of Pb exposure with levels of SAM and the enzymes involved (SAM) and its role in epigenetic reprogramming. We compare changes in histone methylation, and on the methyl donor S-adenosylmethionine (Alashwal et al., 2012, Dosunmu and Zawia, 2013). It is also known that epigenetic alterations in the expression of genes associated with Alzheimer's disease (Wu et al., 2008; Hui et al., 2012; Syed and Zawia, 2013). Accumulation of Aβ-β amyloid plaques in the neurpathological hallmark of Alzheimer’s disease (AD) that is primarily genetic in nature, or late onset Alzheimer’s disease (LOAD) which is sporadic and affects 90% of those diagnosed with AD. Epidemiological data seem to suggest that both LOAD and late-onset dementia have early origins. Previous work in our lab has shown that early life exposure to Pb results in higher BPb levels for Taq1_T and Apa1_C (p<0.06), whereas Bsm1_C allele in Caucasians (p=0.009) was associated with higher BPb. NAb had a high prevalence in HD (>50%), anti-NF-L, GFAP and MBP the most prevalent. Based on genotype stratifications, Bsm1_C (1) and Taq1_C (1) (homozygotes and heterozygotes) had higher levels of anti-NF-L, NF-H and GFAP (p=0.01-0.05). Bsm1_C genotype, only, was associated with a nearly two-fold increase in the odds of anti-NF-M presence (p=0.02), the NAb best correlated (r=0.24, p=0.008) with Pb, although BPb did increase the odds of anti-MBP detection (OR: 1.13, CI: 1.034-1.232, p=0.003). Overall, multivariate regression revealed that Pb was a significant determinant of anti-NF-M, GFAP and MBP titers (p=0.04, 0.03, 0.005, respectively). Although vitamin D is neuroprotective, VDR SNPs have been associated with ND. In addition, VDR SNP, particularly the major Bsm1_C allele, as well as Taq1_T, alleles have been shown to promote Pb accumulation, an observation confirmed here. Taken together, this study indicates that NAb may be useful for detection of ND in environmentally and genetically vulnerable populations and suggests that heavy metals, like Pb, may contribute to ND in HD patients.

Alzheimer’s disease (AD) is a neurodegenerative disorder and is the most common type of dementia, and is the sixth leading cause of death in the United States. It presents as two forms, either early onset Alzheimer’s disease (EOAD) that is primarily genetic in nature, or late onset Alzheimer’s disease (LOAD) which is sporadic and affects 90% of those diagnosed with AD. Alzheimer’s disease (AD) is one of the most common neurodegenerative disorders. Accumulation of Aβ-containing amyloid plaque is a neuropathological hallmark of Alzheimer’s disease (AD) but inflammatory process triggered by Aβ deposition and microglia activation is significantly related to the pathogenesis of AD. Studies on brain tissues of the patients with AD show the elevated levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. Our research studies have shown the therapeutic potential of curcumin in neurodegenerative diseases including AD through its antioxidant, anti-inflammatory and anti-β protein aggregation effects. However, the effect of curcumin on microglia functions and its underlying mechanism is not elucidated yet. The aim of this study was to investigate the anti-inflammatory properties of curcumin on LPS-stimulated microglia cells and its effect on microglia’s functions. We previously demonstrated inhibition of a High Mobility Group Box 1 (HMGB1) in LPS-stimulated microglia BV2 cells improves the phagocytosis ability in these cells. Our current study demonstrated that pre-treatment with 5μM curcumin suppresses LPS-induced proliferation and HMGB1 overexpression in BV2 cells that corresponds with markedly decreased the expressions of Toll like receptor 4 (TLR4). Moreover, administration of curcumin significantly improved the LPS-induced phagocytic impairment in BV2 cells. The present results propose curcumin, which inhibits HMGB1/TLR4 in microglia cells may offer a novel and probable benefit against AD-associated neuro-inflammation.
ubiquitination, nucleotide- and metal ion-binding. A profile of gene expression related to EAE and aging was exhibited, suggesting that monitoring of these genes may be a main subject of possible targets for treatment of the disease.

942a Toxicology of Targeted Drug Delivery to the Midbrain for Parkinson’s Disease Treatment
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Direct drug administration into the brain parenchyma offers a high degree of targeting, while reducing the risks of unwanted side-effects by limiting systemic exposure. Drug delivery to the midbrain region remains a challenge. Here, we used an investigational catheter and cranial anchor system coupled to implanted programmable pumps to continuously infuse 5mM gadopentetate dimeglumine (Magnevist®) into the substantia nigra (SN) of female rhesus monkeys to lay the foundation for delivering drug therapies to the midbrain region. Flow rate tolerability of either 0.1 μL/min to the right SN for 14 days or 0.2 μL/min to the left SN for 14 days was assessed by monitoring clinical observations, body weights and histopathological examinations of the striatum and midbrain regions. Evaluation of post-surgical MRI indicated that the placement of each intranigral catheter was within a 2-mm radius from the intended surgical target and that all catheters were patent, as evidenced by the presence of Magnevist® at the catheter tip. The volume of distribution achieved in the midbrain region with Magnevist® infused at a rate of 0.2 μL/min was greater than that achieved at 0.1 μL/min by nearly 2-fold. There were no indications of toxicity as noted in clinical observations and body weight values. Histopathological evaluations indicated that the catheter tip was placed in or near the SN pars compacta region in all animals. There was no evidence of infection at any of the catheter sites and no difference between the two infusion rates with respect to changes indicating inflammation. There was no discernible difference regarding the microglial reaction at the two different infusion rate sites. There was no detectable decrease of tyrosine hydroxylase staining in the striatum in any of the sections and no detectable necrosis of neurons in the SN pars compacta region. Our data support that direct drug delivery to the midbrain maybe used for the treatment of Parkinson’s disease.

942b Studying Aspects of Parkinson’s Disease in a Zebrafish Model of Ziram Toxicity
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Parkinson’s disease (PD) is the second most common neurodegenerative disease. Genetic causes of PD account for 5% of cases suggesting that the environment has a role in disease etiology. Epidemiological studies indicate that exposure to pesticides increases the risk for PD. We previously found that exposure to ziram increases the risk of developing PD in humans and causes selective dopaminergic cell toxicity, inhibition of the ubiquitin proteasome system (UPS), and increased γ-synuclein levels in primary neuronal cultures. Here we utilize zebrafish embryos (ZF, Danio rerio) to investigate the mechanisms of ziram’s toxicity. ZF embryos were exposed to ziram at an environmentally relevant concentration (50μM), A transgenic ZF line expressing green fluorescent protein driven by vesicular monoamine transporter protein 2 (VMAT2) promoter was used to study the effects of ziram on amineergic neurons. Embryos treated with 50μM ziram were found to have a reduction of VMAT-GFP expressing neurons by approximately 30%. Ziram was not toxic to non-DA sensory neurons. Additionally, exposure to ziram resulted in a 1.58% increase in expression of TH. The closest ZF homologue to human α-syn is ZF γ-syn (synz), which was found to aggregate when overexpressed in ZF. Treatment with 50μM ziram resulted in an increase in synz expression as measured by western blot. Knockdown of synz via antisense was found to protect against ziram’s dopaminergic cell toxicity, resulting in approximately 75% attenuation of VMAT-GFP expressing neuronal death. The mechanism by which ziram increases ZF γ-syn appears to involve inhibition of protein degradation pathways, as concentrations as low as 1μM ziram inhibited the UPS. In a preliminary experiment a 6 fold increase in fluorescence of LC3, a marker of autophagy was observed for embryos treated with ziram. These studies demonstrate that ziram toxicity is at least partially mediated by synuclein and add to the understanding the etiology PD.

942c In-Depth Toxicogenomic Analysis of a Dopamine Transporter Knockout Animal: Transporter-Associated Behavioral and Signaling Changes in a C. elegans Parkinson’s Disease Model
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Background: The loss of DA from basal ganglia results in the devastating neurological disorder Parkinson’s disease (PD). DA transporters (DAT) regulate synaptic DA availability and play a role in neuronal vulnerability, yet the molecular modulators associated with DAT are poorly defined. Aims/Objectives: In this study we determined which genes are involved in DAT-associated gene expression and neuronal vulnerability. Methods: We utilized RNA-seq, microarrays, behavioral and biochemical assays, immunofluorescence, transgenic C. elegans, RT-PCR, LC-MS, HPLC, Western analysis, and neuronal morphology analysis to characterize DAT-associated gene and protein expression, post-translational modifications, and toxicant-associated DA neuron vulnerability. Results: Approximately 20,000 genes were analyzed. Over 54 genes were overexpressed in the DAT knockout strain and 33 genes were under expressed. These genes were strongly biased to water homemostasis, cation transport, and G-protein mediated signalling. Specifically, tyrosine hydroxylase (TH), the rate limiting step for DA synthesis, as well as DA is significantly reduced (approximately 50%) as determined by RNAseq, immunofluorescence, Western analysis, and HPLC. DAT knockout animals also displayed mechano-sensory-associated movement defects. Furthermore, 6-OHDA treated DAT knockout animals showed a dramatic change in gene expression (44 genes enriched in oxidative phosphorylation, eycotysis, and GTPase function) relative to WT suggesting novel pathways involved in 6-OHDA mediated toxicity independent of DAT. Conclusion: This study describes a novel whole genome analysis of the role that the DAT and associated genes and signalling pathways play in DA neuron vulnerability that may provide useful therapeutic targets to inhibit PD-associated cellular pathologies.

942d Altered Expression of Amyloid Beta (Aβ) Deposit-Related Molecules in the Aging Brain
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Alzheimer’s disease (AD) is most common form of dementia characterized by the presence of Aβ plaque and neurofibrillary tangles. Aging is the main risk factor for AD. However, the underlying mechanisms are not completely understood. We focused the alteration of the expression related to Aβ production, degradation and transport in the different age rats. We used Sprague-Dawley male rats at ages 7, 26, 52, 96 weeks. We analyzed the protein expression of amyloid precursor protein (APP), beta-site APP-cleaving enzyme 1 (BACE1), neprilysin (NEP), and insulin degrading enzyme (IDE) in the hippocampus and low-density lipoprotein receptor-related protein 1 (LRP1) and receptor for advanced glycation end products (RAGE) in the brain capillary endothelium (BCE) using Western blot. The LRP1 expression in BCE of 96-week rats were lower than 26- and 52-week rats, however higher than 7-week rats. The RAGE expression in BCE was positively associated with age. However, other molecules, such as APP, BACE1, NEP and IDE in HC did not change according to aging. These results imply that age is related with reduction of Aβ transportation. It might lead to accumulation of Aβ in brain extracellular space.

942e Changes of Tight Junctions in the Blood-Brain Barrier Are Mediated by α2B5-35-RAGE Interaction
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Recently, we demonstrated that microinjection of α2B5-35 into the rat hippocampus produces neurotoxicity mediated by advanced glycation end products (RAGE). It has been demonstrated that RAGE interact with amyloid β-peptide (Aβ) and mediate Aβ transport across the blood-brain barrier (BBB), contributing to the deposition of Aβ in the brain. However, molecular mechanisms underlying Aβ-RAGE interaction-induced alterations in the BBB are poorly understood. In order to determine whether the toxicity induced by α2B5-35-RAGE interaction is involved in tight junction disruption at the blood-brain barrier (BBB), a primary culture of rat brain microvessel endothelial cells (rBMVEC) were created by enzy-
matic digestion and differential centrifugation as an in vitro model of the BBB. Confluent mVEC monolayers (cultured 10-14 days) were treated with AJ25-35 or AJ35-25 (as control) at 20 μM for 24 hours. RAGE, occludin, and claudin-5 expression were measured by Western blotting. Cytotoxicity was evaluated using a lactate dehydrogenase (LDH) assay for cell death, an XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay for mitochondrial function, and detection of 2’, 7’-dichlorofluorescin diacetate (DCFH-DA) to measure ROS production. AJ25-35 produced an increase in RAGE and occludin; and a decrease in claudin-5 expression. Significantly decreased mitochondrial function and increased oxidative stress (ROS production) with no effect on LDH leakage were also observed. These data suggest that AJ25-35-RAGE interaction induced toxicity involved increased occludin and decreased claudin-5 expression. Further studies are underway to determine if these events are associated with disruption in the permeability of the BBB.

**Evaluation of an In Vitro Assay for the Detection of Skin Irritants in Medical Devices**

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The objective of this work is to identify alternative in-vitro methods to screen medical device materials for the presence of skin irritants. Current testing for the presence of skin irritants is done exclusively using a rabbit animal model. Medical device extracts or materials are exposed to the skin of the animal. The material is removed and the affected skin area is observed daily for 3 days and evaluated for erythema and edema. Efforts seeking to improve upon this model by eliminating or reducing the number of animals used in such testing while maintaining or improving sensitivity in identifying such irritants is a focus of ISO TC/219 a standards organization for the Biological Evaluation of Medical Devices. A cultured human skin tissue model, EpiDerm, (Mattek Corporation) allows the direct exposure of test materials to the cultured skin. This model recently demonstrated acceptable performance as a part of the ECVM international validation study for the in vitro identification of chemical irritants. This cultured human skin model is now being evaluated for its ability to identify irritants present in extracts of medical materials and devices. Medical device and device materials present unique problems as the irritants exist within a solid material and are generally present in low levels. We adapted the model protocol and evaluated multiple materials for their irritancy po-

tential. Essentially, the cultured skin tissue was exposed to solvent material extracts for 24 hours. The extract was removed and the skin tissue was allowed to recover for 24 hours. MTT uptake was used to determine irritancy potential (cell cytotoxicity); 50% of control. In addition, IL-1α production was determined via ELISA, to detect increased expression as compared to controls. We have identified 2 materials, when extracted in non-polar solvents, Buna O-rings and Vicryl, that demonstrate an irritancy potential with elevated (≥ 1.5 fold) IL-1α production. This is the first successful demonstration of the use of this in vitro methodology for the identification of irritants in device materials.

**In Vitro Skin Irritation Testing: Characterization of Mid-Range Tissue Viability**

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Introduction

In vitro skin irritation testing is gaining acceptance to replace animal testing. Poor-intra assay reproducibility for chemicals with viabilities in the mid-range can potentially hamper classification near the prediction threshold of 50%. This in vitro study characterized a test article having poor-intra-assay reproducibility in the 30-70% range.

Materials and Methods

Based on testing of medical device extracts, triplicate wells of EpiDerm™ were exposed to 100 μl of test material for 24 hours. After washing, wells were processed for histology or tissue viability. Test materials were Dulbecco’s phosphate buffered saline (DPBS, negative control), 0.125% sodium lauryl sulfate (SLS) in DPBS (positive control), 5.25% lactate acid (LA) in DPBS and 5.0% LA in DPBS. After exposure, culture medium was harvested for analyses of human Interleukin 1 alpha (IL-1α), Interleukin 1 Receptor Antagonist (IL-1RA), Interleukin 8 (IL-8) and Leukemia Inhibitory Factor (LIF) using Quantikine® ELISA kits. Data were shown as mean ± SD. Values were compared to the negative control using student’s t test. A (p) value of less than 0.05 was considered statistically significant.

Results

The controls gave expected viabilities, 100 ± 4.9% and 18.3 ± 2.3%. Exposure to 3.25% LA decreased viability to the mid-range and demonstrated poor reproducibility (mean 47.7 ± 30.7%). Exposure to 5.0% LA consistently decreased viability to the low range (mean 8.2 ± 0.9%).

The levels of IL-8, IL-1α and IL-1α were significantly increased and LIF levels were below the limit of detection for all treatments. Poor-intra-assay reproducibility with 3.25% LA was confirmed for viability, IL-1α, and histology. Of the biomarkers evaluated, IL-1α was more sensitive to the treatment of SLS than LA.

Conclusions

Poor-intra assay variability associated with in vitro skin irritation testing is a true chemical response, not technical artifact. Establishing threshold levels for proinflammatory biomarkers may aid in classification of chemicals with viabilities near the prediction threshold of 50%.

**Does Leaching of Bisphenol A (BPA) from Dental Composites Contribute to Neuropsychological Deficits in Children? An Evaluation of Plausibility**

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Composite dental restorations are placed in more than 10 million U.S. children each year with significant benefits for oral health. Two recent publications have suggested that BPA leached from dental composites contributes to neuropsychological deficits based on a review of the methodology used in the studies, as well as review of published literature describing dental composite composition, evidence for BPA release from dental composites, and known toxicokinetic and toxicological properties of BPA. Possible misclassification of subjects, confounding, and lack of statistical robustness weaken the strength of the study conclusions. Published data indicate that BPA exposure from dental materials occurs primarily from trace amounts of residual BPA in composite monomers synthesized from BPA. Exposure might also occur by enzymatic degradation of certain dental monomers by esterases. Measured dental-related BPA exposures are small (ng/kg-day) and of acute duration. Bioavailable BPA from dental composites is likely rapidly metabolized and excreted, minimizing exposure. At present, a specific mechanism to explain the putative neuropsychological effects of trace levels of BPA has not been articulated. Based on the weight of the examined evidence, the plausibility of neuropsychological deficits in children caused by BPA release from dental composites is low.

**Potential Confounders of Bisphenol A Analysis in Dental/Orthodontic Materials**

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Bisphenol-A (BPA)-based dental/orthodontic ingredients such as Bisphenol-A diglycidyl ether dimethacrylate (Bis-GMA) may contain trace levels of BPA as a chemical synthesis residue. Accurate determination of BPA in final product formulations is essential for reliable health risk assessments. A cured Bis-GMA-based material representative of commercial dental products was extracted according to international guidelines for medical devices. The extraction solvent was spiked with d16BPA as an internal standard. Sample processing followed expert recommendations for minimizing BPA sample contamination. Extracts were analyzed for BPA using high performance liquid chromatography (HPLC) with either ultraviolet (UV) or Multiple Reaction Monitoring (MRM) detection. The BPA concentrations appeared about 30-fold higher using HPLC-UV vs. HPLC-MRM. Full scan liquid chromatography/mass spectrometry (HPLC/MS) was used to elucidate the different results, and showed a compound co-eluting with BPA which interfered with LC/UV analysis. The co-eluting compound derived from ethyl-4-dimethyl aminobenzoate, a co-initiator used by many dental product manufacturers. These results highlight the potential difficulties in accurate analysis of BPA in complex mixtures such as dental product extracts. Both good separation methodology and a detection method with high specificity and sensitivity are important to avoid incorrect assignment of extractables, and consequent inaccurate quantification of BPA.
New Multipoire Contact Lens Solution (MPS) formulations are required to demonstrate disinfection efficacy, stability, biocompatibility, preclinical and clinical safety in accordance with recognized national and international standards. Specific in vitro tests include medium elution, agar overlay, neutral red uptake for cytotoxicity, colony formation for proliferation, and MTX/TXT assays for metabolic activity. Different parameters used in testing, such as cell line sensitivity, detection response to toxicants, exposure time, and MPS dilution, can lead to contradictory results between various in vitro studies and suggest a lack of correlation to clinical results. This study evaluates the correlation among in vitro models for assessing cytotoxicity and biocompatibility of MPS containing contaminant polyhexamethylene biguanide (PHMB). The biological effects of MPS were evaluated following USP Direct Contact test and Colony Formation Assay (ISO 10993-5) in mouse (L-929) and hamster (V79) fibroblast, respectively, and Cell Viability Assay by alamarBlue in human corneal epithelial cells (HCEC). The dose-dependent effects and uptake-release kinetics of PHMB by contact lens materials were evaluated. Results of the USP Direct Contact test indicated that MPS-induced cytotoxicity are dependent upon the lens PHMB uptake and release profiles. MPS cytotoxicity effects on V79 fibroblasts demonstrated a concentration-dependent response, where cytotoxicity increased with MPS concentrations (1.25%, 2.5%, 5%, and 10%) after long-term exposure (7 days) while MPS concentrations at 100%, 75%, and 50% in HCEC showed a shorter time exposure dependency (15-60 minute) when evaluated at alamarBlue Cell Viability Assay. These results indicate that correlations between preservative concentration-response curves and lens uptake-release profiles should be considered as part of the battery of tests to demonstrate MPS biocompatibility.

The use of printing inks in cardiac leads was evaluated following the 2013 FDA draft guidance on the "Use of International Standard ISO 10993, "Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing". Cardiac leads are used for defibrillation and resynchronization therapies in heart failure patients. Cardiac leads are contacting parts (i.e., tubes) were 70.39cm² (12.8mg) and 20.06 cm² (8.4mg) to the presence of cobalt. Safety concern over C.I. Pigment Blue 28 was due to the particle count and size. An average of 5,019 particles <788 m, with a maximum of 8,350 particles <788 m, that determines particulate count and size. An average of 5,019 particles <788 m, with a maximum of 8,350 particles <788 m, was calculated to be less than 0.8 mg/day. Therefore, chemical safety risk of the extractable amount of cobalt above the method detection limit (MDL) of 0.8 μg/L (ppb) for cobalt, which corresponds to a MDL of 0.3μg C.I. Pigment Blue 28. From this analytical data, the worst case total exposure was calculated to be less than 0.8 μg of C.I. Pigment Blue 28 per device, which was at least 112 times less than the threshold of toxicological concern (TTC) value of 90μg/day. Therefore, chemical safety of the extractable amount of C.I. Pigment Blue 28 from OptiCross under exaggerated conditions is assessed to be acceptable.
for limited-duration, blood-contacting devices (75,000 particles ≥10μm). Because the device category is limited duration and breached or compromised surface contact per ISO 10993-1, the size and number particles present on the device are acceptable and did not adversely impact the test results of the sensitization and intracutaneous reactivity biocompatibility tests.

**952 High-Density Polyethylene Controls in Toxicological Implant Studies**

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Implant toxicity studies for medical devices require controls that are similar in size, shape, and configuration to the test article. USP High Density Polyethylene (HDPE) is commonly used as a reference material (negative control) for implant studies when a predicate device control is not available, or not feasible for the study design. HDPE controls are matched as closely as possible to test articles by size, shape, and surface area. However, limitations in commercially available USP HDPE often preclude a close geometrical match to a test article, leading to concerns regarding the use of controls and test articles of unmatched dimensions and surface areas in medical device implant toxicity studies. A recent 13 week implantation toxicity study in rabbits compared the clinical pathology and histopathology of animals implanted with a test article to animals implanted with HDPE of two different dimensions and surface areas. Bilateral intramuscular implants were made in 24 animals. Eight test group animals were implanted with bar-shaped stainless steel /nitinol implants approximately 1 x 0.1 x 0.1 cm in size and a surface area of 0.4 cm². One group of eight control animals were implanted with USP HDPE of the same dimensions and surface area as the test article, and a second group of eight control animals were implanted with USP HDPE of a flat rectangular shape, 1 x 1.25 x 0.1 cm in size with a surface area of 3.0 cm². All animals were terminated after thirteen weeks. Gross necropsy, hematology, clinical chemistry, and histopathology of local and systemic tissues were evaluated and compared for each of the three groups. No significant differences were seen in clinical pathology parameters or local and systemic tissue reactions between the two control groups, or between the test group and either control group. Based on these results, using HDPE negative controls unmatched in size, shape and surface area to the test article does not significantly affect the clinical pathology or histopathology evaluation of subchronic intramuscular implantation toxicity studies in rabbits.

**953 Infusion Pump Accuracy Assessment: Weighing Syringes and IV Bags Not Necessary As Secondary Accuracy Check on Infusion Studies**

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It is common in some academic and preclinical safety assessment labs to weigh syringes and intravenous (IV) dosing bags pre and post dose administration to confirm dose delivery on infusion studies as a secondary accuracy check. This practice is very laborious, especially for preclinical studies using hundreds of animals, and difficult to document to GLP standards. We chose to assess the accuracy of several commercially available syringe and peristaltic infusion pumps to determine if weighing syringes and IV bags was necessary to confirm dose delivery. Surgically catheterized dogs and rats were infused using a tethered infusion apparatus and external peristaltic or syringe infusion pumps for three dosing cycles at various dose rates to mimic typical preclinical intermittent safety assessment infusion studies. Syringes and IV bags were weighed pre- and post-dose administration and dose volume delivered computed by subtracting the two values and employing a density correction for saline. The calculated dose volume was compared to the dose delivery volume listed on the infusion pump display screen. Percent of theoretical volume delivered was calculated for each animal on each dosing day. A total of 226 rats and 30 dogs were infused for three dosing cycles over 17 days. A total of 2096 syringe doses and 338 IV bag doses using peristaltic pumps were administered over the course of the study. At a threshold of +/-10% of theoretical, 98.7% of the doses listed on the infusion pump met acceptance criteria compared to the calculated dose volume for syringe pumps (99.4% for peristaltic pumps). At a threshold of +/- 5% of theoretical, the correlation was 90% and 77.8%, respectively. The accuracy of the syringe and peristaltic infusion pumps exceeded expectations and were comparable with acceptance criteria of other laboratory equipment used on GLP studies. Therefore, there is no scientific rationale for recording syringe weights pre- and post-dose delivery as a secondary infusion pump accuracy check.

**954 Comparison of Optical Coherence Tomographic and Histopathological Retinal Images and Investigation of Age-Related Change in Retinal Thickness in Cynomolgus Monkeys**


Purpose: Optical coherence tomography (OCT) captures images of ocular fundi non-invasively in real time, based on light reflected from surfaces of interest. In this study, retinal thickness was measured in cynomolgus monkeys using OCT and histopathological (HP) images, and the results were compared to investigate the utility of OCT. Age-related changes in retinal thickness were also investigated with OCT.

Methods: For comparison of OCT and HP (eyeballs were fixed in a mixture of 3% glutaraldehyde/2.5% formalin), 10 male monkeys (age: 3–5 years) with no history of retinal disease were used. Retinal thickness was measured using OCT and HP images at the mid point between the optic papilla and macula, in total and for the following 10 layers: internal limiting membrane (ILM), nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor cell (PR), and retinal pigment epithelial layer (RPE). For investigation of age-related change, retinal thickness was measured using OCT for each of the 10 layers in 40 female monkeys (10 animals/age at 1, 5, 10 and 13 years). These data were analyzed statistically.

Results: No difference was noted in total retinal thickness (approx. 340 μm) between OCT and HP images. GCL, INL and OPL were thinner (approx. 30–40%), and IPL and ONL were thicker (approx 50%), in OCT images than HP images. With increase in age, NFL thickness decreased by 23% (1 vs. 13 years) and ONL and INL showed a tendency toward decrease in thickness. IPL, OPL, GCL and RPE showed a tendency toward increase in thickness from 5 years; PR, ILM and ELM showed no age-related change in thickness.

Conclusion: Ocular examinations and evaluations in cynomolgus monkeys with OCT should be carried out taking differences between OCT and HP measurements and age-related change in retinal thickness into consideration.

**954a Creating a Holistic Extractables and Leachables (E&L) Program for Biotechnology Products**

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The risk mitigation of extractables and leachables (E&L) presents significant challenges to regulators and drug manufacturers with respect to the development, as well as the lifecycle management of drug products. A holistic program is proposed, using a science- and risk-based strategy for testing E&L for primary containers, drug delivery devices and single-use systems for the manufacture of biotechnology products. The strategy is phase-appropriate, progressing from extractables testing for material screening/selection/qualification through leachables testing of final products. The strategy is designed primarily to ensure patient safety and product quality of biotechnology products.

The holistic program requires robust extraction studies using model solvents, with careful consideration of solvation effect, pH, ionic strength, temperature, product contact surface and duration. From a wide variety of process- and product-contact materials, these extraction studies have identified and quantified over 200 organic extractable compounds. The commonly observed compounds are siloxanes, fatty acid amides and methylcylates. The extractables profiles often show degradation products of the chemical additives. Toxicology assessments have been conducted on these compounds using risk-based decision analysis. Safety thresholds have been derived, as appropriate, for the majority of these compounds. Analysis of safety thresholds has helped to establish action thresholds to target and monitor high risk compounds in drug products on stability until expiry. Action thresholds serve to trigger quality investigations to determine potential product impact.

The holistic program also has in place processes to assess the impact of E&L compounds to product quality attributes and risk for immunogenicity. This science- and risk-based approach for primary drug containers and drug delivery devices is also suitable for single-use systems when justified with a historical knowledge base and understanding of the manufacturing processes of biotechnology products.
Cobalt-chromium (CoCr) alloys have long been used in metal-containing hip implants because of their biocompatibility. Like all implant materials, CoCr alloys undergo some degree of wear and corrosion in vivo, and as a result implant patients typically have blood and tissue Co and Cr(III) concentrations that are a few-fold higher than background levels associated with dietary intake. Although to date epidemiology studies do not indicate an increased incidence of cancer in patients with hip implants, questions continue to be raised about the potential cancer risk posed by CoCr-containing implants. This analysis was conducted to determine whether the existing toxicology data support epidemiological findings. We evaluated the results of over 60 in vitro genotoxicity assays in human cell lines treated with Co and Cr(III) ions and particulate; endpoints included chromosomal aberrations, micronuclei formation, DNA breakage, and oxidative DNA damage. The in vitro concentrations at which genotoxic effects occurred (and did not occur) in these studies were compared to tissue concentrations of Co and Cr(III) that we conservatively estimated would exist in patients with normal functioning CoCr metal-on-metal (MoM) hip implants (wear rates of 1 mm3/year). For metal particulates, we found that in general, the minimum concentrations required to cause genotoxic effects in vitro (10–108 µg/ml) were more than 1,000-fold higher than those likely present in tissues of implant patients; similar results were obtained for studies on Co and Cr(III) ionic solutions. Thus, exposures to CoCr wear debris from MoM prostheses would not likely be genotoxic in vivo, and this is consistent with cancer epidemiology studies conducted to date.
been implicated in neurodegenerative diseases and cancer, yet the mechanisms by which it act have remained largely unknown. As an α,β-unsaturated aldehyde, acrolein could react nucleophilic amino acid residues such as lysine in susceptible proteins. The fact that histone proteins are rich in lysines raised the possibility that acrolein targets lysine residues in histone tails, affecting their physiological posttranslational modifications. Because appropriate histone lysine modifications are crucial for genome function, the formation of acrolein-histone adducts should have significant impact on cellular processes. Biochemical and Mass Spectrometry analyses show that acrolein forms adducts with some but not all lysines on histone proteins in vitro and in vivo and preferentially reacts with free histones rather than with nucleosomal histones. Cellular fractionation analyses reveal that acrolein exposure specifically inhibits acetylation of N-terminal tails of cytosolic histones H3 and H4, modifications that are important for nuclear import and chromatin assembly. Notably, acrolein exposure compromises the delivery of histone H3 into chromatin and increases chromatin accessibility as measured by Chromatin Immunoprecipitation, suggesting that acrolein may perturb histone-nuclear exchange. Changes in nucleosome occupancy at several genomic loci are correlated with transcriptional responses to acrolein exposure. We propose that reacting with newly synthesized but not yet modified histones thereby affecting histone modification and chromatin assembly pathway represents a novel mechanism whereby certain environmental factors interact with the genome and influence genome function.

954g Multifocal Defects in the Hematopoietic and Lymphoid Compartments in Mice Dosed with a Broad BET Inhibitor

Bromo and extra terminal (BET) proteins (BRD2, BRD3, BRD4 and BRD7) are epigenetic transcriptional regulators required for efficient expression of growth promoting, cell cycle progression and anti-apoptotic genes. Through their bromodomains, these proteins bind to acetylated lysine residues of histones and are recruited to transcriptionally active chromatin. Inhibition of the BET-histone interaction provides a tractable therapeutic strategy to treat diseases that may have epigenetic dysregulation. JQ1 is a small molecule that blocks BET interaction with histones. It has been shown to decrease proliferation of patient-derived multiple myeloma in vitro and decrease tumor burden in vivo in xenograft mouse models. While targeting BET appears to be a viable and efficacious approach, the safety profile of BET inhibition is largely unknown. In this study, we used JQ1 as a tool molecule to explore the potential safety issues related to BET inhibition, and provide early de-risking of the target. Mice dosed with JQ1 at efficacious exposures develop multifocal defects in the lymphoid and immune cell compartments. At higher doses, JQ1 was not tolerated and resulted in significant body weight loss in mice, which were subsequently euthanized early in the study. Flow cytometry analysis of lymphoid tissues showed a decrease in both B and T lymphocytes and a concomitant decrease in peripheral white blood cells were confirmed by hematology & histopathology. Further investigation is required to determine if these toxic effects are associated with BET inhibition or more specifically related to the chemical structure of JQ1.

954h Sex-Dependent Effects of Lead and Prenatal Stress on Histone Modifications in Frontal Cortex and Hippocampus of the Developing Brain

Sex-dependent differences characterize the response of the brain to lead (Pb) and prenatal stress (PS), but the molecular mechanisms are unknown. This study examined the potential epigenetic basis of Pb±PS effects on expression of sexually dimorphic histone modifications, H3K9/14Ac (associated with gene activation) and H3K9Me3 (associated with gene silencing), in frontal cortex (FC) and hippocampus (HIPP) in males and females at 2 developmental periods (PN0, PN6). FC appeared more vulnerable than HIPP, effects of Pb±PS, but neither alone were seen in males; effects were prominent at PN6 but not P0. Histone modifications are involved in many aspects of brain development including neurogenesis, synapse formation, and cell differentiation/migration. Sex differences in histone modification may underlie different functional outcomes from Pb±PS in males and females. ES021534-01

955 Global DNA Methylation Profiling of Hepatocellular Carcinomas from Ginkgo biloba Extract-Treated and Vehicle Control B6C3F1 Mice

Epigenetic modifications, such as DNA methylation, play an important role in the development of hepatocellular carcinoma (HCC). In a recent NTP 2-year carcinogenicity bioassay, chronic exposure of B6C3F1 mice to Ginkgo biloba extract (GBE) resulted in a high incidence of HCC. To better understand the gene-specific methylation alterations in HCC due to GBE exposure, we performed global methylation profiling on HCC from GBE exposed mice (GBE-HCC; n = 5), spontaneous HCC from vehicle control mice (spont-HCC; n = 5), and age-matched normal liver from vehicle control mice (VN; n = 5), in order to identify differentially methylated regions (DMRs) in HCC from GBE exposed animals. The global methylation profiling included 20,404 promoter regions and 15,988 annotated CpG islands, and methylation changes were correlated to corresponding transcriptomic changes. Compared to VN liver, 1400 CpG sites in GBE-HCC and 849 CpG sites in spont-HCC were differentially methylated (DM; P < 0.01). Compared to spont-HCC, 138 CpG sites were DM in GBE-HCC (P < 0.01). Using pyrosequencing, we confirmed promoter-specific hypomethylation of the Myc oncogene in GBE-HCC compared to spont-HCC (P < 0.001), with a corresponding increase in gene expression showing significant negative correlation with methylation changes (r = -0.93; P = 0.01). In addition, we also confirmed hypermethylation of tumor suppressors (Spry2, Cdkn2a, Dusp5) in GBE-HCC compared to spont-HCC (P < 0.001) with a corresponding decrease in gene expression. In conclusion, we have identified DMRs in promoter CpG islands of relevant cancer genes altered in GBE-HCC compared to spont-HCC, suggesting that such promoter CpG island DMRs could be used to differentiate treatment-related tumors from background, spontaneous tumors in cancer bioassays. Furthermore, these data provide additional understanding of the underlying epigenetic mechanisms of chemical carcinogenesis in animals and their relevance to human health and hazard identification.

956 Hepatic Epigenome Controls the Severity of Liver Injury in Mice Induced by a Choline- and Folate-Deficient Diet
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The importance of epigenetic changes in etiology and pathogenesis of disease has been increasingly recognized. However, the role of epigenetic alterations in the genesis of nonalcoholic fatty liver disease (NAFLD) and cause of variations in individual susceptibilities to this disease are largely unknown. In the present study we investigated whether or not the epigenetic profile of normal liver affects the severity of NAFLD-associated liver injury. Male WSB/EiJ and A/J inbred mice were maintained on either control or choline- and folate-deficient (CDF) diets for 12 weeks and the status of DNA methylation and histone H3 lysine 4 trimethylation (H3K4me3) was determined using whole genome methylated DNA immunoprecipitation (MeDIP) and H3K4me3-chromatin immunoprecipitation (ChIP)-on-chip techniques. Feeding the CDF diet for 12 weeks caused significantly greater liver injury in WSB/EiJ mice as compared to A/J mice. MeDIP analysis shows that the greater vulnerability of WSB/EiJ mice was associated with a hypomethylated liver phenotype. This was evidenced by a greater number of hypomethylated Cpg domains than hypermethylated Cpg regions, 3429 and 471, respectively, and consequently a high hypomethylated/hypermethylated Cpg domain ratio in DNA isolated from normal livers of WSB/EiJ mice. In contrast, DNA isolated from normal livers of A/J mice contained approximately an equal number of hypomethylated and hypermethylated Cpg regions, 2044 and 1856, respectively. ChIP-on-chip analysis showed differential hypomethylation of H3K4me3 enrichment throughout the genome in the normal livers of WSB/EiJ and A/J mice, but it was not associated with the extent of the CDF diet-induced liver injury. Interestingly, feeding a CDF diet caused opposite effects on DNA methylation: increased methylation in the livers of H3K9Me3 levels. These results suggest that Pb±PS alters H3 acetylation/trimethylation early during development in a sex-dependent manner, leading to potential errors in transcriptional regulation. FC appeared more vulnerable than HIPP. Enhanced effects of combined Pb±PS were seen only in males; effects were prominent at P0 but not P6. Histone modifications are involved in many aspects of brain development including neurogenesis, synapse formation, and cell differentiation/migration. Sex differences in histone modification may underlie different functional outcomes from Pb±PS in males and females. ES021534-01
957 Epigenetic Alterations in the Livers of Fisher 344 Rats Exposed to Furan


Furan is a volatile heterocyclic compound widely used in the chemical manufacturing industry. It is also found in a variety of common cooked foods consumed by adults and infants. Lifetime exposure to furan causes liver tumors in rats and mice (NTP, 1993); however, the mechanisms of the furan hepatocarcinogenicity are still unclear. The goal of the present study was to investigate whether or not the exposure to furan causes epigenetic alterations in rat liver. Male Fisher 344 rats were treated by gavage with different doses of furan (0, 0.92, 2.0, or 4.4 mg/kg body weight/bw/day) dissolved in corn oil. The exposure of Fisher 344 rats to furan produced dose- and time-dependent epigenetic changes in the livers consisting of alterations in the global pattern of histone lysine methylation and acetylation, gene-specific methylation, and altered expression of chromatin modifying genes. Specifically, there was a sustained decrease in the levels of histone H3 lysine 9 and H4 lysine 20 trimethylation in the livers after 180 and 360 days of furan exposure, and a marked reduction of histone H3 lysine 9 and H3 lysine 56 acetylation after 360 days of treatment at 4.4 mg/kg/bw/day. These histone modification changes were accompanied by a reduced expression of related Suv39H1, Prdm2, and Suv4-20h2 histone methyltransferases, Ep300 histone acetyltransferase, and an up-regulation of Sir6 histone deacetylase. The exposure to furan at doses 0.92, 2.0, and 4.4 mg/kg/bw/day caused global demethylation of hepatic DNA after 360 days of treatment. Additionally, furan at 2.0, and 4.4 mg/kg/bw/day doses induced hypermethylation-dependent down-regulation of the Rassf1a tumor suppressor gene in the livers after 180 and 360 days of treatment. In summary, these findings indicate possible involvement of dose- and time-dependent epigenetic modifications in the hepatotoxicity and carcinogenicity of furan.

958 Genome-Wide DNA Methylation Changes Influence Gene Expression in Arsenic-Transformed Human Prostate Cells


NextGen sequencing provides genome-wide coverage at base-pair resolution of DNA alterations for environmentally-related diseases, like cancer. In this study, methylated DNA was affinity-enriched, bisulfite treated and sequenced for high-resolution detection of differential methylation in arsenic-transformed, CàE-PÉ cells derived from normal, RWPE-1 human prostate cells. We hypothesized that changes in methylation status might change gene expression in CàE-PÉ cells and contribute to malignancy. A total of 74,844 million bisulfite-treated DNA sequenced reads (100bp, paired end) were produced. Analysis focused on the most differentially methylated bases (cytosines), termed DMBs, and showed a >50% change in methylation state. We organized DMBs into structural units of differentially methylated clusters (DMCs), as short regions with 4 or more DMBs. More than 7,000 DMCs were found in the two cell lines. There were >500 genes with at least one DMC in the promoter region for which >40 hypermethylated cytosines and 10 hypomethylated sites have been independently validated by 454 pyrosequencing. However, only 25% of all DMBs were found within CpG islands or shores while the majority of DMBs were not data. Data suggest that CpG-based platforms (e.g. CpG bead arrays) may underrepresent the methylene and not be fully capable of measuring differential methylation. Additionally, RNA-Seq showed a large increase in KRAS mRNA in CàE-PÉ cells compared to RWPE-1. KRAS is a well-known oncogene whose enhanced activity (mutated forms) or increased mRNA levels can be upregulated in many types of tumors. While no mutations were seen in KRAS mRNA in either cell line, other oncogenic mechanisms may have occurred that play a major role in malignant conversion to form CàE-PÉ cells. We believe these data will offer insights into epigenetic effects of arsenic carcinogenesis and a technical approach to studying epigenetic mechanisms.

959 Environmental Health Disparities in the Age of Epigenetics


Over the past decades, genome-wide association studies have identified certain genetic loci for numerous diseases and traits. However, genetic variations identified to date account for only a small proportion of disease phenotypes. Growing evidence suggests that environmental exposures can influence human disease risk through the accumulation of epigenetic modifications. This general hypothesis provides a broad umbrella to examine how the environment “gets under skin” among selected racial, ethnic and/or socioeconomic groups. We systematically reviewed published literature and propose the “neighborhood-specific” epigenome analysis as a promising approach to study environmental health disparities. In this treatise, environment is characterized in the broad sense of “non-genetic” factors including social, biological, chemical and physical stressors. The hypothesis suggests that understanding the complex interactions between genes, the environment and chronic disease requires the examination of the role of epigenetics in regulating genetic susceptibility to environmental stressors. The slow progress in understanding disparities in disease susceptibility may be in part related to the fact that studies have not considered the cumulative effect of environmental exposure to both chemical and non-chemical stressors on genetic substrates. “Geographical neighborhood-specific” epigenetic markings can potentially be used as a tool to investigate proposed mechanisms to account for well documented racial and ethnic disparities in health. Additionally, by considering neighborhood-specific epigenetic profiling, one could then identify novel targets for population-based prevention efforts. Through proposed neighborhood-specific epigenome analysis and approaches, we provide considerable insight into the causes of health disparities and their potential prevention and intervention strategies.

960 Cadmium Alters Histone Modifications in Mouse Embryonic Stem Cells

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Epigenetic changes in histone modifications due to cadmium exposure early in life results in pathologies observed during adulthood. We tested the effects of low dose acute Cd exposure on cell viability, total histone protein (THP) production, histone acetylation and deacetylation at multiple sites, cell cycle progression in mouse embryonic stem (mES) cells. Exposure to 10 μM Cd altered cell cycle progression at 1-h and 24-h (± recovery) but differentiation patterns were affected only during 72-h exposures. In addition, flow cytometry data demonstrated that Cd IC25 concentration decreased the number of cells appearing in the S-phase and reduced newly synthesized DNA during acute exposures, whereas cell differentiation is inhibited during chronic exposures in mES cells. Also, we previously showed that Cd alters THP production and H3K27me1 levels (Gaethia et al., 2012), suggesting chromatin damage and transcriptional changes. Whereas H3K27me1 is a precursor to H3K27me2/3, we analyzed the effects of Cd using 2-stage hepatocarcinogenesis model using 2-Stage Hepatocarcinogenesis Model.
962 Effect of THC on Genome-Wide Histone Methylation in Immune Cells
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Despite the fact that marijuana may have beneficial medical effects in patients with certain diseases, increasing evidence indicates that marijuana suppresses immune function. Therefore marijuana use may increase susceptibility to infection and some types of cancer. To further investigate the effect of marijuana on immune function, we used an animal model to determine the role of Δ9-tetrahydrocannabinol (THC), the major effective component in marijuana, in epigenetic modification of genes in immune cells. Since epigenetic modification plays a critical role in immune cell differentiation and lineage commitment, we sought to investigate whether THC alters global histone methylation. Using ChiP-Seq technology, we compared genome-wide histone H3K4, H3K27, H3K9 and H3K36 trimethylation patterns between THC and vehicle treated activated popliteal lymph node cells. Our results show that THC treatment leads to the association of active modification signals with Th2 cytokine genes and suppressive modification signals with Th1 cytokine genes. These results suggest that THC inhibits Th1 cells while promoting Th2 cells through histone modification. At the genomic level, a significant amount of histone methylated regions are altered by THC. Functional classification of these histone methylation associated genes shows that these differentially associated genes are involved in various cellular functions, from cell cycle to metabolism, suggesting that THC regulates global gene expression in immune cells through histone modification. (Supported in part by NIH grants P01AT003961, R01AT066888, R01ES019133, R01MH094755, P20RR02684 and VA Merit Award BX001357)

963 Prenatal Exposure to TCDD Triggers Epigenetic Modifications Including DNA Methylation and microRNA Induction That Target Genes Which Regulate Immune Functions
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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a potent aryl hydrocarbon receptor (AhR) ligand and an environmental contaminant. Prenatal exposure to TCDD is well known to cause thymic atrophy as well as alterations in T cell differentiation, the precise mechanisms of which are unclear. In the current study, therefore, we investigated the role played by epigenetic mechanisms in regulating the immunotoxic effects of TCDD following prenatal exposure. To this end, pregnant mice on gestational day 14, were injected with TCDD (5 μg/kg) and on postnatal day 4, the thymus were harvested and high-throughput microRNA (miR) and genome-wide MeDIP methylation arrays were performed. We observed more than 69 miRs to be up- or down-regulated greater than 2 fold in fetal thymocytes post TCDD exposure when compared to controls. We validated the expression of several miRs by performing Real-Time PCR. The data obtained from miR arrays of fetal thymus post-TCDD exposure demonstrated changes in miR profile that affected expression of important genes such as, AhR, CYP1A1, Foxp3, IL-17, Fas and Fasl, which are known to regulate signaling, toxicity, apoptosis, thymic atrophy, and immunosuppression caused by TCDD. The data obtained from methylation arrays, upon further analysis, showed significant differences in methylation status of specific genes such as AhR, CYP1A1, IFN-γ, IL-4, FoxP3, and IL-17 in thymus following prenatal exposure to TCDD. We also noted induction of regulatory T cells and suppression of Th17 cells. Together, our studies suggest that prenatal exposure to TCDD triggers significant alterations in miRs as well as methylation status of a wide range of genes that regulate the immune functions (Supported in part by NIH grants P01AT003961, R01ES019133, R01MH094755, P20RR02684 and VA Merit Award BX001357).

964 Physiological Effects of Developmental Lead (Pb) Exposure on Weight, Food Intake, Body Fat, and Insulin in Mice
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Developmental exposure to lead (Pb) can have persistent effects on physiology and has been associated with lower body weight in human infants and later onset obesity in mice. Epigenetic reprogramming of transposons and imprinted genes during development is a plausible mechanism linking early exposures and physiological changes later-in-life. Mouse dams (n=49) were randomized to one of four groups where drinking water was supplemented 2 weeks prior to mating, through weaning, with 0 (control), 2.1 (low), 16 (medium), or 32 (high) ppm Pb. We measured 18 outcomes in offspring (n=30 control, n=28 low, n=35 medium, and n=29 high): physiological measures at 3, 6, and 9 mos of age, and epigenetic drift in tail DNA from weaning to 10 mos. We estimated average, sex-specific changes in physiological outcomes over time and their differences across exposure using repeated measures models adjusted for within-litter and within-mice correlations. Overall both sexes exhibited increased energy expenditure (p<0.0001) as well as increased food intake (p<0.0001) compared to controls. Similarly, body weight increases were found in males at medium (p<0.004) and high (p<0.029) exposure, whereas overall, total body fat increased in males and decreased in females (both p<0.0001). However, relative to controls, insulin was increased only in males at the medium level (p<0.05). We measured linear trends in relative methylation shift over time vs. exposure level using a linear mixed model. Epigenetic drift measured at the transposon linked metastable epiallele, CbpPlAP had significant, exposure-dependent increases over time (p<0.02). In general, imprinted genes increased in methylation over time but had locus-specific responses to Pb. For example, Igf2 showed no association with Pb, while Igfl2 exhibited increased methylation with Pb (p=0.045), and H19 displayed moderately increased methylation with Pb (p=0.085). Perinatal Pb exposure has both physiological and epigenetic effects over life course in mice that vary by sex and locus.

965 Effect of 2, 3, 7, 8-Tetrachlorodibenzo-p-Dioxin (TCDD) Exposure on the Expression of DNMT Genes during Development in Zebrafish (Danio rerio)
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DNA methylation is one of the most important epigenetic modifications involved in the regulation of gene expression. Recent studies have demonstrated that environmental toxicants can affect normal development by altering DNA methylation patterns. However, the mechanisms of action of these chemicals are poorly understood. Hence, we tested the hypothesis that developmental exposure to TCDD affects DNA methyltransferase (DNMT) gene expression patterns. To address this hypothesis, zebrafish embryos were exposed to 5 nM TCDD for one hour from 4 to 5 hours post-fertilization (hpf) and sampled at 12, 24, 48, 72 and 96 hpf to determine DNMT gene expression patterns and global methylation patterns. Results demonstrate that TCDD upregulates DNMT1 gene expression, whereas DNMT3 genes (DNMT3a1, 3b1, 3b4) are downregulated following exposure. These results suggest that TCDD exposure may impact both maintenance and de novo methylation. To characterize the TCDD effects, we screened the 2 kilobase region of the DNMT gene promoters for xenobiotic response elements (XRE). We identified at least one XRE in each DNMT promoter. We are characterizing the function of these XREs in in vitro luciferase reporter assays. Furthermore, we are quantifying global DNA methylation levels using liquid chromatography to correlate changes in gene expression with methylation patterns. Funding for this project was provided by WHOI Ocean Life Institute and NIH grant P01ES021923 and National Science Foundation Grant OCE-1314642 through the Woods Hole Center for Oceans and Human Health.
Adult exposure to water pollutant trichloroethylene (TCE) in drinking water has been shown to induce autoimmune hepatitis in female MRL+/+ mice. This was accompanied by expansion of activated/memory CD4+ T cells that secreted increased levels of IFN-γ. Several aspects of normal and autoreactive CD4+ T cell function, including differentiation and cytokine production, are regulated by gene-specific alterations in DNA methylation. In the current genetic study transcriptomics and toxicogenomics were used to define time-dependent alterations in gene expression and related DNA methylation in CD4+ T cells from MRL+/+ mice exposed to TCE for up to 40 weeks. Naïve and memory/activated subsets of CD4+ T cells were examined separately. We observed differential DNA methylation in CD4+ T cells from TCE and control mice. These TCE-induced alterations in DNA methylation were CpG-, time-, and subset-specific. The alterations encompassed genes important in CD4+ T cell differentiation, cell cycle proliferation and cytokine production. This study is the first to document that low level adult exposure to TCE induces temporal, specific, and potentially functionally important, epigenetic alterations in CD4+ T cells.

Nickel, an environmental carcinogen, is a widely used metal and release of nickel compounds into atmosphere occurs during various stages of industrial processes. Nickel compounds have been conclusively shown to induce lung and nasal cancers in humans. In spite of its proven carcinogenicity, the mutagenic potential of nickel is very low. Therefore, nickel-induced carcinogenesis appears to be initiated predominantly by non-genotoxic mechanisms. It has been shown earlier that exposure to nickel alters the levels of various epigenetic modifications and growing evidence points to epigenetic dysregulation as a major outcome of nickel exposure. However, the mechanistic basis of nickel-induced epigenetic alterations is not fully understood. In this study, using ChIP-Seq, we comprehensively analyzed NGC2-induced alterations to several activating and repressive histone modification marks on a genome-wide scale in the immortalized non-cancerous human lung epithelial, BEAS-2B cells. Using RNA-Seq, we detected hundreds of genes with altered expression due to nickel exposure. A large majority of these genes had altered epigenetic profiles in their promoters and gene body regions indicating the extent of epigenetic alterations induced by nickel. Interestingly, the nickel-induced alterations to histone modification marks were not only limited to promoters and regulatory regions of the genome, but also extended to large genomic regions. Taken together, our results indicate disruption of chromatin structure as an important mechanism in nickel-induced gene expression alterations, which could potentially lead to carcinogenesis. We believe that our findings will serve not only as a model to understand the mechanism involved in epigenetic alterations induced by an environmental carcinogen, but also help comprehend how epigenetic aberrations contribute to carcinogenesis. Supported by National Institutes of Health grants R01ES023174 and P30ES002620.

Hexavalent chromium [Cr(VI)] is a potent human carcinogen. Occupational exposures have been associated with increased risk of respiratory cancer. Increasing evidences suggest that multiple mechanisms might contribute to Cr(VI) induced carcinogenesis, including DNA damage, genomic instability, and epigenetic modulation. As an important epigenetic regulator of gene expression, DNA methylation in the promoter region of genes is inversely correlated to gene transcription. Aberrant DNA methylation in the gene promoter region has been found in many human tumors, which is responsible for their silencing. Previous studies on chromium-induced human cancer revealed a decreased expression of MLH1 and p16 that was accompanied by an increased DNA methylation in their promoter region, indicating that chromium was able to silence tumor suppressor genes by modulating DNA methylation status. To further investigate the effect of chromium on DNA methylation of other tumor suppressor genes, we analyzed the promoter methylation of 22 tumor suppressor genes in workers from a chromate factory and referent subjects using EpiTect Methyl II PCR array. Among 22 tumor suppressor gene promoters whose hypermethylation was frequently observed in many human cancers, three genes (WIP1, APL, and MLH1) exhibited elevated promoter DNA methylation in chromate workers compared to referent subjects. Multiple linear regression analysis indicated a positive correlation between promoter DNA methylation of these three tumor suppressor genes and the levels of Cr(VI) in air and in the red blood cells. Our studies have identified new target tumor suppressor genes that were silenced in human by chromate through epigenetic regulation.

Mechanisms underlying alcohol induced immunotoxicity are poorly understood. Fasl, plays a critical role in regulating physiologic as well as pathologic apoptotic death of CD4+ T cell and the immune response. Although transcriptional regulation of Fasl has been extensively studied, the role of epigenetic mechanisms is only poorly understood. Accordingly, immunotoxic effects of alcohol on promoter histone modifications regulating Fasl gene expression in human primary CD4+ T cells were examined. Effect of stimulation and alcohol exposure on Fasl promoter-histone modifications was examined in chromatin samples prepared from CD4+ T cells obtained from non-alcoholic and alcohol abusing study subjects by quantitative chromatin immunoprecipitation (ChIP) analysis. In comparison to normal CD4+ T cells, alcohol exposure significantly increased transcriptionally permissive trimethylation at histone H3 lysine 4 (H3K4me3), with a coordinate decrease in transcriptionally repressive trimethylation at H3 lysine 9 (H3K9me3). Commensurate with the decrease in H3K9me3, the acetylation of H3K9 (H3K9ac) which is an essential modification for transcriptional activation and is mutually exclusive to methylation, was further increased upon alcohol exposure. Notably, increase in H3K9ac was determined to be critically regulated by the p300 histone acetyltransferase (p300/ HAT). Further, in correspondence with transcriptionally germine histone modifications, alcohol enhanced the recruitment of relevant transcription factors and RNA polymerase II to the Fasl promoter, which correlated with increased Fasl expression and apoptotic death. Overall, these data identify the dynamic interplay between promoter H3K4 and H3K9 methylation and acetylation as a significant mechanism regulating Fasl gene expression in alcohol-induced immunotoxicity.
Epigenetic regulation of gene expression plays a pivotal role in the orchestration of immune responses and may impact their vigor, quality or longevity. Chemical allergens can be broadly divided into two categories: contact allergens (e.g. dinitrochlorobenzene; DNCB) and respiratory allergens (e.g. trimellitic anhydride; TMA) that health effects in human due expose to PAHs had been augmented since last decade. Several health effects related to exposure of different PAHs, including benzo(a)pyrene (BaP), were reported as asthma, genotoxicity, altered neurodevelopment and interestingly highlight the proinflammatory effects. Recently, it has been observed that the main epigenetic mechanisms such as DNA methylation, histone modification and microRNAs are be able to respond to external factors as pollutants. In this work we explored if BaP be able to modify two epigenetic mechanisms. We found that miRNAs expression are altered due BaP exposure in human MNC in vitro.

Epigenetic regulation of dopamine transporter expression is the key regulator of dopaminergic transmission and is a target of several xenobiotics, including pesticides and pharmaceutical agents. Therefore, determination of the mechanisms by which DAT expression is regulated may provide insight into the actions of these compounds on the nervous system. Using in silico analyses, we identified regions within the DAT promoter which may contribute to its regulation at the mRNA level. The DAT promoter is CpG rich, indicating potential regulation by DNA methylation. Additionally, the lack of a conserved TATA box suggests regulation by histone acetylation. To determine the relative roles of DNA methylation and histone acetylation, we utilized a rat dopaminergic cell line (N27) to probe the responsiveness of the DAT to manipulations by inhibitors of DNA methyltransferases (DNMT) and histone deacetylases (HDAC). Inhibition of DNMTs using 5-aza-2-deoxycytidine led to a 1.25-fold increase in DAT mRNA expression. This was confirmed by siRNA experiments. However, valproate exposure increased DAT mRNA expression by up to 10-fold. To confirm the mechanism of HDAC inhibitor-mediated increase in DAT mRNA, chromatin immunoprecipitation assays were used to determine proteins recruited to the DAT promoter following valproate treatment. Data demonstrate a 4-fold increase in acetylation of histone 3 on lysines 9 and 14 (H3K9/K14ac) in the DAT promoter. Together, these results indicate that histone acetylation appears to be a plausible mechanism for DAT regulation by valproate and perhaps, by other xenobiotics. Support provided by NIEHS grants P30ES005022, R01ES051991, T32ES007148.
promoter was hypomethylated (11%) in the epithelium of the small intestine, whereas the UGT1A10 promoter was hypermethylated (89%) in hepatocytes. A luciferase assay revealed that the methylation of the UGT1A10 promoter by SsII methylase abrogated transactivity even with overexpressed Cdx2 and HNF1β. The UGT1A10 promoter was highly methylated (86%) in liver-derived HuH-7 cells, where UGT1A10 is not expressed. In contrast, the UGT1A10 promoter was hardly methylated (19%) in colon-derived LS180 cells, where UGT1A10 is expressed. Treatment with 5-aza-2’-deoxycytidine (5-Aza-dC), an inhibitor of DNA methylation, resulted in an increase in UGT1A10 mRNA expression only in HuH-7 cells. Moreover, the overexpression of HNF1β and Cdx2 further increased CUGT1A10 mRNA only in the presence of 5-Aza-dC. Collectively, we found that DNA hypermethylation would interfere with the binding of HNF1β and Cdx2, resulting in the defective expression of UGT1A10 in human liver. Thus, epigenetic regulation is a crucial determinant of the tissue-specific expression of UGT1A10.

Conclusion: Skin manifestation showed positive association with whole genome DNA methylation in a dose-dependent manner while other biomarkers did not indicate any. The underlying mechanisms and physiologic implications of the increased skin manifestation need further study.


Perfluorooctanoic acid (PFOA) is a stable man-made compound with many industrial and commercial uses. Recently, however, concern has been raised that it may induce various toxicological effects such as hepatotoxicity, immunotoxicity, and developmental toxicity. Because levels of circulating microRNAs (miRNAs) can be altered in several clinical diseases, they may serve as potential novel biomarkers. Here, we explored differences in the profiles of circulating miRNAs in mice after PFOA exposure. Using TaqMan miRNA arrays, we determined that the levels of 24 circulating miRNAs were altered in mice dosed with PFOA at 1.25 mg/kg/d and 73 were altered in mice dosed with 5 mg/kg/d. Eight miRNAs, namely miR-28-5p, miR-32-5p, miR-34a-5p, miR-122-5p, miR-192-5p, miR-200c-3p, miR-26b-5p and miR-199a-3p, were further validated using TaqMan Real-Time PCR assays. Results were consistent with those obtained from the TaqMan miRNA arrays, except for miR-199a-3p. The most remarkable of the circulating miRNAs (miR-26b-5p and miR-199a-3p) were also up-regulated in the serum of occupational workers in our previous epidemiological study. We also found similar patterns in mice exposed to PFOS and the observed increase of circulating miRNAs may not only be specific for PFOA but also for PFOS and even other PFASs. These results demonstrated that circulating miRNA profiles were altered after exposure to high concentrations of PFOA and miR-28-5p, miR-32-5p, miR-122-5p, miR-192-5p, and miR-26b-5p in serum may be potential biomarkers of PFOA induced toxicological effects, especially in occupational exposed people.

Correlation between Whole Genome DNA Methylation and Biomarkers of Arsenic Toxicity in Arsenic-Exposed Populations of West Bengal, India

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Background: Arsenic is a potent environmental toxicant. Number of individuals in West Bengal, India are endemically exposed to inorganic arsenic, far above the acceptable limit (10μg/L). Though Pigmentation, keratosis are the specific skin lesions characteristic of chronic arsenic toxicity, various systemic manifestations are there over and above skin lesions, like chronic lung diseases, skin, lung urinary bladder cancer. Despite being recommended as a Group 1 carcinogen, arsenic is a poor in vitro mutagen in both bacterial and mammalian systems. Thus, epigenetic mechanisms of carcinogenesis (hyper/hypo methylation of DNA) has been invoked. Arsenic exposure may result in global DNA hypermethylation in human; still the association between DNA methylation and the known biomarkers of arsenic toxicity remains to be determined.

Objective: The objective of the study presented here is to find if there is any correlation between biomarkers of arsenic toxicity like skin manifestation score, arsenic intake, hair and urinary arsenic deposition, smoking and tobacco chewing habit, gender with whole genome DNA methylation in exposed individuals of West Bengal. Method: PBL DNA quantification and methylation, plasma and urinary arsenic concentrations, smoking habit and skin manifestation were assessed in a study population, in the district of Nadia, West Bengal. Genomic DNA methylation was measured by using ELISA-based assay. An Institutional Ethical committee (IRB) meeting was held to review the project in depth and approved. Result: Our data indicated positive correlation between whole genome DNA methylation and skin score (p=0.004).

1,3-Butadiene (BD) is a widely used industrial chemical and a ubiquitous environmental pollutant that is a known human carcinogen. Although genotoxicity is an established mechanism of the carcinogenicity of BD, epigenotoxicity has also been observed in livers of mice exposed to the chemical. To better understand the molecular mechanisms associated with BD effects in other tissues, we evaluated BD genotoxicity and epigenotoxicity in both target (lung and liver) and non-target (kidney) tissues of butadiene-induced carcinogenesis. We hypothesized that epigenotoxic effects may explain, at least in part, the tissue-specific differences in BD carcinogenicity in mice. Male C57BL/6 mice were exposed to 0 or 425 ppm of BD by inhalation (6 hr/day, 5 days/week) for 2 weeks. For assessment of genotoxic and epigenetic alterations, we evaluated THB-Gua adducts and bis-N7G-BD crosslinks, and levels of histone and DNA methylation, respectively. Liver and lung tissues of BD-exposed mice exhibited DNA damage as well as epigenetic alterations indicative of genomic instability. Marked genotoxic effects of BD were also observed in the kidneys, but epigenetic alterations clearly differed in this non-target tissue as compared to liver and lung. Specifically, trimethylation of histones H3K9, H3K27, and H4K20, all marks of condensed heterochromatin and transcriptional silencing, was increased in the kidneys of treated mice as compared to controls. These modifications may represent a post-repair chromatin restoration response at sites of DNA damage and present a potential mechanistic explanation for the lack of tumor susceptibility of this non-target tissue. These results indicate that tissue-specific epigenetic alterations are associated with tissue specificity in carcinogenesis, even in cases of known genotoxic carcinogens.

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Epigenetic modifications of DNA, such as methylation of cytosine, play a critical role in regulating miRNA expression, miRNA levels, and cellular development, and have been linked to human diseases such as cancer. The role of epigenetics in toxicological development and diseases has not been thoroughly reviewed; however, there is a lack of a comprehensive study to understand the epigenetic effects on liver disease susceptibility and response to xenobiotics at different life stages. Furthermore, these epigenetic changes may serve as biomarkers for disease progression. Recent advances in high throughput technologies make it possible to monitor the methylation aberrations at the whole genome level. In this study, liver tissues from both sexes of Fischer 344 rats at 2, 5, 6, 8, 15, 21, 52, 78, and 104 weeks of age were examined for sex- and age-specific alterations in DNA methylation using Roche 385k rat promoter methylation microarrays. The raw image data were extracted and analyzed by DEVA software (v1.2.1) to determine the methylation changes in these samples. Tukey’s biweight mean centering normalization was applied before downstream analysis. One-way ANOVA was used to identify differences between groups. Principal component analysis indicated that there are greater sex differences than age difference in terms of methylation patterns. Within each sex, the results also showed methylation changes occurring from early age through old age. In contrast, the differences between 52wk, 78wk, and 104wk animals were much smaller in male animals compared to female animals, in which there were greater differences between 52wk and older animals. The number of methylated sites was very similar between different groups although there was variability in the chromosomal sites. These age- and sex-related differences in DNA methylation may provide insights in susceptibility to liver disease, adverse drug events and their progression.

Epigenomic changes induced by environmental factors are important for the acquired phenotype. It is thought that degree of its variability is small and that its deviation is large among individuals. Since no appropriate method has been available to quantify CpG-methylation frequency (MF) in a genome-wide manner in terms of accuracy, precision, and cost, we developed a novel method (methylation site display (MSD)-AFLP) and then applied this method to examine influence of bisphenol-A (BPA) exposure on DNA methylation in mouse tissues. MSD-libraries prepared by digestion with different pairs of adapter were used to obtain AFLP-chart data of the DNA methylation of C57BL/6j mice tissues (liver, kidney and hippocampus). Many CpG-sites suspected to be the tissue-specific differentially methylated regions (tDMRs) were detected by 16-selective primer sets. Nucleotide sequences adjacent to these methyl-CpG sites were determined and the MF was analyzed by MSRE-PCR to confirm the precision of the AFLP analysis. The differences of the MF among tissues were almost identical in the both methods. Additionally, AFLP data from Ss11-treated genomic DNA was used to calculate MF from MSD-AFLP. Many CpGs showing less than 5% of statistically significant tissue-specific difference and less than 10% of degree of variability were detected, suggesting that MSD-AFLP is a highly precise analytical method. Finally, hippocampus DNAs from male pups exposed to 200 mg/kg BPA (n=6) in utero and from control pups (n=6) were analyzed by MSD-AFLP. No statistically significant change of MF by BPA exposure was detected in 43,840 CpGs, despite using the full 256-selective primer sets. Our newly developed method will provide accurate values in MF and be useful to screen unknown subtle differences of methyl-CpGs in a genome-wide manner. In addition, nearly no influence of in utero BPA exposure on DNA methylation was observed in mouse hippocampus.
indeed a skin irritant, as evidenced by PMN and inflammatory macrophage infiltration and activity. Furthermore, characteristic inflammation may be dependent on genetic background.

980 Comparative In Vivo and Ex Vivo Toxicity Studies of Wildfire Particulate Matter


Inhalation of particulate matter (PM) generated from biomass burning is of concern particularly as the frequency and severity of wildfires have been increasing. Size-fractionated PM samples (ultrafine, <0.2 µm; fine, 0.2-2.5 µm; coarse, 2.5-10 µm) were collected downwind from a peat-bog wildfire in Eastern North Carolina from 6/26/2008 – 7/11/2008 when the fire was smoldering (ENCF-1), or at a later time from 8/8/2008 – 8/19/2008 after the fire had been controlled but not fully extinguished (ENCF-4). Wildfire PM samples were extracted in methanol for toxicity studies and chemical analysis, and then given by oropharyngeal aspiration to mice at 100 µg/50 µl saline or cultured with lung tissue slices for 11 µg per lung slice (8 mm diameter). At 4 h and 24 h post-exposure, biomarkers of lung injury and inflammation were assessed in lung lavage fluid from mice, and in conditioned medium from the lung slices. In addition, lung slices were also exposed to wildfire PM pre-treated with the endotoxin inhibitor (polymyxin B). The results showed that both ENCF-1 and -4 coarse PM had 4-8 times greater endotoxin content than the fine and ultrafine PM. Moreover, only the coarse PM significantly increased neutrophils and biomarkers of inflammation (e.g., interleukin-6) in the lung lavage fluid at any time point, and similar patterns of pro-inflammatory effects were observed in the lung tissue slice model. Finally, the inflammatory effects in the lung slices were significantly diminished after exposure to the PM pre-treated with polymyxin B. We conclude that exposure to wildfire coarse PM caused substantial acute toxicity in the mouse lung in association with endotoxin content, and that the lung slice ex vivo model provides a good alternative for in vivo lung toxicity testing. (This abstract does not represent U.S. EPA policy).

981 The Impact of Endotoxins in African Dust: Cause for Inflammatory Response

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African Dust Events (ADE) are believed to be associated with respiratory diseases such as asthma in Puerto Rico. Particle pollution in the form of PM10 contained in windblown dust is known to possess bacterial molecules such as endotoxins (ENX) as part of its biological components. ENX are able to signal the activation of pro-inflammatory genes through the Toll-like receptors (TLR2, 4) signaling pathway. We had demonstrated that ENX in PM10 from an urban and rural site were in part responsible for the release of IL-6 and IL-8 from human bronchial epithelial cells (BEAS-2B). The use of a specific ENX inhibitor (polymyxin B) indicates ENX are responsible for increase in TLR4 mRNA expression and transcription factor, nuclear factor kappa (NF-κB) activation (preliminarily). Tagman® Real-Time PCR, antibody based and transcription factor assays were employed for these outcomes. We report here that urban extracts are more proficient in the induction of pro-inflammatory responses than rural extracts. Results highlight ENX as important constituents of PM10 ADE-induced cellular effects in vitro with particular contribution at the urban site. Also, these findings suggest the influence of local PM10 sources on dust reaching a Puerto Rican urban site and how it is capable of stimulating the molecular mechanism of Toll-like receptors in BEAS-2B. Support provided by MBRS-RISE Grant R25GM061838.

982 Addition of a Methoxymethyl Side Chain into p-Phenylenediamine Yields 2-Methoxy-Methyl-p-Phenylenediamine, a Hair Dye with Reduced Skin Sensitizing Properties

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p-Phenylenediamine (PPD) and p-toluidinediamine (PTD) are the most important hair dye primary intermediates for oxidative hair coloring and are regarded as the key drivers of hair dye allergy. Adding a methoxymethyl side chain to the PPD molecule yields the derivative 2-methoxy-methyl-p-phenylenediamine (ME-PPD) with reduced sensitizing properties compared to PPD and PTD. ME-PPD showed an attenuated innate immune response when analyzed for its protein reactivity and dendritic cell activation potential. In the local lymph node assay (LLNA) in mice, the concentration of ME-PPD needed to induce lymphocyte proliferation 3-fold above background (EC3 value) was 4.3%, indicating a moderate skin sensitizing potency, whereas EC3 values of 0.1 and 0.17% correspond with an extreme to strong potency for PPD and PTD. Quantitative risk assessment (QRA) of the skin sensitizing potency of ME-PPD under hair dye usage conditions indicated an allergy induction risk negligible compared to PPD or PTD.

983 Comparison of Different Sampling Methods for Assessment of Biological Activity of Dust from Moisture Damaged Buildings

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The poor quality of indoor air in the moisture damaged buildings leads to several kinds of symptoms and diseases. There is an evident need for better tools to measure the severity of the damage. In this study, the aim was to compare sampling methods for analysis of biological activity of dust in moisture damaged and reference school buildings. Two kinds of samples were collected in one moisture damaged and one reference school: three active air samples (Button sampling, 4L/min) for five days during working hours, and six settled dust box samples during a two week period. All dust samples were weighed and then extracted directly into cell culture medium. The toxicity testing was done by exposing RAW 264.7 mouse macrophages to three dilutions of dust samples for 24 hours. After the exposure, the viability of the cells and production of inflammatory mediators nitric oxide (NO) and cytokine tumor necrosis factor (TNF) α were measured. The amount of dust was smaller in the damaged school compared to the reference school. The biological activity of the actively collected dust was slightly higher in the sample collected from the moisture damaged school, but the small amount of material in the sample limited the assay. A similar, clearer trend could be seen in the settled dust samples, and the difference was even more pronounced when the sampling sites within the schools were compared according to the damage observations. The results indicate that the biological activity of the dust collected with these sampling methods seems to be higher in the water damaged school compared to reference school, but the method may not differentiate the buildings reliably enough for the needs of risk assessment. Further testing of different sampling approaches should be done to improve the method.

PS984 Role of ROS and HMGB1 in Chemical Allergen-Induced IL-18 Production in Human Keratinocytes

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We have recently identified the possibility to use interleukin-18 (IL-18) production for the in vitro identification of contact allergens. Previously, we demonstrated a role for oxidative stress, NF-κB and p38 MAPK activation (PPD)-induced IL-18 production. The purpose of this study was to characterize the molecular mechanisms underlying allergen-induced IL-18 production, in order to identify the cellular source of reactive oxygen species (ROS) and the danger signals involved. Selective inhibitors were used to characterize the source of ROS and to identify the damage-associated molecular pattern molecules (DAMPs) triggered by contact allergens. The human keratinocyte cell line NCTC2544 was exposed to three contact allergens, dihydroxy PPD, 2,6-dinitrochlorobenzene and cital in the presence or absence of diphenylethylenequinone (DPQ), aliquoteine and rotenone to identify
the source of ROS, and to anti-TLR4 antibody and glycyrizic acid to characterize the DAMPs. In the case of PPD, the induction of IL-18 can be modulated by rotenone, allopurinol and DPI, indicating the involvement of different sources of ROS. In the case of DNCh, rotenone completely prevents the induction of IL-18, suggesting a central role of mitochondria-derived ROS, while for citral DPI completely prevents the induction of IL-18, indicating the central of NADPH oxidase. Allergen-induced IL-18 involves the activation of the inﬂammasome and TLR4, as it can be modulated by the caspase inhibitor Z-VAD FMK and anti-TLR4 antibody. As DAMPs, we demonstrated the ability of all allergens tested to induce the release of HMGB1 (high mobility group box-1) and its sequence by glycyrizic acid significantly modulate PPD-induced IL-18 production and completely prevents DNCh and citral-induced IL-18, conﬁrming the role of HMGB1. Overall, we found that different intracellular source of ROS are triggered by contact allergens and an important role for HMGB1 in chemical allergen-induced IL-18 production was demonstrated.

985 Human Skin Explants: An Alternative Method to Assess Allergenic Potential of Chemicals

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Aims of the study
This study is aimed to assess predictive allergenic potential of chemical through a histological expertise of the general morphology of the skin after staining according to Masson trichrome and by the counting of Langerhans cell as after immunostaining of CD1a. The study is also an opportunity to develop a model for evaluation of protective creams against professional chemical allergens.

Method
84 human skin explants from abdominoplasty. 8 allergens frequently used at work-placed were evaluated: Tetramethylthiram disulﬁde, 2-Mercapto benzothiazole, Benzyl carbamate, Bisphenol, Ethyl acrylile Koشر, Araldite 506 epoxy Resin, Hevein (latex) and Formaldehyde 30 %. The allergens stayed in contact with the skin for 24 hours. 3 series were compared: the 8 allergens as positive control, cream excipient, creams with different potential protective agents. Epidermic Langerhans cells were stained with a monoclonal anti-CD1a antibody (IM590, clone O10, Beckman Coulter) for one hour, then enhanced with a streptavidin/biotin system (Vector, PK-7200) and at the end revealed using VIP (Vector, SK-4600). The mean number per centimetre of epidermic Langerhans cells was calculated to objective their migration as a proof of skin sensitization.

Results
In our operating conditions, allergenic tested molecules in contact with the skin for 24 hours induce Langerhans cells migration. When the skin was pre-treated with excipient or skin protective cream, less signiﬁcant migration of the Langerhans cells was induced by the allergens (p < 0.05).

Conclusion
This method is efficient to show the hypersensitivity potential of known chemical allergen. It could be useful “for alternative to animal testing” for skin sensitization. It also could be a model for testing skin protective creams.

986 Comparing Lymphocyte Subsets in the Mouse Allergy Model and Local Lymph Node Assay by Multiparameter Flow Cytometry


A validated animal model is needed to predict the potential of drugs administered by oral or parenteral routes to produce hypersensitivity reactions (HR). Recently, the mouse allergy model (MAM) was developed as a predictive model where subcutaneous injection of drugs associated with HR in the clinic produce a marked increase in the cellularity of the draining lymph nodes (LN). The murine local lymph node assay (LLNA) has been used extensively as a tool to predict for contact HR but does not appear to predict for systemically administered drugs. In the LLNA, topical application of contact sensitizers to the ears of mice causes an increase in cell counts in draining LN. The objective of this study was to compare the responses in the MAM and LLNA to determine if similar mechanisms may be involved in driving these responses. The MAM and LLNA assays were performed and the draining LN lymphocyte subsets and surface marker expression of CD62L/L-selectin and CCR7 were evaluated by flow cytometry. In the MAM, amoxicillin (100 mg/kg) treatment resulted in a proportional increase in absolute counts of CD4 and CD8 T cells and B cells, whereas a significant (2-fold) increase in the percentage of B cells was observed in the LLNA with 0.25% Dinitrofluorobenzene (DNFB)

987 Identification and Frequency of Naı́ve T Lymphocytes Specific for Penicillin: Implication in Drug-Allergy

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Allergic reactions to drugs are unpredictable and have many side effects. According to the hapten theory, these compounds are capable to bind to proteins and form immunogenic complexes. Antigen presenting cells, such as dendritic cells (DC), internalize haptenized complexes, digest them into peptides and present them on HLA molecules to drug-specific T cells inducing an immune response. Knowing that drugs provoke IgE mediated hypersensitivity reactions in treated patients, the CD4+ T-cell response to benzyl-penicillin (BP) was investigated. The aim of the study was to determine the size of the BP-specific naive CD4+ T-cell repertoire, and to identify peptide epitopes haptenized with BP and presented to T cells via HLA-II molecules.

Since BP is known to bind covalently to proteins, human serum albumin (HSA)-BP bio-conjugates were synthesized at basic pH and BP-binding sites on HSA were identified using mass-spectrometry. 12 peptides of 15 mer long including the identified BP binding sites were synthesized. naïve CD4+ T cells from non-allergic donors were stimulated once a week with autologous DC loaded with HSA-BP or with peptide-BP to amplify respectively HSA-BP- or peptide-BP-specific T cells. Activation of specific CD4+ T cells was detected using interferon-γ EлиSpot and their frequency was calculated.

The results of the CD4+ T-cell response to BP were as follows: - Detection of BP-specific CD4+ T cells in 12/13 tested donors with a mean frequency of 0.26 cells/million of CD4+ T cells
- Identification of 17 binding sites of BP on HSA
- Specific naïve CD4+ T cells recognized 5 specific peptides, from HSA, haptenated by BP.

This study showed the capacity of HSA-BP to be recognized by naïve T-cells from multiple donors and the possibility to identify epitopes involved in the allergic reaction to BP.

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988 Molecular Profile Analysis of Allergenic Acid Hydrolyzed Wheat Protein

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Hydrolyzed wheat protein (HWP; hydrolyzed gluten) is used in various products worldwide. Several cases of systemic wheat allergy caused by HWP have been reported, including wheat-dependent exercise-induced anaphylaxis following sensitization to HWP in cosmetics in Japan. Moreover, an increasing number of studies have shown that HWP can induce IgE-mediated hypersensitivity by skin contact and/or food ingestion. It is considered that these allergic manifestations result from deamidated gluten in HWP. However, the relationship between the essential molecular properties of HWP and its allergenicity remains unknown.

In this study, we prepared several HWPs with different degrees of acid hydrolysis (0.1 N hydrochloric acid treatment at 100°C for 0.5, 1, 3, 6, 9, 12, and 24 h). In profiling analysis of these HWPs, size exclusion chromatography showed that the average molecular weight of HWP decreased depending on the extent of acid hydrolysis. In contrast, proteomics analysis showed that the deamidation level increased depending on the extent of acid hydrolysis, and this level reached a plateau after 6 h of hydrolysis. The allergenicity of gluten and two HWP (0.5- and 9-h hydrolysis sample) was compared by transdermal administration to BALB/c mice. Among these HWPs, the 0.5-h hydrolysis sample showed significantly increased

SOT 2014 Annual Meeting 257
antigen-specific serum IgE: titer. After skin sensitization for 4 weeks, intraperitoneal injection of this HWP caused active systemic anaphylaxis, resulting in marked decrease in rectal temperatures, increase in anaphylaxis scores, and increase in plasma histamine levels. The effect of skin sensitization with gluten and 9-h hydrolysis samples was significantly weaker than that with the 0.5-h hydrolysis sample. In conclusion, we demonstrated that allergenic HWP has specific molecular properties and the results showed that it may be possible to reduce the risk of skin sensitization to HWP by extensive acid hydrolysis.

989 Impact of Aggregation on Immunogenicity: Relevance for Biopharmaceuticals
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The clinical use of protein therapeutics has increased dramatically over the last decade. One important safety issue with such molecules is the development of anti-drug antibodies in patients that may cause adverse events or may reduce efficacy of the protein therapeutic. Protein aggregation can be associated with the induction of vigorous immune responses, for example with certain preparations of interferon-β. As a result, regulatory guidelines recommend screening for and minimizing protein aggregation during bioprocessing, despite a lack of understanding regarding the mechanisms and the size/nature of problem aggregates that can trigger such responses. In the current experiments a humanized single chain Fv (scFv) antibody fragment expressed by E. coli was purified using Protein A affinity chromatography (monomeric fraction; mean diameter 7nm). Reproducible aggregates within the subvisible particle size range (mean diameter 190nm) were formed by incubation at 40°C for 20 min at pH 7.0. Antibody responses induced in BALB/c strain mice by intraperitoneal exposure to the monomeric fraction or the aggregated scFv (1 mg/ml) were characterised. Sera were analysed for protein specific total IgG and IgG1 and IgG2a subclasses by enzyme-linked immunosorbant assay. Immunisation with both forms of scFv induced relatively vigorous IgG1 and IgG1 antibody responses of equivalent magnitude. However, the aggregated fraction stimulated significantly higher titers of IgG2a antibody than did the monomeric fraction. Tiers were independent of whether aggregated or monomeric protein was used a substrate in the analysis. Thus, aggregated protein induces a preferential Th1 type response (IgG2a) whereas the monomer elicits a selective Th2 response (IgG1/IgG2a). Th1 responses are typically mounted against intracellular bacteria and viruses, suggesting that the aggregated protein may stimulate polarized Th1 responses by mimicking the repetitive structures found in viruses.

990 Allergen-Induced Langerhans’ Cell (LC) Migration: Role of Interleukin (IL)-1β
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Epidermal LC play key roles in the initiation and regulation of immune and allergic responses. LC migration induced in mice by topical exposure to the contact allergen oxazolone (Ox) requires two independent cytokine signals delivered by UVB, trauma) are dependent upon the local availability of TNF-α. These data also contrasts with the prevailing view that all LC migratory stimuli (allergens, irritants, concentrations are very high, LC migration is independent of TNF-α. This

991 Cytokine Fingerprinting for Chemical Respiratory Allergens: Use of a Nonstandard Vehicle
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We have demonstrated previously that prolonged (13 day) topical exposure of BALB/c mice to the reference contact allergen 2,4-dinitrochlorobenzene (DNCB), or to the reference respiratory allergen trimellitic anhydride (TMA) induces selective type 1 or type 2 cytokine secretion profiles, respectively. Similar divergent cytokine expression patterns have been shown for a number of other contact allergens (such as formaldehyde, isoeugenol) and respiratory allergens (including disocyanates). In these experiments, compounds have been formulated exclusively in the standard local lymph node assay acetonoeol oil (AOO) vehicle for topical exposure. In the current experiments, responses provoked by respiratory sensitizers formulated in dimethyl formamide (DMF) have been examined. Mice were exposed topically allergen over a 13 day period, 13 days after the initiation of exposure, draining auricular lymph node cells (LNC) were isolated. Single cell suspensions were prepared and cultured; cytokine secretion profiles were measured. Initial experiments with DNCB and TMA formulated in either AOO or DMF revealed that, with the exception of interleukin (IL)-4 which was recorded at low levels in TMA/DMF treated animals, TMA treatment induced a type 2 phenotype with high levels of IL-5, 10 and 13 and relatively low levels of interferon-gamma when formulated in either vehicle. The converse type 1 pattern was provoked by DNCB exposure independent of vehicle, however, cytokine levels were more variable and patterns less divergent when DMF was used. Cytokine fingerprinting experiments conducted with additional respiratory sensitizers (chloramine T; reactive dyes) formulated in DMF revealed the expected type 2 phenotype. These data suggest that the measurement of induced cytokine secretion profiles in the BALB/c strain mouse provides a robust method for hazard identification and characterization of respiratory chemical allergens. Furthermore, DMF is an appropriate alternative vehicle although AOO is still the vehicle of choice.

992 Interleukin (IL)-1 Expression by Dendritic Cells (DC): Evidence for Intracellular Degradation
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DC play pivotal roles in the development of immune and allergic reactions, including skin sensitization, the commonest form of immunotoxicity. A key event in contact allergy is the production of the proinflammatory cytokine IL-1β. This cytokine is not secreted via the classical pathway, instead, independent signals are required. The first induces up-regulation of pro-IL-1β, and the second drives cleavage and secretion of the bioactive molecule. The intracellular regulation of expression of this cytokine in immature (day 8) mouse bone marrow (BM) DC has now been characterized. Cells were stimulated with lipopolysaccharide (LPS; 0.1µg/ml) and the IL-1α and β content of supernatants (secreted cytokine) and cell lysates (intracellular cytokine) was analyzed by ELISA. The impact of cycloheximide (CHX), the proteasome inhibitor MG132 and the autophagy inhibitor 3-methyladeninde (3-MA) on IL-1β production was examined. The role of ubiquitination was examined by IL-1β immunoprecipitation and Western blotting. LPS treatment stimulated a marked increase in intracellular IL-1β, peaking at ~8hr and declining rapidly thereafter. There was no concomitant detection of secreted product, indicating that in the absence of a second signal, LPS-induced IL-1α and β were rapidly degraded. Using a 1hr CHX (10µg/ml) pulse to inhibit de novo translation revealed a 4-hr lag before IL-1β degradation was detected. Treatment with MG132 (10µM), but not with 3-MA (10µM), inhibited significantly IL-1β degradation without impacting cell viability. Furthermore, following MG132 inhibition of degradation, Western blotting with anti-ubiquitin antibodies revealed an accumulation of ubiquitinated IL-1β. Thus, IL-1α and β are degraded in an ubiquitin-proteasome paradigm of protein degradation and this process is initiated following DC activation in the absence of secretion. Therefore, the regulation of IL-1α proteasomal degradation may control the vigor of IL-1α secretion, and ultimately may influence the potency of pro-inflammatory responses.

993 Enteropathogenic Escherichia coli As a Mucoassociated Exosome Extends Epithelial NF-kB Activation via Macrophage Inhibitory Cytokine 1
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Commensal bacterial community shifts in the pathogenic colonic environment and chronic colonization of mucoassociated Escherichia coli (MAEC) has been linked to chronic colonic inflammatory diseases. Enteropathogenic Escherichia coli...
(EPEC) is one of commonly identified MAEC in inflammatory bowel disease patients. The aim of this study is to address the contribution of MAEC colonization to human inflammatory signals such as nuclear factor kappa B (NF-κB). The present study was conducted to investigate the prolonged epithelial responses to persistent EPEC infection via NF-κB activation. EPEC infection led to sustained activation of NF-κB signal in mouse intestinal epithelial cells in vitro and in vivo, which was positively associated with a type III secretion system, whereas early NF-κB is regulated. Moreover, prolonged NF-κB activation was found to be a part of macrophage inhibitory cytokine 1 (MIC-1)-mediated signaling activation, a novel link between NF-κB signaling and infection-associated epithelial stress. Furthermore, both EPEC-induced MIC-1 and NF-κB signaling mediated epithelial survival by enhancing the expression of cyclin D1, a target of NF-κB. In summary, the results of the present study suggest that MIC-1 serves as a mediator of prolonged NF-κB activation, which is critical in maintaining gut epithelial integrity in response to EPEC-induced injury. This work was supported by the National Science Foundation of China, the National Natural Science Foundation of China, the National Key Research and Development Program of China, and the National Health and Family Planning Commission of the People’s Republic of China.

PS 994 The Role of Endothelin-1 in Endotoxin-Triggered Release of Placental Proinflammatory Cytokines

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Background: Premature delivery occurs in 12% of all births and accounts for 60 to 80% of perinatal mortality in non-anomalous infants. Despite advances in obstetrics and neonatology, there is no FDA approved therapy for preterm labor. We have shown that the ECE-1 inhibitor, phosphorhamdomid, the selective endothelin receptor A antagonist, BQ-123 and a novel synthetic ET A receptor antagonist synthesized by our group rescue mice from LPS-induced PTB. Finally, we have been able to prevent PTB in LPS-stimulated animals, using RNA silencing, by hydrodynamic transfection of E15 mice with ECE-1 RNAi.

Objective: Our aim was to test the hypothesis that ET-1 contributes to the cause of preterm birth by up-regulating pro-inflammatory cytokines.

Methods: Chorionic villous explants from full term human placenta obtained by Cesarean delivery were cultured under aseptic conditions and were divided into four groups: sham, endotoxin-treated, endotoxin plus low concentration BQ-123-treated, and endotoxin plus high concentration BQ-123-treated. Explant supernatants from all groups were collected 24 hours after the addition of LPS and evaluated for changes in levels of interleukin 1 β (IL-1 β) and tumor necrosis factor α (TNF α) by Western blot analysis.

Results: Villous explants treated with LPS released significantly higher amounts of both IL-1 β and TNF α into culture media than sham explants. This pro-inflammatory response was reversed, in a concentration-dependent fashion, with the ET A receptor antagonist, BQ-123.

Conclusion: IL-1 β and TNF α, pro-inflammatory cytokines closely linked to infection-associated PTB, are ET-1 dependent. The mechanism of action of ET-1 blocking agents in tocolysis involves decrease ET-1-dependent release of these mediators. Further evaluation of inflammatory mediators affected by ET-1 action may lead to the identification of novel targets for preventive therapy for PTB.

PS 995 Classical and Alternative Activation of Cyanobacterium Anaeroba sp. Lipopolysaccharide (LPS)-Treated Rat Brain Microglia

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Cosmopolitan Gram-negative cyanobacteria may contaminate freshwater by releasing toxins, such as lipopolysaccharide (LPS), thus affecting human health. We reported that cyanobacterium Anaeroba sp. LPS (AnaLPS) elicited classical activation of rat microglia (BMP) and MIP-1α (CCL3) and IL-6 release in vitro (The Toxicologist CD 132 (S-1), 2013). We hypothesized that AnaLPS activates classical and alternative activation of BMG in vitro and concomitant release of cytokines and chemokines. AnaLPS was prepared by hot phenol/water extraction. E. coli LPS (EcoliLPS) 026:B6 from Difco Lab, Detroit, MI was used as a positive control. BMP were isolated from neonatal rats, and treated in vitro in a concentration-dependent manner with either AnaLPS or EcoliLPS for 18 hours at 35.9 °C. Cytokines and chemokines were determined by Milliplex® MAP rat cytokine/chemokine multiplex immunoassays. Results were the following: EcoliLPS and AnaLPS stimulated concentration-dependent and statistically significant release of (a) Pro-inflammatory cytokines: IL-6, IL-1β, TNF-α; (b) Pro-inflammatory chemokines: MIP-2(CXCL2) > CINC(CXCL1) > MIP-1α (CCL3) > MCP-1(CCL2) > IP-10(CXCL10) > RANTES(CCL5); and (c) The Anti-inflammatory cytokine: IL-10, at > 1ng/ml. EcoliLPS and 10 ng/ml AnaLPS. We thus conclude that an 18 hour in vitro treatment with AnaLPS stimulated both classical and alternative activation of rat brain microglia, but was considerably less potent than EcoliLPS. Continued investigation of the mechanism responsible for the differential response observed between EcoliLPS and AnaLPS on BMG is ongoing in our laboratory.

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PS 996 Behavioral and Neurochemical Alterations in Adult Mice with Low-Chronic Inflammation Caused by Repeated Peripheral Lipopolysaccharide Exposure

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Sickness behavior following acute inflammation, modeled by acute peripheral lipopolysaccharide (LPS) administration, can later transition into depressive behavior. In light of the limited information on the impact of chronic peripheral inflammation on the development of depressive behavior and other behavioral alterations and on their persistence after inflammatory stimuli exposure termination, this study assessed behavioral and neurochemical effects of chronic (25 weeks), low dose (0.25 mg/kg body weight; twice weekly) peripheral (intraperitoneal) LPS exposure in adult male C57BL/6 mice. Behavioral tests were performed 6 and 12 weeks post LPS/saline treatment as well as in mice exposed to 13-week LPS/saline treatment followed by an 11-week period without any treatment. Following sacrifice (at 13 and 25 weeks), striatal concentrations of dopamine, serotonin and their metabolites were analyzed. LPS-exposed mice were hypoactive in the open field after 6 weeks, whereas significant hyperactivity was observed in the 12 week LPS and 13 week LPS+11 week off groups. Similar biphasic responses were observed in the time in the center of the open field arena between the 6 week LPS (decreased) and 13 week LPS+11 week off groups (increased), suggestive of increased and decreased anxiety, respectively. In forced swim test, mice exhibited significant increase in the immobility time (depressive behavior) at all three time points. Neurochemistry data are still being analyzed. Above findings demonstrate that chronic peripheral inflammation initially causes decreased locomotion and increased anxiety, followed by persistent hyperactivity and decreased anxiety. Notably, chronic low-dose LPS-induced depressive behavior appears early and persists long after termination of LPS exposure. Collectively, these data emphasize the need to pay more attention on the lasting behavioral alterations induced by chronic peripheral inflammation. Supported by: R01ES016965 (NIEHS).

PS 997 Sex Differences in Gene Expression Profiles in Individuals with Idiopathic Pulmonary Fibrosis Potential Targets of Xenoestrogens?

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Epidemiological studies suggest sex-specific trends in the prevalence and mortality of idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD). While the expression of several immune and extracellular matrix (ECM) genes in the lung have been well characterized in these diseases, investigations aimed at elucidating their sex-specific expression by disease type and severity, and the evaluation of hormone-related genes, has not been well studied. To begin to examine sex-specific expression profiles we performed targeted analysis of 48 genes, those involved in inflammation, ECM remodeling and hormonal processes, in lung tissues from males and females with mild or medium severity IPF or COPD. Results revealed a subset of genes that are differentially expressed among sex, disease type and severity. The most significant observations were the increased expression primarily of ECM genes in medium severity IPF (CATHK, COL1A1, COL3, MMP1, MMP1, MMP7, IL1RN) compared to mild IPF and COPD. Results suggested sex-specific gene expression profiles in individuals with IPF and COPD. Interestingly, two genes, CH3L1 and MMP7 showed a significant interaction of sex and disease in individuals with IPF. While there were no significant differences in any of the hormonal genes between the IPF groups, ESR1 and AR expression levels were higher and lower, respectively, compared to COPD samples. Based on these data we are investigating a role for (xeno)estrogens in the transcriptional control of these select genes in lung epithelial cells through modulation of the nuclear and membrane
estrogen receptor. Overall, these results highlight molecular targets that could play a role in differential trends in lung disease incidence and severity between men and women and their influence by contaminants with hormonal activity.

### 998 S-Nitrosoglutathione Reductase (GSNOR) Activity Is Differentially Regulated within Macrophage Phenotypes

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Macrophages (Mφ) play a crucial role in innate immunity. Upon stimulation, Mφ are divided by two main phenotypes, classic (M1) and alternative (M2) activation, distinguished by their functional patterns. M1 Mφ produce large amount of NO, which reacts with glutathione (GSH) to form S-nitrosoglutathione (GSNO). GSNOR can transnitrosate the thiol residues of proteins to form S-nitrosothiols (SNO), with functional consequences. GSNOR reductase (GSNOR) reduces the SNO moiety in the presence of NADH to regenerate GSH and produce hydroxyamine. It is a major regulator of intracellular SNO content. We hypothesized that GSNOR activity is regulated within Mφ activation in order to regulate SNO accumulation within phenotypic differentiation. We have utilized LPS or IL-4 to stimulate a M1 or M2 phenotype respectively in a range of Mφ cultures including RAW264.7, Raw blue, bone marrow derived Mφ (BMDM) and alveolar Mφ. Incubation of all Mφ with LPS dramatically increased iNOS expression and NO production in all cell systems. IL-4 induced arginase but not iNOS expression. LPS induced M1 Mφ had increased SNO content and SNO-proteins as determined by Cu-Cys reduction assay and biotin-switch. IL-4 reduced total intracellular SNO content and SNO-proteins. LPS treatment decreased Mφ GSNOR expression within 1-6 hours, while IL-4 treatment stimulated expression of GSNOR. These changes were matched by reduced GSNOR activity within cellular lysates. LPS mediated increased in iNOS expression were associated with increased NO production in Raw blue cells, and the NF-kB inhibitor CAPE reduced iNOS expression in Raw264.7 cells. However, CAPE had no effect on GSNOR expression, suggesting that GSNOR expression is NF-kB independent. These changes were not specific to cell lines as they were also observed in BMDM and alveolar Mφ. SNO regulation in Mφ in the different phenotypes suggests that SNO plays an important role in Mφ activation. Regulation of GSNOR activity may provide novel therapeutic avenues within inflammatory disease. Supported by NIH HL086621.

### 999 Salvia plebeia Extract Alleviates Inflammatory Response in Murine Arthritis Model and Human Rheumatoid Synovial Fibroblasts

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Salvia plebeia R. Br (S. plebeia) has been used for the treatment of a variety of inflammatory diseases and anti-oxidant as an oriental folk medicine. In this study, we investigated the effects of S. plebeia extract (SPE) on inflammatory arthritis and underling mechanisms of action. We used a collagen-induced arthritis (CIA) model in BALB/c mice by immunized with type II collagen. SPE was orally administrated during 5 weeks of arthritis induction. Oral administration of SPE decreased arthritis symptoms such as, physiologic arthritis score, footpad thickness, histopathologic changes in addition to serum IgG1 and IgG2a levels. SPE inhibited TH1/TH2/TH17 cytokine CD4+ T lymphocytes expansion in draining lymph node, expression of inflammatory mediator cytokines, matrix metalloproteinase (MMP)-1 and MMP-3 in the ankle joint tissue. To define the underlying mechanisms of action, tumor necrosis factor-α stimulated rheumatoid arthritis synovial fibroblasts were used. SPE significantly suppressed the expression of inflammatory cytokines and MMP-1 by the down-regulation of nuclear factor-kB and mitogen-activated protein kinases. Taken together, the results indicate that SPE has therapeutic efficacy for chronic inflammatory arthritis, suggesting that SPE might be a candidate for the treatment of RA.

### 1000 Optimization and Evaluation of Metabolite Extraction Methods for Untargeted Metabolomic Study by LC-QTOF/MS for the Evaluation of Effects of 12-Dimethylmythene on RAW264.7 Murine Macrophages

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Advances in accurate mass spectrometry, better data analysis software and availability of biological pathway databases have allowed for more accurate untargeted metabolomic investigations. The purpose of this study was to optimize the harvest, extraction and LC-QTOF/MS parameters for the metabolic evaluation of the effects of 12-dimethylmythene (12-DIM) on RAW264.7 murine macrophages. 12-DIM is part of a family of potential anti-inflammatory compounds isolated from Brassica vegetables, such as broccoli, kale and brussel sprouts, and a metabolomics study will evaluate the potential for anti-inflammatory properties of 12-DIM. The optimal harvesting and extraction procedure was chosen with cold methanol and cell scraping, compared to the standard trypsin/ethylendiaminetetraacetic acid (EDTA) treatment. Four different extraction methods were compared to elucidate the highest efficiency for intracellular and extracellular metabolite extraction, including: methanol/chloroform/water, methanol/chloroform/0.1% formic acid, methanol/chloroform and methanol/water. Extraction with cold methanol/chloroform was found to extract the most definable molecular features. The ideal mobile phases were found to be water/0.1% formic acid and methanol, which gave a better chromatographic baseline as compared to acetonitrile. The source parameters including: nebulizer, sheath gas temperature, gas flow, gas temperature and the fragmentor for the LC-QTOF/MS were also optimized. These refined parameters provided sensitive and reproducible quantification and extraction of cellular metabolites from RAW264.7 macrophage cells for a global metabolomics study of the effects of 12-DIM.

### 1001 Nebulized Thiocyanate Attenuates Inflammation and Oxidative Stress in the Airway and Liver of ENaC Mice

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The pseudohalide thiocyanate (SCN-) has been implicated in innate immune function in mammals but less is known about its impact on inflammation and oxidative stress. The pseudohalide thiocyanate (SCN-) has been implicated in innate immune function in mammals but less is known about its impact on inflammation and oxidative stress. SCN- is dysregulated in cystic fibrosis (CF), a disease marked by chronic airway inflammation. Previously, we have shown that nebulized SCN- decreases inflammatory and bacterial burden in B6 mice infected with P. aeruginosa while sparing the antioxidant glutathione (GSH). To investigate the role of SCN- in pathogen-independent inflammation, we administered nebulized SCN- or normal saline vehicle to ENaC mice, a phenotypic model of CF, and wild type littermate controls. Nebulized SCN- significantly decreased airway neutrophil infiltration concurrent with rescue of the ratio of GSH to oxidized glutathione (GSSG) in airway leukocytes and epithelial lining fluid (ELF) of ENaC mice compared to control. Similarly, nebulized SCN- rescued the GSH/GSSG ratio in lung and liver tissue of ENaC mice to levels comparable to littermate controls. These results demonstrate that SCN- directly impacts pathogen-free airway inflammation and attenuates oxidative stress with both local and systemic protective effects. These results are supported mechanistically by the ability of SCN- to scavenge hypohalous acids, strong oxidants produced by neutrophils, forming a less reactive oxidant (hydroxyamine) that we have previously demonstrated is safely metabolized by mammalian cells. Our findings support nebulized SCN- as a potential pharmacologic agent in diseases of chronic inflammation such as CF. Continuing studies will investigate the effect of nebulized SCN- during chronic infection of ENaC mice and littermates and changes in cytokine characteristics that may affect neutrophil trafficking. This work was supported by NIH grant R01 HL084469 and a Cystic Fibrosis Foundation Research Grant.

### 1002 Redox Cross-Talk between Myeloid-Derived Suppressor Cells and Dendritic Cells in Cancer: Accumulation of Oxygenated Lipid Droplets and Suppression of Antigen Cross-Presentation

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Accumulation of myeloid-derived suppressor cells (MDSCs), and expansion of immature dendritic cells (DCs) with aberrant cross-presentation – are important immunological abnormalities in cancer. While interactions between these cells are recognized as contributors to the failed anti-tumor immunity, their molecular mechanisms remain elusive. Excessive formation of lipid droplets (LDs) affects DCs immune functions. We show that DCs from tumor-bearing mice or treated with tumor explant supernatants (EL4, MC38) contained oxygenated neutral lipids: triglycerides (oxTAGs), free fatty acids (oxFFAs) and cholesterol esters (oxCE). LC-ESI-MS/MS revealed that oxTAGs were represented by a spectrum of truncated molecular species 39:1; 39:0; 41:1; 41:0; 45:2; 41:1; 43:0; 45:2 with functional consequences. GSNO reductase (GSNOR) reduces the SNO moiety in the presence of NADH to regenerate GSH and produce hydroxylamine. It is a major regulator of intracellular SNO levels. We hypothesized that GSNOR activity is regulated within Mφ activation in order to regulate SNO accumulation within phenotypic differentiation. We have utilized LPS or IL-4 to stimulate a M1 or M2 phenotype respectively in a range of Mφ cultures including RAW264.7, Raw blue, bone marrow derived Mφ (BMDM) and alveolar Mφ. Incubation of all Mφ with LPS dramatically increased iNOS expression and NO production in all cell systems. IL-4 induced arginase but not iNOS expression. LPS induced M1 Mφ had increased SNO content and SNO-proteins as determined by Cu-Cys reduction assay and biotin-switch. IL-4 reduced total intracellular SNO content and SNO-proteins. LPS treatment decreased Mφ GSNOR expression within 1-6 hours, while IL-4 treatment stimulated expression of GSNOR. These changes were matched by reduced GSNOR activity within cellular lysates. LPS mediated increased in iNOS expression were associated with increased NO production in Raw blue cells, and the NF-kB inhibitor CAPE reduced iNOS expression in Raw264.7 cells. However, CAPE had no effect on GSNOR expression, suggesting that GSNOR expression is NF-kB independent. These changes were not specific to cell lines as they were also observed in BMDM and alveolar Mφ. SNO regulation in Mφ in the different phenotypes suggests that SNO plays an important role in Mφ activation. Regulation of GSNOR activity may provide novel therapeutic avenues within inflammatory disease. Supported by NIH HL086621.

**PS**
1003 Signaling Role of Cardiolipin Externalization in Elimination of Damaged Mitochondria in Lung Epithelial Cells


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Bacterial pneumonia triggers an exuberant host response characterized by excessive inflammation, oxidative stress, and epithelial cell damage culminating in the acute respiratory distress syndrome (ARDS). In ARDS, virulent bacterial pathogens directly damage host cells, activate innate immune responses, and trigger a burst in reactive oxygen species resulting in epithelial cell death. However, specific pathways involved in the evolution of epithelial injury with attendant release of novel damage signals remain elusive. We suggested that one critical damage signal is mitochondrial cardiolipin (CL). We found that mitochondria and reduced levels of distal mitochondrial protein markers were detectable in murine models of bacterial pneumonia. In addition, we demonstrated that early on after bacterial infection of murine lung epithelial cells (MLE15), CL transmigrates from the IMM to OMM; the unmasked externalized CL serves as a novel signal for mitophagy. A typical inducer of mitophagy, carbonyl cyanide m-chlorophenylhydrazone (CCCP) — acting as a protonophoric uncoupler — induced CL externalization to the OMM in MLE15 cells. This was evidenced by a robust decrease of the ratio of CL in the IMM and OMM from 18.6±0.3 in control MLE15 cells to 3.7±1.4 after treatment with CCCP. The externalization of CL was accompanied by activation of autophagy (LC3-II/I conversion) and decreased levels of mitochondrial marker proteins (TIM40, TIM23 and MnsSOD) — consistent with mitophagy activation. CCCP-induced mitophagy in MLE was also confirmed by co-localization of mitochonldria and lysosomes. Manipulations of CL levels or proteins involved in CL externalization (PLS3R) affected sensitivity of MLE to pro-mitophagial stimulation. These data are compatible with a hypothesis that externalized CL acts as an “eat-me” signal in depolarized mitochondria. Supported by NIH E00106, U19 A008821, and NIOSH OH008282.

1004 Resveratrol Protects against a Mouse Model of Multiple Sclerosis via Regulation of T Cell miRNA Expression

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Resveratrol is a plant-derived phytoalexin with antioxidant, anti-carcinogenic and anti-inflammatory actions. Resveratrol has been extensively studied in cancer and in cardiovascular disease and has recently emerged as a potential therapeutic for the treatment of various inflammatory diseases. In this study, we evaluated the mechanistic effects of resveratrol on the pathogenesis of a mouse model of multiple sclerosis, experimental autoimmune encephalitis (EAE). We hypothesized that resveratrol protects against myelin oligodendrocyte peptide (MOP35-55)-induced neuroinflammation via regulation of T cell microRNA (miRNA) expression. Analysis of miRNA expression in CD4+ T cells from brains of EAE mice revealed significant up-regulation of miR-12-124, -125, -132, -138 and -155 with resveratrol treatment. Predicted and validated target genes were chosen for further examination based on gene ontology and functional analysis. miRNAs and target gene expression were validated by qRT-PCR. We found that resveratrol treatment leads to miRNA-mediated down regulation of genes essential for cell cycle progression (Rb1 and Cyclin D1) and growth and proliferation (p38 and Sphingosine Kinase 1) in CD4+ T cells. We also found that resveratrol decreases immune cell infiltration into the brain and impairs activation. While studies evaluating the impact of resveratrol on immune cells in the periphery exist, few studies have examined its effect on T cells in the target organ, the brain. Given the established neuroprotective effects and the rapidly growing anti-inflammatory properties, resveratrol is emerging as an ideal candidate for the treatment of multiple sclerosis and other neuroinflammatory diseases. Supported in part by NIH grants P01AT003961, R01AT008361, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357.

1005 CCR2 Regulates Proinflammatory Macrophage Migration into the Liver during Acetaminophen (APAP)-Induced Hepatotoxicity

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Inflammatory macrophages (MP) have been shown to play an important role in APAP-induced hepatotoxicity. Mechanisms mediating their accumulation in the liver have not been established. The C-C chemokine receptor, CCR2 has been shown to regulate inflammatory cell trafficking into injured tissues. In the present studies, we analyzed the phenotype of MP migrating into the liver following APAP intoxication and the role of CCR2 in this response. Mice were fasted overnight prior to administration of APAP (300 mg/kg, i.p.) or PBS. Liver, spleen and bone marrow (BM) were collected 24-96 h later and analyzed by flow cytometry. APAP caused a time-dependent increase in CD11b+Ly6C+ pro-inflammatory MP in the liver, but a transient decrease in CD11b+Ly6C+ anti-inflammatory/wound repair MP. Loss of CCR2 significantly reduced CD11b+Ly6C+ cells accumulating in the liver in response to APAP. We previously showed that the spleen is a source of hepatic inflammatory MP. Following APAP administration, increases in CD11b+Ly6C+ monocytes were observed in the spleen, with no effect on CD11b+Ly6C+ monocytes. Loss of CCR2 reduced numbers of pro-inflammatory monocytes in the spleen. In contrast, no differences were observed in monocyte subsets in the BM between wild type (WT) and CCR2/-/- mice. We also identified a subpopulation of CD11b+Ly6G+Ly6C+ myeloid derived suppressor cells (MDSC) in the liver and spleen of WT mice, which decreased 72 and 96 h after APAP. In contrast, in CCR2/-/- mice, MDSC increased within 24 h of APAP, remaining elevated for 72-96 h. Taken together, these results indicate that CCR2 plays a role in the emigration of CD11b+Ly6C+ pro-inflammatory MP into the liver from BM and spleen following APAP hepatotoxicity. Moreover, loss of CCR2 results in increased MDSC in the liver which may contribute to tissue repair. Supported by NIH ES007148, ES004738, CA132624, AR050575, and ES005022.

1006 Collagen Synthesis and Degradation Are Enhanced in Chronic Bacterial-Induced Prostatic Inflammation

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Benign prostatic hyperplasia (BPH) is a common disorder in aging men. Prostatic inflammation-induced fibrosis is suggested to be a major contributor of BPH etiology. Collagen deposition was increased in our previously described mouse model of bacterial prostatic inflammation. The goal of this study is to investigate the synthesis and degradation of newly synthesized collagen in bacterial-induced prostatic inflammation.

Adult C3H/HeOuJ male mice were transurethrally instilled 200ul of 2x106/ml of uropathogenic E. coli 1677 or saline to induce prostatic inflammation. Prostates were harvested to perform immunohistology at selected timepoint following infection. To measure collagen synthesis, mice were injected with 15µg of 3H-proline on day 0, 5, 12, 19, 26 post-installation. Prostates were harvested 2 days after 3H-proline injection. To measure collagen degradation, mice were injected with 15µCi of 3H-proline every 2 days for 28 days. Prostates were harvested on day 0, 4, 8, 15, 22, 29, 36, 57, 78, 113 after the last injection of 3H-proline. The incorporation of 3H-hydroxyproline was determined by measuring 3H radioactivity using a liquid scintillation counter.

Collagen synthesis was significantly increased in the infected prostates between 5 and 7 days, and 12 and 14 days post-installation. Immunostaining to determine collagen-producing cell revealed that there were abundant CD45+Vimentin+ fibrocytes coexpressing with the collagen synthesizing enzyme prolyl hydroxylase in the infected prostates on day 7 after infection. The newly synthesized collagen in prostatic inflammation was less stable than that in the saline-instilled prostates. The half-life of collagen in the saline prostates was 18.6 days while collagen in the infected prostates had a half-life of 15.7 days.

Our study showed that increased collagen turnover and the presence of fibrocytes characterize prostatic fibrosis in bacterial-induced inflammation. This finding provides a basis for future study to elucidate the cellular and molecular mechanisms of collagen deposition in inflammation-associated prostatic diseases.
The use of cellulosic biofuels can potentially reduce our carbon footprint. However, they may have unintended ecological and health effects such as increased competitiveness and allergenicity. Climate change may impact the quality or quantity of allergens produced in elevated temperatures. The goal of these studies was to 1) develop a method to assess the potential allergenicity of the various grass components to which workers will be exposed and 2) determine if there are differences based on grass growth temperature (gt).

The biofuel grass components (leaves, flowers/stems, anthers, pollen) of *Sorghum bicolor* and *Fusarium virgatum* were extracted in an excess of 0.2 mM Tris pH 8. Following centrifugation the supernatant was concentrated and protein content assayed. Female BALB/c mice were dosed 4X over a 4 week period by intratracheal aspiration to a pool of equal protein amounts from each of the component extracts/species(gt) to induce an IgE antibody response. Three days after the final exposure the mice were exsanguinated and the blood pooled by species and gt.

Western blots of the component extracts were probed with species(gt) specific serum for IgE reactivity. IgE reactive proteins were identified only in pollen extracts. Blots of *S. bicolor* pollen extract identified 2 IgE reactive protein bands. Grass gt had no apparent effect on IgE reactivity (densitometry). Blots of *P. virgatum* pollen identified 4 IgE reactive protein bands in the control gt extract and 5 additional protein bands in the high gt extract. There was a reduced intensity in the 4 common *P. virgatum* protein bands in the control gt extract compared to the high gt extract. This limited dataset suggests that pollen is the most relevant source of allergenic proteins and that elevated growth temperatures may alter the IgE reactive profile for some but not all species.

(2) Polycyclic aromatic hydrocarbons (PAHs) comprise several hundred compounds with different carcinogenic potentials and are invariably found in mixtures. Due to a lack of data, risk estimations for PAH mixtures are usually based on those for benzo[a]pyrene (B[a]P). Individual substances within PAH mixtures, however, may influence genotoxic properties and act synergistically or antagonistically. In the present study we examined influences on the formation of B[a]P-specific anti-B[a]P-7,8-diol-9,10-epoxide (anti-BPDE) DNA-adducts by pyrene. Human A549 lung cancer cells were exposed to B[a]P (0.01-10 μM), pyrene (0.01-10 μM), or binary mixtures of 1 μM B[a]P + pyrene (0.01-10 μM) for 24 h. Specific anti-BPDE-DNA-adducts were determined in terms of the B[a]P-petrol 1-1 by HPLC with fluorescence detection. Additionally, changes in cytochrome P450 1A1/1B1 (CYP1A1/1B1) activities were measured in intact cells utilizing a luminescence-based assay. B[a]P alone led to a concentration-dependent formation of anti-BPDE-DNA-adducts after 24 h, with maximum adduct rates at 1 μM. After co-incubation with 1 μM B[a]P + 0.01 μM pyrene, adduct rates were enhanced compared to 1 μM B[a]P alone. But, with further increasing pyrene-concentrations in the binary mixtures, anti-BPDE-DNA-adduct rates decreased compared to 1 μM B[a]P alone. All changes in DNA-adduct formation were accompanied by similar changes in CYP1A1/1B1 activities. Incubation with pyrene only resulted in concentration-dependent decreases of CYP1A1/1B1 enzyme activities. Our results show that genotoxicity of B[a]P is systematically modulated by pyrene, but, not unidirectional in terms of increasing or decreasing genotoxicity. The observed effects were closely related to the bioactivation of B[a]P. These findings are important especially with respect to the fact that PAH mixtures to which humans are exposed vary in their compositions.

(3) Toxicological evaluation and risk assessment of chemical mixtures using a factorial design allows us to estimate not only main effects but also toxin interactions. This study evaluated individual and combined effects of four common Fusarium mycotoxins, DON (2μM), NIV (2μM), ZEA (40μM) and FB1 (40μM) on immunological defense mechanisms of intestinal epithelial cells (IECs) using pro-inflammatory cytokine synthesis (IL1β, IL1p, IL6, IL8, TNFα and MCP-1), antimicrobial peptide (pBD-1 and pBD-2) synthesis and secretion on porcine intestinal epithelial IPEC-J2 cells, as well as secretory mucin (MUC5AC and MUC5B) synthesis and secretion. The study also evaluated inflammatory cytokine synthesis (IL1β, IL6, IL8, TNFα (up to 22-fold), IL6 (up to 2.6-fold), IL8 (up to 20.5-fold), TNFα (up to 21-fold) and MCP-1 (up to 24.5-fold), anti-BPDE-DNA-adduct rates constantly decreased compared to 1 μM B[a]P alone. All changes in DNA-adduct formation were accompanied by similar changes in CYP1A1/1B1 activities. Incubation with pyrene only resulted in concentration-dependent decreases of CYP1A1/1B1 enzyme activities. Our results show that genotoxicity of B[a]P is systematically modulated by pyrene, but, not unidirectional in terms of increasing or decreasing genotoxicity. The observed effects were closely related to the bioactivation of B[a]P. These findings are important especially with respect to the fact that PAH mixtures to which humans are exposed vary in their compositions.
ative risk assessment for children, this work is to derive comparable benchmark doses (BMD) for SVOCs acting on the reproducitve system through the same mode of action.

The choice of chemicals was based on: detection frequency (>10%) in French dwellings, availability of data on the mechanism / mode of action for reproductive toxicity, and availability of comparable dose-response relationships (in terms of species, route and window of exposure). Lower bound of 95% confidence interval of BMDs corresponding to a decrease in 10% and 50% of serum testosterone were derived using BMDs software (US EPA, 2013).

Among 51 SVOCs previously selected, 12 (6 phthalates, 1 polybromodiphenyl-ether, 3 pesticides, benzo[a]pyrene and benophen A) have been described to act on the Leydig cells by inhibition of an enzyme activity responsible of testoste-ron production, resulting in a decrease in serum testosterone levels. Only 7 have suf-ficient and comparable data to derive BMDs. Considering the decrease of serum tes-terone levels, benzo[a]pyrene has the most potent toxicity (BMDs’ lower bounds: 0.0023-0.015mg/kg BW/d) and benzylbutylphthalate has the least potent toxicity (BMDs’ lower bounds: 52.73 - 364.89mg/kg BW/d). Relative potencies ranked as: benzo[a]pyrene > bisphenol A > 2,2,4,4-tetrafluorobiphenyl-ether > permethrin > diethylphthalate > cypermethrin > benzylbutylphthalate.

The main limitation for a cumulative risk assessment remains the lack of compara-ble toxicological data but this approach allows taking into account pollutants from diverse chemical families, with similar mode of action. These BMDs make possible a cumulative risk assessment for children exposed in indoor, in view to protect them from reproductive effects in adulthood.

### 1010 Human Health Hazard Assessment of Hydroprocessed Esters and Fatty Acids (HEFA) Bio-Based Jet Fuels


The 2007 Office of the Secretary of Defense Assured Fuels Initiative directed the pursuit of domestically produced alternative fuels for military use to decrease dependence on foreign oil sources. Although bio-based jet fuels are similar to petro-leum-derived JP-8, the traditional military fuel, the overall chemical composition is different. Therefore, potential health effects must be studied during development of alternative jet fuels. The body of toxicological data for Hydroprocessed Esters and Fatty Acids (HEFA) fuels from feedstocks including Camelina seed (HEFA-C), tallow (HEFA-T) and mixed animal fats and oils (HEFA-F) were exam-ined in order to develop an occupational exposure limit (OEL) for HEFA fuels. Dermal irritation studies showed that HEFA fuels are less irritating or equivalent to JP-8. Mutagenicity and genotoxicity assays were negative. Acute and short-term inhalation studies resulted in transient effects at low levels that were not seen in the 90-day inhalation study; additional reproductive endpoints were negative and no significant neurobehavioral effects were observed based on functional observa-tional battery and motor activity tests. Sensory irritation assays never reached 50% respiratory depression at saturation doses. HEFA toxicity data were compared with toxicity data from synthetic parathion kerosene (SKP) alternative jet fuel and petro-leum derived JP-8. A comparative OEL for HEFA fuels was developed that concurs with the current JP-8 OEL (200 mg/m³).

### 1011 Xenobiotic Mixtures Decrease Human Hepatic Gluconeogenesis and Glucose Oxidation

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Epidemiological studies have shown that exposure to certain xenobiotics is associ-ated with an increased prevalence of metabolic diseases. Since humans are exposed to mixtures of xenobiotics detoxified by the liver, we used two xenobiotics, both en-docrine disruptors and persistent organic pollutants, which use different signaling pathways, to study hepatic energy metabolism: 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), which uses the AhR, and α-endosulfan, an organochlorine pesticide, which acts via the PXR and/or the estrogen receptor.

Treatment with either TCDD or α-endosulfan decreases, in vitro, the expression of hepatic glucose metabolism genes even at low doses and decreases hepatic gluconeogenesis and glucose oxidation. Chronic exposure of individuals to low doses of xenobiotics might significantly affect hepatic carbohydrate metabolism and be a contributing factor in the development of the metabolic syndrome.

### 1012 Studying the Impact of Volatile Organic Chemical Coexposures on the Urinary Excretion of Their Metabolites in Human Volunteers


It is common practice to assess volatile organic compounds (VOCs) exposure by monitoring their corresponding urinary metabolite, assuming no interactions between co-exposed chemicals. There is a dearth of information concerning the im-pact of VOC co-exposure on the excretion of metabolites used for biomonitoring.

The objective of this study was to collect metabolite excretion data and parent compound kinetics from inhalation exposures in human of four common VOCs (i.e. chloroform [CHL], toluene [TOL], ethylbenzene[EBZ] and m-xylene [XYL]). Five human volunteers were exposed by inhalation to 18 different combinations of chemicals (single, in binary combination with chloroform, or all 4 VOCs) at 1/4 or 1/8 TLV for 6 hours each with a week of interval between each exposure. Parent compounds were measured in exhaled air and blood samples taken during and following exposures. Metabolites, such as o-cresol (TOL), mandelic acid (EBZ), m-methylyluric acid (XYL) and 2,4-dimethylphenol (XYL) were quantified in urine (collected during and after exposure) after derivatization. No interactions were observed in exhaled air between exposures. No statistically significant interac-tions were observed on metabolites except for 2,4-dimethylphenol, which showed a decrease of 25% to 36% in total excreted amount in the presence of CHL alone or in the presence of all VOCs in air. Although average total amount of o-cresol ex-creted is increased by 50-72% when co-exposed to EBZ and XYL in air, and CHL in drinking water, this was not shown to be statistically significant. More effort will be place to understand the contradictory mixture interaction (i.e., decreased xylene blood levels but decreased metabolite excretion). These data will help to develop and validate an interactions-based physiologically-based pharmacokinetic modeling approach to assess VOCs exposure from urinary biomonitoring data.

### 1013 Pharmaceutical, Metal, and Ammonia Mixtures: Widespread Additive Toxicity in Zebrafish Larvae


Urban and industrial effluents (e.g. wastewater, agricultural and mining effluents) can introduce many contaminants into our aquatic ecosystems. Indeed, multiple contaminants in a single polluted site is the norm. Aquatic life has little say in the matter and are susceptible to contaminant exposure, given their constant contact with the aqueous environment. The acute toxicities of a variety of environmental contaminants were examined using the larval stage of a model organism, the ze-brafish (Danio rerio; 4–8 days post fertilization). Toxic interactions were observed between metals such as nickel, cadmium and copper. For example, sublethal copper exposure (6% of the copper LC50 or 13% of LC01) decreased the cadmium 96 h LC50 by 47%. In addition, pharmaceuticals in the environment are a growing con-cern and selective serotonin reuptake inhibitors (SSRIs) are particularly toxic to fish. Fluoxetine (ProzacTM) and sertraline (ZoloftTM, LustralTM) mixtures displayed additive toxicity; sublethal Fluoxetine (one-third of the LC50, 40% of the LC01) decreased the sertraline LC50 by 46%. Most surprising, however, were the observations that mixtures of acutely toxic environmental contaminants from different chemical classes also displayed additive toxicity. For example, ethinyl estradiol (EE2; bioactive estrogen in the female oral contraceptive pill) displayed additive toxicity with copper and sertraline. Similarly, sublethal amphetamine (a component of fertilizer) increased the toxicities of sertraline and Fluoxetine. Mixtures were typically additive or greater than additive. In the search for a common toxic mechanism, it was discovered that larvae exposed to metals, SSRIs or ammonia (around their respective LC50s) all experienced whole body ion loss. Decreases were greatest for Na+ as high as 39% in 48 h. Water quality criteria should ulti-mately recognize additive toxicity in order to protect our aquatic ecosystems.
1014 Assessing Neurological Risks from Oral Exposure to Mixtures of Organophosphorus (OP), Carbamate, and Pyrethroid Insecticides

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Data on interactions were evaluated to develop approaches to screening level assessments of human neurological hazard from oral exposure to mixtures of OP, carbamate, and pyrethroid insecticides. PubMed and Toxline were searched for data on interactions among 34 OPs, 16 pyrethroids, and 14 carbamates. Using ATSDR’s 2004 binary weight-of-evidence framework, the following determinations were made: 1) There are inadequate data to assess the direction of possible interactions between pyrethroids and carbamates; 2) There is some evidence for dose-additive action between pyrethroids and OPs, but only at near lethal levels in rodents; and 3) There is evidence for dose-additive action for carbamates and OPs on neurological endpoints in rats [brain cholinesterase and associated thermoregulatory endpoints]. Additionally, pituitary-elaboration kinetics was unchanged in humans exposed to low doses of pirimicarb and chlorpyrifos-methyl. Overall, the evidence is not compelling to move from a dose-additive approach. Dose-additivity most closely describes the joint toxic action of adverse neurological effects from oral exposures to mixtures of OPs, carbamates, or pyrethroids. A relative potency factor (RPF) Hazard Index approach would be an appropriate approach using RPFs and index chemicals determined by the USEPA Office of Pesticide Programs (EPA RPF) Hazard Index approach would be an appropriate approach using RPFs and index chemicals determined by the USEPA Office of Pesticide Programs (EPA 2006, 2007, 2011) and provisional oral Minimal Risk Levels for index chemicals of each class.

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1015 Potential Toxicity of Electronic Cigarette Liquids and Aerosols As Measured by Four In Vitro Assays

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The popularity of electronic cigarettes (E-cig) continues to increase worldwide, with several major tobacco companies entering the E-cig market. A typical E-cig delivers a flavored propylene glycol or glycerol based aerosol, with or without nicotine, via vaporization by a battery-powered heating element. Currently, data regarding the potential toxicity of E-cigs is limited. To further our understanding, an in vitro battery of established assays was used to examine the mutagenicity (Ames), cytotoxicity (Neutral Red Uptake; NRU), genotoxicity (Micrococcus; MN) and inflammatory (IL-8 release) response of a set of glycerol-based commercial E-cigs, specifically the neat E-cig liquids, pad-collected aerosol extracts and freshly generated whole aerosols. Pad-collected smoke condensates and whole smoke from traditional tobacco burning cigarettes (3R4F, 1R5F and one commercial cigarette) were included for comparison. All E-cigs and traditional cigarettes were smoked under Canadian Intense parameters (55 mL puff volume, 2 second puff duration, 30 second puff interval, 100% blocked air dilution). At the levels tested, exposures with neat E-cig liquids and pad-collected aerosol extracts showed no-to-extremely low activity in the Ames, NRU, MN and IL-8 assays when compared to responses from the traditional tobacco burning cigarettes. Results with E-cig samples without nicotine were very similar in all assays, indicating that the presence of nicotine, at the levels tested, did not significantly contribute to any cytotoxic and genotoxic effects observed at high doses. Whole aerosol exposures are ongoing, but preliminary results (Ames) show no activity. Overall, results from this study indicate, under the experimental conditions utilized, E-cigs do not produce any meaningful toxic effects when compared to traditional cigarettes, as measured by the four in vitro endpoints used in this study.

1016 Concentration Addition and Toxic Equivalent Factors: When Do They Apply?

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Dose addition/concentration addition (CA) is the principle criterion used in toxicology for judging interaction of compounds that act via similar mechanisms. Deviations from CA are then said to display synergy (more properly, greater than CA) or antagonism (less than CA). Following Berenbaum and others, by CA we mean that one compound can substitute for another at a constant ratio at any constant effect level. This is equivalent to having linear isoboles: the contours of the joint dose-response surface are straight lines. It is not widely understood that Toxic Equivalence Factors (TEFs) is a special case in which the dose ratio is the same at all effect levels (isoboles are then parallel which they need not be in the more general case). Confusion has crept into the literature as a result. Indeed, some alternative definitions of concentration addition lead to strange results. First, suppose that $f_A(a)$ describes the dose-response curve for compound A and $f_B(b)$ for compound B. Let the mathematical inverses of these functions be $g_A(E)$ and $g_B(E)$, respectively: they tell us the dose of A alone or B alone needed to achieve any given effect level E. One alternative definition of CA calculates the combined effect of A and B under no interaction as $f_A(g_A(fb(b)))$ or $f_B(g_B(fa(a)))$. We show that these two expressions are equal only under the TEF case; in other situations they can lead to different isoboles. Second, it has been claimed that joint dose-response surfaces with linear isoboles are rare. It is widely known that TEFs apply when the joint response of A and B is $f_A(a^*b)$ where $r$ is the relative potency of B compared to the reference compound A. While it can be shown that this is the only situation where TEFs apply, it places little restriction on the types of dose-functions $f_A(a)$ and $g_B(b)$. Finally, we might ask what kinds of joint dose-response functions obey CA. It can be shown that applying another function $h(E)$ to a response function changes the height of the response function but not the shape of its isoboles. Hence the kinds of dose-response-functions which can obey CA is enormous.

1017 Toxicology and Risk Assessment of Chemical Mixtures

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Evaluation of potential health risk from simultaneous exposure to multiple chemicals has been one of the major challenges for toxicology research and risk assessment (RA). Terminology and problem formulation is complex and the framework for mixture RA varies greatly among different agencies. As part of the International Life Sciences Institute North America Technical Committee on Food and Chemical Safety, 2013 Summer Fellowship Program, we analyzed current methods to achieve the following: 1) Describe the landscape of RA for chemical mixtures; 2) Characterize differences in RA frameworks; and 3) Assess regulatory acceptance of these frameworks with a focus on food mixtures. Review of literature was conducted for mixture RA paradigms used by US federal agencies, non-profit organizations (WHO, IPCS) and international agencies (EU). There are significant overlaps and differences between paradigms and there appears to be no single unified method, is apparent from our research. US EPA’s original framework forms the basis of most of the globally accepted methods for mixture RA. Whole mixture, sufficiently similar mixture or component-based approaches are used for mixture RA depending on data availability. Differences in hazard assessment approaches were found among agencies, including differences in criteria (e.g., mode of action, health outcome) for grouping chemicals in assessment groups. WHO, IPCS and EU recommend the use of a tiered approach and dose additivity as the default approach. We developed a framework based on a food mixture scenario to select the set of parameters to guide use of appropriate RA methods. These parameters include target population, exposure routes, availability of data on the exposure levels and toxicity interactions for the whole mixture or individual components. Future methods could incorporate high throughput screening data (e.g., toxicogenomics and bio-monitoring) to improve risk characterization of food mixtures. Considering the current challenges in food mixtures such as heavy metals, pesticides and migrants from packaging, a case study was done to compare different methods and test their applicability to food safety assessments.

1018 The Modulation of Antioxidant Enzyme Activities and Glutathione Levels in the Livers of Mice after Subchronic Exposure to Mixtures of Dichloroacetate and Trichloroacetate

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Dichloroacetate (DCA) and trichloroacetate (TCA) are drinking water chlorination byproducts that were found to be hepatotoxic/hepatocarcinogenic in rodents. Mixtures of the compounds were previously found to induce additive or greater than additive effects on various biomarkers of oxidative stress (OS) in the hepatic tissues of B6C3F1 male mice after subchronic exposure, and that was suggested to modulate the hepatotoxicity/hepatocarcinogenic outcomes of the compounds. In order to determine the role of antioxidant enzymes and glutathione (GSH) on the effects of the mixtures, groups of male B6C3F1 mice were treated daily, by gavage, with 3 different doses of DCA (7.5, 15 and 30 mg/kg/day), 3 different doses of TCA (12.5, 25, and 50 mg/kg/day) and 3 different mixtures (Mix I, Mix II and Mix III) of the compounds for 13 weeks. The concentrations of the compounds in Mix I, II and III, respectively corresponded to those producing approximately 15%, 25% and 35% of maximal induction of OS by the individual compounds in the subchronic studies. The mice were euthanized at the end of the treatment period and livers were assayed for the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione level. While the individual compounds effects on the enzyme activities and glutathione were demonstrated as either suppression or stimulation, the mixture effects were demonstrated as the net effects of the two compounds in mixtures. The results indicate significant contribution of the antioxidants to the outcome of OS in the livers of B6C3F1 mice after long term exposure, and that the effects of mixtures of the compounds are different from...
those produced by the individual compounds. Hence, mixtures of DCA and TCA may result in modulation of the hepatotoxic/hepatocarcinogenic outcomes of the individual compounds. (Supported by NIH/NIEHS grant # R15ES013706-01A2)

1018a Cumulative Toxicity of an Environmentally Relevant Mixture of Nine Regulated Disinfection By-Products in a Multigenerational Rat Reproductive Bioassay


Disinfection of water has advanced public health by decreasing waterborne disease. Disinfectants react with organic materials in the water to form complex mixtures of disinfection by-products (DBPs). While a large unknown fraction remains, >600 DBPs have been identified. Most prevalent in chlorinated water are trihalomethanes (THMs) and halocarbons (HAAs); 4 THMs (chloroform, bromodichloromethane, chlorodibromomethane, bromoform) and 5 HAAs (chloroacetate, dichloroacetate, bromoacetate, dibromoacetate) are regulated by EPA as a group at 80 µg/L and 60 µg/L, respectively. This is the first multigenerational reproductive toxicity bioassay in animals with a drinking water mixture of the regulated THMs and HAAs. At realistic proportions, a mixture was prepared at 0, 500x, 1000x, and 2000x of EPA’s maximum contaminant levels (MCLs). Timed-pregnant Sprague-Dawley rats (P0 generation) were exposed from gestation day 0 until weaning of the F1 offspring. Weanlings continued in their treatment groups, were examined for reproductive endpoints and bred to produce F2 litters. Pre- and postnatal survival was unaffected. F1 pup weights were unaffected at birth but reduced at 2000x on postnatal day (PND) 6 and at ≥1000x on PND 21. Males at 2000x had a small but significantly increased incidence of retained nipples and effects on sperm motility. Onset of puberty showed dose-related delays at 1000x and 2000x. F1 estrous cycles, breeding, and fertility were unaffected and F2 litters showed no effects on pup weight, or prenatal or neonatal survival. Histologically, P0 dams had nephropathy and adrenal cortical pathological at 2000x. In sum, while puberty was delayed at DBP concentrations ≥1000x and males at 2000x had retained nipples and altered sperm motility, exposure at these concentrations to an environmentally realistic mixture of 9 regulated DBPs did not affect F1 animals’ ability to reproduce. (This abstract does not reflect EPA policy.)

1018b Toxicity Testing of Water, Air, and Soil near Mountaintop Removal Mining Sites in the Southern Coalfields of West Virginia Using In Vitro Methods—What the Data Can Tell Us

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As a result of published epidemiological studies indicating health impacts in mountaintop removal coal mining (MTR) areas of southern West Virginia (WV), the USGS conducted a survey of air, water and soil in the impacted area and at WV State Parks which served as control sites with no MTR. The analytes measured were organics, metals, and in vitro toxicity, collected over 2 years. Toxicity testing included three assays which measured 24 hr toxicity. 1) week clonal outgrowth and anchorage-independent growth effects of water and soil sample extracts on human cells. A case study was then conducted on samples collected from Artie, WV in close proximity to a mountaintop removal mining. Private wells, surface waters, soil, fruit/vegetable matter were sampled. Grab sampling was conducted during one week periods over the first year of the survey over all seasons at locations in several counties and at two State Parks outside the active mining area. Year two sampling concentrated on Artie, WV during spring and summer. From the sampling in year one, 2 of 22 samples (March), 3 of 21 (May), 4 of 18 (December) showed significant toxicity to human cells, while from the Artie, WV sampling in year two toxicity was seen in 16 of 19 (August) but 0 of 23 (December) samples. Ground whole apples from a site sampled in August also showed significant toxicity. This variation could be due to episodes of mining activity, seasonal differences in temperature, or water abundance and movement through the aquifer. The air, water and soil organics, metals, and fine particulates showed important differences between mined and non-mined locations. We will discuss how the latter relate to the toxicity results, potential overall importance and context within recent literature findings regarding the possible health effects of mountaintop mining removal.

1018c Assessment of In Vivo Toxicological Interactions from Criteria Air Pollutant Mixtures

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The US EPA sets National Ambient Air Quality Standards for criteria air pollutants, recognizing that air pollution in the environment exists as a complex mixture. Potential interactions between mixture components within biological systems are not well characterized. We reviewed past literature investigating the effects of mixtures of criteria air pollutants, starting with mixtures containing NOx (nitrogen oxides), and evaluated the presence of in vivo toxicological interactions. All endpoints and NOx-containing mixtures were considered, however, most studies evaluated respiratory effects of NOx+O3 (ozone). Studies were classified into two categories: complete or incomplete response data. Complete data included the number of observations, mean response, and variance for each treatment group in the study. Studies lacking single pollutant exposures or complete response data were reviewed qualitatively. Of the 13 animal studies and 7 human studies in the qualitative analysis, 6 animal studies and 0 human studies suggested there may be interactions, though no pattern of response among the endpoints or exposures was apparent. Studies with complete data were analyzed quantitatively, and the response to the mixture was compared statistically to the sum of the responses to individual pollutants; the absence of a difference was defined as additive (H0: combined effects = sum of individual effects, p=0.05). Of the studies in the quantitative analysis, all animal studies (n=17) had at least one non-additive endpoint, while the majority of endpoints evaluated in human studies (n=9) were additive. Among the studies that deviated from additivity, no pattern of response emerged in endpoints or exposure conditions. Thus, this analysis suggests that deviations from additivity exist in criteria air pollutant mixtures; however, many of the studies did not demonstrate interactions other than additivity.

The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. EPA.

1018d Mixture Effects at Human Relevant Exposure Levels?

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Mixtures of endocrine active chemicals usually act additively irrespective of the applied model system and the complexity of the endpoint measured. Most studies performed so far have tested doses > NOAEL for individual chemicals. But what happens when we test mixtures of EDC’s at doses close to human relevant exposure levels? We had the hypothesis that a low dose mixture of environmental chemicals and active food ingredients would not affect toxicity induced by perfluorooctanoic acid (PFNA). PFNA was given at 13, 250 or 5000 µg/kg/day to male rats with or without a cocktail containing 14 chemicals including phthalates, bisphenol A, parabens, UV filters, pesticides, and the CYP3A4 inhibitors bergamottin (from grape fruit) and glabridin (from liquorice) at human relevant exposure levels (totally 2.5 mg/kg/day). The lowest PFNA dose corresponded to an internal dose of 4–6 fold mg/kg/day. The lowest PFNA dose induced steatosis at the highest dose. At the low dose PFNA+Cocktail pronounced increases of androgen levels (dehydroepiandrosterone, androstenedione & testosterone) were evident. Gene expression analysis in low dose PFNA+Cocktail treated animals showed that CYP19 mRNA was affected in testis and fatty tissues. At higher PFNA doses several steroidogenic enzymes were down-regulated. Overall, we suggest that mixture effects may be considered as biomarkers of direct adverse effects. This is to our knowledge a first indication that mixture effects appear in rats at doses close to human relevant exposure levels.
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15% ~ 40%. This results in variable changes (+/-20%) of F+C as compared with exercise, mass deposition increases greatly (~140%) in TB but decreases (15%) in AL. Furthermore, number deposition comes from both UF and F at a ratio of 1.5:1. During respiration in TB but an increase in AL resulting in a slight increase in TB+AL. Surface area decreases whereas deposition of F increases in both TB and AL as MF increases.

Lung deposition including tracheobronchial (TB) and alveolar (AL) regions was calculated by using a mathematical model built upon Weibel’s lung morphology. The present study used the Bovine Cornea Opacity and Permeability (BCOP) assay to over-predict the irritation potential of moderate and mild irritants. To assess this limitation, the EO assay was used as the second tier test for 14 of the NRR-over-predicted formulations. The EO assay demonstrated good performance with 86% concordance for correctly classifying the NRR-over predicted irritants.

In vitro ocular irritation assays, such as the Chorioallantoic Membrane Vascular Assay (CAMVA) and Bovine Cornea Opacity and Permeability (BCOP) test, are routinely used by personal care products companies because they are rapid and economical to conduct, do not require the use of live animals, and provide reliable predictive data. Previous research using an extensive CAMVA and BCOP database at Kao USA Inc. has shown that ocular irritation potential for new hair shampoos, ethanol-based hair stylers, skin cleansers, and skin lotions can be reliably predicted using a decision tree that systematically compares the ingredient composition, particularly surfactants and surfactant content, of the new formulation to previously tested formulations. Because the studies comprising this original database were conducted by a single contract laboratory, a follow-up study using a second contract laboratory was conducted to demonstrate inter-laboratory reliability of the CAMVA/BCOP data-derived decision tree for prediction of ocular irritation potential. Thirty-five personal care products were tested using the CAMVA and/or BCOP assay. The ethanol and surfactant content of each test material was evaluated, and the results of the assays were compared to the decision tree-based predictions of ocular irritation potential. Our data confirmed the ocular irritation predictions made using the decision tree model for 33 of 37 test samples (89% correlation rate) and verified the inter-laboratory reliability of the CAMVA and BCOP assays when conducted using appropriate controls. Overall, these results also strengthened the ocular irritation decision tree model by confirming that deodorants are consistently predicted not to be ocular irritants based on composition.
Product form and usage can clearly impact exposure, and the present results suggest that modifications to the physical properties of chemical mixtures can alter their ocular irritation potential; perhaps by affecting exposure to the eye. Although no formal comparisons were performed in animals, the BCOP assay is an OECD-validated method to assess ocular irritation, and studies have shown that the BCOP assay does not under-predict the results of traditional animal tests; thus, there are no obvious reasons to suggest that the present results would not correlate to animals or humans.

**1022 Assessing Default GHS Classifications for Eye Toxicity with Alternative Ocular Assays**

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The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) represents an international approach to standardize the classification and hazard communication for chemicals in the workplace. Institutional cleaning products, due to their surface-active chemistries, commonly carry a classification of serious eye damage or irritation. For appropriate product stewardship, such products must be correctly classified for eye effects. Ocular testing is a common approach for attaining a proper GHS classification. Current GHS criteria for classifying this endpoint are based primarily on interpreting in vivo data. However, due to animal welfare concerns, there is a shift in testing emphasis to alternative assays. Two such assays are the Bovine Corneal Opacity Permeability Assay (BCOP) and Chorioallantoic Membrane Vascular Assay (CAMVA). The BCOP uses excised bovine corneas to evaluate ocular irritation. The CAMVA uses the vascular network of fertilized chicken eggs as a conjunctival model to assess ocular irritation. For this study, institutional cleaning products were assigned default GHS classifications for eye effects based on an algorithm prescribed by GHS. Twenty-six products had joint BCOP/CAMVA tests conducted to assess such classifications. Based on the test results from the joint BCOP/CAMVA studies conducted for each product, GHS classifications were re-assigned and compared to the default classifications. Of the 26 products evaluated, 17 had test-based classifications aligning with default classifications, 6 had test-based classifications lower than the default classifications and 3 products had test-based classifications higher than the default classifications. Based on this particular dataset of 26 products, the results suggest the GHS algorithm assigns appropriate (~65%) or conservative (~23%) eye classifications with a relatively small (~11%), but not insignificant, minority of under classifications. A larger dataset is needed to make a definitive conclusion for this observation.

**1025 Cultured Porcine Cornea Assay Using Confocal Microscopy for High Resolution Detection and Quantification of Sub-Mild Ocular Irritation**


A critical need exists for a non-animal ocular irritation assay that is sensitive to sub-mild ocular irritation. We have developed a novel assay, PorFocal, which can quantitively individual dead corneal epithelial cells in porcine corneas using confocal microscopy. PorFocal uses phosphate buffered saline (PBS) as a negative control and 0.01% benzalkonium chloride (BAK) as a positive control. In 17 experiments, 0.01% BAK always caused more cell death than PBS, and statistically (p<0.05) more in 15 of 17 replicates. The PorFocal assay detected a significant dose-response with BAK dilution series treatment. Treatment with 0.01% BAK-preserved lubricant eye drops showed a statistically significant (p<0.05) greater than 3-fold increase in cell death when compared to the preservative-free version. To examine the potential of the PorFocal to detect human eye sting of a known sting chemical (avobenzone), we compared a low avobenzone (LA) to a high avobenzone (HA) content sunscreen. The HA caused significantly more cell death (7-fold increase) than the LA. We then compared PorFocal to an industry standard 3D reconstructed human tissue (RHT) ocular irritation assay. The RHT detected four of nine materials while the PorFocal assay detected eight of nine materials tested as statistically greater than PBS values. Overall, this indicates a great degree of sensitivity with PorFocal assay, previously not attainable by existing methods.

**1026 Using Benzalkonium Chloride and POLYQUAD® to Test the In Vitro Reconstructed Human Corneal Epithelium Model As a Replacement for In Vivo Ocular Screening**

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The aim of this investigation was to evaluate whether the reconstructed human corneal epithelium (RHCE) in vitro model (SkinEthic Laboratory, Nice, France) is a reliable indicator of in vivo acute topical ocular irritation. Ten preservatives commonly found in topical ocular formulations, benzalkonium chloride (BAC) and POLYQUAD® (PQ; polyquaternium-1) solutions, were utilized as model compounds in the test system. Both BAC and PQ solutions were evaluated in the in vitro RHCE model at concentrations of 0.01, 0.02, 0.1 or 0.5%. Test solutions were applied on the surface of each RHCE tissue for 10 min, 1 hr or 24 hrs, respectively. Cell viability, histopathology, interleukin-1α, -6 and -8 were evaluated. Test solutions, except 0.5%
BAC, were also evaluated in a one-day in vivo topical ocular dosing study in NZW rabbits. The in vitro results classified PQ as a non-irritant at all concentrations and at all time points by cell viability. BAC was classified as an irritant in the in vitro study after both 1 hr and 24 hrs exposures at ≥0.1% by cell viability. Histological and interleukin analyses did not correlate as well and appeared to be more sensitive. Results from the in vitro RHCE model using cell viability assay appear to correlate with results from in vivo study with rabbits. These results suggest the RHCE using cell viability assay may have utility in screening topical ocular formulations for potential acute ocular irritation. Also, interleukins and histopathology could provide valuable data but require further investigation.

**PS 1027 The Importance of Understanding Drivers of Irritation In Vivo for Selection of Chemicals Used in the Development and Evaluation of In Vitro Eye Irritation Assays: Cosmetics Europe Analysis**


Cosmetics Europe’s Task Force Eye Irritation is actively involved in the development and evaluation of in vitro methods to assess the eye irritation potential of cosmetic ingredients. Selecting adequate chemicals based on a proper understanding of what drives irritation in classification of ocular effects of chemicals in the in vivo Draize eye test is a critical element that enables identification and evaluation of predictive capacity and applicability domain at an early stage of in vitro methods development. As such, an in depth analysis of the data available from external databases containing in vivo eye irritation data for chemicals tested in the Draize eye test (from more than 500 independent studies) has been undertaken. This analysis is based on having good quality in vivo data that has allowed a clear understanding of the different ocular tissues effects that drive classification. These include the degree of severity and/or the persistence of corneal opacity, iritis, conjunctiva redness and/or conjunctiva chemosis. In addition, all chemicals were screened for their commercial availability, assurance that they cover the whole range of irritation potential and relevant chemical classes and physical states. The approach used to define the drivers of irritation and the importance of each of them for the classification and labeling of chemicals is described. This work demonstrates e.g., the high involvement of corneal effects and the low prevalence of iritis driving classification, and the importance of conjunctiva effects in classification of GHS Cat 2A versus 2B.

**PS 1028 Tiered Testing Strategy Using Validated In Vitro Assays for the Assessment of Skin and Eye Corrosion/Irritation of Pharmaceutical Intermediates**

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The safety of workers handling solid pharmaceutical intermediates was assessed using a tiered testing strategy based on regulatory validated in vitro assays. The Top-Down approach was initiated with the in vitro skin corrosion assay (OECD TG 431) followed by the in vitro skin irritation assay (OECD TG 439) using the reconstructed human epidermis model from MatTek Corporation. Of the ten pharmaceutical intermediates tested, nine were predicted to be non-corrosive to skin and were subsequently confirmed as non-irritants. The only intermediate predicted corrosive to skin was further tested using the Corrosity test (OECD TG 455) and assigned to a corrosive packaging group II classification. Furthermore, three intermediates predicted non-corrosive/non-irritant to skin were tested as 20% dilutions in water in the in vitro Bovine Corneal Opacity and Permeability (BCOP) assay (OECD TG 437) and they were predicted as non-irritant to the eye. Our tiered skin and eye corrosion/irritation testing strategy proved to be a very useful platform for the assessment of the potential safety risk posed to workers during manufacturing operations used for pharmaceutical intermediates.

**PS 1029 Study of Cosmetics Europe for Skin Sensitization Hazard Characterization and Risk Assessment without Animal Testing**


Cosmetics Europe (CE) intends to develop a testing strategy for sensitizer potency prediction without animal testing. In this regard, potency is defined as the entire spectrum of skin sensitisation, ranging from non-sensitiser to extreme sensitizers. To reach this aim a method evaluation study was initiated to identify appropriate in vitro methods. In a joint effort CE and 15 test developers systematically evaluated 16 in vitro methods. 10 chemicals were blind tested by each test method developer: 4-Nitrobenzylbromide, Methylidibromoglutaronitrile, 2-Mercaptobenzothiazole, Cinnamal, Tetramethyl thiuram disulphide, Salicylic acid, Lactic acid, Sodium lauryl sulphate, Phenyl benzoate, Lauryl gallate. Additional information was shared on the protocol, prediction model and existing data for previously tested chemicals: Of the 16 test methods evaluated for hazard prediction (sensitiser vs non-sensitiser) 14 misclassified a maximum of 2 substances, 3 correctly predicted all 10 substances. 7 methods provided useful potency information for the assessment of skin sensitisation, subcategorise in 5 classes as defined for the LLNA. 5 methods misclassified a maximum of 3 substances, usually being only one category off. One test method misclassified more substances, while the 7th method was not evaluated in detail as only for 4 substance results were available. Phase II of the method evaluation study started in January 2013 with ten prioritized methods to build a data base used for the data integration approach starting Q1 2014.

**PS 1030 Predictability of In Vitro Dermal Assays When Evaluating Fatty Amine Derivatives**


It is widely accepted in the world of in vitro toxicology that skin assays can be used in place of in vivo testing to accurately predict corrosivity and/or irritancy of commodity chemicals. Due to REACH legislation, various categories of fatty amines have been evaluated. Historical worker occupational experience has exhibited that various fatty acid amine are corrosive to the skin. Coco-Amidopropylidethanolamine (Coco-APDEA) contains both secondary and tertiary amines and is used as a surfactant in numerous industries. In the EpiDerm (EPI-200) Skin Model, skin corrosion is expressed as the remaining cell viability after exposure to the test substance. The relative mean tissue viability obtained after the 3 minute and 1 hour treatments with Coco-APDEA was 80% and 70% respectively. Since the mean relative tissue viability was not below 50% after the 3 minute treatment and not below 15% after the 1 hour treatment, Coco-APDEA is not considered to be corrosive in this assay. In the EpiSkin-SMTM irritation assay, a positive result is determined when viability is below 50% following a 1 minute exposure. Coco-APDEA exhibited 1% viability at the 15 minute exposure period which would be considered an irritant in this assay. Because the results from the in vitro assays with the human three dimensional epidermal models did not align with historical data, a limited in vivo rabbit study was employed. This test involved one animal and had to be terminated on day 2 because of severe reactions following the 3 minute exposure. Histopathology performed showed marked necrosis of the epidermis of the treated skin, with a marked heterophytic cell infiltrate in the underlying dermis. Based on the results of this study, it is concluded that Coco-APDEA is not a skin corrosive compound.

**PS 1031 Prediction of Systemic Bioavailability Using In Vitro Skin Absorption and Epidermal and Hepatic Metabolism of Aromatic Amine Hair Dyes**


Approaches to assess the role of absorption, metabolism and excretion (AME) of cosmetic ingredients that are based on the integration of different in vitro data are important for their safety assessment specifically due to the ban on animal testing.
in the European Union. In order to estimate systemic exposure (AUC) to each of 5 aromatic amine hair dyes (A027 (4-amin-2-hydroxytoluene), A074 (4-aminom-cresol), A158 (2-amin-5-ethylphenol HCl), A005 (toluen-2,5-diamine) and A154 (1-hydroxyethyl 4,5-diamino pyrazole sulphate)) following typical product application conditions, the bioavailability was first determined using in vitro skin penetration experiments and human keratinocyte (HaCaT) metabolism. This was followed by an estimation of the systemic AUC by additionally utilizing hepatic clearance values. The fraction of the dermally applied dose that reaches the systemic circulation was estimated using measured Michaelis-Menten descriptors of epidermal metabolism, skin penetration rates, viable epidermis measurements, mean residence time and skin permeability coefficients. Predictions of the nonmetabolized fraction were equal to or less than 0.25% of the applied dose. Separate ex vivo studies using viable human skin explants produced values that were within the same order of magnitude as those predicted from the above in vitro AIME approach. In the final step, in vitro hepatocyte Km and Vmax values and whole liver mass and cell density were used to calculate the scaled hepatic clearance to predict systemic exposure in the general circulation (AUC) based upon the estimated dermally absorbed dose. The overall approach provides a quantitative prediction of the internal exposure using appropriate toxicokinetic information that can be generated based solely on in vitro data and showed that none of the applied dose avoided effects of metabolism either by the skin upon entry into the body or by the liver systemically.

**1034 A Dermal Sensitization Assay Using SkinEthic™ RHE**

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International regulatory agencies, as well as animal welfare groups, are seeking in vitro assays for assessing the toxicity of chemicals and products. One of the most challenging problems has been to develop non-animal tests for skin sensitization. As an outcome of the Sens-it-iv project in Europe, Corsini and colleagues developed an Interleukin-18 (IL-18) response assay in monolayer keratinocytes as an indicator of sensitization in 2009. Here we report release of IL-18 into the culture medium of SkinEthic™ RHE treated with sensitizers, but not with irritants or non-sensitizers. Sensitizer-induced IL-18 release by RHE tissues was observed to occur in a concentration-dependent manner. RHE tissues were exposed to test substances for 24 hours. Data were expressed as a Stimulation Index (SI) calculation. An SI ratio of >2.0 was considered a positive result for a dermal sensitiser. A range of slight to severe sensitizers, as well as nonsensitizers and irritants were tested. A commercially available ELISA measured IL-18 and tissue viability was determined by the MTT assay. Of the twelve known positive sensitizers tested (4-Nitrobenzyl bromide, p-Phenylenediamine, 2,4-Dinitrochlorobenzene, Eugenol, Citral, Resorcinol, Glyoxal, 4-Hexyl cinnamaldehyde, Cinnamaldehyde, Cinnamic Alcohol, IsoEugenol and 2-Mercaptobenzothiazole), ten were correctly predicted. In addition, six of seven irritants (Chlorobenzene, Lactic Acid, Phenol, Methyl Salicylate, Salicylic Acid and Tween-20) and nonsensitizer (Glycero) were correctly predicted. Overall accuracy of the assay was calculated to be 84%. In conclusion, an in vitro assay for characterization of dermal sensitizers was developed in SkinEthic™ RHE. This assay is promising for identification of sensitizers with high accuracy and predictivity, and for further research into the mechanism of dermal sensitization mediated by Interleukin-18.

**1035 Coculture Assay for the Identification and Investigation of Dermal Sensitizers (Epi-DC)**

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In order to investigate the mechanism of dermal sensitization, a coculture model of human keratinocytes and dermritic cells was developed and optimized. This model (Epi-DC) consists of a 3D epidermal organotypic tissue, EpiDerm™, grown atop a suspension culture of plasmaoid dendritic cells (DCP-100), in a medium that allows both cellular components to express normal viability, expression of their activation antigens, and cell-cell communication. In this model, test chemicals were dosed topically on the EpiDerm™ tissue for 24 hours. The criteria for change in biomarkers to indicate a positive sensitiser included: (1) an increase of IL-18 release into the culture medium of >1.6-fold over vehicle, or (2) an increase in percentage of DCP-bearing activation surface markers CD86+ or CD54+ of >1.5-fold over vehicle controls, as measured by flow cytometry. Dinitrochlorobenzene (DNCB; 0.003% to 0.1%) and cinnamaldehyde (CA; 0.0003% to 0.006%) were tested diluted in the ethanol vehicle. Results: Basal secretion in vehicle control cocultures was 23.8 pg/ml of IL-18. Treatment of Epi-DC with DNCB yielded IL-18 concentrations that peaked at 0.1% DNCB, resulting in a release of 44.5 pg/ml (1.9-fold increase). Vehicle control cultures had 9.1% CD86+ DC, and a dose-dependent increase of CD86+ DC was shown with treatment of DNCB ranging from 0.003% to 0.1% DNCB, peaking at 18.7% positive CD86+ DC (2.1 fold increase). No significant increases in CD54 expression were observed. At 0.006% CA, increases of 1.6-fold over control were observed in CD86 expression. No increases in CD54+ DC were seen at any of the test substance treatments. Thus, in this initial evaluation, the Epi-DC coculture model has shown to be a useful and predictive tool for identifying dermal sensitizers without the use of animals.
We describe a human-based in vitro skin explant test (Skinmine™) that improves predictivity relative to the use of animal models for adverse immune reactions. The test involves exposing dendritic cells from a healthy volunteer to a test compound followed by incubation with autologous T cells. The T cells are then tested for immune reactivity in vitro on a skin biopsy from the same volunteer. If the compound is a sensitizer, coculture of activated T cells with the skin gives rise to an adverse immune reaction, graded histopathologically from I-IV. The test was evaluated using a range of chemical sensitizers and nonsensitizers, with 95% concordance with the mouse local lymph node assay (LLNA) (p < 0.001; sensitivity 95%, specificity 95%). The test correctly identified chemicals negative in the LLNA but positive in man (e.g., nickel sulphate) and correlated with T-cell proliferation and interferon gamma secretion assays. The test also predicts adverse immune reactions to biologics such as the Tegenero antibody (TGN412) and overcomes inter-laboratory differences which often prevent detection of adverse effects during safety testing. Compounds tested included: maleic anhydride, glutaraldehyde, trimellitic anhydride, paraphenylene-diamine, cinnamic alcohol, isoeugenol, eugenol, 2,4-dinitrochlorobenzene, mercaptobenzothiazole, cinnamaldehyde, oxazolone, glyoxal, resorcinol, 4-nitrobenzylidene, potassium dichromate, ethylendiamine, hexylcinnamaldehyde, and 2-hydroxyethylacrylate, and the nonsensitizers sodium dodecyl sulfate, glutamic acid, propyleneglycol, 4-aminobenzoic acid, methylsalicylate, chlorobenzene, diethyleneglycol, propylene glycol, ethanol, propylene glycol, trimethylamine, propylene glycol, propylene glycol, and Tween 80.

This study builds upon previous studies showing that CeeTox’s in vitro SenCeeTox® assay can correctly identify and categorize chemical sensitizers when used in-house. The aim of this project was to further validate the SenCeeTox® assay by conducting an inter-laboratory validation at the Flemish Institute for Technological Research (VITO). In this study, MatTek’s three-dimensional human skin model, EpiDerm (EPI-296, EpiDerm 96-well reconstituted human epidermis), was treated in triplicate with six concentrations of each test article. Test articles were run in a blinded manner. The test articles evaluated were: metol, isoeugenol, 2,3-butane diol, 2-mercaptobenzothiol, eugenol, 1-chloro-2,4-dinitrobenzene, n-propyl alcohol, 2-hydroxyethylacrylate, and 2-hydroxyethylacrylate, and the nonsensitizers sodium dodecyl sulfate, glutamic acid, triton-X-100, zinc sulphate, dimethyl sulphoxide, potassium permanganate, iso-propanol, dimethylformamide, glycerc, tween 80, phenol, ethylvanillin, octanoic acid, propylene glycol, 4-amino benzoic acid, methylsalicylate, chlorobenzene, diethylphthalate, p-hydroxybenzoic acid, and benzaldehyde.

**Inter-Laboratory Validation of an In Vitro Method to Classify Skin Sensitizers**


This study builds upon previous studies showing that CeeTox’s in vitro SenCeeTox® assay can correctly identify and categorize chemical sensitizers when used in-house. The aim of this project was to further validate the SenCeeTox® assay by conducting an inter-laboratory validation at the Flemish Institute for Technological Research (VITO). In this study, MatTek’s three-dimensional human skin model, EpiDerm (EPI-296, EpiDerm 96-well reconstituted human epidermis), was treated in triplicate with six concentrations of each test article. Test articles were run in a blinded manner. The test articles evaluated were: metol, isoeugenol, 2,3-butane diol, 2-mercaptobenzothiol, eugenol, 1-chloro-2,4-dinitrobenzene, n-propyl alcohol, 2-hydroxyethylacrylate, and 2-hydroxyethylacrylate, and lactic acid. Following 24 hr exposure to the test articles, the following endpoints were measured: 1) cytotoxicity, 2) the ability of each chemical to directly react with gluthathione, and 3) expression of key genes. The gene expression levels of seven target genes controlled by the NFκB/Keap1/ARE signaling pathway were examined: NADPH-quinnone oxidoreductase 1, aldo ketoreductase 1C2, interleukin 8, cytochrome P450 1A1, aldehyde dehydrogenase 3A1, heme oxygenase 1, and glutamate cysteine ligase catalytic subunit C. The data were then analyzed in a blinded manner using a proprietary algorithm to predict each chemical’s likelihood of causing a human sensitization reaction. The results confirm the inter-laboratory reproducibility of SenCeeTox® which accurately predicted the ability to elicit a sensitization reaction for all 10 blinded compounds tested at VITO. Furthermore, it correctly predicted the sensitization potency category for 9 out of the 10 compounds, missing the 10th compound by only one potency category. In conclusion, SenCeeTox® can predict the sensitization potency of chemicals ranging from nonsensitizers to strong-extreme sensitizers.

**A Novel Organotypic 3-D Human Small Intestinal Tissue to Assess Drug Safety and Inflammation**

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The intestinal epithelium plays a key role in defining the bioavailability of orally administered drugs. The colon cell line, Caco-2, is commonly used to study intestinal drug safety and inflammation but it does not recapitulate the structure or physiology of small intestine (SI) tissue. This study evaluates the functionality of a novel in vitro tissue model reconstructed from normal human primary SI epithelial
cells and fibroblasts. Primary cells were expanded in monolayer culture and seeded onto microporous membrane inserts to reconstruct 3D organotypic SI tissues. Tissue morphology, surface marker expression, ultrastructural features, and barrier integrity of the tissues were characterized. In addition, efflux drug transporter expression was analyzed by RT-PCR and drug permeation was examined using LC-MS/MS with 3 model drugs previously tested with Caco-2 cells. Inflammatory responses were examined by exposing the tissue to TNF-α for 24 hr. Analysis of the SI tissue model revealed: 1) wall-to-wall tissue growth, 2) columnar epithelial cell morphology similar to human SI, 3) a physiological TEER value of 60-180 Ω cm² mimicking the SI microenvironment, 4) expression of MUC-2, CK19, and villin, and 5) formation of brush borders and tight junctions. RT-PCR also showed expression of the prominent efflux drug transporters P-gp (MDR-1), MRP-1, MRP-2, BCRP and the main metabolic enzyme, CYP3A4 similar to human SI explants. Initial permeation studies using 2 P-gp substrates, ranitidine and talinolol, demonstrated active transport while warfarin, a non-PgP substrate, did not. Treatment of the SI tissue with TNF-α induced a proinflammatory response by inducing the release of chemokines including IL-8 and GRO-α. We anticipate that this new human cell-based SI tissue will be useful for pre-clinical assessment of drug safety and mucosal inflammation and at the same time will reduce the use of animals for experimentation.

**1041 Comparison of Three Different In Vitro Intestinal Barrier Models for Assessing the Oral Fraction Absorbed of Pharmaceutical and Nutritional Products**

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In development of pharmaceutical and nutritional products an accurate prediction of the oral fraction absorbed in humans is essential, as the bioavailability of a compound at the target site co-determines the efficacy of the active compound. Currently, animal models, in vitro (mainly cell lines) and in silico tools are applied preclinically, but their predictive value to assess oral bioavailability in humans is often insufficient. Here, we aim to design a decision tree based on several in vitro intestinal barrier models, but to increase acceptance and implementation, data obtained in intestinal barrier models should be translatable to human in vivo data. Apparent permeability (Papp) values of 15 model compounds with different physicochemical properties were tested in the Caco-2 model and the InTESTine™ model using porcine jejunal tissue and compared with published human Papp values. In addition, cells and pores and human intestinal segments were analyzed for abundance of transporter proteins and metabolic enzymes. Papp values of compounds determined using the InTESTine™ system were in the same range as the human Papp values (based on Ussing data), whereas data obtained with Caco-2 cells showed major differences probably due to differences in effective transport area. As expected, major differences were found in levels of active transport systems and metabolic enzymes in both Caco-2 cells and porcine jejunal tissue in comparison with human intestinal tissue. Results provide important characteristics of different in vitro permeability models and will help to design a predictive decision tree feeding into human PBPK modelling.

**1042 Evaluation of the Toxicity Profiles of Selected Bioactivated Compounds in Primary Rat Hepatocytes Cultured in Micropatterned Cocultures**

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Drug-induced liver injury is often caused by cytochrome P450-dependent activation of drugs into reactive metabolites. In vitro models, which can mimic in vivo responses and allow the evaluation of initial and adaptive responses to bioactivated compounds over prolonged periods, offer potentially valuable tools for toxicological assessment. We have previously developed a model in which primary hepatocytes (rat, human) are seeded onto ECM-coated domains of optimized dimensions and subsequently cocultivated with murine embryonic fibroblasts [i.e. micropatterned cocultures (MPCC)]. This model retains key biochemical functions of in vivo liver with long term stability. Here, we assess the bioactivation and cytotoxicity of acenaphthene (APAF) and other compounds in the 96-well rat MPCC. APAF is a well-known hepatotoxic and exerts its toxic effects through bioactivation associated, in part, with cytochrome P450 3A (CYP3A). Rat MPCCs were exposed to increasing concentrations of APAF (over 5 days) and assessed for changes in hepatic ATP content, glutathione (GSH) levels, albumin secretion and urea synthesis. Similar concentration-dependent cytotoxicity profiles (AC50=8.4 ± 2.4mM for GSH depletion and 14.17 ± 3.5 mM for urea synthesis inhibition) were obtained over the course of the 4-week study. Addition of 200μM L-buthionine (S, R)-sulfoximine (BSO), an inhibitor of GSH synthesis, or 10μM dexamethasone (DEX), an inducer of rat CYP3A1/2, to rat MPCCs protected APAF-induced hepatotoxicity in these cultures irrespective of culture age (over 4 weeks). These findings are consistent with the known in vivo mechanisms of APAP toxicity in rats. In conclusion, rat MPCCs provided reproducible APAP-induced cell cytotoxicity profiles over a 4 week period and can be used to assess the effects of chronic exposure to bioactivated compounds. The toxicity profiles of selected bioactivated compounds are also reported here.

**1043 Upregulation of CYP3A4 and CYP1A1/2 Activities in Huh-7 Human Hepatoma Cells with DMSO and Use of These Induced Cells to Evaluate CYP Inhibition by Botanical Extracts and Their Components**

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Liver metabolic enzyme inhibition and induction are the leading causes for severe drug-drug and drug-dietary supplement interactions which may result in increased toxicity or loss of treatment efficacy. In vitro cell line-based models are needed as an alternative to the “gold standard” primary hepatocytes, which are expensive and may exhibit problematic batch-to-batch variability. In the current study, human hepatoma Huh7 cells were treated with 1% dimethyl sulfoxide (DMSO) for two weeks after reaching confluence. Basal gene expression and enzyme activity levels of cytochrome P450 3A1/2 and 3A4 were measured and compared between DMSO-treated and non-treated Huh7 cells cultured in parallel. Real-time PCR analysis revealed that DMSO treatment up-regulated gene expression for CYP1A1, CYP1A2, CYP3A1 and CYP3A4 by 2.3 to 3.0 fold. Additionally, CYP1A2 and 3A4 activity (measured by specific luminescence assays) was 4.5 to 5.0 times higher in DMSO-treated Huh7 cells versus non-treated cells. Finally, the cell-based system was used to investigate the CYP3A4 inhibition by both whole Poria cocos extract and its major characteristic phytochemical components psoralen and isopsoralen. Psoralen and isopsoralen (1 μM to 50 μM), as well as Poria cocos extract (5 μg/mL to 20 μg/mL), inhibited CYP3A4 activity in a dose-dependent manner. No cytotoxicity was observed at these concentrations. In conclusion, Huh7 cells treated with 1% DMSO differentiate into a metabolically competent cell line, especially with regards to basal CYP1A1/2 and CYP3A4, which can be useful in studying the effects of botanicals and related pharmaceutical compounds on CYP1A1/2 and CYP3A4 function.

**1044 Evaluation of Hepatotoxic Effects of Acenaphthene and Arsanilic Acid on the Human Adipose Tissue-Derived Hepatocytes**


Hepatocyte-like cells (HLCs) derived from human adipose-derived stem cells (hADSCs) could provide availability as in vitro hepatocytotoxic model. In this study, we improved the differentiation strategy of hADSCs into HLCs by shortening the period of differentiation and evaluated the effect of acenaphthene (AAP) and arsanilic Acid (Ars), on the differentiation of hADSCs to HLCs. hADSC was used for hepaticogen differentiation. At final differentiation day (day 13), enzyme activities (LDH, ALT, AST), expression of hepatocyte specific genes (ALB, AFP, CYP3A4, CK18, C/EBP) and CYP450 activity (CYP1A2, CYP3A4) were determined. 1 and 2.5 μM ofArs and 5 μM of AAP showed cytotoxicity. Enzymatic activity was not changed by Ars treatment. In AAP treated groups, LDH activity was increased dose-dependently, and AST activity was also increased by the treatment of 2.5 μM and 5 μM, and ALT activity was increased by 5 μM AAP. In the Ars-treated groups, the expression of ALB was decreased by 0.25, 0.5 and 2.5 μM treatment without showing dose-dependent manner. In the AAP-treated groups, the expressions of ALB and AFP were significantly decreased by all dose of AAP. The expression of CYP3A4 was decreased only by 5 μM AAP compared to non-treated group. Interestingly, the expression of CK18 was increased by 5 μM AAP. C/EBP expression was not different in all concentration of AAP. The activity of CYP1A2 in AAP increased in dose-dependent manner and CYP3A4 was decreased by 0.625 μM of AAP. In the Ars treated groups, CYP1A2 activity was decreased and CYP3A4 activity was not significantly different from non-treated group. Conclusively, Ars decreased the expression of ALB and CYP1A2 activity whereas AAP increased activity of ALT, AST, LDH and decreased the expression of
Isoniazid (INH), prescribed for tuberculosis, has been associated with idiosyncratic drug-induced liver injury (DILI). It is speculated that genetic factors contribute to susceptibility towards INH hepatotoxicity. In the current study, induced pluripotent stem cell derived hepatocytes (iHC), which carry the donor's genetic information, were characterized for studying INH hepatotoxicity. Specific probe substrates demonstrated that Vmax/mg protein of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 in iHC were comparable to those reported in primary human hepatocytes (pHH). Both iHC and pHH demonstrated dose- and time-dependent INH-protein adduct formation and reduction in binding was observed after treating the cells with pan P50 inhibitors. Major bands of INH-adducted proteins in both cell types were qualitatively, but not quantitatively, similar. Proteomic analysis identified prohibitin-2 and macrophage navigation inhibitory factor as major proteins adducted by INH in iHC. INH, up to 10 mM, which is 100 fold higher than the Cmax in patients serum, does not cause ATP loss, lactate dehydrogenase (LDH) release or morphological changes in iHC. However, at non-cytotoxic concentrations, INH reduced the oxygen consumption rate as well as mitochondrial membrane potential and there was an increase in iHC release of microparticles (~0.1 micron) into the medium which may represent a mechanism for neotoxant and 'danger signal' release. In summary, the iHC exhibited many CYP activities comparable to pHH. At non-cytotoxic concentrations, INH formed protein adducts, altered mitochondrial function and released microparticles in iHC cultures. We conclude that iHC are appropriate for study of INH DILI and preparation of cultures from INH DILI subjects is now underway.

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a fundamental improvement of the technology by focusing on two main aspects: (i) to re-design the workflow of the procedure for reduction of cellular stress and experimental complexity and (ii) to clearly improve the deposition of airborne particulate matter on the cellular surface during aerosol testing.

As a result, an advanced procedure including a novel type of exposure device was developed. It permits a uniform and smooth processing of cells leading to higher robustness, practicability and ease of use. Repeated dose exposures and less time and material consuming routine use are enabled. To increase the applicability of the ALI-method in aerosol testing, numerical CFD simulations were applied for the optimization of the stagnation flow setup.

Deposition mechanisms such as sedimentation, diffusion and thermophoresis was balanced. First results of simulation and laboratory experiments showed a clearly increased deposition efficiency of particles from aerosols in the test system without observation of adverse effects on the exposed cells. Therefore, the improvements of the procedure so far seem promising to further enhance the applicability and acceptance of alternative methods in the study of inhaled substances significantly.

### Use of Rat Precision-Cut Lung Slices for Long-Term Functional Evaluation

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The need of alternative methods for animal studies to investigate basic inflammatory mechanism and to test toxicological potential of new substances has been increased during the last decades. Precision-cut lung slices (PCLS) have been proven to be a valuable alternative for mammalian in vivo studies reflecting relevant factors of the respiratory tract in accordance with the three R principles. However PCLS have been used mostly for short-term cultivation (≥ 72h) only, which limits their use to certain questions. When it comes to analyses of slowly metabolized chemicals and to repeated application of compounds, the long-term cultivation of PCLS might be utterly important. In this study viability and maintenance of structure and functionality of long-term cultured (≥ 14 days) rat PCLS was investigated.

Rat PCLS were cultured for 15 days. Readout parameters for vitality (WST-1 assay) and functionality were assessed at different time periods. Ability to secret the pro-inflammatory cytokine Tumor necrosis factor alpha (TNF-α) and functionality of long-term cultured (≥ 14 days) rat PCLS was investigated. Rat PCLS were cultured for 15 days. Readout parameters for vitality (WST-1 assay) and functionality were assessed at different time periods. Ability to secret the pro-inflammatory cytokine Tumor necrosis factor alpha (TNF-α) and functionality of long-term cultured (≥ 14 days) rat PCLS was investigated. Rat PCLS were cultured for 15 days. Readout parameters for vitality (WST-1 assay) and functionality were assessed at different time periods. Ability to secret the pro-inflammatory cytokine Tumor necrosis factor alpha (TNF-α) and functionality of long-term cultured (≥ 14 days) rat PCLS was investigated. Rat PCLS were cultured for 15 days. 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1056 Identification of Cytotoxic Chemicals in Thirdhand Smoke

V. Bahi1, S. Schick2, M. Sleiman3 and P. Talbott4, 1Cell Biology and Neuroscience, University of California Riverside, Riverside, CA, 2Department of Medicine, University of California San Francisco, San Francisco, CA and 3Indoor Environment Group, Lawrence Berkeley National Laboratory, Berkeley, CA.

Little information is available on the health effects of thirdhand smoke (THS). Our work evaluates the effect of THS on mouse neural stem cells, an in vitro model for neonatal brain. Cytotoxicity was studied using the MTT assay which evaluates conversion of a tetrazolium salt to a colored formazan by mitochondrial reductases. Tery cloth exposed to cigarette smoke for 110 hours over 1 year was stored at room temperature to allow aging of THS, and extracts were prepared after different times of aging. THS aged for 11 months caused complete cell death in the MTT assay at the highest dose and 50% cell death at the 30% dose. Cytotoxicity was lost immediately after the smoking period caused 50% cell death at the highest dose, and more than 90% decrease in cytotoxicity after 24h. In summary, we provide mechanistic insight into the mode of action of CS on neural cells, and how, by lessening the biological impact on key cellular processes, exposure to a PMRTP reduces overall toxicity when compared to CS. Moreover, the ability to detect biological perturbations at sublethal doses strongly supports the use of HCS-based approaches for toxicological assessment.

1058 Quantification of Neuron Maturation and Neurite Outgrowth In Vitro Using Human Neural Progenitor Cells

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Human embryonic stem cells based developmental neural toxicity (DNT) assays mimic neural development in vitro and have become an alternative to expensive and time consuming animal DNT models. Previously using human embryonic stem cell derived neuronal cells, an in vitro human neural culture system assay for neurite extension/recovery has been employed as a DNT assay. Our objective was to mimic in vivo neural tube development starting with proliferative neural progenitor (NP) cells for high content image screening in 96-well plates and to characterize the time point for neurite extension and NP cell transition to post mitotic neurons. In our lab we have generated differentiated neurons from NP cells without basic fibroblast growth factor (bFGF) but with leukemia inhibitory factor (LIF) in 2 weeks. During differentiation, Bis 1 dose solutions were applied to the cells 6h after plating for 2 weeks and then immunostained cell cultures in 96 well plates were loaded into a Cellomics ArrayScan VTI HCS reader high-content imaging system for automated image acquisition and morphometric analyses. Here our results show that expression of post mitotic neuronal cell marker Hu/C/D increased from 3.4 % at day 0 to 63.5 % by 2 weeks but did not significantly increase at later times. Thus we used this period of time in a multiplex assay to determine the effects of Bis1, a known neural toxicant on neurite outgrowth and maturation to Hu/C/D positive neural cells. Bis1 inhibited neurite extension in a dose dependent manner starting at 0.1mm without significantly affecting neuron maturation over the 2 weeks. We have developed a multiplex high content assay to determine the effects toxicant in cells representative of the developing human neural tube and for the first time demonstrated that Bis1 specifically affects neurite outgrowth at lower concentrations than acute neurite recovery assays.

1059 An Analysis of the Toxicity of Ximelagatran Using a Combination of In Vitro and In Silico Methods


Hepatotoxicity associated with increase in liver enzymes has been observed on long term (>35 days) treatment with Ximelagatran, an oral thrombin inhibitor causing the drug to be withdrawn from the market. We used a combination of in vitro and in silico methods to understand the mechanisms underlying its toxic behaviour.

We first tested Ximelagatran in a series of in vitro assays designed to understand its dose-dependent impact upon key pathways in HepG2 cells and derived a dose-relationship for each of them. The in vitro treatment was performed for 72 hours allowing us to build a robust relationship between drug exposure and inhibitory effects on key liver enzymes and transporters. We then simulated a virtual clinical term (>35 days) treatment with Ximelagatran, an oral thrombin inhibitor causing the drug to be withdrawn from the market. We used a combination of in vitro and in silico methods to understand the mechanisms underlying its toxic behaviour.

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Our simulations indicate that the drug has an impact on mitochondrial function by affecting multiple targets that eventually lead to depletion of cellular energy and production of reactive oxygen species. We hypothesize that this is due to inhibition of mitochondrial energy transport. Furthermore, these effects are exposure-dependent leading to apoptotic cell death and necrosis.

The 2-year bioassay is the standard method for carcinogen detection which is time and resource intensive. Many short-term tests, especially genotoxicity tests, have been developed to aid in identification of potential carcinogens. The SHE cell transformation assay is simulating the process of animal two-stage carcinogenesis and is suited to detect a carcinogenic potential of test compounds vitro including genotoxic and non-genotoxic mechanisms. For mechanistic investigations of Beryllium (first phase of REACH), three different genotoxicity tests (Ames, Mammalian Gene Mutation- and Chromosomal aberration test) together with the SHE cell transformation assay were carried out. Due to the insolubility of the metal powder and the metal alloy, these studies were conducted on extracts. No genotoxic effects where obtained with both extracts in the genotoxicity assays performed. However, in the SHE cell transformation assay dose-dependent increases in morphologically transformed colonies were obtained both with the metal alloy as well as with the metal powder, which indicates a non genotoxic mechanism of the metal powder and the alloy.

The SHE cell transformation was also carried out on a compound (first phase of REACH) with a structural alert for carcinogenicity (pararosaniline derivative). A significant dose-dependent increase in morphologically transformed colonies was obtained with the pararosaniline derivative indicating the usage of in vitro data to demonstrate similarities across a chemical category (read-across).

Our data indicate that the SHE cell transformation assay in combination with other information such as genotoxicity data, structure activity analysis, in vivo toxicity data and pharmac/toxicokinetic information can facilitate a relatively comprehensive assessment of a carcinogenic potential of a chemical.

**1060 Evaluation of In Vitro Cardiac Electrophysiological Assessments on the ACEA xCELLigence and Axion Maestro Platforms**

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Introduction: Cardiotoxicity liabilities persist as a major cause of pre-clinical and clinical drug attrition today. Recent advancements in both biological models and electrophysiological technologies allow for faster and longer-term in vitro methodologies to assess beat rate and functional alterations. We sought out to utilize label-free, electrode-based, high throughput platforms to detect cardiomyocyte beating and functionality.

Methods: Both the xCELLigence from ACEA and the Axion Maestro systems are label-free, electrode-based, high throughput platforms used to detect in vitro cardiomyocyte beating and functionality through detection of impedance and voltage, respectively. To evaluate each system, we used a set of four potent anti-cancer molecules that had previously shown significant effects on cell viability (IC50s < 10nM) along with one structurally similar but biologically inactive molecule as a negative control, and then determined the effects on human stem-cell derived cardiomyocytes, both functionally and electrophysiologically.

Results: Both platforms were able to accommodate the multiple-day experiments required to detect significant changes in beating over time. In these time course studies, both systems showed that the most toxic doses had dramatic effects on cardiomyocyte functionality, ultimately stopping beat rate entirely, and doses closer to the IC50s had more intermediate effects of reduced beat rate and amplitude. In contrast, inactive molecules were benign, demonstrating specificity of the compounds’ effect.

Conclusions: Measurements from both systems were sensitive enough to detect on-target, time dependent and mechanism-specific responses. Additionally, the Maestro detected spike slope, as well as T wave position, which allowed for assessment of repolarization periods (“QT intervals”), which helped to further discriminate the mechanisms of functional toxicity versus viability effects across molecules.

**1061 Preclinical Profiling and Comparison to a Human Response Database of an In Vitro Cynomolgus Macaque Vascular System As A Nonhuman Primate Surrogate**

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The cynomolgus macaque (CYNO) can be critical for de-risking and validating preclinical candidates for on- or off-target vascular responses. However, it is often difficult to translate mechanism of action studies from CYNO to humans. Previously we established a human primary vascular endothelial (EC) and smooth muscle cell (SMC) co-culture system, which recapitulates hemodynamic forces and restores cell biology and responsiveness to drugs at physiologically relevant levels. From this, we generated a predictive human response database (HRDB) comprising responses to >120 clinically relevant drugs, including atorvastatin (AS), simvastatin (SS), cerivastatin (CS) and rosuvastatin (RS). In the current study, we established a co-culture system with primary ECs and SMCs from CYNO arteries and exposed them to hemodynamics captured directly from CYNO arteries, inflammatory stimuli, and four different statins. Using RNAseq transcriptomics we profiled responses observed in the CYNO co-culture system and compared this to the HRDB. As in the HRDB, the expression of CYNO vascular genes (KLF-2, KLF-4, eNOS) that serve an antiangioprotective function was significantly increased by healthy regional hemodynamics compared to atheroprotective hemodynamics. The addition of advanced inflammatory conditions dramatically increased the expression of genes associated with oxidative damage (SOD2) and inflammation (MCP-1, IL-8) in both the CYNO system and the HRDB. Treatment with 3 out of 4 statins (AT, SS, CS) restored KLF-2 and KLF-4 under advanced inflammatory conditions; the exception was RS, which failed to restore these protective genes. A transcriptomic analysis will be presented comparing and contrasting the responses to statins of the human and CYNO systems. In summary, the CYNO vascular system described here mirrors many well-known responses of the human vascular system, and should provide a valuable preclinical tool for understanding mechanisms of action.

**1062 The Use of the SHE Cell Transformation assay for the Safety and Risk Assessment of Compounds under the EU REACH Regulation**


Chemically induced vascular toxicity during embryonic development can result in a wide range of adverse prenatal outcomes. We constructed an embryonic vascular disruption adverse outcome pathway (AOP) based on molecular initiating events corresponding to genetic models with phenotypic evidence of abnormal embryonic vascular development in the Mouse Genome Informatics Database. ToxCast high throughput screening data for 25 assays mapping to targets in the AOP were used to prioritize ~1000 chemicals for their potential to disrupt vascular development. A subset of 38 predicted vascular disruptive chemicals (pVDCs) or non-pVDCs were selected for targeted testing in zebrafish (D. rerio). To test computational predictions, TG(Ik(lGFP) zebrafish embryos were used to visualize and quantify blood vessel formation during development. Manual and automated methods of vessel quantification were developed, and the assay was evaluated with anti-angiogenic reference compounds PTK787 and AG1478, small molecule inhibitors of VEGFR2 and EGFR, respectively. The functional consequence of developmental vascular toxicity was assessed in larval and juvenile zebrafish. The assay was used to test the effects of 38 pVDCs and non-pVDCs including pesticides, flame retardants, and endocrine active compounds. The test chemicals were also evaluated in a functional angiogenesis assay comprised of a human endothelial cell and fibroblast co-culture system. Chemical rankings were well correlated among the predictive signature and zebrafish and in vitro tubulogenesis assays. Taken together, the zebrafish assay meets a critical need for an in vitro platform that can assess predictions generated by computational models of developmental vascular toxicity. This abstract does not necessarily reflect EPA policy.
Prolonged exposure to cadmium (Cd) in cigarette smoke has been associated with adverse health effects involving damage to pulmonary and cardiovascular tissue. Cd is known to induce oxidative stress, inhibit DNA repair, and interfere with essential metals. A recent study with Calu-3 cells also described its ability to disrupt tight junctions (TJs), possibly by a direct effect on adherens junction proteins. Herein, we report a novel mechanism of Cd-mediated TJ disruption in an in vitro human air-liquid interface (ALI) airway tissue model derived from normal primary human bronchial epithelial cells. Cultures were exposed basolaterally to 10-100 μM CdCl2 for 16 h, which, while considerably higher than the ~15 nM Cd found in smokers' blood, was non-cytotoxic to ALI cultures based on the MTS assay. Treatment with 100 μM CdCl2 resulted in the collapse of barrier function as demonstrated by Trans-Epithelial Electrical Resistance (TEER), immunofluorescence staining with TJ markers, ZO-1 and occludin, and histopathology staining. PCR array analysis of human TJ genes indicated that CdCl2 exposure altered the expression of several groups of genes encoding proteins associated with TJ assembly. Immunoblotting of select junction interacting proteins demonstrated down-regulation of their expression, suggesting that disruption of junctional complex functions is essential for maintaining TJ integrity is a possible mechanism for Cd toxicity. Furthermore, inhibition of kinase signaling using specific inhibitors prevented the down-regulation of junction interacting proteins, inhibited the up-regulation of tyrosine phosphorylated occludin, and preserved the integrity of TJs. Our findings indicate that acute doses of Cd likely disrupt TJ integrity through kinase activation and occludin hyperphosphorylation. Whether or not Cd causes similar toxicity with chronic, low level exposures experienced by smokers remains to be determined.

Recent changes in regulatory restrictions and social views against animal testing have accelerated development of reliable alternative tests for predicting skin sensitizing potential and potency of many chemicals. Lately, the approach of using a battery of in vitro tests instead of a single in vitro test has been suggested as a replacement for animal tests. In this study, we created a dataset of 66 test chemicals with human sensitizing potential data, human cell line activation test (h-CLAT), direct peptide reactivity assay (DPRA), and in the silico prediction system DEREK Nexus. DPRA, based on the covalent binding between proteins and haptenbs, and h-CLAT, emulating dendritic cell activation in the skin during sensitization, have been successfully pre-validated under ECVAM. For DEREK, 21 out of 66 chemicals have been used as the training dataset. The results of these tests were converted into a score from 0 to 2, taking into account the integrated testing strategy concept. For the 66 test chemicals, the total battery score between 0 and 5 was calculated by the sum of individual scores. When we set the positive criteria as more than a score of 2, the score-based battery system provided an accuracy of 94%, sensitivity of 96%, and specificity of 85% for the human sensitizing potential. We next developed a tiered approach, weighing the predictive performance of the h-CLAT and DPRA. The h-CLAT indicated a higher sensitivity than the DPRA. Thus, we determined the h-CLAT was a good first step for a tiered approach. The tiered system provided an accuracy of 89%, sensitivity of 98%, and specificity of 60%. Both battery systems showed a higher sensitivity compared with the h-CLAT or DPRA alone. In summary, our data not only demonstrates that the h-CLAT could be part of a non-animal test battery together with the DPRA and other methods for the assessment of skin sensitization but also supports the practical utility of a tiered system where h-CLAT and DPRA are the first screening methods for skin sensitization.

High-throughput screening assays are generating information regarding the toxicity of thousands of untested environmental chemicals. However, current methods are unable to test volatile organic compounds (VOCs). In addition, mechanistic information is needed to link effects at molecular targets to adverse outcomes in whole organisms. This study is designed to address these issues using the Drosophila Genetic Reference Panel (DGRP), a set of genetically well-characterized strains of fruit flies. The goals of the project are to determine whether fruit flies can be used to screen VOCs for toxicity, and to identify adverse outcome pathways associated with acute exposure. The first phase of this study was to determine dose-effect functions using behavior endpoints for 3 VOCs that differ in their mode of action. In a second phase, behavioral response profiles across strains of DGRP flies will be determined at the EC20 of each VOC and mapped to genetic markers of effect. We used a hybrid line of flies derived from ~40 DGRP lines to identify an EC20 for each chemical. Individual flies were placed in 5mm dia. glass tubes and exposed for 4 hours at various concentrations of toluene vapor (n= 16/sex/dose); controls received air. Real-time locomotor activity was observed at 10-minute intervals for both sexes. Males were overall more active than females. Toluene decreased activity in both

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Cerebral microvascular endothelial cells have been reported to serve as a vascular niche for the recruitment of neural stem cells, regulation of breast tumor metastasis to the brain, and promote the self-renewal of cancer stem like cells in glioblastoma multiforme (GBM). We previously reported the effect of the nonocplanar PCB153 on human brain endothelial cell line IC1M5/D3. PCB153 exposed IC1M5 showed a significant increase in stem cell markers CD34, CD133, and VEGFR-3. Exposure to PCBs may severely compromise normal function and lead to increased level of stem cell markers that participate in the development and promotion of brain tumors. We tested our hypothesis using in vitro 3D endothelial spheroid cell model that may serve as a rapid and cost-effective alternative to animal testing. The goal of the current study was to develop a 3D in vitro model to evaluate the effects of another PCB congener 126. PCB126 belongs to the coplanar dioxin-like group of PCBs that act through an aryl hydrocarbon receptor (AhR) mechanism. We show that PCB126 (100ng/ml) significantly increased the size of 3D endothelial spheroids over a 48h time period in the 3D endothelial cell clone. MTT assays confirmed sferoid cell viability and flow assisted cell sorting (FACS) showed significantly less apoptosis in spheroids exposed to PCB126. Both stem markers VEGFR3 and CD133 were significantly increased from PCB126 exposure as shown in FACS studies and Western blot. Our results indicate that PCB126 may contribute to brain tumor angiogenesis by enhancing VEGFR3 signaling. For instance, elevated tissue levels of the isomers VEGF3 and VEGFD have been shown in human brain and tumor derived endothelial cells receiving anti-VEGF therapy. Since these isomers are known to contribute their effects via VEGFR3 signaling, our findings support the hypothesis that environmental pollutants may angiogenically activate the brain microvasculature and exacerbate brain cancer or other brain disorders through their effects on the neurovascular niche.

Biphenol A (BPA) and its analogs (BPAF, BPS) are well-known endocrine disruptor compounds (EDCs). We have previously shown that BPA induces estrogen receptor (ERα) but not androgen receptor (AR) activation in luciferase reporter assays. BPA also represses androgen-induced reporter activity in transiently transfected CV-1 cells. Nuclear receptor (NR) target gene transcription levels are dictated by ligand-binding, altered receptor conformation and subsequently modulation of recruitment of coregulators proteins, which modify chromatin accessibility for RNA polymerase. The NR-interacting surfaces of coregulators contain highly conserved helical LxxLL (NR-box, coactivators) or LxxK (NR-box, corepressors) motifs. In order to study the con-regular expression of the BPA-bound ERxR or AR, we applied MARCoNi which mimics NR-coregulator interaction in vitro. Crude lysate of U2OS cells transfected with EGFP-tagged full-length NR were incubated with a peptide array of 154 CoR- and NR-boxes. The latter serves as a sensor for NR conformation, and hence activity status as a function of ligand. To investigate and compare the mechanisms of action by which compounds modulate ERxR activity we quantified the ERxR binding profile of BPA, BPA, BPF, 17β-estradiol (E2, the natural ligand) or solvent (2% DMSO) only. Compound response profiles, i.e. compound-induced log-fold change of ERxR binding, were subjected to hierarchical clustering and demonstrated clear differences between compounds and their ability to modify ERxR conformation. All four compounds induced repulsion of a similar set of coregulators. ERα interaction with a second set of coregulators is enhanced by E2 and BPS (with partial efficacy), largely unaffected by BPA, and moderately decreased by BPAF.

Biphenol A (BPA) is used to make resilient epoxy coatings for food cans. These coatings are required to protect food quality. Although science-based regulatory bodies, including the U.S. FDA and the European Food Safety Authority, conclude that BPA is safe for use in making food-contact coatings, the market has begun to de-select materials containing BPA because it is has very weak agonist activity in the ERα.
activity toward the estrogen receptor. After years of research, no polymers other than epoxies have been identified that are able to fulfill all integrity demands of light metal food packaging.

Alternate bisphenols that technically could be used to replace BPA in epoxy coatings include bisphenol F, S, M, B, AP and AF. Unfortunately, these and most other common bisphenols, like BPA, are weakly estrogenic. This fact has led some policy makers to believe that all potential BPA replacement bisphenols are estrogenic. Using drug-discovery techniques such as QSAR, followed by rigorous in vitro testing, it was possible to identify and verify that some bisphenols exhibit no estrogenicity whatever, and a subset of these non-estrogenic bisphenols can be used to make robust epoxy food contact coatings. Valspar identified 4,4’-methylenebis(2,6-di-tert-butylphenol), CASN 118-82-1, and 2,2’-methyleneendiphenol, CASN 2467-02-9, as likely to have no estrogenicity using a simple QSAR technique in ChemDraw® that measures the immutability of the methylene bridge angle at low temperature. Molecules selected in this way were negative in the yeast estrogen screen (YES) assay. Bisphenols that were negative in the YES assay, and from which epoxy coatings could be made, were further tested and found to be uniformly negative in the U2OS ER green fluorescent protein redistribution assay, the ER Calux® assay, and even in the low-specificity MCF-7 cell proliferation assay (EScreen).

It can be concluded that not all bisphenols are estrogenic. Furthermore, some of these non-estrogenic bisphenols can be used to make robust food-contact epoxy coatings with performance that is comparable to BPA-based polymers.

**1065 Interpreting Estrogen Screening Assays in the Context of Potency and Human Exposure Relative to Natural Exposures to Phytosterogens**

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Indication of exposure to potentially endocrine active substances can be detected in urinary biomonitoring studies. While EPA and OECD have validated in vitro & in vivo screening assays to measure interaction of substances with estrogen, androgen & thyroid pathway components, to date, methods to contextualize such results in terms of potencies & actual human exposures are lacking. Although urinary concentrations of substances can be compared on a mass per volume basis, such comparisons are not biologically meaningful because a receptor response is dependent upon potency and the concentration of the active moiety at the receptor. To understand endocrine screening results in terms of potency & human exposure, we propose a method that entails 1) calculating a benchmark dose (BMD) for a response measured in an endocrine screen; 2) estimating the human urinary concentration upon potency and the concentration of the active moiety at the receptor. Using this method, we can correlate estrogenic activity of chemicals in vitro with urinary biomonitoring studies.

**1066 Adaptation of T47D-KBLuc and MDA-kb2 to Screening and Comparison to Other Receptor Reporter Platforms**


Mineral oils (MO) used as ingredients for many printing inks are complex mixtures of hydrocarbons. Although migration of MO from paper into food has been shown, the toxicological relevance of these findings is elusive. Therefore we investigated the estrogenic potential of mineral oil using different in vitro assays. Proliferative effects of MO in the estrogen responsive human breast cancer cell line MCF7 were assessed in the E-screen, and endogenous estrogen responsive gene expression was quantified by real-time PCR. Transactivation assays were performed in HE-Lu9903 containing the human estrogen receptor (ER) and an estrogen responsive element (ERE)-driven luciferase as well as in yeast containing ERE and an ERE-dependent green fluorescent protein. MO primarily consists of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). These two fractions were separated by preparative HPLC and also assessed in the bioassays. MO in a thousand fold dilution stimulated MCF7 proliferation but did not induce reporter gene expression neither in yeast nor in HE-Lu9903 cells. Furthermore, expression of the estrogen responsive gene PGR and TFF1 in MCF7 cells was induced after 24 h exposure to total MO. The estrogenic effects were derived from the MOAH fraction, as exposure of MCF7 cells to this fraction induced gene expression and increased proliferation. In conclusion estrogenic action of aromatic substances derived from MO could be shown in vitro.

**1067 Estrogenic Activity of Aromatic Substances from Mineral Oil Used in Printing Inks**

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Lower chlorinated PCBs undergo oxidative metabolism to hydroxylated PCBs with a higher potential to cause xeno-estrogenic effects. While previous studies have demonstrated that PCBs are substrates of cytosolic phase II enzymes which produce PCB sulfates as a new class of metabolites. Because very little is known about the biological and metabolic disposition of this class of metabolites, 8 sulfat monoesters which are either derived from PCB3, a congener shown to be biotransformed to sulfates, or are derivatives of a paint-specific congener or from OH-congeners shown to interact with sulfotransferases (i.e. 2, 3, 2’, 3’ and 4’PCB3 sulfate, 4PCB11, 4PCB39, and 4PCB53 sulfate) and 4’OH-PCB3 as positive control. These compounds were tested for estrogenic and anti-estrogenic, androgenic and anti-androgenic potential at concentrations from 100 µM to 1 µM in the E-and A-screen as well as evaluated for cytotoxicity. These screens are quantitative assays which identifies potential xenogen-receptors. Only 4’OH-PCB3 is highly cytotoxic, while the 4PCB53 and 3PCB3 sulfates are weakly cytotoxic. Regarding endocrine disruption, our data suggest that there is a structure-activity relationship, with the 4’OH-PCB3 (1 uM) and its sulfate ester (100 µM) as most active estrogenic compounds followed by 4PCB3 sulfate, 4PCB39 and 4PCB53 sulfates (100 µM). The same compounds and 3PCB3 sulfate with lower concentrations also exhibited anti-estrogenic activity. 4’OH-PCB3 is the most potent androgenic compound, visible at the highest concentration tested, followed by 3’PCB3 sulfate and 4PCB53 sulfate. Also weakly androgenic are 4PCB11 sulfate, 4PCB39 sulfate and 4’OH-PCB3. Anti-androgenicity was observed with 2’PCB3 and 3’PCB3 sulfates at very low concentrations. Endocrine-disrupting chemicals often display U-shaped or inverted U-shaped nonmonotonic dose-response curves. Similarly no classical dose-response
curves were seen in our experiments. These preliminary data suggest that these PCB sulfate monoester have very low cytotoxic potential in human breast cancer cells as well as very low potential to act as endocrine disrupting agent.

**1069** Pharmacophore Model of Natural Phytoestrogens Which Induce Estrogen Receptor Alpha/Beta Heterodimerization

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Estrogen plays an important role in the normal development and growth of the mammary gland. The binding of 17β-estradiol (E2) and other estrogenic compounds to the estrogen receptor (ER) leads to its dimerization and consequent genomic transcription and cascade of biological activities. ERα and ERβ have been shown to exhibit a “Ying and Yang” relationship in regulating breast cancer cell growth: ERα promotes cell growth while ERβ inhibits it. At the receptor level, ERβ appears to negatively regulate the proliferative role of ERα through the formation of ERαβ heterodimers. The objective of this study is to identify natural estrogenic compounds which promote the formation of ERαβ heterodimers, thereby activating ERβ while dampening ERα activity, which might have a protective effect in breast cancer. We initially screened 37 phytoestrogenic compounds from four flavonoid subclasses in a two-step screening process that include activation of ER gene transcription using an ER-dependent reporter assay and dimerization using the Bioluminescent Resonance Energy Transfer (BRET) assay where 6 slightly selective ERαβ heterodimer inducing compounds were identified. In silico studies were then performed to identify the pharmacophore that confers ERαβ heterodimeric specificity in order to design high affinity ligands. We have proposed to test the cancer preventive effects of compounds that induce the ERαβ heterodimer in the E2-induced breast cancer model of AC1 rats.

**1070** Disruption in the Hypothalamus Neonatally Exposed to P-Tert-Octylphenol Is Essential for Induction of Early Occurrence of Persistent Estrus, a Feature of Delayed Effect in Rats


Neonatal exposure to estrogenic compounds induces delayed effect characterized as early occurrence of persistent estrus (Takahashi et al., 2012), but its mechanism has not been understood. The delayed effect is a big concern for offspring toxicity because reproductive toxicity studies couldn’t detect it. We investigated which organ, the hypothalamus or the ovary, is essential for the delayed effect of estrogenic compound. Female Donryu rats were treated with 100 mg/kg p-t-oc-tylphenol (OP) sc 3 times during 5 days after birth (PND5), which treatment is inducible delayed effect, and 8 times of the same manner as PND5 during 15 days (PND15) to induce typical androgenization. The bilateral ovaries in the PND5 or PND15 OP treated or the control groups were dissected at the 4-week of age, and transplanted into the subcaps of ovarioctomized rats at the same age in each group. Estrus cyclicly were checked up to 17 weeks of age and compared to rats with intact ovaries in the corresponding groups. The transplanted ovaries were morphologically examined. In the control group, most of rats received the donated ovaries from control and all treated groups showed normal cycle within 4 weeks after the transplantation, and corpora lutea (CL) were observed in all donors ovaries. In the PND5 group, normal cyclicly changed to persistent estrus 1.5 months after the transplantation in the rats donated the ovaries from control and PND5 groups, the status being similar to corresponding ovary-intact animals. In the PND15 group, all rats with and without the transplantation showed persistent estrus up to 8 weeks of age. Microscopically, few CL were observed. These results indicate that any disruption in the hypothalamus exposed to OP neonatally at delayed effect inducible dose plays an essential role for early occurrence of persistent estrus.

**1071** Pituitary Proliferation Is Affected by Bisphenol A Exposure, but Recovers upon Its Removal


Endocrine disrupting chemicals (EDC) including alkylphenols, bisphenols, diethylstilbestrol (DES) binds directly to ER LBD and enable ER dimerization and estrogen response element (ERE) mediated transcription of target genes. While these EDCs have the required pharmacophore to induce agonist conformation of ER, many EDCs that are currently classified as weak estrogens produce considerable endocrine damage even at low exposure. Plasticizers, alkylphenols and phthalate esters, do not share estrogen phamacophore but are misclassified as compounds binding to ER-LBD. Here we report our integrated in silico and in vitro structure-activity analyses of phalate esters and their ability to module ER-ERE based transactivation by acting on a non-ligand binding site. Compounds were assessed for their ability to compete with fluorescent-labeled estradiol and FITC-tagged peptides binding to non-ligand binding sites using a fluorescence polarization based high throughput screen. Computational screening of library of 87,000 EDCs against pseudoreceptor-based pharmacophores yielded a focused library of ER binding compounds. We identified 1870 chemicals bound to ER-LBD and 276 bound to a specific non-ligand binding site near the estrogen receptor. Bisphenol A (BPA) impacts the hypothalamic-pituitary-gonadal (HPG) axis, affecting neuroendocrine differentiation, reproductive capacity, and puberty. Although the pituitary gland is a central component, little is known about its response to BPA exposure. We previously demonstrated that environmentally relevant BPA treatment during the embryonic period leads to increased pituitary proliferation and gonadotropin cell numbers in female mice. It is unknown whether the effects of prenatal exposure to BPA would cause persistent increases in progenitor proliferation or if the critical window of postnatal pituitary expansion is directly impacted by BPA treatment. Therefore, we used two hypotheses. First, that embryonic treatment of BPA would have lasting effects into the early postnatal period. To test this, mice were dosed with 0.5 or 50 mg/kg/day BPA from embryonic day 10.5 to postnatal day (PND)0 and collected at PND4. No differences were seen in proliferation or gonadotropin number between groups, indicating removal of BPA restored the pituitary to normal conditions. Second, we hypothesized that BPA would have a direct effect on the postnatal pituitary. To test this, we cultured intact PND0 pituitaries with 0.05 or 50 μg/mL of BPA, 10 nM 17β estradiol, or ethanol for 48 hours. QRT-PCR revealed that mki67 (a proliferation marker) decreased with both doses of BPA, however estradiol raised mki67 levels. Progesterone receptor (Pgr) mRNA, a known target of estrogen signaling, was raised in the presence of estradiol, but unchanged by BPA. Interestingly, levels of Esr1 were decreased with BPA treatment, but unaltered by estradiol. From these data, we conclude that BPA affects proliferation and gene expression in the postnatal pituitary in a manner dissimilar to estradiol. These studies also highlight that Influence of the HPG axis or time of dosing may be contributing to pituitary effects of BPA. Supported by P01 ES022848, R01 DK076647, T32 ES007326.
There is growing concern worldwide on the effect of endocrine disrupting chemicals (EDCs) on aquatic organisms and wildlife. Aquatic organisms are particularly vulnerable to exposure because their ecosystems act as sinks for a wide variety of pollutants. Vitellogenin expression, gonad histological alterations are amongst specific biomarkers for monitoring the effects of EDC’s in fish. Vitellogenin expression in male, female and juvenile fish and gonad histopathological alterations were evaluated in Cichlids: Tilapia zillii, T. guineensis, Sarotherodon melanorham, S. galileaus, Oreochromis niloticus, Hemichromis fasciatus and Chromidotilapia gutentheri from Eleiyele reservoir to assess endocrine disruption in fish. Vitellogenin-B2a (VTG-B2a) gene expression in the liver of fish was detected using RT-PCR. Interssex was observed in 53.3% of sample of fish which of 29.0% were adult and 71.0% juvenile male fish. The highest occurrence of interssex was in T. zillii and histological appearance of interssex included the development of primary oocytes along with mature spermatocytes (juveniles/matured spermatocytes within testicular tissue. A significant expression of Vtg-B2a gene was observed in all adult male and some juvenile male fish suggesting exposure to EDCs. VTG gene expression correlated with pathological presentation of intersex observed in adult/juvenile male fish. Further studies to quantify the level of VTG expression by RT-qPCR is on-going but available data indicate that Cichlids could be sentinel species for evaluating the presence of EDCs in lotic waters because of their commercial importance to the population structure of lakes. This is a first report of responsiveness to EDCs in a man-made lake in Nigeria.
Orally Subchronic Exposure to Benzo[a]pyrene Alters Reproductive Hormone Profile

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The objective of this study was to assess whether benzo[a]pyrene (B[a]P) alters reproductive hormones, which in turn leads to decreased spermatozoa quality and spermatogenic cell levels. Hsd: ICR (CD1) 10-week old males were orally exposed to B[a]P at 1, 10, 50, and 100 mg/kg/day of body weight for 30 and 60 days. At the end of the experiment, mice were anesthetized and reproductive tissues, i.e. testes, seminal vesicles, and epididymis, were collected. Spermatogenic cells and mature spermatozoa were recovered from the testes and cauda epididymis, respectively. Spermatozoa quality, including concentration, morphology, motility and viability, was examined. Testosterone, estradiol, follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels in serum were determined using the Enzyme Immunoassay/Enzyme Linked Immunosorbent Assay (ELISA). Mice exposed to B[a]P at 50 mg/kg/day and 100 mg/kg/day for 30 days and 60 days exhibited a significantly decrease in serum testosterone and estradiol levels as compared with the control, while FSH and LH remained stable. The levels of testosterone and estradiol were positively correlated with decreased spermatozoa motility (p<0.025 and 0.014, respectively), viability (p<0.002 and 0.018, respectively), and morphology (p<0.007 and 0.018, respectively), but the hormone levels did not correlate with spermatozoa levels. The testosterone and estradiol levels did not correlate with the levels of spermatogonia, pachytene spermatocytes, round spermatids, and elongated spermatids. Subchronic exposure to B[a]P could modulate the production of testosterone and estradiol, which subsequently influence spermatozoa quality.

Testing Mixtures of Antiandrogens In Vivo at Human-Relevant Exposure Levels

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Endocrine disruption has become an important topic of public concern. Despite an increasing amount of public attention, little is understood about whether human-relevant doses of endocrine disrupting chemicals (EDCs) affect homeostasis. To address these concerns we performed pre- and post-natal reproductive toxicity studies to measure the developmental toxicity of low single- and mixture-doses of three substances with an anti-androgenic mode of action: vinclozolin, flutamide and prochloraz. Doses were selected to mimic a LOAEL, a NOAEL for endocrine effects, and the acceptable daily intake (ADI) for each compound, which were then combined together into three mixtures of the LOAELs, NOAELs, and ADIs. In addition to standard regulatory parameters, a number of molecular endpoints were also incorporated within the same study design. Thus, single- and mixed-exposures can be compared from molecular to pathological levels in one and the same animals.

1078  Testing Mixtures of Antiandrogens In Vivo at Human-Relevant Exposure Levels

Calçatina and Estrogen Replacement Therapy in Menopausal Women

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Aims: To determine the effects of estrogen replacement therapy (ERT) on the concentration of sex hormone-binding globulin (SHBG) in menopausal women. Methods: We studied 16 postmenopausal women (ages 51 to 71 years) who were randomly assigned to either ERT (Estraderm TTS, Ciba-Geigy, Basle, Switzerland) or placebo. Blood samples were obtained at baseline and after 3, 6, 9, and 12 months of treatment. SHBG levels were measured by a competitive protein-binding assay. Results: SHBG levels increased significantly from baseline to month 3 (p<0.01). There was no significant difference in SHBG levels between the ERT and placebo groups. Conclusions: ERT increases SHBG levels in postmenopausal women. However, the clinical significance of this finding is unknown.

Mechanistic Investigation of Testicular Tumor Formation in Rats Treated with the SGLT2 Inhibitor Canagliflozin

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Canagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor induced Leydig cell tumors (LCTs) in a 24-month rat carcinogenicity study in male rats at dose levels ranging from 10 to 100 mg/kg/day. There were no corresponding preneoplastic lesions in the 3-month or 6-month repeated dose toxicity studies. No tumor development was present in the mouse carcinogenicity study with this compound. A 7-month repeated dose toxicity study was conducted in two groups of 30 male Sprague-Dawley rats receiving vehicle or 100 mg/kg/day canagliflozin by oral gavage to investigate the potential mechanism of LCT formation. Blood samples were collected monthly for measurement of luteinizing hormone (LH) and testosterone (T) and pathology of testis and secondary sex organs was assessed at the end of the study.

Compared to vehicle, serum LH concentrations were increased after 1 month of dosing and generally remained elevated throughout the dosing period. Circulating testosterone levels were unchanged at all time points in comparison to the vehicle. There was a decrease in the weight of the secondary sex organs in the canagliflozin treated group with no change in testis weights. Histopathological examination showed a slight increase in interstitial cell hyperplasia in the canagliflozin treated rats and this was correlated with the increased LH levels. Disruption of the hypothalamic-pituitary-gonadal axis, resulting in sustained increases in LH levels is an established mechanism of toxicity described for a number of non-genotoxic compounds causing LCT’s in male rats. Clinically, no increases in LH were seen in male subjects treated with canagliflozin for 12 weeks. Therefore the formation of LCT’s in male rats treated with canagliflozin is not relevant to humans.

The Fungicide Prochloraz Induces Transporter Breast Cancer Resistance Protein BCRP (ABCG2) in Human Mammary and Adrenocortical Cells

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The imidazole fungicide prochloraz is widely used in horticulture and agriculture, e.g. as a post-harvest anti-mold treatment of fruits and vegetables. Prochloraz is a known endocrine disruptor causing developmental toxicity with multiple mechanisms of action. In this abstract, we report a new mechanism by which prochloraz may exert toxicity on a wide range of cells. BCRP is an efflux transporter, with a broad substrate specificity ranging from xenobiotics such as chemotherapeutics, antibiotics, and the dietary carcinogen PhIP (2-amino-1-methyl-6-phenylimidazo-pyridine) to endogenous substances such as estrone-3-sulfate and DHEA-S. BCRP is widely expressed, e.g. in the intestine, liver, kidney, testis and brain, where it can restrict the cellular uptake of xenobiotics and facilitate the excretion of endogenous substances. BCRP is also present in the mammary gland, where it may cause secretion of xenobiotics into breast milk and dairy. The aim of the present study was to investigate the effects of prochloraz on BCRP gene expression in two human cell models, representing mammary tissue (MCF-7) and adrenocortical tissue (H295R). Cells were treated with 0.1-10 μM prochloraz for 24 h. RNA was isolated and relative BCRP gene expression measured by real-time RT-PCR. All experiments were conducted using non-toxic levels of prochloraz as determined by MTS assay. We found that prochloraz is able to induce BCRP in both cell lines in a dose dependent manner. The gene expression of BCRP was increased 1.5- to 3-fold. In addition to the previously reported mechanisms for prochloraz-induced toxicity, our results show a novel mode of action for prochloraz, which might be
important in a wide range of tissues. A prochloraz-induced stimulation of BCRP could alter both the milk excretion of BCRP substrates, and the steroid availability via increased efflux of estrone–3-sulfate and DHEA-sulfate from endocrine tissues.

1082 Polybrominated Diphenyl Ether Congener 47 Increases Aldosterone Secretion in a Human Adrenocortical Cell Line

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Elevated circulating aldosterone levels are associated with hypertension, thrombosis formation, cardiac hypertrophy, and congestive heart failure. Polybrominated diphenyl ethers (PBDEs) and their metabolites have been previously shown to alter various endocrine biosynthetic pathways including thyroid hormone, estrogens, and androgens. Additionally, PBDEs have been shown to accumulate in the adrenal gland. Therefore, we characterized the effect of PBDE-47 on aldosterone secretion in a human adrenocortical cell line. HAC15 cells were exposed to vehicle or various concentrations of PBDE-47 (10 nM-100 μM). After 72 h, cell viability, aldosterone secretion, and gene expression of enzymes and cofactors involved in aldosterone synthesis was examined. The only concentration of PBDE-47 that affected cell viability was 100 μM. PBDE-47 induced basal aldosterone secretion at 10 and 100 μM (in ng/mL: vehicle, 9.0±1.7; 10 μM PBDE-47, 70.2±9.8; 100 μM PBDE-47, 78.5±19.8; n=4, p<0.05). PBDE-47 also increased Ang II-stimulated aldosterone secretion at 10 μM, but decreased Ang II-stimulated aldosterone secretion at 100 μM (in ng/mL: 137.9±6.7; 10 μM PBDE-47, 200.4±9.3; 100 μM PBDE-47, 80.1±20.6; n=4, p<0.05). Gene expression of several enzymes and cofactors involved in aldosterone synthesis were increased by 10 μM PBDE-47. These data indicate that PBDE-47 disrupts the regulation of aldosterone secretion and provides further evidence that PBDEs are potential endocrine disruptors.

1083 NQO1, a Component of the Plasma Membrane Electron Transport System, Regulates Redox Status in Clonal Pancreatic Beta Cells and Primary Islets


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Plasma membrane electron transport (PMET) is a ubiquitous system which transports electrons out of cells while re-oxidizing intracellular reduced equivalents NADH and NADPH. Recently, NQO1, NAD(P)H-dependent quinone reductase 1, well known for its role in 2-electron reduction of quinones, was shown to be a mediator of PMET in pancreatic beta cells, where it helps to support glucose metabolism and subsequent insulin secretion following exposure of these cells to elevated glucose levels. Our previous work showed that NQO1 positively regulates glucose-stimulated insulin secretion (GSIS), suggesting a role for NQO1 in glucose metabolism and homeostasis. In the current work, we characterized the role of NQO1 in the PMET pathway of pancreatic beta cells and its role in the alteration of intracellular oxidative state in the presence of the redox cycling pro-oxidant menadione. Adenoviral-mediated over-expression of NQO1 resulted in the increase of the NQO1 protein and NQO1 enzymatic activity, and reduced the level of menadione-dependent ROI production in both clonal INS-1 832/13 cells and isolated islets. In islets isolated from global NQO1 knockout mice, menadione-dependent ROI production was elevated. This is consistent with NQO1’s role in detoxification of menadione in other tissues via two-electron reduction.

According to the environmental obesogen hypothesis, exposure to some environmental contaminants may initiate or exacerbate the development of obesity and its associated health consequences. Obesity is now regarded as a risk factor for colon cancer. Perfluorinated compounds (PFCs) are used in applications such as clothing, carpets, food packaging, fire extinguishers etc. We examined effects of exposure to the PFC chemicals PFOA and PFOS during critical periods of development (in utero) in the intestinal cancer mouse model C57BL/6-Min/+ (multiple intestinal neoplasia). The dams were exposed to PFOA or PFOS (0.01, 0.1 and 3.0 mg/kg bw/day) by oral gavage on gestational days 1-17. Body weight of the pups was recorded from birth until termination at 11 weeks and presented as area under the curve (AUC). The small intestine was fixed, stained with 0.2% methylene blue, and tumors scored by an inverse light microscope. Plasma glucose levels (non-fasted) were measured by glucometer at age 6 and 11 weeks, to study the hypothesis of disrupted glucose regulation as a link between obesity and intestinal tumor development. After 3.0 mg/kg bw/day PFOA very few live pups were obtained, whereas this dose of PFOS and the other two doses of both chemicals gave enough mice for statistical analysis. No obesogenic effect of PFOA or PFOS was observed up to 11 weeks of age. Compared with mice exposed to water, mice treated with 0.1 mg/kg bw PFPOA had significantly lower AUC on days 3-18 and weeks 3-11, and also compared with mice treated with 0.01 mg/kg bw PFPOA on weeks 3-11, indicating a toxic effect of PFOA. None of the doses of PFOA or PFOS increased the plasma glucose levels at either time point or increased the number of small intestinal tumors compared with mice given the vehicle distilled water. Conclusion: Exposure to PFOA or PFOS in utero did not have obesogenic effect, at least not up to 11 weeks of age, and did not increase blood glucose levels or intestinal tumorigenesis in Min mice.
Hydroxylated polychlorinated biphenyls (PCBs) and polychlorinated dibenzylo ether (PBDEs) are detected in human plasma as the metabolites of PCBs and PBDEs, their configuration are similar to that of thyroid hormone, and their effect on thyroid hormone system are concerned. Iodotyrosine deiodinase (IYD) has an ability to salvage iodide from iodotyrosine that is generated as byproducts of thyroid hormone biosynthesis. This enzyme has also been discovered to act as a dehalogenase of bromo- and chlorotyrosine. The aim of this study was to investigate the possible influence of halogenated compounds on thyroid hormone metabolism, IYD-inhibitory activity by 44 halogenated compounds, PCBs, PBDEs, agrochemicals, antiparasitics, pharmaceuticals and food colorants, were examined in vitro using microsomes of HEK-293T cells expressing recombinant human IYD. Among them, 22 halogenated phenolic compounds inhibited IYD activity. Rose bengal was the most potent inhibitor, followed by erythrosine B, phloxine B, benzamorone, 4'-hydroxy-2,2',4,5'-tetraiodobiphenyl ether, 4-hydroxy-2,3',4',5'-tetraiodobiphenyl ether, 4-hydroxy-2,3,4,5'-pentachlorobiphenyl, 4'-hydroxy-2,2',4,5'-tetraiodobiphenyl ether, triclosan, and 4'-hydroxy-2,2',3,4',5'-pentabromobiphenyl ether. However, among PCBs and PBDEs without a hydroxyl group, including their methoxylated metabolites, none inhibited IYD activity. These results suggest that halogenated compounds may disturb thyroid hormone homeostasis via inhibition of IYD, and that the structural requirements for IYD-inhibitory activity include halogen atom and hydroxyl group substitution on a phenyl ring.

Purpose: A previous epidemiological study showed dose-dependent increase in TSH level in the serum of the workers exposed to 1-bromopropane (1-BP). Hyperthyroidism-like signs were observed in rats exposed to 1-BP, such as irritant behavior as well as decreased cholesterol and increased protein levels in plasma. The present study investigated effects of exposure to 1-BP on transthyretin (TTR), which is a carrier of thyroid hormone from blood to cerebrospinal fluid (CSF), as well as the level of thyroid hormone in the CSF and blood to understand the possible involvement of thyroid hormone in the toxicity of 1-BP to the central nervous system. Methods: Forty-eight male F344 rats were divided into four equal groups and exposed to 1-BP at 0, 200, 400 and 800 ppm 8 h/d for four weeks. Distribution of TTR protein in the brain was examined by immunohistochemistry. The levels of TTR mRNA in the choroid plexus and liver were measured by quantitative real time PCR. Free triiodothyronine (Free-T3) in the CSF and plasma was measured using ELISA kit. Total triiodothyronine was also measured in the plasma. Results: Four-week exposure to 1-BP at 200 and 400 ppm significantly increased mRNA of TTR in the choroid plexus of the lateral ventricle and fourth ventricle, but reduced that at 800ppm. The level of TTR mRNA didn’t show any change in hippocampus. Free-T3 increased significantly at 400 and 800 ppm in the CSF after four-week exposure to 1-BP but didn’t change in the plasma. Free thyroxine (T4) in the plasma significantly decreased in all the 1-BP exposure groups. Conclusion: The study showed increase in free T3 in the CSF of the rats exposed to 1-BP, which may explain the irritating behavior as well as low cholesterol and high protein in serum of rat exposed to 1-BP.

**1087a** Enhancement of the Endocrine Disruptor Knowledge Base for Assessing Endocrine Activity of Untested Chemicals

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Endocrine disruptors (EDs) are exogenous chemicals with potential to disrupt the endocrine system. EDs came under international scientific discussion and debate some two decades ago, sparking regulatory attention. Accordingly, FDA developed the Endocrine Disruptor Knowledge Base (EDKB) and made it publically available. Since then, the EDKB has been widely used by the scientific community. It was recently updated with more comprehensive data and corresponding predictive models. Effects mediated by the estrogen receptor homodimer complex are a primary ED concern. Since many EDs exhibit estrogenic activities, affecting normal estrogen signaling pathways, we first updated the estrogenic data and augmented the EDKB with a comprehensive database of chemicals assayed for estrogenic activity. The database contains over 21,000 estrogenic activity data points across more than 8,000 compounds assayed among 11 species. It also contains protein subtypes and domains used in the assays. Original data were converted into the same units, such as logRBA for competitive receptor binding. Quality control was performed to ensure data quality. It was built using Instant JChem and subsequently instantiated in a web-based software system for users to browse, search and export data. It enables search across multiple fields such as chemical structure, substructure, chemical name, assay type, species, activity range, and references using logical operators. Additionally, data for binding affinity to α-Fetoprotein (AFP) for 125 chemicals are included. The AFP 3D structure was modeled using homology and molecular dynamics (MD) simulations, and AFP-ligand binding modes elucidated for 13 binders. With the recent enhancements, the EDKB provides the scientific and regulatory communities a free source to search endocrine activity data and to develop predictive models for predicting endocrine activity of chemicals for which no data are available.
1087c International Validation of Two Human Recombinant Estrogen Receptor (ER alpha) Binding Assays


An international validation study has been successfully completed for 2 competitive binding assays using human recombinant ERs. Assays evaluated included the Freyberger-Wilson (FW) assay using a full length human ER, and the Chemical Evaluation and Research Institute (CERI) assay using a ligand-binding domain of the human ER. Twenty three compounds were tested in 6 laboratories for the FW assay and 5 for the CERI assay, which included three controls (used with every run), 9 uncoded, and 14 coded chemicals across 3 subtasks. The overall goal of this validation study was to demonstrate the ability of each of the two assays to reliably classify the test chemicals as binders or non-binders. Laboratories had little trouble with the ER binders that produced a full binding curve when using either the CERI or FW assays. As is typical with all ER competitive binding assays, the weak binders proved to be more challenging. However, overall results from both the FW and CERI assays were consistent and in agreement with expected classifications regardless of the form of the hER (i.e., full length ER versus an ER ligand binding domain) or the subtle differences in the protocols for conducting each assay. The reproducibility and accuracy for classification of chemicals as potential ER binders and non-binders using the FW and CERI hER binding assays were comparable to that of the U.S.EPA’s existing ER binding test guideline OPPTS 890.1250, while providing an improved, higher throughput method that does not require animal tissue as the source of receptor. An OECD test guideline is currently being drafted. This abstract does not reflect US EPA policy. Parts of this work were supported by the German Bundesministerium für Bildung und Forschung (BMBF) under Grant 0311154N.

1087e Triclosan-Induced Cell Growth Was Reversed by a Phytoestrogen, Kaempferol, via Regulating Cell-Cycle Related and Apoptosis-Related Genes in MCF-7 Breast Cancer Cells

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Triclosan is one of endocrine disrupting chemicals (EDCs) which are scattered with environment agents, such as toothpastes, deodorant and cleaning supplies. As a phytoestrogen, kaempferol is one of bioflavonoids, which has been found at various of vegetables including broccoli, tea and tomato. Although kaempferol may have anti-cancer activity, its exact mechanism is under investigation in the induction of apoptosis and inhibition of cell proliferation or angiogenesis. In this study, we examined the anti-proliferative effects of kaempferol in triclosan-induced cell growth in MCF-7 breast cancer cells. A proper concentration of kaempferol or triclosan was determined in MCF-7 cells measured by MTT assay. In this study, kaempferol significantly reduced the viability of MCF-7 cells compared to a negative control treated with DMSO, and that kaempferol reversed triclosan-induced MCF-7 cell growth at 50 μM. To confirm that kaempferol inhibited triclosan-induced cell growth, we examined the transactional levels of cell growth and apoptosis-related markers, i.e., cyclin D, p21, cyclin E, p27 and bcl-2, and bax genes, using reverse transcription (RT)-PCR. The expression levels of cyclin D, cyclin E and bax/bcl-2 ratio were increased, while that of p21 and p27 mRNA was decreased by triclosan in MCF-7 cells. In addition, kaempferol reversed triclosan-induced gene expressions in an opposite manner. Taken together, these results indicated that kaempferol may inhibit the growth of MCF-7 cells via regulating of cell cycle and apoptosis-related genes. In addition, EDCs-induced progression of breast cancer may be suppressed by a phytoestrogen, i.e., kaempferol, in a specific manner. [This work was supported by National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of the government of the Republic of Korea (2013R1A1A2059092)].

1087d Comparison of In Vitro and Ex Vivo Thyroid Hormone Synthesis Inhibition Results and In Vitro Outcomes for a Series of Benzothiazoles


Assessing how in vitro data may be used to predict adverse effects in vivo is critical as efforts are advanced to incorporate in vitro assays into a risk assessment framework. Within the context of a thyroid hormone (TH) synthesis inhibition adverse outcome pathway (AOP), in vitro, ex vivo and in vivo assays were used to study the TH disrupting potential for a series of benzothiazoles: benzothiazole (BTZ), 2-mercaptobenzothiazole (MBT), 5-chloro-2-mercaptobenzothiazole (CMBT), 2-aminothiazole (ABT), 2-hydroxybenzothiazole (HBT), and 2-(methylthio) benzothiazole (MTBT). A thyroid peroxidase (TPO) inhibition assay was used to determine the activity of these chemicals in vitro. The rank order potency for TPO inhibition was MTBT>CMBT>ABT>BTZ>HBT. MTBT did not inhibit TPO activity. The benzothiazoles were tested further in Xenopus laevis thyroid gland explant culture with inhibition of TH release as the endpoint. Toxicity was assessed as decreased glandular ATP, MBT inhibited TH release at non-cytotoxic concentrations and with similar potency to methimazole. The benzothiazoles with greatest potency for T4 release inhibition were MBT, CMBT, and HBT, with IC50s of 3, 30, and 133 μM, respectively, but all benzothiazoles showed some inhibitory activity. Benzothiazoles were further assessed in vivo in a 7-d X. laevis tadpole assay. MBT and CMBT were the most potent for affecting endpoints of thyroid hormone synthesis inhibition, whereas others showed little or no effect. Both MBT and CMBT significantly increased sodium iodide symporter (NIS) mRNA indicative of compensatory TSH stimulation in response to decreased circulating TH. Taken as a whole, these results indicate the utility of in vitro assays for queuing chemicals for further testing, but illustrate the need for caution in interpreting results of in vitro or ex vivo inhibition assays especially where toxicity may be a confounding factor. This abstract does not necessarily reflect U.S. EPA policy.

1087f In Vitro Metabolism of Tamoxifen in Human, Rat, and Fish Microsomes


Results from an in vitro study comparing biologically-active metabolites in the plasma of Wistar rats and cunner fish (Tautogolabrus adspersus) treated with tamoxifen indicate notable differences in circulating metabolite concentrations between these two species. After a single oral dose of tamoxifen (25 mg/kg), the predominant metabolite observed from 1 to 72 hrs in rats was N-desmethyltamoxifen while in cunner it was 4-hydroxytamoxifen. The predominant metabolite in humans has been reported to be N-desmethyltamoxifen. To investigate if these species differences could be predicted using an in vivo assay, hepatic microsomes from cunner fish, Wistar rats, and humans were used. The experiment was designed to measure metabolic activity in all 3 species at 37°C. Toxicity was assessed on Wistar rats and cunner fish using LC-MS/MS. The highest concentrations of N-desmethyltamoxifen and 4-hydroxytamoxifen at all time points were observed in rat microsomes. In rat and human microsomes, N-desmethyltamoxifen was the predominant metabolite at both 18°C and 37°C. While formation of 4-hydroxytamoxifen was minimal with human microsomes, this metabolite doubled from 1 to 2 hrs and in fish microsomes (18°C). Unlike in vivo results, fish microsomes produced about twice as much N-desmethyltamoxifen as 4-hydroxytamoxifen during 4 hrs in the in vitro assays. Thus, though these in vitro results are similar to in vivo studies reported for human and rats, they do not reflect in vivo results observed for cunner fish. This abstract does not necessarily reflect U.S. EPA policy.
1087g  Fenhexamid Regulated the Transcripts of Aryl Hydrocarbon Receptor, Aryl Hydrocarbon Receptor Nuclear Translocator and Cell Cycle Related Genes in Human BG-1 Ovarian Cancer Cells Expressing Estrogen Receptors

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Fenhexamid is antifungal agents used in agricultural applications, which are present at measurable amounts in fruits and vegetables. Fenhexamid has been reported to act as an anti-androgen in an androgen receptor reporter assay in engineered human breast cancer cells. Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor, which translocates into nucleus and dimerizes with aryl hydrocarbon receptor nuclear translocator (ARNT). In this study, we examined the effects of fenhexamid, a pesticide, on the expression of AhR, CYP1A1, CYP1B1, ARNT, and p21 by RT-PCR analysis. To evaluate the ability of cell viability, BG-1 cells were cultured with a negative control (0.1% DMSO), 17b-estradiol (E2; 1x10^-9 M), 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD; 1x10^-8 M), or fenhexamid (1x10^-5 - 1x10^-8 M). E2 as a positive control markedly increased BG-1 cell proliferation compared to DMSO. In addition, TCDD and fenhexamid increased BG-1 cell proliferation at the concentration of 1x10^-8 M and 1x10^-5 M, respectively. The transcriptional level of p21 was reduced at 6 h by E2, TCDD, or fenhexamid, while its level was reversed at 24 h following their treatments. The mRNA expression of AhR was reduced by E2, TCDD, or fenhexamid in a time-dependent manner, while its level was reversed in the presence of alpha-naphthoflavone, an AhR inhibitor. In contrast, the transcriptional level of ARNT appeared to be increased by E2, TCDD, or fenhexamid. Taken together, these results indicate that fenhexamid may regulate AhR, ARNT, and p21 to induce cell growth in BG-1 ovarian cancer cells expressing estrogen receptors. A further study will continue to examine disruptive effects of pesticides in estrogen receptor expressing cells or tissues. [This work was supported by National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of the government of Republic of Korea (2013R1A1A2059092).]

1087f  Effects of In Vivo Exposure to Tamoxifen on a Nontarget Species, the Marine Fish Cunner (Tautogolabrus adspersus)


Tamoxifen is an endocrine-active pharmaceutical that is used worldwide to treat certain breast cancers. Because tamoxifen has been detected in aquatic environments, a study was undertaken to investigate its biological effects in a non-target species, the marine fish cunner (Tautogolabrus adspersus). Effects of a 17-day exposure to two different oral doses of tamoxifen (0.5 and 5 mg/kg fish weight) on reproduction in spawning cunner were examined relative to controls. Results show mean egg production in tamoxifen-treated cunner was significantly less than controls. No significant effects were observed in female or viable eggs relative to controls, although a downward trend for both endpoints was noted. After the 17-day exposure, activity of the steroidogenic enzyme aromatase in brains and ovaries from these fish was also evaluated. Ovarian aromatase activity was significantly increased in cunner treated with 5 mg/kg of tamoxifen. There were no significant differences in brain aromatase activity or gonadosomatic index in either sex compared to controls. In a separate experiment, formation of biologically active metabolites over time (from 1 to 72 hours) after a single oral dose of tamoxifen (25 mg/mg) was compared in Wistar rats versus cunner. The predominant metabolite in plasma from rats was N-desmethyltamoxifen, while in plasma from cunner it was 4-hydroxytamoxifen. This difference suggests that the effects of exposure to tamoxifen could be magnified in cunner, since 4-hydroxytamoxifen is considered to be about 100 times more efficacious than tamoxifen or N-desmethyltamoxifen. Overall, differences in circulating metabolites between species and a significant reduction in egg production in spawning cunner treated with tamoxifen both suggest that exposure to tamoxifen could present a tangible ecotoxological risk to fish such as cunner. This abstract does not necessarily reflect USEPA policy.

1087i  Subchronic Toxicology of Tetrabromobisphenol A (TBBPA) in CD® Rats


This study was conducted to evaluate the subchronic toxicity of the flame retardant tetrabromobisphenol A (TBBPA) in CD® rats. Three treatment groups, ten rats/group, were gavaged daily at 100, 300, or 1000 mg/kg/day for 90 days. Recovery animals (five rats/sex) were included in the control and high-dose groups and evaluated 6-weeks post-treatment. In addition to routine observations, organ weights, hematology, and clinical chemistry, a Functional Observational Battery (FOB) was conducted pretest and at Week 12. Motor activity was also evaluated during Week 12. Moreover, thyroid hormone levels (TSH), T3, and T4, were evaluated at 33 days, termination, and recovery. T4 levels were statistically significantly lower in males in all dose groups relative to controls at Day 33 (-26.2%, -31.0%, -31.7% at 100, 300, 1000 mg/kg, respectively) and at Day 90 (-35.8%, -33.4%, -39.3% at 100, 300, 1000 mg/kg, respectively). T4 levels were comparable to controls in the 1000 mg/kg recovery animals. T4 levels were statistically significantly lower in females in all dose groups relative to controls only at Day 33 (-22.5%, -24.1%, -22.0% at 100, 300, 1000 mg/kg, respectively). No changes were noted at Day 90. No changes in TSH or T3 levels relative to controls were noted in either sex at any time point. No toxicologically significant changes were observed in any of the other parameters. The reduction in serum T4 levels was not accompanied by evidence of toxicity or adverse effects, and the animals were clinically normal. Moreover, because the decrease in T4 levels was not of sufficient magnitude to alter mean serum TSH or T3 levels, thyroid histopathology, thyroid weight, or other parameters indicative of thyroid pathology (e.g., body weight), the decrease in serum T4 levels was not considered adverse. Thus, in this rat 90-day oral toxicity study with TBBPA, the No Observed Adverse Effect Level (NOAEL) was at least 1000 mg/kg/day, the highest dose tested. Supported by the NAIFRA Panel of the American Chemistry Council.

1087j  Adverse Human Reproductive Outcomes and Body Burdens of Endocrine Disrupting Compound—Polychlorinated Biphenyls

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Inconsistent evidence exists for an association between polychlorinated biphenyls and adverse human reproductive outcomes. Therefore, the goal of our study was to determine if risk of reproductive and endocrine dysfunction in humans is increased due to exposure to endocrine disrupting compound - polychlorinated biphenyls. The CDC’s NHANES data was used to examine exposure levels of PCBs and the association between blood levels of PCBs and self-reported reproductive health outcomes among U.S. women. The study population ranged from 3109 to 3192 female participants (12 years of age and older) of the National Health and Nutrition Examination Survey who provided blood samples between 1999 and 2004. Individual levels of 7 PCB congers (PCB 074, 099, 118, 138, 153, 170, and 180), the sum of dioxin-like PCB congers (PCB 074, 118), and the sum of non-dioxin-like PCB congers (PCB 099, 138, 153, 170, and 180) were used in conjunction with data obtained from the medical and reproductive health questionnaires. Self-reported reproductive health outcomes were obtained from the interviews of female participants 12 years of age and older. The following outcomes were evaluated using categorical data: age at first live birth, age at menarche, age at menopause, having undergone a medical procedure, uterine fibroids, endometriosis, parity, having a baby with low birth weight, and menstrual cycle regularity for the past 12 months. Separate analyses showed that body burdens of PCBs in blood were significantly higher among women with breast cancer, women with a history of uterine fibroids, and women who had undergone surgical removal of their uterus and/or ovaries when compared to the rest of the study population. While these results do not provide any evidence of causal associations, it is noteworthy that women with these reproductive outcomes have significantly higher body burdens of PCBs than women without them.

1087k  Developmental Exposure of Zebrafish to Dieldrin Alters Gene Expression Associated with Energy Homeostasis

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Environmental exposure to xenobiotics has been suggested to modify energy balance and increase the risk for obesity, metabolic syndromes, and diabetes incidence. The importance of developmental exposure to pollutants and the associated risk for disease later in life is not clear. Reproductive development and energy homeostasis are tightly linked. Our previous studies have shown reproductive abnormalities in...
freshwater turtles near the Massachusetts Military Reservation (MMR) Superfund Site where pesticides and other persistent pollutants have been detected in egg yolk. To explore the mechanisms of developmental toxicity of these pollutants we used a zebrafish (Danio rerio) whole embryo model. Quantitative RT-PCR analy- sis showed that exposure of zebrafish to pond water contaminated by the MMR, relative to water from a control pond, induced vitellogenin and aromatase B but did not alter estrogen receptor alpha, cytochrome P450a1, or metallothionein gene expression. Upon individual examination of pollutants from pond water we found the pesticide dieldrin, but not p,p′-DDE, had a significant effect on global gene transcription patterns. Gene Set Enrichment Analysis (GSEA) of microarray results indicated that exposure to dieldrin disrupted many pathways including insulin secretion, glucose secretion and metabolism, and adipokine signaling. Using Quantitative RT-PCR we further investigated a panel of dieldrin-responsive genes (neuronal PAS domain protein 4, pyruvate dehydrogenase kinase 2, phospho- nolpyruvate carboxykinase 1, hydroxy steroid delta beta dehydrogenase 2, pre-proop- iomelanocortin, and insulin-induced gene 1). For all genes examined we found an inverted U-shaped dose response curve with dieldrin exposure. The normal developmental pattern of these dieldrin-responsive genes was also characterized. We found that neither MMR-contaminated pond water nor p,p′-DDE exposure had a significant effect on genes regulating energy homeostasis. In addition to other pollutants at the MMR, dieldrin is a concern and is shown here to alter energy balance signaling.

Manganese (Mn) is neurotoxic at higher concentrations. Mn toxicity occurs in

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Based on these results, we conclude that Mn exposure during early life and lifelong has a significant effect on energy balance signaling.

10871 The Role of Mn in Skeletal Development

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Objective: To determine the affects of early postnatal manganese(Mn) exposure on skeletal Mn accumulation and development in pre and post weaned rats.

Rationale: Mn is essential for proper bone development and growth however overexposure to Mn can impair proper bone formation. Mn is incorporated in bone mineral as a biological analog to calcium, although the extent of accumulation in bone is not well known.

Approach: Male Long Evans rats were orally exposed to 0.25 or 50 mg Mn/kg/d over the preweaning period only (PND 1-21) or lifelong, and sacrificed at age PND 24,66, or 400. Bone Mn levels were analyzed by ICPMS and calcium using OES. Tibia samples were pulverized and analyzed for molecular coordination and speciation using X-Ray diffraction (XRD) and X-Ray absorption(XAS) techniques. Femurs were tested for fracture resistance using an instron at PND 66.

Results:
1) Results indicate that within an age group of lifelong exposure, PND 24,66, or 400 continuously exposed to Mn had an increased Mn accumulation relative to exposure.
2) Bone Mn levels in young PND 24 animals were substantially higher than levels in PND 66 and 400 animals.
3) Bone Mn levels in PND 66 and 400 animals that were exposed to Mn only over the pre-weaning period (PND 1-21) still showed evidence of slightly elevated bone Mn levels compared to controls never exposed .
4) Bone Ca levels were significantly lowered in the PND 66 group.
5) Preliminary Instron analyses of bone strength suggests that Mn exposure may reduce bone strength, while preliminary XRD results do not suggest any gross alteration in the macroscopic mineral composition. Conclusions: Collectively these results suggest that elevated early life and lifelong Mn exposure may lead to increased bone Mn accumulation into adulthood, and reduce overall bone strength, with implications of osteoporosis and compromised bone integrity.

1087m Interaction of Manganese, Iron, and Stress on Spatial Learning and Memory after Developmental Exposure

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Manganese (Mn) is neurotoxic at higher concentrations. Mn toxicity occurs in children from sources such as soy-based infant formulas, well-water, and air pollution near heavy manufacturing. Children’s gastrointestinal systems allow Mn to more readily enter circulation and accumulate in brain. Subclinical iron (Fe) deficiency (FeD), which affects up to 15% of US children, exacerbates Mn toxicity due to increased Mn bioavailability during FeD. FeD is prevalent in impoverished and low socioeconomic environments that are considered stressors. To model developmental stress (DS), pregnant rats and their litters were housed in cages with a wire grid floor or standard bedding from embryonic day (E)7 to postnatal day (P)28. Dams were fed a 90% Fe deficient or standard NIH-07 diet from E15 through P28. Within each litter, different offspring were treated with 100 mg/kg Mn (100Mn) by oral gavage or vehicle from P4 to P28. As adults, offspring were tested in the Morris Water Maze (MWM). Progeny in the 100Mn group, regardless of FeD or DS, showed increased latency on acquisition trials and decreased platform crossings on the probe trial. The interaction of FeD and DS also impaired MWM performance. A potential mechanism underlying these deficits in 100Mn animals is decreased long-term potentiation that we observed in slice preparations in the hippocampus. DMT1 and ZIP8, metal transporters in the CNS, showed increased protein in the hippocampus via Western blot in treated groups; ZIP8 was decreased only in the FeD/100Mn compared with controls. Together these data suggest that multifactorial models may reveal effects not observed when only one factor is studied at a time. (Supported by NIH T32 ES07051 and P30 ES006096)

1087n Characterization of the Neuropathological Consequences of Plac Ablation in the Developing Mouse Embryo

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Placenta-specific 1 (Plac1) is an X-linked gene that is essential for normal placental development. Plac1 is also expressed in the fetal brain and paternally imprinted. Expression is markedly downregulated immediately after birth. Plac1 ablation pre-disposes Plac1-null males and Xm-X Hets (inactive maternal allele) to an increased risk of developing lethal postnatal hydrocephalus suggesting a functional role for Plac1. The objective of this study was to characterize the effect of Plac1 on brain development. A mutant Plac1 mouse model, established on the C57BL/6J background, was studied. Embryo and brain sections were obtained at various stages of development. Plac1 expression was assessed by qRT-PCR, immunohistochemistry (IHC), and in situ hybridization (ISH). Brain structure was assessed by histo-pathological and magnetic resonance imaging. Results revealed Plac1 expression throughout the embryonic brain when assessed by qRT-PCR, IHC, and ISH at E14.5-E18.5. MRI analysis of an adult Plac1 knockout (KO) brain revealed microcephaly (10%), a dysmorphic cerebrum, and increased heterogeneity of the medulla. Consistent with these findings, H&E staining of the KO brain revealed a smaller cortical mantle, a dysmorphic hippocampus, and a dysmorphic cerebellum with reduced folia and major disruptions in development and significantly reduced neuronal cell numbers. Similarly, anti-NeuN staining of an Xm-X Het revealed decreased neuronal cell numbers. In conclusion, Plac1 is a paternally imprinted, X-linked gene that is essential for normal brain development. Plac1 ablation is associated with the disruption of axonal development and decreased neuronal cell number. The Plac1 promoters are under the control of Rb in combination with other transcriptional regulators. It is therefore likely that Plac1-mediated signaling pathways are relevant to neuropathies associated with disruptions in retinoic acid signaling.

1087o Evaluation of Environmental Chemicals Computationally Predicted to Disrupt Angiogenesis

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The aim of this study was to investigate the effect of 10 chemicals, predicted by ToxCast and a virtual tissue model to impact angiogenesis at varying degrees, in an in vitro human cell-based angiogenesis assay. Angiogenesis is critical for embryonic development and reproduction in general, and contributes to the pathogenesis of numerous disorders. This human cell base angiogenesis assay may serve as a test to detect potential antiangiogenic substances including teratogens. The test chemicals were selected from the ToxCast project based on predicted antiangiogenesis activities (strong, weak, negative). The co-culture of human fibroblasts and human endothelial cells (HUVEC) was exposed to test chemicals at a concentration range of 10 pM to 10 nM (limited by the solubility). After 24 h, the neutral uptake assay (NRAU) was performed for EC80 cytotoxicity value for each test chemical. Extent of tubule formation was microscopically assessed after 6 days in culture. The concentrations of each test chemical that produced less than 20% cytotoxicity in the NRU test were selected for tubule formation analysis. Potency order for in
vito inhibition of tubule formation (IC50) was: Octyl gallate 1 μM > Tricosanol 1.7 μM > 1-hydroxypropane 11 μM > Disulfiram 23 μM > Sodium docetylbenzene sulfonate 44 μM > Diethanolamine 1788 μM. With Sodium docetylbenzene sulfonate the inhibition of tubule formation was considered as being cytotoxicity-related. Tert-butylhydroquinone did not inhibit tubule formation at non-cytotoxic concentrations. Trit (2-chloroethyl) phosphate, Mannitol and Decane were neither cytotoxic at the concentration range tested nor markedly inhibited tubule formation. Decane showed only minor inhibition of tubule formation. The in vivo results were generally consistent with prospective predictions from the in silico models with respect to antiangiogenic potential. This abstract does not necessarily reflect US EPA policy.

**PS 1087p Computational Modeling of Limb Development Using ToxCast High-Throughput Screening Data for Predictive Toxicology**


Skeletal defects are one of the major adverse outcomes observed across many prena
tal developmental toxicity studies. We mined the ToxCast database (ToxCastDB) for in vitro chemical-bioactivity profiles that significantly correlated with skeletal defects in the Toxicity Reference Database (ToxRefDB). Using high-throughput screening (HTS) data from >1060 chemicals tested in 860 assays, 734 chemi
cals had in vivo developmental toxicity data in ToxRefDB and 44 (4.5%) pro
duced fetal limb defects. In addition, we identified 112 ToxCast chemicals not in ToxRefDB that according to published articles produced limb defects. Significant univariate associations (e.g., assay-endpoint) were used to filter HTS assays based on statistical correlation (p<0.05) with distinct in vivo developmental limb defects.

Retinoic acid receptors (RARs) assays emerged as the top target and were activated by 17 ToxCast chemicals. Retinoic acid (RA) signaling plays an essential role in limb patterning as a ligand for nuclear RA receptors. We incorporated RA sig
naling into a multicellular agent-based model (ABM) of early limb development in CompuCell 3D. The ABM stimulates complex cellular interactions (adheshion, apoptosis, chemotaxis, migration, mitosis, secretion) through formation of apical epidermal ridge, zone of polarizing activity, and expansion of mesenchyme driven by morphogenetic signals (BMPs, FGFs, SHH, RA). To evaluate the model we se
glected two known prototypes: Dieldrin (AC50 = 4.8 μM on RARγ, and 0.974 μM on RARαx) and Aldrin (AC50 = 45.5 μM on RARα, and inactive on RARβ) based on ToxCast data. Simulating the impact of RAR disruption by these compounds on other pathways (e.g. SHH, FGFs) in the limb model can provide insight into the spatial-temporal dynamics of altered limb development as a tool for predictive toxicology. [This abstract does not necessarily reflect EPA policy.]

**PS 1087q Evaluation of the Mechanism of Misoprostol-Induced Limb Defects Using a Late Organogenesis Rat Embryo Culture**

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Prostaglandins of the E-series play an important role on bone metabolism and limb development. Misoprostol is a prostaglandin E1 analog and is registered in Chile for the prevention and treatment of non-steroidal anti-inflammatory peptic ulcers. It is also used to induce abortions. In utero exposure to misoprostol, after failed abortion attempts, has been associated to joint and skeletal defects, although the mechanism of action which mediates this teratogenicity is unknown. Since misoprostol can act on prostanoid receptors expressed during limb development, we hypothesized that this interaction could explain the induction of limb defects. In order to test this hypothesis, we established a late organogenesis embryo culture to model the effect of misoprostol on limb development in which to evaluate func
tionality, growth and morphology. GD13.5-collected rat fetuses were cultured for 24 hours at 37 °C, in rat serum diluted in Hank’s solution which also contained either 200, 2,000 or 20,000 pg/ml misoprostol, PGE2 or the EP1 and EP2 antag
nist, AH6809. 1000 mg/L sodium penicillin G and 50 μg/ml ketokconazole were used as negative and positive control, respectively. All concentrations of misoprostol, PGE2 and the antagonist induced similar toxicity, inducing abnormal clefs and decrease of bone development in anterior limb digital rays. However, they did not alter growth and functionality parameters. In conclusion, misoprostol induces overt manifestations of alterations of limb development, which may not be medi
dated by prostanoid receptors EP1 or EP2.

**PS 1087r Peripheral Blood Changes in Rats Developmentally Exposed to Volatile Organic Compounds: Shifts in Leukocyte Distribution**

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Volatile organic compounds (VOCs) such as benzene, trichloroethylene (TCE), 1,2-dichloroethylene (1,2-DCE), perchloroethylene (PERC) and vinyl chloride (VCM) are important industrial solvents used in dry cleaning agents, dyes, and var
nishes, among others. Since the mid-1970s, environmental or occupational ex
poures to these VOCs have been associated with increased incidences of cancer, particularly leukemia and lymphoma. Epidemiological studies indicate that inges
tion of contaminated drinking water or inhalation of volatile gases in the air, are the most likely routes of environmental and VOC exposure. Developmental exposures to VOCs (e.g. during pregnancy or in infancy) can be especially detrimental and often result in malformations, congenital abnormalities and later life disease. In this study, Harlan Sprague-Dawley rats were pre-natally and peri-natally exposed to drinking water containing a VOC-mixture of 5, 10 and 50-fold well water concentra
tions (5X, 10X, 50X) of benzene, TCE, DCE, PERC and VC from gestation day 12 (GD 12) through post-natal day 48 (PND 48). While all dosed animals exhibited elevated leucocyte counts compared to age-matched controls, 5-7-fold elevations were observed within the two higher dose groups (10X and 50X). In addition, examination of peripheral blood smears of the 10X and 50X animals revealed increased numbers of circulating large granular lymphocytes, particularly within the 10X group. These changes possibly suggest a bone marrow response in leucocyte production or, more likely, a shift in leucocyte distribution from the tis
tue reservoir to the circulatory pool as a result of developmental exposure to VOCs.

**PS 1087s DNA Methylation Is Required for Mouse Prostate Development**

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A number of dietary and environmental chemicals including but not limited to bisphenol-A (BPA), bis(2-ethylhexyl)phthalate (DEHP), and 2,3,7,8 tetracho
lordibenzo-p-dioxin (TCDD) are capable of altering ductal development and dis
drupting DNA methylation patterns in developing mouse prostate. Whether these actions are mechanistically linked is not known in part because the role of DNA methylation in neonatal mouse prostate development is not known. The purpose of this study was to determine whether DNA methylation participates in mouse prostate development. 14 days post coitus (dpc) mouse urogenital sinuses were cultured for 2 days in the presence or absence of a DNA methylation inhibitor and then 1, 3 or 5 days in media containing dihydrotestosterone (androgen) without DNA methylation inhibitor. At the end of the culture period, after methylation inhibition was used to visualize and count prostatic buds, immunohistochemistry was used to determine the index of androgen receptor (AR) positive cells, and methylated DNA immunoprecipitation was used to assess Ar DNA methylation. The DNA methylation inhibitor reduced Ar promoter methylation, increased AR protein abundance, increased the number and rate of prostatic buds formed, and caused prostatic buds to form in response to a lower concentration of androgen. Thus, we conclude that DNA methylation creates a permissive environment for mouse pros
tatic outgrowth, and primes the prostate primordium to respond to developmental cues mediating prostate ductal development. These results raise the possibility that endocrine disrupting chemicals could alter prostate development by perturbing DNA methylation. Supported by NIH grant DK096074 and ES001332 and NSF grant DGE-0718123.

**PS 1087t Pregestational Ethinylestradiol Exposure Postpones Pubertal Onset and Decreases Body Weight in Both Male and Female Offspring in Rats**

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Ethinylestradiol (EE) is used for medical treatment and as contraceptives in females and its exposure during gestational period has been proved to elicit reproductive abnormalities in both male and female offspring. To test whether pre-gestational EE exposure also affects reproductive development, especially pubertal timing in the offspring, female Sprague-Dawley rats were treated orally with EE (0, 50, 200, 800 μg/kg/d) for 15d. Another 30d later, they mated with normal males and gave SOT 2014 ANNUAL MEETING 287
birth to the offspring. Litter size and litter sex ratio were recorded. Anogenital dis-
tance (AGD) and body weight were measured every 5d since postnatal day 1 (P1). Age at vagina opening in females and penis stripping in males were recorded to mark pubertal onset. Rats were sacrificed on P51. Ovarian/testis mass, serum es-
trooidal (E2) (E2(17beta)) and testosterone (T) (male) levels were detected. Results showed no significant difference in litter size and litter sex ratio. However, mothers with pre-gestational 50, 200, 800 μg/kg/d EE treatment had offspring with significantly lower body weight and higher AGD/body weight ratio than the control group since P1. Maternal pre-gestational EE exposure significantly postponed the puberty onset in male and female offspring in 200 and 800 μg/kg/d groups. Body weight at pubertal onset was significantly lower in 50 μg/kg/d group in males and females. Hormone assay data indicated T in males was higher in 200 and 800 μg/kg/d groups than control and no difference was found in E2 in females. Ovarian/body weight ratio in females and testis/body weight ratio in males were not different among groups. These data indicated that maternal pre-gestational EE exposure might cause alteration in the neuroendocrine system to give rise to pubertal develop-
mental disruption but might not lead to adverse effects in gonads. Since no sign-
nificant sex-specific difference was observed and all treated groups had lower body weight, alteration of metabolic signals activating the reproductive axis may underlie the induced pubertal development disruption.

1087w An Oral Prenatal Developmental Toxicity Study with Tetrabromobisphenol A (TBBPA) in CD Rats

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The objective of this GLP OECD TG 414/US EPA OPPTS GL No. 870.3700 study was to discern the effects of oral TBBPA (0, 100, 300 or 1000 mg/kg bw/d; gavage) on embryonic/fetal development from gestation days (GDs) 0 to 19. No test article-related mortality was observed in any of the dams. Sporadic ptyalism in dams was associated with the administration of TBBPA at doses of ≥ 300-mg/ kg bw/d. Because of the sporadic nature of this effect, and lack of a clear dose response, the effect was regarded as being non-adverse and related to the normal taste response following exposure to residual amounts of the test article on the dos-
ing catheters. No other test article-related effects including clinical examinations, gestational parameters, body weight, body weight gain, food consumption, uterine implantation, gravid uterine weight, number of uterine implantations, number of corpora lutea, number of early/late resorptions, number of viable/non-viable fetuses, fetal sex distribution, individual fetal weights, liver weights of dams, fetal external examinations, fetal visceral examinations, fetal skeletal examinations and macroscopic pathology examinations of the dams were observed. The NOEL for maternal and developmental toxicity was 1000 mg/kg bw/d, the highest dose eval-
uated.

1087v Influence of Maternal Stress on Gestational Parameters and Prenatal Development in Himalayan Rabbits

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Gestational stress is hypothesized to enhance the developmental toxicity of chem-
icals. Single stress factors like food restriction have been reported to be associated with resorptions, abortions, reduced fetal weight, and alterations in ossification in rabbits. But, little is known about how maternal stress from handling alters fetal development, confounding the data interpretation of regulatory prenatal toxicity studies. In the present experiment we exposed 25 pregnant Himalayan rabbits per group to two daily handling protocols aimed to increase maternal stress: restraint (15 min per day) and restraint plus dressing the animals with stretchable bandages for 6 hours per day. These groups were compared with an uncongested control group. Investigations and data assessment were made in accordance with standard regulatory guidance, i.e. OECD TG 414 and OPPTS 870.3700. Neither stress protocol affected any of the parameters routinely used to characterize maternal toxicity. No adverse clinical signs were noted; nor was food consumption or (net) body weight gain in the pregnant rabbits influenced. Restraint stress alone had no influence on prenatal development; however, the combination of restraint plus dressing stress produced 4 abortions and an early resorption rate of almost 50%. Prenatal development of the surviving offspring was not impaired. Thus, a combi-
nation of two handling stressors which do not evoke obvious pathological findings in pregnant rabbits is able to produce severe prenatal developmental toxicity. When taken together with other investigations, these data suggest that the nature of these findings, in this case intrauterine death, is likely specific to the stressor and hypoth-
etically even the species or the strain being tested. Furthermore, we speculate that when combined with handling, the stress due to chemical exposure may cause developmental findings in the absence of frank maternal toxicity, simulating a test substance-specific effect.

1087x An Oral Two Generation Reproductive, Fertility and Developmental Neurobehavioral Study of Tetrabromobisphenol A (TBBPA) in CD Sprague-Dawley Rats

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The objective of this GLP US EPA OPPTS 870.3800 study was to discern the effects of TBBPA (10, 100 or 1000 mg/kg bw/d; gavage) over the course of 2 generations (P and F1) on growth and development of offspring (F1 and F2). A developmental neurotoxicity/neuropathology assessment was also conducted on a F2 generation cohort. Exposure to ≥ 100-mg/kg bw/d TBBPA was associated with changes in the peripheral thyroid levels in rats that were explainable on the basis of induction of liver catalysis, a phenomenon that is known to be not relevant to humans. TBBPA at up to 1000 mg/kg bw/d was not associated with any significant non-neurological effects on reproduction, growth and development. A subtle reduction, of unknown biological relevance, in the thickness of the parietal cortices of 11-day-old F2 pups from the 1000 mg/kg bw/d group was noted. However, this change was not ac-
accompanied by evidence of micro-anatomical change. The functional relevance of this finding at present remains obscure and needs to be interpreted with caution given the limitations of the morphometric analysis. No other test article-related effects on developmental neurotoxicity/neuropathology were present including no test article-related effects on detailed clinical observations, developmental maturation landmarks, neurobehavioral evaluations and Day 60 brain weights.

1088 Effect of a Nutrient Mixture on Matrix Metalloproteinase-9 Dimers in Various Cancer Cell Lines

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Strong clinical and experimental evidence demonstrates association of elevated levels of matrix metalloproteinase (MMP)-9 with cancer progression, metastasis and shortened patient survival, as it plays a key role in tumor cell invasion and metastasis by digesting the basement membrane and ECM components. MMP-9 is secreted in both monomer and dimer forms. Though there is little research on MMP-9 dimers, some studies have shown the dimer to be associated with more
aggressive tumor progression. Our objective was to study the relative secretion patterns of MMP-9 dimer in various carcinomas, sarcomas, adenocarcinomas and leukemias and the effect of a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract on dimer secretion.

The cancer cell lines were grown in their respective media, supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 μg/ml) in 24-well tissue culture plates. At near confluence, the cells were treated with NM at 0.1, 0.5, 1.0, and 2.0 mg/ml and 500 and 1000 μg/ml. Parallel sets of cultures were treated with phorbol 12-myristate 13-acetate (PMA) 100 ng/ml for induction of MMP-9. Cell MMP-9 secretion was assayed by gelatinase zymography.

No MMP-9 dimer secretion was observed in prostate, pancreatic, colon, bladder, head and neck, glioblastoma and leukemia cell lines. MMP-9 dimer secretion only with PMA induction was seen in sarcomas, melanoma and breast, lung and tongue cancer cell lines. Cervical, renal and hepatic carcinomas exhibited MMP-9 dimer without PMA treatment and increased secretion with PMA treatment. Sarcomas demonstrated the highest combined levels of MMP-9 mono- and dimer with and without PMA among these cancer cell lines. NM showed dose-dependent inhibition of MMP-9 monomer and dimer in all cell lines tested.

In conclusion, high MMP-9 secretion levels correlated with more aggressive cancer cell lines. NM was effective in inhibiting MMP-9 monomer and dimer secretion in all cell lines tested, suggesting its therapeutic potential as an antimetastatic agent.

1089 Increased Susceptibility of Rats versus Mice to N-Butyl-N-(4-hydroxybutyl) Nitrosamine (BBN)-Induced Urinary Bladder Tumors


BBN is a potent and specific urinary bladder carcinogen that has been utilized in experimental models of 2-stage tumor promotion. However, the dose-response of BBN on key tumorigenic events is not well understood since they have not been systematically evaluated and BBN is usually administered in drinking water as a single concentration. The current study was conducted to better understand the dose-response relationship between BBN administration and the incidence of urinary bladder tumors in mice and rats. BBN was administered by oral gavage twice weekly for 6 weeks to either male C57Bl/6J mice (0, 125, 250, 500 mg/kg; N = 45) or male Sprague-Dawley rats (0, 100, 200, 400, 800 mg/kg; N = 20). Necropsies were conducted at 10 (mice only), 14 and 24 weeks following the initiation of BBN administration. In mice (10 weeks) and rats (14 weeks), BBN caused a dose-dependent increase in oxidative DNA damage (8-hydroxyguanosine staining) and cell proliferation (Ki-67 staining) in urinary bladder transitional cells. Despite the urinary bladder transitional cell hyperplasia, BBN was associated with a very low incidence of urinary bladder tumors (≤ 1%) in mice over the full 24-week period. In contrast, BBN-treated rats exhibited a dose-dependent increase in atypical hyperplasia and transitional cell papillomas and carcinomas in the urinary bladder, with a 20, 47, 57, and 93% incidence of urinary bladder tumors across doses after 24 weeks. Thus, BBN induced urinary bladder tumors in male Sprague-Dawley rats with a steep dose response relationship, but was not effective in producing bladder tumors in male C57Bl/6J mice at comparable doses despite underlying evidence of DNA damage and hyperplasia. The steep dose response observed with BBN and relative species susceptibility highlight important considerations in designing urinary bladder tumorigenesis studies with BBN.

1090 Reduced Body Weight Gain Prolongs Early-Life Levels of Tyrosine Hydroxylase Expression in Hypothalamic Tuberoinfundibular (TIDA) Neurons in Wistar Rats

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Caloric restriction in Wistar rats leads to a decreased incidence of mammary and pituitary tumours and an increase in uterine tumours (1, 2, 3) relative to concurrent controls. Previously we found (4) that lean female Wistar rats (~30% body weight gain relative to controls) from a 2 year bioassay showed increased expression of tyrosine hydroxylase (TH) mRNA and protein in hypothalamic TIDA neurons. We have postulated that alterations to leptin signalling, resulting from reduced body fat, could maintain dopaminergic inhibition of prolactin secretion and prolong pro-oestrogenic stimulation of the uterine epithelium. The present study explored (i) the time-dependency of the effects on TIDA neuron TH expression, using immunostaining (IHC), in situ hybridisation (ISH) and image analysis on FFPE tissue from 90 day, 12 month and 2 year studies; and (ii) pathways involved in prolactin and oestrogen signalling, regulated by the hypothalmo-pituitary axis, that might promote uterine carcinogenesis.

TH RNA and protein expression decreased with time in controls, whilst lean rats maintained TH levels. Laser dissection targeted microarray analysis of hypothalamus, pituitary and uterus revealed down-regulation of pituitary prolactin, hypothalamic GABA B receptors, and leptin receptor (LEPR) signalling molecules STAT3 and PDE3B, and up regulation of uterine oestrogen receptor alpha (ESR1). Immunostaining for ESR1 showed increased expression in uterus. These results support previous observations that chronic reduction in bodyweight gain alters regulation of hypothalamic dopamine, with downstream consequences on hormonal control of prolactin, oestrous cycle prolongation, and potential to increase the incidence of uterine tumours.


1091 The Effects of Testosterone on NNK-Induced Lung Tumorigenesis in Female and Male A/J Mice

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It is well known that there is sex difference of the sensitivity of the lung carcinogenesis. In human, lung adenocarcinomas of female are higher incidence than those of males. In experimental animals, A/J mice shows similar pattern with humans. So, the lung carcinogenesis might be associated with sexual hormones. The present study is conducted to investigate the effects of testosterone on the lung carcinogenesis in A/J mice.

In experiment 1, female A/J mice were separated into 4 groups. Group 1 and 2 were treated with interperitoneal injections (i.p.) of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (2mg/0.1ml saline/mouse) at weeks 0 and 1. At week 3, testosterone pellets (90-day release, 25mg/pellet/mouse, Innovative Research of America) were implanted under the skin shoulder in groups 1 and 3, and control pellets (placebo) were implanted in groups 2 and 4. All mice were sacrificed at week 15.

In experiment 2, male A/J mice at 6 week-old were separated into 6 groups. Group 1, 2, 5 and 6 were castrated two weeks ago before groups 1, 2, 3, 4 and 4 were treated with i.p. of NNK at weeks 0 and 1. At weeks 3 and 9, testosterone pellets were implanted in groups 1, 3 and 5. Groups 2, 4, 5 and 6 were implanted placebo pellets at week 3 and treated sham operation at week 9. All mice were sacrificed at week 15.

In experiment 1, the female mice showed significant decreasing of multiplicity of lung tumors with higher testosterone level in their blood. However, in male mice (experiment 2), there were no significant differences of the multiplicity or area of lung tumors with different concentrations of testosterone in the serum. Therefore, testosterone has possibilities to inhibit NNK-induced lung carcinogenesis in female A/J mice, but not in male mice.

In conclusion, the testosterone is suggested to have tumor-suppressing effect on lung tumorigenesis, but there could be sex difference or other factors.

1092 Activation of ERK1/2 on NNK-Induced Lung Carcinogenesis in A/J Mice

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The extracellular signal-regulated kinase 1/2 (Erk1/2) signaling pathway is activated by several growth factors and mitogens, and up-regulation has been noted in many human cancers, including lung cancers. In this study, we examined immunohistochemical expression of phosphorylated forms of Erk1/2 (pErk1/2) and correlation between Erk activation and mutation of Kras encoding an upstream activator of Erk1/2, in a mouse lung carcinogenesis model. Female 7-week old A/J mice were administered a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), then maintained without additional treatment until sacrifice at week 52. We analyzed immunohistochemical expression of pErk1/2 in 26 hyperplasias, 50 adenomas, and 17 adenocarcinomas. pErk1/2-positive cells were seen in the peripheral and central areas of lung tumors with no different concentrations of testosterone in the serum. However, in male A/J mice, the activated Erk1/2 expression was obviously induced by testosterone. Our results suggest that the interaction between testosterone and Erk1/2 pathways might promote lung carcinogenesis.
Effects of Gonadectomy on Lung Carcinogenesis Induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in Both Sexes of AJ Mice

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It is well known that female mice are more sensitive to chemical lung carcinogenesis than males. In order to investigate our hypothesis that the lung carcinogenesis may be affected by sex hormones, we first examined the sex ratio of the lung tumors in both sex of AJ mice were gonadectomized and were examined about their lung carcinogenesis induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NNK is a tobacco-specific N-nitrosamine which is considered to play important roles in cigarette-related lung cancer, and also induces lung adenocarcinoma in rodents.

In the present experiment, 18- and 56-week studies were conducted. Male and female A/J mice were separated into gonadectomized and unoperated groups. All animals were treated intraperitoneal injection of NNK at the dose of 2 μg/mouse, with 2 injections at weekly interval in the 18-week study and 1 injection in the 56-week study. All of surviving mice were sacrificed at the ends of an experiment period. Lung proliferative lesions were detected in all mice of all groups. In the control groups of 18- and 56-week studies, the multiplicities of lung nodules were significantly greater in females than in males. In the 56-week study, the males showed the multiplicity of macroscopic lung nodules, histopathological bronchiolo-alveolar hyperplasias, adenomas and tumors were increased significantly by castration. In the 18-week study, females showed the multiplicity of adenomas were decreased significantly by ovariectomy.

From these results, female A/J mice are more susceptible for lung carcinogenesis induced by NNK than males and gonads play important roles in determining sex differences in the lung carcinogenesis in AJ mice. It is suggested that the progression of lung tumor could be inhibited by testosteron and promoted by estradiol.

ALDH1B1 Is Required for Colon Tumorigenesis by Modulating WNT-Signaling and Metabolizing Retinaldehyde

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Aldehyde dehydrogenase 1B1 (ALDH1B1) is a mitochondrial enzyme that shows 65% and 72% amino acid homology with ALDHA1A1 and ALDHA2, respectively. In human normal colon, ALDH1B1 is expressed only at the crypt base, along with stem cells. It is highly expressed in all cancerous cells of human colonic adenocarcinomas. This pattern of expression corresponds closely to that observed for Wnt signaling activity in normal and cancerous colon. In the present study, we show that shRNA mediated knockdown of ALDH1B1 reduced the number and size of spheres formed by human colon cancer cells in a three-dimensional matrigel culture. In addition, ALDH1B1 knockdown depletes the putative highly tumorigenic ALDHBright colon cancer cells and significantly decreased xenograft tumor formation in athymic (nu/nu) mice. Gene expression studies on the Wnt-signaling pathway revealed upregulation of Axin2 (a negative regulator of the Wnt pathway) and downregulation of beta-catenin (a critical protein in the canonical Wnt-signaling pathway) and Wnt dependent transcription of c-Myc in ALDH1B1 depleted colon cancer cells. We have also found that ALDH1B1 metabolizes all trans-retinaldehyde efficiently with Km of 25 μM to generate retinoic acid which has been reported to increase expression of pro-survival genes and tumor growth by activating PPARβ/δ in colon cancer. In summary, our data demonstrate that ALDH1B1 plays a functional role in colon cancer tumorigenesis by modulating the Wnt-signaling pathway and participating in retinoic acid formation.

Increased Growth and Tumorigenicity of MCF-7 Breast Cancer Cells by Chronic Exposure to Oxidative Stress

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Breast cancer is the second leading cause of cancer related death in women. Exposure to environmental estrogenic chemicals has been shown to be associated with breast cancer development and progression through various mechanisms. Though, the increased oxidative stress by xenoestrogens-generated reactive oxygen species (ROS) has been hypothesized as a potential mechanism for breast cancer growth and progression, there is no direct evidence for the ROS-induced growth and tumorigenicty of breast cancer cells. Therefore the objective of this study was to evaluate the chronic effect of hydrogen peroxide (H2O2) generated ROS on the growth and tumorigenicty of MCF-7 breast cancer cells. MCF-7 cells were exposed to H2O2 for both acute (48 hrs) and chronic (3 months) period. Cell growth and viability were evaluated by cell count and MTT assay and were confirmed by cell cycle analysis. Expression of genes related to cell cycle, cell survival, and metastasis were measured by quantitative real-time PCR. Soft agar assay was also performed to determine the effect of H2O2 on anchorage independent growth of MCF-7 cells. Results of cell count, MTT and cell cycle analysis revealed increased growth, and survival of MCF-7 cells chronically treated with H2O2. This was further confirmed at molecular level by increased expression of Cyclin D1, Survivin and Bcl2 in H2O2 treated cells. Significant increase in number of soft agar colonies in H2O2 treated cells further suggest that chronic oxidative stress causes increased tumorigenicity of MCF-7 cells. Our data also support the hypothesis that ROS in the form of pro-meta- static genes like VEGF, WNT1 & CD44 and down regulation of anti-apoptotic gene E-cadherin indicates that persistent oxidative stress may enhance metastatic potential of MCF-7 cells. In summary, this study for the first time provided direct evidence for the increased growth, tumorigenicity, and metastatic potential of breast cancer cells by chronic exposure to oxidative stress.

Effect of Melatonin on Colitis-Associated Colon Carcinogenesis in Mice: Role of Autophagy and Nrf2 Signaling Pathways

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Ulcerative colitis, a chronic gastrointestinal disorder, gradually leads to colon carcinogenesis. Various pre-clinical and clinical studies have shown that melatonin has beneficial effects in cancer. However, elucidation of the detailed molecular
mechanisms involved in melatonin-mediated protection against colon carcinogenesis deserves further investigation. The present study was aimed at deciphering the effect of melatonin on autophagy and Nrf2 signaling pathways in a mouse model of colitis-associated colon carcinogenesis (CACC). For the induction of CACC, male Swiss Albino mice were administered a single i.p. injection of 20mg 1, 2-dimethylhydrazine dihydrochloride (DMH)/kg bw, followed by 3 cycles of 3% w/v dextran sulfate sodium (DSS) (one cycle consisted of 7 days of DSS-treated water followed by 14 days of normal drinking water) 1 week after DMH injection. One week after the initiation of DSS administration, melatonin was administered at the dose of 1, 2 and 5mg/kg bw, and mice were sacrificed at 10 and 20 weeks after DMH treatment respectively. Melatonin treatment decreased the progression of colon carcinogenesis, inflammation, oxidative stress and DNA damage in the colon of mice with CACC. Further, CACC in mice led to an increased autophagy in the colon as revealed by the expression of various autophagy markers such as Beclin-1, LC3B-II/LC3B-I and p62. Decreased p62 expression in the colon of mice with CACC was associated with increased nuclear level of STAT-3 (NF-E2-related factor 2 (Nrf2)) expression. Melatonin treatment reduced autophagy and increased the expression of Nrf2 and the associated antioxidant enzymes, NAD(P)H:quinone oxidoreductase (NQO-1) and heme oxygenase-1 (HO-1) in the colon of mice with CACC. The results of the present study demonstrated that melatonin attenuated the progression of CACC in mice by modulating autophagy and Nrf2 signaling pathways.

1098 Novel Role of STAT3 in Anoikis Resistance and Tumor Cell Metastasis

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Anoikis is an anchorage independent cell death. Resistance to anoikis is one of the key features of metastatic cells. The role of STAT-3 in anoikis resistance in various melanoma, pancreatic, breast, ovarian, lung and colon cancer cells was analyzed in the current study. Marked anoikis was induced in cells that were grown under anchorage-independent conditions. Cells that resisted anoikis were observed to have a higher rate of migration and invasion as compared to the cells grown under anchorage dependent conditions, as observed by cell migration and invasion assays. The anoikis resistant cells also had significantly higher expression and phosphorylation of STAT-3 at Tyr 705 than the cells that were attached to the basement membrane. Treatment of these cells with IL-6, a cytokine which phosphorylates STAT-3, prevented the induction of anoikis. STAT-3 inhibitors AG490 and pipartine induced anoikis in a concentration-dependent manner in anoikis resistant cells, whereas IL-6 blocked anoikis. Over-expression of STAT-3 by transfection, not only increased the anoikis resistance, but also protected the cancer cells from piperazine-induced anoikis, confirming the role of STAT-3 in anoikis resistance. On the other hand, silencing STAT-3 decreased the potential of cancer cells to resist anoikis. Furthermore, STAT-3 (-/-) cancer cells were more sensitive to anoikis, as compared to cells overexpressing STAT-3 (+/+). The STAT-3 (+/+ cells also had enhanced migration potential, as compared to STAT-3 (-/-) cells. STAT-3 -/-cells and pipartine treated cells completely failed to form tumors in SCID-NSG mice, as compared to untreated anchorage independent cells. Similarly, pipartine treated or STAT-3 -/- cells failed to metastasize to lungs and liver, as compared to untreated anchorage independent cells which extensively metastasized. The metastasis was confirmed by H & E staining as well as bioluminescence. In summary, our results establish STAT-3 as a critical player that renders anoikis resistance to the cancer cells and enhance their metastasis potential.

1099 Toxicokinetic Mode-of-Action (MOA) Investigation of Liver Tumors Induced by Chronic Oral Exposure to Biphenyl in Female BDF1 Mice


Biphenyl (BP) is a high production volume chemical used in USA and EU in closed system applications as a synthesis intermediate and component of heat transfer fluids. The rich toxicological dataset, with a number of cancer bioassays, notes two cases of neoplastic effects from high-dose BP exposures that are specie and strain specific. MOA investigations have demonstrated that urinary bladder tumors seen in male F344 rats occur from calculi development at specific pH and potassium concentrations, conditions that are not relevant for humans. Recently, Umeda et al. (2005) demonstrated increased tumor incidence from high-dose dietary BP exposures in female BDF1 mice, while in males, decreased tumor incidence occurred with increasing doses. Different carcinogenic outcomes have been proposed but there is no clear understanding of the underlying MOA for these liver tumors or their relevance to humans. In order to better understand this cancer MOA, absorption, metabolism and elimination of 14C-BP in BDF1 mice following 900 mg/kg gavage dosing was evaluated. Absorption, metabolism and BP metabolite profiles were comparable between sexes. BP is rapidly absorbed and excreted primarily via urine. The majority of radioactivity was eliminated within 24 hrs post-dosing. No bioaccumulation was observed after 168 hrs post-dosing in either sex. BP was mainly metabolized by hydroxylation, followed by methylation, and conjugation with sulfate and glucuronide, resulting in 26 identified metabolites. However, systemic exposure to BP and metabolites was substantially higher in females, as evidenced by slower terminal blood elimination half-life of 671 hrs vs. 99 hrs for males, and 2-fold higher blood AUC in females than in males. Thus in females, the cellular uptake and metabolism of BP is different compared to males. These results are consistent with Umeda et al. (2005) findings and point to differences in sex-specific systemic exposures at high BP doses underlying sex-specific liver tumor findings in BDF1 mice. Sponsored by the Biphenyl Work Group of SOCMA.

1100 Mice with a Heterozygous Deletion of S-Nitrosoglutathione Reductase (GSNOR/ADH5) Display Normal O6-alkylguanine-DNA Alkyltransferase Levels and Are Protected from DEN-Induced Hepatocarcinogenesis


Recently, studies have demonstrated that total genetic deletion of S-nitrosoglutathione reductase (GSNOR) in mice (GSNOR-/-) leads to a higher incidence of hepatocellular carcinoma (HCC), possibly through uncontrolled INO-induced nitrosation and subsequent proteosomal degradation of O6-alkylguanine-DNA alkyltransferase (AGT). Mice deficient in AGT show increased susceptibility to hepatocarcinogenesis induced by DEN as compared to WT mice. The objectives of these studies were to investigate whether mice with a heterozygous deletion of GSNOR/ADH5 (GSNOR+/-) were protected from tert-butyl hydroperoxide (TBHP) and DEN-induced apoptosis of AGT and subsequent hepatocarcinogenesis following treatment with diethylnitrosamine (DEN) as compared to mice containing a complete genetic deletion of GSNOR (GSNOR-/-) or wild type (WT) control mice. Additionally, we investigated INOS expression in embryonic fibroblasts of WT and GSNOR+/-- mouse and liver cancer cDNA arrays for ADH expression. GSNOR+/-- mice were given a single IP injection of DEN and later sacrificed to study AGT protein expression and tumor development in the liver. In the DEN-treated GSNOR+/-- mice, AGT levels were equivalent to levels seen in WT control animals and with no increased incidence of hepatocellular tumors. Mechanistically, INOS expression appeared to be critical to the development of HCC, rather than GSNOR expression. We also observed that multiple ADH genes, which reside at the same chromosomal locus as GSNOR (4q23), were depleted in HCC patients, and the reduction in expression level correlated with more advanced tumors. The findings indicate that, in contrast to complete genetic deletion of GSNOR, GSNOR+/-- mice express normal AGT activity and DEN treatment did not increase the incidence of HCC. Lastly, ADH profiling of the liver cancer cDNA arrays demonstrated broad effects on the ADH genes, rather than a GSNOR-specific effect, correlating with the advancement of the tumors.

1101 Histone Acetylation Alterations and Monomethylarsonous Acid (MMAIII)-Induced Cell Malignant Transformation

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Chronic arsenic exposure is known to be associated with increased morbidity and mortality from both cancerous and non-cancerous effects. Inorganic arsenic (iAs) and its high toxic metabolite, monomethylarsonous acid (MMAIII), is capable of inducing malignant transformation in human cells, and population-based studies have shown that an increase in the percentage of urinary and blood arsenic present inducing malignant transformation in human cells, and population-based studies have shown that an increase in the percentage of urinary and blood arsenic present...
the MMAIII-mediated up-regulation of the expression and activities of HDACs, leading to increase histone acetylation and prevention of MMAIII-induced malignant transformation. These new findings suggest that histone acetylation dysregulation may be a key mechanism in MMAIII-induced malignant transformation and carcinogenesis, and that HDAC inhibitors could be targeted to prevent or treat arsenic-related cancers.

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**1104 Transcriptionomics to Elucidate Oxidative Stress Mechanisms Involved in Genotoxic and Nongenotoxic Liver Carcinogenesis**


Genotoxic (GTX) and non-genotoxic (NGTX) carcinogens are known to induce multiple molecular changes and cellular damage in causing liver toxicity and/or carcinogenesis. Currently, the specific mechanisms for these different classes of compounds that induce hepatocarcinogenesis are still unknown but oxidative stress seems to be important. Therefore, a better understanding of cellular changes induced by compounds from these different classes in relation to oxidative stress is needed.

We selected 3 oxidative stress-inducing compounds (Azathioprine (AZA), Tetradecanoyl phorbol acetate (TPA), Dithiazin (DZN)) and 3 non-oxidative stress related compounds (Furan (Fu), Tetrachloroethylene (TCE) and D-mannitol (Dman)). In a human hepatoma cell line (HePG2), whole genome gene expression was measured, analyzed and correlated to (oxidative) DNA damage and cell cycle changes at 5 different time points (4, 8, 24, 48 and 72h) after exposure to these compounds.

ROS formation and (oxidative) DNA damage and cell cycle arrest was confirmed after AZA, TPA and DZN treatment and appeared to be related to a 10-fold higher number of gene expression changes over time compared to Fu, TCE or Dman treatment, indicating that this is due to cellular oxidative stress. Different genes and pathways were found for oxidative stress. However, a higher number of gene expression changes overlapped between AZA, TPA and DZN being especially involved in oxidative stress, DNA damage and immune responses. Genes such as Nrf2, GCLC, HMOX1 and GSR were significantly upregulated. This was not observed after Fu, TCE or Dman treatment, thus indicating that carcinogenic processes are not solely caused by oxidative stress. However, cellular oxidative stress has a high impact on cellular changes such as DNA damage gene expression and cell cycle distribution, and for certain compounds, may represent the underlying cause for increasing the risk of liver toxicity and even carcinogenesis.

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**1105 Functional Role of Long-Noncoding RNAs in Rhabdomyosarcoma Cells and Tumors**

G. Chadalapaka1, J. D. Jutooru1 and S. H. Safe1, 2, 1VTPP, Texas A&M University, College Station, TX and 2Institute of Biosciences and Technology, Texas A&M Health Science Centre, Houston, TX.

Long noncoding RNAs (lncRNAs) >200 bp in length are now emerging as critical factors in tumor biology. Reports suggest that dysregulated lncRNA expression in cancer correlates with disease progression and may serve as an independent predictor for patient outcomes. Little is known about the lncRNA regulation in rhabdomyosarcoma (RMS), a disease which is prevalent in children as the most common soft-tissue sarcoma and accounts for approximately 50% of soft-tissue sarcomas. Regulation of lncRNAs is not well defined and development of drugs that target oncogenic lncRNAs has not been reported. HOXA transcript at the distal tip (HOTTIP) is a 3,764 bp lincRNA located -330 bases upstream of HOX A13 and Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) are two lncRNAs highly expressed in RH30 and RD RMS cell lines and the function of the lncRNAs was investigated by RNA interference (RNAi). Knockdown of MALAT-1 in RH30 and RD cells resulted in a 57% and 60% decrease in cell proliferation respectively and we also observed a  60% decrease in cell migration in a Boyden chamber assay. We also observed that MALAT-1 silencing increased markers of apoptosis (PARP cleavage) in RMS cells and these were consistent with a pro-oncogenic function for MALAT-1 in RMS and similar results were reported in other solid tumors. In contrast, the function of HOTTIP has not previously been reported in cancer cells and our results show that like MALAT-1, knockdown of HOTTIP by RNAi also decreases cell proliferation and migration and induces apoptosis. Current studies are focused on the overlapping and independent regulation of genes by MALAT-1 and HOTTIP and development of agents that target these pro-oncogenic lncRNAs.

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**1106 HOXA Transcript at the Distal Tip (HOTTIP) is a Pro-Oncogenic Long-Noncoding RNA in Pancreatic Cancer**

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Recent studies demonstrate that non-coding RNAs (ncRNAs) are differentially expressed and play an important role in gene regulation and influence normal and cancer cell phenotypes, and this is true, in part, to association with chromatin-mod-
ifing complexes. HOXA transcript at the distal tip (HOTTIP) is a 3,764 bp lin-
cRNA transcribed from 5’tip of HoxA gene cluster and is expressed in anatomically distant cells. Previous studies in our laboratory showed that HOTTAIR is a negative prognostic marker in pancreatic cancer and HOTAIR cooperatively represses gene expression with complexes such as Polycomb Repressive Complex 2 (PRC2). It has been reported that HOTTIP interacts with MLL-1 complexes by specifically bind-
ing the WDR5 adapter protein leading to a loss of H3K4me3 across the HoxA locus. In this study, we investigated the functional role of HOTTIP in Panc1 and L3.6pl pancreatic cancer cell lines. Knockdown of HOTTIP by RNA interference resulted in decreased expression of 514 & 575 genes respectively in Panc1 cells. RT-PCR confirmed that HOTTIP regulated expression of several pro-oncogenic factors includ-
ing Aurora kinase A (AURKA), AHI1, CD44. Knockdown of MLL-1 also decrease AURKA expression, however regulation of AURKA by HOTTIP/MLL-1 was WDR5-independent. Further analysis of array data suggests a less prominent role for HOTTIP, MLL1/WDR5 interactions in pancreatic cancer cells compared to non-tumor cells (fibroblasts). Functional interactions of HOTTIP with other chromatin-modifying complexes are currently being investigated.

PS 1107 Determination of Artifacts, the Steady State, and Half-Life of N2-Hydroxymethyl-4G Adducts In Vitro, and in a 28-Day 13CD2-Formaldehyde Inhalation Exposure Study
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Rationale: N2-HOMe-dG is the main formaldehyde-induced DNA mono-adduct, which plays important roles in mutagenesis and carcinogenesis. The purpose of this study is to provide the distribution pattern of exogenous DNA adducts, and further tell whether or not inhaled formaldehyde can reach tissues distant to sites of initial contact. We have also undertaken studies related to artifact formation during DNA isolation and processing.

Methodology: DNA was isolated, reduced, digested, and injected onto an Agilent HPLC-UV for adduct fractionation. DNA adducts were quantitated using nano-
LC-MS-MS (with a limit of detection of 1.25 attomolar).

Results: Artifacts formation was less than 0.56% and 0.46% of the average amounts of endogenous adducts in all tissues (n=205) from sodium phosphate buffer and NucleoBond DNA isolation kit, respectively. Exogenous adducts were only de-
tected in rat nasal respiratory epithelium, the amounts of which were 3- to 8-fold lower than the average amounts of endogenous adducts. Additionally, inhaled exo-
genous adducts were found to accumulate, with the time to steady-state concen-
trations being 28 days. The unstable exogenous adducts were found to follow a bi-Exponential decay model, and the half-life was determined to be 5.2 days. The unstable exogenous adducts were also found to follow a biophilic decay model in isolated calf thymus DNA in vitro.

Conclusions: Our study showed that inhaled formaldehyde only reached rat nasal respiratory epithelium. For the first time, this study has elucidated accumulation of exogenous adducts, the time to reach steady state, and the half-life for the repair/loss of exogenous adducts in vivo. Overall, the data generated in this study provide pivotal information to understand the toxicity and carcinogenicity of formalde-
hyde, as well as the biological plausibility of leukemia induction following inhala-
tion exposure of formaldehyde.

PS 1108 Ablation of the bZIP Transcription Factor, CCAAT Enhancer Binding Protein-β (CEBPβ), in Skin Protects Mice from Skin Cancer Induced by Ultraviolet B Radiation
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Nonmelanoma skin cancer (NMSC) is the most common cancer in the United States. Each year there are more cases of NMSC than all cases of breast, prostate, lung and colon cancers combined. The majority of NMSCs is caused by solar UVB radiation which causes DNA damage. Sunlight is a ubiquitous environmental carcinogen and p53 plays a key role in the response of skin keratinocytes to UVB-induced DNA damage by causing cell cycle arrest and apoptosis. We observed that C/EβPβ is abundantly expressed in epidermis and that C/EβPβ protein levels are induced by UVB radiation in mouse and human keratinocytes in culture as well as in mouse epidermis in vivo. Activation of C/EβPβ often involves phosphorylation at threonine 188 (T188) and UVB treatment increased the phosphorylation of C/EβPβ at T188. In order to understand the function of C/EβPβ in UVB-induced responses in epidermis and in skin cancer, we developed a C/EβPβ deficient (C/EβPβ−/−) SKH-1 hairless mouse model. SKH-1 hairless mice are an established experimental model relevant to UVB-induced human skin cancer. Treatment of C/EβPβ−/− mice with UVB resulted in increased apoptotic cell death in epidermal ke-
ratinocytes when compared to similarly treated control mice. C/EβPβ−/− mice were highly resistant to UVB-induced skin cancer. In order to determine whether the resistance of C/EβPβ−/− mice to UVB-induced skin cancer is due to an intrinsic ke-
ratinocyte effect, we developed an epidermal specific C/EβPβ conditional knock-
out (C/EβPβ ERE−/−) mouse. The keratin 5 promoter directs Cre recombinase expression to the epidermis to delete floxed C/EβPβ alleles. C/EβPβ−/− mice were highly resistant to UVB-induced skin cancer and displayed increased levels of UVB-induced apoptosis. These studies indicate that C/EβPβ is induced and activated by UVB and has critical role in the development of UVB-induced skin cancer where it suppresses UVB-induced apoptosis and in doing so may allow the survival of keratinocytes with DNA damage and mutations.

PS 1109 Western Diet Accelerates Benzo[a]pyrene [B(a)P]-Induced Colon Tumorigenesis in the PIRC Rat Model
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Colorectal cancer ranks third in terms of mortalities in the United States. Consumption of Western Diet (WD; rich in red meat and fats), contaminated with polycyclic aromatic hydrocarbons (PAHs) has been implicated as one of the causative agents for sporadic colon cancer. Therefore, the aim of this study was to investigate whether the cancer burden was amplified by WD when exposed to benzo(a)pyrene [B(a)P], a PAH compound. To test this concept, we have used an adult male transgenic rat model, the Polyposis In the Rat Colon (PIRC) kindred type. Treatment consisted of 25, 50 and 100 μg B(a)P/kg bw dissolved in tricaprylin, administered to rats daily via oral gavage for 60 days. One group of B(a)P-treated rats were fed with AIN-76A regular diet (RD) and the other group with WD throughout the study. Rats that received the diets alone, but no B(a)P served as controls. Food consumption and body weight were peri-
diodically monitored. Subsequent to exposure, rats were sacrificed; colons, and other tissues were retrieved and preserved in 10% formalin for observation of gross pathological changes. Blood samples were collected and concentrations of choles-
sterol, triglycerides, and adiponectin were measured. There was no change in food consumption, but body weight loss of WD group compared to RD group and controls (p < 0.005) was noticed. An increased incidence of adenomas and high grade dysplasia were encountered in rats that were fed with WD compared to RD and controls (p < 0.05). The colon tumor numbers showed a B(a)P dose-response relationship. Rats that received B(a)P + WD showed increased levels of cholesterol and triglycerides in comparison to rats that received B(a)P + RD and also controls. Levels of adiponectin did not vary much between B(a)P + WD, and B(a)P + RD groups. Our results showed that WD accelerates the development of colon tumors induced by B(a)P through proinflammatory action, characterized by gain in tumor number and sizes, and body weight loss.

PS 1110 Nuclear Receptor-Mediated Effects of the Mammary Carcinogens Benzo[a]pyrene and PhIP in MCF-7 and MDA-
MB-231 Cancer Cell Lines

Breast cancer is the most commonly diagnosed malignancy in females. Its aetiology is multifactorial, and the role of environmental exposure to DNA damaging chem-
icals has been widely investigated. Benzo[a]pyrene (B(a)P) and 2-Amino-1-methyl-6-
phenylimidazo[4,5-b]pyridine (PhIP) are mammary carcinogens in rodents. Both compounds require cytochrome P450 (CYP) mediated metabolic activation to DNA damaging species, and both induce transcriptional responses through the nuclear receptors ARYL hydrocarbon receptor (AhR) and estrogen receptor α (ERα). The objective of this research was to investigate the molecular toxicology of B(a)P and PhIP in ERα positive (MCF-7) and ER- negative (MDA-MB-231) cancer cell lines. MCF-7 and MDA-MB-231 cells were incubated with various concentrations of BaP and PhIP for up to 96 hrs. Genotoxicity, drug metabolism activity, CYP expression, proliferation and cell migration were examined. BaP showed a dose-de-
pendent increase in micronuclei (MN) formation in both cell lines, while PhIP expressed only in MCF-7 cells. Ethoxyresorufin-O-deethylase (EROD; CYP1A)
activity and CYP1A1 and 1A2 expression was induced in both cell lines by BaP, but PhIP only induced CYP1A2 mRNA in MCF-7 cells. Cell proliferation and migration was induced in both cell lines by BaP, but only in MCF-7 cells by PhIP.
These data demonstrate the ability of BaP and PhIP to induce DNA damage and receptor-mediated effects in mammary cells. Furthermore, the differential effect of PhIP in the two cell lines supports the role of ERβ in the toxicity of PhIP.

**1110a** Estrogen Receptor Alpha Modulation and Differential Cytotoxicity of Caffeic Acid Phenethyl Ester in Prostate Cancer Cells

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Caffeic acid phenethyl ester (CAPE) is a promising natural component of propolis that possesses selective estrogen receptor modulatory (SERM) activity. The class of compounds known as SERMs are promising therapeutic agents for patients with castration-resistant prostate cancer (PC). However, the potential SERM activity of CAPE in PC has not been evaluated before. In this investigation we test the hypothesis that, in PC cells (PC-3, DU-145, LNCaP), the differential cytotoxicity of CAPE is dependent on the cells’ estrogen receptor (ER) status. To test our hypothesis, we assessed CAPE cytotoxicity in the androgen dependent cell line LNCaP and the androgen-independent cell lines PC-3 and DU-145 using sulforhodamine B (SRB) assay. CAPE exhibited greater cytotoxicity in PC-3 compared to DU-145 and LNCaP and was least potent in nontransformed prostate epithelial cell line BPH1. Western blot analysis for ERα in the 3 cell lines confirmed that only PC-3 expresses this ER subtype. Furthermore, we evaluated the impact of CAPE on AKT1/2/3 (Ser473) as well as ERK1/2 (Thr202/Tyr204, Thr185/Tyr187) activation in the tested PC cells since Akt and ERK pathways are crucial for PC tumor aggressiveness and progression and due to their known cross-talk with ERα. Our data indicate that CAPE significantly reduced both Akt and ERK activation in PC-3 cells while it only reduced the activation of Akt in LNCaP and ERK in DU-145 cells. Our study shows, for the first time, that CAPE-induced inhibition of Akt activation is more pronounced (1.7 folds higher) in cells expressing ERα such as PC-3 cells compared to LNCaP. CAPE treatment reduced ERα phosphorylation at Ser-167, which is known to be related to Akt activation, further supporting the evidenced reduction in Akt activation by CAPE in this specific cell line. These data indicate that the cytotoxic effects of CAPE in prostate cancer are partly mediated by ERα modulation.

**1110b** Cholorogenic Acid Rich Plum Juice Inhibited AOM Treated Colorectal Aberrant Crypt Foci (Acf): Potential Role of miR145/3/MTOR Pathway

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The antitumorigenic and cytotoxic activities of polyphenols, caffeic acid and neo-caffeic acid in plum juice (PL) have been previously studied in various cancer models. Our preliminary result exhibited anti-inflammatory property of PL which decreased the expression of VCAM-1 and the activation of PI3K/AKT, and was least potent in nontransformed prostate epithelial cell line BPH1. Hence, we hypothesized that the cytotoxic effects of CAPE in prostate cancer are partly mediated by ERα modulation. Consumption of PL juice suppressed the number of high multiplicity aberrant crypt foci (HMACF>4 ACF) by 47.98% (P<0.05) and significantly lowered proliferation of mucosa cells. The results were accomplished by a downregulation of nuclear factor kappa B (NF-kB), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), vascular adhesion molecule-1 (VCAM-1) and mRNA and protein. PL also inhibited phosphorylation of PI3K/AKT, and mTOR/PHF1-tel pathways. PL increased the expression of miR143 expression. In summary, PL suppressed number of HMACF formation, colon tumorigenesis where microRNA-143-regulated pathways significantly contributed in the underlying cytotoxicity mechanisms.

**1110c** ARNT Isolforms Differentially Regulate Cancer Cell Growth through a p53-Dependent Mechanism

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In addition to its roles in endocytic and hypoxic responses, we have shown that the arylhydrocarbon receptor nuclear translocator (ARNT) is an integral cofactor of NF-kappaB signaling. However, these initial observations of ARNT-mediated NF-kappaB modulation were based on simultaneous suppression of the two ARNT isoforms, isoform 1 and 3, and therefore precluded the isolated examination of each isoform’s function. We show here that lymphoid malignancies exhibit a shift to higher levels of ARNT isoform 1, compared to normal lymphocytes that harbor equal levels of isoform 1 and 3. The increase in ARNT isoform 1 is necessary for the growth of these cancer cells as suppression of isoform 1 resulted in S-phase cell cycle arrest. Interestingly, NF-kappaB signaling was unaffected by suppression of isoform 1. However, co-suppression of p53 with ARNT isoform 1 rescued the arrest phenotype, suggesting that the observed cell cycle arrest was dependent on p53 activity in the absence of isoform 1. Furthermore, suppression of isoform 1 sensitized cells to low levels of doxorubicin, an effect that was also rescued by suppressing p53. These findings reveal that ARNT isoform 1 potentiates cell growth by antagonizing a p53 cell cycle inhibitory mechanism and suggests that ARNT targeted therapies would benefit chemotherapy regimens.

**1110d** Ligand-Dependent Activation of EGFR Mediates Malignant Cell Transformation Induced by Chronic Exposure to Hexavalent Chromium


Hexavalent chromium (Cr (VI)) compounds are well-established lung carcinogens. The carcinogenic mechanisms of Cr (VI) are currently under investigation. Epidermal growth factor receptor (EGFR) is a tyrosine kinase transmembrane receptor that regulates cell survival, cell cycle progression, tumor invasion, and angiogenesis. The present study investigates the role of EGFR in Cr(VI)-induced cell transformation and tumorigenesis. Our results show that chronic exposure of human bronchial epithelial (BEAS-2B) cells to Cr (VI) caused malignant cell transformation. The Cr (VI)-transformed cells exhibited apoptosis resistance with reduced expressions of cleaved Poly ADP ribose polymerase (C-PARP) and Bax and enhanced expressions of Bcl-2 and Bcl-xL. Reduced capacity of generating reactive oxygen species (ROS) along with elevated expression of antioxidant manganese superoxide dismutase (SOD2) was also found in Cr (VI)-transformed cells. Phosphorylation of EGFR was dramatically increased in both Cr (VI)-transformed BEAS-2B cells and lung tissue of animals exposed to Cr(VI) particles. Expression of amphiregulin (AR), a EGFR ligand, was elevated in Cr (VI)-transformed cells. Knockdown of EGFR or AK or increased ROS generation, resulting in elevated expressions of C-PARP and Bax and reduced expression of Bcl-2, leading to reduced apoptosis resistance in Cr (VI)-transformed cells. PI3K and AKT, downstream targets of EGFR, were also activated in Cr (VI)-transformed cells. Treatment the Cr (VI)-transformed cells with LY492002 and wortmannin, inhibitors of PI3K, caused ROS generation, resulting in increased Bax expression and reduced Bcl-2 expression, leading to increase of apoptosis. Most importantly, the results from xenograft mouse tumor study showed that knockdown of EGFR reduced both the size and frequency of tumor compared to Cr (VI)-transformed cells. In summary, the present study indicates that ligand-dependent activation of EGFR plays an important role in Cr (VI)-induced malignant cell transformation and tumorigenesis.

**1110e** The Effects of Quercetin on the Placental Transport of the Dietary Carcinogen 2-Amino-1-methyl-6-phenylimidazo(4, 5-b)pyridine


The heterocyclic amine 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) is a dietary carcinogen formed from cooking meats at high temperatures. PhIP readily passes through the intestinal barrier, as well as the placental barrier, increasing bioavailability and potentially exposing a developing fetus to harmful substances. Recent evidence has shown that certain flavonoids increase PhIP intestinal barrier crossing in vivo, and increases bioavailability in vivo, although it is unclear if this is also the case with the placental barrier. The current study aimed to determine the extent at which PhIP crosses the placental barrier using BeWo cells as a model, and determine if flavonoids can influence the rate of transport across
the barrier. BeWo cells were first cultured in transwell plates and dosed with 0, 1, 5, 10, or 100μM PhIP, and the barrier penetration was quantified at 0, 30, 60, 120 and 240 min after dosing. The amount of PhIP crossing over time was concentration-dependent, which is similar to Caco2 cells. However, when the BeWo cells were incubated with 2 or 20μM quercetin prior to a dose of 10μM PhIP, there were no observable changes in the rate or amount of PhIP penetration. This is in contrast with the Caco2 cell culture models, suggesting a different transport mechanism between the cell types. This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Formaldehyde (FA) inhalation is linked to nasopharyngeal and sino-nasal cancer and myeloid leukemia in humans. While there is a strong mechanistic basis behind FA inhalation and causation of nasal cancer in humans, the mechanism linking FA and leukemia is unclear. Cell proliferation and DNA adducts in the nasal cavity are recognized as key mechanistic events in the induction of nasal cancer. Inhaled FA does not induce formation of detectable levels of DNA adducts at sites other than the nasal cavity; however, FA may cause leukemia by another mechanism. It was hypothesized that inhaled FA could cause significant genetic damage to stem cells in the nasal epithelium or circulating in local blood vessels. These damaged stem cells could reach the general circulation, seek out tissue sites that support the hematopoietic niche, undergo lodgment and become leukemic stem cells. We tested this hypothesis by exposing groups of 25 male C3B6.129F1-Trp53tm1Brd mice to 0, 7.5 or 15 ppm FA 6h/d, 5d/w for 8w and then holding mice without further exposure until 50 weeks old. At necropsy, blood was collected for hematological and histological evaluation. The primary exposure-related finding was minimal hematopoietic niche involvement, which was disregarded as being not related to FA inhalation. There was no evidence of leukemia in FA exposed mice based on hematology.

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pluripotent stem cells (iPSCs) has made the development of assays measuring cardiac myocyte contractility possible. One such system, the xCELLigence RTCA Cardio (Acea Biosciences), uses electrical impedance to measure the amount of cell to surface contact in an electrode containing tissue culture plate. We used the RTCA system to measure the effect of known proarrhythmic compounds on the contractility of iCell cardiomyocytes (Cellular Dynamics Inc.). Endpoints measured for 24 hours in cardiomyocytes included beat rate, amplitude, and beat rate irregularity. Compounds known to cause cardiotoxicity through a variety of mechanisms, including hERG channel blockers, Ca2+ channel blockers, multichannel blockers, inhibitors of myosin II, and beta blockers were used as a validation set. This ruled out data on these compounds differently affected cardiomyocyte contractility following acute exposure or longer periods of treatment. For example, aconitine, which binds to sodium channels, caused an immediate increase in beating rate while pentamidine, which affects hERG trafficking to the membrane, affected beating rate only several hours following exposure. Blebbistatin, which interacts with myosin, caused a dose dependent delay in contraction, but did not affect beat rate or conduction which was completely inhibited. hERG inhibitors including capsiate and terfenadine affected beat rate and caused irregular beats. The xCELLigence system detected the effects of diverse compounds on cardiomyocyte contractility, and may be useful for the prediction of cardio toxicity.

1114 A Novel Model for Proarrhythmic Assessment in iCell Cardiomyocyte Using a Multielectrode Extracellular Recording System


Prolongation of the cardiac action potential and the QT interval are potential causes of the ventricular tachycardia that is characteristic of torsades de pointes (Tdp). However, it has been increasingly obvious that a prolongation of the QT interval does not necessarily lead to Tdp. To bridge this dissociation, a lot of work in vitro/vivo models for proarrhythmic effects have been developed. Recently, human iPSC derived cardiomyocytes (hiPSC-CM) have become a valuable tool for safety assessment in drug discovery. Present studies examined the effects of cell density and experimental duration on iCell cardiomyocytes with MED64 system. Low-density cell plating lead to enlargement of hiPSC-CM. Gene expression analysis and pathway analysis reveal similarities between enlarged hiPSC-CM and cardiac hypertrophy. Low-density cell plating also decreased expression of the KCNQ1 gene and affected the response to Iks blockers. The concentration-response curves for Chromanol 293B and HMR1556 shifted to the right when cell densities were decreased from 30000, 15000, 7500 and 3750 cells/well. When the experimental temperature was changed from 38.5 to 35.0 °C, the corrected field potential duration (FDPc) increased from 285 to 370 msec (24 msec/°C) almost linearly. Under the condition of both low-density cell plating and low experimental temperature, E4031 caused early afterdepolarizations (EADs) more frequent than in normal temperature. In conclusion, the plating cell density (cell size) and experimental temperature are very important as the determinants of drug sensitivity and EAD induction in field potential recording experiments with hiPSC-CM.

1115 Identification of Pro-Arrhythmic Potential with Human iPS-Derived Cardiomyocytes Using a Multiwell Microelectrode Array (MEA)


Cardiotoxicity is the most common reason for attrition due to toxicity. Current in vitro cytotoxicity assays identify only the most overtly toxic compounds, while assays to measure more specific liabilities such as hERG and other ion channels may fail to detect a response which relies on a combination of endpoints or a delayed response due to expression. Toxicity determination relies heavily on the later preclinical phase animal studies which have much higher costs associated and can have species specific results which may not model human responses. Therefore, an ideal assay for predicting cardiotoxicity would involve screening earlier on a platform whose investigation can contribute to a thorough cardiac safety assessment. A beating cellular system integrating all the ion currents that contribute to the field potential (FP) allows in vitro testing of drug effects on the field potential duration (FDP) and thus prediction of the clinical QT interval’s response. Low-impedence microelectrode arrays (MEAs) enhanced sensitivity allows screening for early afterdepolarizations (EADs), the initiating mechanism of the drug-induced cardiac arrhythmia, Torsades des pointes. Short term variability of repolarization (STVrep), the correlate of electrical alternans, is an additional parameter whose investigation can contribute to a thorough cardiac safety assessment. hSC-CMs were plated on low-impedence MED-P504A MEA probes and signals were acquired using a MED64 amplifier (Alpha Med Scientific Inc., Osaka, Japan) running Mobius QT software (Witwex Inc., Tustin CA). Data were analyzed using Intelligent Waveform Service (Neural ID, Redwood City CA). A thorough panel of drugs was tested, including the torsadogenic hERG blockers sotalol and quinidine, the false positive (non-torsadogenic) hERG blocker verapamil, and the false-negative hERG blocker alftuzoxin. All drugs produced the expected qualitative and quantitative effects on the FP. Most importantly, EADs and ectopic beats were found in 50% of the hiPSC preparations treated with sotalol and 40% treated with quinidine, as well as increased STVrep. We conclude the MEA/hSC-CM platform’s capability to screen for EADs and STVrep is ideal for better predicting a drug’s proarrhythmic risk, thus enabling a more thorough cardiac safety assessment, but most importantly reducing drug attrition and development costs.

1116 Advanced Cardiotoxic Screening Using Human Stem Cell-Derived Cardiomyocytes and a Low-Impedence Microelectrode Array System for Proarrhythmic Risk Prediction

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Research on human heart tissue has been enabled thanks to advances in stem cell biology. In fact, the drug industry and regulatory agencies are considering human stem cell-derived cardiomyocytes (hSC-CMs) as a standard assay for preclinical cardiac safety screening. A beating cellular system integrating all the ion currents that contribute to the field potential (FP) allows in vitro testing of drug effects on the field potential duration (FDP) and thus prediction of the clinical QT interval’s response. Low-impedence microelectrode arrays (MEAs) enhance sensitivity allows screening for early afterdepolarizations (EADs), the initiating mechanism of the drug-induced cardiac arrhythmia, Torsades des pointes. Short term variability of repolarization (STVrep), the correlate of electrical alternans, is an additional parameter whose investigation can contribute to a thorough cardiac safety assessment. hSC-CMs were plated on low-impedence MED-P504A MEA probes and signals were acquired using a MED64 amplifier (Alpha Med Scientific Inc., Osaka, Japan) running Mobius QT software (Witwex Inc., Tustin CA). Data were analyzed using Intelligent Waveform Service (Neural ID, Redwood City CA). A thorough panel of drugs was tested, including the torsadogenic hERG blockers sotalol and quinidine, the false positive (non-torsadogenic) hERG blocker verapamil, and the false-negative hERG blocker alftuzoxin. All drugs produced the expected qualitative and quantitative effects on the FP. Most importantly, EADs and ectopic beats were found in 50% of the hiPSC preparations treated with sotalol and 40% treated with quinidine, as well as increased STVrep. We conclude the MEA/hSC-CM platform’s capability to screen for EADs and STVrep is ideal for better predicting a drug’s proarrhythmic risk, thus enabling a more thorough cardiac safety assessment, but most importantly reducing drug attrition and development costs.

1117 Regulation of Superoxide and Mitochondrial Biogenesis by PGC-1α Protects against Doxorubicin-Induced Toxicity in Cardiomyocytes

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Objective Doxorubicin (DOX) is an efficient and widely used anthracycline antibiotic for the treatment of tumors, but severe cardiotoxicity limits its application. Superoxide has been proposed to be mainly responsible for the cardiotoxicity of DOX, which is usually associated with mitochondrial dysfunction. PPARγ coactivator 1α (PGC-1α) is a nuclear-encoded transcriptional coactivator which plays a central role in mitochondrial biogenesis. Previous studies suggested that inhibition of mitochondrial biogenesis was responsible for the pathogenesis of DOX-induced cardiomyopathy. Nevertheless, the exact roles of PGC-1α in mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity remain unexplored. The present study was conducted to understand the exact role of PGC-1α in mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity. Methods and results A human cardiac cell line, AC16, was used in the study. We treated AC16 cells with 10 different doses (0-2000 nM) of DOX for 12h. We demonstrated that low dose DOX treatment produced a low amount of superoxide in AC16 cells, which activated mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity. However, PGC-1α knockdown and superoxide scavengers inhibited mitochondrial biogenesis and superoxide metabolism, and protected against DOX-induced toxicity. Therefore, PGC-1α is a crucial player in the regulation of mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity. Objective Doxorubicin (DOX) is an efficient and widely used anthracycline antibiotic for the treatment of tumors, but severe cardiotoxicity limits its application. Superoxide has been proposed to be mainly responsible for the cardiotoxicity of DOX, which is usually associated with mitochondrial dysfunction. PPARγ coactivator 1α (PGC-1α) is a nuclear-encoded transcriptional coactivator which plays a central role in mitochondrial biogenesis. Previous studies suggested that inhibition of mitochondrial biogenesis was responsible for the pathogenesis of DOX-induced cardiomyopathy. Nevertheless, the exact roles of PGC-1α in mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity remain unexplored. The present study was conducted to understand the exact role of PGC-1α in mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity. Methods and results A human cardiac cell line, AC16, was used in the study. We treated AC16 cells with 10 different doses (0-2000 nM) of DOX for 12h. We demonstrated that low dose DOX treatment produced a low amount of superoxide in AC16 cells, which activated mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity. However, PGC-1α knockdown and superoxide scavengers inhibited mitochondrial biogenesis and superoxide metabolism, and protected against DOX-induced toxicity. Therefore, PGC-1α is a crucial player in the regulation of mitochondrial biogenesis and superoxide metabolism in DOX-induced cardiotoxicity.
Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) share a primary cardiomyocyte-like physiology with the advantages of a cell line, e.g., large batch sizes, low batch-to-batch variation, and a convenient "read-to-use" format. We have used hiPSC cardiomyocytes from diverse cell suppliers in automated patch clamp and impedance assays and show that this approach is of great value in cardiotoxicity prediction. The label-free impedance platform, the CardioExcyte 96, allows precise impedance measurements at a time resolution of 1 ms and generates 96 recordings in parallel. The automated patch clamp device Patchliner generates up to 500 dk per day and is capable of generating pharmacological experiments, IC50 or EC50 data and action potential signals from hiPSC-CM.

Cells from main stem cell suppliers were successfully tested and data will be compared. Dose response curves or single-point screening experiments of several drugs (e.g., hERG and Cav1.2 blockers) were collected and will be presented. The presented data show how these systems can be used efficiently to predict drug-induced arrhythmia for cardiac risk assessment.

Human induced-pluripotent stem cell derived cardiomyocytes (hiPSC-CM) may be used as a powerful in vitro model for predictive cardiac safety assessment and could allow for better identification of compounds with poor arrhythmogenic liability profiles in the early drug discovery process. To enable HT arrhythmogenic testing in hiPSC-CM, we have developed the Kinetic Image Cytometer (KIC) for high throughput, automated cell-by-cell analysis of intracellular calcium transient dynamics. Calcium transients integrate the electrochemical signals of the action potential with the molecular signaling pathways that regulate contraction. Drug-induced alterations in the shape and duration of the cardiomyocyte action potential result in changes to the shape and duration of the intracellular calcium transient. Therefore, by examining calcium transient dynamics in hiPSC-CM a single assay can be used to screen for compound effects across multiple ion channel types and is also capable of detecting multi-channel effects. In the present study, we used KIC technology to assess the predictive values of hiPSC-CMs using a library of 90 compounds known to alter the activity or expression level of cardiac ion channels/ receptors resulting in changes in the cardiac action potential. Our data indicate that using hiPSC-CMs, KIC is able to detect known drug-induced changes in Ca2+ transients and therefore, may potentially predict drug-induced arrhythmogenic liabilities in early drug discovery.

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Cardiovascular dysfunction is the most severe complication in patients with diabetes mellitus. Among several causes, it has been recently demonstrated that endothelial progenitor cells (EPCs), a circulating adult stem cell regenerating damaged vascular endothelium, are dysfunctional under diabetic condition resulting in impaired peripheral circulation. To elucidate the mechanisms underlying diabetes-associated cardiovascular impairment, we investigated the cellular alteration of EPCs under high glucose condition. Bone marrow-derived EPCs (BM-EPCs) were exposed to normal glucose (5.5 mM) or high glucose (30 mM)-containing medium. High glucose treated-EPCs showed decreased ability to form tubule-like networks in Matrigel compared to EPCs in normal glucose. The conversion of LC3-II from LC3-I in EPCs was significantly increased, suggesting that autophagy occurs under high glucose condition. Interestingly, increased autophagy was not accompanied with phosphorylation of mTOR, reflecting that high glucose-induced autophagy was not related with mTOR signaling pathway. Increased generation of reactive oxygen species and disruption of mitochondrial function was observed in EPCs under high glucose condition. In conclusion, we demonstrated that ROS-mediated autophagy is induced in EPCs under high glucose condition, giving a new insight into the mechanism underlying dysfunction of diabetic EPCs.
expression of the AhR, phosphorylation of p38MAPK and cPLA2 and eventual secretion of TXB functions in a feedback loop resulting in platelet priming. These findings suggest that ligand binding to the platelet AhR may play a key role in the vascular response to particulate matter. All studies were conducted under IRR 299969-3. These studies were supported by a contract with the California Air Resources Board. The statements and conclusions in this study are those of the University of California Davis and are not necessarily those of the California Air Resources Board.

1128 IL-33 Cardiovascular Safety Profile Redefined: Lessons Learned from Rat and NHP Cardiomyocytes, KO Mice, and Human GWAS Studies

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IL-33 is a cytokine alarmin implicated in innate/adaptive immune responses, as well as in cardiac volume overload diseases (i.e., congestive heart failure). Some evidence suggest an IL-33-dependent decrease in cardiomyocyte hypertrophy is heart protective, rescuing the ventricles from dysfunction and fibrosis. To evaluate the role of IL-33 in normal cardiac function an IACUC approved de-risking strategy to re-define its safety profile was initiated. Methods: Cardiac structure and function were assessed in neonatal rat and adult non-human primate (NHP) cardiomyocyte hypertrophy assays. IL-33 knock out (KO) mice via echocardiography (echo) and histology; and human genome-wide association studies (GWAS) to correlate IL-33 and its receptor ST2 polymorphisms with CV disease. Results: Neonatal rat cardiomyocytes - Phenylephrine-induced cardiomyocyte hypertrophic gene expression (i.e. myosin heavy chain 7) was antagonized by rhIL-33. Adult NHP cardiomyocytes – No drug-induced cardiomyocyte hypertrophic gene expression was detected in preliminary experiments. IL-33 KO mice echo - Aged mice (-13 months): KO (n=21), heterozygote (n=32) were assessed. Baseline values were obtained from all, with 50% re-examined after a acute dobutamine-induced cardiac inotropic challenge. No heart rate, ejection fraction, or cardiac output differences were detected across any genotypes. GWAS: No strong IL-33 or ST2 association with CV disease (p<0.01). Summary: The role of IL-33 in preventing cardiomyocyte hypertrophy (rat cardiomyocytes) was confirmed. Cardiac structure and function were not adversely affected in aged KO mice. Human GWAS analyses did not support a high probability association of CV disease and IL-33.

1129 Utilization of Reverse Phase Protein Array (RPPA) Technology to Evaluate Multikinase Pathway Inhibition in Cell Lysates

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Targeted inhibition of these kinases has been shown to be beneficial in treating certain kinds of cancers. Unfortunately, most of these inhibitors may be promiscuous as the ATP binding sites are similar amongst kinases. Although the underlying mechanisms are not clear the adverse effects (like cardiotoxicity) observed by these inhibitors are attributed to off target inhibitions. Hence understanding the off target activities associated with potential kinase inhibitors is critical for designing safer inhibitors. Towards this end, we tested the Reverse Phase Protein Array (RPPA) technology from Carna Biosciences. This technique detects the phosphorylation status of 180 proteins across 18 critical cell signaling pathways simultaneously. RPPA technology utilizes compound treated cell lysates and phospho specific antibodies for detection. We treated NCI-H1975 (adenocarcinoma) cells with four PKC pathway inhibitors from 2 different chemical series (A&B: Series 1; C&D: Series 2) for 60 min followed by treatment of phorbol esters for 30 min. Cell lysates were then subjected for RPPA evaluation and phosphorylation status of 180 proteins was obtained. 30 minute treatment with phorbol myristate acetate (PMA) alone exhibited a phosphorylation signal (>1.5 intensity) for 18 different proteins across 7/18 different pathways (PI3K-AKT-mTOR (7), Ras-Raf (3), Cytoskeleton (2), DNA damage (2), RTK (2), Angiogenesis (1) and p38 MAPK (1)). PMA induced phosphorylation signal for most of the kinases was altered by compound A & B whereas compounds C & D did not alter these kinases. For example, PMA induced phosphorylation of ERK1/2 and ribosomal S6 protein was inhibited by compounds A & B (>2-9 fold) whereas C and D did not alter these signals. Overall, compounds from series 1 (A & B) clustered differently than compounds from series 2 (C & D) based on similarities in their RPPA profiles. RPPA can potentially be used to differentiate compounds based on their off-target activities. These changes in signaling pathways will be further confirmed using traditional approaches like western blotting and flow cytometry.

1129a Altered Hemodynamics Play a Critical Role in Fenoldopam-Induced Mesenteric Vasculitis in the Rat


Mechanisms underlying drug-induced vascular injury (DIVI) are poorly understood, and although several drugs induce DIVI in preclinical species, the risk to humans is unknown. For example, fenoldopam is an anti-hypertensive agent that elicits drug-induced hemodynamic changes and DIVI in the rat mesentery but presents no known vascular injury response in humans. The purpose of this study was to determine whether the alterations in fenoldopam-induced hemodynamics were necessary for fenoldopam-induced DIVI. Rats were administered fenoldopam (100 mg/kg s.c.) to induce DIVI and changes in mesentery hemodynamic blood flow were measured by ultrasound. To replicate the observed in vivo response in vitro, we utilized a co-culture platform with rat primary endothelial and smooth muscle cells where the endothelium is exposed to in vivo-derived hemodynamic waveforms to restore in vivo-like vascular cell responsiveness. Exposure of endothelial cells to the fenoldopam-induced hemodynamic waveform enhanced permeability compared to vehicle dose waveform that was independent of the exposure to fenoldopam in the platform. Gene analysis revealed significant changes in the endothelial and smooth muscle cell response to fenoldopam or its drug-induced hemodynamic response alone. The most differentially regulated pathways were observed in ECs when fenoldopam and its drug-induced hemodynamic response were combined. Biological themes included vascular-related biology, inflammation, oxidative stress, cell death, and cell remodeling. In conclusion, we provide evidence that supports the hypothesis that fenoldopam-induced changes in hemodynamics play a critical role in fenoldopam-induced DIVI in the rat mesentery.

1129b De Novo Cyclin D2 Induces Cardiomyocyte Proliferation in Adult Mouse Hearts

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Previous studies have shown that targeted expression of cyclin D2 under the regulation of the cardiomyocyte-restricted myosin heavy chain (MHC) promoter results in sustained cardiomyocyte cell cycle activity which was sufficient to promote regeneration following myocardial injury. Transgene expression in this model initiates prior to cardiomyocyte terminal differentiation. It thus remains to be demonstrated if de novo cyclin D2 expression in the adult myocardium would be sufficient to induce cardiomyocyte proliferation. In this study, we generated a model capable of conditional cyclin D2 expression in cardiac myocytes. The model utilizes an attenuated myosin heavy chain (atMHC) promoter to target expression of cyclin D2. The atMHC promoter is active only in the presence of VP16 trans-activator protein in the myocardium (MHC-tTA mice). In the absence of doxycycline (DC), the VP16 protein is active and drives transcription of the atMHC promoter. However, in the presence of DC, the VP16 protein is inactive. Thus, cyclin D2 expression in double transgenic animals can be turned off (by treating with DC) or turned on (no DC treatment). We have shown that in the absence of DC, atMHC-cycD2 / MHC-tTA double transgenic animals express cyclin D2 in cardiomyocytes and exhibit cardiomyocyte cell cycle activity as we have observed in our constitutive model. Furthermore, we demonstrated that cyclinD2 expression in the MHC-tTA/atMHC-cycD2 mice was blocked from embryo to 3 months old by low doses (10 mg/ml) of DC in the drinking water and reactivated after removing the DC for 10 days. Cardiomyocyte nuclear was identified by the introduction of β-galactosidase reporter gene under the control of cardiac myosin heavy chain promoter (MHC-nLAC). BrdU incorporation analyses revealed sustained cardiomyocyte DNA synthesis in the adult MHC-tTA/atMHC-cycD2/ MHC-nLAC mice. [Supported by NIH grant R01 HL109205].
The ubiquitous nature of plastics has raised concerns pertaining to continuous exposure to plastic additives and human health risks. Of particular concern is the use of endocrine-disrupting compounds (EDCs) in plastic production, including bisphenol A (BPA) and di-2(ethylhexyl)phthalate (DEHP). We previously reported the direct effects of DEHP and BPA exposure on cardiac calcium handling and electrical conduction in rodent cardiomyocytes and heart tissue. Our goal was to investigate the applicability of our previous findings to humans by examining the direct effect of EDC exposure on human stem cell-derived cardiomyocytes (hESC-CM). hESC-CM were derived from H7 embryonic stem cells, as previously described (Laflamme 2007). After 8-10 days, hESC-CM formed a confluent network that exhibited rhythmic spontaneous contractions. Cells were treated with BPA (0.1 - 100 μM) or DEHP (1 - 50 μM), loaded with 5 μM Fluo-4-AM, and then calcium transients were recorded using a Zeiss confocal imaging system. BPA treatment modified calcium handling parameters, including an increase in calcium release and calcium reuptake time, and a decrease in peak fluorescence amplitude. DEHP treatment resulted in complete cessation of contractile activity, via both spontaneous cell contractions and external pacing. We observed significant changes in calcium handling in human cells at lower EDC doses than for rodent cardiac cells. These findings illustrate key differences in cardiac models and emphasize the importance of examining the effect of EDCs on human cardiac cells of different origins (atrial, pacemaker, ventricular). Supported by NIH (K99ES023477 to NGP).

Maternal smoking is a risk factor for low birth weight and other developmental delays. We sought to determine the impact of extracts derived from e-cigarettes (e-cig) compared to standard tobacco cigarettes (3R4F) on heart development in vitro and in vivo. Human embryonic stem cells were used as a model for in vitro cardiac development and were differentiated using monolayer cardiac differentiation. Cells were exposed to purified nicotine or extracts from e-cig or tobacco cigarettes containing 1.7, 3.4, 6.8, and 13.7 μM nicotine, or vehicle control throughout differentiation. On day 14 control cells had an intrinsic beating rate of 39.5 ± 2.7 beats per minute (bpm), cardiomyocyte cell yield of 2.5x10^6 ± 4.3x10^5 cells, and cardiomyocyte purity of 83.6 ± 1.1% cTnT + cells by flow cytometry. Cells exposed to purified nicotine or e-cig extracts showed no effect on intrinsic beating rate, cardiomyocyte yield, or cardiomyocyte purity under all concentrations tested. In contrast, there was no survival of cells exposed to 13.7 μM nicotine from tobacco cigarettes. Furthermore, cells exposed to 1.7, 3.4, and 6.8 μM nicotine showed reduced intrinsic beating rate (4.5 ± 0.9 bpm), but no effect on cardiomyocyte yield or purity. Cardiomyocytes exposed to 1.7, 3.4, and 6.8 μM tobacco nicotine showed significantly increased levels of α-smooth muscle actin, consistent with cardiomyocyte immaturity. To determine if these data correlate in vivo, zebrafish embryos were raised in 3.4, 6.8, and 13.7 μM e-cig, tobacco extract or control from 0-72 hours post fertilization. Tobacco-exposed fish showed significant decrease in heart rate, survival, general development and increased incidence of heart malformation in a dose dependent manner. In contrast, e-cig exposed fish closely resembled the time-matched controls. These data suggest that nicotine alone or e-cig extracts do not have a marked impact on early heart development. However, smoke extracts generated from tobacco markedly impact cardiac development by reducing cardiac beating rates and delaying cardiomyocyte maturation.

Cytochrome P450 2J2 (CYP2J2) is the major human P450 isoform expressed in cardiac tissue. CYP2J2 is unique among other cytochrome P450 enzymes in that it is a drug metabolizing enzyme with a known endogenous function. CYP2J2 is responsible for oxidizing arachidonic acid to epoxyeicosatetraenoic acids (EETs), which are protective in maintaining cellular function in various organs. In the cardiomyocyte, EETs are electrophysiologically active and regulate L-type Ca2+, Na+ and ATP dependent K+ (KATP) channels, which are important in the repolarizing phase and control the QT-period during the cell’s electrical cycle. Therefore any reduction in EET levels due to chemical inhibition of CYP2J2 is expected to have a toxic effect on the cardiomyocyte. In this study, the inhibition of CYP2J2 by five drugs (terfenadine, grepafloxacin, cisapride, danazol and sertindole) known to cause cardio toxicity and especially QT prolongation was determined in a recombinantly expressed enzyme as well as adult human cardiomyocytes. The effect of terfenadine, on the action potential duration in freshly isolated mouse cardiomyocytes was also determined using patch clamp. Data demonstrates that at pharmacological concentrations of 0.1-1.0 μM, the above drugs efficiently inhibit CYP2J2 (by ~80%) both in recombinant enzyme and in adult cardiomyocytes. In freshly isolated mouse cardiomyocytes, terfenadine (0.2μM) increased the action potential duration (ADP90) from 52 to 70s. This increase in ADP was completely reversed following the addition of 50nM 11,12-EET. However, 11,12-EET was not able to reverse terfenadine inhibition of hERG using CHO cells (over-expressing hERG) in an IC50 shift assay. This indicates that CYP2J2 mediated EETs, play a protective role against drug induced QT-prolongation but probably through a hERG independent pathway. Consequently, it is prudent to avoid CYP2J2 inhibition, and reduction of EETs in the cardiomyocyte, when developing new chemical entities as potential therapeutic.

The tryptophan metabolites indole, indole 3-acetate and tryptamine were identified in mouse cecal extracts and fecal pellets by mass spectrometry using a triple quadrupole ion trap mass spectrometer coupled to a binary pump HPLC. Levels of indole (200-400 μM), tryptamine (10-20 μM), and indole 3-acetate (10-40 μM) were variable in both extracts. The AHR agonist and antagonist activities of these microbially-derived compounds were investigated in CaCo-2 intestinal cells as a model for understanding their potential effects on colonic tissue which is highly aryl hydrocarbon (AH)-responsive. Activation of AH-responsive genes such as CYP1A1 demonstrated that tryptamine and indole 3-acetate were AHR agonists.
agonists, whereas indole was an AHR antagonist in CaCo2 and breast cancer cells. Moreover, chromatin immunoprecipitation (ChIP) assays showed that indole inhibited TCDD-induced binding of the AHR complex to dioxin response elements on the CYP1A1 promoter. These results demonstrate that the tryptophan metabolites indole, tryptamine and indole 3-acetate modulate AHR-mediated responses in CaCo2 cells, and concentrations of indole that inhibit AHR antagonist activity (100-250 μM) are detected in the intestinal microbiome and impacts on intestinal function are being investigated.

**1131 NR4A1 Antagonists Inhibit Breast Cancer Cell Growth, Survival, and Migration**

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The orphan nuclear receptor NR4A1 (Nur77/TR3) is overexpressed in many solid tumors, and results of RNA interference (RNAi) studies suggest that NR4A1 functions as a pro-oncogenic factor. However, previous studies in breast cancer cells on the activity of NR4A1 are equivocal and we have investigated the function of this receptor in breast cancer cells by knockdown of NR4A1 (siNR4A1) or treatment with a C-DIM/NR4A1 antagonist activates caspases-8 and -7 and induced cleaved PARP expression in MCF-7, MDA-MB-231 and SKBR3 breast cancer cells. Transfection of erbB2-overexpressing SKBR3 cells with siNR4A1 also decreased cell proliferation by >30% after 72 hr and cell migration in a Boyden chamber assay was decreased by >80%. NR4A1 regulates cell survival and mTOR pathways and maintains low levels of oxidative stress in pancreatic and lung cancer cell lines, and treatment with C-DIM/NR4A1 antagonists or transfection with siNR4A1 decreased survival (induced apoptosis), inhibited mTOR, and induced stress in breast cancer cells. However, it was also evident that the expression of these well-characterized NR4A1-regulated pathways and the effects of NR4A1 inactivation were also highly cell context-dependent among a panel of breast cancer cell lines (MCF-7, SKBR3 and MDA-MB-231). Nevertheless, the C-DIMs/NR4A1 antagonists were effective antineoplastic agents in breast cancer cell lines and inactivated one or more NR4A1-regulated pathways.

**1132 AhR Knockout Rats Are Insensitive to Changes in Tissue Pathology and Serum Chemistry Markers following 4-Week Repeated-Dose Exposure to TCDD**

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Persistent activation of the aryl hydrocarbon receptor (AhR) is believed to play a key role in the mode-of-action for TCDD-induced rat liver tumorigenesis. To evaluate the AhR-dependence of early pathological changes prior to tumour formation, we conducted a four-week TCDD repeated-dose study in adult female AhR knockout (AhR-KO) and wild type (WT) rats and investigated alterations in target tissue pathology and serum chemistry markers. Beginning at 8 weeks of age, AhR-KO and WT rats (n = 20 rats/dose/genotype) were dosed by oral gavage (4-5 doses / week, 19 total doses) with varying concentrations of TCDD in corn oil (0, 3, 22, 100, 300, 1000 ng/kg/day). Liver, lung, thymus and kidney were examined for treatment- and genotype-related gross and histopathological effects. Serum was isolated from blood samples and analyzed using a standard rat clinical chemistry panel. Treatment-related increases in the severity of hepatic and thymic pathology were observed in WT rats only. In the liver, these included hepatocytic vacuolization and hypertrophy and increased inflammation. Treatment-related increases in serum ALP, AST, cholesterol, globulin, total bile acids and total bilirubin as well as decreases in serum triglycerides, glucose and A/G ratio were also observed in WT, but not AhR-KO rats. These data demonstrate that AhR-KO rats are insensitive to early changes in liver function and histopathology induced by repeated exposure to TCDD.

**1133 Epithelial Aryl Hydrocarbon Receptor Contributes to Intestinal Host-Microbe Homeostasis**

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Recent evidence demonstrates a role of the aryl hydrocarbon receptor (AHR) within the immune compartment of the intestine in establishing tolerance to commensal microbes and suppressing pathogenic infection. However, the contribution of epithelial AHR in maintaining host-microbe symbiosis has thus far not been examined. Using C57Bl/6j mice Ahrfx/fx & epithelial Ahr knockout (IEAhr) models together with 16s quantitative PCR (qPCR), we have investigated the effect of epithelial Ahr ablation upon the composition of the intestinal microbiota. The data reveal a 2-fold increase in ileal bacteria of IEAhr mice when compared to Ahrfx/fx counterparts. ELISA data revealed that bacterial outgrowth observed with IEAhr mice was associated with a 50% increase in fecal IgA, suggesting a humoral response to the increased bacterial population. qPCR analysis supports the notion of a humoral response in IEAhr mice with increased expression of Iif together with its receptors Tnfsf13c, Tnfsf13a and Tnfsf17. Furthermore, ileal expression of the acute phase proteins Saa2/3 were increased in IEAhr mice. Additionally, elevated hepatic Scl1 was observed, indicative of a systemic response. Coincident with the elevated bacterial population and the inflammatory response, we observed a 10-fold decrease in segmented filamentous bacteria (SFB). The association of SFB with the ileal epithelium educates the immune system with regard to commensal bacteria, prompting tolerance through the Th17/Treg system, limiting outgrowth/pathogen expansion. We therefore examined ileal Il17a mRNA expression in IEAhr and Ahrfx/fx mice. Data revealed that Il17a expression reflected the decrease in SFB. The deficit in Il17a expression suggests that IEAhr mice may exhibit a reduced capacity to respond to opportunistic pathogens. Here, we propose that ablation of epithelial Ahr restricts SFB colonisation, thereby decreasing microbial tolerance, which leads to bacterial expansion, dysbiosis and an inflammatory response. Funding:NIHES004869/019964.

**1134 Characterisation of the Hepatic Effects of Phenobarbital in Constitutive Androstane Receptor (CAR, NR113) Knockout Rats**

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The administration of Constitutive Androstane Receptor (CAR, NR113) activators to rats and mice induces hepatomegaly that is characterised by hepatocellular hypertrophy (due to the proliferation of smooth endoplasmic reticulum and concomitant induction of enzymes belonging to the CYP2B and 3A families) and hyperplasia (increased semi-conservative DNA synthesis and cell proliferation). Previous studies utilising CAR knockout (KO) mice have demonstrated that the presence of an active CAR is necessary for the induction of this hepatomegaly. This study was designed to investigate the effects of the CAR-activator phenobarbital (PB) in CAR KO rats (SAGE Labs, Boyertown, PA). Male Wild Type (WT) and CAR KO rats (n=5 per group), which had been implanted with osmotic pumps containing BrdU to allow determination of replicative DNA synthesis (S-phase), were administered PB (500 ppm) in the diet for 7 days. Other groups of WT and CAR KO rats received control diet. PB treatment of WT rats resulted in a 1.2-fold increases in liver weight and livet/ body weight ratios, centrilobular hepatocellular hypertrophy, a 5-fold increase in hepatocellular S-phase labelling index and a 3-fold increase into total microsomal P450. The microsomal CYP2B catalysed reactions pentoxyresorufin-O-depentyl- hepatic S-phase labelling index and a 3-fold increase into total microsomal P450. The microsomal CYP2B catalysed reactions pentoxyresorufin-O-depentylation (PROD) and benzyloxyresorufin-O-debenzylation (BROD) were induced 100-fold and 40-fold respectively. These changes were accompanied by increases in CYP2B1 (2600-fold) and CYP2B2 (1120-fold) mRNA. None of these PB-induced effects were observed in the CAR KO rats. The use of Whole Genome Expression Microarrays demonstrated 343 differentially expressed genes in the PB-treated WT rats versus the WT control group (selection criteria of ≥1.5 fold change and a p value < 0.05). There were 200 differentially expressed genes in the PB-treated CAR KO rats compared to the untreated control CAR KO rats. In conclusion, an active CAR is required for phenobarbital-mediated hepatomegaly in rats and only 40% of the genes regulated by PB appeared to be CAR-dependent.
1135 Effects of Perfluoroalkyls on the Activations of Human CAR3, PXR, and TR Receptors In Vitro


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Research studies have shown that several perfluoroalkyl substances (PFASs) have the potential to activate certain xenosensor nuclear receptors (NR) involved in regulating metabolic processes. Pleiotropic responses following activation likely are responsible for many effects observed in toxicological studies, and key differences in expressions and responses on activation are known to exist. Although many studies have investigated the potential of PFASs to activate NR1C1 (PPARα), few studies have examined potential for activation of NR1I3 (CAR3) and NR1I2 (PXR). Notably lacking are investigations into the ability of PFASs to activate the thyroid receptors, NR1A1 (TRα) and NR1A2 (TRβ). We investigated the potential of 5 PFASs (perfluorooctanesulfonate (PFOS, 2 samples), perfluorohexane-sulfonate (PFHxs), perfluorobutanesulfonate (PFBS), perfluorobutyrate (PFBA), and perfluorooctanoate (PFOA)) to activate human CAR3, PXR, and TRα using chimeric systems with a luciferase reporter plasmid at PFAS concentration ranging from 0.4 to 100 μM. The same PFASs as well as perfluorononanoate (PFNA) and perfluorodecanoate (PFDA) were tested for human TRα and TRβ activation in another similar system with luciferase reporter plasmid at PFAS concentrations of 10 μM. In these assays, PFOS, PFNA, and PFDA were also tested for activation at 1, 10, and 100 μM. No human TTR or TRα activation or repression was observed with any PFAS at any concentrations tested. Weak activations that were lower in magnitudes than the respective positive controls were observed with CAR3 (PFOS, 100μM) and PXR (PFHxs, 33 and 100 μM; and PFOS, 100 μM). These PFAS concentrations (equivalent of 13,000 – 50,000 ng/mL) were several orders of magnitude higher than general population. Based on these observations and in light of known human exposure levels, the PFASs included in this study are not expected to act via direct activation of the studied NR1 sub-family receptors.

1136 Activation of Aryl Hydrocarbon Receptor (AHR) Synergistically Induces Lipopolysaccharide (LPS)-Mediated Transcription of Proinflammatory Chemokine (c-c motif) Ligand 20 (Ccl20)

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Chemokines, a family of chemotactic cytokines, dictate recruitment of leukocytes, and play a critical role in immune homeostasis and tumorigenesis. Ccl20, a pleiotropic chemokine, also known as macrophage inflammatory protein-3α or Eotaxin-1, was originally identified as a chemoattractant for lymphocytes and dendritic cells. Ccl20 is highly expressed in pathological conditions such as rheumatoid arthritis, colon and pancreatic cancers and sepsis. It is the ligand for CC chemokine receptor 6 (Ccr6), which is expressed on the surface of dendritic cells, memory T cells and naïve B cells. LPS, a component of outer membrane of gram-negative bacteria, activates inflammatory signaling in macrophages, leading to enhanced secretion of pro-inflammatory cytokines and chemokines, such as Ccl20, via NF-kB-mediated activation. It is known that during inflammation, AHR can crosstalk with p65 (NF-κB) via NF-κB. Therefore, we examined the role of AHR in LPS-mediated activation of Ccl20. Primary peritoneal macrophages (1×10⁶ cells) from C57BL/6 (Ahrb) and Abr null mice (Abr-/-) were isolated and treated with AHR agonists 2.3.7,8-pentachlorodibenz-α-p-dioxin (TCDD) or indolo[3,2-b]carbazole (ICZ), followed by stimulation with pro-inflammatory LPS. Quantitative real time PCR revealed that the activation of Ahr in LPS-mediated activation of Ccl20. Primary peritoneal macrophages (1×10⁶ cells) from Ahrb or Abr-/- mice not only induces the basal levels of Ccl20 but also synergistically promotes LPS-mediated activation of Ccl20 mRNA. Studies performed in 1×10⁶ cells from Abr-/- mice revealed that the basal/synthetic effects to be Ahr-dependent. ELISA performed on supernatants from Ahrb 1×10⁶ cells exposed to TCDD and LPS mirrored the mRNA expression, albeit without synergy. Overall, these findings suggest that activation of AHR plays an underlying role in regulating the Ccl20 transcription in 1×10⁶ cells, and likely contribute towards AHR agonist-mediated inflammation. (Funded by: ES004869 and ES019964)

1137 Aryl Hydrocarbon Receptor: Insights into Its Role in Metabolic Homeostasis

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Obesity is a multi-factorial disease governed by a complex combination of genetic, lifestyle, and environmental inputs. Obesity is often associated with hyperglycemia, hypertension, hepatic steatosis, endotoxemia, inflammation, and cancer. Numerous studies indicate these pathologies are influenced by the Aryl Hydrocarbon Receptor (AHR). Recent evidence demonstrates that AHR has the capacity to attenuate de novo cholesterol and fatty acid synthesis in the liver. However, AHR's role in obesity appears to be complex and requires further investigation. Here, we have examined the effect of different allelic variations and conditional knockout murine models of Ahr upon metabolic homeostasis and associated gene expression. We identified that male mice harboring the low-affinity Ahrb allele are 20% more glucose tolerant than their high-affinity Abr-/- counterparts. Additionally, Abr-/- mice exhibit a 2-fold increase in liver triglyceride content, accompanied by a relevant increase in the expression of fatty acid synthesis genes (Fasn, Acaca, Srd1, and Sreb1L1). Furthermore, we observed a 3-fold increase in hepatic Fgf21 (fibroblast growth factor 21) expression among AHR-deficient mice in comparison with Abr-/- mice. Similarly, liver-specific, conditional AHR-knockout mice recapitulated the effect seen in Abr-null mice, exhibiting a 2.7-fold increase in Fgf21 expression when compared to their Abr-/- counterparts. This suggests that AHR exerts liver-specific control over Fgf21 gene expression. Fgf21, an important regulator of the response to fasting, is known to ameliorate obesity when administered to various genetic and diet-induced murine models of the disease. Interestingly, the Fgf21 promoter contains several putative dioxin response elements (DREs). We hypothesize that AHR is involved in the regulation of this key hormone through direct repression on the promoter. This project is supported by NIH grants ES004869 and ES019964.

1138 Selective Aryl Hydrocarbon Receptor Modulator (SAHRM) SGA360 Attenuates Lipopolysaccharide (LPS)-Mediated Septic Shock

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Septic shock is a pathological condition predominantly associated with endotoxin (LPS) producing gram-negative bacteria. It requires immediate medical attention since it is the leading cause of death in patients admitted to intensive care units worldwide. LPS activates Toll-like receptor 4 leading to enhanced secretion of proinflammatory cytokines and chemokines. Overexpression of these proinflammatory mediators contributes to multi-organ dysfunction syndrome, leading to death. Aryl hydrocarbon receptor (AHR), a ligand activated transcription factor, has recently been implicated to possess immuno-modulatory effects. We have previously shown that SGA360 can be used to mitigate cytokine-mediated pro-inflammatory gene expression. Thus this study was conducted to evaluate the role of AHR in both in vivo and in vitro models of septic shock. C57BL/6J (Ahrb), Abr-/-, and Ahrb-/- mice were injected with SGA360 at -1h and +12h relative to a lethal dose of LPS. Animals from all three genotypes exposed to LPS alone showed median survival of 28h, 29h and 30h respectively. However, Ahrb mice exposed to SGA360 followed by LPS showed a significant increase in median survival to 42h. Abr-/- and Ahrb-/- mice exposed to SGA360 followed by LPS showed poor median survival rate (27h and 20h). Macrophages have been shown to play a critical role in LPS-mediated inflammation. Thus, in order to study the ability of SGA360 to selectively modulate AHR, primary peritoneal macrophages (1×10⁶ cells) isolated from Ahrb and Abr-/- mice were pretreated with SGA360 followed by activation with proinflammatory LPS. Quantitative real-time PCR revealed that SGA360 mitigated expression of multiple inflammatory genes induced by LPS only in Ahrb mice. These findings suggest that selective modulation of AHR may be a viable therapeutic approach to mitigate inflammatory signaling associated with septic shock. (Funded by: ES016964)

1139 Adaptation of the Human Ah Receptor to Bind Simple Indoles

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Recent evidence has shown that specific tryptophan catabolites are ligands for the aryl hydrocarbon receptor (AHR) and are important for the maintenance of intestinal health. The activation of AHR promotes gut immunological homeostasis.
and increased epithelial barrier function. Through characterization and analysis of multiple tryptophan catabolites, we have identified a discrepancy between the ability of mouse versus human AHR to bind simple indole molecules. Computer modeling of the ligand-binding domain (LBD) of the mouse and human AHR reveal significant differences, consistent with a 10-fold decrease in affinity for TCDD observed with the human AHR, relative to the murine Ah allele. The abundant microflora tryptophan catabolite indole (20 μM) was found to increase relative luciferase activity 6-fold in a dioxin response element driven luciferase reporter human HepG2 assay, similar to results observed with 10 nM TCDD. Conversely, in a mouse axolotl reporter line, indole failed to exhibit agonist activity compared to TCDD. qPCR analysis revealed tryptophan catabolite 3-methyl indole to display agonist activity specific to human AHR similar to that of indole, while isomers 1-methyl indole and 2-methyl indole failed to exhibit agonist activity. These results suggest that the human AHR has evolved to bind simple indoles produced in the gut. This project is supported by NIH grants ES004869 and ES019964.

1142 An Aryl Hydrocarbon Receptor from the Salamander Ambystoma mexicanum Exhibits Low Responsiveness to 2, 3, 7, 8-Tetrachlorodibenzo-p-Dioxin

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Sequence and structural features of the aryl hydrocarbon receptor (AHR) can underlie species- and population-specific differences in its affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other agonists. These differences often explain the varying degrees of TCDD toxicity exhibited by distinct animal groups. Frogs, for example, are dramatically less sensitive to TCDD toxicity than many other vertebrates, and we have previously demonstrated that frog AHRs bind TCDD with low affinity. In Xenopus laevis AHR1B, weak TCDD binding results from the combination of three residues in the ligand binding domain: A354 and A370, along with N325. In the current study we seek to determine whether this mechanism of low TCDD responsiveness is shared by AHRs of other amphibian groups. To this end, we used RT-PCR to isolate a cDNA encoding an AHR from a salamander, the Mexican axolotl (Ambystoma mexicanum). It encodes a polypeptide of 95.5 kDa that shares 59% identity with Xenopus laevis AHR1B and 50% identity with mouse AHR. Phylogenetic analysis reveals that the axolotl AHR is orthologous to vertebrate AHR1s. Ambystoma AHR contains the same amino acid residues in the positions that confer low TCDD affinity to frog AHRs (N335, A364 and A380), suggesting that it also binds TCDD weakly. We tested this hypothesis with transactivation assays employing a luciferase reporter gene governed by the enhancer region from mouse CYP1A1 (pGudLuc6.1). For axolotl AHR, the EC50 for reporter gene induction by TCDD was 45 nM, even lower than frog AHR1B (23 nM) and dramatically less than a chimeric frog AHR containing the mouse ligand binding domain (0.2 nM). Taken together, the low TCDD responsiveness and the sequence conservation with the frog AHR ligand binding domain suggest that axolotl AHR binds TCDD with low affinity. We predict that Ambystoma mexicanum salamanders will exhibit resistance to the toxic effects of TCDD and other xenobiotic AHR agonists. Funded by the NIH (R15 ES011130) and the Kenyon Summer Science Scholars Program.

1143 1, 1-Bis(3′-indolyl)-1-(aromatic)methanes Are NR4A1 Antagonists That Inhibit Rhabdomyosarcoma Cell Growth and Survival

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Rhabdomyosarcoma (RMS) is the most common tissue sarcoma that is primarily observed in children and adolescents. Cytotoxic drugs are the major chemotherapies used for RMS treatment and these are accompanied by toxic side-effects. The nuclear orphan receptor NR4A1 (Nur77/TR3) is a pro-oncogenic factor that is expressed in RMS cells, and we investigated the effects of a series of 1,1-bis(3′-indolyl)-1-(p-substituted phenyl)methane (C-DIMs) analogs that exhibit NR4A1 antagonist activity on the growth and expression of selected pro-oncogenic factors in RD and RH30 RMS cells. EC50 values for growth inhibition (2 days) by the p-hydroxyphenyl (DIM-C-pPhOH), p-cyanophenyl (DIM-C-pPhCN), and p-carboxymethyl (DIM-C-pPhCO2Me) were 14, 6.4 and 10 μM, respectively, for RH30 cells and 16, 4.4 and 11 μM, respectively, for RD cells. NR4A1 regulates multiple pro-oncogenic pathways/genes and acts as a coactivator for multiple specificity protein 1 (Sp1)-regulated genes through NR4A1-Sp1 interactions, and several Sp-regulated genes are critical for RMS cell and tumor growth. Treatment of RMS cells with 15-22.5 μM DIM-C-pPhOH significantly decreased expression of bcl-2, survivin, epidermal growth factor receptor (EGFR), cyclin D1 and c-Myc proteins, and the mechanisms of NR4A1-Sp1 interactions and their inactivation by C-DIMs are being investigated. Thus, NR4A1 antagonists are a novel class of relatively non-toxic agents for clinical treatment of RMS and for application of combined drug therapies.
The aryl hydrocarbon receptor (AhR) is expressed in many tissues and is mainly studied in the context of environmental chemicals and toxins. For example, benzo[α]pyrene (BaP) and TCDD are well-established activators of the AhR, whereas endogenous ligands of the AhR are still uncertain. In its function as an antioxidant, vitamin E (VE as alpha-tocopherol) is oxidized to alpha-tocopherylquinone (TQ), which is widely considered to be biologically inert. Our early studies suggested that TQ may be biologically active as an activator of the AhR in prostate cancer cells (i.e. LNCaP cells). Thus, the rationale for this study was to examine TQ as an activator of the AhR and the completion of gene expression analyses, TQ was found to produce more than 3,800 gene expression changes (greater than 2-fold), whereas VE treatment resulted in 1,200 gene expression changes. Gene expression pathway and network analyses identified a clustering of AhR-responsive genes affected by TQ, but not VE, which was validated using qPCR for specific AhR-responsive genes (e.g., CYP1A1). Activation of AhR by TQ was further examined using an XRE/DRE-responsive luciferase reporter system where TQ, but not VE, stimulated activity. Experiments using RNAi to down-regulate AhR activity showed that the TQ-induced up-regulation of CYP1A1 expression was indeed mediated by the AhR. Finally, primary cultures of AhR knock out mouse embryonic fibroblasts (MEFs) were established. CYP1A1 expression in AhR wild-type (WT) and knock out (KO) MEF cells was determined after treatment with TQ. KO MEF cells showed little change in the expression of CYP1A1, while WT MEF cells responded to TQ treatment with increased CYP1A1 expression, establishing that the AhR is necessary for TQ-mediated up-regulation of CYP1A1. Details on the mechanism of TQ’s activation of the AhR remain unclear. However, we have shown that quinone structures, as present in TQ, are effective at activating the AhR. Results from these studies provide intriguing support for TQ as an endogenous agonist of the AhR.

1145 Pharmacological and Electrophysiological Characterization of Ascaris suum Homopentameric Nicotinic Acetylcholine Receptors in Xenopus laevis Oocytes
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Nicotinic acetylcholine receptors (nAChRs) belong to the family of ligand-gated ion channels (LGICs) and are found in both vertebrate and invertebrate species. The nAChRs in nematode parasites are pharmacologically different from mammalian host nAChRs allowing nematode selective compounds (anthelmintics) to be used for treatment and prophylaxis. Unfortunately, the widespread use of anthelmintics has given rise to the development of resistance. There is then, an urgent need to characterize the different nAChRs present in parasitic nematodes to provide a better understanding of their pharmacology for drug development and to find ways of overcoming resistance. Using molecular cloning and the two-electrode voltage clamp electrophysiological techniques, we have cloned and expressed two nAChR subunit genes (acr-16 and acr-21) from Ascaris suum in Xenopus laevis oocytes. Our results showed that ACR-16 or ACR-21 on their own can reconstitute a functional nAChR in the presence of an ancillary factor, RIC-3. Pharmacological characterization of homomeric receptors produced by ACR-16 or ACR-21 revealed that these receptors have distinct properties. The homomeric ACR-16 receptor is more sensitive acetylcholine than to nicotine and insensitive to levamisole and pyrantel. On the other hand, the homomeric ACR-21 receptor is more sensitive to nicotine than to acetylcholine but also insensitive to levamisole and pyrantel. These previously uncharacterized receptors are potential target sites for new anthelmintic compounds and are not anticipated to show cross-resistance with existing anthelmintics.
sylated proteins. Correspondingly, ensemble FRET spectroscopy measurements indicate that glycosylated and non-glycosylated proteins induce ‘open’ and ‘closed’ P-domain conformations respectively. The co-chaperone Erp57 influences substrate-binding kinetics and induces a ‘closed’ P-domain conformation, even in the presence of a glycosylated substrate. Together with analysis of the interactions of CRT with cellular proteins, these findings indicate that recruitment of monoglycosylated proteins to CRT is kinetically driven, while the P-domain and co-chaperone binding contribute to stable CRT-substrate interactions. Substrate sequestration in the cleft between the glycan binding site and P-domain is a likely mechanism for CRT-assisted protein folding.

**1149 Quantitative Profiling of Environmental Chemicals and Drugs for Farnesoid X Receptor Activity**

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Farnesoid X receptor (FXR), a bile acid sensor, exerts protective function in numerous diseases including cholestasis, diabetes, liver regeneration, and cancer. Despite the extensive interests in FXR ligands in drug discovery, little is known regarding potential FXR-mediated toxicity effects from xenobiotic chemicals. Here we describe the profiling of approximately 10K environmental chemicals and drugs in modifying FXR signaling and associated cytotoxicity. The FXR beta-lactamase assay was used to screen the Testox10K compound library, containing environmental chemicals, clinically-approved drugs and known bioactive small molecules, in a 1536-well plate format at 15 concentrations in triplicate runs. 435 potent and reproducible hits were identified and grouped into several clusters based on their chemical structure similarity and known biological function. Many environmental chemicals including synthetic hormones, pesticides, and industrial chemicals showed FXR antagonist activity. Some drugs acted as agonists, antagonists, or partial agonists of FXR. Several clusters of compounds identified from the screening were also found to be active against other functionally related nuclear receptors. These results not only provide directions for prioritizing chemicals for further testing FXR-mediated toxicity but also suggest novel signaling pathways for future mechanistic studies. Supported by EPA Interagency Agreement Y3-HG-7026-03. This work was reviewed by EPA and NTP and approved for publication but does not necessarily reflect official agency policy.

**1150 Thalidomide Increases Cytochrome P450 Activity and Drug Metabolism in Liver through Direct Activation of Nuclear Receptor CAR and PXR**

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Thalidomide (TC) is a widely used antiangiogenic drug in the treatment of several cancers, including multiple myeloma. However, the mechanism of TC action in liver cancer is not well understood. Previously, we have shown that TC is a potent activator of both CAR and PXR in human liver cancer cells. In this study, we investigated the effects of TC on the expression of CYP3A4, a key enzyme involved in the metabolism of many drugs. Our results indicate that TC significantly increases the expression of CYP3A4 in a dose-dependent manner. This effect is not mediated by classical CAR activation, but rather by direct activation of PXR. These findings suggest that TC may have potential therapeutic applications in liver cancer, and that the modulation of CYP3A4 activity by TC may be a target for further investigation.

**1151 Novel Interaction between PXR and CAR1 in Cytochrome P450 Induction**

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PXR and CAR are nuclear receptors that regulate the expression of genes involved in drug metabolism. In this study, we investigated the interaction between PXR and CAR1, two key nuclear receptors that participate in the induction of several CYPs including CYP2B6, CYP3A4, and CYP2C9. More than 20 splice variants of CAR transcripts have been identified. CAR1 is the main isoform found in hepatocytes; however, CAR1 shows strong constitutive activity when expressed in cell lines. In contrast, two other less abundant splice variants, CAR2 and CAR3, display no constitutive activity and can transactivate CYP2B6 and CYP3A4 upon ligand binding. In order to investigate the molecular mechanism of CAR1-mediated induction in hepatocytes, we assembled secreted alkaline phosphatase (SEAP) reporter gene constructs using native promoter sequences from CYP genes. These reporters were co-transfected in HepG2 cells with combinations of CARs, PXR, and HNF4α/β by STEP reverse transfection. The reporter constructs were functionally verified by expressing PXR or CAR3 and stimulated with corresponding ligands. In the presence of CAR1, both the CYP2B6 and CYP3A4 reporters exhibited the expected strong constitutive activity which masked the induction effect of CAR ligand C7TC7. Interestingly, the co-expression of PXR and CAR1 drastically suppressed the basal constitutive activities of CAR1 on both reporter genes. As a result, the effect of C7TC7 activation of CAR1 on CYP2B6 reporter became much more prominent (>5-fold). Although C7TC7 binding to CAR1 or CAR3 triggered only around 2-fold induction of CYP3A4 reporter gene, the co-activation of CAR1 and PXR led to a much more robust (>11-fold) induction of CYP3A4 than by activation of either nuclear receptor alone indicating a synergistic effect. This study emphasizes the significance of nuclear receptor interactions in the regulation of gene expression and illustrates the importance of using native promoter sequences instead of short synthetic regulatory elements in reporter gene assays. (Supported by NIH grant ES019807)

**1152 Effects of PPAR-Alpha Activation on Liver Toxicity in Mice Exposed to Trichloroethylene**

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Trichloroethylene (TCE) is an environmental and occupational toxicant and is classified as 'carcinogenic to humans' by both the US EPA and IARC. It has been hypothesized that peroxisome proliferator-activated receptor alpha (PPARα) activation, mediated by oxidative metabolites of TCE, is an important mechanistic event for liver cancer in mice. Although it is known that trichloroacetic acid (TCA), a major metabolite of TCE, may activate PPARα, uncertainties remain regarding the association between the extent of oxidative TCE metabolism, PPARα activation, and toxicity pathways that may be independent of PPARα. This study investigated oxidative TCE metabolism in the context of PPARα status (wild-type, Ppara-null, and humanized Ppara) in the mouse. Male and female mice of each genotype were treated with TCE (400 mg/kg/day in 5% Alkamuls EL-620 in saline vehicle) by oral gavage for 4 weeks. Liver and serum were collected at 5 hrs after the last dose. Quantification of TCA, trichloroacetic acid (TCA), and trichloroethanol (TCHO) was performed. In addition, ALT, liver-to-body weight ratio, hepatocellular proliferation, expression of peroxisome proliferator marker genes (Ppara, Acox1, and Cyp4a10), inflammation, and steatosis were evaluated. We found that (1) there were significant differences in TCE metabolism between different genotypes; (2) expression of Acox1 and Cyp4a10 significantly increased in both wild-type and pPpara mice, but not in Ppara-nulls; (3) spontaneous steatosis was observed in Ppara-null vehicle-treated, but not in mice treated with TCE; (4) mild increase in inflammation in TCE-treated group compared to vehicle was observed in Ppara mice. In conclusion, these data provide additional mechanistic information for understanding the linkages between oxidative TCE metabolism, PPARα activation, and liver toxicity of TCE. This work was supported by the Superfund Basic Research Program grant P42 ES005948.
1153 A Central Role of Aryl Hydrocarbon Receptor in High-Fat Diet-Induced Hepatic Steatosis

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The aryl hydrocarbon receptor (AhR) is a ligand-activated basic helix-loop-helix/period-AhR nuclear translocator-single minded transcription factor which mediates a wide range of physiological and toxicological effects upon exposure to endogenous and exogenous ligands. Recent studies have shown that activation of AhR induces hepatic steatosis through upregulation of fatty acid translocase that is responsible for fatty acid transportation into the liver. The aim of this study is to determine whether AhR knockout protects against high-fat diet-induced hepatic steatosis.

6-week old wild-type (WT), AhR heterozygous (AhR+/−) and AhR knockout (AhR−/−) male mice were exposed to a normal chow diet (NCD, 10% fat diet) or a high-fat diet (HFD, 60% fat diet) for 14 weeks. AhR+/− as well as AhR−/− mice were protected from HFD-induced hepatic steatosis, impairment of the insulin signaling pathway, and inflammation, as well as systemic insulin resistance. In addition, the expression of Cyp3a fatty acid translocase, key lipogenic genes, fatty acid synthase (Fas) and acetyl-CoA carboxylase (Acc), peroxisome proliferator-activated receptor-ε (PPARε) and fatty acid oxidation key enzymes, carnitine palmitoyl transferase-1 (CPT-1) and acyl-CoA oxidase (ACO) were lower in AhR−/− and AhR+/− mice compared to WT mice after HFD feeding. Since fatty acid oxidation was decreased as an adaptation to decreased de novo fatty acid synthesis, the expression of fatty acid oxidation markers suggested that the protective effects on HFD-induced hepatic steatosis in AhR−/− as well as AhR+/− mice resulted from upregulation of Cyp3a fatty acid translocase expression, thereby increasing fatty acid transportation into the liver. These findings elucidate an important role for the AhR in HFD-induced hepatic steatosis, and the AhR signaling pathway could become a potential therapeutic target for fatty liver disease.

1154 Profiling of Compound-Induced Modulation of CAR-Coregulator Interactions As a Means to Differentiate between Direct and Indirect CAR Activation

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The nuclear receptor (NR) superfamily member constitutive activated receptor (CAR) controls the expression of a large battery of genes encoding xenobiotic metabolizing enzymes and transporters. CAR can be activated by at least two mechanisms: direct binding (e.g. CITCO) and subsequent recruitment of coregulator proteins which regulate target gene chromatin accessibility, or an indirect mechanism involving interference with a signaling pathway that inhibits the ability of CAR to translocate to the nucleus (e.g., phenobarbital (PB) and phenytoin (PN)). Although a large number of chemicals activate CAR, use of structure-activity relationships to predict CAR activation has been hampered due to the lack of an assay that in conjunction with classical trans-activation assays, allows chemicals to be classified as indirect or direct activators. As a potential solution for this caveat, we applied a peptide microarray with 154 coregulator-derived NR-interaction motifs, (CoR)NR boxes, which serves as a sensor for CAR conformation and activity status as a function of ligand. Human CAR LBD-coregulator interactions were profiled in the presence of PB, PN, or CITCO, clomizolamze (antagonist) or solvent (2% DMSO). Compound response profiles, i.e. compound-induced log-fold change of CAR binding, were subjected to hierarchical clustering and demonstrated clear differences between compounds and their ability to modify CAR conformation. A large number of significant interactions between CAR and coregulators were stimulated with CITCO. Many of the same interactions were significantly decreased with clomizolamze. PB and PN exposure led to very few significant interactions indicating that they do not activate CAR directly. In summary, coregulator-NR interaction assays are promising new tools to identify chemicals that activate nuclear receptors by different mechanisms. (This abstract does not reflect EPA policy)

1155 Ethanol-Induced Hepatic Expression of Cytochrome P450 (Cyp) 2b10 Is Dependent on PPAR/B/D

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Because of the significant morbidity and lethality due to alcoholic liver disease (ALD), there remains a need to elucidate the mechanisms how ALD is caused. This will allow for the development of new non-invasive biomarkers of ALD that could lead to new approaches for the prevention and treatment of ALD. It was previously shown that PPARβ/δ has an important function in the ALD, and that PPARβ/δ could prevent some ethanol-induced hepatic effects as shown by differences in the urinary metabolome. Thus, the present study global gene expression patterns to begin to identify potential genes that may contribute to the differences in urinary metabolites modulated by PPARβ/δ. Chronic ethanol treatment causes increased hepatic Cyp2b10 mRNA and protein in wild-type mice, but this was not observed in Pparβ/δ-null mice. This suggests that induction of CYP2B10 by ethanol is regulated by PPARβ/δ, and this could be involved in the etiology of ALD modulated by PPARβ/δ. Interestingly, nuclear and cytosolic sub-localization of ethanol inducible (CAR), a trans-activation factor known to regulate Cyp2b10 expression, was different between each wild-type or Pparβ/δ-null mice. However, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), a transcription factor which participates in CAR transactivation of the Cyp2b10 gene, was up-regulated in wild-type mice, and this effect was not observed in Pparβ/δ-null mice. These results suggest that PPARβ/δ regulates CYP2B10 expression independently of CAR activation, not by affecting CAR translocation directly. Ongoing studies are evaluating the role of Cyp2b10 in ALD and how PPARβ/δ influences the metabolism of endogenous compounds due to this drug metabolizing enzyme (Supported by AA018863 and ES016013).

1156 Compromised Adaptive Upregulation of Mitochondrial Metabolism and Accumulation of Hepatic Lipids Associate with Alcoholic Liver Damage in PPARα/-/- Mice

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Alcohol alters hepatic metabolism, and chronic alcohol consumption can result in alcoholic liver disease (ALD). Peroxisome proliferator-activated receptor-ε (PPARε), a ligand activated transcription factor modulates expression of genes that regulate fatty acid catabolism. The specific PPARα target genes that modulate ALD are unclear. There is remarkable similarity between the histopathology of ALD in Pparα-/- and in early ALD in humans. Importantly, PPARα expression and activity are thought to be lower in human liver than mouse liver. Hence, the Pparα-/- mouse is a good model to investigate ALD. In this study, Pparα-/- mice were used to elucidate whether PPARα is activated or repressed by chronic alcohol consumption. Hepatic transcriptomic and metabolomic analyses were used to examine the alterations of genes and metabolites associated with pathological changes associated with ALD. The changes triggered by chronic alcohol consumption in Pparα-/- mice were compared with those in wild-type mice. The results showed that in the presence of PPARα, energy metabolism pathways in mitochondria, namely the fatty acid β-oxidation, the TCA cycle and electron transfer chain, were induced transcriptionally in response to two-month alcohol feeding. In contrast, these responses were greatly reduced in the absence of PPARα expression. In line with the transcriptional modifications of these metabolic pathways, lipidomics profiling results showed consistent accumulation of triglycerides in Pparα-/- mice as compared to controls. A significant increase in the number of metabolites and a strong induction of fibrogenesis genes were also observed exclusively in alcohol-fed Pparα-/- mice as compared to controls. These observations indicate that PPARα plays a protective role in response to chronic alcohol consumption by adaptive transcriptional activation of this receptor.

1157 A Novel Pregnane-X-Receptor (PXR) Knockout Rat Model: Characterization and Evaluation of the Role of PXR in Hepatic Lipidosis

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The PXR plays a well-established role in drug metabolism and protection against toxic metabolites such as bilirubin, however its role in lipid metabolism is less well understood. Previous studies in mice have linked PXR activation to induction of hepatic lipidosis, although the consequences of PXR activation in the rat and human remain unclear. To further understand the role of PXR in lipid metabolism, we evaluated the phenotype a novel Sprague Dawley rat PXR knockout (KO) model constructed using zinc finger nuclease technology. PXR KO rats were fertile and were of similar body weight to age-matched wild type rats. There were no differences observed by light microscopy in the majority of tissues examined, however there was a consistent decrease in hepatosplenomegaly and lymphopoeisis, which was reflected by a decrease in hematocrit and circulating red and white blood cells (all lineages), decreased bone marrow cellularity and decreased lymphocytes in the spleen. Altered basal energy metabolism in the PXR KO rats was indicated by mild decreases in serum glucose and cholesterol but no change in serum triglycerides or non-esterified fatty acids. Consistent with published studies in mice, treatment of...
wildtype rats with a PXR activator (pregnenolone-16alpha-carbonitrole (PCN)) induced centrilobular hepatic hypertrophy consistent with enzyme induc- tion and peripoportal hepatic microvesicular vacuolation, which stained posi- tively for adipophilin confirming the vacuoles contain lipid. An increase in hepatic triglycerides further confirmed accumulation of hepatic lipid by PXR activation in the rat. When the PXR activator PCN was administered to PXR KO rats, no hepaticcellular hypertrophy or vacuolation (and no increase in hepatic triglyc- eride content) was evident, thus confirming that PXR induction is associated with increased hepatic lipid in rats. These results are similar to those reported in mice, and further suggests PXR activation is associated with changes in hepatic lipids in rodents.

**1158 Aryl Hydrocarbon Receptor-Dependent Induction of Liver Fibrosis by Dioxin**

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The contribution of environmental pollutants to liver fibrosis is an important and poorly explored issue. In vitro studies suggest that the environmental pol- lutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other Aryl hydrocarbon Receptor(AHR) ligands induce several genes that are known to be up-regulated during liver fibrosis. Our aim was to determine whether exposure to such pollutants can lead to fibrosis and to characterize the mechanisms of action. Mice were treated for 2, 14, or 42 days, once a week with 25µg/kg of TCDD. Gene and pro- tein expression, in vitro and in vivo, as well as liver histology were investigated for each treatment. Treatment of mice with TCDD for 2-weeks modified the hepatic expression of markers of fibrosis such as collagen-1a1 and -smooth-muscle-actin. This is not observed in Ahr KO mice. Following 6-weeks of treatment, histol- ogical features of murine hepatic fibrosis became apparent. In parallel, the levels of inflammatory cytokines and of markers of activated fibroblasts were found to be up-regulated. Interestingly, we also found increased expression of genes of the TGF beta pathway and a concomitant decrease of miR-200a levels. Since the transcription factors of the Snail family were shown to be involved in liver fibrosis, we stud- ied their regulation by TCDD. Two members of the Snail family were increased, whereas their negative targets, the epithelial marker E-cadherin and Claudin1, were decreased. Further, the expression of mesenchymal markers were increased. Finally, we confirmed that Snai2 is a direct transcriptional target of TCDD in the human hepatocarcinoma cell line, HepG2. The AHR ligand, TCDD, induces hepatic fib-rosis by directly regulating pro-fibrotic pathways.

**1159 Identification of Environmental Chemicals Which Could Contribute to Nonalcoholic Fatty Liver Disease by Nuclear Receptor Activation**

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Environmental chemical exposures have previously been associated with nonal- coholic fatty liver disease (NAFLD). Activation of xenobiotic receptors includingpregnan-xenobiotic-receptor (PXR) and constitutive androstane receptor (CAR) by environmental chemicals may influence energy metabolism. We hypothesize that PXR activation is a mode of action for toxicants in NAFLD. The purpose of this study is to identify and validate environmental chemicals which interact with PXR or CAR and could thus contribute to NAFLD. EPA-ToxCast Phase I (320 chemicals) was screened for compounds associated with receptor activation. Human (h) and murine (m) PXR and CAR activation were examined for selected compounds from ToxCast Phase I in addition to organochlorine pesticides which were previously associated with human NAFLD. These compounds were validated in vitro for PXR/CAR activation using transient transfection assays in HepG2 cells. Nearly 2/3 of chemicals screened positive for PXR activation. 67 compounds and 102 compounds were found to activate hPXR by NGCG and Attagene assays respectively. The CelleDirect assay identified 202 compounds that were found to change CYP3A4 (PXR target gene) expression. Among chemicals selected for validation in cell-based reporter assays, trans-nonachlor, chlordane, DDE, DDT, lindane and alachlor activated h/m PXR. Diedrindrin activated mPXR only. Diedrin, trans-nonachlor, DDT, lindane and alachlor also activated hCAR but not nCAR. Potential PXR interactions were identified for nearly 2/3 of ToxCast Phase 1 chemicals. However, not all chemicals identified by the ToxCast screening assays proved to be xenobiotic receptor agonists. We postulate that the environmental chemicals that activate PXR may contribute to NAFLD through the effect of this receptor on energy metabolism which may differ in humans and rodent models.

**1160 Involvement of Constitutive Androstane Receptor (CAR) in the Liver Hypertrophy and Hepatocarcinogenesis Induced by Three Fibrates in Mice**

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Fibrates are known as PPARα agonists and induce hypertrophy and tumors in mouse liver. To clarify the involvement of CAR in the mechanisms of liver hyper- trophy and tumors induced by fibrates, 6-week-old male C3H (wild) and CAR knockout (KO) mice were fed a diet containing 2,000 ppm clofibrate (Clo), 2,400 ppm fenofibrate (Feno) or 5,000 ppm bezafibrate (Beza). Four-week-treatment of three fibrates induced centrilobular (Clo) or diffuse (Feno and Beza) liver hyper- tropy in wild mice, while CARKO inhibited the hypertrophy of Clo only. In all treated groups of both genotypes, Cyp2b10 expression was markedly increased in the liver. All fibrates increased Cyp2b10 expression in wild mice and the expression significantly decreased by CARKO in all treated groups. However CYP2P10 pro- tein was clearly observed in the centrilobular areas only in wild Clo group, indicat- ing a discrepancy between mRNA and protein expression. These data suggest that CAR might involve in Clo-induced liver hypertrophy. In 27-week-feeding treat- ment with characteristic three fibrates after diethylstilbestrol initiation, the numbers of basophilic altered foci and/or adenomas were significantly increased in wild mice, but not CARKO, indicating that CAR might involve in induction of basophilic foci and adenomas induced by these three fibrates. On the other hand, CARKO did not affect the increased numbers of eosinophilic altered foci and/or adenomas, which were induced by each fibrate in wild mice. In conclusion, not only PPARα but also CAR plays an important role in liver hypertrophy of Clo and in the hepa- tocarcinogenesis process of Clo, Feno and Beza.

**1161 Ginkgo biloba Extract Is Nongenotoxic In Vivo and Constitutive Androstane Receptor Is Involved in Its Hepatocarcinogenesis**

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Ginkgo biloba extract (GBE) is commonly used as a herbal supplement aiming to improve brain function and memory. A recent National Toxicology Program (NTP) study of GBE (TR578) reported concerns of genotoxicity and clear evid- ence of liver hypertrophy and hepatocarcinogenicity in mice. To understand the mode of actions of hepatocarcinogenesis of GBE, we investigated the genotoxicity of GBE in vivo and constitutive androstane receptor (CAR) involvement in liver hypertrophy/carcinogenesis, using wild-type and CAR knockout (CARKO) mice. Our studies of liver comet assay and bone marrow micronucleus assay showed negative results, even at 2,000 mg/kg GBE by gavage for 3 days. This indicates that GBE is non-genotoxic in vivo.

In a 4-week GBE dietary treatment of doses up to 10,000 ppm, a dose-dependent hepatic hypertrophy accompanied by CYP2B10 expression was induced in wild-type mice. In CARKO mice, only the highest dose of GBE induced same alterations as wild-type mice, indicating that GBE-induced liver hypertrophy is mainly CAR-mediated.

In a two-stage hepatocarcinogenesis model initiated by diethylnitrosamine administration, a 27-week treatment with GBE at 10,000 ppm resulted in an increased number of liver tumors and altered foci in wild-type mice. In contrast, the lesions were far less frequent in CARKO mice. These results demonstrate that GBE has a certain potential to induce mouse liver tumors through a non-genotoxic mechanism, and that the mode of action for hepa- tocarcinogenesis of GBE is CAR-mediated.

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SOT 2014 Annual Meeting 307
Bile acid (BA) homeostasis is dysregulated during pregnancy as a result of reduced signaling of the Farnesoid X Receptor (Fxr), which can lead to hypercholesterolemia and liver diseases such as intrahepatic cholestasis. To determine whether Fxr function could be pharmacologically rescued during pregnancy, pregnant C57BL/6 mice were treated with vehicle or 100 mg/kg GW4064 (GW), a synthetic Fxr agonist, on gestation days 13 and 14, and livers and intestines were collected for qPCR and western blot analysis. In pregnant mice, the mRNAs of hepatic transporters Ntcp, Oatp1a1/4, Mrd2, Bsep and Oatp mRNAs as well as Cypl7a1 and Mrpl mRNAs by 20 to 150%. Expression of Cyp7a1 mRNA was reduced in GW-treated pregnant mice to levels lower than virgin controls. In addition, GW restored expression of Cyp7a1, Bsep and Cypl7a1 proteins. In the ileum, treatment of pregnant mice with GW increased Fgf15 and Ostb mRNAs between 2- and 3-fold. Pregnancy also decreased the expression of the Fxr target gene and transcription factor, Shp, in liver and ileum by 65% and 98%, respectively. GW returned Shp expression to levels of virgin controls in the liver, and elevated expression by 4-fold in the ileum. These data demonstrate that pharmacological activation of Fxr during pregnancy may partially restore the down-regulation of key bile acid and xenobiotic transporters, as well as additional target genes in the Fxr pathway. Supported by R01ES020522, T32ES007148, and P30ES050522.

**1162 Activation of the Farnesoid X Receptor Restores Hepatic and Intestinal Bile Acid Synthetic Enzyme and Transporter Expression in Pregnant Mice**

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Fetal complications of Intrahepatic Cholestasis of Pregnancy (ICP) frequently include perinatal mortality. The severity is positively related to maternal serum total bile acids, but the genetic etiology remains unresolved. Respiratory Distress Syndrome (RDS) occurs in 28.6% of newborns of ICP patients. ABCB11 is a hepatic export pump for bile acids. Abcb11 KO mice showed elevated serum bile acids. Therefore, we hypothesized that Abcb11 KO female mice might be a model for ICP.

Offspring from KO dams showed 100% mortality, regardless of paternal genotypes. Necropsy only revealed atelectasis in neonates from KO dams. Gene and protein expression of pulmonary cell type markers did not change. Histopathological analysis did not reveal any defect in developmental lungs of neonates from KO dams. However, electron microscopy revealed super-coiled surfactant in the lungs of neonates from KO dams. The lipid components of pulmonary surfactant did not differ between neonates of WT and KO dams. But serum bile acids were significantly higher in KO dams during pregnancy and accordingly, higher in their neonates. However, only taurine conjugated muricholic acid (T-MCA) was elevated in the lungs of neonates from KO dams. Our data suggests that elevated T-MCA affects pulmonary surfactant, leading to poor oxygenation. This is consistent with our findings that secretion of total bile acids in postnatal newborns is significantly elevated after birth.

To rescue the mice, we backcrossed Abcb11 KO into either CAR or PXR-null background. Abcb11/CAR double KO mice rendered 100% neonatal mortality rates. In contrast, maternal absence of PXR with Abcb11 produces strong neonatal rescue with about 60% of neonates. The studies have identified Abcb11 as a major gene that accounts for neonatal RDS of cholestatic mothers. The Abcb11 KO female mice could be animal model to develop therapeutic approach.

**1163 An Animal Model of Intrahepatic Cholestasis of Pregnancy**

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To determine whether Fxr function could be pharmacologically rescued during pregnancy, pregnant C57BL/6 mice were treated with vehicle or 100 mg/kg GW4064 (GW), a synthetic Fxr agonist, on gestation days 13 and 14, and livers and intestines were collected for qPCR and western blot analysis. In pregnant mice, the mRNAs of hepatic transporters Ntcp, Oatp1a1/4, Mrd2, Bsep and Oatp mRNAs as well as Cypl7a1 and Mrpl mRNAs by 20 to 150%. Expression of Cyp7a1 mRNA was reduced in GW-treated pregnant mice to levels lower than virgin controls. In addition, GW restored expression of Cyp7a1, Bsep and Cypl7a1 proteins. In the ileum, treatment of pregnant mice with GW increased Fgf15 and Ostb mRNAs between 2- and 3-fold. Pregnancy also decreased the expression of the Fxr target gene and transcription factor, Shp, in liver and ileum by 65% and 98%, respectively. GW returned Shp expression to levels of virgin controls in the liver, and elevated expression by 4-fold in the ileum. These data demonstrate that pharmacological activation of Fxr during pregnancy may partially restore the down-regulation of key bile acid and xenobiotic transporters, as well as additional target genes in the Fxr pathway. Supported by R01ES020522, T32ES007148, and P30ES050522.

**1163a Exposure to Aryl Hydrocarbon Receptor Antagonist Alpha-Napthalene Blocks Diet-Induced Obesity and Nonalcoholic Fatty Liver Disease**

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Previous studies have indicated a role for the aryl hydrocarbon receptor (AHR) in mediating obesity. Mice with the Ahdr variant low-affinity allele have been shown to become less obese when fed a high-fat diet compared to mice with the high-affinity Ahdr allele. In the current study, we hypothesize that high doses of known AHR antagonist alpha-napthalene (NF) will also prevent diet-induced obesity. To test this, C57BL/6 mice were fed either a standard mouse diet, a “Western diet” containing additional cholesterol 45% of kilocalories come from fat, or these two diets containing 2% NF. Following 26 weeks of diet exposure, no adverse response to the NF was observed, and no differences in food consumption were associated with the NF diet. At 26 weeks’ diet, 16 mice per group per sex were sacrificed. NF had a near-complete effect on blocking obesity. Body weights at sacrifice for females were 22.0 ± 1.4 g (control), 28.7 ± 3.3 g (Western), 23.6 ± 1.8 g (control + NF), and 24.5 ± 2.2 g (Western + NF). The difference between Western diet and Western diet + NF was significant, P value < 0.05. Furthermore, histological examination of livers indicate fat accumulation indicative of non-alcoholic fatty liver disease (NAFLD) was widespread in Western diet groups, but was not widely detected in any other group. Differences in fat accumulation were also detected via MRI imaging and fat pad weights taken at sacrifice. From these data we conclude that inhibition of the AHR via dietary exposure to high levels of a known antagonist can block pathologies associated with the Western diet, including obesity and NAFLD. This further affirms a possibly major role of the AHR in regulating the body’s response to diet.

**1163b Amphiregulin (AREG) and Transforming Growth Factor-alpha (TGF-α) Play Opposing Roles in 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD)-Induced Proliferation and EGFR Signaling in Skin**

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In the epidermis, the balance between differentiation and proliferation is modulated by epidermal growth factor receptor (EGFR) signaling. EGFR activation promotes proliferation and is dampened under differentiating conditions. Previous studies show that TCDD, calcium (Ca), and TCDD/Ca cause time- and treatment-dependent down-regulation of [125I]-EGF binding, however the mechanism and implications of this down-regulation on epidermal homeostasis and EGFR signaling have not been fully addressed. In this study, we sought to identify whether secreted ligands drive EGFR down-regulation and the impact of these ligands on EGFR signaling, ligand responsiveness, and cellular proliferation in primary neonatal human epidermal keratinocytes (NHEKs). A 72 h treatment with TCDD, Ca, or TCDD/Ca reduced [125I]-EGF binding to 77%, 41%, and 32% of control cells respectively without loss of EGFR protein. TCDD and TCDD/Ca-treated NHEKs showed no change in basal ERK activity but significantly enhanced responsiveness to exogenous ligand. TCDD, Ca, or TCDD/Ca caused elevated secretion of TGF-α compared to control cells while all NHEKs secreted AREG in a time-dependent manner. Using neutralizing antibodies, we showed that altering secreted ligands had no effect on total EGFR protein. TGF-α had an anti-proliferative effect and neutralization led to significantly decreased [125I]-EGF binding. Conversely, neutralization of AREG led to decreased cell number in TCDD, Ca, and TCDD/Ca-treated NHEKs. Neutralization of both ligands reversed treatment-dependent EGFR down-regulation in TCDD-, Ca-, and TCDD/Ca-treated cells, supporting that this down-regulation is modulated through the secretion of both TGF-α and AREG, which leads to an alteration, though not a loss, of EGFR signaling. TGF-α and AREG play opposing roles in downstream NHEK proliferation suggesting that TCDD pathology is in part due to imbalancing ligand-driven EGFR signaling.
This study was conducted in male Swiss Webster mice to further confirm our previous findings on heteroprotection of thioacetamide (TA)-primed mice against acetaminophen (APAP)-induced lethality. The objective was to examine the role of annexin A1, an endogenous inhibitor of phospholipase A2, in heteroprotection by TA against APAP-induced liver failure and mortality. Male Swiss Webster mice were primed with a low dose of TA (40 mg/kg in 10 ml normal saline/kg, intraperitoneally (i.p.)) 36h before challenge with a lethal dose (600 mg/kg 0.4% NaCl pH 8.2, i.p.) of APAP. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) were measured as biomarkers of liver injury, and plasma secretory phospholipase A2 (sPLA2) activity was measured to monitor the expansion of APAP-initiated liver injury. Hepatic glutathione (GSH) depletion following APAP treatment was measured in TA primed and non-primed mice. TA priming resulted in marginal decrease (~25%) in Cyp2e1 enzyme level, but the extent of rise in ALT and AST and decrease in hepatic GSH after APAP overdose was not affected due to TA priming. Moreover, TA priming did not affect the rise in plasma sPLA2 level at 2h following APAP treatment; however, no further rise in sPLA2 was observed at later time points in TA primed mice as compared to non-primed mice. TA priming resulted in overexpression of annexin A1 at 24 to 48h. Inhibition of annexin A1 biosynthesis using protein synthesis inhibitor cycloheximide (CHX) (40 mg/kg in 5 ml distilled water/kg, i.p.) at 1h before TA priming resulted in unabated expansion of APAP-induced liver injury and led to 100% mortality in stark contrast to 100% survival in APAP-overdosed TA primed mice that did not receive CHX intervention. In conclusion, annexin A1 overexpression in proliferating hepatocytes abolishes the expansion of liver injury after a lethal APAP overdose in mice.

This study was designed to test whether inhibiting secreted phospholipase A2 (sPLA2), a death protein, with a specific inhibitor, BPPA [5-(4-benzoxylphenoxy)-4S-(7-phenylheptanoylamino) pentanoic acid], prevents the expansion of liver injury initiated by a lethal overdose of acetaminophen (APAP) in mice. Male Swiss Webster mice (25-30g) were treated with a lethal dose of APAP (600 mg/kg, ip, in warm 0.45% NaCl pH 8.2) followed by a single dose of either BPPA (20 mg/kg, ip, in DMSO 3 ml/kg) or DMSO vehicle (3 ml/kg, ip) alone injected at 2, 4 or 8h after APAP administration. Survival and mortality were recorded over the next 14 days. Plasma alanine aminotransferase (ALT) and sPLA2 activities were measured in the mice on alternate days from days 1 to 13. ALT and sPLA2 activities increased sharply in the mice treated with TA either alone or followed by DMSO (3 ml/kg, ip) led to 80% mortality. In contrast, mice treated with BPPA at 2 and 4h after the APAP administration exhibited similar rise in sPLA2 and ALT activities on day 1 declining thereafter and suffered only 10 and 30% mortality, respectively. Covalent binding of 14C-APAP-derived reactive metabolite to liver protein, hepatic glutathione (GSH) depletion, and hepatic GSH:GSSG ratio did not change after the APAP overdose in the mice receiving the BPPA intervention. Ninety, 70, and 60 percent of the mice treated with BPPA at 2, 4, and 8h after the lethal dose of APAP, respectively, survived. We conclude that timely intervention by the administration of the sPLA2 inhibitor, BPPA, prevented the expansion of APAP-induced liver injury and death of the mice after the lethal challenge with APAP.
Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that pos-positively regulates the expression and activity of cytoprotective genes during periods of oxidative stress. Literature has shown that some of these genes are highly expressed in livers of female than male mice. This may explain previous reports of greater tolerability by female mice to acetaminophen (APAP) hepatotoxicity than males. The hypothesis for this study is that resistance to APAP toxicity by female mice is due to higher basal and inducible expression of Nrf2 and Nrf2-dependent genes. Here, we examined differences in basal mRNA and protein expression for Nrf2 and Nrf2-dependent genes in naïve (fed) male and female mice and overnight-fasted mice following vehicle or APAP treatment. Alamine aminotransferase (ALT) activity was also measured as an indicator of hepatotoxicity. Hepatic mRNA and protein levels were measured by quantitative PCR and western blotting, respecti-respectively. Contrary to our hypothesis, results show that basal Nrf2 mRNA expression was significantly lower in livers of fed female than male mice. Consistent with this, basal Nrf2 protein expression was lower in livers of both fed and over-night-fasted female than male mice, with significance observed in livers of fed mice. However, greater induction of the Nrf2-dependent gene NAD(P)H quinone oxidoreductase 1 (Nqo1) and multidrug resistance-associated protein transporter 4 (Abcc6 or Mrp4) was observed in livers of overnight-fasted female than male mice. Similarly, Nqo1 protein expression was significantly higher in livers of both fed and overnight-fasted female mice. As expected, ALT activity was significantly elevated in both WT and Nrf2-null male mice following APAP treatment, but no significant increases in ALT were observed in either genotype of female mice. This unaltered tolerance by female mice lacking Nrf2 indicates that other factors, rather than Nrf2, are responsible for the lower susceptibility of female mice to APAP hepatotoxicity. Supported by NIH DK069557.
ALF using an incremental dose model. C57BL/6 mice were treated with either 300 mg/kg (APAP300, regenerative dose) or 600 mg/kg (APAP600, non-regenerative dose) APAP. Liver injury and regeneration were studied over a time-course of 0 to 96-hr. Mice treated with APAP300 developed extensive liver injury followed by significant regeneration resulting in resolution of injury by 48-hr. In contrast, APAP600 group exhibited significant progression of injury with substantial decrease in regeneration. The inhibition of regeneration in APAP600 group was associated with decreased cyclinD1 mRNA and protein expression. Further analysis of upstream signaling revealed that growth factors signaling pathways (EGFR/c-Met, downstream MAPKs) were dose-dependently activated and remain highly activated even at APAP600, where regeneration was inhibited. However, canonical Wnt/β-catenin and NF-κB signaling were activated only in APAP300 where regeneration was stimulated. Next, we investigated role of Wnt/β-catenin in further detail. ChIP analysis revealed increased binding of β-catenin to cyclinD1 promoter specifically at APAP300 correlating with higher cyclinD1 induction. Furthermore, over-expression of stable form of β-catenin (M565) in mice resulted in induction of cyclinD1, improved liver regeneration and decreased progression of injury following APAP overdose. Overall, our study identified several potential pathways and confirmed role of Wnt signaling in regulation of liver regeneration after APAP overdose.

1174 Strain Difference in Carbamazepine-Induced Hepatotoxicity


Carbamazepine (CBZ) is a widely used antiepileptic drug, and severe hepatotoxicity is reported in a small population of the patients who took CBZ. Recent publications suggest that the CBZ-induced hepatotoxicity is immunity-mediated. The present study investigated effects of mouse strain-specific immunological back ground on CBZ hepatotoxicity. CBZ was administered to 5 strains mice, ICR (frequently used in general toxicology study), C57BL/6 (Th1 dominant) and BALB/c (Th2 dominant). These mice were given CBZ at 400 mg/kg/day for 4 days and 800 mg/kg for subsequent 1 day. After 24 hr of the final dosing, all survived animals were necropsied and laboratory tests were performed. Hepatotoxicity, such as necrosis of hepatocytes along with increases in plasma AST, ALT or LDH, was observed in 0/10, 5/10 and 8/10 mice in ICR, C57BL/6 and BALB/c, respectively, among which the severity was worst in BALB/c. Blood TCR-β values were comparable in all 3 strains. However, RT-PCR analysis using liver revealed highly increased gene expression of pTCRβ, CCL2 in C57BL/6 and BALB/c. Furthermore, there was also increased gene expression of TNF-α, IL-6 and IL-10 also increased. These responses were strongest in BALB/c. In addition, an increased gene expression of liver Hemeoxygenase-1 (HO-1) was observed in the hepatotoxicity-induced mice. Since HO-1 is reported to present in dendritic cells in the liver, HO-1 might play some role for CBZ-induced hepatotoxicity. Taken together, there were clear strain differences in incidence and severity of CBZ-induced hepatotoxicity. The different hepatotoxicity responses between the strains were correlated to different immunological responses in the liver and BALB/c was most sensitive.

1175 Assessment of the Effects of Perfluorononanoic Acid on Hepatic Homeostasis

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Polyfluorinated compounds (PFC) are compounds where all or most hydrogen atoms are replaced by fluorine atoms. Due to their physical-chemical properties, PFCs are used in more than 200 industrial and consumer applications of which lubricants, fire-fighting foams, and emulsifiers for the production of fluoropolymers are a few examples. Perfluorononanoic acid (PFNA) is a nine-carbon perfluorinated compound, used as a surfactant and emulsifier. Due to the long half-life and occurrence in both human and animal serum, PFNA is a frequently detected PFC’s in human serum. Over the last decade (1999-2010), researchers have found a three-fold increase in PFNA concentrations in blood from the US population. Due to the long half-life of PFNA and thus the tendency to bio-accumulate, there is an increased concern about the human exposure to these compounds. PFNA is between the four most frequently detected PFC’s in human serum. To study the potential effects of PFNA on hormonal and hepatic homeostasis, we dosed adult male Wistar rats with three doses of PFNA (0.0125, 0.25, and 5 mg/kg bw/day) once daily for 14 days by gavage. After euthanization, we sampled blood and various tissues and isolated mRNA from liver samples for Agilent rat gene expression microarrays. A metabolomics analysis was performed on the blood samples using LC MS/MS. The identified perturbed targets/signaling pathways were validated using RT qPCR and immunohistochemical staining of liver slices. The highest PFNA dose caused steatosis and general toxicity. We found significant changes in plasma levels of androstenedione and testosterone at the highest dose and corticosterone at the lowest dose. Initial overrepresentation analyses of the gene expression microarray data using Gene Ontology categories suggested effects of PFNA on energy metabolism and peroxisome proliferation along with mitochondrial and endoplasmic reticulum function. This is e.g. indicated by misregulation of genes involved in mitochondrial fatty acid β-oxidation. This is in agreement with the findings from the metabolomics analysis, showing decreased levels of lipids in the plasma samples.

1176 Tolerance to Acetaminophen (APAP) Hepatotoxicity in a Mouse Model of Autoimmunization


APAP overdose is the leading cause of acute liver failure in the U.S. Rodents treated with low hepatotoxic doses of APAP become resistant to toxicity from subsequent treatment with higher doses of APAP (APAP autoprotection). Although this phenomenon is also seen in patients who repeatedly take supratherapeutic doses of APAP, the underlying mechanism(s) is not known. Previous gene microarray studies revealed a pronounced induction of liver Fmo3 mRNA expression in our mouse model of APAP autoprotection. Here, we characterized the gene regulation and protein expression of liver Fmo3 during APAP hepatotoxicity. The functional consequences of Fmo3 induction were also investigated. Plasma and livers were collected from male C57BL/6 mice over a period of 72h following a single dose of APAP (400 mg/kg) to measure Fmo3 mRNA and protein expression. Although Fmo3 mRNA levels increased significantly following APAP treatment, its protein expression marginally changed. By contrast, both Fmo3 mRNA and protein expression were significantly higher in mice pretreated with APAP (400 mg/kg) and re-exposed to a higher dose (600 mg/kg). In contrast to naïve C57BL/6 male mice, females mice have about 80-times higher basal Fmo3 mRNA and are highly resistant to APAP hepatotoxicity. Co-administration of APAP with the Fmo3 inhibitor methimazole rendered female mice susceptible to APAP hepatotoxicity, with no changes in susceptibility detected in male mice. Furthermore, a human hepatocyte cell line (HC-04) clone overexpressing human FMO3 showed enhanced resistance to APAP cytotoxicity. Taken together, these findings establish for the first time induction of Fmo3 protein expression and function by xenobiotic treatment. Our results also indicate that Fmo3 expression and function plays a role in protecting the liver from APAP-induced toxicity. Although the mechanism(s) of this protection remains to be elucidated, this work describes a novel function for this enzyme. Supported by NIH DK069557.

1177 Altered Expression of Hepatic Xenobiotic Metabolism Systems in Transport Deficient (TR-) Rats

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TR- rats are characterized by a spontaneous mutation in the biliary transporter, multidrug resistance-associated protein 2 (Mrp2; Abcc2), and generally recognized that there is a compensatory increase in the expression of the basolateral transporter Mrp3 (Abcc3) in these rats. The purpose of this work was to characterize global hepatic gene expression changes in TR- rats, with emphasis on xenobiotic metabolizing systems. Liver mRNA profiles were evaluated in TR- and control Wistar rats (AllDirex®; Rat Genome 230, 2.0 GeneChips; p < 0.01), and results were confirmed by RT-PCR. For Phase I metabolic enzymes, Cyp450 reductase, Cyp2b1, and Cyp2a2 increased (≥ 1.5x), whereas Cyp2c40, Cyp2b2, and Cyp7a1 decreased (≤ 2.7x). For phase II conjugating enzymes, Ugt1a1 increased along with several glutathione transferases including, Gsta5, Gstm2, Gstp1, and Gstt1. Gsta5 was the most significantly increased in this family (9.6x). As expected, Abcc3 mRNA was increased, along with an increase in Abca1 (3.9x). Hepatic expression of numerous solute carriers (Slc family) was altered, with 7 members significantly up-regulated. Of these transcripts, expression of Slc35c2, a transporter for GDP-fucose, was elevated (6x). An additional 11 Slc genes were down-regulated, including the sucrose transporter Slc5a6 (4.4x) and the Na+/phosphate exchanger, Slc34a2 (8.8x). These results indicate that hepatic expression of numerous genes involved in Phase I, II and III metabolism are altered in Mrp2-deficient TR- rats. The potential contribution of these changes (e.g. altered enzyme activity or transport of solutes) to the disposition of endogenous and exogenous compounds should be considered when this model is used to assess the role of Mrp2 in biliary excretion and toxicity of drugs and environmental chemicals.
Acetaminophen (APAP) overdose causes severe and occasionally fatal liver injury. Numerous drugs that attenuate APAP toxicity have been described. However, these compounds frequently protect by cytochrome P450 inhibition, thereby preventing the initiating step of toxicity. We have previously shown that pretreatment with allopurinol can effectively protect against APAP toxicity, but the mechanism remains unclear. In the current study, C57BL/6 mice were administered allopurinol 18h or 1h prior to APAP overdose. Administration of allopurinol 18h prior to APAP overdose resulted in an 88% reduction in liver injury (serum ALT) 6h after APAP, however, 1h pretreatment offered no protection. APAP-cytochrome adducts and glutathione depletion kinetics were similar with or without allopurinol pretreatment. The phosphorylation and mitochondrial translocation of c-Jun-N-terminal kinase (JNK) has been implicated in the progression of APAP toxicity. In our study we showed equivalent early JNK activation (2h) however late JNK activation (6h) was attenuated in allopurinol treated mice, which suggests that later JNK activation is more critical for the toxicity. Additional mice were administered oxypurinol (primary metabolite of allopurinol) 18h or 1h pre-APAP, but neither treatment protected. This finding implicated an aldehyde oxidase (AO)-mediated metabolism of allopurinol, so mice were treated with hydralazine to inhibit AO prior to allopurinol/APAP administration, which eliminated the protective effects of allopurinol. We evaluated potential targets of AO-mediated preconditioning and found increased hepatic metallothionein 18h post-allopurinol. These data show metabolism of allopurinol occurring independent of P450 isoenzymes preconditions the liver and renders the animal less susceptible to an APAP overdose.

The role of allopurinol in APAP-induced liver injury is well documented. However, the mechanism of its protective effect is unclear. In our study, we investigated the protective effect of allopurinol using freshly isolated PHH, isolated from either unused tissue or surgical waste. PHH were exposed to 5mM, APAP hepatotoxicity using freshly isolated PHH, isolated from either unused tissue from donor livers or material from liver resections. PHH were exposed to 5mM, 10mM and 20mM APAP over a period of 48 hours, and multiple parameters were assessed. As evidenced by the release of alanine aminotransferase (ALT), APAP dose-dependently induced significant hepatocellular necrosis starting from 24h, which correlated with the clinical onset of human liver injury after APAP overdose. Interestingly, cellular glutathione was depleted rapidly during the first 3h, which was similar to the murine model. APAP also resulted in early formation of APAP-protein adducts (measured in whole liver homogenate and in mitochondria) and mitochondrial dysfunction as shown by the loss of mitochondrial membrane potential after 12h. Furthermore, APAP time-dependently activated c-Jun N-terminal kinase (JNK). More specifically, JNK phosphorylation occurred in the cytosol and mitochondria 1h after APAP exposure, while phosphorylated JNK subsequently translocated to the mitochondria. Both pretreatment and co-treatment with JNK inhibitor SP600125 reduced JNK activation and partially attenuated cell death at 24h and 48h after APAP. Our data demonstrate for the first time detailed mechanistic events in human hepatocytes and mitochondrial dysfunction, in addition to the role of JNK in determining the progress of APAP induced hepatotoxicity. TLR4 are major receptors for extracellular histone mediated sterile inflammation, injury and death in mouse model of APAP. The aim of this study was to elucidate the possible role of TLR4 blockers in preventing APAP induced hepato-fetal injury. TLR4 blockers were used in APAP-induced rat hepatotoxicity in order to elucidate the protective role of TLR4 in APAP-induced liver injury.

Acetaminophen (APAP) overdose is the most prevalent cause of drug-induced liver injury in western countries. Extensive studies have been conducted to investigate the mechanism of injury after APAP overdose in various biological models, including mice, rats and hepatoma cell lines. However, it remains ill-defined as to how the injury progresses in humans. Primary human hepatocytes (PHH) represent the gold standard model of studying drug toxicity, and here we investigated APAP hepatotoxicity using freshly isolated PHH, isolated from either unused tissue from donor livers or material from liver resections. PHH were exposed to 5mM, 10mM and 20mM APAP over a period of 48 hours, and multiple parameters were assessed. As evidenced by the release of alanine aminotransferase (ALT), APAP dose-dependently induced significant hepatocellular necrosis starting from 24h, which correlated with the clinical onset of human liver injury after APAP overdose. Interestingly, cellular glutathione was depleted rapidly during the first 3h, which was similar to the murine model. APAP also resulted in early formation of APAP-protein adducts (measured in whole liver homogenate and in mitochondria) and mitochondrial dysfunction as shown by the loss of mitochondrial membrane potential after 12h. Furthermore, APAP time-dependently activated c-Jun N-terminal kinase (JNK). More specifically, JNK phosphorylation occurred in the cytosol and mitochondria 1h after APAP exposure, while phosphorylated JNK subsequently translocated to the mitochondria. Both pretreatment and co-treatment with JNK inhibitor SP600125 reduced JNK activation and partially attenuated cell death at 24h and 48h after APAP. Our data demonstrate for the first time detailed mechanistic events in human hepatocytes and mitochondrial dysfunction, in addition to the role of JNK in determining the progress of APAP induced hepatotoxicity.
Acorinel accumulation, activation of pro-apoptotic stress kinase-JNK, ER stress, and apoptotic cell death was examined in vitro in alcohol-exposed rat hepatic cells (H4IIEC), and in vivo in a mouse model of alcohol consumption. Exposure to alcohol led to substantial accumulation of acorinel adducts both in vitro and in vivo. This was accompanied by phospho-activation of JNK and upregulation of ER stress transcription factors ATF3 and ATF4, and the pro-apoptotic protein, GADD153/CHOP. Cellomics analysis revealed that accumulation of acorinel adducts correlated with (i) disruption of mitochondrial membrane potential; (ii) release of free calcium; and (iii) cell death in hepatocytes. This study demonstrates that acorinel is likely to be a major culprit in the ER stress and hepatotoxicity associated with alcohol consumption. Acorinel scavengers may have therapeutic potential in alleviating the adverse effects of alcohol consumption, and we are actively investigating this concept.

1183 Mechanism of Altered Metformin Distribution in Diabetic Nonalcoholic Steatohepatitis
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Metformin is an anti-hyperglycemic agent that is widely prescribed for type-2 diabetes and is currently being investigated for the treatment of other insulin resistance-related disorders including nonalcoholic fatty liver disease (NAFLD). NAFLD is a highly prevalent chronic liver disease with stages ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). NASH, which contains various cellular stress responses including lipid accumulation, inflammation, oxidative stress and fibrosis, alters transporter expression and drug disposition. Metformin elimination is transporter-dependent, mediated by Oct2, Oct1 and Matel in the liver and kidney. The purpose of this study was to determine whether metformin demonstrates altered expression and tissue distribution in diabetic and NASH mouse models. A single oral dose of [14C]metformin was administered to C57BL6 and diabetic ob/ob mice, each fed either a control diet or a methionine and choline deficient (MCD) diet to model NASH. ob/ob (diabetic) mice exhibited increased hepatic Matel1 and decreased renal Oct1 expression compared to WT, and diet-induced NASH in diabetic mice increased mRNA expression of hepatic Oct1. Both diabetes and NASH decreased Oct2 and Matel mRNA expression in the kidney. For metformin distribution, diabetic mice had increased plasma area under the curve (AUC) and liver and kidney metformin levels compared to WT. In both WT and ob/ob mice, presence of NASH increased metformin exposure, with NASH mice exhibiting increased plasma AUC and muscle concentrations compared to WT, and diabetic NASH mice exhibiting increased plasma AUC, muscle and liver concentrations compared to diabetic alone. Overall, diabetes and NASH additively increased plasma AUC and retention of metformin in liver and muscle compared to WT control. These results indicate that diabetes and NASH alter transporter expression and shift the disposition profile of metformin, potentially increasing the risk of drug toxicity.

1184 Altered Transcription Initiation and Exonization of the First Intron of the Human Liver ABC4 Gene in Tissue Specimens from Cases of Acetaminophen Overdose
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Liver injury caused by overdose of the analgesic and antipyretic drug acetaminophen (APAP) is a serious human health problem. The precise molecular events mediating initiation, progression and recovery from APAP liver injury remain unclear. In our previous studies, mRNA and protein induction of the eflux transporter ABC4 gene was found to be associated with APAP liver injury and recovery. In this study, we examined potential changes in transcription start site (TSS) profile and intronic exonization of the ABC4 gene in human liver specimens from APAP overdose cases. Using 5’RLM-RACE analysis (RNA ligase mediated rapid amplification of cDNA 5’-end) and genotyping of ABC4 mRNA intronic exons, we found multiple ABC4 TSSs distributed in a dispersed fashion in liver samples from APAP overdose patients. Similarly, multiplicity and dispersion of ABC4 TSSs was also observed in normal livers. However, the frequency and location of ABC4 TSSs in APAP livers were noticeably different from those in normal livers. We also found two types of ABC4 mRNA variants with different exonisation of intron 1 in both normal and APAP livers. Lower levels of 5’ unapped mRNA variants containing a longer intronic exon, while higher levels of 5’ capped mRNA variants containing a shorter intronic exon were identified in APAP livers. This indicates that during APAP toxicity, degradation of ABC4 mRNA variants containing a longer intronic exon is enhanced while degradation of variants containing shorter intronic exon is repressed. In silico analysis showed that ABC4 mRNA variants containing the shorter intronic exon encode for a 39-amino acid peptide that is approximately1/35th the length of the full peptide. The biological significance and function of these ABC4 mRNA variants remains to be further investigated. Taken together, our findings provide new insights into the mechanism of ABC4 gene regulation at the transcriptional and posttranscriptional level in association with APAP hepatotoxicity. Supported by NIH Grant DK069557.

1185 Nr2f Protects against Furosemide-Induced Hepatotoxicity
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Furosemide is a diuretic drug, but its reactive intermediates lead to acute liver injury in mice. Given the essential role of Nr2f as a cellular defense regulator, we investigated whether Nr2f would protect against furosemide-induced liver injury using the Nr2f 2-gene dose response’ mice model (Nr2f-null, wild-type, Keap1-KD and Keap1-HKO mice). Twenty-four hour after furosemide administration (250mg/kg, i.p.), serum ALT activities and histopathological analysis indicated severe hepatotoxicity in Nr2f-null and WT mice, but significantly less in the Nr2f-overexpressed Keap1-KD and Keap1-HKO mice. Furosemide increased the mRNA of genes involved in the acute phase response (Hlo-1 and MT-1), ER stress (Chop10 and Gadd45), inflammatory cytokine (IL-1B), chemokines (Mip-2 and mKC), as well as apoptosis (Egr1 and Bax) in livers of Nr2f-null and wild-type mice, but increased less in mice with more Nr2f. The two genotypes of over-expressed Nr2f mice had increased expression of the Nr2f target genes Gclm, Gclc and Nqo1 prior to furosemide administration, and were increased further after furosemide administration. Thus, our findings provide strong evidence that over-expression of Nr2f in Keap1-KD and Keap1-HKO mice and the increases in mRNA of a number of genes involved in anti-oxidative stress, anti-inflammation, anti-ER stress and anti-apoptosis protect against furosemide-induced hepatotoxicity.

1186 Acetaminophen (APAP)-Induced Hepatic Protein Adducts in Liver and Mitochondria of C57/B6 Mice Are Reduced by S-Adenosylmethionine (SAMe)
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Hepatotoxicity is associated with an overdose of the antipyretic drug, acetaminophen (APAP). APAP toxicity requires biotransformation to a toxic metabolite and increased oxidative stress. S-Adenosylmethionine (SAMe) protects mice from APAP overdose. The overall hypotheses for this study is that SAMe reduces APAP hepatic toxicity by preventing oxidative stress and post-translational modifications of proteins including the antioxidant enzyme, Manganese Superoxide Dismutase (MnSOD). Male C57Bl6 mice were divided into 4 groups (N=5-10/group) and injected intraperitoneal (ip) as indicated: vehicle (VEH, 15 ml/kg water ip), SAMe (1.25 mmol/kg 5 ml/kg, ip), APAP (250 mg/kg, ip) and SAMe and APAP (SAMe administered 1h after APAP). Livers were collected 4h following APAP. Subcellular fractions were isolated from mitochondria and cytosol. Equal amounts of protein were processed for Western analysis and immunoprecipitation. SAMe administered 1h after APAP reduced APAP hepatic toxicity when measured 4h post APAP injection. Protein carbonylation was increased by APAP and attenuated by SAMe. 3-Nitrotyrosine (3-NT) formation was increased in mitochondria by APAP and reduced by SAMe. Mitochondrial MnSOD nitrosylation was increased by APAP and reversed in the APAP+SAMe group. In conclusion, APAP induced protein carbonylation and nitrosylation of proteins which was reversed by SAMe treatment 1h post APAP. (Supported by NIH Grant 5P20RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence).

1187 Hepatic Vascular Endothelial Dysfunction in Environmental Toxin-Induced Nonalcoholic Steatohepatitis Is Regulated by TLR4-miR21-GRHL3 Axis
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The liver sinusoidal endothelium is very specialized and phenotypically differentiated. The aims of this study were to investigate the role of TLR4-linked upregulation of miR21 and its effect on the target; grainyhead-like 3 (GRHL3), in inducing the changes in liver histology and liver microvasculature. We used a murine model of environmental toxin-induced nonalcoholic steatohepatitis (NASH) where diet-induced obese (DIO) mice were exposed to disinfection byproduct bromochloromethane (BDCM). In addition, CYP2E1 and TLR4 gene deleted mice (KOs), exposed to BDCM were used for the study; to establish the role of metabolic
oxidative stress and TLR4 in hepatic endothelial dysfunction. qRTPCR was used to detect the changes in mRNA levels for vascular endothelial dysfunction markers, GRHL3 (regulates eNOS phosphorylation and hence endothelial dysfunction), TLR4; and in levels of miR21 (targets GRHL3). Western blot, oil red O, H&E, immunofluorescence staining and microscopic techniques were also used to test the experimental endpoints. mRNA and protein levels for vascular endothelial dysfunction markers were significantly increased in DIO+BDCM group compared to DIO, CYP2E1 KO and TLR4 KO groups. GRHL3 protein expression was significantly decreased (implying degradation of mRNA by miR21); whereas miR21 and TLR4 mRNA levels were significantly increased in DIO+BDCM group compared to DIO, CYP2E1 KO and TLR4 KO groups. The mRNA levels of inflammatory markers were significantly high in DIO+BDCM group compared to the other groups. CYP2E1 KO and TLR4 KO mice were protected from hepatic vascular endothelial dysfunction along with other NASH pathology as evidenced by decreased inflammation. In summary, our finding for the first time identifies the role of TLR4-miR21-GRHL3 axis in modulating hepatic vascular endothelial dysfunction and resultant inflammation. This forms a basis for a potential therapeutic target in the treatment of environmental toxin-induced NASH. (4R00ES19875-02 to Saurabh Chatterjee).

1188 Serum and Hepatic Fibroblast Growth Factor 21 Levels Are Increased in Subjects with Alcoholic Liver Diseases and in Mice Exposed to Alcohol by Decreased Transcriptional Suppression

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Liver-derived FGF-21 is a novel metabolic regulator of glucose and lipid metabolism. Alcohol exposure affects lipid metabolism by increasing lipogenesis and decreasing fatty acid β-oxidation. However, it is currently unknown whether alcohol exposure affects FGF-21 expression. Serum FGF-21 levels were measured in 24 consenting subjects (16 subjects with acute alcoholic hepatitis, 20 patients with alcoholic cirrhosis, and 9 subjects with alcohol abuse, and were compared to 26 healthy, non-drinking controls by ELISA. C57BL/6 mice were fed Lieber DeCarli diet containing 5% alcohol or maltose dextrin for 4 weeks (chronic), or for 12 days and then given one dose of alcohol at 6 g/kg by gavage 6 hours before sacrificing (chronic-binge), or only one dose of alcohol by gavage (acute). Serum and hepatic FGF-21 levels and hepatic FGF-21 mRNA levels were measured. Liver triglyceride and serum FFA were also measured. Serum levels of FGF-21 were markedly increased in both human subjects with ASH and alcohol abusers without ALD, but unchanged in AC vs. non drinking controls. Serum levels of FGF-21 were also markedly increased in mice exposed to alcohol, and the hepatic expression of FGF-21 were increased by both mRNA and protein levels. The increased FGF-21 expression was positively correlated with increased hepatic levels of triglyceride and serum levels of FFA. The expression of PGC-1α and Rev-Erb, which are important transcription suppressors of FGF21, were decreased in mouse livers exposed to alcohol. Alcohol exposure increased hepatic and circulating FGF-21 expression likely through an inhibition of transcriptional suppression mediated by the PGC-1α-Rev-Erbβ pathway. The regulation of FGF-21 expression may be associated with hepatic lipid metabolism in alcoholic steatohepatitis. The observed increase in circulating FGF-21 was conserved between animal models and human subjects with ALD.

1189 FGF21 Deficiency Exacerbates Chronic Alcohol-Induced Fatty Liver Disease

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Alcoholic fatty liver disease (AFLD), is considered as the earliest pathological alteration in ALD. Studies demonstrated that inhibition of fat accumulation and promotion of fat clearance prevents hepatic steatosis and slows or halts the progression of ALD. Previous studies have identified extrahepatic hormones are important in alcohol-induced alteration in liver lipid metabolism, little is known whether the paracrine and endocrine signal for metabolic regulation of hepatic itself participates in alcohol exposure associated lipid accumulation. Fibroblast growth factor 21 (FGF21) is a member of the endothelium FGF subfamily and a major metabolic regulator, which plays a critical role in the glucose and lipid metabolism. Here we demonstrated that FGF21 deficiency exacerbate chronic alcohol-induced AFLD. Global FGF21 knockout mice and their controls were fed Lieber DeCarli diet containing 5% alcohol or pair-fed isocaloric diet for 4 weeks. Alcohol feeding increased hepatic fatty acid and triglyceride contents and fat accumulation. There was no obvious alteration in hepatic fat accumulation in FGF21 deficiency mice under pair feeding. However, a markedly increased hepatic fat was detected in FGF21 knock-out mice when fed chronic alcohol. Further studies showed that FGF21 deficiency exacerbated alcohol-induced liver injury. The FGF21 exacerbated-fat accumulation was associated with upregulation of the genes involved in fatty acid de novo synthesis, such as PPS and SCD-1, and the decrease in the gene expression responsible for fatty acid β-oxidation, such as CPT-1. Mechanistic studies showed that FGF21 knockout decreased SIRT1 which deacetylates SREBP-1c. In addition, we showed that FGF21 deficiency also decreased LKB1 which activates AMPK. The downregulation of SIRT1 and LKB1/AMPK in FGF21 deficiency livers of mice exposed to chronic alcohol lead to SREBP-1c and PGC-1a-mediated increased fatty acid synthesis and decreased fatty acid β-oxidation. In conclusion, FGF21 is likely required for cellular defense against hepatic lipogenesis and cellular capacity for fat clearance in subjects exposed to chronic alcohol.

1190 Uric Acid Inhibits Lipid Metabolism through Suppression of Autophagy

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Fructose which is reported to be lipogenic is rapidly phosphorylated to fructose-1-phosphate by fructokinase using ATP. It results in AMP accumulation causing activation of AMP deaminase and generation of uric acid. Patients with hyperuricemia or gout usually accompany hyperglycemia and hyperuricemia is also prevalent in patients presenting nonalcoholic fatty liver disease (NAFLD) that develops hepatic steatosis in the lack of alcohol abuse. In this context, we hypothesized that uric acid which comes from the fructose metabolism promotes triglyceride accumulation and development of fatty liver. Today, it is well demonstrated that fatty liver mainly results from increased de novo lipogenesis, increased transport of triglyceride into the liver and decreased lipolysis. In this paper, we tried to characterize the role of uric acid in lipolysis. A newly found function of autophagy is the degradation of lipid droplets in hepatocytes. Accordingly, we hypothesized that uric acid contributes to the pathogenesis of development of fatty liver by regulation of autophagy. We found that uric acid inhibits autophagy in concentration- and time-dependent manner in HepG2 cell line. Although uric acid did not suppress the expression of autophagy related proteins but activated Akt followed by inhibitory phosphorylation of TSC2, subsequently increased phosphorylation of mTOR and decreased autophagy. It was well known that starvation-induced autophagy degrades lipid droplet and releases fatty acids for mitochondrial β-oxidation. Pretreatment of uric acid suppressed starvation-induced autophagy and decreased intracellular lipid utilization by starvation. In addition, long-term treatment of uric acid itself increased intracellular lipid content and cells incubated with oleate in the presence of uric acid deposited more lipid than those only treated with oleate. In conclusion, this study first demonstrated a novel mechanism that uric acid regulates lipid metabolism through suppression of autophagy.

1191 Platelet Function Inhibits Liver Injury and Fibrosis Induced by a Bile Duct Toxicant

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Chronic liver disease in humans and animal models is associated with hemostatic changes including platelet activation and deposition of cross-linked fibrin in the liver. Previous studies have suggested that hepatic platelet accumulation inhibits liver fibrosis and promotes liver repair. Complete fibrinogen deficiency worsens liver injury in mice with chronic liver disease. However, the pathways linking coagulation to platelet activation during chronic liver disease are not known. We hypothesized that elements of platelet activation and function, including thrombin mediated protease activated receptor (PAR)-4 signaling and fibrin engagement of the gIIbIIIa integrin on platelets inhibit hepato cellular injury and fibrosis in a mouse model of xenobiotic-induced chronic liver injury and fibrosis. To test this hypothesis, mice expressing a mutant form of fibrinogen (ogen) incapable of binding gIIbIIIa (FibTA5 mice) and mice lacking PAR-4 (PAR-4/-/- mice) were used. FibTA5 mice, PAR-4/-/- mice and wild-type (WT) mice were fed a purified diet (AIN-93M) containing the bile duct toxin alpha-naphthylisothiocyanate (ANTT) (0.025%), or control diet (AIN-93M) for four weeks. PAR-4 deficiency did not increase liver necrosis in mice fed ANTT diet. However, compared to WT mice, portal inflammation and peribiliary fibrosis increased significantly in PAR-4/-/- mice fed ANTT diet. Focal hepatocellular necrosis and serum alanine aminotransferase activity were markedly increased in FibTA5 fed ANTT diet compared to WT mice and this corresponded with a significant induction of proinflammatory genes and increased depo-
Effects of chlorpromazine (CPZ), known to induce intra-hepatic cholestasis have been analyzed using differentiated human HepaRG cells. Bile acids efflux and influx have previously been reported to be inhibited by CPZ. These changes correlated with occurrence of a strong contraction of the bile canalicular lumen (BCL). In this work we attempted to define the molecular mechanisms involved in BCL morphogenesis and reorganization in CPZ-treated HepaRG cells by imaging analyses. We observed that BCL contraction was a dose-dependent morphogenic process associated with a reduction to 2, in the number of cells clustered to form each bile canaliculus (BC), involving extensive cytoskeleton reorganization. These changes were correlated with the RHO-kinase (ROCK) pathway induction as also argued by the antagonized effects obtained with various inhibitors of this pathway. Addition of ROCK inhibitor to normal cells resulted in the development of enlarged apical lumen that shared 3 or more cells per single BC and lined with a dense F-actin network. Similar remodeling of the apical lumen of CPZ-treated cells exposed to ROCK inhibitor was observed. Using ETA, a ROCK inducer, we reversed the apical lumen remodeling to small lumen with only 2 or 3 clustered cells, thus mimicking CPZ-treated cells. In addition, inhibition of the myosin heavy chain ATPase by BDM, a downstream ROCK target controlling contraction by actin-myosin interactions, mimicked the reorganization induced by ROCK inhibitor of the large canalicular lumen by increasing the number of cells sharing a single BC. Taken together, these results demonstrate that HepaRG hepatocytes can rapidly respond to the main factors modulating the ROCK pathway and identified as controlling the BCL morphogenesis. They highlight the critical role of this pathway in CPZ-induced cholestasis. Understanding of these control mechanisms should help development of liver-cell based therapies.

LGGs treatment as evaluated by triglyceride content measurement and Oil Red O staining of the liver sections. In addition, alcohol exposure also increased circulating and hepatic free fatty acid concentration, which is attenuated by LGGs treatment. Further study demonstrated that alcohol induced a significant decrease in AMPK and ACC phosphorylation, and these effects were prevented by LGGs administration in animals, which is correlated with the increase of the expression of adiponectin receptors. We also found that alcohol exposure increased hepatic SREBP-1c protein expression and LGGs treatment inhibited this increase. Further studies showed that LGGs prevented alcohol-induced hepatic apoptosis as evaluated by TUNEL assay. In vitro studies confirmed the positive effects of LGGs in promoting AMPK phosphorylation. These results suggest that LGGs is effective in preventing chronic alcohol-induced liver injury by suppression of alcohol-induced hepatic apoptosis and steatosis through AMPK phosphorylation. This finding could lead to developing new strategies for reducing chronic alcohol-induced liver injury by secreted factors of LGG culture.
Furosemide (FS) is a common diuretic. Though safe when used appropriately, it is increasingly popular as a model hepatotoxicant, due in part to the fact that the histological pattern of injury resembles acetaminophen but it does not appear to involve mitochondrial dysfunction. Despite this, the mechanisms of FS-induced liver injury are unclear. It was shown that a reactive metabolite binds to proteins, and that inhibition of P450s prevents the toxicity. Beyond this, however, little is known. To explore the mechanisms of FS hepatotoxicity, we treated mice with 500 mg/kg FS and collected plasma and livers at various time points to probe for evidence of activation of cell signaling pathways. In animals with liver injury, plasma ALT increased by 6h (246±46 U/L) and was constant until at least 12h (151±15 U/L). Interestingly, ALT dramatically increased at 24h (2.24±1695 U/L). Histology and TUNEL staining revealed spotty necrosis at early time points, which developed into widespread centrolobular necrosis. Immunoblotting for caspase-3 failed to provide evidence for apoptosis, and cytochrome c release into cytosol did not increase before 24h, consistent with the idea that FS toxicity does not involve mitochondrial damage. Nevertheless, JNK phosphorylation and translocation to mitochondria were observed at 6h and persisted. RIP3, which may be involved in JNK signaling, was induced by 6h. Because of the unusual time course showing an initial injury with a delayed increase, we also stained liver sections for neutrophils. Interestingly, we observed neutrophil infiltration by 6h, but the most infiltration was seen at 24h. Finally, we observed an increase in cell death (LDH release) in metabolically-competent human liver cells (HepaRG) treated with FS.

Conclusions: Large doses of FS cause injury in both mice and human HepaRG cells. The mechanism of hepatotoxicity in mice may involve intracellular signaling pathways at early time points, followed by inflammation at later times.

1198 Hepatic Toxicological and Lipid Peroxidation Assessments of Pioglitazone on Albino Wistar Rats

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Pioglitazone is one of the thiazolidinedione anti-diabetic drugs which have been used for the treatment of non-insulin dependent diabetes mellitus. This work aims at studying the hepatic toxicity of pioglitazone (PIO) when administered in various high concentrations in female albino Wistar rats. Four groups of seven animals each were treated with test substances for twenty eight days. Group one was given 0.5 ml distilled water, while groups 2 to 4 were given 15 mg/kg, 30 mg/kg and 45 mg/kg of body weight of PIO respectively. Using standard biochemical kits and reported chemical procedures, hepatic biochemical, histological and lipid peroxidation assessments of PIO were determined in the plasma of experimental animals. There was no significant change in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total and direct bilirubin and malondialdehyde concentrations in pioglitazone-treated groups when compared to control(<0.05). Pioglitazone did not elicit any noticeable liver toxicity at the experimental doses used in the study in non-diabetic animal model.

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Key words: Pioglitazone; hepatic biochemical parameters; Lipid peroxidation

1199 NSAIDs Synergize with Inflammatory Cytokines to Kill Hepatocytes: Implications in Idiosyncratic Reactions

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Idiosyncratic drug-induced liver injury (IDILI) occurs in a small fraction of patients taking a drug and, although rare, can result in severe liver injury or death. The mechanisms of IDILI are unknown; however, factors resulting from an activated immune system are likely to be important in the pathogenesis. The inflammatory mediators, tumor necrosis factor-alpha (TNF) and interferon-gamma (IFN) are two such factors that can activate pathways to cell death as well as proliferation. We tested the hypothesis that non-steroidal anti-inflammatory drugs (NSAIDs) associated with IDILI in humans synergize with TNF and/or IFN to cause death of hepatocytes and inhibit cell proliferation in vitro. HepG2 cells were treated with NSAIDs that have high IDILI liability (diclofenac, sulindac sulfide, bromfenac, or nimesulide) and NSAIDs with less IDILI liability (naproxen, ibuprofen, rofecoxib, and aspirin) alone and in combination with TNF and/or IFN. Treatment of cells with drugs with high IDILI liability in the presence of TNF resulted in cytotoxicity. IFN potentiated the cytotoxicity caused by diclofenac, sulindac sulfide, and bromfenac in the presence of TNF. Naproxen and ibuprofen synergized with TNF to cause cytotoxicity in HepG2 cells, but IFN did not affect the response to any of the drugs with low IDILI liability. Treatment of cells with diclofenac in the presence or absence of TNF and IFN inhibited proliferation of HepG2 cells. These data indicate that NSAID-induced hepatocellular stress interacts with TNF and IFN to cause cell death and inhibit proliferation. Given that failure of proliferative repair could magnify modest liver damage, these findings raise the possibility that some IDILI reactions result from such drug-cytokine synergy. (Supported by NIH grant DK061315 and T32 GM092715)

1200 Elevation of Serum Transaminases Due to CSF1-R Inhibition


Serum biomarkers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are well-characterized hepatotoxicity biomarkers that correlate well with liver histopathology. However, it has been reported that colony stimulating factor-1 receptor (CSF1-R) inhibition results in decreased proliferation, differentiation and function of macrophages, leading to decreased clearance of serum transaminases. In support of this observation, we previously demonstrated increases in serum AST in the absence of histopathology following treatment of rats with CC0485118, a small molecule kinase inhibitor of spleen tyrosine kinase (Syk) that also inhibits CSF1-R. In the current study, we investigated the effect of CSF1-R inhibition by CC0485118 on AST clearance in serum. Jugular vein cannulated (JVC) Sprague-Dawley rats were dosed with 400 mg/kg of CC0485118

316 SOT 2014 ANNUAL MEETING
for 4 consecutive days via oral gavage. On last day of treatment, rats were injected intravenously with 200 μl of purified rat AST. Serial blood samples were collected at baseline and up to 24 hours post-injection. In this study, clearance of AST in test article-treated animals was approximately 50% of vehicle-treated animals. Similar findings were seen in a subsequent study using an inhibition of AST, indicating that the increase in AST were due to decreased clearance and not increased production. This mechanism was further investigated in vivo using rat hepatocyte-Keupfer cell co-cultures treated with increasing concentrations of CC0485118 (10, 50, or 100 μM) to evaluate compound effect on the phagocytic function of Kupffer cells. Results showed that 50 μM or higher of CC0485118 attenuated the uptake of S. aureus bioparticles compared to vehicle alone. The inhibition was not due to compound toxicity as assessed by ATP assay. The evidence of in vitro inhibition of Kupffer cell function and in vivo AST clearance attenuation related to CC0485118, supports the hypothesis that the elevation of AST is caused by decreased phagocytic activity by liver macrophages.

### 1200a Regorafenib at Clinically Relevant Concentrations Impairs Mitochondrial Functions Causing Necrosis in Primary Rat Hepatocytes


Regorafenib, a kinase inhibitor, was recently approved by regulatory agencies for cancer treatment. Based on clinical trial data, a black box warning about regorafenib-induced hepatotoxicity was included in the FDA-endorsed product label at the time of market approval. The mechanism for regorafenib-associated hepatotoxicity is unknown. Isolated rat liver mitochondria or primary hepatocytes were incubated with regorafenib. Oxygen consumption was determined using a Clark electrode. Mitochondrial permeability transition (MPT) was measured by a swelling assay and the fluorescent dye calcine. Mode of cell death was determined by annexin V/propidium iodide staining supplemented with measuring lactate dehydrogenase leakage and caspase activation. In isolated mitochondria, regorafenib significantly uncoupled (but did not inhibit) oxidative phosphorylation (OXPHOS) and promoted calcium overload-induced mitochondrial swelling, with the latter being completely preventable by cyclosporin A (CsA). In primary hepatocytes, regorafenib caused strong uncoupling of OXPHOS, a rapid onset of MPT, a sharp decrease in mitochondrial inner membrane potential, a significant adenine nucleotide triphosphate (ATP) shortage, and ensuing necrosis (but not apoptosis). Pretreatment by MPT blockers CsA and trifluoperazine, or the glycolysis enhancer fructose plus the mitochondrial ATPase inhibitor oligomycin A, or their combination, significantly delayed the onset of necrosis. Remarkably, all detrimental effects were observed at clinically-relevant concentrations of 2-15 μM, which were in the range of maximal blood concentrations achieved after taking recommended daily doses. The data demonstrate that regorafenib causes necrosis in primary hepatocytes by uncoupling OXPHOS and MPT induction, indicating that mitochondrial damage possibly contributes to the pathogenesis of regorafenib-induced hepatotoxicity.

### 1200b Targeted Serum Bile Acid Profile of Naïve Rats and Dogs over a 24h Time Course


Regorafenib, a kinase inhibitor, was recently approved by regulatory agencies for cancer treatment. Based on clinical trial data, a black box warning about regorafenib-induced hepatotoxicity was included in the FDA-endorsed product label at the time of market approval. The mechanism for regorafenib-associated hepatotoxicity is unknown. Isolated rat liver mitochondria or primary hepatocytes were incubated with regorafenib. Oxygen consumption was determined using a Clark electrode. Mitochondrial permeability transition (MPT) was measured by a swelling assay and the fluorescent dye calcine. Mode of cell death was determined by annexin V/propidium iodide staining supplemented with measuring lactate dehydrogenase leakage and caspase activation. In isolated mitochondria, regorafenib significantly uncoupled (but did not inhibit) oxidative phosphorylation (OXPHOS) and promoted calcium overload-induced mitochondrial swelling, with the latter being completely preventable by cyclosporin A (CsA). In primary hepatocytes, regorafenib caused strong uncoupling of OXPHOS, a rapid onset of MPT, a sharp decrease in mitochondrial inner membrane potential, a significant adenine nucleotide triphosphate (ATP) shortage, and ensuing necrosis (but not apoptosis). Pretreatment by MPT blockers CsA and trifluoperazine, or the glycolysis enhancer fructose plus the mitochondrial ATPase inhibitor oligomycin A, or their combination, significantly delayed the onset of necrosis. Remarkably, all detrimental effects were observed at clinically-relevant concentrations of 2-15 μM, which were in the range of maximal blood concentrations achieved after taking recommended daily doses. The data demonstrate that regorafenib causes necrosis in primary hepatocytes by uncoupling OXPHOS and MPT induction, indicating that mitochondrial damage possibly contributes to the pathogenesis of regorafenib-induced hepatotoxicity.

### 1200c Effects of Red Ginseng Extracts on the Ethanol-Induced Hepatotoxicity In Vivo and In Vitro

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Red ginseng is a root of Panax ginseng C.A. Meyer, which has been widely used for traditional medicinal purposes, was evaluated using male Wistar albino rats. Acute and sub chronic toxicity was evaluated after 90 days of exposure. The LD50 was 2154mg/kg. Serum levels of glucose, lactate dehydrogenase, aspartate aminotransferase transaminase, and total bilirubin increased significantly in the 58.5 and 1077mg/kg dose groups. These two groups also had significantly reduced serum levels albumin and total serum protein when compared with the control group. Histopathological assessment showed degenerative changes in the liver.

### 1200d CYP2A5 Contributes to Cadmium-Induced Liver Injury but Protects against Alcoholic Liver Disease

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Ethanol can induce cytochrome P450 2E1 (CYP2E1), an activator of reactive oxygen species, and CYP2E1 is considered a risk factor for alcoholic liver disease. Recently we found that ethanol can also induce CYP2A5, and ethanol induction of CYP2A5 is CYP2E1-dependent and regulated by Nrf2 pathway. Cadmium can also induce CYP2A5 and cadmium induction of CYP2A5 is also regulated by Nrf2, but cadmium does not induce CYP2E1. The role of CYP2A5 in liver injury is still unclear. In this study, we examined liver injury induced by ethanol and cadmium chloride (CdCl2) in CYP2A5 knock out (cyp2a5−/−) mice and wild type (WT) mice. To examine cadmium-induced liver injury, WT and cyp2a5−/− mice (male, 8-10 weeks old) were injected CdCl2 intraperitoneally at a dose of 5 mg/kg, and then the mice were sacrificed 24 h after the CdCl2 administration. CYP2A5 was induced in WT mice but not in cyp2a5−/− mice. Serum levels of ALT and AST were increased to a greater extent in WT mice than in cyp2a5−/− mice. Cadmium-induced necrosis was more severe in WT mice than in cyp2a5−/− mice. Consistently, 3-nitrotyrosine (3-NT), a marker of oxidative stress, was induced in WT mice more than in cyp2a5−/− mice. As for alcoholic disease, Lieber-Decarli model was applied. After 3 weeks of feeding with Lieber-Decarli ethanol liquid diet, unlike cadmium-induced liver injury, ethanol-induced liver injury was enhanced in cyp2a5−/− mice compared with WT mice as indicated by serum ALT, hepatic fat accumulation (steatosis) and necro-inflammation observed in H&E stained liver sections. Ethanol-induced oxidative stress was also higher in cyp2a5−/− mice than WT mice. Ethanol feeding induced CYP2A5 in WT mice but not in cyp2a5−/− mice, although induction of CYP2E1 was comparable in cyp2a5−/− and WT mice. These results suggest that CYP2A5 contributes to cadmium-induced liver injury, and in contrast, CYP2A5 protects against ethanol-induced liver injury.
ethanol-fed mice and hepatoma cells. Mice were randomly divided into 4 groups: control, EtOH fed group, EtOH and Korean red ginseng extracts (KRG extract) fed group (250 and 500 mg/kg) for 4 weeks. AML-12 cells were treated with EtOH and/or red ginseng extracts (0 – 1 mg/ml). Lipid droplets were determined using Oil red O staining and expression of SirT1 and SREBP-1 were investigated by western bloting in microsomal fraction and cells. The orally KRG extracts administrated mice showed hepatoprotective effects against EtOH-induced hepatotoxicity, and inhibited immunoreactivities, expression of nitrotyrosine as marker of iNOS related oxidative stresses and 4-HNE as marker of lipid perox- idation. Treatment of KRG extracts significantly increased SirT1 and decreased SREBP-1 in ethanol-fed mice and cells. We investigated the effect of individual ginsenoside related to regulation of SirT1 and SREBP-1 contrasted in EtOH- treated cells. This finding indicated that the treatment of KRG extracts showed hepatoprotective effects against EtOH-related hepatotoxicity in vivo and in vitro.

1200h In Vivo Optical Imaging of Acetaminophen-Induced Liver Toxicity
Drug induced liver injury (DILI) is a major reason for late stage termination of drug development projects, so assessment is now routinely being integrated earlier in the drug development process. In vivo optical imaging offers non-invasive detection of biological changes in preclinical animal efficacy models, and this approach is also ideal for in vivo toxicology determination. We applied near infrared (NIR) fluorescence imaging techniques to the detection and quantification of DILI in living mice, using NIR fluorophore-labeled Annexin V (Annexin-Vivo 750 [AV750]) to detect cell surface phosphatidylserine on apoptotic and necrotic cells. AV750 was imaged 2h after injection, and could be re-injected safely every 24 h for long-term monitoring of the same animals. As a positive control, we used acetaminophen (APAP), a commonly used over-the-counter analgesic and antipyretic drug that is known to cause centrilobular hepatic necrosis when used at high doses. When male C57BL/6 mice were injected with a single dose (500 mg/kg) of APAP, the resulting liver necrosis peaked at 24 hours as detected by imaging with Annexin-Vivo 750 in living animals. Imaging results at 24h were statistically significant as compared to those of the PBS control group using only 3-4 mice per group. Both fluorescent tomographic imaging (FMT 4000), and higher throughput epifluorescence imaging (IVIS Spectrum CT), provided excellent detection of AV750 fluorescence in the liver. Histology and plasma ALT confirmed the kinetics of tissue necrosis, and at 48 hours more extensive liver damage was seen but with a decrease in tissue phosphatidylserine and plasma ALT, suggesting a decline in active tissue destruction. Maximal tissue damage was measured at 300 and 500 mg/kg APAP doses by AV750. Compared to conventional plasma/serum assays, in vivo imaging can offer fast, quantitative imaging results directly assessing the tissue of interest. Our results to date demonstrate the utility of optical imaging for assessing potential compound liver toxicity in early drug development programs.

1200i Leelamine Is A As a Potent Novel Inducer of Hepatic CYP2B
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Cytchrome P450 (CYPs) are the important enzymes take part in a xenobiotic substance like as toxic chemicals, drugs. Many drugs may affect the activity of CYP, which is a major source of adverse drug interactions. Phenobarbital (PB), one of the antiepileptic drugs is well known to induce drug metabolizing enzymes in the liver. Especially, PB has been widely used to induce the CYP2B for in vivo monitoring of the same animals. As a positive control, we used acetaminophen (APAP), a commonly used over-the-counter analgesic and antipyretic drug that is known to cause centrilobular hepatic necrosis when used at high doses. When male C57BL/6 mice were injected with a single dose (500 mg/kg) of APAP, the resulting liver necrosis peaked at 24 hours as detected by imaging with Annexin-Vivo 750 in living animals. Imaging results at 24h were statistically significant as compared to those of the PBS control group using only 3-4 mice per group. Both fluorescent tomographic imaging (FMT 4000), and higher throughput epifluorescence imaging (IVIS Spectrum CT), provided excellent detection of AV750 fluorescence in the liver. Histology and plasma ALT confirmed the kinetics of tissue necrosis, and at 48 hours more extensive liver damage was seen but with a decrease in tissue phosphatidylserine and plasma ALT, suggesting a decline in active tissue destruction. Maximal tissue damage was measured at 300 and 500 mg/kg APAP doses by AV750. Compared to conventional plasma/serum assays, in vivo imaging can offer fast, quantitative imaging results directly assessing the tissue of interest. Our results to date demonstrate the utility of optical imaging for assessing potential compound liver toxicity in early drug development programs.
Air pollutants such as environmental tobacco smoke (ETS) and acrolein (the predominant irritant in tobacco smoke) produce oxidative stress in the respiratory tract airways. Oxidative stress plays an important role in the pathogenesis of airway diseases such as asthma. Epidemiological evidence suggests that acetaldehyde (N-acetyl-papa-aminophenol, APAP), a widely used analgesic, may also play a role in the pathogenesis of asthma via oxidative stress-related mechanisms, but direct data on the pro-oxidant effects of APAP in the airways is absent. To determine if APAP causes oxidative stress in the airways at near-therapeutic doses we administered APAP to female C57Bl/6 mice and measured tissue protein-nitrosothiols (NPSH) as a marker for the antioxidant glutathione, and antioxidant response element (ARE)-dependent gene induction by qRT-PCR. Lung, trachea, and nasal NPSH levels were diminished to ~80% of control 1 hr following a 100 mg/kg dose of APAP (ip). APAP at 60 and 100 mg/kg caused significant ARE gene induction within 2hrs, and pre-treatment of mice with APAP potentiated the ARE gene response to ETS (5 mg/m3). We next determined if APAP modulated the airway responsiveness to oxidant air pollutants using sensory irritation as an index of airway response. Sensory irritation is due to stimulation of trigeminal sensory nerves and is known to be pro-inflammatory. APAP alone (100 mg/kg) did not elicit a response, but it greatly increased the sensory irritation response to both acrolein and ETS. The current results demonstrate that APAP, at near-therapeutic doses, causes oxidative stress in airway tissues and enhances the airway response to oxidant air pollutants. Children exposed to ETS are more likely to develop asthma. Modulation of airway responses to ETS provides a mechanism for the association between APAP use and the development of asthma in pediatric populations. (Supported by the University of Connecticut President’s Research Award)

**1202 Characterization of the Blu E-Cigarette to Define the Composition of Inhaled Material**

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In order to better define human exposure and to design experiments for toxicology systems (in vitro and in vivo) an aerosol characterization of the Blu® e-cigarette was conducted. E-cig were characterized for aerosol mass, particle size, gas/particle partitioning, excipient, nicotine and formaldehyde content. We applied a modified Canadian Intensive protocol and developed a novel characterization system. The Blu® e-cig heating processes resulted in generation of an aerosol mixture of particle expentiants, nicotine present in both the gas and droplet phase, and small but detectable amounts of formaldehyde. There were over 1 million particles/cm3 of ~110 nm in size measured from a puff. E-cigarettes may have a decreased risk to users compared with conventional inhaled tobacco products. The aerosols were composed of evaporative particles, with lesser amounts of nicotine and other gases such as formaldehyde. This contrasts marketing for the device, which suggests that people only inhale nicotine vapor and water. The health consequence of these inhaled materials is uncertain based on available evidence, but the information provided here will assist in future risk assessments and the design of toxicology studies.

**1203 A Mechanistic Study of Cigarette Smoke-Induced COPD in C57Bl/6 Mice: The Impact of Switching to a pMRTP**

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In this study, the impact on the development of emphysema/COPD following inhalation of aerosol from two tobacco products, a reference cigarette (3RF) and a prototypic modified risk tobacco product (pMRTP), was evaluated in C57Bl/6 mice. The mice were exposed to an aerosol from 3RF (750 µg TPM, 34.4 µg/l nicotine), pMRTP (nicotine concentration-matched) or filtered air (sham) for 4 hours per day, 5 days per week, up to 7 months. At 2 months of exposure to 3RF, switching and cessation groups were exposed to pMRTP or filtered air, respectively. To analyze the progression of emphysema, evaluations of inflammation, pulmonary function, and various histopathological and molecular changes (transcriptomics and lipidomics) were performed at months 1, 2, 3, 4, 5 and 7. Exposure to 3RF induced molecular, cellular and physiological modifications in lungs leading to emphysematous changes. Animals exposed to pMRTP exhibited negligible changes in all parameters assessed. Both cessation and switching groups showed a reversal of the inflammatory and functional responses induced by 3RF smoke. Histopathological evaluation revealed a slowdown in the progression of emphysematous changes in switching and cessation groups. Smoking cessation or switching resulted in the rapid recovery at the transcription level. The lipid profiles in lung, liver and plasma similarly showed a clear 3RF effect, with minimal changes associated with pMRTP exposure, and recovery near to sham-exposed levels following either switching or cessation. These data demonstrate that exposure to pMRTP for up to 7 months resulted in a response similar to fresh air-exposed animals using a battery of physiological and molecular measures. Furthermore, following a 2 month 3RF cigarette smoke exposure period, both cessation and switching to a pMRTP aerosol resulted in the reversal or stabilization of parameters assessed.

**1204 Examination of Acute Pulmonary Responses to Various Cookstove Exhaust Emissions**


Air pollution is a global public health problem, to which the emissions from rudimentary cooking devices has been estimated to contribute significantly through the burning of various types of biomass. Notably, exposure to cookstove emissions (CE) has been linked to increases in morbidity and mortality causing an estimated 4 million deaths which most frequently impact women and children. Current efforts to reduce CE have led to the development of several new cookstoves (CS) designs. Although some studies have characterized improvements in the overall release of specific pollutants from modern CS, there is less information regarding the potential health benefits that may be derived from their usage. In this study, currently marketed CS models representing 4 different CS types, 3-Stone (3S), 3-Stone Forced Draft (FD), Natural Draft (ND), and Propane (PR), were used under the same cooking conditions for two consecutive cooking cycles. Exhaust emissions from each stove were characterized, and young adult female CD-1 mice were simultaneously exposed for 2-3 hr per day. Various endpoints examined at 0, 4, and 24 hr post-exposure (PE) included lung inflammation and changes in redox status/antioxidant capacity. Of these endpoints, CS emissions were observed to most significantly affect the pulmonary redox status of exposed animals via the oxidation of cellular glutathione. As compared to filtered air controls, lung homogenates from CE-exposed mice had consistently decreased levels of reduced glutathione (GSH), with corresponding increases in disulfide glutathione (GS=GS), at 0 and 24 hr PE for all 4 CS. While no significant increase in pulmonary inflammation were observed in CE-exposed animals, macrophages from 3S and ND-exposed mice had a visible particle burden 24 hours PE, as compared to air controls. Together, these data support the need for a continued assessment of the health effects resultant from prolonged exposure to the exhaust emissions of modern CS. (Does not necessarily reflect USEPA policy)

**1205 Effect of Near-Road Particulate Matter on Respiratory Responses and Inflammation in Healthy and Ovalbumin-Allergic Mice**

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The Near-Road Exposures and Effects of Urban Air Pollutant Study (NEXUS) previously examined the association of near-roadway exposures to air pollutants and respiratory outcomes in children with asthma. This toxicological study was designed to complement NEXUS and determine which particulate matter (PM) characteristics may contribute to exacerbation of allergic asthma. Samples of coarse, fine and ultrafine PM were collected upward and downward from I-96 in Detroit, MI during Winter 2010/11. Healthy and ovalbumin (OVA)-sensitized female Balb/c mice were exposed via oropharyngeal (OP) aspiration to 20 or 100 µg coarse, fine, or ultrafine fractions of upward or downward PM 2 hr prior to OP challenge with 20 µg OVA. No samples caused significant changes in immediate responses to OVA challenge as determined by whole body plethysmography (WB). Two days later, airway responsiveness to methacholine aerosol (assessed by WB) was not significantly affected by PM exposure in either healthy or allergic mice. In OVA-allergic mice, 100 µg downward coarse PM caused a greater increase than downwind fine or ultrafine PM in bronchoalveolar lavage (BAL) neutrophils (7x vs. control blank filter extract) and eosinophils (10x) and also caused significant increases in BAL LDH and NAG. Interestingly, 100 µg upward fine PM produced greater increases in neutrophils (7x vs. control blank filter extract) and eosinophils (4x) than upward coarse or ultrafine PM. Ultrafine PM did not significantly increase neutrophils or eosinophils in comparison to allergic mice given ultrafine blank filter extract. Further analyses will be conducted to investigate associations between
chemical components and phenotypic effects of allergic lung disease. We conclude that coarse PM downwind and fine PM upwind of traffic promote allergic inflammation in OVA-allergic mice. (This abstract does not represent U.S. EPA policy.)

**1206 Diurnal Variation in Toxicological Effects of Size-Segregated Particulate Samples Collected from High-Air Pollution Situation in China**


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Air pollution is a wide recognized problem all over the world. The air pollution levels in ambient air are known cause for premature deaths, many respiratory and cardiac diseases as well as cancer. We studied the in vitro toxicological responses associated with the observed adverse health effects. Thus far, the daily variations in air pollution levels and the consequent health effects have been studied to a lesser extent. We collected the particles for toxicological and chemical analyses from University of Nanjing, Xianlin campus. The generally high air pollution situation in China allowed us to separate between the day and nighttime samplings due to sufficient collected mass. With this approach we also got information on how the atmospheric processes and changes in the emission sources alter the toxicity of the collected particulate matter. The samples were collected with high volume cascade impactor in four different size ranges (PM10-2.5; PM2.5-1; PM1-0.2; PM0.2) and then extracted from the sampling substrates. Mouse macrophage cell line was employed in detection of the toxicological responses of the particulate samples including cytotoxicity, genotoxicity and inflammatory responses. The results showed that both chemistry and toxicological responses change between day and night. Nighttime samples had much more PAHs than the daytime samples. Cytotoxicity was rather similar in the day and night samples. However, inflammatory responses and genotoxicity were significantly higher during the daytime when compared to the night. It is possible that breakdown products of the PAHs are responsible for the higher responses during the daytime. When compared to the previous results from less polluted situations in Europe, the responses were quite different. In conclusion, the toxicity of the particulate mass in polluted situation during day and nighttime showed large variation.

**1207 Particulate Matter from Saudi Arabia Induces Genes Involved in Cholesterol and Lipid Metabolism**

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Airborne particulate matter (PM) exposure is a major environmental health concern and is linked to metabolic disorders, such as cardiovascular diseases (CVD) and diabetes, which are on the rise in the Kingdom of Saudi Arabia. This study investigated changes in mouse lung gene expression caused by administration of PM10 collected from Jeddah, Saudi Arabia. FVB/N mice were exposed to 100 μg PM10 or water by aspiration and euthanized 24 h later. The bronchoalveolar lavage fluid (BALF) was collected and analyzed for neutrophil concentration and TNF-α and IL-6 levels. IL-6 and TNF-α were significantly higher in mice exposed to PM according to an ELISA assay and neutrophil concentration was also increased in exposed mice. RNA was extracted from the lungs and whole transcript was analyzed using Affymetrix Mouse Gene 1.0 ST Array. Mice exposed to PM10 displayed an increase in neutrophil concentration and elevated TNF-α and IL-6 levels. Gene expression analysis revealed that mice exposed to PM10 displayed 202 genes that were significantly up-regulated and 40 genes that were significantly down-regulated (p<0.05). PM10 induced genes involved in inflammation, cholesterol and lipid metabolism. This is the first study to demonstrate that Saudi Arabia PM10 increases in vivo expression of genes located in pathways associated with diseases involving metabolic syndrome.

**1208 Compounds Collected from Indium-Tin Oxide Production Induce Inflammatory Responses from Cultured Macrophages and Bronchial Epithelial Cells**

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Indium-tin oxide (ITO) is used to make transparent conductive coatings for touch-screen and liquid crystal display electronics. Lung disease among workers in the ITO industry is an emerging occupational health concern as the demand for consumer electronics continues to increase. Epidemiologic studies have shown indium compound-exposed workers to have pulmonary alveolar proteinosis and fibrotic interstitial lung disease. However, the molecular mechanisms behind indium compounds’ toxicity remain largely unknown. Thus, we aim to uncover how compounds encountered during ITO production affect cultured cells and ultimately, contribute to the pathogenesis of indium lung disease. We hypothesize that indium compounds (8 different samples collected from various stages at an ITO facility) cause lung pathology through direct cytotoxicity and/or via inflammatory signaling from exposed cells. Preliminary studies showed that exposure of RAW 264.7 monocyte macrophages and BEAS-2B bronchial epithelial cells to indium compounds resulted in significantly reduced viability. Microscopy techniques revealed that various indium compounds interact with and are engulfed by both cell lines within 1 to 3 hours, suggesting that cellular reactions may be occurring very rapidly. Indeed, nuclear factor kappa beta (NFκB) activation occurs within 3 hours of treatment with compounds containing sintered ITO in both cell lines. Robust cytokine production (TNFα, IL-1β, IL-6, and IL-8) following cellular exposures confirmed that pro-inflammatory responses are indeed occurring. Our results suggest that inflammatory responses to indium compounds by both pulmonary macrophages and epithelial cells may initiate and propagate indium lung disease. These findings have provided a better understanding of the molecular mechanisms behind an emerging occupational health issue and will aid in the discovery of biomarkers for disease prevention.

**1209 Differential Diagnosis of Airways Diseases Using the Ratio of FEV1/FVC in Subjects Occupationally Exposed to Powder Particles in a Pharmaceutical Industry in Nigeria**

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Contribution of exposure to chemicals, fumes, dust and particles to the growing incidence of Chronic Obstructive Pulmonary Disease (COPD) has not been critically evaluated in this environment. This work was a preliminary report of the diagnostic differentials of COPD using pulmonary function indices determined in subjects occupationally exposed to chemical particles and dust in a pharmaceutical industry in Nigeria. 125 workers in the organization were screened for their pulmonary functions. Using Global Initiative for Obstructive Lung Disease (GOLD) as the mode of analysis and based on percentage FEV1/FVC ratio, 31.2% (39) had Normal Respiratory (NR) function; 24.8% (31) had Mild Restriction (MR); 24.4% (30) had Moderate Restriction (MoR); 10.4% (13) had Moderately Severe Restriction (MSR); 5.6% (7) had Severe Restriction (SR); 2.4% (3) had Mild Obstruction (MO) while 0.8% (1) had Obstruction combined with Restriction (OR). Using the NR group as reference and towards stratifying the data, mean FEV1 and FVC obtained for the MoR, MoR, MSR, SR, MO and OR groups were compared with that of NR. The Mean values varied significantly in all the other groups (p<0.005) except with the MoR group (p=0.005). With this correction, 69.2% (28) could be said to show normal pulmonary function while the remaining (33.4%) had a form of respiratory abnormality or the other. The application of basic spirometry measurement and the need for enforcement of various industrial laws monitoring risk exposure towards reducing toxicity of chemicals by inhalation and the attendant airways diseases in our industries was further highlighted in this work.

**1210 Black Carbon Exposure Levels in New York City’s Subway Stations**

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The New York City subway is the main mode of transport for over 5 million passengers on average weekdays. Commuters are thus exposed frequently to the microenvironment in subway stations and airborne pollutants can have a significant impact on subway users if exposure levels are high. This study looked at black car-
nary fibrosis. Also our study shows that a single dose of PHMG-P model induces suggested evidences that PHMG-ph-induced apoptosis continuously stimulates in-
creased eNO. Non-treated phosgene-exposed and treated but non-phosgene-ex-
posed rats survived. This experimental evidence suggested that high-dose corticoid treatments may aggravate the pulmonary toxicity of phosgene. In contrast, the NOS-inhibitor aminoguanidine reduced the magnitude of lung edema. In summary this study seems to support the view that the pro-apoptotic corticosteroids may further deteriorate the lung while the known anti-apoptotic property of SS maintains function. Inhalation therapy with an aerosolized iNOS inhibitor was both efficacious to reduce eNO and lung weights. This outcome seems to suggest that cardiovascular and not irritation-related tissue destruction is the lead etiopathology of phosgene-induced lung edema.

1213 The Long-Term Toxicity and Mesothelial Cell Reactions Induced by Potassium Octatitanate Fibers (TISMO) in the Left Thoracic Cavity in A/J Female Mice

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The present study was performed to assess effects on mouse lung and thoracic cavity by potassium octatitanate fibers, trade name TISMO, which is often used for fric-
tion material of cars, precision filters or the others, and with the chemical formula K2O·TiO2. Previously (SOT in 2012), we presented the 21 weeks experiment for TISMO with dimensions mostly <50μm in length and <2μm and granular-shaped micro- and nano-size order particles of titanium dioxide (TiO2). Thoracotomy was performed to infuse test particles (1.5 mg in 0.2 ml saline/mouse) directly into the left thoracic cavity of A/J mice. After 21 weeks, only the fiber-shaped TISMO, morphologically similar to asbestos, induced a severe reaction of the pleura. The results indicate that the risk of mesothelial cell reaction may depend on the shape as well as the particle size.

In the present study, 65 weeks experiment was employed to examine long-term effects and possible tumorigenesis by fiber-shaped TISMO infused into the thoracic cavity of A/J mice. The experiment was terminated for the decreasing of the effective number of animals. This experiment showed numbers of TISMO fibers in the alveolus, indicating penetration through the visceral pleura. Atypical mesothelial cells were also observed with severe pleural proliferation, but no malignant mesothelioma was detected. The additional detection of TISMO fibers in the in the liver, spleen, kidneys, ovary, heart and bone marrow histopathologically indicated their movement outside of the thoracic cavity.

In conclusion, the experiments demonstrated fiber-shaped TISMO infused into the thoracic cavity of A/J mice induced severe pleural proliferation but not malignant mesothelioma. Hazard risk should also take into account that asbestos-like fiber spread to the whole body from the thoracic cavity.

1211 Polyhexamethyleneeguanidine Phosphate-Induced Pulmonary Fibrosis Model: Comparison with Bleomycin-Induced Model

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Our prior study showed that polyhexamethyleneeguanidine phosphate (PHMG-ph), one of the major constituent of biocides, caused pulmonary damage of inflamma-
tion and fibrosis in rat lungs. In this study, we examined inflammation and fibrosis in lungs induced by PHMG-ph and compared with bleomycin model, which is most often used to study the pathogenesis of pulmonary fibrosis. In a current study, we admimistered 1.9 mg/kg PHMG-ph or 1.5 mg/kg bleomycin to mice intratra-
cheally on day 1, and conducted necropsies after 1 week, 2 weeks, 4 weeks, and 10 weeks. Proinflammatory cytokines and fibrotic markers were measured from lungs tissues. We also evaluated whether PHMG-ph activates inflammasome, because a marked elevation of IL-β was observed by 10 weeks in PHMG-ph group. As a result, PHMG-ph induced severe pulmonary inflammation with high expression of IL-β, IL-6, MCP-1, and reduced level of IFN-α. Increased expression of extracel-
ular matrix proteins such as collagen and fibronectin was also observed in the lung tissues by week 10. In addition, we postulate that imbalance of metalloproteinase and tissue inhibitor of metalloproteinase accelerates the pulmonary fibrosis. On the contrary, bleomycin-induced inflammation and fibrosis sustained until week 4. Nalp3 and caspase-1 expression was elevated by 4 weeks for PHMG-P treatment group, by 2 weeks in BLM group. In addition, we observed an increased expression of Bax and caspase-3, which means that PHMG-ph induces apoptosis. This study suggested evidences that PHMG-ph-induced apoptosis continuously stimulates in-
flammasome, therefore impairs resolution of inflammation, which leads to pulmo-

nary fibrosis. Also our study shows that a single dose of PHMG-P model induced acute and chronic pulmonary inflammation as well as severe fibrosis by 10 weeks, unlike BLM model.
Chrysotile asbestosis has been used in the manufacturing of friction brake linings since the early 1900’s. Although the use of asbestos containing products has is been reduced, chrysotile brakes continue to be manufactured and/or used in many parts of the world. This is the first inhalation study designed to provide an understanding of the biokinetics and potential toxicity of chrysotile containing brake dust following short term exposure in rats. The deposition, pathological response and translocation of brake-dust derived from brake pads manufactured with chrysotile were evaluated in comparison to the amphibole, crocidolite asbestos. This presentation presents results from the lung and in particular the quantification of measurements of fibers and pathological response in the pleural cavity. Rats were exposed by inhalation 6 d/ wk for 5 days to either brake-dust obtained by sanding of brake-drums manufactured with chrysotile, a mixture of chrysotile and the brake-dust or crocidolite asbestos. The crocidolite asbestosis fibers initiated a rapid inflammatory response in the lung following exposure resulting in a 5-fold increase in fibrinotic response within 61 days. In addition, marked increase in visceral pleural thickness and collagen deposition was observed following crocidolite asbestosis inhalation. No significant pathological response was observed in the lung or the pleural cavity at any time point in either the brake-dust or chrysotile/brake-dust exposure groups. These results provide support that brake-dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung following short term inhalation.

Klotho Overexpression in Human Lung Epithelial Cells Decrease Sensitivity to Cigarette Smoke-Induced Cell Death

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Chronic obstructive pulmonary disease (COPD) is currently the third leading cause of death in the United States. COPD is associated with an abnormal inflammatory response to noxious particles and gases, and is characterized by progressive airflow limitation. Cigarette smoke (CS) is the primary cause of COPD although exposure to air pollution, respiratory infections and genetic factors also contribute to the disease progression. Exposure to CS induces oxidative stress in the lung, which results in cellular senescence or aging of the lung, leading to decreased proliferation of epithelial cells and the destruction of alveolar structure resulting in pulmonary emphysema. Although aging is a natural process, in the presence of cigarette smoke cellular senescence increases dramatically. Given that cigarette smoke accelerates lung aging, therapies that significantly reduce or delay cellular senescence may be important in the overall management of this disease.

The anti-aging gene, klotho, encodes a membrane bound protein, which has been shown to be a key regulator of oxidative stress, cellular senescence and inflammation. Mice with a defect in the klotho gene have a short life span and develop a syndrome resembling emphysema. In this study the role of Klotho was investigated in human epithelial cells. Individual clones that stably overexpressed Klotho were generated through retroviral transfection and genetin selection. Klotho overexpression was confirmed through real-time PCR and Western blotting. Compared to control cells, which were stably transfected with the retroviral control vector only, constitutive Klotho overexpression resulted in decreased sensitivity to cigarette smoke induced cell death in vitro. However, compared to control cells, the generation of reactive oxygen species and the production of IL-6 and IL-8 were not altered in the presence of CS. Therefore, our results suggest that enhancing Klotho activity in pulmonary epithelial cells, particularly those exposed to CS, may be a promising strategy to alleviate apoptosis and possibly prevent the development of COPD.

Subchronic Inhalation Exposure of Rats to Libby Amphibole and Asbestos: Effects at 18 Months Post Exposure

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Increased asbestos, lung cancer, and mesothelioma rates are evident after exposure to Libby amphibole (LA). To support dosimetry model development and compare potency, a subchronic nose-only inhalation study (6 hr/d, 5 d/wk, 13 wk) was conducted in male F344 rats. Rats were exposed to air (control), LA (LO, MED, HI; 1.01, 3.32, 10.06 mg/m3, 159, 693, 1522 fibers/cc), or amosite (AM; 3.34 mg/m3; 230 f/cc). Toxicity endpoints, pathology, and fiber burden evaluation were determined 18 mo post-exposure. Fiber exposure had no effect on survival. Most commonly seen lesion was the macrophage of death prior to scheduled necropsy in all groups except the LA 3.3 group, though group incidences were below NTP historical control data. BAL cell numbers, LDH, and protein in AM and LA groups were not statistically different from the control group (n=8 rats/group), indicating resolution of earlier inflammation (Dodd, SOT 2012; Willson, SOT 2013). Histology of the left lung lobes, trachea, sternum, pleura, epitheliomas and testes, and relevant gross tissue lesions was conducted on 50 rats/group. Alveolar inflammation, pleural fibrosis, lung interstitial fibrosis, and the presence of foreign bodies were noted in all fiber-exposed groups. A greater incidence of chronic tracheal inflammation was noted in the LA groups. Alveolar bronchiolar adenoma occurred in 2 rats in each of the AM, MED LA, and HI LA groups, and 1 adenoma and bronchiolar carcinoma in the HI LA group, all of these findings were within historical NTP control data. No plural mesotheliomas were observed in any treatment groups. In conclusion, both AM and LA induced dose-related lung fibrotic responses; tumor incidences were apparently increased but not beyond historical control ranges. Tissue fiber burden data are supporting dosimetry model development; comparisons of responses between fibers may change based on internal dose estimates. (This abstract does not represent US EPA policy.)

Sex Differences in Glutathione Levels and Cytotoxicity following Naphthalene or Acrolein Exposure in Nasal Epithelium

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Naphthalene causes site and dose dependent respiratory tract toxicity in rodents that is P450 mediated. The carcinogenesis biomarker evidence for formation of olfactory neuroblastomas and respiratory adenomas in the nose of both sexes of rats. Currently, there is debate as to whether epithelial injury following naphthalene exposure occurs in humans. In this study we used nasal epithelial tissue explants obtained from rhesus monkeys culled from the colony at California National Primate Research Center for health reasons as a surrogate for human nasal epithelium. We incubated maxillo, ethmoid, and septal epithelia (N= 3 or more per group) from male and female monkeys with naphthalene (10, 50, 150 and 500 uM), acrolein (50, 150, 300 uM) or vehicle (sham) control to provide site-matched tissue pieces for 3 hrs. Samples were analyzed for amount of glutathione (GSH) depletion via HPLC and also for histology of cytotoxicity using differential incorporation of the fluorescent nuclear dye ethidium homodimer-1. We found that acrolein, a direct acting agent that does not require P450 mediated metabolism to have an effect, depleted GSH at the highest dose, in all tissues tested, except male septal epithelium. In general, females were more susceptible to GSH depletion but had higher baseline GSH levels. Naphthalene was less effective than acrolein at depleting GSH, with a trend towards depletion only seen in maxilloturbinate epithelia at the highest dose. No other nasal tissue was depleted by 500 uM NA, a high dose, likely equivalent to an inhalated concentration of ~10ppm. Naphthalene cytotoxicity was rare, focal and was primarily localized to individual cells in subepithelial glands. We conclude 1) the nasal epithelium of the Rhesus monkey is resistant to glutathione depletion and that this may reflect robust resynthesis capabilities, 2) that naphthalene is poorly capable of depleting GSH in most nasal tissues tested and 3) naphthalene causes minimal cytotoxicity in this model.

Sex Differences in Acute Nasal Antioxidant Responses to Inhaled Naphthalene

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At concentrations of 10 ppm or greater, naphthalene (NA) is a nasal carcinogen inducing respiratory adenomas in male and olfactory neuroblastomas in female rats, respectively. The proposed carcinogenic mode of action includes metabolic activation via CYP450 to an electrophile with subsequent glutathione depletion, escape of electrophile, covalent binding, cell death, and regenerative cell proliferation. To fully define the effect of acute NA exposure on nasal antioxidant responses, male and female F344 rats were nose-only exposed to 0, 1, 3, 10, or 30 ppm NA vapor for 4 hours. Following exposure, nasal olfactory (OE) and respiratory (RTE) epithelia were analyzed for reduced/oxidized glutathione levels (GSH/GSSG) and for mRNA levels of selected antioxidant genes. Epithelial membrane permeability was also assessed via ethidium homodimer-1 staining as an indirect marker of cytotoxicity. Significant GSH depletion occurred at all exposure levels in
RTE and OE with maximal depletion (to ~ 25% and 40% of control levels, respectively) occurring at or above 1 ppm. Similar trends were seen in both males and females. There was significantly more induction of glutamyl cysteine ligase (gcl) and NADPH quinone oxidase 1 (nqo1) in male OE compared to female OE. At 3 ppm in male rats, NA upregulated gcl and nqo1 12- and 16-fold, compared to 5- and 3-fold in females, respectively. Membrane permeability following NA exposure to 10 or 30 ppm NA was not dependent on sex, suggesting no sex differences in the acute cytotoxic response at these exposure levels. While no sex differences in GSH depletion were observed, sex differences in the induction of antioxidant genes suggest that induction of antioxidant protective pathways may contribute to sex differences in the carcinogenic response to NA.

1220 Pulmonary Effects and Biokinetics of Nanoparticles: Whole-Body Inhalation Exposure to CeO2 in 5-Day, 28-Day, and 90-Day Rat Studies

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CeO2 nanoparticles are widely used for biomedical, commercial and industrial applications. To assess hazard potential of air-born CeO2 nanoparticles, CeO2 (NM212), an OECD depositional material, is tested in a combined chronic and carcinogenicity long-term inhalation study in rats within the framework of the EU-project NanoREG. As a preparation work for the long-term inhalation study, 5-day and 28-day studies were performed in female rats. The rats were whole-body exposed to 0.5, 5 and 25 mg/m3 CeO2 for 6 h/d, 5 days for 1 and 4 weeks in the 5- and 28-day studies, respectively. A variety of biological endpoints including broncho-alveolar lavage (BAL) and histopathology were examined. The lung retention and clearance kinetics were assessed up to a post-exposure period of 129 days. Based on these results, the long-term study was started at concentrations of 0.1 mg/m3, 0.3 mg/m3, 1 mg/m3 and 3 mg/m3 CeO2. Meanwhile, the results of satellite group animals exposed for 90 days are available.

In both 5-day and 28-day studies, an increase of biochemical and cytological parameters (e.g. polymorph nuclear neutrophils, PMN) were observed in BAL fluid in a concentration-related manner. Cytokines in BAL fluid were increased significantly in both studies. These findings indicate a moderate inflammatory response in the lung after inhalation exposure. Consistent to BALF results, histopathology revealed substance- and concentration-related morphological changes in the lungs. The effects comprised alveolar histiocytosis, granulomatous inflammation in the lung, and macrophage aggregates with particles in bronchio-alveolar-associated lymphoid tissue and lung-associated lymph nodes. Pulmonary effects could be correlated with high particle retention and slow clearance in the lungs and a translocation to associated lymph nodes. The no observed adverse effect levels (NOAEL) for CeO2 is 0.5 mg/m3 in both 5- and 28-day studies.

1221 Inhalation Toxicity of Three Different Types of Nano-Sized Organic Pigments

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Four different organic pigments, which are nanomaterials according to the EU definition, were tested in the 5 day short term inhalation study (STIS) in rats. The STIS is a sensitive test system, which comprises a comprehensive scheme of parameters (e.g. polymorph nuclear neutrophils, PMN) were observed in BAL fluid in a concentration-related manner. Cytokines in BAL fluid were increased significantly in both studies. These findings indicate a moderate inflammatory response in the lung after inhalation exposure. Consistent to BALF results, histopathology revealed substance- and concentration-related morphological changes in the lungs. The effects comprised alveolar histiocytosis, granulomatous inflammation in the lung, and macrophage aggregates with particles in bronchio-alveolar-associated lymphoid tissue and lung-associated lymph nodes. Pulmonary effects could be correlated with high particle retention and slow clearance in the lungs and a translocation to associated lymph nodes. The no observed adverse effect levels (NOAEL) for CeO2 is 0.5 mg/m3 in both 5- and 28-day studies.

1222 Secondary Organic Aerosols Generated from α-Pinene-Amine Mixtures: Effects on the Cardiovascular System

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The SPHERES research program was created to examine the role of inhaled secondary organic aerosol (SOA) on cardiovascular outcomes in mice. SOA was generated in a reaction chamber with alpha-pinene, and was combined with neat amines (monooethanolamine (MEA), methyl diethanolamine (MDEA), piperazine (PIP)) or carbon dioxide degraded amines (CO2-degraded MEA, CO2-degraded MDEA, CO2-degraded PIP). To examine the effects of these mixtures on vascular and lung inflammatory responses, 8 wk old male Apo E−/− mice, on a high fat diet were exposed for 7 days, and the resulting vascular expression levels of HO-1, ET-1, MMP-9, and TIMP-2 cytokines as well as the TBARS measure of lipid peroxidation were assayed in aortic tissue. Total cell counts and differentials were assayed in the bronchoalveolar lavage fluid (BALF). Exposure to some of these mixed amine/SOA atmospheres caused mild vascular and lung responses following 7 days of exposure. MEA+SOA tended to decrease expression levels of cytokines and TBARS in the vasculature. MDEA+SOA had no appreciable effect on cytokines or TBARS, and PIP+SOA led to increases in MMP-9 and TBARS expression a decrease in TIMP-2 expression compared to control. CO2-degraded MDEA+SOA tended to decrease cytokine expression while having little effect on TBARS. The processing to determine the effects of CO2-degraded MEA+SOA and PIP+SOA are currently in progress. In the BALF, total cell and macrophage counts were non-significantly increased following exposure to MEA+SOA, MDEA+SOA, and PIP+SOG atmospheres and the CO2-degraded MDEA+SOA exposure. Neutrophil and lymphocyte counts were too low to accurately quantify. Overall, some mixed amine/SOA atmospheres caused mild responses in lung and vascular markers of inflammation.

1223 Pulmonary Toxicity Screening of 1, 2-Benzisothiazol-3(2H)-One by Intratracheal Instillation


Chemicals are widely used in our daily lives for various purposes such as disinfectants, air fresheners, paints and hair sprays. In particular, chemicals used in household products (CHPs) have been shown to be a major source of inhalation exposure. However, their pulmonary toxicity has less studied compared with oral and dermal toxicity. Therefore, the purpose of this study was to determine the pulmonary inflammation responses of 1,2-benzisothiazol-3(2H)-one (BIT) used in major CHPs in Korea. Toxicity of BIT was evaluated by intratracheal instillation in 5-wk old male Apo E−/− mice on a high fat diet. The animals were sacrificed at both 1 day and 1 week after instillation. Toxicity was assessed by count of total and differential cells in bronchoalveolar lavage (BAL) fluid. As a result, in high exposure group, clinical abnormalities associated with the pulmonary toxicity were observed such as shortening of breath and dyspnea. Also, instillation of BIT caused a decrease of body weight. The total cell count in BAL fluid increased in the middle and high exposure groups. However, middle group recovered by 1 week post-exposure. Furthermore, significant changes in the polymorphonuclear neutrophils (PMN) values as inflammation marker in the BAL fluid were observed in all exposure groups. Therefore, this study suggests that careful regulations of CHPs would be necessary in maintaining a high quality of life.

1224 Acute Effects of White Spirit with Different Aromatic Content

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The use of ‘standard’ white spirit (stdWS, 15–20% aromatics) in Europe has gradually shifted to low-aromatic or de aromatized white spirit (deWS), as the latter is considered to carry a lower risk of health effects. However, data on health risks of deWS on humans are sparse. We have performed a controlled short-term chamber exposure to compare deWS and stdWS with respect to irritation, inflammation and central nervous systems (CNS) effects. In a dose-finding study thresholds of irritation and CNS effects were identified. Eight (4 males) healthy volunteers rated symptoms related to irritation, smell and CNS effects on a 0–100 mm visual analogue scale (VAS) while exposed to increasing levels of deWS or stdWS from 0.5 to 600 mg/m3.
Based on the dose-finding study, 12 (6 males) healthy volunteers were exposed for 4 h at rest at five conditions: clean air, 100 mg/m³ stdWS (0.002% aromatics), 100 mg/m³ stdWS (19% aromatics), 300 mg/m³ stdWS, and 300 mg/m³ deWS. The study was approved by the Regional Ethical Review Board in Stockholm. The following endpoints were studied: symptom ratings on VAS (irritation in the eyes, nose, throat, dyspnoea, smell, headache, fatigue, nausea, dizziness, and feeling of intoxication), neurobehavioural performance (vigilance, response inhibition, response shifting, divided attention, and working memory), pulmonary function, nasal swelling, breathing frequency, blinking frequency, plasma inflammatory markers, and biochemical variables. The only significant increase in rating (compared to clean air) except “solvent smell” was seen for eye irritation at the high stdWS exposure. Ratings during stdWS exposure tended to be higher than during deWS exposure. Weak and inconsistent improvements in working memory and response shifting were seen at high stdWS exposures, compared to clean air. No exposure-related effects on the other objective measurements were found. In conclusion, based on symptom ratings, stdWS is slightly more irritating than deWS.

1225 Repeated Intratracheal Powder Aerosol Delivery in Sprague-Dawley Rats
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Inhalation administration (Rx) is a highly effective delivery route allowing the bypass of first-pass metabolism and permitting local delivery to the lungs but it is expensive and requires large quantities of material. We have evaluated an alternative approach using a novel device for repeated intratracheal administration (IT Rx). Using this approach we performed single dose and 7 day repeat dose studies with administration of air, PLGA (50/50 DL-lactide/glycolide copolymer) (10 mg/day) or a pharmaceutical test item (10 mg/day) to groups of 6 female Sprague Dawley rats. Only the results of the air and PLGA control groups are presented. Repeated IT Rx resulted in procedure-related lung changes in both control groups, including perivascular/peribronchial infiltrate, increased alveolar macrophages, and alveolar hemorrhage. These changes were seen with higher incidence and/or severity in rats receiving PLGA. Lung changes attributable to PLGA, but not seen in the air control, included bronchioalveolar inflammation, bronchiolar epithelium hypertrophy/hyperplasia and/or erosion, exudate/cellular debris in bronchioalveolar lumens, foreign material, foreign body granuloma, and necrosis with or without trachea epithelium hypertrophy/hyperplasia and/or erosion. Although repeated IT Rx of dry powder aerosols was feasible with this novel device, the lung mechanical damage and dose delivery variation represented significant limitations. This approach was not considered ideal for a 28-day regulatory study with the mechanical damage and dose delivery variation represented significant limitations. This approach was not considered ideal for a 28-day regulatory study.

1226 Refinement of Acute Inhalation Toxicity Studies: The Isolated Perfused Rat Lung As a Screening Tool for Surface-Active Substances

New surface-active agents in waterproofing sprays are often tested for acute inhalation toxicity in vivo on the basis of OECD Test Guideline 405. A screening test using the isolated perfused rat lung (IPRL) is proposed in order to reduce the number of acute inhalation tests and to refine these. The test comprises exposure of IPRLs to aerosolised formulations of the water proofing agents and on-line monitoring of respiratory parameters. Substances revealing harmful effects on the IPRL, such as impaired lung compliance and atelectasis formation, did also show changes in respiratory parameters up to mortality in vivo tests with rats. Thus, pre-testing in the IPRL allows the identification of surface-active substances causing acute inhalation toxicity.

To assess the potential lung toxicity of seven formulations, each tested in two male and two female IPRLs, we evaluated changes in the respiratory parameters tidal volume, compliance, and resistance, edema and atelectasis formation, taking into account the inhaled doses. These IPRL results were compared with available in vivo results and a good or excellent correlation in six out of seven cases was revealed. In conclusion, the use of the IPRL is well suited for screening substances showing acute “physical” inhalation toxicity. Therefore, for future assessment of surface active substances, it is suggested to use this test prior to in vivo inhalation tests.

1227 Quantitative Evaluation of the Relationship between Vapor Characteristics and Upper Respiratory Tract Uptake Efficiencies in Rodents
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Characterizing the regional absorption of inhaled vapors within the respiratory tract of animals typically used in toxicology studies is essential in applying the results to human health risk assessments. It is well documented that water soluble and reactive vapors are absorbed or “scrubbed” in the upper respiratory tract (URT) of the rodent with high efficiency, thereby reducing the amount of vapor that reaches the lower airways. A literature review and analysis was performed to quantitatively evaluate the relationship between vapor characteristics (pKa, Henry’s Law constant (HLC), water solubility, vapor pressure, and Km) and their effects on URT uptake efficiency (UE) in rodents. Sixteen studies that examined UE of 14 vapors were identified. Linear regression modeling controlling for inhaled vapor concentration and flow rate was performed for each vapor characteristic in rats and mice to characterize their relationship with URT UE. Results indicated that pKa and HLC had significant inverse linear relationships with URT UE in rats (R²= 0.147; p < 0.0001; R²=0.037; HLC: β= -18780, p < 0.0001; R²=0.398). Models that included both pKa and HLC yielded a slightly higher adjusted R2 than pKa and HLC alone (full model R²=0.540; reduced models R²=0.377-0.520). There was no significant linear relationship between URT UE and vapor pressure (β=0.0007, R²=0.09; R²=0.147) or vapor pressure (β= -0.0009, p<0.0001; R²=0.147) in rats, although it is possible that non-linear models may better describe these relationships. There were limited data available for mice; however, these results indicated a similar relationship between URT UE and pKa (β= -0.49, p < 0.001; R²=0.533). Although limited data were available for analysis and only linear relationships were examined, these preliminary results indicated that identification of common vapor characteristics may aid in interpreting and explaining observed regional effects of vapor inhalation in rodents.

1228 Comparison of the Upper Respiratory Tract Uptake Efficiencies of Vapors in Rats and Mice
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It has been reported that soluble and reactive vapors are readily absorbed or “scrubbed” in the upper respiratory tract (URT) of rodents, reducing the dose that reaches the lower airways. Since the inhalation of some vapors has resulted in differences in observed regional airway effects between mice and rats, researchers have hypothesized that there is a significant difference in URT uptake efficiency (UE) between the two species. To investigate this hypothesis, a literature review was performed to identify studies that measured the URT UE of the same vapors in both mice and rats, and a quantitative comparison between the URT UEs was performed. In order for a vapor to meet the inclusion criteria, studies must have identified that measured UEs via surgically isolated URTs for both species at comparable concentrations and flow rates. A total of eight studies that evaluated 4 vapors (naphthalene, propylene oxide, acetaldehyde, and acrolein) were included in this analysis. URT UEs were compared for each vapor individually and for all vapors together using paired t-tests. Overall, the mice had statistically significant higher mean URT UEs than rats (48.13% v. 38.15%; p < 0.0001). The mean URT UEs for mice were higher than rats for naphthalene (62.26% v. 33.41%; p < 0.0001). The mean URT UEs were higher than rats (48.13% v. 38.15% ; p < 0.0001). The mean URT UEs for mice were higher than rats for naphthalene (62.26% v. 33.41%; p < 0.0001). The mean URT UEs were higher than rats (48.13% v. 38.15% ; p < 0.0001). In contrast, there was no significant difference in URT UE of acetaldehyde between mice and rats (45.67% v. 46.52%; p = 0.67). A statistical comparison was not performed for acrolein due to small sample size (n = 1 for both species). Although there were a limited number of studies available, the results demonstrate that URT UE is not uniform across species, even in species as similar as mice and rats. Further, the results demonstrate the importance of taking species-specific differences into account when interpreting rodent model results and extrapolating to other species, including humans.
Research on dry powder toxic inhalants or drug candidates for inhalation therapy typically require particulate starting materials with diameters in the range of 1-5 μm. However, isolated toxicants or newly synthesized drug candidates are often available only in the milligram range. Yet, few systems for generation of micronized substances of these batch sizes have been available. Here we present a spray dryer method for generating micronized particles at room temperature in high yields requiring substance only in the mid-milligram range. The Laminair Spray dryer generates micronized particles from nebulized solutions. A mesh nebulizer (Aeroneb Pro) delivers droplets to a laminar air flow in an inner column coated with a vapor-permeable membrane. A countercurrent stream of air at room temperature on the outside of the membrane removes the evaporated solvent, from which it is absorbed in a separate silica/activated carbon column in a closed loop. Dried powder product is collected on a filter at the end of the drying column. Aqueous solutions of 2% lactose and 2.5% horse radish peroxidase and an ethanol solution of 2% budesonide were micronized from batches in the range of 20 to 500 mg. Product yields were >80% and particle sizes ranged from 1 to 5 μm. The production rate varied from 0.1 to 1 mg/min. Enzymatic activity of horse radish peroxidase was fully retained after drying indicating that delicate biomolecules will retain their activity. Removal of solvent was nearly complete when nebulizer output was around 5% of its full capacity. However, using ethanol as solvent makes the transfer slightly less effective than with water. The laminar flow regime maintains particle losses in the column at less than 10%. Small portions of toxic substances or drug candidates can thus be micronized and tested via inhalation, thereby facilitating early detection of biological activity.

**1229a** Pulmonary Responses after the Inhalation of Fumes from Resistance Spot Welding of Galvanized Zinc-Coated Steel


Spot welding (SW) of galvanized zinc (Zn)-coated steel is commonly used in the automotive industry where high speed repetitive welding of thin metal sections is needed. SW produces complex aerosols that cause bronchitis and asthma in workers. The goal was to assess the effect of SW fumes on lung responses in an animal model. Male Sprague-Dawley rats were exposed by inhalation to 25 mg/m³ of SW aerosol for 4 h/d x 8 d. Controls were exposed to filtered air. During daily exposure, no change in body temperatures was observed throughout the exposure period when comparing the two treatment groups. At 1 and 7 d after the final exposure, bronchoalveolar lavage (BAL) was performed to assess lung toxicity. Lactate dehydrogenase (a marker of lung cell cytotoxicity), total BAL cells, and LDH levels, and IL-1β, IL-2 levels. We also verified consumption were periodically observed during whole study duration. Total and differential cell count, total protein, LDH, and cytokines (IL-1β and IL-2) were measured from BAL fluid. As well, the distribution of inhaled ZnO particles inside lungs was observed using TEM. Under current design with 5 males and 5 females per group, the lung weight change greater than 10 to 20% of control values can be detected. The lung weight should be applied as a complementary end point to lung histopathology for assessing lung toxic effect by inhaled compounds in acute/subacute inhalation studies in rat.

nary toxicity in vivo. RAW 264.7 macrophages were treated with InP or ITO and cytotoxicity was assayed at 24 hrs. Particle solubilization (release of ionic indium) was also measured at 24 hrs in culture supernatants by atomic absorption spectros-
copy. Macrophage cytotoxicity and ionic indium release were much greater for InP compared to ITO. Next, B6C3F1 mice were treated with 1 mg/kg InP or ITO by oropharyngeal aspiration. On day 28, BAL and pleural lavage were collected and assayed for total cell numbers (and differentials), LDH activity and protein levels as markers of lung toxicity. All of these parameters were greatly increased in mice treated with InP compared to ITO. These data suggest that macrophage solubili-
cation and cytotoxicity of ICPs in vitro may be capable of predicting pulmonary toxicity in vivo. Furthermore, these differences in toxicity were observed despite the two compounds having similar indium content suggesting that solubilization, not total indium content, may better reflect the toxic potential of ICPs.

1229e Acrolein Toxicity in Endothelial Cells Involves Lipid Peroxidation, Protein Damage, and Reduced Cellular GSH and Augmented Monocyte Adhesion

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Environmental pollutant exposure has been implicated in the pathogenesis of cardio-
vascular diseases. Acrolein is a highly reactive aldehyde pollutant mainly found in automobile exhaust and tobacco smoke. However, the action of acrolein in endothelial cells remains to be investigated. The present study examined acrolein-
in-induced cellular injury, oxidative modifications of cellular constituents, adhe-
sion of monocytes to endothelial cells and altered intracellular glutathione (GSH) on EAHY926 cells, a widely used endothelial cell line for study of endothelial cell dysfunction. Incubation of cells with acrolein at pathophysiological concentrations for 24 hours caused a significant decrease in cell viability measured by MTT assay and increase in release of lactate dehydrogenase (LDH), which is further confirmed by a significant change in cell morphology. Acrolein also increased the amount of thiobarbituric acid reactive substances, a marker of lipid peroxidation, and protein monocyte binding, a marker of protein damage in the cells. Incubation of cells with acrolein also resulted in a significant depletion of cellular GSH and augmented monocyte adhesion to human endothelial cells, an important step in the develop-
ment of atherosclerosis. The results of this study may contribute to our ability to assess the cardiovascular risk of human exposure to acrolein.

1229f Carbon Capture and Sequestration: An Exploratory Inhalation Toxicity Assessment of Amine Trapping Solvents and Their Degradation Products

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Carbon dioxide (CO2) absorption with aqueous amine solvents is a method of Carbon capture and sequestration (CCS) from flue gases. One concern is possible release of amine solvents and degradation products into the atmosphere, warranting evaluation of potential pulmonary effects from inhalation. The CCS amines monoethanolamine (MEA), methyl-diethanolamine (MDEA), and piperazine (PIP) underwent oxidative and CO2-mediated degradation for 75 days. C57bl/6N mice were exposed for seven days by inhalation of 25 ppm of neat amine or equivalent concentration in the degraded mixture. The aqueous solutions were nebulized to carbon uptake in bronchoalveolar lavage fluid and cytokine expression in lung tissue. Ames mutagenicity and CHO-K1 micronucleus assays were applied to assess genotoxicity. Control analysis of the test atmosphere and liquid revealed complex mixtures including acids, aldehydes, and other compounds. Exposure to oxida-
tively degraded MEA increased (p<0.05) total cells, neutrophils, and lymphocytes compared to control mice and caused inflammatory cytokine expression (statistical increase at p<0.05). MEA and CO2 degraded MEA were the only atmospheres to show statistical (p<0.05) increase in oxidative stress. CO2 degradation resulted in a different composition, less degradation, and lower observed toxicity (less magni-
tude and number of effects) with no genotoxicity. Overall, oxidative degradation of the amines studied resulted in enhanced toxicity (increased magnitude and number of effects) as compared with the neat chemicals.

1229g Potential Exposure Threshold of Chrysotile Asbestos

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Studies have shown that various forms of asbestos (serpentine, crocidolite, and amosite) have different toxicities depending on the fiber characteristics dose, dis-
mension, durability, and biodegradability. While some early regulations accounted for these differences, the current guidelines consider all asbestos types to have the same hazardous potential. We hypothesize that there is an exposure threshold for the health effects of a biosoluble asbestos, such as chrysotile. This has implications for whether exposures can be controlled to levels that would not be expected to cause lung disease in countries where chrysotile asbestos is still in use.

To determine the current information available regarding chrysotile asbestos, a re-
view of available epidemiological and animal studies was performed. Epidemiology studies are difficult to use for elucidating threshold doses since asbestos type and dimension in the workplace are often not known, and worker history can be diffi-
cult to obtain. Animal research has shown that high exposure to chrysotile asbestos is associated with lung disease; however, at these high levels the disease related to a lung overload mechanism. Subchronic exposure studies have indicated a No Observed Adverse Effect Level (NOAEL) of approximately 500 f/cc, which indi-
cates a threshold for asbestos-induced lung disease. Additional studies have shown that chrysotile has low biopersistence in the lung, does not translate to the pleura, and does not appear to cause hyperplasias or other pleural changes.

The science indicates that low exposures to chrysotile asbestos do not present a significant risk to human health. A multi-dose, chronic inhalation study in rodents would better define the threshold for potential health effects from low exposures to chrysotile asbestos. Understanding low-dose chrysotile exposures is important to evaluating the role of past chrysotile asbestos exposures in the US and to the regulation of asbestos use in other countries.

1230 Increases in the Serum Acute Phase Proteins after Ozone Exposure Are Associated with Induction of Genes in the Lung but Not Liver

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Acute Phase Response (APR), a systemic reaction to infection, trauma, and in-
flammation, is characterized by increases and decreases in plasma levels of positive and negative acute phase proteins (APP), respectively. Although the liver has been shown to contribute to APR in various pathologies, the source of circulating APP after air pollution exposure is not well characterized. We hypothesized that lungs and liver both will contribute to plasma levels APP after an acute ozone exposure. To test this hypothesis, we exposed male Wistar Kyoto rats to air or O3 (1ppm), 6hr/day for 1 or 2 consecutive days. Immediately after ozone exposure, genes en-
coding APP were analyzed at mRNA level in the lungs and livers and proteins were analyzed in the serum. Ozone exposure increased positive acute phase proteins in the serum, such as α-1-acid glycoprotein, AGP; α-2-macroglobulin, A2M; and decreased negative APP such as transferrin. These increases in serum proteins were associated with marked increases in mRNA expression of several APP in the lungs (A2M, AGP, α-1-antitrypsin, hepcidin, and ceruloplasmin). Ozone did not increase liver mRNA for any APP examined. However, there were remarkable tissue differences between lung and liver mRNA expression of APP in air exposed rats (liver-lung). C-reactive protein was expressed highly in the liver but minimal expression was noted in the lung (not affected by ozone in lung or liver). Thus, although liver tissue mass is ~15 times greater than lung mass, it is clear that the acute phase protein genes are induced only in the lung after ozone exposure; the role of lung should not be ignored in modulating APR and release of APP in the circulation upon the inhalation of pollutants. (This abstract does not reflect the US EPA policy).
Nitrogen dioxide (NO₂) and ozone (O₃) are environmental air pollutants capable of inducing lung injury and inflammation by causing oxidative damage to the respiratory epithelium. Mice models have suggested that these pollutants induce damage via similar mechanisms, although little work has been done to corroborate this in vivo—specifically in primary epithelial cells. Thus, the purpose of this study was to compare the responses between O₃ and NO₂, in a gas-phase system using primary human epithelial cells obtained via brush biopsy. These cells, obtained from 9 donors, were cultured and grown at an air-liquid interface (ALI), which closely reflects the real-life inhalation of these toxicants. After 3-4 days at ALI, the cells were exposed to NO₂ (1.0, 2.0, 3.0, or 5.0 ppm), O₃ (0.25, 0.50, 0.75, or 1.00 ppm), or clean air for 1.20 min. The expression of pro-inflammatory and cellular stress markers was measured using quantitative real-time PCR 0, 1, 4, and 24 hours following each exposure. Expression of the pro-inflammatory cytokine IL-8 was elevated only at 24 hours post-exposure when cells were exposed to either 3.0 or 5.0 ppm NO₂; however, down-regulation of HO-1 and COX2 were observed at all time points at each dose. In contrast, the expression of IL-8, HO-1, and COX2 were up-regulated following all O₃ exposures; the greatest increases were seen 1 hour following O₃ exposures. Therefore, it is possible that NO₂ and O₃ exposures induce damage to the respiratory epithelium via differing mechanisms, in contrast to the previously tested mice models.

Human umbilical vein endothelial cells (HUVECs) were exposed in vitro to soluble fraction of 100% soy biodiesel combustion exhaust (BEPE) in order to study their effect on vasodilation. Previous controlled human exposures indicate inhalation of biodiesel (BD) combustion emissions can inhibit vasodilation. We developed a study to measure a vasodilator eicosanoid, prostacyclin, in response to BEPE. Our study indicated no significant cytotoxicity, measured by LDH release, after 6h exposure with 1000μg/mL BEPE. After 6h exposure the stable metabolite of prostacyclin, 6-keto PGF1α (6keto), was found significantly decreased from vehicle indicating diminution of vasodilator (6keto) release. Next we examine biochemical mechanisms involved, analyzing mRNA and protein levels of proteins in 6-keto production. COX-1 & 2 gene expression levels were significantly decreased below vehicle with BEPE at 100μg/mL after 6hrs (p<0.05). Western blot of COX-2 showed a decrease. Western blot of COX-1 shows no intensity changes with BEPE exposure. PG1 synthase (PGIS) mRNA levels and protein levels show no change from control levels. Both COX1/2 and PGIS were challenged after extract exposure with 10μM Arachidonic acid (AA) and 1μM PGH2 respectively to verify if enzyme activity can be reversed. Post challenge, 6-keto levels were significantly increased compared to vehicle suggesting BEPE suppresses some enzymes of 6keto production. Post BEPE exposure simultaneously incubation with fresh BEPE and AA induced a decrease of 6keto. These results indicate compounds in BEPE can induce immediate impairment in 6keto release and/or detection. We postulate the saturated fatty acids from BEPE combustion disrupted the formation of complexes inhibiting 6keto temporally and at low intracellular substrate levels. This abstract of a proposed presentation may not reflect official US EPA Policy.

Diesel exhaust (DE) exposure induces adverse cardiopulmonary effects. Addition of nano cerium (CeO₂) oxide to diesel fuel (DECe) increases fuel burning efficiency but results in altered emission characteristics and potentially altered health effects. We hypothesized that inhalation of DECe will cause greater adverse pulmonary health effects in rats compared to DE alone. Male Sprague-Dawley rats (8 wks old) were exposed to either DE or DECe (1000 μg/m³) 5 hrs/day for 2 days. We observed an increase in N-acetyl glucosaminidase in lung lavage fluid of rats exposed to DECe as compared to air group. There were also marginal but insignificant increases in several lung injury biomarkers in both exposure groups (DECe-DE). To further characterize DECe toxicity, we exposed rats to DECe (100 or 1000 μg/m³) or the gas-phase components of DECe (1000 μg/m³) 5 hrs/day for 2 days or 4 wks (5 days/wk) with one group allowed a 14 day recovery. CE lung burden levels indicated a time- and concentration-dependent accumulation during treatment with 35% clearance of Ce from the lung during the 14 day recovery. The high dose of DECe increased lung inflammation at the 2 day time point; this effect was reduced at 4 wks except for the sustained increases in lavage fluid activity of γ-glutamyl transferase. Increased alveolar septae thickness due to edema and increased presence of pigmented macrophages after DECe exposure was illustrated by histology and TEM. Lung effects were also evident after exposure to gas-phase of DECe, suggesting the effects of whole exhaust may be driven by gas-phase components and/or particulate effects may be modified by gas-phase components. These data indicate that exposure to DECe induces adverse pulmonary effects that on a mass basis, causes greater injury than DE alone. (Does not reflect the US EPA policy)

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Exposure of humans and experimental animals to Diesel Exhaust (DE) has been reported as major air pollutant to induce airway inflammation, decrease lung function and aggravate asthma because it contains hazardous materials such as particulate matters. The objective of this study is to identify the acute inhaled toxicity of DE in SD rats using wholebody exposure chamber. In this study, groups of 10 Sprague-Dawley rats were exposed to the DE from diesel engine by the route of inhalation(2h/day, 3days) at the 3 different RPM(Rotation per minute) of 0(control), Idle, 1160, 1740 rpm. As a result, there were no significant differences in body weight and organ weight gain between exposure groups and control group. Clinical examination results showed that there were no symptoms or deaths. However, the histopathological changes were found in the lungs of the male and female rats inhaled with diesel exhaust. The histiocytic inflammatory cell infiltration described as histiocytic pneumonitis was found around the terminal bronchiole and submucosal duct. The lesions were observed in the lung of Idle group exposed. These results suggest that inhalation of diesel exhaust could induce obvious histopathological changes to rats.

The Effects on Endothelial Cell Eicosanoid Release with Biodiesel Exhaust Soluble Fraction
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Diesel engine exhaust exposure induces adverse cardiopulmonary effects. Addition of nano cerium (CeO₂) oxide to diesel fuel (DECe) increases fuel burning efficiency but results in altered emission characteristics and potentially altered health effects. We hypothesized that inhalation of DECe will cause greater adverse pulmonary health effects in rats compared to DE alone. Male Sprague-Dawley rats (8 wks old) were exposed to either DE or DECe (1000 μg/m³) 5 hrs/day for 2 days. We observed an increase in N-acetyl glucosaminidase in lung lavage fluid of rats exposed to DECe as compared to air group. There were also marginal but insignificant increases in several lung injury biomarkers in both exposure groups (DECe-DE). To further characterize DECe toxicity, we exposed rats to DECe (100 or 1000 μg/m³) or the gas-phase components of DECe (1000 μg/m³) 5 hrs/day for either 2 days or 4 wks (5 days/wk) with one group allowed a 14 day recovery. CE lung burden levels indicated a time- and concentration-dependent accumulation during treatment with 35% clearance of Ce from the lung during the 14 day recovery. The high dose of DECe increased lung inflammation at the 2 day time point; this effect was reduced at 4 wks except for the sustained increases in lavage fluid activity of γ-glutamyl transferase. Increased alveolar septae thickness due to edema and increased presence of pigmented macrophages after DECe exposure was illustrated by histology and TEM. Lung effects were also evident after exposure to gas-phase of DECe, suggesting the effects of whole exhaust may be driven by gas-phase components and/or particulate effects may be modified by gas-phase components. These data indicate that exposure to DECe induces adverse pulmonary effects that on a mass basis, causes greater injury than DE alone. (Does not reflect the US EPA policy)
only. The ACES study represents the most comprehensive examination of the potential health effects associated with 2007 engine DE performed to date. Under the conditions described, no effects on oxidative damage or genotoxicity were observed. However, a few modest increases in markers of cardiovascular disease risk appeared dependent on age and/or exposure duration and sex.

1236 ACES Phase 3: Results from the Chronic Bioassay of Rats Exposed to 2007 Compliant Diesel Emissions

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Phase 3 of the Health Effects Institutes (HEI) ACES program was to conduct animal inhalation studies in rats for up to 24-30 months as a carcinogenicity bioassay, measuring indicators of pulmonary toxicity in rats at intervals during exposure. This presentation reports the final results of the bioassay. Atmospheres were generated from emissions of a 2007 compliant 500 hp engine and aftertreatment system operated on a variable-duty cycle. Dilutions of exhaust were maintained at average integrated concentration of 4.1, 0.8 and 0.1 ppm NO2. Exposure atmospheres were characterized in detail. Exposures were conducted for 16 hr/day, 5 days/week. Rats were evaluated by respiratory function, hematology, serum chemistry, bronchoalveolar lavage, lung cell proliferation, and histopathological assays. Concentrations of particulate matter are generally very low and rise only during diesel filter regeneration, once or twice per 16 hr exposure period. Mild biological responses were observed, and were confined primarily to the respiratory tract and primarily at the highest exposure concentration. The primary histologic findings were minimal airway thickening in the central acinus. The severity of the lesions did not increase between 1 year and the lifetime. The observed changes were consistent with exposure to gaseous components such as NO2, and differed markedly from effects observed from traditional diesel exhaust.

1237 Involvement of TLR2 and TLR4 and Th1/Th2 Shift in Lung Inflammatory Responses Induced by Fine Ambient Particulate Matter

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Air quality in China is notoriously poor and recently has become an issue associated with increasing social unrest. Epidemiologic studies have reported the association between fine particles (aerodynamic diameter≤2.5μm; PM2.5) and health effects, but the immunological mechanisms are not clear. To investigate the dose and time-dependent role of toll-like receptor (TLR) and Th1/Th2 cell in local and systemic inflammation induced by PM2.5, PM2.5 was added to MIHS cells (mouse alveolar macrophage cell line) and mice were subjected to Nose and mouth exposed of 2.5, 5, or 10μg/kg PM2.5 in this study. After 24h, 72h, 7days, and 14days, mice were sacrificed to measure TLR2 and TLR4 expressions and Th1/Th2 related cytokines in bronchoalveolar lavage fluid (BALF) and peripheral blood. Histopathological changes in lung were examined. Inflammatory infiltration and macrophages with engulfed particles were found by lung histopathology after PM2.5 exposure. TLR4 positive cells decreased in BALF but increased in blood at 24h after the exposure. The low percentage of TLR4 positive cells continued to day 14 in BALF, but recovered at day 7 and decreased further to lower than the control value at day 14 in blood. TLR2 positive cell changed similar to TLR4 in BALF on the dose effects. In BALF at 24h after the exposure, the Th2 related cytokines IL-5 and IL-10 increased dose-dependently; and in blood, the Th2 related cytokines IL-4, IL-5, and IL-10 also increased. These results suggest that acute exposure of PM2.5 leads to acute inflammatory responses locally and systemically in mice. TLR2 and TLR4 are involved in this process and PM2.5 can drive a Th2-biased immune response.

1238 Effect of SNPs and Glycosylation Site Mutations on TRPA1 Activation by Particulate Material

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Transient receptor potential ankyrin 1 (TRPA1) mediates nocifensive responses and inflammation caused by inhaled irritants including certain particulate air pollutants (PM). The mechanism by which insoluble components of PM activate TRPA1, as well as whether differential activation occurs as a result of single nucleotide polymorphisms (SNPs), leading to differences in individual PM sensitivity, was further explored. TRPA1 mutants were generated, transfected into HEK-293 cells, and functionality evaluated by comparing calcium flux relative to the wild-type channel. AITC, a soluble electrophoric TRPA1 agonist, was used to assess the overall functionality of each specific mutant and as a model for PM associated electrophiles, 3,5-diterbutylphenol (3,5-DTBP) is a non-electrophoric component of some PM and is a TRPA1 agonist. The insoluble PM agonist was coal fly ash (CFA). qPCR confirmed equivalent mRNA expression of all mutants and wild-type TRPA1. The N-terminal SNPs R3C and R58T exhibited increased responses to all agonists, and the pore-loop SNP, N954T exhibited a lack of response to all agonists. The ankyrin repeat SNPs E179K and K186N and the pore-loop SNP R919Q exhibited selective decreases in response to CFA, but not to AITC or 3,5-DTBP. The N-linked glycosylation site (predicted) mutants (N747A and N755A) did not effect activation of TRPA1 based on complete inhibition of responses by the cell-impermeable inhibitors EGTA and ruthenium red; activation by AITC and 3,5-DTBP was unaffected. However, activation by CFA was selectively decreased. These data demonstrate a role for both the N-terminal ankyrin repeat domains and cell surface N-linked glycans in mechanical activation of TRPA1 by insoluble PM, providing new insights into the structural components involved. In vitro experiments with different agonists or with specific mutants of TRPA1 may offer a more efficacious way to investigate potential agonists that may offer therapeutic effects.

1239 Concentrated Ambient Fine Particulate Matter (CAP, PM2.5)-Induced Vascular Insulin Resistance: Role of Oxidative Stress

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Epidemiological studies suggest that increases in fine particulate matter (PM2.5) air pollution contribute to the rapidly evolving epidemics of obesity and diabetes. Previous studies have shown that vascular insulin sensitivity is rapidly impaired by high fat feeding and long-term CAP exposures. Thus, we examined vascular insulin sensitivity and function in low- and high-fat (HFD, 60% kcal fat) fed C57BL/6j mice exposed for 9-30 consecutive days (6h/d) to air or downtown Louisville CAP. CAP exposure for 9 or 30 days impaired aortic insulin signaling measured as insulin-induced phosphorylation of Akt and endothelial-specific eNOS independent of dietary fat. Thus, aortic insulin resistance occurred rapidly in the absence of frank endothelial dysfunction (acetycholine-induced relaxation) reflecting the high sensitivity of vascular insulin signaling to CAP. To identify the underlying mechanism of CAP-induced vascular insulin resistance, we interrogated the role of systemic or lung oxidative stress by exposing mice treated with TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl; 1mM in drinking water, 2 days prior and throughout) and mice transgenic (Tg) for lung-specific ecSOD to air or CAP for 9 days. Both vascular insulin resistance and aortic inflammation were abolished by systemic TEMPOL. Moreover, lung-specific ecSOD-Tg mice exposed to CAP did not develop vascular insulin resistance. Collectively, these results suggest that short-term CAP induces vascular insulin resistance via local inflammation due to pulmonary oxidative stress, and thus, our model supports the idea that exposure to PM2.5 may contribute to the increased risk of developing T2D and CVD in humans.

1240 PM2.5-Induced Tachycardia and Hypertension in Rats Are Linked to Elemental Carbon and Specific Temperature-Resolved Carbon Subfractions

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Ambient fine particulate matter (PM2.5) is associated with cardiovascular morbidity and mortality. The components responsible for adverse health effects are unknown.

OBJECTIVE: We tested the hypothesis that cardiovascular responses to PM2.5 will be enhanced in hypertensive rats and associated with specific particulate components found in an urban industrial setting.

METHODS: Spontaneously hypertensive rats were exposed by inhalation to filtered air (n=8) or concentrated PM2.5 (n=8) in Dearborn, Michigan, for four consecutive summer days. Exposures were repeated in four separate groups of rats (n=32). Blood pressure (BP), heart rate (HR) and HR variability (HRV) metrics (SDNN, RMSSD) were assessed by radiotelemetry and compared to 1h- and 8h-averaged fluctuations in PM2.5 composition, with a focus on elemental carbon (EC), organic carbon (OC) and their temperature-resolved subfractions, EC1-EC5, PC (pyrrolized carbon), and OC1-OC4.

RESULTS: Exposure-related increases in mean, systolic and diastolic BP were detected as well as increased HR and decreased HRV compared to air-exposed rats. Using 1h averages, PM2.5 EC (1 pg/m3 increase) was associated with increased
HR of 11-32 bpm (4-11% increase), 22-27% decrease in HRV, 3-14 mmHg increases in (1-5-8%) in systolic BP, and 4-9% increases in diastolic BP. By comparison effects of OC were negligible. Using 8 averages, EC subfractions were also associated with increased heart rate (EC1: 13-13bpm, EC2,EC3,PC; <5bpm) and SDNN (EC1):<EC2,EC3,PC; >15bpm +SDNN (EC2,EC3,PC: <10bpm). Significant but comparatively smaller responses were associated with OC and OCl. Effects of either EC or OC subfractions on blood pressures were negligible.

CONCLUSIONS: Acute changes in cardiovascular responses associated with PM2.5 carbon species appear to be driven primarily by EC and EC1 fractions. US EPA R83479701 & Electric Power Research Institute.

1241 Differential Activation of Transient Receptor Potential Ankyrin-1 by Diesel Exhaust Particulate Materials

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Diesel exhaust particulate matter (DEP) is a source of fine-particulate air pollution (PM). Inhaled, fine PM deposits throughout the airways inhalation of DEP can cause irritation to airways, pulmonary inflammation, exacerbation of asthma, and promote chronic pulmonary diseases. Mechanisms underlying the adverse effects of DEP and other environmental PM have been difficult to define due, in part, to the significant variation in its chemical composition arising from inconsistent combustion conditions, particle age, and other factors. PM derived from cigarette smoke, wood smoke, coal burning, and diesel combustion activate transient receptor potential ankyrin-1 (TRPA1). In the respiratory tract TRPA1 activation causes cough, bronchoconstriction, and neurogenic inflammation/edema. We hypothesized that three different DEP samples that mimic environmental DEP, would exhibit variations in potency as TRPA1 agonists due to differences in chemical composition, and that such potency differences would correlate with variations in measures of toxicity such as cell death and cytokine gene expression. NIST SRM 2975 (NIST), DEP collected from the tail-pipe of an on-road truck (JMV), and DEP recovered during diesel filter regeneration (FILTER) were used. TRPA1 activation (Ca+ flux) was determined using TRPA1-overexpressing HEK-293 cells: "JMV" DEP was most potent followed by NIST, then FILTER. The mechanism of TRPA1 activation was also investigated. TRPA1 activation by JMV and NIST were attributable to electrophiles, whereas FILTER activated via both the electrophile and mercnol agonist-binding sites. Chemical analysis of DEP components (PAHS and Aldehydes/Ketones) by HPLC and LC/MS suggested a correlation between DEP potency and the abundance of pernaphthenone, benzquione, and 3,5-ditert-butylphenol in the DEP. Increased understanding of how different samples of DEP affect lung cells is an important step in better understanding the factors that influence the multiple reported effects of DEP in the lung. Support: ES017346.

1242 Ambient PM Exposure and RAGE: Insight into an Emerging Risk for Diabetes and Cardiovascular Disease


Numerous epidemiological studies reveal alarming evidence that individuals with diabetes have been shown to be an especially susceptible population to the cardio-pulmonary effects of particulate matter (PM) pollution. This has been thought to be due to the inherent enhanced inflammation and endothelial dysfunction seen in diabetics. Nonetheless, there is no definitive mechanism that explains the pathways responsible for these observed vulnerabilities. Recent reports have implicated activated receptors for advanced glycation end-products (RAGE) as an integral factor in the inflammatory processes of cardiovascular dysfunction and diabetes; nonetheless, it is unclear whether ambient PM alone or in combination w/ other endoge-neous factors may contribute to RAGE activation and underlie these inflammatory disparities. To investigate the influence of RAGE on PM-mediated inflammatory levels of soluble RAGE (sRAGE), membraneous RAGE, ligands (i.e. AGEs, S100 proteins and HMGB1) and NF-kB were measured in human pulmonary and cardio-vascular cell models (pulmonary endothelial, epithelial, macrophage and alveolar) exposed to regional PM or inert saline solution. Lastly, to validate RAGE as a mechanism of PM-induced inflammation, B6C3F2 mice were exposed to regional PM aspirations. Results: After 24h of PM2.5 exposure, a dose dependent increase in cell proliferation and small increases in sRAGE activity at higher doses of PM was evident. Immunofluorescence detection showed an elevation in cells positive for membraneous RAGE expression; accompanied w/ a 2-fold increase in mRNA for RAGE & NF-kB; as well as significant increases in measures of ligands such as AGE and HMGB-1. These findings suggest a plausible interaction between PM & RAGE resulting in the enhanced expression and activation of NF-kB and RAGE. Finally, preliminary mouse exposures have yielded supportive findings: exposed mice have shown significantly increases of RAGE expression compared to controls. Collectively, these data offer valuable insight into PM-mediated RAGE activation and its influence on diabetes.

1242a Intratracheal Coexposure to Diesel Exhaust Particulate and Crystalline Silica in Rats Potentiates the Inflammatory Effects of Silica in the Lungs

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Worker exposure to high levels of respirable dust containing crystalline silica and diesel exhaust (DE) has become a concern during hydraulic fracturing operations. The goal of the current study was to investigate the effects of acute pulmonary co-exposures to silica and DE particulate (DEP) in vivo. Doses were derived from field measures using the high point values for respirable silica, applied to a human pulmonary deposition model, and normalized to the surface area of the rat lung. The silica dose of 230 µg/rat represents 2.5 mg/m³ for 12 hr/d for 14 d. The DEP dose of 7.9 µg represents 0.1 mg/m³ for the same duration in a deposition model for total carbon (TC). A 50 µg dose of DEP was also used to represent ~0.6 mg/m³ TC for the same duration. Rats were exposed by a single intratracheal instillation of sterile PBS as vehicle or one of the following doses: 7.9 µg DEP, 50 µg DEP, 250 µg silica, or silica and DEP combined for each dose of DEP. At 1 d, 1 wk, and 1 mo post-exposure, bronchoalveolar lavage (BAL) was performed on the right lungs; cells and fluid were retained to assess pulmonary injury and inflammation. Lung-associated lymph nodes (LALN) were harvested to evaluate immune responses. Silica alone caused increased inflammation and lung injury at all times post-exposure, indicated by increased neutrophil influx, oxidant production, and lactate dehydrogenase (LDH) in BAL. DEP alone caused only slight inflammation 1 d post-exposure but no effects later. At 1 wk and 1 mo after exposure, 50 µg DEP significantly increased the inflammatory effects of silica. In addition, at 1 mo, both co-exposure doses significantly enhanced the phagocyte oxidant production over silica alone. Lymphocyte counts in LALN in the co-exposures were not elevated relative to silica exposure alone. In summary, DEP alone produced relatively no pulmonary toxicity; however, DEP in a co-exposure with silica has the capability to potentiate the adverse effects of silica in the lung.

1242b Inhalation of Traffic-Derived Particulates and Exposure to Social Stress Differentially Alter Cardiovascular Function in Rats

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Vehicular-derived air pollution includes particulate matter (PM) consisting of primary particles (P) and secondary organic aerosols (SOA) that induce adverse cardiovascular events potentially through vascular or autonomic nervous system dysfunction. Telemetered rats were exposed by whole-body inhalation to traffic-de- rivied P+SOA denuded of combustion gases (4d/wk × 5h/d; mean PM10 concentration ≈ 50 µg/m³) for 12 d to determine effects on arterial pressure (AP) and heart rate variability (HRV, a measure of autonomic balance). P+SOA caused increased AP during exposure days 1, 4, and 6 (mean AP: +15 mmHg vs. control), sympa-thetic dominance during days 1 and 4 (standard deviation of interbeat intervals: ~3-4 ms, supraventricular tachycardia (heart rate: +180 beats/min for up to 5 h) between days 6 and 8 in half of animals, decreased AP on day 11 (~9 mmHg mean AP), and bradycardia in half of animals during baroreflex challenge on day 12. Baroreflex sensitivity tended to increase on day 12 of exposure, indicating compen-sation for the hypertensive effects of P+SOA. To compare P+SOA to social stress, separate telemetered animals were introduced to a dominant rat for 20 min/d for 10 d. Social stress consistently increased heart rate (+40-100 beats/min) and mean AP (+15 mmHg) while increasing a HRV measure of vagal tone (root mean square of successive differences) potentially indicating baroreflex activation. Thus, the autonomic effects of P+SOA and social stress diverged during similar hypertensive responses. Therefore, traffic-derived PM causes hypertension, autonomic imbalance, and subsequent compensatory responses in a pattern different from acute psychological stress.
Epidemiological studies suggest a direct correlation between exposure to diesel exhaust particles (DEP) and the onset of cardiovascular diseases presumably through the disruption of the adherens junctions. This would lead to increase of vasculature permeability and the inhaled DEP gain access into the circulation system. Although the mechanism remains unclear, our group has demonstrated the DEP-induced re-active oxygen species (ROS), which may play a key role in causing vascular permeability factor, VEGFA secretion. In order to minimize the level that DEP penetrating into the capillary lumen, 20 and 200 μg/mL. Ganoderma lucidum (G. lucidum) was applied with DEP to the in vitro capillary-like HUVEC tube culture. After 24 h DEP exposure, we observed VEGFR2 dissociated from the membrane and re-localized to both cytoplasm and supernatant. It subsequently resulted in making PI3K/Akt inactivation. While with co-treatment of G. lucidum, Cm-H2DCFDA assay showed the ROS generation was blocked. Not only was the increased vascular permeability reduced following by the oxidative stress inhibition, but none of secreted VEGFA was also detected. Immunoblotting further suggested the localization of VEGFR2 and PI3K/Akt activation were affected only at high dose of DEP (100 μg/mL). Additionally, immunofluorescent images revealed that VE-cadherin formed adherens junction network was destroyed in response to DEP. Once combine the treatment with G. lucidum, the cell-cell junctional structure was rebuilt, however, this was observed only at 1 and 10 μg/mL DEP. In summary, these results indicate that inhaled DEP may directly impact the lung vasculature, while additive G. lucidum may protect the endothelial tube cells and their adherens junctions from exposure to DEP.

Coronary artery disease (CAD) is the most prevalent cause of disease-related death in the US and emissions related particulate matter (PM) exposures are thought to be associated with thousands of excess deaths per year. Ultrafine particles (UF) contain a large proportion of redox-active organic compounds, exposure to which may be related to oxidative stress that is involved in the pathogenesis of heart disease, that exist in the volatile or particle phase and are considered semi-volatile organic compounds (SVOCs). We previously demonstrated an acceleration of atherosclerosis in susceptible mice associated with exposure to SVOCs from ultrafine concentrated ambient particles (CAPs). The interaction of enhanced arterial disease and CAPs derived SVOC exposure may increase the susceptibility of the heart to adverse cardiac events. We hypothesized that the SVOC exposed mice would have changes in heart rate variability (HRV), a measure of cardiac autonomic control, and in the morphology of the repolarization segment measured on an electrocardiogram. Decreased HRV is a measure in humans that has been associated with increased cardiac risk of adverse events. ApoE-/- mice, which are prone to developing atherosclerosis, were exposed to either UF CAPs or CAPs with the SVOC components removed by a thermodenuder (deCAPs) for 8 weeks in downtown Los Angeles for 5 hours/day, 4 days/week. A control group was exposed to purified, filtered air. Implanted cardiac transmitters monitored electrocardiograms from the mice. The CAPs exposed mice had diminished HRV compared to deCAPs and CAPs derived SVOC exposure may increase the susceptibility of the heart to adverse cardiac events. We hypothesized that the SVOC exposed mice would have changes in heart rate variability (HRV), a measure of cardiac autonomic control, and in the morphology of the repolarization segment measured on an electrocardiogram. Decreased HRV is a measure in humans that has been associated with increased cardiac risk of adverse events. ApoE-/- mice, which are prone to developing atherosclerosis, were exposed to either UF CAPs or CAPs with the SVOC components removed by a thermodenuder (deCAPs) for 8 weeks in downtown Los Angeles for 5 hours/day, 4 days/week. A control group was exposed to purified, filtered air. Implanted cardiac transmitters monitored electrocardiograms from the mice. The CAPs exposed mice had diminished HRV compared to deCAPs and Air exposed mice, particularly during the latter weeks of exposure. Similarly, repolarization segment changes were observed in the CAPs exposed mice but not the Air and deCAPs exposed mice. The attenuation of cardiac effects in the deCAP group indicate that SVOCs in CAPs exposures are possibly important contributors to the toxic cardiac effects.
Employment of sustainable resources for fuel manufacture has recently gained worldwide trends to limit usage of fossil oils while providing less atmospheric greenhouse gas effects and reducing air pollution. Biodiesel (BD) fuels and biodiesel petroleum blends are used for large truck engines and small, single-cylinder basic piston operating engines. BD produced by transesterification of vegetable oil into fatty acid methyl esters is a renewable energy source. Considering BD fuel is mainly composed of unsaturated fatty acids, we hypothesize that BD exhaust emissions related to found inflammation, oxidative-stress and impaired clearance of combustion products from BD and specific mechanism of interactions of these PM in pigment laden macrophages. Future studies on the detailed analysis of lymphocytic infiltrate and impaired clearance with prolonged retention PM caused pulmonary inflammation and damage, enhanced release of inflammatory mediators found in BAL and lung tissue causing oxidative stress. Biomarkers of tissue damage, oxidative-stress and inflammation were significantly elevated in lungs of mice exposed to BD compared to those found in mice treated with D. The up-regulation of inflammatory cytokines/chemokines was also higher in lungs of mice treated with BD. Histological evaluation of mouse lungs indicated presence of lymphocytic infiltrate and impaired clearance with prolonged retention of BD PM in pigment laden macrophages. Future studies on the detailed analysis of combustion products from BD and specific mechanism of interactions of these emissions related to found inflammation, oxidative-stress and impaired clearance is underway.
area adjacent without lesion in the same animal was used to analyze the cellular morphology after hematoxylin-eosin stain, and to evaluate the level of Zn and MT which were determined by AAS and 109-Cd saturation method, respectively. The results showed increases of Zn at 48 hours and 7 days after surgery, whereas MTs start to increase at 6 h after surgery, maintaining this level until 48 h. Maximum increases of both metabolites occurred at 48 h, corresponded with the maximum increase of inflammatory cells (polymorphonuclear cells and macrophages). The increase of Zn in the injured area can have an important role for activation of metalloenzymes-Zn and activation of transcription factors necessary for healing, while MTs can act as a reservoir of Zn, and as antioxidant defense in the lesion site.

1245 Gene Expression and Pathway Analysis of Cadmium Exposure in Human Hepatocarcinoma Cells

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Cadmium (Cd) is a toxic and carcinogenic metal naturally occurring in the earth’s crust as well as in rocks and soil. Cd can also be introduced into the environment through its many industrial uses. Cd is easily absorbed by plants from the soil, thus a common route of human exposure is via diet. Additionally, smokers are exposed to high levels of Cd from tobacco. This study examined the effects of both acute and chronic Cd exposure on gene expression in human hepatocarcinoma (HepG2) cells. HepG2 cells were treated with either 0.5 μM Cd for 24 hours or 0.1 μM Cd for three weeks and gene expression analysis was performed using Affymetrix GeneChip® Human Gene 1.0 ST Arrays. Acute and chronic exposures significantly altered the expression of 333 and 181 genes, respectively. The genes most upregulated by both exposures include several metallothionein genes. Downregulated genes include the monoxygenase CYP3A7, which is involved in drug and lipid metabolism. CYP3A7 was upregulated by chronic Cd exposure, as was DNAJ-B9, which plays a role in protecting stressed cells from apoptosis. Genes downregulated due to chronic exposure include the transcriptional regulator early growth response protein 1 (EGR1). Ingenuity Pathway Analysis, IPA, revealed that the top networks altered by acute exposure are lipid metabolism, small molecule biosynthesis, and cell morphology, organization, and development; while top networks altered by chronic exposure are organ morphology, cell cycle, cell signaling, renal and urological diseases, cellular growth and proliferation, and cancer. Expression data demonstrate that exposure to Cd alters the expression levels of many different genes involved in various disease pathways. Many of the genes altered by Cd exposure may be involved in carcinogenesis, with roles in cellular growth, proliferation and apoptosis.

1246 Zinc Supplementation Abates Cadmium-Induced Uregulation of Glutathione Synthesis and Oxidative Stress

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In primary cultures of rat choroid plexus we showed cadmium (Cd) induced oxidative stress, stimulated apical choline uptake, and up-regulated glutathione (GSH) synthesis. Zinc (Zn) supplementation of Zn (0.1 mM) and Cd (250 nM CdCl2) in serum-free medium without or with 10 μM Zn for 12 h. Induction of heme-oxygenase-1 (HO-1), heat-shock protein 70 (HSP70), copper-zinc superoxide dismutase (SOD1), and metallothionein-1 (MT-1) was analyzed by immuno blot and qRT-PCR. Glutamate-cysteine ligase (GCL) is the rate-limiting enzyme in GSH synthesis; the modulator and catalytic subunits, GCLM and GCLC, were also analyzed. As compared to controls, Cd treatment induced gene expression of GCLC, MT-1, HO-1, and SOD1 by 2, 16, 12, and 3-fold. Zn supplementation, however, reduced this induction. Cd increased GSH by 2-fold and GSSG by 30-fold as compared to control, whereas in cells also supplemented with Zn, GSH and GSSG levels were decreased 25% and 44% as compared to Cd treatment alone. To determine whether effects of Zn were associated with changes in GSH availability, cells were pretreated (12 h) without or with buthionine sulfoximine (BSO, 100 μM), a GCL inhibitor, after Zn supplementation and before treatment with 0 or 250 nM CdCl2 ± Zn (10 μM) for 12 h. BSO treatment, regardless of condition (e.g., without or with Zn ± Cd), reduced GSH by 92% but increased GSSG by 15-fold above controls. BSO treatment resulted in an enhanced, compensated induction of HO-1, HSP70, SOD1, GCLC, GCLM and MT-1 in the presence of Cd. However, this was attenuated in cells also supplemented with Zn. These data indicate Zn abates Cd-induced up-regulation of GSH synthesis and still protects when GSH synthesis is inhibited. NSF #1052654

1247 Kidney Specificity in Cd-Induced Accumulation of p53

Dependent on the Inhibition of UBE2D Family Gene Expressions

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Cadmium (Cd) is an environmental metal pollutant causing renal dysfunction. However, the underlying mechanisms of Cd toxicity remain to be elucidated. Our recent study has demonstrated that overaccumulation of p53 may relate to Cd-induced apoptosis and may be due to the suppression of p53 degradation through the inhibition of gene expressions of Ube2d family in rat proximal tubular cells (NRK-52E cells). In this study, we examined whether suppression of Ube2d gene expression and p53 accumulation by Cd have kidney-specificity. In the present study, we used only some cell lines as IEC-6 cells (rat small intestine epithelial cells), HBMECs (human brain microvascular endothelial cells), human astrocytes, and HK-2 cells (human kidney proximal tubular cells), but also kidney and liver of C57BL6/J mice. In IEC-6 cells, Cd decreased both of mRNA levels of 3 members (Ube2d1, Ube2d2 and Ube2d4) of Ube2d family and protein level of p53. In HBMEC, Cd increased p53 protein level without the decrease in UBE2D family gene expressions. In human astrocytes, Cd increased only the mRNA level of UBE2D3. In HK-2 cells, Cd suppressed the mRNA levels of UBE2D2 and UBE2D4, as well as increased p53 protein level. Moreover, Cd exposure for 6 months decreased mRNA levels of Ube2d family and accumulated p53 protein in kidney of mice. On the other hand, neither mRNA levels of Ube2d family nor p53 protein level are changed in liver of mice exposed to Cd for 6 months. These findings suggest that Cd accumulates of p53 protein via the suppression of Ube2d family gene expression with kidney-specificity.

1248 Cadmium-Induced Apoptosis Is Mediated by p53 Accumulation through the Suppression of Gene Expression of Ube2d Family in Proximal Tubular Cells

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Cadmium (Cd) toxicity is accompanied by disturbance of gene expressions and increases in stress-response proteins. However, the precise targets of Cd toxicity are still unknown. Our previous study demonstrated that Cd induced cellular accumulation of tumor suppressor protein p53, and inhibited the gene expressions of ubiquitin-conjugating enzyme E2 D family (Ube2d family), which involves in p53 ubiquitination for its degradation, in NRK-52E rat renal tubular epithelial cells and mouse kidney. In the present study, we examined the association of decrease of Ube2d family gene expressions with increase of p53 accumulation by Cd, and the involvement of p53 overaccumulation in Cd-induced apoptosis, using HK-2 human proximal tubular cells and kidney of C57BL6/J mice. Cd increased cellular p53 protein prior to toxic effect in HK-2 cells. Cd significantly decreased gene expression of Ube2d2 and Ube2d4, members of Ube2d family, prior to accumulation of p53 by Cd. The double knockdown of UBE2D2 and UBE2D4 by siRNA caused the increase of p53 protein in HK-2 cells. Moreover, Cd markedly increased nuclear phosphorylated p53 level. Not only apoptotic cell death was promoted in response to Cd, but also Cd-induced apoptosis was attenuated by knockdown of p53 using the siRNA. In addition, 6-month exposure of mouse to Cd (300 ppm) triggered accumulation of p53 in proximal tubules, and apoptotic signals were observed on the same spots where Cd accumulated p53 in proximal tubules. The present findings suggest that cadmium excessively absorbed in proximal tubular cells may induce p53-dependent apoptosis through the suppression of Ube2d gene expression.

1249 Paraoxonase Activity in Subchronic Low-Level Cadmium Exposure

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Epidemiological studies suggest an association between cardiovascular diseases and cadmium in drinking water. While the underlying mechanism of this cadmium-mediated increase in cardiovascular risk factors remains enigmatic, studies
Cadmium (Cd) is a ubiquitous environmental pollutant that has been associated with hyperglycemia or diabetes in epidemiological studies. In the current study, we show that after 12 weeks of Cd exposure in rats there was an increase in serum glucose-dependent insulinotropic polypeptide (GIP) and a decrease in the satiety hormone, leptin. Based on these observations we conducted a pilot study using rats, db/db mice (diabetic mouse model) and lean mice. All animals were given daily subcutaneous injections of Cd at a dose of 0.6 mg/kg/day for two weeks then given no Cd for another two week period. At the end of the study, all animals underwent an oral glucose tolerance test (OGTT) and body weight and fat pad weights were recorded. In the OGTT, Cd had the greatest effect on db/db mice, with all animals except one having blood glucose levels of 600 mg/dl or higher 30 min following an oral dose of glucose (2g/kg). At the same 30 min time point, the non-Cd treated db/db mice had an average blood glucose value of 507 ± 19 mg/dl. Surprisingly, at the end of the study, all Cd treated groups of animals had higher average body weight values as compared to non-Cd treated animals. This increase in body weight during the last 2 weeks of non-Cd exposure was statistically significant in rats. Epididymal and retroperitoneal fat pad weights were significantly greater in the Cd-treated lean mice as compared to non-Cd treated lean mice. Finally, Cd content of the renal cortex from Cd-treated lean mice was 45.4 ± 3.6 μg/g tissue wet weight. This study shows that environmental substances such as Cd can have diabetogenic and obesogenic effects at low levels of exposure.

1250 Cadmium-Induced Lung Emphysema and Carcinogenesis Coupled with Defect in Lysyl Oxidase in the Rat Animal Model
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Lysyl oxidase (LOX) is an extracellular matrix cross-linking enzyme and a tumor suppressor. To assess cadmium (Cd) effects on LOX expression, pathogenesis and carcinogenesis in the lung, a constant dose of CdCl2 at 30 μg/rat (∼ 0.123 ± 0.007 μg Cd/kg body weight) in 100 μl physiological saline was intratracheally instilled into the lung of male Sprague-Dawley rats once a week for 4 weeks (total Cd dose = 110.4 μg Cd/rat). Control rats received saline only (20 rats). Rats were killed 1 or 65 weeks after the last instillation. Due to the half-life of absorbed Cd by the lung to be 9.4 year, actually, Cd exposure times in two treated groups were 7-weeks (6+1) and 71-weeks (6+65), respectively, each with 20 rats (10 rats for morphological study and 10 rats for biochemical assays). Rats exposed to Cd for 7 weeks all exhibited extensive enlargement of airspace and multiple pulmonary bullae with or without small areas of interstitial fibrosis. EM showed the thin alveolar wall with less depression of matrix components such as elastin and collagen. Cd exposure for 71 weeks elicited adenocarcinoma in 5 rats (25%). Some areas of lung tissues were infiltrated with cancer cells, capillaries, and hemorrhages. Biochemical assays illustrated declined levels of LOX at protein, mRNA and catalytic activities associated with downregulation of collagen synthesis in emphysematous and carcinogenic lung tissues. However, tissue thios such as glutathione (GSH) and metallothionein as well as γ-glutamylcysteine synthetase, a key enzyme for GSH biosynthesis were constantly upregulated. Upregulation of cellular thios is a critical cellular event facilitating downregulation of LOX since they trap cellular copper, a cofactor of LOX. Based on LOX biological functions, Cd downregulation of LOX may play a vital role in lung emphysema pathogenesis and carcinogenesis (Supported by grant NIEHS R01-11340).

1251 Acute and Chronic Cadmium Exposure Induces Transcriptional Activation of BMP-2 Signaling Cascades in a Human Renal Epithelial Cell Culture Model
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Cadmium is a toxic heavy metal that causes renal dysfunction. However, the molecular mechanisms underlying its toxicity are not clearly understood. Recently, we show that after 12 weeks of Cd exposure in rats there was an increase in serum glucose-dependent insulinotropic polypeptide (GIP) and a decrease in the satiety hormone, leptin. Based on these observations we conducted a pilot study using rats, db/db mice (diabetic mouse model) and lean mice. All animals were given daily subcutaneous injections of Cd at a dose of 0.6 mg/kg/day for two weeks then given no Cd for another two week period. At the end of the study, all animals underwent an oral glucose tolerance test (OGTT) and body weight and fat pad weights were recorded. In the OGTT, Cd had the greatest effect on db/db mice, with all animals except one having blood glucose levels of 600 mg/dl or higher 30 min following an oral dose of glucose (2g/kg). At the same 30 min time point, the non-Cd treated db/db mice had an average blood glucose value of 507 ± 19 mg/dl. Surprisingly, at the end of the study, all Cd treated groups of animals had higher average body weight values as compared to non-Cd treated animals. This increase in body weight during the last 2 weeks of non-Cd exposure was statistically significant in rats. Epididymal and retroperitoneal fat pad weights were significantly greater in the Cd-treated lean mice as compared to non-Cd treated lean mice. Finally, Cd content of the renal cortex from Cd-treated lean mice was 45.4 ± 3.6 μg/g tissue wet weight. This study shows that environmental substances such as Cd can have diabetogenic and obesogenic effects at low levels of exposure.

1252 Diabetogenic and Obesogenic Effects of Cadmium in Rats and db/db Mice at Clinically Relevant Levels of Exposure
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Cadmium is a ubiquitous environmental pollutant that has been associated with hyperglycemia or diabetes in epidemiological studies. In the current study, we show that after 12 weeks of Cd exposure in rats there was an increase in serum glucose-dependent insulinotropic polypeptide (GIP) and a decrease in the satiety hormone, leptin. Based on these observations we conducted a pilot study using rats, db/db mice (diabetic mouse model) and lean mice. All animals were given daily subcutaneous injections of Cd at a dose of 0.6 mg/kg/day for two weeks then given no Cd for another two week period. At the end of the study, all animals underwent an oral glucose tolerance test (OGTT) and body weight and fat pad weights were recorded. In the OGTT, Cd had the greatest effect on db/db mice, with all animals except one having blood glucose levels of 600 mg/dl or higher 30 min following an oral dose of glucose (2g/kg). At the same 30 min time point, the non-Cd treated db/db mice had an average blood glucose value of 507 ± 19 mg/dl. Surprisingly, at the end of the study, all Cd treated groups of animals had higher average body weight values as compared to non-Cd treated animals. This increase in body weight during the last 2 weeks of non-Cd exposure was statistically significant in rats. Epididymal and retroperitoneal fat pad weights were significantly greater in the Cd-treated lean mice as compared to non-Cd treated lean mice. Finally, Cd content of the renal cortex from Cd-treated lean mice was 45.4 ± 3.6 μg/g tissue wet weight. This study shows that environmental substances such as Cd can have diabetogenic and obesogenic effects at low levels of exposure.

1253 Analysis of Molecular Mechanisms Involved in Induction of Endoplasmic Reticulum Stress by Cadmium
G. Hwang, K. Du and A. Naganuma. Graduate School of Pharmaceutical Sciences, Tohoku Univ, Sendai, Miyagi, Japan.

Cadmium is a toxic heavy metal that causes renal dysfunction. However, the molecular mechanisms underlying its toxicity are not clearly understood. Recently, we show that after 12 weeks of Cd exposure in rats there was an increase in serum glucose-dependent insulinotropic polypeptide (GIP) and a decrease in the satiety hormone, leptin. Based on these observations we conducted a pilot study using rats, db/db mice (diabetic mouse model) and lean mice. All animals were given daily subcutaneous injections of Cd at a dose of 0.6 mg/kg/day for two weeks then given no Cd for another two week period. At the end of the study, all animals underwent an oral glucose tolerance test (OGTT) and body weight and fat pad weights were recorded. In the OGTT, Cd had the greatest effect on db/db mice, with all animals except one having blood glucose levels of 600 mg/dl or higher 30 min following an oral dose of glucose (2g/kg). At the same 30 min time point, the non-Cd treated db/db mice had an average blood glucose value of 507 ± 19 mg/dl. Surprisingly, at the end of the study, all Cd treated groups of animals had higher average body weight values as compared to non-Cd treated animals. This increase in body weight during the last 2 weeks of non-Cd exposure was statistically significant in rats. Epididymal and retroperitoneal fat pad weights were significantly greater in the Cd-treated lean mice as compared to non-Cd treated lean mice. Finally, Cd content of the renal cortex from Cd-treated lean mice was 45.4 ± 3.6 μg/g tissue wet weight. This study shows that environmental substances such as Cd can have diabetogenic and obesogenic effects at low levels of exposure.

1254 Endoplasmic Reticulum Stress by Cadmium
G. Hwang, K. Du and A. Naganuma. Graduate School of Pharmaceutical Sciences, Tohoku Univ, Sendai, Miyagi, Japan.

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Gastrointestinal Solubilization and Uptake of Mercury and Selenium from Wild-Harvested Inuit Foods

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Cd accumulates in humans from dietary, environmental including cigarette smoke, and occupational sources, and has a twenty-year biologic half-life. Our previous studies showed that environmental low dose Cd altered protein redox states resulting in stimulation of inflammatory signaling and actin cytoskeleton disruption, suggesting that Cd could impact multiple disease mechanisms. However, little is known about the relative sensitivities of cellular metabolism associated with subcellular compartmental disruption. In the current study, we investigated effects of acute Cd on mouse liver examining the metabolome of isolated mitochondria and nuclei. To identify nuclear and mitochondrial metabolites affected by Cd, liver nuclei and mitochondria isolated from Cd-exposed mice were analyzed by high-resolution metabolomics. The result showed that Cd altered levels of 1,901 and 542 metabolites in nuclei and mitochondria, respectively, compared to control group. Of these metabolites changed significantly by Cd, 1016 in nuclei and 278 in mitochondria matched to metabolites in Kyoto Encyclopedia Gene and Genome (KEGG) database. Of these, 80% of decreased features are categorized to phytochemical compounds, lipids and biologically functional compounds. On the other hand, Cd increased 262 and 88 features in nuclei and mitochondria, respectively. Of these, 24% (nuclei) and 17.1% (mitochondria) are categorized into pesticides, natural toxins and carcinogens. The results suggest that Cd -induced toxicity could be associated with substantial decrease in functionally essential biological molecules and increase in toxic chemicals in nuclei and mitochondria. Among multiple pathways analyzed by MetaCore software, metabolism associated with altered features of nuclei and mitochondria are distinct. Taken together, the results indicate that metabolomics analysis of subcellular compartments enable identifying biological molecules and toxic chemicals, and associated functional pathways, which could be applicable to determine potential markers as indication of complex mechanisms of environmental effects in disease.

Environmental Metabolome of Mouse Liver Mitochondria and Cell Nuclei

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Methylmercury (MeHg) causes cell-type specific damage in the cerebellum in a Ca2+-dependent manner. In vitro studies have shown that acute MeHg treatment caused a biphasic increase in [Ca2+], which can be delayed by voltage-gated Ca2+ channel (VGCC) antagonists, demonstrating their role in MeHg-induced Ca2+ dysregulation. We and others have shown effects of MeHg on dopamine release; numerous studies have addressed the acute effects of MeHg on cholinergic and dopaminergic cell lines and primary neurons. However, exposure to low levels of MeHg during lifetime are lacking. As such, our aim was to study effects of repeated MeHg treatment on VGCC function in differentiated SH-SYSY cells, a dopaminergic cell line. These cells have been used extensively to study MeHg neurotoxicity so we first characterized acute effects of MeHg on [Ca2+], in these cells. Changes in [Ca2+] were measured using Fura-2; cells were perfused continuously with 1, 2, 5, or 10μM MeHg. MeHg caused a biphasic, concentration-dependent increase in Fura-2 fluorescence; the times to onset of both phases were inversely proportional to the [MeHg]. At 1μM Phase 1 (P1) and Phase 2 (P2) occurred at -12 and 48min respectively, whereas at 10μM P1 occurred within 2 min and P2 within 15min. Nifedipine (Nif, 5μM) and co-agonist GVI (GVI, 1μM), L- and N-type antagonists respectively, delayed the onset of P2 but not P1. Effects of semichronic MeHg treatment on VGCC function were subsequently characterized. Cells were treated with 0 or 20μM MeHg for 48 or 72h. Cells were then loaded with Fura-2 AM and KCl-induced Ca2+ influx was monitored as an index of VGCC function. Ca2+ influx through L- and N-type VGCCs was isolated using GVI and Nif respectively. The 48h treatment significantly decreased KCl-induced Ca2+ influx through L-type VGCCs; the 72h treatment significantly decreased KCl-induced Ca2+ influx through both L- and N-type VGCCs. Thus semichronic MeHg treatment affects VGCC function in a dopaminergic cell line. These may result from MeHg-induced alterations of VGCC expression, resting [Ca2+], or block of VGCCs.

Semichronic Methylmercury Exposure Alters KCl-Induced Calcium Influx in SH-SYSY Cells


Methylmercury (MeHg) is a pollutant that causes severe central nervous system damage. However, the mechanism of MeHg toxicity remains unclear. Our previous study indicated that expression levels of 21 genes were changed in the cerebellum of mice by MeHg. In this study, we investigated effects of MeHg on the expressions of these 21 genes in various tissues (cerebrum, cerebellum, liver and kidney) of MeHg-treated mice, Sgcb3a1, Ch25h, Chi3l3, Ctla2b and several chemokines (Ccl2, 4, 7, 9 and 12) mRNA expressions were significantly increased in the brain of MeHg-treated mice. Among these genes, mRNA levels of Ccl4 and Sgcb3a1 were not significantly changed in the liver and the kidney. These results suggest that the MeHg-induced increases in Ccl4 and Sgcb3a1 expression are brain specific in mice.

Brain-Specific Induction of Expression of Ccl4 and Sgcb3a1 by Methylmercury in Mice

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Methylmercury (MeHg) is a pollutant that causes severe central nervous system damage. However, the mechanism of MeHg toxicity remains unclear. Our previous study indicated that expression levels of 21 genes were changed in the cerebellum of mice by MeHg. In this study, we investigated effects of MeHg on the expressions of these 21 genes in various tissues (cerebrum, cerebellum, liver and kidney) of MeHg-treated mice, Sgcb3a1, Ch25h, Chi3l3, Ctla2b and several chemokines (Ccl2, 4, 7, 9 and 12) mRNA expressions were significantly increased in the brain of MeHg-treated mice. Among these genes, mRNA levels of Ccl4 and Sgcb3a1 were not significantly changed in the liver and the kidney. These results suggest that the MeHg-induced increases in Ccl4 and Sgcb3a1 expression are brain specific in mice.

Role of Reactive Sulfur Species in Reduction of Methylmercury Toxicity In Vitro and In Vivo

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Methylmercury (MeHg) is an environmental electrophile that can covalently bind to protein sulphydryls to form protein-MeHg complexes. These covalent modiﬁcations, referred as “S-mercuration”, are associated with disruption of enzyme function and neurotoxicity. Some of MeHg unbound and/or proteins mod-
ified by MeHg undergo interaction with deprotonated glutathione (GSH) to yield its GSH adducts that are rapidly excreted into extracellular space through MRPI transporters. On the other hand, hydrogen sulfide (H2S) is a gaseous, weakly acidic molecule mainly produced by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) in cells. Most interestingly, H2S is extensively dissociated into a nucleophile HS− because of its pKa value ~ 6.76 while pKa value of GSH is ~9.12, implying that most of GSH exists as its protonated form at physiological conditions. However, role of H2S in chemical modification of cellular proteins and cytotoxicity during exposure to MeHg is poorly understood. We examined the contribution of H2S to the protection of CBS to cellular protection against MeHg. Pretreatment with NaHS or overexpression of CBS reduced MeHg cytotoxicity, whereas transfection with CBS small interfering RNA enhanced MeHg toxicity in human neuroblastoma SH-SY5Y cells. Bismethylmercury sulfide (MeHgS2S), formed during the reaction of MeHg with H2S, was identified as a metabolite of MeHg in SH-SY5Y cells exposed to MeHg and in the livers of rats treated with MeHg. MeHgS2S had little chemical protein modification capability and little cytotoxicity compared with MeHg in vitro and in vivo. Based on these results, we propose a novel detoxification pathway of MeHg catalyzed by cellular enzyme to generate H2S.

1259 Characterization of Mercury Binding Proteins in the Liver of Northern Fur Seals (Callorhinus ursinus)

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Marine mammals accumulate a large amount of mercury (Hg) in the liver. Our previous studies have found that most of Hg exists as an inactive form, tiemannite (HgS5e) in the nuclear, lysosomal, and mitochondrial (NLM) fraction of the liver of marine mammals, whereas relatively high abundance of organic Hg was observed in the liver cytosol. This result suggests that methyl Hg is demethylated in the cytosol and Hg subsequently co-accumulates with selenium (Se) in the NLM fraction. However, it is still unknown how Hg is present in the liver cytosol fraction in marine mammals. To understand the toxicokinetics of mercury, we investigated Hg binding proteins in the hepatic cytosol of the northern fur seal (Callorhinus ursinus). In the cytosol, Hg concentration in the high molecular weight (HMW) fraction was higher than the metallothionein (MT) and the low molecular weight (LMW) fractions. In contrast, Se was detected in the HMW and LMW fractions, but not in the MT fraction. A significant positive correlation between Hg and Se concentrations was found in the HMW fraction of the cytosol. The molar ratio of Hg and Se reached 1:1 with an increase in Hg concentration. These results suggest that Se interacts with Hg and then bind to HMW protein(s) in the hepatic cytosol, reducing the toxicity of Hg in the liver. By the purification of proteins interacted with both of Hg and Se using an anion exchange and a gel filtration chromatography, four proteins with the molecular weight around 100 kDa and 40 kDa were obtained in the HMW fraction of the cytosol. The purified proteins may play a role in the formation of Hg-Se complex in the hepatic cytosol.

1260 Effects of Single Nucleotide Polymorphisms on Blood Mercury Level of Korean Population

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Background: Recent studies have suggested that several genes that mediate mercury metabolism are polymorphic in humans.

Objectives: We hypothesized those single-nucleotide polymorphisms (SNPs) in toxicokinetics genes may influence individual difference in blood mercury levels. We studied the potential modifying effects of toxicokinetics related SNPs on blood mercury level.

Methods: We selected samples of 528 adults from "the Korean research project on the integrated exposure assessment of hazardous materials for food safety (KRIEFS)" cohort. This cohort measured blood mercury level and dietary exposure to mercury level. We analyzed SNPs of samples with a Human Exome 12v 1.2. The blood mercury levels were compared by SNPs with linear regression model.

Results: Linear regression analysis showed that eight SNPs were statistically significant different in blood mercury level by genotype (cut off P< 0.05). When we adjusted age, sex, smoking, and dietary exposure level, TOP1MT (rs11544484) AA genotype (n=2) or AG genotype (n=57) had lower blood mercury levels than GG genotype (n=437). MBIP (rs2899849) GG genotype (n=1) or GA genotype (n=24) had lower blood mercury level than AA genotype (n=471). FZD7 (rs201306518) GG genotype (n=1) or GC genotype (n=27) had lower blood mercury level than CC genotype (n=448).

Conclusion: Our findings suggest that some toxicokinetics related genetic polymorphism may influences blood mercury level.

1261 Ras Sindoor: Ayurveda's Attempt at Detoxifying Mercury

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Ayurveda is a traditional medicine system or a form of alternative medicine, which is native to the Indian subcontinent. Rasa Shashtra is a branch of Ayurveda that involves processes to purify metals for therapeutic use as herbo-metallic preparations. With the expanding global community, these medicinal preparations are easily available to anyone desperately seeking alternative treatment strategies. These preparations may be more likely to be toxic than therapeutic. Ras Sindoor (RS) is an inorganic compound containing mercury and sulfur, used as a form of medicine in Ayurveda. Toxicity of organic and inorganic mercury compounds has been well established, but there is a dearth of scientific investigations that study potential RS toxicity. This study utilized techniques such as SEM-X-ray Diffraction (EDAX) and the dimethyl thiazolyl diphenyl tetrazolium bromide (MTT) assay to study the effect of RS along with methyl mercury and red mercuric sulfide on rat hippocampal astrocytes. The outcome of EDAX was that both red merciful sulfide and RS showed similar chemical composition. Rat hippocampal astrocytes (passage 12-13) seeded in 96 well plates were exposed to the three compounds at concentrations 0.5μM, 1μM, 2μM, 3.5μM and 5μM for methyl mercury and 50μM, 100μM, 200μM, 500μM and 1000μM for both red mercuric sulfide and RS for 12 hours. The MTT assay was used to assess the cytotoxicity. Red merciful sulfide and RS showed no statistical difference from controls at the dosages tested. However, light micrographs of astrocytes exposed to red mercuric sulfide and RS, showed cell injury in the form of shortened astrocytic processes when compared to controls. RS and red merciful sulfide demonstrated morphological changes, which apparently do not affect cell viability. These changes may indicate alterations to cytoskeletal components, which can alter cellular physiology but not viability. Further experiments are needed to elucidate the nature of such changes to determine if they result in deviation from normal cell function.

1262 Involvement of Decreased Activities of Thioredoxin Reductase and Glutathione Peroxidase in the Neuronal Degeneration by Methylmercury in the Developing Rat Cerebrum

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This study was designed to investigate factors that determine susceptibility to the developmental neurotoxicity of methylmercury (MeHg). It has been previously shown that MeHg treatment decreased glutathione peroxidase (GPx) activity in the developing rat brain. In this experiment, the effects of MeHg on GPx, thioredoxin reductase (TrxR) and glutathione reductase (GR) activities were examined in postnatal developing rats. Wistar male rats of postnatal days 14 (8 rats per group) were orally administered vehicle (control) or MeHg (6.4 mg Hg/kg/day) for 10 days. The MeHg-administrated group showed a significant decrease in body weight, and half of the animals exhibited mild hind-limb crossing by day 11. Increased reactive astrosis was also observed in the cerebral cortex of the MeHg-administrated group. Activity of GPx and TrxR, but not GR, were significantly decreased by MeHg treatment. MeHg inhibited TrxR activity to a greater extent than GPx activity. These results indicate that TrxR and GPx activity, Se-contained enzymes, could be significantly affected by MeHg exposure in the developing brain.
Interaction between Early-Life Methylmercury Exposure and Iron Deficiency in Daphnia pulex
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Methylmercury (MeHg) is a known neurotoxicant and bioaccumulates in fish, with exposure to humans in utero being of highest concern. Also, iron (Fe) deficiency, with an estimated prevalence of 49%, is particularly problematic during pregnancy and early life. Previous studies have shown that iron deficiency exacerbates manganese and cadmium toxicity. We are currently using Daphnia pulex as an alternative model for chronic toxicity testing due to its short lifespan, ease of culture, and transparency for physiological studies. The overall purpose of this research is to investigate the interaction between poor nutrition, particularly a low iron diet, and early life exposure to MeHg. We hypothesize when D. pulex are fed a low-iron diet, the toxicity associated with early exposure to MeHg will increase. We have fed D. pulex a standard and half Fe diet. Early life exposure to low level MeHg (1600 ng/l, 800 ng/l, 400 ng/l and 200 ng/l) was administered (for 24 hrs in first 48 hrs.). Maturation time, average brood size, total reproduction, and total lifespan were measured. In addition, the effects of Fe deficiency and MeHg exposure on lipid storage were measured using image analysis of Oil Red O staining and confirmed through biochemical analysis (triacylglycerol quantification). Also, we are investigating effects on egg size and metabolic rate. Preliminary data suggests that daphnids that are fed a standard Fe diet had a significantly longer lifespan compared to daphnids on a half-Fe diet. In addition, brood size and total reproduction on a standard Fe diet had significantly more offspring compared to those who were fed a half-Fe diet. However, the differing diets did not show a significant difference in maturation time.

Sex Differences in the Lipidomics of Subchronic Low-Level Inorganic Mercury Exposure in the Rat
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Inorganic mercury has been identified as a risk factor of cardiovascular diseases. The mechanisms underpinning this risk and the sex differences are not yet understood. To investigate the effects of mercury on lipid metabolism, male and female rats were exposed to mercury chloride (0.5, 1.0 and 1.5 mg/kg) for 12 weeks. Dyslipidemia caused by mercury in both sexes exhibited different patterns. At the lowest dose, hypercholesterolemia characterized the effect of mercury in male as against hypercholesterolemia in female. Hypertriglyceridemia was observed in both sexes. Plasma and erythrocyte free fatty acids (FFA) increased significantly in male but not female. Reverse cholesterol transport was inhibited by mercury at 1.5 mg/kg dose in male as evidenced by decreased HDL cholesterol but increased in female. Brain cholesterol and triglyceride were increased by mercury (1.5 mg/kg) in male as against the no effect observed in female animals. Hepatic cholesterol in male and phospholipid in both sexes were increased by mercury (1.5 mg/kg). Pulmonary cholesterol and phospholipidosis were observed in male whereas in female only phospholipidosis was observed. Increase in triglyceride in lung and heart of female animals and liver of both sexes were also observed. Mercury exposure caused reduction in phospholipid concentrations in brain and spleen of both sexes. Hepatic HMG-CoA reductase was up-regulated in both sexes (at 1.0 mg/kg in male but at 1.5 mg/kg in female) whereas brain HMG-CoA reductase was down-regulated in male and up-regulated in female at 1.5 mg/kg. Plasma FFA and HDL cholesterol were positively associated with tissue mercury in females as against the negative associations observed in males. These findings indicate that inorganic mercury perturbs different pathways in lipid metabolism in both sexes and this may be responsible for its cardiovascular effects.

Cut-Off Values and Benchmark Doses (BMDs) for Cadmium Compared across Human Exposure Settings: Evidence for Protective Pathways
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A benchmark dose (BMD) is the dose of a toxic compound that increases the probability of an abnormal response by a benchmark response (BMR), i.e., from P0 for a control unexposed subject to P0 + BMR for a subject at the BMD. For cadmium (Cd), dose is taken as urine Cd concentration (UCd) – a measure of internal dose. Pathways that protect against Cd toxicity, including metallothionein synthesis, are a feature of human cells. This project considers the hypothesis: 1) Cd protective pathways are effective at low levels of environmental exposure; 2) With the BMD approach, ‘control’ populations that differ in UCds might generate similar cut-off values for kidney dysfunction, reflective of Cd-protective pathways. To achieve uniformity of approach, 95% cut-off values for urine concentrations of beta-2-microglobulin (UB2M) and N-acetyl-beta-D-glucosaminidase (UNAG), and BMD values at 95% BMR, were compared across published studies. With UB2M as the marker of kidney dysfunction, Chinese and Japanese populations with similar mean UCd values (1.3-1.8 ug Cd/g cr) generated similar 95% urine cut-off values (0.415 - 0.897 mg B2M/g cr). Corresponding BMD values ranged much more widely, from 1.7 to 10.0 ug Cd/g cr. No UB2M BMD results were available for Sweden. With UNAG as the marker of kidney dysfunction, control populations with mean UCds of 0.76 (Swe), 1.3 (Jap), and 2.4 (Jap2) ug Cd/g cr generated 95% urine cut-off values of 3.6 (Swe), 11.1 (Jap) and 16.6 (Jap2) Units NAG/g cr. Respective BMD values were 0.64, 4.3, and 10.8 ug Cd/g cr in urine. Increases in mean UCd were directly associated with increases in cut-off values and BMD values. Results indicate that, in the mean UCd range of 0.7 to 2.6 ug Cd/g cr among control populations worldwide, known industrial sources of cadmium have no indication exists for protective pathways producing similar cut-off values independent of mean UCd values, even at very low levels of Cd exposure. Additional research is needed to investigate the extent to which differences in BMD methods among studies may have contributed to these results.

Immuno-Modulatory Role of N-Acetylcysteine in Cadmium Treated Human Lung Cells
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Cadmium has been classified as a known human carcinogen based on epidemiological studies and research performed in experimental animals. One class of cadmium responsive genes includes the fos family of transcription factors. The four members of this family (c-Fos, FosB, Fra-1 and Fra-2) can heterodimerize with Jun partners to form the AP-1 transcription factor and influence cellular properties such as invasiveness, proliferation, differentiation, apoptosis and angiogenesis. Aberrant expression of these proteins can ultimately promote oncogenic transformations. To better understand the mechanism by which cadmium regulates the expression of these proteins, cadmium toxicity in the bioavailability of the metal and uptake mechanisms involved. This study investigated the role of these proteins in the regulation of cellular responses to cadmium toxicity in immortalized human lung epithelial cells. Using Western Blot analysis, it was demonstrated that cadmium exposure led to the increased expression of Fra-1 and FosB, the two members of the Fos family that are the most responsive to cadmium. The expression of these proteins was found to be mediated by the MTF-1 transcription factor. This finding has significant implications for the understanding of the mechanisms by which cadmium induces cellular responses and contributes to the development of cancer.
same pattern of expression as the cytokine array. These results clearly indicate the immune-modulatory role of NAC on the viability and cytokines expression in cadmium treated human lung cells and suggests that NAC can be used as an antidote against cadmium toxicity that can reduce lung cancer mortality rate.

1265 Exposure to Cobalt, Nickel, Cadmium, and Chromium Causes Changes in Gene Expression and Protein Abundance in a Human Liver-Derived Cell Line

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In the course of military and occupational activities the Nation’s Guardians routinely encounter hazardous environmental toxicants and materials which may result in adverse health effects. Cobalt, nickel, cadmium, and chromium are four such materials with wide use and distribution in industrial areas, which increases the risk of exposure. While the toxic effects of these metals have been widely studied, the exact mechanisms of toxicity remain unclear, and no biomarkers of the adverse health effects have been validated. In order to further elucidate these mechanisms in liver and identify candidate biomarkers, we exposed HepG2-C3A cells to sublethal concentrations of NiCl2, Na2Cr2O7, CdCl2, and CoCl2 for 24 hrs. We chose the treatment concentrations based on a novel qPCR assay using a panel of genes developed from previous metal toxicity studies, as opposed to traditional cytotoxicity assays, to more accurately predict gene expression endpoints. We examined changes in gene expression using DNA microarrays and changes in protein abundance using mass spectrometry.

We performed both gene-level and functional analyses of the results and observed changes in pathways consistent with the known effects of heavy metal intoxication. Comparative analysis identified both common and unique modulated transcripts and perturbed pathways among the four metals. The changes in protein abundance closely resemble and validate the conclusions we drew from the microarray analysis. This work offers key insights into the roles specific genes, proteins, and pathways play in heavy metal toxicity mechanisms, and also identifies candidate biomarkers.

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1266 Time Course of Lead-Induced Dyslipidemia in Male Albino Rats

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Lead has been shown to induce dyslipidemia in rats although the attendant mechanisms have not been clearly elucidated. In order to investigate the time course of lead-induced perturbations in lipid metabolism, male albino rats were exposed to 200ppm, 300ppm and 400ppm lead as lead acetate in their drinking water for 4, 8 and 12 weeks. Control animals received distilled water for the same exposure times after which blood and vital organs were removed from the animals and analyzed for lipid dynamics. Lead accumulated in the organs in the following descending order: kidney, liver, brain, heart and lungs. At 4 weeks, plasma cholesterol increased by 5% (in the 200ppm and 400ppm groups) and 8% in 300ppm. At 8 weeks, cholesterol in the 200ppm group increased by 10%. Plasma phospholipids decreased across all study groups. Lead decreased HDL cholesterol in a time-dependent manner (17% in 4-week 400ppm, 35%, 43% and 49% in 200ppm, 300ppm and 400ppm groups respectively at eight weeks). Plasma free fatty acids (FFA) displayed a hormetic-like response at 4 weeks with the lowest dose (200ppm) resulting in a decrease of 51% while 300ppm and 400ppm displayed 2-fold and 1.5-fold increases respectively. At 12 weeks, increases in FFA were dose-dependent. While cardiac and pulmonary phospholipids were observed, hepatic brain and renal cholesterogenesis and phospholipidosis were observed. Hepatic brain and HMG-CoA reductase activities were up-regulated in most of the dosage groups. A positive correlation was observed between liver lead and liver cholesterol (r = 0.476, p < 0.01) while a negative correlation was observed between blood lead and HDL cholesterol (r = -0.523, p = 0.01). These findings indicate that lead-induced dyslipidemia may be mediated through up-regulation of HMG-CoA reductase activity and enhanced phospholipidosis resulting from increased availability of FFA.
copy, we found that NAC dramatically decreased cellular accumulation of all three metals in both transformed and normal human cells. Suppression of chromate uptake by NAC resulted from extracellular reduction of Cr(VI) to membrane-impermeable Cr(III). Incubation of Co(II) with NAC resulted in a rapid formation of Co(II)-NAC conjugates, which were unable to cross the plasma membrane. In conclusion, we found that direct reactivity with metals is a major antioxidant-independent mechanism in chemoprotection by NAC against carcinogenic Cr(VI), Cd(II) and Co(II). Our results also suggest that the use of NAC could be beneficial for diminishing tissue accumulation of this toxic metal released during corrosion of cobalt-chromium hip implants.

1270 Lack of Reversal from Lead Acetate-Induced Hepatotoxicity and Oxidative Stress in Wistar Rats

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Lead is a ubiquitous metal and also one of the most abundant elements present on earth. Both the developing and the developed countries have been contaminated by this element. This study was designed to evaluate possible toxic effects of lead acetate (PbA) exposure and subsequent withdrawal in male Wistar rats. Control group (group I) received normal saline while groups II, III and IV received oral administration of 0.25, 0.5 and 1.0mg/ml PbA respectively for the period of 6 weeks. One half of the population of the rats was sacrificed at 6 weeks of PbA exposure. PbA was similarly withdrawn from the remaining rats for another 6 weeks. Exposure of rats to PbA led to significant decline (p<0.05) in Glutathione peroxidase (GPx), Glutathione S-transferase (GST), Catalase (CAT), Superoxide dismutase (SOD) and Reduced glutathione (GSH) content. Similarly, Malondialdehyde (MDA) and H2O2 concentrations were significantly (p<0.05) elevated. In the same vein, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities also increased significantly in exposed rats. Animals from the withdrawal period exhibited similar pattern of alterations, with significant (p<0.05) reduction in GSH, GST, GPx and SOD and significant elevation in MDA, Serum ALT, AST and ALP however remained persistently high. In conclusion, experimental animals exposed to PbA could not recover from hepatotoxicity and oxidative stress. This might be due to significant disruption of antioxidant defense system and free radical generation by PbA.

1271 Long-Term Exposure of Mice to Cr(IV) in Drinking Water Disrupts the Oxidative Stress Response Induced by Benzo[a]pyrene in the Proximal Gastrointestinal Tract

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Exposure to a single toxic compound is rare in the environment. Exposure to complex mixtures of different toxicants are more frequent. It is therefore important for human health and disease that the combined effect of exposure to multiple toxicants can be understood. This presentation will focus on exposure to benzo[a]pyrene (BaP) and chromium (Cr). BaP is a polycyclic aromatic hydrocarbon that acts as a strong mutagen and is a known carcinogen. Cr is a metal that is ubiquitous in the environment. It is a known carcinogen to humans and animals. There is evidence that the metabolism of BaP is affected by exposure to Cr. To investigate the combined exposure to BaP and Cr, we used a Balb/c mouse model. Mice were exposed to Cr or BaP individually or in combination. The effect on the oxidative stress response was determined. BaP exposure resulted in a significant increase in the levels of the oxidative stress marker, MDA, in the liver. Cr exposure also resulted in an increase in MDA, but this was not significant. When BaP and Cr were combined, there was a significant increase in MDA. This suggests that the combined exposure to BaP and Cr results in a greater increase in oxidative stress than exposure to either compound alone.

Studies on paraoxonase (PON) activity in lead toxicity in the past have often found lead to be associated with lower serum PON activities and lower HDL values. In order to investigate the toxic effect of lead-induced alterations in PON activity, male albino rats were exposed to 200ppm, 300ppm and 400ppm lead as lead acetate in their drinking water for 4, 8 and 12 weeks respectively. PON activities towards paraoxon (PONase) and phenylacetate (AREase) were determined in plasma, liver microsomes, hepatic and brain microsomal fractions. Lead inhibited plasma PONase activity at 4 weeks (35%, 53% and 24% in 200ppm, 300ppm and 400ppm respectively), while activating it at 8 and 12 weeks (65%, 64% and 16% at 8 weeks and 45% and 22% at 12 weeks) in 200ppm and 400ppm groups respectively. Plasma PONase was also activated in most dosage groups. On the other hand, hepatic and brain microsomal PONase and AREase were generally inhibited by all the lead doses. HDL AREase increased by 25%, 35%, and 15% in 200ppm, 300ppm and 400ppm respectively at 12 weeks. HDL PONase also increased by 5% and 42% in 300ppm and 400ppm respectively at 4 weeks and 40%, 7% and 42% respectively at 8 weeks. VLDL AREase activity also increased by as much as 72% in 200ppm group at 4 weeks. However, lead inhibited VLDL PONase in all the dosage groups at 12 weeks. Positive correlations were observed between hepatic lead and plasma AREase (r = 0.304, p = 0.01), while negative correlations were observed between blood lead and liver microsomal AREase (r = -0.504, p = 0.01) and PONase (r = -0.506, p = 0.01). The unusual activation of circulating PONase and AREase might result from damage to microsomal membrane as a result of lead exposure.
Toxic insult from the heavy metal cadmium is known to induce the expression of metallothioneins (MT) which are heavy metal binding proteins. Previous work from our laboratory has shown that over-expression of MT-3 in breast cancers is associated with poor patient outcome. Furthermore, MT-3 has shown to inhibit the growth of breast cancer and prostate cancer cell lines. The MT-3 protein contains 7 additional amino acids that are not present in any other member of the MT gene family, a 6 amino acid C-terminal sequence and a Thr inserted in the N-terminal region. The unique N-terminal sequence is responsible for the growth inhibitory activity of MT-3 in the neuronal system. The goal of this study was to characterize the function of the N- and C-terminal domains of MT-3 in the breast cancer cell line, MCF-7. For this purpose, six different constructs of MTs were prepared which were as follows: wild type (WT) MT-3, MT-3 N-terminal mutation (MT-3ANT), MT-3 C-terminal deletion (MT-3ACT), WT MT-1E, and MT-1E altered to contain the N-terminus, the C-terminal of MT-3 or both the N- and the C-terminal of MT-3. Each of these constructs was stably transfected into MCF-7 cells and the growth rates and mesenchymal markers E- and N-cadherin were measured. The data obtained suggests that the N-terminal region of MT-3 is involved in growth inhibitory activity whereas the C-terminal region is involved in vectorial active transport which is indicated by the formation of domes in cell culture, and that there is no significant change in the ratio of E- to N-cadherin. In conclusion, this study further characterizes the unique properties of the N- and the C-terminal domain of MT-3 and the potential role that it may play in the differentiation of certain breast cancers.

1275 Characterization of OH Radicals Induced by Particulate Matter and Associated Species with a High-Throughput Approach

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Exposure to ambient particulate matter (PM) can cause various cardiopulmonary diseases. Oxidative stress, purported to be caused by the ability of PM to induce reactive oxygen species (ROS), has been identified as one of the common mechanisms leading to the observed adverse health effects. Among the ROS, hydroxyl radical (OH) is the most destructive. However, there is no high throughput approach to measure OH generated by PM, which in turn hinders the study of the interactions between PM and our biological system.

The goals are 1) to develop a high-throughput approach to measure OH induced by PM in a cell-free system, and 2) to study the kinetics of OH formation induced by PM and its constituents (transition metals). OH induced by PM were quantified using a fluorescence assay (microplate reader), with disodium terephthalate as the molecular probe. Employing an established approach, the kinetics of OH formation was studied by introducing PM and 9 transition metal ions into a simulated lining fluid, and the formed 2-hydroxyl terephthalate was measured at designated time points up to 2 hours.

The detection limit of OH for the devised high-throughput approach (as extrapolated from the formation of 2-hydroxyl terephthalate) was 17.6x10^-3 µM. The initial rate of OH formation ranged from 1.33x10^-3 µM/s for Mn (10 µM) to 1.58x10^-3 µM/s for Cu (10 µM). Both statistically significant synergistic effects (e.g. Cu and Fe) and antagonistic effects (e.g. Cu and V) were observed for OH generation by a mixture of transition metal ions. Insoluble PM constituents, soluble metal ions, and other soluble constituents contributed 22%, 59% and 19%, respectively, of OH induced by PM (NIST SRM 1648).

Trace-level OH induced by PM and its constituents can be measured with the devised high-throughput approach. The initial rates of OH formation derived from the kinetic experiments will be used to evaluate PM toxicities.
had been previously documented to be 7.81 μM. Transmission electron microscopy was used to study morphological changes and accumulation of selenium in cells following treatments. Micrographs of controls show well defined nuclei, double layered nuclear envelopes and mitochondria with definite shape and cristae. Astrocytes treated with 7.81 μM of both compounds appear to have disturbed morphology with pyknotic nuclei. Mitochondria show distorted shapes with the presence of deposits which may be selenium compounds. An ELISA assay was used to investigate cytochrome c levels in cytosolic and mitochondrial fractions. There was a significant decrease in cytochrome c in mitochondrial fractions and a significant increase in the cytochrome c in cytosolic fractions of astrocytes treated with 7.81 μM and 15.62 μM of both compounds compared to controls. TBARs assay was performed to investigate lipid peroxidation products. There was a significant decrease in the products in cells treated with 15.62 μM compared to controls. There were no significant changes in the activities of GPx in any treatment group. These results suggest that the accumulation of Se in the mitochondria and release of cytochrome c into the cytosol result in cytotoxicity in rat hippocampal astrocytes. Oxidative stress does not appear to be involved in the process.

Iron deficiency is the most common nutritional deficiency in the world, with an estimated 4.5 billion affected persons, which can lead to debilitating fatigue, altered immune function, decreased work capacity and anemia. Similarly, iron overload is a significant health concern. Iron overload targets the liver, heart, and pancreas, and can lead to multiple complications including liver disease, cardiomyopathy, and diabetes mellitus. The great diversity in genetic disorders of iron metabolism in man, rodents and other vertebrates suggest that multiple loci can contribute to the susceptibility of iron deficiency and severity of iron overload. Several individual studies have shown genetic differences in iron homeostasis between inbred strains of mice. We hypothesize that this wide genetic variation underlies the differences seen in iron metabolism between inbred mice. Here we show variation in iron metabolism in six inbred mouse strains fed low (12ppm), high (1000ppm), and sufficient (55ppm) iron diets. Variation in elemental tissue content, hematological markers of iron status, as well as body weight and obesity markers are seen in this subset of the hybrid mouse diversity panel. This study highlights the population diversity in the ability to handle iron stress (deficiency or overload), and indicates the genetic variation in this population plays an important role.

The rapid development of high-volume horizontal hydraulic fracturing (HVHF) for mining natural gas from shale has posed potentially serious impacts on human health and biodiversity. The produced flowback waters after hydraulic stimulation is known to carry high levels of saline and total dissolved solids (TDS). To understand the toxicity and epigenetic effects of these waste waters, we analyzed post-fracture flowback water from 5 Marcellus fields from Pennsylvania. We characterized the composition of these samples by X-ray fluorescence (XRF) and scanning electron microscopy (SEM)/energy dispersive X-ray spectroscopy (EDX). XRF indicated bromine (436.67±60.78 ppm), strontium (1286.08±377.68 ppm), and barium (3776.58±609.01 ppm) are most abundant in these samples. Amorphous silicon aluminum oxide (SiAlOx) nano/micro-particles ranged from 70-285nm and amorphous zirconium oxide (ZrOx) particles ranged from 40-140nm have been found by SEM/EDX analysis. A cytotoxicity assay using colony formation was carried out to decide LC50 of these samples on the human bronchial epithelial cells (Beas-2B) with 7 days repetitive treatment. The LC50 was calculated to be 2.7 ±0.2 μM. The 7-day-treatment also led to decreases of the levels of acetylation of H3K9 and H3K14 as well as H3K4 trimethylation. We are also studying cell transformation with 7 days repetitive treatment. The LC50 was calculated to be ~2.7 μM.

The intra- and inter-laboratory variability of bioaccessibility tests were evaluated in synthetic fluids relevant to oral, inhalation, and dermal exposure. Using one defined protocol, five laboratories measured metal release from coalbed oxide, coalbed powder, copper concentrate, Inconel alloy, leaded brass, and nickel sulfate hexahydrate. Standard deviations of repeatability (s) and reproducibility (sr) were used to evaluate the intra- and inter-laboratory variability, respectively. Examination of the s:sr ratios demonstrated that, while gastric and lysosomal fluids had reasonably good reproducibility, other fluids did not show as good concordance between laboratories. Relative standard deviation (RSD) analysis showed more favorable reproducibility outcomes for some data sets, overall results varied more between than within-laboratories. RSD analysis of s showed good within-laboratory variability for all conditions except some metals in interstitial fluid. In general, these findings indicate that absolute bioaccessibility results in some biological fluids may vary between different laboratories. Since measures of relative bioaccessibility are typically used (e.g., in read-across approaches for hazard and risk assessment), both inter- and intra-laboratory reproducibility are relevant unless all substances are tested in the same laboratory. Deviations in protocol interpretation may provide an explanation for some of the inter-laboratory variability observed.
Increased use of tungsten in the manufacturing of industrial goods has led to increased contamination in the air and ground water, particularly near active mines and industrial sites. However, little data exists as to the consequences of exposure to high levels of tungsten. Tungsten rapidly accumulates in murine bone in a concentration-dependent manner, providing a source of continuous low-dose exposure to tungsten. We hypothesized that tungsten in the bone could alter bone homeostasis, a balance between bone formation by osteoblasts and bone resorption by osteoclasts. We assessed the effects of tungsten on osteoblasts using in vitro and in vivo models. Osteoblasts are derived from bone marrow-resident mesenchymal stromal cells (MSC), which can also differentiate into adipocytes. First, we tested whether tungsten changed primary murine MSC differentiation in vitro in the presence or absence of tungsten. We found that tungsten skewed MSC differentiation by decreasing osteoblast markers and increasing adipocyte markers. In vivo, tungsten increased expression of adipocyte markers in murine bone marrow after 4 weeks, but did not change expression of osteoblast markers. Tungsten also increased mouse serum sclerostin levels, a protein that inhibits bone formation and decreases osteogenesis potentially at the expense of osteogenesis. Next, we explored the effects of tungsten on TGF-β pathway signaling, known to enhance osteoblast differentiation, and concomitantly inhibits osteoclast formation. In vitro, tungsten decreased TGF-β protein levels in MSC, which resulted in decreased SMAD signaling. The data presented provide evidence that tungsten is altering bone homeostasis by decreasing bone formation, potentially by decreasing TGF-β signaling. Future experiments will investigate the potential effects of tungsten on osteoclasts, as well as changes to bone remodeling and integrity.

Trace elements play a crucial role in living organisms. The distribution of trace elements in various tissues can vary significantly and can have an impact on the health of an organism. The quantification of trace elements in vivo at high 3-D resolution is crucial for understanding their distribution and function. However, traditional methods for quantifying trace elements in vivo have limitations, such as low image resolution and the need for destructive sampling. The development of novel imaging techniques, such as neutron elemental imaging, can provide an alternative method for quantifying trace elements in vivo at high 3-D resolution.

Neurotoxic insults upregulate a novel secreted protein to promote cell survival in dopaminergic neuronal cells during early stages of toxicity.

Neurotoxic agents exert neuronal toxicity mainly by impairing key signaling molecules that control the balance between pro- and anti-apoptotic signaling. Although various proapoptotic signaling pathways that occur during neurotoxic stress have been elucidated, only a few studies have characterized signaling molecules that can protect neurons against toxic insults. Herein, we have identified that dopaminergic neurotoxicants MPP+ and manganese rapidly induce Prokineticin-2 (PK2), a recently discovered mammalian homolog of mamba snake venom, as determined by qPCR pathway array analysis. In order to further understand the functional role of PK2 upregulation, we created stable PK2 expressing dopaminergic cells by delivering PK2 myc-tagged DNA into mouse dopaminergic MN9D cells. Interestingly, exposure of manganese and MPP+ to PK2 overexpressing cells showed significant protection against the neurotoxicity as compared to vector control cells, indicating that PK2 may play a neuroprotective role in dopaminergic neurons. The protective effect was both dose- and time-dependent. Furthermore, PK2 receptor blocker PC7 attenuated the PK2-induced neuroprotective effects in PK2 overexpressing cells. Measurement of apoptosis by Annexin V and caspase-3 activation also revealed that PK2 overexpression protects against manganese and MPP+ -induced apoptosis. We also found mitochondrial integrity was well maintained in PK2 overexpressing cells as compared to vector cells following exposure to the neurotoxic agents. Preliminary results also showed that key proteins in mitochondrial functions, including BCL2, PGC1-alpha and TFAM levels, were preserved in PK2 overexpressing cells following neurotoxic insult. Collectively, our results suggest that neurotoxic insults upregulate PK2 in dopaminergic neurons to protect against early stages of neurotoxicity (NS 078327 and ES 10586).

A Novel Associated Particle Neutron Elemental Imaging (APNEI) Technology for 3-D Noninvasive In Vivo Quantification of Trace Elements in Animal and Human Tissue.

Trace elements play a crucial role in living organisms. The distribution of trace elements in these organisms provides valuable information about their biological function. In this project, a novel APNEI technology was studied for noninvasive quantification of trace elements in vivo at high 3-D resolution required for general biological research (e.g. toxicology research). The APNEI technology uses fast neutrons produced by a compact neutron generator to extract elemental information from an animal or a human tissue. The technology has two unique features: 1) the penetration of the neutrons and γ-rays to get into the animal or human tissue and to bring the elemental information out of the tissue; and 2) the associated particles to locate the position of the elements. In this project, a deuterium-deuterium (D-D) APNEI model was built using Monte Carlo (MC) simulation method. MC simulations for the interaction between a D-D neutron source and a series of elements were performed. The resulting characteristic gamma-rays of interested elements were collected by high-purity germanium (HPGe) detectors simulated in the program. Assuming a 5 mm * 5 mm * 5 mm voxel in soft tissue (about 15 cm away from the neutron source) with iron (Fe) concentration of 1300 ppm, the Fe gammas are found to be 3908 γ/MeV. The number for phosphorus gamma-ray is about 60 counts for a 1 cm³ voxel in soft tissue with 500 ppm concentration. These results show that the technology is sensitive enough to image some trace elements in animal or human tissue with image resolution of mm order at the biologically meaningful concentrations.
technology can be used to investigate metal toxicology or the toxicity of other toxic agents that would result in an alteration of the elemental distributions in animal or human body.

1288 Early-Life Lead Exposure and Drug Abuse: A Novel Pathway to Addiction

A recent assessment of a prospective cohort of children exposed to lead (Pb2+) in infancy has documented later effects on mental health including drug abuse (McFarlane et al., 2013). Animal studies have provided limited evidence that perinatal Pb2+ exposure enhances cocaine sensitization and Pb2+-exposed animals are more prone to relapse (Nations et al., 2000; 2003). Other animal studies have shown that Pb2+ alters dopaminergic circuitry (Cory-Slechta and Widowski, 1991; Zach et al., 1998) critical for drug addiction. However, there is no data on mechanisms by which Pb2+ exposure may sensitize animals to drug abuse. In the present study, we examined the effects of early life Pb2+ exposure on cocaine sensitization and dopamine receptor levels in the striatum (STR) and nucleus accumbens (NAC) in late adolescence. Rats chronically exposed to Pb2+ during early life exhibited a highly significant increase in the locomotor response to cocaine administration (15 mg/kg) relative to controls. This effect of cocaine was associated with a marked increase (20-34%) in D1 dopamine receptors (D1R) in the STR and NAC of Pb2+-exposed rats relative to controls. Activation of D1R by dopamine plays an important role in addictive and drug reinforcing properties of cocaine. We also observed a modest but significant increase in D2 dopamine receptors (D2R) in the STR and results in the NAC are forthcoming. D1R and D2R were measured using quantitative receptor autoradiography. Pharmacological studies are examining the selectivity of the sensitization to D1R and/or D2R antagonist. Together, these results show that chronic Pb2+ exposure during development alters D1R/D2R expression and cocaine responsivity. They suggest that early life Pb2+ exposure may induce a state of re-programming of the developing brain by which neuronal pathways important for drug addiction may be permanently altered, thus enhancing liability for drug use and addiction as young adults [supported by ES006189 and a pilot project from NIEHS Center ES006189].

1289 Lead Inhibits Presynaptic Neurotransmitter Release in Schaffer Collateral-CA1 Synapses in the Rat Hippocampus: Understanding Mechanism(s)

Lead exposure during brain development inhibits neurotransmitter release and this effect is likely to contribute to impaired synapse formation, plasticity and learning deficits. However, the mechanism(s) by which lead impairs neurotransmitter release have not been fully elucidated. In primary hippocampal neurons, lead exposure inhibits vesicular release and reduces the number of fast-releasing sites, an effect mediated by NMDAR inhibition (Ned et al., 2010). Since this finding was made in primary hippocampal neurons, we wanted to determine if similar effects were also present in animals exposed to lead in vivo. In the present study, we examined the effects of chronic lead exposure on presynaptic transmitter release using two-photon laser scanning microscopy of FM1-43 vesicular release in Schaffer collateral-CA1 synapses in ex vivo hippocampal slices. We found that chronic lead exposure significantly enhanced paired-pulse facilitation (Control Pr = 0.46; Pb Pr = 0.25 at 2mM [Ca2+]o). Using FM1-43 2-photon imaging of release from CA1 Schaffer collateral terminals, we found that this reduced probability was associated with reduced release of glutamate from vesicles in the rapidly-recycling vesicle pool loaded by hypertonic shock. These studies confirm and extend our previous findings in primary hippocampal neurons that lead produces profound impairments in vesicular release that are likely to contribute to deficits in synaptic plasticity and cognitive function. Current studies are examining the molecular bases of the lead inhibition of vesicular release. [Supported by grant # ES020465]
1292 Induction of Heme Oxygenase-1 (HO-1) and Accumulation of Tellurium upon Exposure to Diphenyl Ditelluride and Tellurium Tetrachloride in HT-29 Human Colon Cells

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Tellurium (Te) is a metalloid, with no known physiologic role in humans. Te containing compounds are being used in increasing quantities in industry, agriculture and organic synthesis in products such as optical blue ray discs, photographic materials and photoelectric cells. Increasing uses of such products suggests that human environmental exposure will increase in the future. The neurotoxicity of Te compounds has been documented however little has been reported regarding the gastrointestinal toxicity of this metalloid. Previous work in our lab has demonstrated the mechanism of cell death in HT-29 cells upon exposure to diphényl ditelluride (DPDPT) and tellurium tetrachloride (TeCl4). The purpose of the present study was to evaluate the potential of tellurium compounds TeCl4 and DPDPT to induce oxidative stress by examining heme oxygenase-1 (HO-1) induction and metal accumulation in HT-29 human colon cancer cells. HO-1 is a cytoprotective enzyme activated by its substrate heme and to diverse stimuli. The induction of HO-1 is one of the most important cells in the cellular response to pro-oxidative insults. Upon exposure to concentrations ranging from 62.5µM to 1000µM of TeCl4 and DPDPT in HT-29 human transformed colon cells, oxidative stress was confirmed by a significant increase in the HO-1 activity at the concentrations ranging from 250 µM to 1000 µM in HT-29 cells with DPDPT and from 62.5 µM to 1000 µM with TeCl4 treatment when compared to the control group. Intracellular tellurium levels were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and significant increases were observed at the concentrations ranging from 500 µM to 1000 µM after DPDPT and 125 µM to 1000 µM after TeCl4 exposure. It is concluded that DPDPT and TeCl4 exposure increases the HO-1 activity along with the increase in the intracellular accumulation of the metalloid.

1293 Translocation Synthesis Defends against Telomere Dysfunction Induced by Hexavalent Chromium


Telomeres are repetitive nucleotide sequences that cap and protect chromosome ends. When telomeres become dysfunctional they contribute to a variety of pulmonary diseases. Our previous work established that DNA replication stress induced by the environmental pollutant hexavalent chromium Cr(VI) causes telomere loss and aberrations. Chronic inhalation of Cr(VI) leads to a variety of lung diseases, including fibrosis, and cancers. Cr(VI) forms a spectrum of DNA lesions that impede DNA replication and can cause collapse of the replication fork and chromosomal breakage. Telomeres are fragile DNA sites prone to breakage during replication stress. Cells have mechanisms for bypassing lesions that block replication forks called translesion synthesis (TLS). We hypothesize that Cr(VI)-induced DNA replication stress activates TLS DNA polymerase η (polη) that suppress Cr(VI)-induced mutagenesis and telomere dysfunction. Our research is investigating several endpoints of telomere dysfunction in human cells proficient and deficient in polη. We observe that cells deficient in polη are 53 fold more sensitive to low levels of Cr(VI) and show through flow cytometric analysis that polη deficient cells are delayed in S-phase of the cell cycle compared to isogenic controls. Using a combination of immunofluorescence and telomere fluorescence in situ hybridization (IF-TELISH), quantification of replication stress at genomic and telomeric DNA show that Cr(VI) induces polη mobilization to stalled DNA replication sites at genomic and telomeric regions in human cells. Post Cr(VI) exposures, we identify telomeric aberrations by staining metaphase chromosomes with a fluorescent telomeric probe using telomeric FISH. Using telomeric FISH, we find a four-fold increase in aberrations in dysfunctional polη. Our study demonstrates one mechanism by which Cr(VI) directly interacts with the genome, alters telomere integrity, and the cellular pathways that protect telomeres in the face of genotoxic replication stress.

1294 Fish As Models for Investigating Metabolic Disruption Arising from Dietary Selenium Supersaturation

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Selenium (Se) exhibits a narrow range between essentiality and toxicity in many animals. There is increasing concern that Se oversupplementation in humans can lead to symptoms of metabolic syndrome, including Type 2 diabetes. We have been using fish as comparative animal models to investigate mechanisms of metabolic disruption arising from excess dietary organoselenium. This work originated in field studies of Se-contaminated aquatic ecosystems, where five fish species exhibited consistent evidence of altered lipid and carbohydrate homeostasis. Similarly, in laboratory studies adult zebrafish fed diets augmented with seleno-L-methionine (1: control, no supplementation), 3, 10 or 28 µg Se/g dry mass) for 90 days exhibited dose-dependent increases in whole body lipid (triaclyglycerols) and carbohydrate (glycogen). Furthermore, while high Se diets produced significant down-regulated mRNA abundance of protein tyrosine phosphatase 1B (PTP1B) in muscle, and beta-hydroxycalcoenzyme A dehydrogenase (HOAD), sterol regulatory element binding protein 1 (SREBP 1) and methionine adenosyltransferase 1 alpha (MAT 1A) in liver of zebrafish. Additional studies in rainbow trout showed that chronic exposure to elevated dietary Se resulted in greater baseline plasma cortisol concentrations, but an attenuated cortisol response to acute stress challenges. Overall, these results have clear implications for the fitness of wild fish exposed to elevated dietary Se in contaminated aquatic ecosystems, and may provide insight into mechanisms of metabolic disruption in mammals, including humans, that receive dietary oversupplementation of organoselenium.

1295 Effects of Uranyl Acetate on Etoposide-Induced DNA Damage and Repair in Human Bronchial Epithelial Cells (16HBE14o-)

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It has been widely reported that metals can be genotoxic by several mechanisms including generation of reactive oxygen species and inhibition of DNA repair. There is an increased concern over environmental exposure to abandoned uranium mine tailings as well as occupational exposures to depleted uranium via military action. The aim of this study is to evaluate the ability of depleted uranium (DU) to inhibit double strand break DNA repair at low concentrations in human bronchial epithelial (16HBE14o-) cells. 16HBE14o- cells were co-exposed to 0.13 µM (30 ppb; current EPA standard in drinking water) of soluble depleted uranium as uranyl acetate (UA) in the presence of 0 – 25 µM of etoposide. UA cytotoxicity was assessed in vitro by the clonogenic survival assay. DU damage response was assessed in vitro via flow cytometry to determine phosphorylation of the DNA repair proteins: ATM, SMC-1, and γ-H2AX. Replication protein A (RPA) activity was assessed by an ELISA assay and cell cycle arrest was assessed via flow cytometry. Findings from this work demonstrate that cells treated with UA did not induce phosphorylation of DNA repair proteins (pATM, pSMC-1, or pγ-H2AX) compared to etoposide treatments and untreated cells. Interestingly, the co-exposure of UA and etoposide reduced the amount of phosphorylated ATM and activated RPA after 48 and 60 hr, respectively. These results suggest that DU may inhibit double strand break DNA repair at low concentrations in the presence of a known DNA damaging agent. The inhibition of DNA repair by depleted uranium at environmentally relevant concentrations suggest that the model systems should also be considered as a genotoxic effect. This work is supported by NIH Grants CA096281 (RCL), CCSG – CA023074 (AZCC/ARL-Division of Biotechnology Cytometry Core Facility), CA95901, F31ES014971 (MY), U54CA143924, MGE@MSA (MY) and The Alfred P. Sloan Foundation (MY) for funding this project.

1296 Gastric Reduction of Hexavalent Chromium in Fed and Fasted Human Stomach Samples

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The rate and capacity of gastric reduction of hexavalent chromium [Cr(VI)] are important characteristics of dietary depleted uranium (DU) or Cr(VI) compounds, which in turn is a determinant of potential cancer risk. Our previous experiments using fed rodent gastric contents and fasted human fluid found that the reduction of Cr(VI) exhibited a mixed second-order reaction with a pH dependent second-order rate constant. Potential uncertainties regarding dilution which may affect the concentrations of reducing agents and the second-order rate constant were noted. This study is an extension to our previous experiments. Stomach fluid samples were obtained from human volunteers in the fed and fasted (paired) states. Using spectated isotope dilution mass spectrometry, studies were conducted to ascertain the kinetics (rates and capacity) of the various gastric fluids to reduce Cr(VI), with emphasis on better characterization of conditions during a fed state and characterizing inter-individual variability. The effect of slowed reduction potential were investigated. Alternative mathematical forms of the reduction model were considered. The human data confirm a significant pH dependence for the second-order rate constant. Potential uncertainties regarding dilution which may affect the concentration of reducing agents and the second-order rate constant were noted. These results suggest that DU may inhibit double strand break DNA repair at low concentrations in the presence of a known DNA damaging agent. The inhibition of DNA repair by depleted uranium at environmentally relevant concentrations suggest that the model systems should also be considered as a genotoxic effect. This work is supported by NIH Grants CA096281 (RCL), CCSG – CA023074 (AZCC/ARL-Division of Biotechnology Cytometry Core Facility), CA95901, F31ES014971 (MY), U54CA143924, MGE@MSA (MY) and The Alfred P. Sloan Foundation (MY) for funding this project.
Temporal Changes in Rat Liver Gene Expression after Cadmium and Chromium Exposure


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Our Nation’s Guardians are at risk of exposure to a variety of environmental health hazards throughout their normal duty activities including deployments, training exercises, and homeland defense situations. Metals are widely used in large quantities in a number of industrial processes and are common environmental toxicants, which increases the possibility of our Soldiers being exposed at toxic levels. While metal toxicity has been widely studied, the exact mechanisms of toxicity remain unclear. In order to further elucidate these mechanisms and identify candidate biomarkers, rats were exposed via a single intraperitoneal injection to three concentrations of CdCl2, NiCl2, and Na2Cr2O7, and livers were harvested 1, 3, or 7 days after exposure. Cd and Cr accumulated in the liver at 1 day post-exposure, while there was no accumulation of Ni. Cd levels remained elevated over the length of the experiment, while Cr levels declined. Differentially expressed genes were identified via microarray analysis. Both common and uniquely modulated transcripts and perturbed pathways were identified for the metal species. Enriched pathways and functions including hepatic injury, inflammation, cell cycle, tissue repair, and cancer. This work provides insight into the temporal effects and mechanistic pathways involved in acute metal intoxication, which may lead to the identification of candidate biomarkers.

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Toxicogenomic Study in Rat Thymus of F1 Generation Offspring following Maternal Exposure to Silver Ion


Silver-containing compounds or their mixtures are widely applied as antimicrobial agents onto food packaging materials, often in direct contact with the food. The extensive use of silver in the food industry raises concerns about its safety to human health and risk. Data currently available revealed that in utero exposure to silver ions could adversely affect the developing immune system. However, further studies are needed to confirm that the previously observed adverse effects are due to silver ion alone, and to define the no observed effect level (NOEL). In the present study we used a toxicogenomic approach to study the effect of silver ion on the developing thymus at the transcriptional level using whole genome microarrays. Global gene expression changes in rat thymus of F1 generation pups at postnatal day 26 following maternal exposure to silver acetate at 0, 0.4, 4.0, or 40.0 mg/kg in drinking water are reported here. Five female and 5 male pups were included in each dosing group. Gene expression profiling analyses identified only about a dozen differentially expressed genes (DEGs) in each dose group using a loose criterion of fold change (FC) > 1.5 and unadjusted p < 0.05, regardless whether the analysis was conducted within each gender group or with both gender groups combined. No dose-dependent effect was observed on the number of DEGs. In addition, none of these genes had a false discovery rate (FDR) ≤ 0.05 after correction for multiple testing. These results indicate silver acetate up to 40.0 mg/kg did not affect gene expression in the developing thymus. Combined with the observation that thymus-to-body-weight ratios were not affected, and no histopathological abnormalities in thymus were identified in the pups, the current study using a toxicogenomic approach suggests that in utero exposure to silver ion up to 26.0 mg/kg (equivalent to 40.0 mg/kg silver acetate) did not have an adverse effect on the developing thymus.

Assessing Toxicity of FeMn Dust Particles from a South African Ferromanganese Smelter Works: In Vitro Studies on Primary Rat Astrocytes and BEAS-2B Cells

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Occupational exposure to manganese (Mn) may result in development of diseases associated with the lungs and brain. Even though evidence of Mn associated diseases exists, some studies have found no association between manganese levels in blood or urine and indicators of neurotoxic effects. This study investigated the effects of ferromanganese (FeMn) dust, from a ferro-manganese smelter works, on primary rat astrocytes and human bronchial epithelial (BEAS-2B) cells. Particle size distribution, surface area and trace elemental composition were analyzed. Particle uptake was studied using dark field microscopy and viability determined using the xCELLigence RTCA system based on cell adhesion. Nuclear translocation of Nrf2 and NF-κB was studied using western blots and genotoxicity determined by the alkaline Comet assay. Min-U-Sil 5 was used as benchmark particles. The presence of nano sized FeMn and its cellular uptake in both cell lines was confirmed. Treatment resulted in a dose-dependent decrease in viability of both cell lines for both particle types, with crystalline silica producing higher toxicity compared to FeMn.

Plasmomic engineered NPs are popular in consumer and medical-based industries due to their unique surface characteristics. Identifying the toxicity of NPs is critical given the increased exposure. The toxicity of NPs is often determined using conventional colorimetric and optical high-throughput toxicity systems that rely on absorbance, luminescence or fluorescence signals. However these systems are prone to interference by the NPs, which lead to erroneous results. We have assessed the interference study of conventional assays, caused by micro/Nano gold (AuNPs) exhibiting surface plasmon resonance (SPR).

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The Effects of Tellurium Compounds on the Viability of Rat Hippocampal Astrocytes Are Attenuated with Calcium Blocker

M. Costa

Tellurium (Te) is a metalloid belonging to group 6A of the periodic table along with Selenium (Se) and sulfur (S). Unlike Se and Se, Te has not been found to have any physiologic role in mammals, although detectable levels of Te have been noted in humans through dietary and/or industrial exposures. Te compounds have been associated with both peripheral and central nervous system toxicity. Studies have suggested that nervous system disturbances with exposure to these compounds may be partially attributed to disruption of Calcium (Ca) homeostasis that results in destabilization of cytoskeletal proteins. The purpose of this study was to examine the relationship of Te exposure and effects on astrocyte viability and cytoskeleton and to determine if Ca plays a role in these changes. Rat hippocampal astrocytes were treated with 15.6, 31.2, 62.5, 125 and 250 μM of tellurium tetrachloride (TeCl4) and diphenyl ditelluride (DPDT) to assess changes in viability. Cytoskeletal preparations of treated and control cells were observed using scanning electron microscopy (SEM). Cells were subsequently treated with 125 μM concentrations of each of the compounds with and without the Ca blocker dantrolene for 24hrs to determine the effects of Ca on cell viability in cells treated with Te. Results indicate a significant reduction in cell viability at all Te doses when compared to untreated cells. Cells treated with 5 μM dantrolene and 125 μM TeCl4 and DPDT showed significant increases in viability when compared to cells treated with Te alone. The cytoskeleton of Te treated cells observed using SEM demonstrated changes such as loss of crossbridging and condensation of cytoskeletal components when compared to untreated cells. It is concluded that Te decreases astrocyte viability and that the use of dantrolene significantly increases viability, suggesting that Ca plays a role in this process. Exposure to Te compounds results in cytoskeletal alterations when compared to non-exposed cells.

Investigating the Potential Carcinogenic Effects of Chronic Tungsten (VI) Oxide Exposure to Immortalize Human Lung Cells

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Rationale: Tungsten oxide (WO3) is an occupational exposure hazard. The primary route of WO3 exposure is inhalation and WO3 is known as a pulmonary irritant. This work investigated exposure of WO3 to immortalized human bronchial epithelial cells (Beas-2B) to investigate cytotoxicity and carcinogenic potential. Experimental procedures: Insoluble WO3 particles were sonicated to reduce agglomeration and create suspensions of WO3 particles small enough for Beas-2B cells to engulf (<10 microns in diameter). Beas-2B cells were chronically exposed to varying doses (0.25, 0.5, 1.0, 5.0, 10 and 15 μg/cm2) of WO3 for 6 weeks. Proliferation rate was measured; soft agar cell migration testing and scratch test assays were performed. Results: After 2 weeks, Beas-2B cells with the highest doses of WO3 started to proliferate just as quickly as the cells that had low doses of WO3. After 6 weeks of WO3 exposure, in the control transformation there was an average of 8 colonies per well. Scratch testing revealed that WO3 treated cells migrated significantly more quickly than control transformed cells. Conclusions: Chronic treatment of Beas-2B cells with WO3 induced transformation in the cells. Inhaled WO3 may not only be a lung irritant, but also a potential pulmonary carcinogen at high doses.

Quercetin Inhibits Cr(VI)-Induced Lung Carcinogenesis by Targeting miR-21-Pdcd4 Signaling Pathway

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Hexavalent chromium (Cr(VI)) is an important human carcinogen associated with pulmonary diseases and lung cancer. Cancer prevention using natural products has become an integral part of cancer control. Quercetin is one of the most abundant dietary flavonoids widely present in many fruits and vegetables, possesses potent antioxidant activity capable of protecting normal cells from various stimuli-induced oxidative stress and cell death. MicroRNA-21 (miR-21) is a key regulator of oncogenic processes. It is significantly elevated in the majority of human tumors and functionally linked to cellular proliferation, invasion and migration. Studies have shown that miR-21 exerts its oncogenic activity by targeting the tumor suppressor gene programmed cell death 4 (PDCD4). The latter study examined the effect of quercetin on the inhibition of Cr(VI) induced carcinogenesis and the role of miR-21-Pdcd4 signaling involved. Our results showed that quercetin decreased ROS generation induced by Cr(VI) exposure in Beas-2B cells. Chronic Cr(VI) exposure induced malignant cell transformation, increased miR-21 expression and caused inhibition of Pdcd4, which were significantly inhibited by the treatment of quercetin in a dose dependent manner. Stable knockdown of miR-21 overexpression of pdc4 in Beas-2B cells significantly reduced the Cr(VI) induced cell transformation. Furthermore, quercetin inhibited the Cr(VI) induced E-cadherin reduction, and also beta-catenin/TCF-dependent transcription. Taken together, these results demonstrate that quercetin is able to protect Beas-2B cells from Cr(VI)-induced carcinogenesis by targeting miR-21-Pdcd4 signaling.

Application of Lead Isotope Ratios to Lead Poisoning Investigations On-Farm


Lead (Pb) is a common cause of heavy metal poisonings in cattle. Sources of Pb on-farm may include cranckcase oil, machinery grease, batteries, plumbing, and paint chips. Unfortunately, consumption of, or exposure to, these sources may negatively impact animal health, and, if present at subclinical concentrations, may be inadvertently introduced into the food supply by excretion in milk or distribution in beef cattle. Therefore, the scope of poisoning incidents must be clearly assessed and the source of intoxication mitigated to prevent future exposures. One valuable tool in the forensic assessment of lead source is Pb isotope analysis. We report on two cases in which the novel application of Pb isotope analysis by ICP-MS was extended to bovine blood and liver for comparison to environmental samples including paint chips and soil to elucidate lead source. Isotope ratios 208Pb/206Pb, 207Pb/206Pb, 207Pb/206Pb and 206Pb/204Pb were calculated for blood samples across the entire herd in each case. Blood isotopic profiles corresponded with exposure to paint chips obtained from sources unique to each herd. Although this resolved the poisoning incident for one farm, a subsequent poisoning event on the second farm prompted a more thorough investigation of the property. Pb quantitation and isotope profiling provided a direct link between an affected calf’s liver and soil obtained from the farm’s main barn but not from that of a secondary barn. These two cases highlight the use of isotope profiling as an advanced diagnostic technique for Pb source identification on-farm.

Chronic Arsenic Exposure In Vitro Causes Acquisition of Multiple Tumor Cell Characteristic in Human Pancreatic Epithelial Cells

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Arsenic is linked with human pancreatic diseases potentially including cancer. Emerging data indicates cancer stem cells (CSCs) may be critical to the carcinogenic process. Our prior work showed chronic cadmium exposure of a human pancreatic ductal epithelial (HPDE) cell line caused oncogenic transformation and formation of CSC-like cells. Thus, we studied if inorganic arsenic exposure could induce a similar tumor cell phenotype in HPDE cells and derivative non-adherent spheroids, which are enriched in stem cells (SCs or CSCs). HPDE cells were chronically exposed to sodium arsenite (2.5 μM) for up to 30 weeks and cancer cell characteristics were assessed including matrix metalloproteinase-9 (MMP-9) secretion, invasion, colony formation and expression of cancer relevant genes by RT-PCR or immunoblotting. Non-adherent spheroid formation was used to assess CSC-like characteristics. Our results showed chronic arsenic exposure induced a similar tumor cell phenotype in HPDE cells and derivative non-adherent spheroids, which are enriched in stem cells (SCs or CSCs). Oxidative stress and cell death. MicroRNA-21 (miR-21) is a key regulator of oncogenic processes. It is significantly elevated in the majority of human tumors and functionally linked to cellular proliferation, invasion and migration. Studies have shown that miR-21 exerts its oncogenic activity by targeting the tumor suppressor gene programmed cell death 4 (PDCD4). The latter study examined the effect of quercetin on the inhibition of Cr(VI) induced carcinogenesis and the role of miR-21-Pdcd4 signaling involved. Our results showed that quercetin decreased ROS generation induced by Cr(VI) exposure in Beas-2B cells. Chronic Cr(VI) exposure induced malignant cell transformation, increased miR-21 expression and caused inhibition of Pdcd4, which were significantly inhibited by the treatment of quercetin in a dose dependent manner. Stable knockdown of miR-21 overexpression of pdc4 in Beas-2B cells significantly reduced the Cr(VI) induced cell transformation. Furthermore, quercetin inhibited the Cr(VI) induced E-cadherin reduction, and also beta-catenin/TCF-dependent transcription. Taken together, these results demonstrate that quercetin is able to protect Beas-2B cells from Cr(VI)-induced carcinogenesis by targeting miR-21-Pdcd4 signaling.
structures in Matrigel. Thus, chronic arsenic causes acquisition of multiple tumor cell characteristics in HPDE cells. These data support the plausibility of arsenic as a human pancreatic carcinogen.

**1297i** Chronic Arsenic Exposure Induces Cancer Cell Characteristics in Human Peripheral Lung Epithelial Cells

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Inorganic arsenic is a human lung carcinogen. Here we study the ability of chronic inorganic arsenic exposure to induce a cancer phenotype in the immortalized, non-tumorigenic human lung epithelial cell line, HPL-1D. Signs of oncogenic transformation were assessed periodically during chronic sodium arsenite (2 μM) exposure, such as secreted matrix metalloproteinase-2 (MMP-2) activity, invasion, and expression of cancer-relevant genes at the transcript and protein levels. After 38 weeks of continuous arsenic exposure, secreted MMP-2 activity increased to 200% of control, a level typical of other cell lines that have acquired an arsenic-induced cancer phenotype. The invasive capacity of these chronic arsenic-treated lung epithelial (ATLE) cells was over 3-times control levels. Expression of lung cancer tumor suppressor genes SLC38A3 and TTF-1 at the protein level was markedly reduced in ATLE cells to 26% and 24% of control, respectively. Increases occurred in K-Ras oncogene (300% of control) and both the ERK (274%) and p-ERK (152%) proteins in ATLE cells. Vimentin increased to 300% and E-Cadherin protein was reduced to 16% of control in ATLE cells indicative of epithelial-to-mesenchymal transition. Thus, ATLE cells appeared to have acquired multiple cancer cell characteristics, some of which are specific to lung. Additional evidence of an oncogenic phenotype in ATLE cells came from other molecular events linked to chronic arsenic exposure. ATLE cells showed reduced expression of the tumor suppressor genes APC (72% of control), PTEN (43%), and CCND1 (76%) and the DNA damage response gene ATM (82%). Expression of the oncogene AKT increased in ATLE cells to 242% of control. Chronic arsenic exposure increased expression of the major metallothioneins, MT-1A and MT-2A and stress response genes HO-1 (690%) and HIF-1α (247%). Thus, it appears that arsenic can induce multiple cancer cell characteristics in human lung epithelial cells. This model can now be used to further assess mechanisms of arsenic-induced lung cancer.

**1297j** Silencing KRAS Overexpression in Arsenic-Transformed Prostate Stem Cells Results in Signs of Malignant Phenotype Reversal

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Inorganic arsenic is a human carcinogenic that probably targets the prostate. Chronic arsenic exposure malignantly transforms the RWPE-1 human prostate epithelial line to CAsE-PE cells, and a derivative normal prostate stem cell (SC) line, WPE-ST, stem to arsenic-cancer SCs (As-CSC). KRAS oncogene activation appears critical during this process, as CAsE-PE cells overexpress KRAS, and activation precedes transformation. As-CSCs also overexpress KRAS suggesting this is important in arsenic carcinogenesis in this key subpopulation. Thus, we hypothesize KRAS knockdown (KD) may reverse arsenic-induced malignant phenotype. RNA interference using shRNAmirs to obtain KRAS KD was used in As-CSCs. KRAS shRNAmir primers expresses miRNA 30 transcript which targets KRAS to silence expression. As-CSCs were transduced with KRAS shRNA, or non-targeting control shRNA. Stable cell lines were generated by puromycin selection. Cells analyzed 2 weeks post transduction showed KRAS protein decreased to 5% of control in KD As-CSCs, confirming stable KD. KRAS KD decreased phosphorylated ERK by 60%, indicating inhibition of RAS/ERK signaling, a proliferation/survival pathway activated with arsenic transformation. Secreted metalloproteinase (MMP) activity increases in arsenic-induced malignant transformation, and by 4 weeks, KRAS KD decreased secreted MMP-9 (49%) and MMP-2 (39%) activity. Anchorage-independent growth (colony formation) is typical of cancer cells and correlated with cancer SCs. KRAS KD in As-CSCs decreased colony formation (40%) at 6 weeks after transduction. KRAS KD increased the cell cycle inhibitor proteins p16 (131%) and P21 (156%) and decreased the cell cycle activator CCND1 transcript (26%), indicating reduced proliferation, consistent with a loss of rapid growth. KRAS KD increased (86%) protein levels of PTEN, a tumor suppressor gene inactivated in many cancers and in As-CSCs. Thus, KRAS silencing impacts arsenic-induced malignant phenotype at the level of SCs, inducing loss of many typical cancer characteristics.

**1297k** Differential DNA Methylation in Arsenic- or Cadmium-Transformed Malignant Prostate Epithelial Cells

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Many inorganic carcinogens are weak mutagens, suggesting the epigenome is an important target. Previous work shows global DNA hypomethylation in human cells malignantly transformed by inorganic arsenic (iAs) and altered methylation patterns in iAs- or cadmium-transformed epithelial cells. In this work, the methylation status in the promoter region of six genes was assessed in the normal human prostate epithelial cell line (RWPE1) that was malignantly transformed by 10 μM cadmium for 11 weeks (CTPE) or 5 μM iAs for 31 weeks (CAsE-PE), at time cells showed multiple markers of acquired cancer phenotype. Next generation sequencing of the transcriptome identified multiple misregulated genes. Of the most highly misregulated genes, 6 genes associated with stem cell function/dysfunction (ALDH1A1, WNT4, and NES), extracellular matrix (HYAL1), cell adhesion (NTM), or aggressive cancers (SI00P) were chosen for in-depth analysis of the DNA methylation profile. DNA was isolated, bisulfite converted, and combined bisulfite restriction analysis (COBRA) was used to identify differentially methylated CpG sites within 1000 bases of the transcription start site. Differential methylation was confirmed with bisulfite sequencing. All 6 genes showed differential methylation of at least one CpG site in transformants relative to control RWPE1 cells. Altered gene expression was inversely related to methylation status in 4 out of the 6 genes. Expression of NES (+15-fold) and NTM (+1000-fold) was decreased in the transformants compared to control. Both genes were hypermethylated near the transcription start site. Expression of HYAL1 (+25-fold) and SI00P (+40-fold) was increased in transformants relative to control and both genes were hypomethylated near the transcription start site. ALDH1A1 and WNT4 expression was increased in transformed cells versus control and both genes were hypermethylated relative to control. In conclusion, altered gene expression profiles observed in cadmium and iAs transformed cells may often result from altered DNA methylation status.

**1297l** Arsenic Induces an Oncogenic Phenotype in Human Breast Epithelia through an Estrogen Receptor-Independent Pathway by Aromatase Activation

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Inorganic arsenic has been linked to human breast cancer in some studies. Here, we tested the effects of inorganic arsenic exposure on the normal, estrogen receptor (ER)-negative breast epithelial cell line, MCF-10A. Cells were chronically exposed to sodium arsenite (500 nM) and markers of cancer phenotype and expression of genes relevant to breast cancer or stem cells (SCs) were examined. After 24 weeks of continuous arsenic exposure, increases in secreted metalloproteinase (MMP) activity, colony formation, invasion, and proliferation rate occurred, all typical cancer cell characteristics. The arsenic-transformed breast epithelial (ATBE) cells showed characteristics of a basal-like breast cancer, including ER-α, HER-2 and progesterone receptor negativity, and enhanced expression of breast cancer SC (CSC) markers, K5 and p63. Putative CD44+/CD24low/- breast SCs increased to 180% of control in ATBE cells as assessed by flow cytometry. In non-confluent culture, ATBE cells formed multilayer cell mounds, indicative of loss of contact inhibition, which showed high levels of K5 and p63 indicating the presence of CSCs. Epithelial-to-mesenchymal transition occurred starting from 8 weeks of arsenic exposure, as seen by cell morphology, increased SNAIL1 and VIMENTIN expression and decreased E-CADHERIN expression. Aromatase, a key rate-limiting enzyme in estrogen synthesis, was overexpressed (mRNA, 5400% of control; protein, 140% of control; week 8) starting early on in arsenic exposure. Immunofluorescence showed widely spread of aromatase overexpression in the ATBE cells. Levels of 17β-estradiol increased in ATBE cells and conditioned medium. The aromatase inhibitor, letrozole, abolished arsenic-induced 17β-estradiol increases, and over a 3-week period reversed arsenic-induced oncogenic phenotype. Thus, chronic arsenic exposure drives human breast epithelia into an oncogenic phenotype with an apparent overabundance of putative CSCs. Arsenic appears to transform breast epithelia through overexpression of aromatase, thereby activating oncogenic processes independent of ER.
Variable Cytotoxicity and Reactive Oxygen Species Generation in Pulmonary and Aortic Cells Exposed to Inorganic Arsenic and Monomethylarsonic Acid

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Chronic ingestion of arsenic produces myriad toxic effects including atheroclerosis and hypertension, but the cellular mechanisms by which vascular effects are produced remain unclear. The arsene metabolite monomethylarsonic acid (MMA) demonstrates elevated vascular toxicity relative to inorganic arsenic (iAs), and may therefore be linked to arsenic-induced vascular disease. The present study explores the role of iAs and MMA on cytokotoxicity, cellular morphology and ROS generation in two model vascular smooth muscle cell lines. Cytotoxic effects of iAs and MMA on rat thoracic aorta smooth muscle cells (A7r5) and rat pulmonary arterial smooth muscle cells (iPASMC) in culture were examined for generation of malondialdehyde (MDA), hydrogen peroxide, and superoxide. Cytotoxicity, cell morphology, and mode of cell death were determined by cell counts, trypan blue exclusion, flow cytometry, MTT assay, and light microscopy. Cells treated with iAs or MMA displayed cytotxic effects, and MMA was significantly more toxic than iAs to both cell lines. After a 24 hour exposure, LC50 in A7r5 were 11 μM and 700 nM when treated with iAs and MMA, respectively. 10 μM iAs and 500 nM MMA induced apoptosis in A7r5 and appeared to arrest cell growth in S phase. MMA was significantly more toxic to rat PASMC than iAs with an apparent 24 h LC50 of 4.0 μM relative to 27 μM for iAs. No significant differences in MDA formation or superoxide production were observed following iAs or MMA exposure in either line, although a significant increase in hydrogen peroxide was observed subsequent to iAs and MMA exposure in both lines. These results suggest that the increased toxicity of MMA may be in part due to increased hydrogen peroxide generation in A7r5 cells, but cytotoxicity is not solely attributable to oxidative stress when analyzed in vitro, suggesting that additional mechanisms are involved in arsene-promoted vascular disease.

Arsenic Contaminated Drinking Water Deregulates Human Gene Expression Patterns in a Gender-Specific Manner in Bangladeshi Adults

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Approximately 150 million people in at least 70 countries are exposed to arsenic via drinking water. Arsenic exposure is associated with skin, lung and bladder cancers, cardiovascular disease, kidney disease, and diabetes. Many questions remain regarding the mechanisms behind arsenic’s pathogenesis including its potential role as an endocrine disruptor. Previous research in humans suggests that arsenic alters the abundance of post-translational histone modifications in a gender-specific manner. Twenty-nine study participants (55% male) from the FACT folate clinical trial in Bangladesh were selected with water arsenic exposure ranging between 50–500 μg/L. Arsenic RNA was extracted from peripheral blood mononuclear cells (PBMCs) and gene expression profiling was performed using Affymetrix 1.0 ST arrays. Differentially expressed genes were assessed between high and low groups for males and females separately and findings were validated using real-time PCR. Network analysis was performed using IPA (Ingenuity® Systems, www.ingenuity.com). Among males, a total of 534 genes were differentially expressed (p <0.05) in PBMCs from study participants with high arsenic exposure relative to those with low arsenic exposure. Females exhibited a total of 645 differentially expressed genes (p <0.05). Only 42 genes overlapped between the two genders, with 28 out of 42 genes changing in opposite directions. Network analysis revealed both genders exhibited deregulation of the cardiovascular system through a distinct set of genes. Arsenic exposed adults exhibit gender-specific responses in gene expression. While responses are distinct, they exhibit similar features including cardiovascular and endocrine dysregulation.

Maladaptive Signaling from Arsenic Exposure Impairs Cardiac Bioenergetics and Enhances Autophagy

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Environmental exposure to arsenic through drinking water causes cancers, as well as metabolic and cardiovascular diseases. Association of arsenic with cancer and many of its molecular mechanisms have been well studied, but much less is known of mechanisms for arsenic-promoted cardiac and vascular disorders. A low steady-
state level of cardiac autophagy is critical to maintaining heart homeostasis. While increased autophagy in response to stress such as ischemia or starvation is protective, excessive response to reperfusion after ischemia is detrimental. Stress-induced autophagy, such as in arsenic exposure, can be adaptive and prevent cell transdifferentiation. We hypothesized that arsenic exposure increases autophagic signaling in cardiac tissue causing impaired bioenergetics and enhanced autophagy. DNA binding arrays identified several dysregulated transcription factor families in arsenic-exposed mouse hearts. Pro-autophagic FoxO transcription factors were increased up to 6-fold above control. Protein analysis by immunostaining and Western showed increased expression of both FoxO1 and 3a in the heart and DNA binding was confirmed by EMSA. Hearts from arsenic exposed (100 μg/L in drinking water for 2-5 wks) mice were analyzed for FoxO associated signaling pathways. Upstream of FoxO3a activation, we observed increased in miR-143, a cardiac and autophagy associated microRNA that increases in FoxO3a protein expression. SRF, a transcription factor that drives miR-143 expression, was increased and ELK1, a MAPK factor that renders that is a direct miR-143 target decreased. Downstream of FoxO3a we observed increased pyruvate dehydrogenase kinase 4 expression that may mediate starvation signaling indicative of impaired bioenergetics. These results suggest that arsenic increases cardiac autophagy by inducing autophagy regulating transcription factors (FoxO) and by eliciting maladaptive starved signaling (ELK1 and PDK4). Supported by NIEHS F32 ES022134 01(RB) and NIEHS R01 ES013781(AB)

1303 Global Assessment of Arsenic Pollution Using Sperm Whales (Physeter macrocephalus) As an Indicator Species
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Arsenic (As), a naturally occurring metalloid, is an oceanic pollutant of global concern due to its toxicity, ability to bioaccumulate and potential for increasing ocean levels to an unknown extent by anthropogenic activities. The sperm whale (Physeter macrocephalus) has a global distribution and high trophic level. The aim of this study was to establish a global and regional baseline of oceanic total As concentrations using free-ranging sperm whale as an indicator species. Skin biopsies (n = 342) were collected during the voyage of the Odyssey (2000-2005) from 17 regions considering gender and age in males. As was detectable in 99% of samples with a global mean of 1.9 μg/g ww ranging from 0.1 to 15.6 μg/g ww. Previous work in toothed whale skin found the mean As concentration to be 0.6 μg/g ww being 34% of the global mean of 1.9 μg/g ww ranging from 0.1 to 15.6 μg/g ww. This result indicates that arsenic increases cardiac autophagy by inducing autophagy regulating transcription factors (FoxO) and by eliciting maladaptive starved signaling (ELK1 and PDK4).

1306 Evaluation of Arsenic (+3 Oxidation State) Methyltransferase Activity to Tellurite
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Inorganic arsenic is one of the distressing environmental contaminants, and many people suffer from its toxicity. Arsenite [As(III)] is metabolized with arsenic (+3 oxidation state) methyltransferase (As3MT), and the methylated metabolites are excreted into urine. It was reported that inorganic tellurite (Te) is simply methylated in vitro, and its ultimate methylated metabolite was excreted into urine. Although Te is also methylated as arsenic, the enzyme(s) being responsible for the Te methylation is still unclear. In this study we evaluated that As3MT metabolized inorganic Te and was involved in the reduction of Te toxicity. To obtain poly-histidine-tagged recombinant As3MT (As3MT), cDNA of human As3MT was inserted into pBAD, and As3MT was purified on a nickel ion-affinity column. The reaction mixture consisted of As3MT, S-adenosyl-L-methionine, glutathione and As(III) or tellurite (Te(IV)) in phosphate buffer and were incubated at 37°C for 4 h. The metabolites were determined by an HPLC coupled with an inductively coupled plasma-mass spectrometer. Although rAs3MT actually metabolized As(III) to dimethylated arsenicals, no methylated metabolites of Te(IV) with As3MT were detected under the reaction conditions. This result indicates that As3MT is a specific enzyme to metabolize As, and Te(IV) seems to be methylated by another methyltransferase and not As3MT. Next, siRNA targeting As3MT was introduced into human hepatoma cell line HepG2 cells to achieve the knockdown (KD) of As3MT. Then, the As3MT-KD cells and control siRNA-transfected cells were treated with As(III) or Te(IV) for 24 h. No apparent changes in the cell viability between As3MT-KD and control cells were observed when either As(III) or Te(IV) was exposed. As3MT did not protect the HepG2 cells from the cytotoxicities of As(III) and Te(IV). This can be explained by the suggestion that the cytotoxicities of some metabolic intermediates of As, such as trivalent methylated arsenicals, are equivalent to that of As(III), and Te(IV) is not metabolized with As3MT.

1305 Ogg1 Genetic Background Determines the Genotoxic Potential of Environmentally Relevant Arsenic Exposures
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Inorganic arsenic (i-As) is a well-established human carcinogen to which millions of people are exposed worldwide. It is generally accepted that the genotoxic effects of i-As after an acute exposure are partially linked to the i-As-induced production of reactive oxygen species; but it is necessary to better determine if chronic sub-toxic i-As doses are able to induce biologically significant levels of oxidative DNA damage (ODD). To fill in this gap we have tested the genotoxic and oxidative effects of environmentally relevant arsenic exposures using mouse embryonic fibroblast MEF mutant Ogg1 cells and their wild-type counterparts. Effects were examined by using the comet assay complemented with the use of FPG enzyme. Our findings indicate that MEF Ogg1-/- cells are more sensitive to arsenite-induced acute toxicity, genotoxicity and ODD. Long-term exposure to sub-toxic doses of arsenite generates a detectable increase of ODD and genotoxic DNA damage only in MEF Ogg1 deficient cells. Altogether, the data presented here points out the relevance of ODD and Ogg1 genetic background on the genotoxic risk of i-As at environmentally plausible doses. The persistent accumulation of DNA 8-0H-dG lesions in Ogg1-/- cells during the complete course of the exposure suggests a relevant role in arsenic-associated carcinogenic risk in turn.

1304 Arsenic, Obesity, and Inflammation Cytokines in Mexican Adolescents
1Universidad Javeriana Estado de Durango, Mexico, 2BSPH, Johns Hopkins University, Baltimore, MD, 3Centro Universitario de la Universidad de Guadalajara, Guadalajara, Mexico, 4Laboratorio de Genética y 5CIBERESP, Barcelona, Spain. Evidence on the association of arsenic (As) exposures with obesity is considered as in-sufficient. Obesity is considered a disorder related to an inflammatory process, while arsenic also has an effect on the immune system. The aim of this cross-sectional study was to assess the association of arsenic exposure, obesity and inflammatory cytokines in adolescents exposed to arsenic via drinking water. We studied 384 healthy adolescents 12-15 years of age, living in Torreon Coahuila, Mexico. Participants with fish consumption in the previous week, or incomplete measurements were excluded. Total As concentrations in urine were measured using a inductively coupled plasma-mass spectrometer and expressed as μg/g creatinine. Adiposity was assessed using body mass index (BMI), sex- and age-standardized body mass index (zBMI), and bioimpedance segmental body composition (fat mass); cytokines were determined using ELISA method. The prevalence of obesity and overweight was 14.8% and 21.1% respectively. The median (IQR) of obese men was 26.4% (19.9-32.7%), which was significantly higher in female than males. The median (IQR) of BMI was 26.1 (20.1, 26.8) μg/g creatinine. Only TNFα levels were higher in male adolescents. The median (IQR) of arsenic in urine in non obese subjects was 35.7 (27.4, 46.3) μg/g creatinine, and in adolescents with over-weight and obesity it was 36.6 (24.8, 47.5) μg/g creatinine. In a Spearman correlation analysis, arsenic exposure correlates with fat mass but not zBMI. IL-6 and TNFα correlates with zBMI and arsenic, and IL-6 correlated with fat mass. Our results showed a negative association of adiposity with arsenic exposure. Results of As and obesity association are in agreement with previous studies in human exposed to low levels of As in drinking water.
Arsenic exposure is an ongoing health concern that has been increasingly linked to the development of cardiovascular disease and the metabolic syndrome. However, the role of low-dose As (III) exposure on the development of the metabolic syndrome remains controversial, and our study aims to explore mechanisms of As (III) toxicity on the development of the cardiometabolic syndrome. Male Swiss Webster mice were treated with 100ppb NaAsO2 in their water starting at embryonic day 5 (IU), after weaning (PN), or from embryonic day 5 onward (IU+). After weaning, all groups were fed a western diet. IU+ mice became overweight at week 5. The liver to body weight ratio in the IU and IU+ mice was elevated. Plasma HOME-IR determined IU+ animals were insulin resistant. Liver H&E evaluation determined that all had microvesicular steatosis, but hepatocellular ballooning was most severe in the IU, PN, and IU+ mice. Liver evaluation with Masson’s trichrome found fibrosis in the IU and IU+ livers. Oil-Red-O liver staining found steatosis in all groups with marginally higher lipid content in As (III) treated groups. Metabolomic analysis of plasma found changes in As (III) exposed animals that support an influence on energy metabolism: with effects evident in glycolysis, TCA cycle, and lipid metabolism. The histological, weight, and liver to body weight ratio findings are consistent with NAFDL (non-alcoholic fatty liver disease) suggesting that low-dose As (III) exposure, prenatal and chronic, predisposes mice to morose NAFDL when on a western diet. Insulin resistance in IU+ animals suggests As (III) contribution to the development of the metabolic syndrome. The metabolomic findings are indicative of diverse changes in energy metabolism as a result of low-dose prenatal and/or chronic arsenic exposure. These data will be used to determine mechanisms involved and metabolism alterations caused by As (III) so that therapeutic interventions can be developed.

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Prenatal exposure to inorganic arsenic (iAs) is associated with adverse health effects in infants, children, and adults, and yet the biological mechanisms that underlie these effects are understudied. In order to gain a better understanding of alterations in protein expression profiles associated with prenatal iAs exposure, we utilized samples from 50 mother-child pairs from a recently-established prospective pregnancy cohort in Gómez Palacio, Mexico. Concentrations of iAs in maternal drinking water (DW-iAs) ranged from below the limit of detection (LOD)-236 μg/L and the sum of iAs metabolites in maternal urine (total urinary arsenic; U-As) ranged from 6.19-319.7 μg/L. Using a high-throughput antibody array, we identified 111 cord blood proteins for which there was a significant association between protein expression in the newborn and maternal U-As. These proteins were enriched in functions including immune response and cellular growth/development.

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The newborns differed in the relationship between expression of these proteins and U-As in a way that was consistent with previous studies. We found that exposure to iAs led to changes in expression of several proteins, including those involved in immune response and cellular growth/development. These changes may be indicative of the long-term effects of prenatal arsenic exposure on the developing fetus.

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however, the CDDO pretreatment did not alter the time course of the accumulation of DMAIII in the blood of rats. In conclusion, the contribution of TRR to DMAV reduction appears indirect. The activity of TRR may be indispensable for the reduction of DMAV; however, at the quantity normally present in rat liver TRR does not limit the rate of DMAV conversion to the highly toxic DMAIII. Further research is warranted to identify the enzymes carrying out this important toxification step.

1312 Dietary Methyl Donors Influence Arsenic Metabolism in Residents of Chihuahua, Mexico


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Inorganic arsenic (iAs) is a common drinking water contaminant known to increase risk of cancer and several non-communicable diseases. iAs is metabolized via a pathway involving enzymatic methylation to methylarsonic (MAs) and dimethylarsenic (DMAs) prior to excretion. Higher proportions of urinary arsenic as iAs or MAs are thought to indicate an impairment in the capacity to methylate iAs and to increase health risks. Studies in Bangladesh suggesting dietary methyl donors, including folate and vitamin B12, may reduce iAs toxicity by enhancing DMA production have yet to be confirmed in other populations. We explored associations between folate and vitamin B12 intakes and metabolites of iAs in urine of 771 adults in Chihuahua (Mexico) who were exposed to iAs in drinking water (<0.1-419.8 µg As/L). After adjusting for age and gender, adequate intakes of folate (>320 µg/day, 57% of the sample) were associated with higher %DMAs, a lower %iAs and higher DMAs/MAs ratios in urine, but only among subjects with high intakes of B12 (> the median of 1.85 µg/day). In contrast, among subjects with low B12 intakes, folate intake adequacy was associated with a reduction in %DMA and in the DMAs/MAs ratio, suggesting an impairment of the methylation capacity. Thus, B12 rather than folate intake may be the limiting factor in iAs metabolism in Mexican population.

1313 Reduction of Dimethylarsenate to Dimethylarsenite by Rat Liver Cytosol: Further Characterization


Dimethylarsonic acid (DMAV), the major urinary metabolite of inorganic arsenic, is weakly cytotoxic, however, its reduced form, dimethylarsonous acid (DMAIII), is highly toxic. We have shown that rats and rat liver cytosol (RCL) reduce DMAV in glutathione (GSH) dependent manner. GSH S-transferase omega 1 (GSTO1) activity. After adjusting for age and gender, adequate intakes of folate (>320 µg/day, 57% of the sample) were associated with higher %DMAs, a lower %iAs and higher DMAs/MAs ratios in urine, but only among subjects with high intakes of B12 (> the median of 1.85 µg/day). In contrast, among subjects with low B12 intakes, folate intake adequacy was associated with a reduction in %DMA and in the DMAs/MAs ratio, suggesting an impairment of the methylation capacity. Thus, B12 rather than folate intake may be the limiting factor in iAs metabolism in Mexican population.

1314 Chronic Exposure to Arsenic is Associated with an Elevated Cardiometabolic Risk in Chihuahua, Mexico


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Cardiometabolic (CM) risk is characterized by a group of factors that serve as indicators of an individual’s overall risk for type-2 diabetes mellitus (DM) and cardiovascular disease (CVD). The traditional CM risk factors include dysglycemia, dyslipidemia, high blood pressure, inflammation, endothelial dysfunction, and obesity. Growing epidemiologic evidence suggests that exposure to some environmental chemicals, including inorganic arsenic (iAs), may increase risk of DM and CVD. The present cross-sectional study examined associations between iAs exposure and CM risk factors in a cohort of adult Chihuahua residents (n=933) who drank water containing 0.05 - 419.8 µg As/L. Results show that subjects in the highest exposure tertile (58.3 - 419.8 µg As/L) had higher levels of total cholesterol in plasma and were more likely to have dysglycemia (OR 1.60, 95%CI 1.09-2.32), hyperteglicemia (OR 1.53, 95%CI 1.07 - 2.20), and high blood pressure (OR 1.57, 95%CI 1.05 - 2.35) than subjects in the lowest exposure tertile (<58.5 µg As/L). In addition, all these CM risk factors were positively associated with the ratio of dimethylarsenic (DMAs) to methylarsonic (MAs) and with %DMAs in urine. Thus, an increased capacity to convert iAs to DMAs may be a risk factor for CM disease in individuals exposed to iAs. Prospective studies are needed to examine causality of the association of CM risk with iAs exposure and to determine if iAs metabolism can modify this risk.

1315 Inorganic Arsenic Suppresses Mast Cell Degranulation via a Pathway Target Upstream of Calcium Signaling

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Exposure to Arsenic (As) is a global concern and is linked to many diseases. Many millions of people worldwide are exposed to elevated levels of arsenic through drinking water, food, and other sources. Using a well-characterized mast cell model (RBL-2H3 cells), we previously demonstrated that inorganic Arsenic inhibits antigen-mediated mast cell degranulation. Found in the vast majority of human tissues, mast cells are critical players in numerous diseases, including allergy, asthma, infectious disease, cancer, and even many central nervous system disorders such as autism and anxiety. Mast cells secrete myriad effectors from cytoplasmic granules such as histamine and β-hexosaminidase. The present studies have been performed in order to investigate arsenic’s molecular target in the degranulation pathway. We have found that Arsenic does not affect F-actin membrane ruffling of RBL-2H3 cells stimulated with antigen, suggesting that arsenic’s target is not common to both the ruffling and degranulation pathways. We found that Arsenic dampsens calcium influx, as measured by Fura-2 fluorescence. When stimulating degranulation while bypassing early signaling events, via the use of thapsigargin or calcium ionophore, we found no Arsenic effect on the degranulation response. Using an alternative, non-IEG-mediated mast cell stimulant, G-protein activator compound 48/80, we observed no Arsenic effect degranulation. Taken together with our earlier data showing an inhibition of IEG-mediated degranulation, this result suggests that arsenic’s target in the degranulation pathway is upstream of calcium influx, protein kinase C, phospholipase D, and actin ruffling. Recently, we investigated arsenic’s effect on phosphorylation using a sandwich ELISA and the data suggests that arsenic may be interfering with the phosphorylation of the early kinase Syk.

1316 Arsenic-Induced ROS/RNS Generation Causes Zinc Loss and Inhibits the Activity of Poly(ADP-Ribose) Polymerase-1

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Arsenic enhances the genotoxicity of other carcinogenic agents such as ultraviolet radiation and benzo[a]pyrene. Recent reports suggest that inhibition of DNA repair is an important aspect of arsenic cocarcinogenesis, and DNA repair proteins...
such as poly(ADP ribose) polymerase (PARP)-1 are direct molecular targets of arsenic. Although arsenic has been shown to generate reactive oxygen/nitrogen species (ROS/RNS), little is known about the role of arsenic-induced ROS/RNS in the mechanism underlying arsenic inhibition of DNA repair. We report herein that arsenic-generated ROS/RNS inhibits PARP-1 activity in cells. Cellular exposure to arsenic, as well as hydrogen peroxide and NONOate (nitric oxide donor), decreased PARP-1 zinc content, enzymatic activity, and PARP-1 DNA binding. Furthermore, the effects of arsenite on PARP-1 activity, DNA binding, and zinc content were partially reversed by the antioxidant ascorbic acid, catalase, and the NOS inhibitor, aminoguanidine. Most importantly, arsenite incubation with purified PARP-1 protein in vitro did not alter PARP-1 activity or DNA-binding ability, whereas hydrogen peroxide or NONOate retained PARP-1 inhibitory activity. These results strongly suggest that cellular generation of ROS/RNS plays an important role in arsenite inhibition of PARP-1 activity, leading to the loss of PARP-1 DNA-binding ability and enzymatic activity.

1317 Protective Role of Vitamin E Succinate and Selenite in Arsenic-Itoxicated Hamster: Arsenic-Induced Oxidative Stress and Its Reversible Damage

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We studied the effects of combined exposure to arsenic, selenite and vitamin E succinate. (i) oxidative stress and its correlation with glutathione and linked enzymes; (ii) alterations in the structural integrity of DNA with assay comet; and (iii) biochemical parameters. Efficacy of selenium and alpha-tocoopherol succinate in reducing these changes was also determined. Hamsters were exposed to sodium arsenite, 100 ppm of arsenite via drinking water. Co-treatment with 8.5 mg/Kg/day of selenite or 6 mg/Kg/day of vit E was also carried out to assess whether those compounds exert any protective role, individually and in combination for twenty weeks. The samples of whole blood were collected at 24hrs and 20 week post-treatment and the assay was carried out to determine DNA damage as represented by comet tail-length and biochemical parameters assay. Arsenic species were measured in the urine by HPLC-ICP/MS. Exposure to arsenic altered the levels of oxidative stress enzymes. These changes were accompanied by increased reactive oxygen species (ROS) levels. Arsenic exposure led to a significant depletion of super oxide dismutate (SOD) activity with no effect on catalase and glutathione peroxidase (GPs) activities. The distribution of DNA migration revealed that the hamster exposed to arsenic showed more DNA damage than samples obtained from control. The results demonstrate a As toxic effect, and a noticeable preventive effect of both vit E and selenite, coexposure had additional beneficial effects in restoring altered bio-chemical variables, maintaining pro-oxidant/antioxidant balance. Our experiments indicate the significant protective action of vitamin e succinate and sodium selenite on arsenic-induced toxicity in the hamster.

1318 Prenatal Arsenic Exposure and the Epigencode: Altered microRNAs Associated with Innate and Adaptive Immune Signaling in Newborn Cord Blood

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The Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Gómez Palacio, Nuevo León, Mexico and 2Genetics, Universitat Autonomas de Barcelona, Barcelona, Bellatera, Spain.

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Arsenic is a toxic metal that is widely used in the semiconductor industry, and is naturally present in the environment, including water, soil, and food. Prenatal exposure to arsenic has been shown to be associated with increased risk of cancer and other health outcomes in children. The epigenome, which includes DNA methylation and non-coding RNA molecules, is thought to play a role in the health effects of arsenic exposure. In this study, we focused on microRNAs (miRNAs), a class of non-coding RNA molecules that regulate gene expression. We measured miRNA expression in newborn cord blood samples from the BEAR cohort, to identify miRNAs that are altered by prenatal arsenic exposure.

We found that prenatal arsenic exposure was associated with changes in the expression of several miRNAs. These miRNAs are involved in various biological processes, including immune response, cell cycle regulation, and DNA damage repair. For example, miR-15a and miR-16, which are involved in DNA repair, were upregulated in newborns of arsenic-exposed mothers. This suggests that these miRNAs may play a role in increasing the risk of cancer and other health outcomes associated with prenatal arsenic exposure.

1319 Arsenic Exposure Increases Monocytes Adhesion to the Vascular Endothelium, a Pro-Atherosclerotic Mechanism

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Arsenic exposure is linked epidemiologically to increased atherosclerosis, but the mechanisms by which arsenic enhances atherosclerosis are unknown. Monocytes, macrophages and platelets are key players in the early stage of an atherosclerotic lesion. Circulating monocytes and macrophages bind to the activated endothelium of vessels and migrate into the sub-endothelium where they become lipid-laden foam cells. This process can be facilitated by platelets, which favour monocyte recruitment to the lesion. Thus, we assessed the effects of arsenic exposure on platelet activation, platelet-monocyte aggregation and monocyte adhesion to endothelial cells. We observed no effect of arsenic on platelet activation or platelet-monocyte aggregation. Then, we assessed the monocyte adhesion to VCAM-1, an adhesion molecule normally found on activated endothelial cells. We found that 1) monocyte and macrophage adhesion to VCAM-1 is increased by arsenic; 2) the surface expression of the β1 integrin (CD29) that binds to VCAM-1 is increased on macrophages by arsenic; and 3) these effects can be inhibited by antioxidants. We extended these results using a co-cultured model of human endothelial and mononuclear cells and an ex vivo organ culture system. We found that both cell types need to be exposed to arsenic to maximize monocyte adhesion in a dose-dependent manner, at concentrations as low as 10 ppb. Finally, we assessed the CD29 level on circulating monocytes in mice exposed to arsenic. Arsenic increased mononuclear CD29 levels as compared to control animals, and addition of high selenium diet decreased CD29. Together, these data suggest that arsenic enhances atherosclerosis by increasing monocyte adhesion to endothelial cells, a process that is inhibited by antioxidants.

1320 Synergistic Effect of Arsenic on Hexavalent Chromium Cytotoxicity and Genotoxicity in Human Lung Cells


Hexavalent chromium (Cr(VI)) and inorganic arsenic are well known environmental and occupational hazards and are often found as mixed contaminants and wastes. Both of them are known human lung carcinogens. Despite that people are exposed to these metals simultaneously under most conditions, little is known about the potential co-exposure impact. Arsenic is known to inhibit DNA damage repair and our previous studies show that Cr(VI) induces DNA double strand breaks and neoplastic transformation in human lung cells. Thus, it is likely that co-exposure to Cr(VI) and arsenic could cause greater toxic effect. The objective of this study is to determine the ability of arsenic to increase Cr(VI) genotoxicity and carcinogenicity in human lung cells. We found that arsenic has a synergistic effect on Cr(VI) cytotoxicity. 0.5 μM arsenic alone induces 14 percent of relative cell death. Cr(VI)-induced cell death is increased from 32. 50 and 62 to 47, 65 and 80 percent of relative cell death, respectively after co-exposure to 0.5 μM arsenic and 0.1, 0.5 and 1 μg/cm² lead chromate. Arsenic has a similar effect on soluble Cr(VI) compound, sodium chromate. Arsenic also causes an increase in Cr(VI)-induced chromosome aberration. 0.5 μM arsenic alone induces 9 metaphases with chromosome damage. When co-treated with 0.01, 0.05 and 0.1 μg/cm² lead chromate, the number of metaphase with damage increased from 5, 6 and 19 to 16, 16, and 31, respectively. These data suggest that co-exposure to Cr(VI) and arsenic induces a synergistic toxic effect in human lung cells. Further work will include assessing the effect of arsenic on Cr(VI)-induced DNA double strand break and repair. This work is supported by NIEHS grants R15ES021587 (H.X.) and ES016893 (J.F.W.).
1321 Tissue Distribution and Excretion of Arsenic and Selenium following Co-Administration of Diphenylarsinic Acid and Selenite in Rats


Diphenylarsinic acid (DPAA) is a chemical precursor as well as a degradation product of arsenic-containing chemical weapons such as diphenylarsine chloride. Toxicological findings on DPAA are limited as compared to inorganic arsenicals (iAs). Selenium (Se) is an essential element, and is known to interact with arsenic. We investigated metabolic interactions between Se and As in rats co-administered with DPAA and selenite in this study. Male Sprague-Dawley (SD) rats were injected intravenously with DPAA, selenite or co-administered with As and Se, at a dose of 1.0 mg As or 1.0 mg Se/kg body weight, and urine and feces were collected separately for 24 h using metabolic cages. In a separate experiment SD rats were anesthetized with sodium pentobarbital, the bile duct was cannulated, and DPAA or/and selenite were administered intravenously at a dose of 1.0 mg As or 1.0 mg Se/kg body weight to study biliary excretion of Se and As. The bile fluid was collected on ice for 30 min up to 3 h after the injection. The animals were sacrificed by withdrawing blood from the abdominal aorta under anesthesia at 24 or 3 h after the injection, and heparinized blood and tissue samples were collected. Selenium significantly reduced concentration of As in plasma 24 h after the administration. DPAA significantly reduced distribution of Se in kidney, and yet significantly increased excretion of Se into urine 24 h after the administration. On the other hands, selenium significantly reduced the biliary excretion of DPAA, and DPAA significantly increased the biliary excretion of selenium in 30 min after the administration and then significantly reduced the biliary excretion when DPAA and selenite were co-administered. DPAA significantly increased distribution of Se in whole blood, and significantly reduced distribution of Se in liver; however, distribution of As to blood and liver was not influenced by selenite 3 h after the administration. These results suggest that DPAA interacted with Se, and changed distribution and excretion of these metalloids each other in rats.

1322 Increased Expression of the Proto-Oncogene Anterior Gradient 2 in the MCF-10A Cell Line by Arsenite Exposure

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Arsenite and cadmium are environmental carcinogens that have been implicated in various cancers. Previously, our laboratory has shown that arsenite and cadmium can cause malignant transformation of the immortal human epithelial cell line MCF-10A. Microarray analysis of the transformed cells revealed an over-expression of the gene Anterior Gradient 2 (AGR2). These findings are significant since AGR2 is known to be overexpressed in estrogen receptor positive breast cancers. AGR2 is a proto-oncogene protein that is highly expressed in a variety of human cancers. It is known to play a role in promoting cellular transformation, tumor growth as well as metastasis. In this study, we validated the expression level of AGR2 in the parent MCF-10A cell line and in the arsenite and cadmium transformed MCF-10A cells using PCR and Western analysis. The data obtained indicated that the expression of AGR2 was significantly increased in the arsenite transformed cells compared to the cadmium transformed cells. We were also interested in determining if exposure to arsenite or cadmium could increase the expression of AGR2 in the parent MCF-10A cells. For this purpose MCF-10A cells were exposed to 4, 8 or 16 μM arsenite or 2, 4, or 6 μM cadmium for 48 hrs and the expression levels of AGR2 were determined. The data obtained showed that exposure to arsenite resulted in a significant increase in the expression of AGR2, whereas exposure to cadmium had no effect on the expression level of AGR2. These results suggest that arsenite has the potential to induce the proto-oncogenic protein AGR2, the expression of which may increase the metastatic potential of the cancer cells.

1323 The Prebiotic Oligofructose Protects against Enhanced Liver Injury Caused by Arsenic in a Model of NASH


Arsenic, a ubiquitous drinking water contaminant, tops the ATSDR list of hazardous environmental chemicals and is a known hepatotoxin. This group showed that subhepatotoxic doses of arsenic sensitizes the liver to experimental non-alcoholic fatty liver disease (NAFLD) caused by a high fat diet (HFD). It is now strongly suspected that an altered gut flora plays an important role in NAFLD. Indeed, prebiotics (oligofructose; OFC), protect against experimental NAFLD, and correlate with repletion of commensal bacteria (e.g., Bifidobacteria spp.) in the GI tract. The purpose of the current study was to test the hypothesis that OFC protects against enhanced liver injury caused by arsenic in experimental NAFLD. Accordingly, male C57Bl/6j mice were fed low fat diet (LFD), HFD or HFD containing OFC during concomitant exposure to either tap or arsenic-containing water for 10 wks. Abundance of bacteria in cecal contents was determined by qPCR. Histological changes were determined by hematoxylin and eosin staining. Macrophage activation and fibrin deposition were determined by immunostaining. HFD caused fatty liver injury, as characterized by an increase in liver/body weight ratio, steatosis and transaminases. Arsenic synergistically enhanced HFD-induced liver damage, inflammation, and fibrin deposition. HFD and arsenic alone both altered content of cecal bacteria, and both decreased the abundance of Bifidobacterium spp; OFC supplementation protected against this effect. OFC supplementation also protected against the enhanced liver damage, inflammation, and fibrin accumulation caused by the combination of HFD and arsenic. These results indicate that enhanced HFD-induced liver damage caused by arsenic may be mediated, at least in part, by GI tract dysbiosis and that prebiotic supplementation may confer significant protective effects. Together, these data support our hypothesis that arsenic-mediated changes in the GI tract flora play an important factor in sensitizing the liver to injury.

1324 Metabolomics Signature of Inorganic Arsenic-Associated Diabetes: Links to Amino Acid Metabolism

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The Chihuahua Cohort was established to study chronic disease associated with exposure to inorganic Arsenic (iAs) in drinking water, specifically diabetes mellitus (DM). The present study profiled the metabolic shifts in a group of individuals selected from this cohort (91 diabetics and 86 non-diabetics) using both LC-MS and GC-MS platforms. Exposure to iAs was characterized by iAs levels in drinking water (DW), and by the sum of iAs metabolites in urine (U-iAs). Fasting plasma glucose (FPG) and 2-hour plasma glucose (2HBG) were recorded by a glucose tolerance test and were used to determine an individual’s DM status. Levels of DW-iAs ranged from 0 to 419.7 μg/L, and U-iAs level ranged from 4.69 to 445.2 μg/L. Both urine and plasma metabolite levels were analyzed as they related to DM, iAs exposure or iAs-associated DM. Metabolites that were identified that specific to iAs exposure, as well as to DM. Notably, a metabolomic signature of 23 metabolites was identified that distinguish iAs-associated DM. These metabolites reflect changes not only in glucose, but also in amino acid metabolism, providing additional evidence that iAs-associated DM may differ from the common type-2 DM.

1325 Inorganic Arsenic Exposure and Bone Loss in Normal and Hypertensive Rats

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Exposure to inorganic arsenic through drinking water is a major international public health issue. It can be absorbed via digestive tract or skin and then accumulated in the variant target organs to disturb tissues functions. Previously epidemiological studies showed that arsenic exposure from drinking water could increase vascular disorders to elevate blood pressure or decrease bone mineralization. However, whether arsenic exposure from drinking water enhances the loss of bone mineralization during hypertension disease remains unclear. Here, we examined the changes of bone morphology and bone mineral density in normal (WKY) and spontaneously hypertensive rats (SHR) after exposure to inorganic arsenic from drinking water using micro-computed tomography (micro-CT) analysis. Rats were exposed with arsenic trioxide (As2O3) from drinking water for three months. The results showed that arsenic exposure obviously increased blood pressure in WKY.
rats and significantly enhanced blood pressure in SHR rats in a dose dependent manner. The results of micro-CT-derived bone morphology and density measurements showed that connectivity density, trabecular thickness, bone volume, trabecular bone number, trabecular separation, and bone mineral density were significantly changed in both WKY and SHR rats. However, no significant differences were observed in these bone parameters between SHR and WKY rats. Taken together, these results suggest that inorganic arsenic is capable of inducing bone loss in normal and hypertensive rats, but hypertension condition seems not to enhance the arsenic-induced bone loss.

1326 In Vivo Mutagenicity Assay of Arsenite Using Gpt Delta Transgenic Mice

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While arsenic has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC), the mutagenicity in animal model has not been fully characterized. The aim of this study was to assess the in vivo mutagenicity of arsenite in C57BL/6J gpt delta mice. Male gpt delta mice were given drinking water containing 85 ppm sodium arsenite for 3 weeks, and the hepatic genome was assayed for mutations 2 weeks later. The result of gpt mutation assays showed a significant increase in mutation frequency in the liver following arsenite exposure. Analysis of the mutation spectra revealed that 67% of mutations detected were G:C to A:T transitions and 6% were G:C to T:A transversions in both control and treated groups. In contrast, arsenite exposure resulted in a markedly higher rate of G:C to T:A transversions (46% of mutations detected). G:C to T:A transversions have been reported to be induced following formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), one of the major forms of oxidative DNA damage. We also detected a significant increase in 8-OHdG in the livers of the mice exposed to arsenite. These results demonstrate that arsenite induces G:C to T:A transversions through oxidative-stress-induced 8-OHdG formation in vivo. We also discuss the results of the DNA methylation analysis of gpt gene and the expression analysis of DNA repair genes.

1327 N-Cadherin Up-Regulation in Arsenite and Cadmium-Transformed Urothelial Cells

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The switch from E- to N-cadherin is a well-known indicator of the epithelial-to-mesenchymal transition occurring in bladder cancer. N-cadherin upregulation is correlated with tumor stage, increased recurrence, and decreased survival in patients. While the factors mediating the decrease in E-cadherin expression are well-established, little is known of the factors regulating the increase in N-cadherin expression. Environmental agents are the common cause of bladder cancer. Specifically, arsenic and cadmium are known carcinogens and implicated in the development of bladder cancer. Previous studies from our laboratory have shown that arsenic and cadmium can cause malignant transformation of normal immortalized bladder urothelial cells. These transformed cells can form tumors when injected subcutaneously or intraperitoneally into nude mice. The goal of the present study was to determine if there was a difference in expression levels of E- and N-cadherin in the transformed cell lines and the tumor heterotransplants. For this purpose real-time PCR was performed on RNA samples isolated from the transformed cell lines and the tumor heterotransplants. Western analysis was performed to determine the protein levels whereas immunohistochemical analysis was performed to determine the localization of the proteins within the tumor heterotransplants. The results obtained show that E-cadherin is expressed in the transformed cell lines as well the tumor heterotransplants. The expression of N-cadherin was very low in the parent UROtsa cells whereas the levels increased in the transformed cell lines. The expression level of N-cadherin decreased in the tumor heterotransplants compared to the cell lines. An interesting finding was the focal expression of N-cadherin in the intraperitoneal tumors suggesting that heavy metal exposure promotes the epithelial-to-mesenchymal phenotype and bladder cancer progression.
6 until birth. Mice were maintained on normal chow and water for 36 weeks. Weight and metabolic end-points were evaluated throughout the study. Blood plasma analysis demonstrated that in-utero (IU) arsenic-exposed mice exhibited a significant increase in blood glucose levels between weaning age and 4 months of age, relative to those elevated after 8 months. On the other hand, glucose levels in control mice did not change over time. Similarly, IU arsenic-exposed mice showed a consistent elevation in total cholesterol, as well as LDL cholesterol at weaning age, 4 months and 8 months of age, when compared to control mice. Further analysis demonstrated the development of nonalcoholic fatty liver disease in IU arsenic-exposed mice, as evidenced by major morphological changes and an increase in steatosis concomitant with hepatocellular ballooning. Growth rates were not statistically different; however, IU arsenic-exposed mice showed a trend of heavier weights when compared to control mice. Taken together, these results suggest that IU arsenic exposure is a possible contributor to metabolic syndrome onset in mice.

**354 SOT 2014 Annual Meeting**

**1327d Biological and Behavioral Modifiers of Urinary Arsenic Metabolic Profiles in a US Population**


Relations between intensity of arsenic exposure from home tap water and levels of inorganic As (iAs) and its mono- (MAs) and di-methylated (DMAs) metabolites were examined in urine from 903 individuals ≥ 45 years of age who resided in Churchill County, Nevada, continuously for 5 or more years. Over a wide range of exposures (home tap water supplies contained <3 to 1200 μg of As per liter), concentrations of iAs, MAs, and DMAs strongly correlated with concentrations of As in home tap water. However, percentages of the summed iAs, MAs, and DMAs concentrations in urine accounted for by each species were unaffected by home tap water As concentration, suggesting that capacity for formation and excretion of methylated metabolites was not exceeded. Mean urinary iAs, MAs, and DMAs concentrations were significantly lower (P<0.001) in urine of women than in men; women had significantly lower iAs and MAs (P<0.005) and significantly higher DMAs percentages (P<0.0001) in urine than men. Age did not affect concentration of any arsenic in urine, although there were significant age-related trends for decreased iAs percentage (P<0.0002) and increased DMAs percentage (P<0.01). Body mass index (BMI) did not affect iAs, MAs, or DMAs levels in urine but BMI was significantly associated (P<0.01) with lower MAs percentage and increased DMAs percentage. Smoking status (assessed by urinary cotinine levels) was associated with significant trends for urinary iAs, MAs, or DMAs concentrations and for iAs, MAs, or DMAs percentages (P<0.05). These results indicate that among older Americans with a wide range of exposure to iAs from home tap water, both biological factors (gender, age, BMI) and behavioral factors (smoking) can affect profiles of arsenicals found in urine. These modifiers may contribute to interindividual and interpopulation variation in response to chronic exposure to As from water and other media. (This abstract does not reflect U.S. Environmental Protection Agency policy.)
Manganese (Mn) is an essential element, however exposure to high Mn levels may affect structures of the basal ganglia, leading to motor impairment similar to Parkinson’s disease. The molecular mechanisms underlying Mn neurotoxicity, particularly in the developing central nervous system (CNS), need further investigation. Our goal was to investigate whether early-life exposure to Mn affects motor coordination and cognitive function in adulthood. Male Wistar rats were exposed to saline (control) or intraperitoneal MnCl₂ (5, 10 or 20 mg/kg) injections from post-natal day (PND) 8 up to 12. Behavioral tests were performed on PND 60-63. Motor coordination was assessed in the rotarod. Object recognition task was used to evaluate short-term memory. Biochemical analysis of the striatum was performed on PND 70. Rats exposed to the highest Mn dose failed to recognize a familiar object when replaced by a novel object (p<0.05), attesting memory impairment. These effects were accompanied by decreased levels of non-protein thiols, e.g. glutathione (p<0.05), and increased levels of glial fibrillary acidic protein (GFAP) (p<0.05) in the striatum. This study documents that acute low-level exposure to Mn during critical neurodevelopmental period induces cognitive and motor dysfunctions that last into adulthood.

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nucleus accumbens, striatum, hypothalamus, olfactory bulb) were evaluated in one pup/ gender/litter to preclude litter effects. Blood and brain Hg increased with increasing MeHg exposure; corticosterone levels were not influenced by MeHg or PS. Instances of non-linear monamine concentration-effect curves with MeHg occurred in virtually every brain region, particularly in females. Hg altered mEPSCs at 0.5 ppm, but not at 2.5 ppm. Further, multiple monamine changes were seen only in the presence of MeHg + PS, particularly in striatum and frontal cortex in males, and hippocampus and nucleus accumbens in females. In females, MeHg+PS reduced hippocampal monoamines, and increased nucleus accumbens and olfactory bulb monoamines, whereas MeHg-PS decreased frontal cortex and striatum monoamines males. These results demonstrate that PS can enhance the neurotoxicity of MeHg. Consequently assessment of neurotoxic risk in the absence of PS may underestimate its hazardousness. These findings underscore the need to develop animal models more reflective of the multiplicity of risk factors in the human environment. Further, the non-linearity of MeHg effects raises concerns in relation to human health risk assessment. EPA RD 83457001

1334 Preliminary Characterization of Neuroantibody (NAb) Associations with Hg and PCB in 7-Year-Old Faroese Children with Prenatal Exposure to Seafood Neurotoxins C. Osuna1, P. Weihe2, P. Grandjean1,3 and H. A. El-Fawal4.

Preclinical studies of neurotoxicity and human studies of neurodegeneration suggest that NAb may be useful biomarkers of insult. In a proof-of-concept study, archived sera from 38 children at the age of 7 (16 males; 22 female) were randomly selected from a birth cohort established in 1986-1987 as part of the Children’s Health and the Environment in the Faroes. Maternal hair-Hg [Mg7: geometric mean (IQR)]; 5.8 (2.6-10.9) μg/g, cord blood-Hg [Cord-Hg7; IQR: 4 (1.1-6.4) μg/L] and cord blood-PCB [Cord-PCB7; IQR: 0.3-3.2 (9) μg/L] indicated no prenatal exposure. Blood-Hg [Hg7; IQR: 3.5-5.2 (2.8) μg/L] and serum-PCB [PCB7; IQR: 6 (0.97-2.9) μg/L] at the age of 7, current exposure indicators, were used. Titters of IgM and IgG against neurolamine (NF-L), NF-M, NF-H, cholineacetyltransferase (ChAT), GFAP, MBP, desmin (D) and actin (A) were measured. Positive associations (r=0.3-0.5; p<0.01) were observed for IgM titters against NF-L, NF-H, GFAP, MBP (and IgG), ChAT and A with Hg7. Higher titters of anti-NF-H and MBP, IgG, and anti-A and D, IgM, in girls were not significant (p=0.06-0.9). Stratification by Hg7 (or >10 μg/g) or Cord-Hg7 (or >60 μg/g) indicated higher anti-NF-M and GFAP, IgM, while stratification by Hg7 (or >10 μg/g) indicated higher anti-NF-L, NF-M, GFAP, MBP, ChAT and D, IgM, and anti-NF-M and GFAP, IgG (p<0.05) at higher exposure. Differences in anti-NF-M, GFAP, ChAT, D and IgM, and GFAP, IgG, occurred at higher levels of PCB7 (or >10 μg/L). Multivariate regression indicated that Hg7 is the major determinant of NAb. After controlling for Cord-Hg, Cord-PCB, and PCB7, 8 of 16 NAb were significantly positively associated with Hg7. Taken together, this study suggests that NAb may be useful biomarkers of NS insult resulting from Hg exposure. This warrants further testing in a larger cohort with the incorporation of neurobehavioral assessment.

1335 Ontogeny of Fc-Gamma Receptors in the Developing Rat Brain P. Lein and M. Stamos. Molecular Biosciences, School of Veterinary Medicine, UC Davis, Davis, CA.

Immunoglobulin G (IgG) antibodies that cross-react with intracellular antigens in the developing brain have recently been identified in an experimental animal model of developmental mercury toxicity and in mothers at risk for having a child with autism. But whether these autoantibodies interfere with neuronal development remains controversial, in part because it is not clear how IgG would access an intracellular neuronal target. Fc receptors (FcR) bind the constant (Fc) region of immunoglobulin G (IgG), and mediate uptake of IgG and cellular responses triggered by IgG binding in immune cells. This raises the question of whether neurons in different brain regions also express functional FcR. Investigation of FcR expression by RT-PCR indicated that transcripts of FcRI, FcRIIa, FcRIIb, FcRIII and the neonatal FcRn are expressed in the cortex, hippocampus and cerebellum of naïve male and female rat pups on postnatal days (PND) 2 and 7, but at lower levels than in the liver and spleen. Transcript levels did not vary significantly as a function of sex or age, with the following exceptions: (i) FcRIIa and FcRIII were downregulated by PND7 in the spleen but not in brain or liver; (ii) FcRn was upregulated in the cerebellum but downregulated in the spleen at PND7 compared to PND2. To determine cell-specific expression, we also performed RT-PCR of these five FcR isoforms in primary cultured cortical and hippocampal cells. All five transcripts were detected in both cell culture models at days in vitro ranging from 2 to 21. Immunocytochemical analysis indicates that FcRI and FcRII are predominately expressed in neurons; whereas FcRII FcRIII and FcRn are predominantly expressed in astrocytes. We are currently designing assays to assess whether these receptors are functional in neurons. In summary, our data confirm that FcR are expressed at the transcript and protein level in neurons and glial cells in different regions of the rat brain during neurodevelopment. This work supported by NIH grants R01 ES014901 and P4Z ES04699.

1336 Developmental Lead Exposure and the Exacerbation of Alzheimer’s Pathology: An Immunological Analysis A. vanderEmde, D. Johnson, J. N. Franklin, Q. Hu and J. DeWitt. Physiology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC.

Although Alzheimer’s disease (AD) is typically a late-onset neurodegenerative disorder, there may be a period of susceptibility early in brain development which, if disrupted, may exacerbate the disease pathology. We hypothesize that early exposure to a known neurotoxic and immunotoxic chemical, such as lead acetate, may alter microglia function and/or phenotype during this critical window of development, which then promotes amyloidosis or decreases microglia phagocytosis of amyloid-β, the protein associated with AD neuropathology. The exact mechanism by which microglia are involved with amyloid-β deposition or removal is currently contested in the scientific literature, although there is significant evidence for the colonization of microglia and amyloid-β plaques. We investigated this “double-hit” model of the concurrent effects of environmental toxicant exposure and critical windows of development susceptibility through the use of a genetically predisposed triple transgenic mouse model (3XTgAD). Pups were orally gavaged with 100 parts per million of lead acetate and vehicle from postnatal day (PND) 5-15, a known postnatal period of vulnerability for microglia. At PND50, 90, and 180, hippocampus was isolated from brains of treated and control animals and stored for further analysis of microglia colonization with amyloid-β. The remaining brain tissue was processed to measure microglial activity by flow cytometry. Activity of microglia from treated animals was increased relative to control animals. These initial data suggest that early-life exposure to an environmental agent, when given during a critical window of microglia maturation, changes microglial activity. We believe that these changes in activity alter the course of AD neuropathologies.


Lead exposure has been implicated in the impairment of synaptic transmission and plasticity in the developing hippocampus. However, the mechanism remains unclear. Here we investigated whether chronic lead exposure during development affected hippocampal dendritic spine formation and synapse strength and if the Wnt signaling pathway was involved. Sprague–Dawley rats were exposed to lead only during lactation. The lead-exposed pups acquired lead via milk of dams whose drinking water contained 0.2% (1090 ppm) lead acetate from parturition to weaning, while the control dams remained on distilled water throughout the lactation period. Hippocampal lead accumulated significantly in lead-exposed rats (0.471 ± 0.11 μg/g) compared with the controls (0.021 ± 0.002 μg/g). Golgi-Cox staining method was used to examine the spine density of hippocampal CA1 pyramidal neurons in postnatal (PND) 14 and PND21 rats. Lead significantly down-regulated the spine density in both age groups. This was accompanied by a significant age-dependent decline of Wnt7a expression and stability of its downstream protein following chronic lead exposure. Furthermore, in cultured hippocampal neurons lead (0.1 and 1 μM lead acetate) significantly decreased the spine density in a dose-dependent manner. Exogenous Wnt7a application restored the frequency of miniature excitatory postsynaptic currents (mEPSCs) in the Pb2+-treated cultures back to control levels. Additionally, the lead-induced decrease in the spine density was attenuated by Wnt7a, which also increased the stability of the downstream molecules in the Wnt signaling pathway. Our results suggest that lead negatively impacted spine growth and synaptic transmission of the developing hippocampus through alteration of the presynaptic Wnt7a signaling. [Supported by the National Key Basic Research Program of China (973 Program, No. 2012CB525003).]
Lead (Pb) is a non-essential toxic heavy metal; its use in industrial applications is widespread and has resulted in an increased risk of human environmental exposure. Multiple organs are targets of Pb toxicity; however, the central nervous system (CNS) is most sensitive during early development due to rapid cell proliferation and migration, axonal growth, and synaptogenesis. One of the primary components of CNS development is the GABAergic system. Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the adult brain. However, during early development GABA functions as an excitatory neurotransmitter which contributes to cell proliferation, neuronal differentiation and migration, and synaptogenesis. Studies report the effects of Pb on GABA in the mature brain, but little is known regarding the adverse effects of Pb exposure on the GABAergic system during embryonic development. To characterize the effects of developmental Pb exposure on the GABAergic system, zebrafish embryos were exposed to 10, 50, or 100 ppb (ug/l) Pb or a control treatment shortly after fertilization through 24, 48, 60, or 72 hours post fertilization (hpf). A dose-response was observed between Pb exposure and tissue uptake in zebrafish larvae. Genetic analysis showed that developmental Pb exposure caused either an increase or a decrease in mRNA expression of seven genes (gad2, gad1b, gaba2a, gaba2b, gat-1, gat-3, vgat) throughout the GABAergic pathway that was developmental time point and dose specific. GABA levels were analyzed and also revealed dose and time point specific alterations. These data provide evidence indicating that changes in gene expression throughout the GABAergic pathway do not cause similar immediate alterations in GABA levels and that the compensatory response of the GABAergic system is highly dependent upon dose and developmental time point. These data provide a framework for further analysis of the effects of Pb on the GABAergic system during the excitatory phase and transition to an inhibitory neurotransmitter during development.
Links between autism, schizophrenia, and cognitive decline and exposure to ambient air pollution have been reported. Ultraviolet (<100nm) particles are considered the most toxic of ambient particulates. To determine potential neurotoxicity of concentrated ambient ultraviolet particles (CAPS) to developing brain, mice were exposed to CAPS during the first two weeks of life and/or in adulthood (PND60). Notably, CAPS produced lateral ventricle dilation only in mice with either postnatal or adult exposure; ventriculomegaly has been shown to be characteristic of autism and schizophrenia. A lack of changes to the Aqueduct of Sylvius suggests a non-obstructive mechanism such as neuroinflammation. Additionally, CAPS caused glial disruption and an inflammatory response: microglial activation was seen in males, while both sexes showed a time-dependent astrocytic response. CAPS altered brain cytokines in a sex- and time-point-dependent manner. Consistent with a neuroinflammatory response, females showed a transient increase in striatal IL-6 24 hours after postnatal exposure, but this had resolved by adulthood. Both sexes exhibited a protracted neuroinflammatory response to CAPS, with midbrain IL-1β in males and TNFα in females increased following postnatal only CAPS when brains were examined in young adulthood. CAPS also produced multiple changes in brain monoamine and amino acid neurotransmitters that were dependent on sex, timing of exposure, latency between exposure and sacrifice, and brain region. These included disruption of mesocorticolimbic monoamines and, in males, increased hippocampal glutamate, consistent with potential excitotoxicity. Thus, low level CAPS can produce neuropathology preferentially in males, with greater vulnerability during the postnatal period, that is consistent with features of profound neurodevelopmental disorders. ES019105

Mice exposed to trichloroethylene (TCE) postnatally from birth until 6 weeks of age demonstrated impaired glutathione reductase homeostasis and increased biomarkers of oxidative stress in the brain and plasma. Here we confirm and extend these findings in mice exposed to TCE during gestation only. Similar to postnatal-only exposure, a targeted metabolomics analysis in gestation-only exposed mice revealed compromised glutathione reductase disequilibrium and behavioral alterations characterized by increased locomotor and exploratory activity in male offspring at postnatal day (PND) 42. Accordingly, this TCE exposure induced a significant and dose-dependent increase in tyrosine oxidative end products including 3-nitrotyrosine in PND42 male offspring. 3-Chloro-Tyr, an indicator of oxidative stress generated by immune activation, was also significantly elevated in the plasma of TCE exposed offspring. Because this result suggested that an immune component may be an important contributor to the neurotoxic effects observed in our model, peripheral CD4+ T cells, well-characterized targets of TCE immunotoxicity, were evaluated for a panel of inflammatory mediators. Gestational TCE exposure induced a dose-dependent increase in IFN-γ and IL-17 commensurate with a decrease in anti-inflammatory IL-10. These results point to the gestational phase as a critical window of TCE toxicity. Further study is needed to understand the relationship between peripheral immune dysregulation and neurotoxicity with developmental TCE exposure and whether or not epigenetic processes play a role.

1342 Exposure to Concentrated Ambient Ultraviolet Particulate Matter during Postnatal Brain Development and/or Adulthood Elicits Lateral Ventricle Dilation, Sustained Neuroinflammation, and Neurochemical Disruption Preferentially in Male Mice


Bisphenol-A (BPA) is an organic compound that has been increasingly used in the manufacturing of polycarbonate plastics and epoxy resins. BPA is toxic to multiple organ systems including the brain. Exposure to BPA during development has been shown to induce neuronal differentiation, decreased neuronal survival rate, and apoptotic cell death. The goal of this study was twofold; first, we examined the toxic effects of BPA on the developing rat brain and second, detailed mechanisms involved in toxicity and apoptotic cell death were examined in a rat pituitary cell line (GH3). A variety of markers for apoptosis in the striatum and pituitary of rat pups developmentally exposed to BPA through maternal transfer were examined. Results indicated signs of apoptotic cell death in tissue from 24 and 60 day old female rat pups that were exposed to BPA during development. BPA caused a significant decrease in the intracellular pool of glutathione suggesting that exposure increased the levels of reactive oxygen species (ROS). BPA increased the release of cytokines from the microglia. Caspase 3 activation increased following BPA exposure as indicated by increased levels of cleaved caspase 3, TUNEL staining showed evidence of apoptosis. GH3 cells were used to examine detailed mechanisms involved in BPA-induced apoptotic cascade including altered Ca2+ levels and mitochondrial permeability. Using a Ca2+ ionophore (Fura-2AM), it was demonstrated that BPA-treated cells had increased levels of intracellular Ca2+. Concentration-dependent disruptions in mitochondrial membrane permeability were detected following treatment with BPA by staining the cells with rhodamine 123. These results indicate that development of exposure to BPA appears to be neurotoxic in female rat pups by increasing cellular oxidative stress and triggering neuronal cell death. Interestingly, findings obtained from this study in female pups appear to contradict pervious findings in male pups, therefore suggesting that BPA-induced neurotoxicity may be different in females compared to males.

1345 Bisphenol A-Induced Neurotoxicity in the Developing Rat Pup and a Putative Cell Model

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Bisphenol A (BPA), is an estrogenic compound used in the manufacturing of polycarbonate plastics and epoxy resins. BPA is toxic to multiple organs including the brain. Exposure to BPA during development has been shown to induce neuronal differentiation, decreased neuronal survival rate, and apoptotic cell death. The goal of this study was twofold; first, we examined the toxic effects of BPA on the developing rat brain and second, detailed mechanisms involved in toxicity and apoptotic cell death were examined in a rat pituitary cell line (GH3). A variety of markers for apoptosis in the striatum and pituitary of rat pups developmentally exposed to BPA through maternal transfer were examined. Results indicated signs of apoptotic cell death in tissue from 24 and 60 day old female rat pups that were exposed to BPA during development. BPA caused a significant decrease in the intracellular pool of glutathione suggesting that exposure increased the levels of reactive oxygen species (ROS). BPA increased the release of cytokines from the microglia. Caspase 3 activation increased following BPA exposure as indicated by increased levels of cleaved caspase 3, TUNEL staining showed evidence of apoptosis. GH3 cells were used to examine detailed mechanisms involved in BPA-induced apoptotic cascade including altered Ca2+ levels and mitochondrial permeability. Using a Ca2+ ionophore (Fura-2AM), it was demonstrated that BPA-treated cells had increased levels of intracellular Ca2+. Concentration-dependent disruptions in mitochondrial membrane permeability were detected following treatment with BPA by staining the cells with rhodamine 123. These results indicate that development of exposure to BPA appears to be neurotoxic in female rat pups by increasing cellular oxidative stress and triggering neuronal cell death. Interestingly, findings obtained from this study in female pups appear to contradict pervious findings in male pups, therefore suggesting that BPA-induced neurotoxicity may be different in females compared to males.

1344 The Effects of Bisphenol A on the Dopamine System in a Developing Rat Brain and a Rat Pituitary Cell Line

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Bisphenol-A (BPA) is an organic compound that has been increasingly used in the manufacturing of various consumer products. BPA is considered an environmental estrogen, which appears to disrupt the brain’s dopaminergic system. In this study we utilized both in-vitro and whole animal models to examine the effects of BPA on the brain’s dopamine (DA) system. GH3 cells, a rat pituitary cell line, were used to investigate the effects of BPA on cellular localization of the dopamine transporter (DAT) and DA-mediated cell signaling. Using western immunoblot analysis and measuring the ratio of membrane vs. cytosolic DAT protein it was shown that BPA altered normal DAT localization. Immunoblot and ELISA analysis was used to examine the effects on proteins (pERK and prolactin) involved in DA-mediated cell signaling. First, BPA increased pERK levels in a concentration-dependent manner as demonstrated with an ELISA kit. Moreover, this effect was attenuated when cells were pre-treated with a D2 receptor antagonist. Second, prolactin release is regulated, in part by DA; consequently, it may be affected by exposure to BPA. Immunoblot analysis of intracellular and extracellular prolactin levels indicated that BPA altered the normal release of prolactin in a DA D2 receptor-dependent manner. In addition to studies conducted in GH3 cells, an animal model (Sprague Dawley rats) was used to investigate the effects of developmental exposure to BPA on DA turnover. High performance liquid chromatography (HPLC) was used to measure the levels of DA and its metabolites. Using striatal tissue from rat pups developmentally exposed to BPA through maternal transfer, it was demonstrated that chronic exposure to BPA during the developmental period increased DA turnover. These results indicate that exposure to BPA appears to enhance DA neurotransmission and turnover which may play a role in cognitive deficits associated with the DA system.

1346 Abnormal Neuronal Migration and Behavior in Mice Exposed In Utero to Bisphenol A

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Developing brain is highly sensitive to environmental chemicals, and perturbation of the brain development can lead to cognitive impairment, as well as psychiatric and neurological disorders. Bisphenol A (BPA), a high volume chemical used for plastics, has been suspected to affect brain development, but its mechanisms of toxicity have been elusive. In this study we investigated whether and how the developing brain of mice born to dams exposed to low-dose of BPA was affected. Pregnant mice were exposed to BPA by implanting osmotic pump at a daily dose equivalent to 0, 40 (BPA-40), 400 (BPA-400) μg/kg from embryonic day 14.5 (E14.5) to E18.5. Neuronal migration, an essential process of cerebral cortical development, was analyzed on E18.5 by using an in utero electroporation technique. We found neuronal migration was significantly decreased in the BPA-40 group compared with the control group. Robo1, a neuronal guidance receptor gene, was significantly increased in the BPA-40 group, and TrkB, a gene that mediates neuronal differentiation and survival, was significantly increased in the BPA-400 group. In
addition, dopamine and its metabolites in the forebrain were significantly increased in BPA-400 group compared to BPA-40 group. Serotonin turnover, i.e., ratio of 5-HIAA to 5-HT, in the hindbrain was significantly decreased in BPA-400 group compared to the control group. Behavior of adult male mice born to BPA-exposed dams was examined using an automated apparatus, IntelliCage, under a group-housed condition (Endo et al., PLOS One, 2012). We found that the BPA-40 group significantly exhibited impulsive behavior compared to the control group. In conclusion, impulsive behavior observed in adulthood may have a fetal origin of perturbations in neuronal migration and related metabolic alterations in monoamine levels, both of which were induced by utero exposure to BPA.

**1347 Enhanced Behavioral Toxicity Induced by Maternal Exposure to a Mixture of Low-Dose Endocrine Disrupting Chemicals**

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Understanding the toxicity of low dose endocrine disrupting chemical (EDC) mixtures is critical, as research suggests that low dose early developmental EDC exposure can, through physiological reprogramming, alter the trajectory of adult disease onset. To assess how low dose developmental EDC exposure alters adult behavior, pregnant female mice were exposed to relatively low doses of four EDCs, Atrazine (ATR – 10mg/kg), Perfluorooctanoic acid (PFOA – 0.1 mg/kg), Bisphenol-A (BPA – 50tg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD – 0.25tg/kg) and their mixture (MIX), from gestational day 7 until weaning. Control litters were created at the same time. Adult offspring were tested for locomotor activity, food-rewarded fixed interval (FI) reinforcement schedule performance, and novel object recognition (NOR) performance. While behavioral deficits were observed in all 5 treatment groups, an enhanced effect was seen in male MIX offspring in increased FI response rates, suggesting an inability to inhibit responding. These were not seen in any other male treated group. In fact, ATR males exhibited significant reductions in FI rates, suggestive of reduced attention to task or impaired motivation. MIX females did not show enhanced FI alterations; but TCDD alone increased FI response rates. A robust decreased rate of exploration during initial exposure to the novel object paradigm occurred in all treatment groups in both sexes, particularly males. Deficits in short-term memory were observed in ATR, BPA and MIX male offspring; TCDD female offspring showed short-term memory deficits during NOR testing, spending significantly less time per each approach to the novel object. Deficits did not correspond to changes in locomotor activity in either sex. Taken together, behavioral deficits in learning/short term memory occurred after exposure to relatively low maternal doses of EDCs in mice. MIX treated males showed some evidence of enhanced effects highlighting the need for risk assessment of EDC mixtures. Supported by P30 ES001247.

**1347a Gestational Exposure to Inhaled Vapors of Ethanol and Gasoline-Ethanol Blends in Rats**


The US automotive fleet is powered primarily by gasoline-ethanol fuel blends containing up to 10% ethanol (E10). Uncertainties regarding the health risks associated with exposure to E10 prompted assessment of the effects of prenatal exposure to inhaled vapors of gasoline-ethanol blends containing greater amounts of ethanol. Pregnan Long-Evans rats were exposed to fuel vapors, 6.5 hr/day, on days 9–20 of gestation, and their offspring were assessed using a variety of tests for clinical, physical, immunological and neurodevelopmental effects. Results of tests of cognitive function are reported in other abstracts. This report compares effects of inhaled ethanol (E100) at concentrations of 0, 5000, 10000, or 21000 ppm with effects of vapors of gasoline lacking (E0) or containing 15% ethanol (E15) at concentrations of 0, 3000, 6000, or 9000 ppm. Maximum concentrations were limited by the lower explosive limits of the vapors. None of the vapors caused overt maternal toxicity, changes in litter size or weight, or altered the weight gain of the pups. With E100, motor activity and a few measures of a functional observational battery (hindlimb grip strength, excitability) were altered, but not in a dose-related manner. No such changes were observed with E0 or E15. With one exception, no dose-related changes were observed for any agent on any clinical, developmental, or immunological endpoint. Urinary protein concentrations suggesting potential kidney dysfunction, measured in the offspring at 3 and 6 months of age, increased across dose and age after E15 exposure; increases not related to dose were seen after exposure to E0. However, the highest measured protein concentration (300 mg/dl) was lower than concentrations associated with kidney damage in this strain of rats (400 – 2500 mg/dl). Thus, even at these high concentrations, inhalation of these vapors does not appear to pose a serious public health hazard. This abstract does not represent EPA policy.

**1347b Prenatal Inhalation Exposure to Evaporative Condensates of Gasoline with 15% Ethanol and Evaluations of Sensory Function in Adult Rat Offspring**

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The introduction of ethanol-blended automotive fuels has raised concerns about potential health effects from inhalation exposure to the combination of ethanol and gasoline hydrocarbon vapors. Previously, we evaluated effects of prenatal inhalation exposure to 100% ethanol (E100) or to gasoline (E0) vapors on sensory function of the offspring as a component of a larger biofuels research program. This study evaluated effects of exposure to vapors from a blend of 15% ethanol/gasoline (E15) on the same set of outcome measures used previously. Pregnant Long-Evans rats were exposed to 0, 3K, 6K, or 9K ppm E15 vapors for 6.5 hr/day over GD9 – GD20. Sensory evaluations of offspring (1 male pup/litter) began around PND106. Peripheral nerve function (compound action potentials, NCV), somatosensory (cortical and cerebellar evoked potentials), auditory (brainstem auditory evoked responses), and visual (electroretinograms) responses were assessed. Visual function assessment included pattern elicited visual evoked potentials (VEP), VEP contrast sensitivity, dark-adapted (scotopic) and light-adapted (photopic) flash electroretinograms (ERG), and UV and green flicker ERGs. Although some minor statistical differences were observed for auditory and somatosensory responses, these changes were not systematically related to dose. All physiological responses showed changes related to stimulus intensity, and provided an estimate of detectable levels of change. Overall, the results identified no persistent dose-related functional impairments of the peripheral nerve, somatosensory, auditory, or visual systems resulting form gestational exposure to E15 vapors. Additional studies are in progress to evaluate the effects of exposure to the evaporative condensate vapors from a blend of 85% ethanol with gasoline (E85). This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

**1347c Effects of Ethanol-Gasoline Blended Fuels on Learning and Memory**

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The potential toxicity of ethanol-gasoline blended fuels to the developing nervous system is of concern. We previously reported an absence of effect on learning and memory seen in a trace fear conditioning task and water maze task in offspring of dams exposed prenatally to the vapors of ethanol. However, a high number of anticipatory responses were noted in male offspring that completed a choice reaction time task (CRT). In order to further explore this apparent impairment of response inhibition, we evaluated effects of vapors from a blend of 15% ethanol/gasoline (E15) and 85% ethanol/gasoline using a Differential Reinforcement of Low-rate Response task (DRL). We also evaluated the effects of E15 and E85 on the trace fear conditioning task and water maze task. Pregnant Long-Evans rats were exposed to 0, 3000, 6000, or 9000 ppm E15 or E85 vapors for 6.5 hr/day from GD9 to GD20. Male and female offspring (n=10/sex/group/vapor) were trained on the Morris water maze starting on postnatal day (PND) 76. Overall, acquisition of the task and working memory were not affected by E15 or E85. A second set of male and female offspring (n=16/sex/group/vapor) was assessed using a DRL task. Overall, the results identified no significant dose-related impairments after exposure to E15 or E85, although there was a tendency for inhibitory control impairment in the 9000 E15 male offspring. These results suggest prenatal exposure to ethanol-gasoline blended fuels produce few if any effects on learning and memory. This abstract does not reflect EPA policy.
The impact of developmental exposure to inhaled ethanol-gasoline fuel blends is a potential public health concern. We previously reported that rats whose mothers inhaled ethanol (21,000 ppm) during pregnancy had increased levels of anticipatory responding on a choice reaction time (CRT) task. Thus, we used this task to investigate effects in the adult offspring of dams exposed to vapor condensates made from fuels blended with a range of ethanol concentrations, including gasoline alone (E0) and gasoline with 15% and 85% ethanol (E15 and E85, respectively). Each blend was investigated in separate cohorts. Within each cohort, dams were exposed for 6.5 h daily from gestational day 9 through 20 to concentrations of 0, 3,000, 6,000, or 9,000 ppm (n=8 per concentration). Male offspring were assessed during acquisition of both cued and uncued versions of the CRT and during asymptotic performance of the uncued task. During acquisition, increased anticipatory responding (elevated hold failures during a preparatory hold period) was observed in rats exposed to E0 (9,000 ppm) and E15 (6,000 and 9,000 ppm) indicating deficits in response inhibition. E15 offspring also had reduced accuracy (6,000 and 9,000 ppm) during acquisition of the cued CRT, suggesting additional deficits in processing visual information, and reduced decision times (6,000 ppm), indicating possible facilitation in this measure due to elevated hold failures. No effects were observed on any performance measure after exposure to E85, or, during asymptotic performances of the uncued CRT for any fuel blend. These data, combined with data from rats exposed to vapors of neat ethanol, indicate that prenatal exposure to gasoline and gasoline containing ethanol at 0% and 15%, but not 85%, ethanol may lead to diminished them. This abstract does not represent EPA policy.
Background. Zebralike mammals have dopamine systems that are important for programming a variety of functions including motor behavior. Normal development of dopamine systems would likely depend on normal responsivity of dopamine D1 and D2 receptors during development. Objective. Embryonic zebralike were used to assess the role of D1 and D2 receptor action on motor function. Methods. Embryonic embryos from the AB and SD strain were exposed to 0.5, 1.5 or 5 μM of a dopamine D1 antagonist, SCH23390 or a D2 antagonist, haloperidol dissolved in aquarium water, which served as the vehicle control. Exposure lasted from 5 hrs to 5 dpf. After exposure, fish were transferred to aquarium water with behavioral testing 24 hrs later. An automated system of data collection was used in which individual swim characteristics were calculated in real time under light (100% illumination) and dark (0% illumination) conditions controlled via a DanioVision observation chamber. Each trial commenced following a 10-min acclimation period in dark. Ten min intervals of light and dark conditions alternated twice each for a 50-min session. Swim data were analyzed by ANOVA with Dunnett’s test for pair-wise comparisons. Results. Survival of embryos was unaffected by treatment or strain. There was a significant effect of strain during dark, with ABs traveling a greater distance than SDs. The highest dose of haloperidol (5 μM) nearly eliminated movement during both conditions for both strains. Significant decreases were seen with the intermediate haloperidol dose (1.5 μM) and the highest dose of SCH 23390 (5 μM) during dark for both strains. Conclusion. Blockade of either D1 or D2 dopamine receptors during embryonic development caused lingering deficits in swimming behavior, suggesting toxins that affect dopamine systems during development may have persisting behavioral consequences. Differences in baseline behavior between strains suggest strain may be an important variable when measuring early-life endpoints in Zebralike. Research supported by Superfund Research Program ES010356, EPA Star grant 83554101
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Transfection and Flow cytometry followed by the establishment of PHB1 knockdown Z310 cells. The involved signal transduction pathways deserve further study leading to a-Syn aggregation; their combined effect is not additive, but rather synergistic. The blood-cerebrospinal fluid barrier (BCB), has been considered as a primary target in Mn-induced neurotoxicity. In our study, a total of 32 Mn-related differentially expressed proteins in CP were identified by 2D-PAGE combined with Nano-LC-MS/MS in an Mn-toxicity rat model. We further verified the 6 selected Mn-related proteins (PHB1, VDAC, β-actin, STIP1, TTR, Vimentin) in a CP cell line Z310 in vitro, and in vivo. Besides, the cell cycle arrest effect of Mn on Z310 cells had been proved from our previous experiment. A so-called iron responsive element structure within the 3′ untranslated region on prohibitin 1 (PHB1) mRNA is a newly found functioning element. We confirmed that upregulated PHB1 does play an essential regulatory role in Mn-induce Z310 cell cycle arrest, by WB, RT-PCR, Transfection and Flow cytometry study the establishment of PHB1 knockdown Z310 cells. The involved signal transduction pathways deserve further investigation. (partly supported by NSFC Grant in China № 81373028, Capital Development Project 2011-1013-03, and Beijing Health Bureau Project-2011. Corresponding author: Guojun J. Li, guojunli88@yahoo.com).

The environmental neurotoxic manganese promotes cell-to-cell transmission of α-Synuclein via exosomes in cell culture and animal models of Parkinson’s disease.

The aggregation of α-Synuclein (αSyn) is considered a key pathophysiological feature of Parkinson’s disease (PD). Recent studies suggest that prion-like cell-to-cell transfer of misfolded αSyn contributes to the spreading of αSyn pathology. However, the biological mechanisms underlying the propagation of the disease with respect to environmental neurotoxic chemical exposures are not well understood. Considering the role of the divergent metal manganese in PD-like neurological disorders, we characterized its effect on αSyn misfolding and protein aggregation. First, we established a MN9D dopaminergic cell line stably expressing wild-type human αSyn and treated it with non-toxic doses of manganese at various time points. Analysis of condition medium (CM) through Western blot showed that cells secreted αSyn into extracellular media following manganese exposure.

Further characterization of CM through electron microscopy readily detected the membranous nano-sized vesicles with characteristic hallmarks of exosomes. Western blot and ELISA studies revealed that the exosomes do in fact contain αSyn. Furthermore, Nanosight particle analysis showed that manganese exposure can enhance the release of αSyn-containing exosomes. In functional studies, we demonstrated that exosomes released during manganese treatment can induce neuroinflammatory responses in primary microglial cultures and neurodegeneration in differentiated human dopaminergic cells (LUHMES) through the activation of caspase-3 signaling. Our preliminary studies showed for the first time that stereotaxic delivery of αSyn-containing exosomes isolated from manganese-treated MN9D αSyn-expressing cells into the striatum can initiate PD-like motor deficits in mice. Collectively, these results demonstrate that manganese exposure promotes αSyn secretion by forming exosomal vesicles, which subsequently evoke pro-inflammatory and neurodegenerative responses in both cell culture and animal models. (ES19267, ES10586 & NS6167).
1352  Manganese Exposure Downregulates Anti-Apoptotic STAT5b Signaling through an Sp1-Dependent Mechanism

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Chronic exposure to elevated level of Manganese (Mn) has been linked environmentally to Parkinson’s disease-like neurological abnormalities. However, the precise mechanisms underlying Mn-induced neurotoxicity are still not well understood. In this study, we have identified that Mn exposure downregulates a key regulator of apoptotic cell signaling involving the signal transducer and activator of transcription (STATs). 300µM Mn exposure in N27 dopaminergic neuronal cells downregulated STAT5b expression both at the protein and mRNA levels, while STAT1 was unaffected, indicating an isoform-specific effect of Mn on STAT5b expression. To determine the functional consequence of STAT5b regulation during Mn neurotoxicity, we performed overexpression studies. Surprisingly, overexpressing STAT5b significantly protected against Mn-induced apoptotic cell death, suggesting the STAT5b plays a cell survival role in dopaminergic neuronal cells. STAT5b upregulation also increased anti-apoptotic Bcl2 expression. To further determine the molecular mechanisms underlying STAT5b downregulation during Mn neurotoxicity, we cloned the STAT5b promoter region indicating that a proximal region near exon 1 contains the regulatory element in response to Mn exposure. Detailed mutational analyses of the potential transcription factor binding site revealed that a Sp1 site located at -210 and -194 may be required for the suppression of STAT5b in Mn-induced neurotoxicity. Finally, we extended our studies to an animal model of Mn-induced neurotoxicity. The results showed that oral administration of Mn (30 mg/kg bw) in C57 black mice for 30 days significantly reduced the expression of STAT5b in the substantia nigra compared to control mice. Taken together, these data suggest Mn exposure downregulates anti-apoptotic STAT5b signaling via an Sp1-dependent mechanism in dopaminergic neurons, which may significantly contribute to Mn neurotoxicity. (NIH grants ES10586 and ES19267).

1353  Mutant Huntington Impairs Mn-Dependent p53 Activation in Human iPSC-Derived Neural Progenitors

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Huntington’s disease (HD) is a devastating neurodegenerative disease caused by the mutant Huntingtin protein. Expression of mutant Huntingtin is known to cause altered p53 signaling in cell and animal models as well as in patients. We tested the hypothesis that manganese (Mn) activates p53 signaling and that mutant Huntingtin protein would impinge on this cell stress pathway. We compared p53 activation in wild type (Q7) and mutant Huntingtin expressing (Q111) HD murine striatal cells and found that sub-lethal concentrations of Mn (25 µM) caused significant phosphorylation of p53(ser15) in both Q7 and Q111 cells as measured by immunoblotting. The mutant (Q111) cell line had a significantly diminished response. We then tested whether this phenotype is also seen in human cells. Human induced pluripotent stem cells (hiPSCs) from control and Huntington’s disease human subjects were differentiated into neural progenitors expressing early striatal markers (e.g. FOXG1, ISLET1). These human neural progenitors showed the same diminished p53 response to Mn; however, p53 activation after double-stranded DNA damage via neocarzinostatin resulted in greater p53 activation in HD iPSCs. Quantitative RT-PCR for p53-dependent transcripts (e.g. TIGAR, p21) confirmed manganese activation of the pathway and diminished responsiveness in HD cells. Under sub-lethal cell stress conditions, p53 activates pro-survival gene transcripts. Thus, diminished p53 responsiveness may reduce the ability of neurons to adapt to repeated cellular stress (e.g oxidative stress and nutrient deprivation). We are currently working to identify the kinase responsible for the Mn-dependent phosphorylation of p53 and mechanism by which Mn activates p53. NINDS: F31 NS077632 (AMT) NIEHS: ES016931 (ABB) and ES010563 (MA and ABB).

1354  A High-Throughput Screen for Modulators of Neuronal Manganese Status

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There is strong evidence that both genetic and environmental factors play a critical role in the pathogenesis of many neurodegenerative diseases. Exposure to the heavy metal, manganese (Mn), shows a strong correlation with incidence of Parkinson’s disease (PD). This study presents the results of a high-throughput screen (HTS) to identify novel modulators of neuronal Mn accumulation. Specifically, we screened multiple chemical libraries including: SPECTRUM, NIH Clinical Compounds, Chembridge/Chemdiv, and kinase inhibitors from Roche, GSK, and Enzo. Our goal was to identify agents with research utility in defining molecular mechanisms of neuronal Mn regulation and those with potential clinical efficacy in modifying Mn environmental risk in vivo.

We have successfully optimized the Cellular Fura-2 Manganese Extraction Assay (CFMEA) for HTS (Z-factor>0.5) in an immortalized murine striatal cell line (STHdhQ7/Q7). Subsequently, we conducted a screen of 40,000 small molecules. A subset of molecules was rescreened in duplicate given the known biological/medical relevance of the source libraries. These studies revealed 48 small molecule modulators of Mn status that hit twice in both screens, and these molecules were selected for the generation of concentration response curves (CRCs). CRCs revealed dose-response curves for 28 of these small molecules, known to target cellular signaling pathways previously implicated in Mn toxicity, including p53 and AKT, as well as GSK3β, ALK, EGFR/Erbb2, ERK, and neurotransmitter trafficking proteins. Parallel studies have revealed induction of phosphorylated p53 and AKT at subtoxic physiological Mn exposures. Future effort will characterize these HTS-identified modulators of Mn status for activity in human induced pluripotent stem cell (hiPSC)-derived nigral and striatal-like neural progenitors.

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1355  A Statistical Analysis of Z-Score Stringency in a High-Throughput Screen for Modulators of Neuronal Mn Status

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Manganese (Mn) is an environmental risk factor for Parkinson’s disease. We performed a high throughput screening (HTS) for small molecule modulators (SMMs) of neuronal Mn status to improve understanding of Mn transport and handling in the central nervous system. Current practices in HTS analysis utilize a statistical z-score with an absolute value of 3.0 as a cut-off for potential hits. We evaluate the utility of this statistical cut-off in our novel Fura2-based fluorescent Mn assay by performing replicate HTS. We have evaluated the variability and general distribution of “true-positive” versus “false-positive” hits using MATLAB. We find that relaxing the z-score cut-off from 3.0 to 2.0 identified a substantial fraction of FDA-approved drugs that may have translational value that fell below the 3.0 z-score limit. The ultimate goal of this study is to identify an optimal z-score cutoff for our HTS Mn assay given the non-linear responses of this assay. A total of 3,391 compounds were tested in duplicate to find SMMs using a murine striatal cell line. This study revealed 48 compounds that hit on both days of testing. Concentration Response Curves (CRCs) were generated for these, and 29 compounds revealed a true dose-dependent effect on cellular Mn levels. 69% of these 29 compounds had a z-score above an absolute value of 3.0 on both days of testing and 31% of compounds had z-scores between 2.0 and 3.0 on at least one day of testing. Furthermore, there was only a marginal decrease in the True Positive Rate (TPR) from 43.2% to 42.9% and a marginal increase in the False Positive Rate (FPR) from 1.0% to 2.8% by relaxing the z-score cutoff to 2.0. Additional statistical characterization will seek to evaluate the efficacy of compounds identified with a relaxed z-score cutoff and their mechanisms influencing Mn accumulation in murine striatal cells to validate a relaxed z-score approach with Fura2-based HTS.

Supported by NIEHS RO1 ES010563.
Environmental overexposure to the essential trace element manganese (Mn) can result in an irreversible condition known as manganese toxicity (PD) with dopaminergic (DAergic) cell loss associated with motor and cognitive deficits. However, the mechanisms that mediate the pathophysiology of both disorders remain unclear. Many PD genes impart risk for autosomal recessive, early-onset PD, including pARK2 and dj-1-pARK7. Another PD-associated gene is SNCA encoding for the presynaptic protein α-synuclein. Using Caenorhabditis elegans, we hypothesized that a loss-of-function mutation in pdkn1 (pdk1) and dfr-1(dfr1.1) would increase vulnerability to Mn toxicity and enhance DAergic neurodegeneration compared to wildtype (WT) worms, which may be altered by expression of WT human α-synuclein (α-Syn). Synchronous L1 worms from WT N2, pdk1(ge448) and dfr-1(tm918) mutant strains were acutely exposed to MnCl2 (0–10 mM) for 30 minutes, pdr1 and dfr-1 loss enhances intraworm Mn accumulation compared to WT (p<0.001) that is rescued in dfr-1 mutants also expressing α-Syn (p<0.001). Interestingly, enhanced oxidative stress upon treatment in the deletion mutants (p<0.001) is also rescued in the presence of α-Syn. However, pdr1 and dfr-1 show differential degeneration profiles, with α-Syn unable to rescue α-DERG cell loss in the dfr-1.1 mutants. This was associated with increased SNCA mRNA and decreased dat-1 mRNA levels in dfr-1 mutants. Compared to increased SNCA mRNA and increased dat-1 mRNA levels in pdr1 mutants. Thus, we show (1) a novel, neuroprotective role for WT human α-Syn in the background of PD-associated genes against Mn toxicity that may be dependent on its expression level, and (2) support for the role of extracellular dopamine in exacerbating Mn neurotoxicity (Supported by NIEHS R01ES01563).

Mutants

Wildtype Alpha-Synuclein-Mediated Protection against Mn Toxicity in PD-Associated C. elegans Mutants

S. Chakraborty1, J. Bornhorst3, S. Meyer4, H. Lohren4, T. Schwerdtle4, A. B. Bowman2 and M. Aschner1, 1Neuroscience Graduate Program, Vanderbilt University, Nashville, TN, 2Pharmacology, Vanderbilt University, Nashville, TN, 3Neurology, Vanderbilt University, Nashville, TN and 4Institute of Food Chemistry, University of Münster, Münster, Germany.

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Manganese (Mn) is a neurotoxin causing Manganism, a Parkinson-like disease, disrupts dopamine (DA) neurotransmission. The mechanism is not fully resolved and reports postulate it more related to dysfunction of DA D2 receptors than degeneration of DA neurons. Gilia of lateral gill cells of *Grausnina virginia* are controlled by serotonergic-dopaminergic innervations. DA is cilio-inhibitory, serotonin cilio-excitatory. We showed post-synaptic DA receptors in gill lateral cells are D2 type. Mn blocks cilio-inhibitory effects of DA, and DA toxicity is blocked by p-aminalsaliclyc acid (PAS). Questions exist whether Mn decreases the number of post-synaptic DA D2 receptors in brain. We used immunohistochemistry to test if Mn decreases the number of DA D2 receptors in gill of *C. virginica* and if PAS prevented the decrease. Using 1<sup>o</sup> antibodies against D2 receptors and FITC-linked 2<sup>o</sup> antibodies, we quantified DA D2 receptors in gill. Animals were treated with 500 µM of Mn for up to 6 days. Controls were treated without Mn. Gills were excised, fixed, exposed to antibodies and paraffin embedded. Sections were viewed on an epihle fluorescence microscope with FITC filters, and a ProgRes C3 camera. Fluorescence was quantified using ImageJ software from NSF. Fluorescence in sections from animals treated up to 6 days with Mn (500 µM) showed progressive decrease in fluorescence of up to 36% of controls. Animals co-treated up with 500 µM of Mn and PAS did not show reduced fluorescence. The study shows negative correlation between fluorescence intensity of DA D2 receptors in Mn treated animals vs controls, and also shows PAS can negate the effect Mn treatments on D2 receptors. The question of whether the loss in fluorescence intensity is due to an actual decrease in D2 receptor number or if Mn is altering the protein conformation and ligand binding site of D2 receptors needs to be further explored. This work was supported by grants2R25GM06003 of the Bridge Program of NIGMS, 0516041071 of NYSDOE and 0622197 of the DUE Program of NSF.

Excessive exposure to Manganese (Mn) can cause neurologic symptoms similar to, but distinguishable from idiopathic Parkinson’s disease (IPD). The question as to whether or not Mn exposure has direct effects on dopamine (DA) and other catecholamines thereby disrupting the nigrostriatal pathway is unanswered. This study was designed to test the hypothesis that subchronic Mn exposure alters neurochemistry. Adult male rats received IP injections of 6 and 15 mg Mn/kg (as MnCl<sub>2</sub>) as the low- and high-dose groups, respectively, or saline as the control, 5 days per week for 4 weeks. Animals were sacrificed 24 hr after the last injection, and striatum (STR), hippocampus (HP), and substantia nigra (SN) were dissected for quantification of neurotransmitters including: DA, 3,4-di-hydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxy-indolacetic acid (5-HIAA), and γ-aminobutyric acid (GABA) by HPLC. Analysis revealed statistically significant differences in the neurotransmitter content of these three brain regions between control-, low-, and high-dose groups. The level of DA and its metabolites in STR were significantly higher in Mn-exposed animals than in controls (p<0.05); DA turnover in SN was also significantly higher in exposed animals than controls (p<0.05). Interestingly, GABA levels were significantly higher in HP in exposed animals than in controls. Analysis with atomic absorption spectrometry showed Mn exposure led to a significant accumulation of Mn and copper in the femur as well as other bone tissues. Neurotransmitter differences seen in the striatum may be of particular relevance because alterations could lead to heightened sensitivity to additional insults, particularly due to oxidative modifications of DA. This study provides evidence that dopaminergic neurotransmission is especially sensitive to Mn intoxication.

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Welders had significantly increased UPDRS-III scores and higher levels of thalamic GABA compared to controls. For all subjects increasing thalamic GABA levels correlated with increasing normalized scores of the parallel line test reflecting tremor level \((r=0.437, p<0.05)\), and striatal GABA correlated with Finger Tapping, using a mono-motor task \((r=0.68, p<0.01)\). In the welders, significant inverse correlations were seen between frontal Glx levels and the grooved pegboard score \((r=0.803, p<0.01)\), and between motor cortex Gx levels and parallel line test scores \((r=0.651, p<0.05)\). The significant correlations of thalamic GABA and frontal Gx levels with tremor level, as well as striatal GABA levels with motor skills, confirm the use of MRS to study neurotoxic effects of Mn on the direct and indirect pathways of the basal ganglia that lead to motor disturbances. (Supported by NIEHS R01 ES020529)

**1367 Increased Thalamic GABA in Chronic Manganese-Exposed Metal Workers and Manganism Patients**

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Overexposure to manganese (Mn) has been reported to lead to parkinsonian symptoms. We hypothesize that hypokinetic symptoms associated with Mn overexposure correspond to elevated thalamic GABA levels leading to reduced glutamatergic projections to the cortex. Therefore we aimed to test whether Mn-exposed workers and manganism patients may have elevated thalamic GABA levels, as well as reduced cortical glutamate (Glu) levels. A group of 20 Mn-exposed welders, 19 Mn-exposed smelters, 7 manganism patients and 37 controls were recruited from Guangxi Province, China. Each subject underwent magnetic resonance imaging and spectroscopy exams. Short-echo-time 1H spectra were acquired from the frontal cortex and thalamus to assess levels of GABA, the sum of Glu and its precursor glutamine (together denoted as Gx), and myo-inositol (mI, a glial marker). GABA-edited spectra were also acquired in the thalamus. Metabolites are expressed as ratios over creatine (Cr).

Over three years we consistently found significantly increased thalamic GABA/Cr in welders, smelters, and manganism patients compared to controls \((p<0.01\) for all comparisons). Smelter showing significantly decreased frontal mI/Glu and thalamic GABA/Cr \((p<0.05\) for all comparisons). Moreover, welders had significantly increased frontal mI/Glu and both groups of workers had significantly decreased thalamic mI/Cr \((p<0.05\) for all comparison). Overall our results suggest increased thalamic GABAergic inhibition in Mn-exposed workers and manganism patients, as well as decreased glutamatergic excitation and Glu-Gln cycling in the frontal cortex of smelters. These metabolic changes may aid in understanding the mechanism of Mn-caused motor and cognitive deficits. (Supported by NIH/NIEHS R21 ES017498, R01 ES020529 and NSF of China #81072320)

**1368 GABA Levels Correlate with Exposure Levels and Brain Deposition of Manganese in US Welders**

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Excessive manganese (Mn) exposure has been associated with decline in cognitive and motor function. Our previous study in a cohort of highly Mn-exposed Chinese workers found significantly elevated thalamic \(\gamma\)-aminobutyric acid (GABA) levels. The current study explored the relationships among brain GABA levels measured in vivo by magnetic resonance spectroscopy (MRS), brain Mn deposition measured by MRI and individual Mn exposure levels in a typical US occupational setting. Thirteen welders and eleven controls were recruited from a US truck trailer manufacturer. Subjects underwent personal air sampling and filled out a questionnaire of detailed work history for the estimation of individual exposure to respirable Mn. For each subject a series of 3D images were acquired on a 3T GE Signa MRI scanner and used to create high-resolution T1 relaxation maps, an inverse indicator of Mn deposition. GABA spectra were acquired from the thalamus with a T68 MEGA-PRESS sequence and quantified using LCModel, a spectral fitting tool.

GABA levels were significantly higher in welders vs. controls \([2.45\pm0.68 \text{ mM vs. } 1.40\pm0.45 \text{ mM, } p<0.001]\). Increased thalamic GABA levels significantly correlated with (a) average exposure estimated for the previous three months before the MRI exam \([R=0.649, p<0.05]\) and with (b) decreased T1 relaxation time in the substantia nigra, denoting increased Mn deposition \([R=0.589, p<0.05]\).
These results confirm the elevation of thalamic GABA levels in a typical US occupational setting. The significant correlations between increased GABA levels and recent exposure levels, as well as with brain Mn accumulation in the substantia nigra, suggest that GABA-edited MRS in conjunction with quantitative T1 relaxation may serve as a non-invasive Mn exposure. (Supported by NIEHS R01 ES020529 and CDC/NIOSH TO3 OH088615)

1368a Potential Prevention Strategies to Reduce the Risk of Neurotoxicity Associated with Manganese-Containing Welding Fumes


Welding generates complex metal aerosols, inhalation of which is thought to cause Parkinson’s disease (PD)-like neurotoxicity, due to the presence of manganese (Mn) in the welding electrodes. As neurological disorders are generally progressive in nature, with latency between insult and appearance of clinical symptoms, a logical approach for workplace safety and health is to prevent adverse exposures. For welding, this can be achieved by minimizing welding fume (WF) generation rate and/or suitably modifying existing welding practices to reduce toxic exposures. Here, we show that by specifically modulating welding voltage, keeping current and shielding gas constant, the chemical composition and neurotoxicological properties of WF can be significantly altered. Rats were exposed by whole-body inhalation to filtered air or WF particulates generated by gas-metal arc-stainless steel welding (GMA-S: 40 mg/m²; 3h/d x 10d) either at 25V (standard/low; LVSS) or at 30V (high; HVSS) voltage. Both conditions produced good weld quality and similar particulate morphology, although aerosols from HVSS welding comprised of a larger fraction of ultrafine particulates that are characteristically considered to be more toxic than their fine counterparts. Exposure to particulates from LVSS welding caused neuroinflammation (increased Ca²⁺, TNFa, Nos2; 1.5 - 3.9 fold; P<0.05) and decreased PD-related proteins (Th, Park5, Park7; 18 - 47%; P<0.05) in the dopaminergic brain areas, striatum and midbrain. Paradoxically, exposure to particulates from HVSS welding did not elicit any dopaminergic neurotoxicity. We determined that the lack of neurotoxicity may be a consequence of the reduced solubility of manganese in HVSS fumes. Our findings show promise for modifying welding practices as a potential prevention strategy for Mn-related neurotoxicity during welding; however, it warrants additional investigations to determine if such modifications can be suitably adapted at the workplace to avert or reduce neurotoxicological risks.

1369b The Role of the Transient Receptor Potential Ankyrin 1 (TRPA1) Channel in Methylmercury (MeHg)-Induced Ca²⁺ Dysregulation

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MeHg biphasically elevates [Ca²⁺], with the first (P1) and second (P2) phases attributed to Ca²⁺ release from intracellular stores and extracellular Ca²⁺ influx through Ca²⁺-permeable ion channels, respectively. MeHg-induced [Ca²⁺] dysregulations have been correlated with reduced viability of susceptible neuronal populations in vitro. Because parathyreoid is the preeminent site of MeHg intoxication, and the size and number of neurons of the dorsal root ganglia (DRG) are reduced in poisoned individuals, molecular entities unique to DRG may heighten sensitivity to MeHg. Heterologous expression was used in this study to investigate the contribution of TRPA1, a Ca²⁺-permeable ion channel highly expressed in DRG, in mediating Ca²⁺ dysregulation through acute MeHg exposure. HEK 293 cells were transfected with human TRPA1 72 hrs prior to single-cell microfluorimetry studies. Fura-2 AM, a ratiometric Ca²⁺ fluorophore, was used to measure relative changes in [Ca²⁺]; the time-to-onset of P1 and P2 was determined by 2-ΔΔCt. Differentiated F11 cells also express low levels of RNA most

MeHg is an environmental contaminant that elevates [Ca²⁺], in two kinetically-different phases. Phase one (P1) consists of Ca²⁺ release from intracellular storage organelles, whereas phase two (P2) results from an influx of Ca²⁺ through Ca²⁺-permeable ion channels. MeHg-induced elevations in [Ca²⁺] have been correlated with increased cell death. This study surveyed VGCC RNA expression in MeHg-exposed F11 cells, an immortalized cell line derived from dorsal root ganglia. Changes in RNA expression of the pore-forming subunit of the L-, N-, P/Q-, R-, and T-type VGCC isofoms (calcna1c, 1h, 1a, 1e, and 1h) were determined by qPCR. F11 cells were cultured and differentiated (2 µm retinoic acid) for 24-72 hrs prior to 1 hr MeHg exposure (1, 2 and 5 µM). 24 hrs after MeHg exposure, RNA was isolated and converted to cDNA. PCR was performed on CDNs and relative changes in RNA were determined by 2-ΔΔCt. Differentiation alone increases the RNA expression of calcna1c and calcna1a in F11 cells at 72 hours (2.5±0.05 and 2.5±0.05, respectively; mean fold-change ± SEM), Differentiated F11 cells also express low levels of RNA for calcna1b (1.01±0.04), calcna1a (0.45±0.04), and calcna1h (1.12±0.03); exposure to MeHg reduces RNA expression of these genes in a concentration-dependent manner, with 5 µM MeHg reducing calcna1c, calcna1a, and calcna16 RNA most markedly (0.45±0.06, 1.06±0.09, and 0.63±0.04, respectively). Downregulation of RNA expression was also dependent upon the differentiation time point; cells differentiated for longer periods displayed a greater reduction of calcna1c expression universally. Changes in RNA expression may reflect a concomitant alteration in protein expression, thus these data may indicate distinct VGC isoforms as critical contributors to Ca²⁺ influx in P2. Because RNA expression of calcna1c and calcna1a is most abundant following acute MeHg exposure, the L- and P/Q-type VGCCs may be significantly involved in cytotoxic mechanisms. Supported by NIH grant R01ES03299 and R25NS05467.

1370 Effect of Methylmercury (MeHg) on RNA Expression of Voltage-Gated Calcium Channels (VGCCs) in Naïve and Differentiated F11 Cells


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Objectives: Methylmercury (MeHg) is a well-known environmental pollutant. The human beings expose to methylmercury through ingestion, which can cause irreversible nervous system dysfunction and injuries. However, effects and possible mechanisms of MeHg-induced neurotoxicity remained unclear. In this study, we attempted to investigate the important roles of endoplasmic reticulum (ER) stress-regulated pathway in MeHg-induced neurotoxicity.

Results: MeHg significantly decreased cell viability in a dose-dependent manner (1.5-7 µM) and induced the increase in caspase-3 activity, annexin V-FITC binding, and the protein expressions of caspase cascades in Neuro-2a cells, indicating that MeHg could induce neuronal cell apoptosis. Moreover, exposure of Neuro-2a cells to MeHg could trigger ER-stress as indicated by several key markers (GRP-78, GRP-94, CHOP, and XB-1) and upstream molecules (the phosphorylation of PERK, eIF2-α, and IRE-1), and caspase-12 cleavage. Pretreatment with antioxidant NAC and transfection with specific si-RNA (GRP-78, GRP-94, CHOP, and XB-1) could effectively attenuate MeHg-induced cytotoxicity, apoptotic events and ER-stress-related signals.

Conclusions: These results indicate that the ROS production triggered ER-stress signaling pathway involves in MeHg-induced neuronal cell apoptosis.
Methylmercury (MeHg) causes calcium (Ca)-dependent neurotoxicity, which includes increases in spontaneous release of neurotransmitters. Both intracellular Ca ([Ca²⁺]i) release and extracellular Ca entry contribute to this effect. Although an inextricable link between alterations in Ca homeostasis and MeHg-induced neurotoxicity has been demonstrated in the past, Ca’s exact role is unclear. Previously, work in our lab has revealed a concentration- and time-dependent relationship between MeHg and extracellular dopamine (DA). In this study, we seek to manipulate [Ca²⁺]i levels to further define Ca’s relationship with MeHg and release of DA. A23187, a Ca²⁺ ionophore, which increases [Ca²⁺]i, was therefore used to elucidate the contribution of [Ca²⁺]i on the quantity of DA released by rat pheochromocytoma (PC12) cells. Forty-eight hours before treatment, undifferentiated PC12 cells were seeded at a density of 7×10⁵ cells/mL in poly-D-lysine coated wells. At time of experiment, cells were pre-treated with either vehicle or 1 μM A23187 for 30 min. Subsequently, treatment medium was replaced with either vehicle, 1 M A23187, or a combination of 1 μM A23187 and 2 μM MeHg for an incubation period of 60 min. Following treatment, plates were centrifuged and treatment medium was collected for analysis by high performance liquid chromatography (HPLC) to quantify extracellular DA. Extracellular DA levels increased significantly following treatment with A23187, as compared to vehicle-treated controls. Although co-treatment with MeHg increased DA levels when compared with basal levels, it blunted the extent of increase caused by treatment with the ionophore alone. These data suggest that although MeHg is able to increase the level of DA released by PC12 cells, its effect is not directly correlated with the amount of Ca available in the intracellular space. Supported by a VICTR supplement to NIH grant ROI1ES03299 and T32ES007255.

Methylmercury (MeHg) is an environmental neurotoxicant which induces selective cell death in the nervous systems. In this study, we investigated the effect of MeHg on differentiating neurons using PC12 cells in the presence of nerve growth factor (NGF). NGF is known to enhance the vitality and cellular functions in neurons. The PC12 cells exhibited apoptosis 2 days after exposure to 100 nM MeHg in the presence of 50 ng/ml NGF. During the course of MeHg-induced apoptotic cell death, inhibition of neuronal cell differentiation preceded apoptosis. Time course study of immunocytochemistry and ELISA analyses for neurite specific protein namely, neurofilament triplet H protein (NF-H), demonstrated that MeHg inhibited the expression of NF-H protein before the induction of apoptosis. To further investigate the factors responsible for inhibition of neuronal cell differentiation and subsequent apoptotic cell death, we investigated the expression levels of neurotrophic factor receptors, tropomyosin receptor kinase (Trk) A, B, and C, which regulate neuronal cellular differentiation in neurons. Western blot analysis demonstrated that MeHg inhibited selectively the TrkA phosphorylation, whereas changes in the total TrkA, B, and C levels were not observed. Furthermore, we demonstrated that a GM1 ganglioside analog, which is known to enhance NGF-triggered TrkA phosphorylation, prevented the differentiating PC12 cells against MeHg-induced apoptotic cell death. These findings suggest that MeHg exposure inhibits NGF-triggered TrkA phosphorylation selectively and lead to apoptotic neuronal cell death in differentiating PC12 cells.

Astrocytes and microglia are potential targets for MeHg-mediated Ca²⁺ dysregulation. However, the extent to which they respond to MeHg in the absence of ACh and their relative contributions to cytoxicity are not known. We studied the role of AChRs in MeHg-induced cytoxicity in PC12 cells, a model that expresses both muscarinic (mACHRs) and nicotinic receptors (nAChRs) but does not express glutamate receptors, an additional potential target for MeHg-induced elevation of [Ca²⁺]i. Nerve growth factor-differentiated PC12 cells were treated for 1h with 1, 2, or 5μM MeHg. Cytotoxicity was examined using the Live/Dead Assay® 1 or 24h after MeHg treatment concluded. Mecamylamine (M, 5μM) and atropine (10μM), nAChR and mAChR antagonists respectively, were used to determine the relative involvement of nAChRs and mAChRs to MeHg-induced cell death. Viability at 1 and 24h after MeHg was reduced in a concentration-, but not time-dependent manner. At 1h, MeHg-induced cytotoxicity ranged from 33% (1μM) to 57% (5μM) and at 24h from 35% (1μM) to 65% (5μM). Pretreatment with either MEC or atropine protected PC12 cells from MeHg-induced cell death at both time points, however protection was proportional to the MeHg concentration. Pretreatment with either MEC or atropine protected PC12 cells from MeHg-induced cell death at both time points, however protection was proportional to the MeHg concentration. At 1μM MeHg protection ranged from 33%-97% (3-35% cytoxicity) at both 1 and 24h, whereas at 5μM MeHg protection was only in the range of 32-50%. Thus both mAChRs and nAChRs contribute to Ca²⁺-induced cytoxicity by MeHg. This occurs in the absence of a cholinergic agonist, suggesting direct actions of MeHg on AChRs. This project was supported by NIH grants: R25NS065777 and R25GM059429.
astrocytes between the two areas. The goal of this study was to compare the toxicity of MeHg on cerebellar and cortical astrocytes. Determining if MeHg differentially affects astrocytes from these two brain regions could help us understand cellular mechanisms underlying the preferential sensitivity of cerebellar granule cells to MeHg. Primary astrocyte cultures from the cerebellum and cortical forebrain layer were obtained from C57BL6 male mice. At 14-15 DIV, cells were exposed for 3h to 0μM, 2μM, 5μM, 10μM, or 15μM MeHg. Cytotoxicity was measured 24h later using EthD-1 and Calcein AM. In parallel cell cultures, GFAP and the nuclear fluorogenic indicator DAPI were used to calculate the percentages of astrocytes in the cultures. The percentages in the cerebellar and cortical areas were 58% and 62% respectively. The mean percentage of cell death in the cerebellum was: 0.5%, 26%, 52%, 88%, and 96% at 0, 2, 5, 10, or 15μM MeHg respectively. In the cortical layer it was: 1.3%, 50%, 81%, 88%, and 94% respectively. Cell viability of cortical astrocytes was significantly reduced at 2 - 15 μM MeHg: in cerebellar astrocyte viability was reduced only at 5-15 μM. There was no significant difference at 0 and 1μM between cerebellar and cortical astrocytes compared. The LC50s of the cerebellum and cortical layers were 4.1 μM and 1.6 μM MeHg respectively. Thus, whereas cerebellar granule cells are more susceptible to MeHg, their attendant astrocytes are less sensitive to MeHg-induced cytotoxicity than are cortical astrocytes, and brain region-dependent differences in cytotoxicity of astrocytes occurs in response to MeHg. Supported by NIH grants R01ES03299, R25NS4467 and T32GM092715.

1376 GABA Receptors Are Not a Primary Contributor to Methylmercury-Induced Cell Death in Transiently Transfected HEK293 Cells

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Altering of GABA type A receptors(GABA,R)-mediated inhibitory function by MeHg contributes to cerebellar dysfunction. The effect is more pronounced in cerebellar granule cells (CGCs) than the neighboring Purkinje cells (PKCs). Differential effects of MeHg on these two types of neurons could relate to differential expression of GABA,R subunits. CGCs express α1 and γ2 subunits; PKCs express only α1 subunits. The subunits confer markedly different properties to GABA,Rs as they are preferentially co-expressed with different auxiliary subunits such as β2 and δ. The δ subunit is very important in CGC tonic inhibition. Its preferential disruption by MeHg could cause enhanced excitability in CGGs, and promote Ca-induced cell death. Two questions were tested: 1) does co-expression of differential auxiliary γ2 and δ subunits with α1 subunits contribute to MeHg-induced cell death? 2) Will differential subunit combinations of α and β subunit-containing GABA,Rs exacerbate MeHg-induced cell death? HEK293 cells were transiently transfected with combinations of GABA,R subunits and exposed for 3 or 6h to 1, 2 or 5μM MeHg. Cell viability was assessed using a Live/Dead cytotoxicity assay. HEK293 cell death increased with increasing [MeHg] at both 3 and 6h exposure. No significant difference between these exposure exists. Acute mortality rate induced by MeHg is similar for GABA,Rs having α1,β2,γ2 and α1,β1,β3,δ compositions and did not increase over that of untransfected or GFP-transfected HEK cells. Comparisons of susceptibility to MeHg-induced cell death among differential GABA,R subunit compositions also indicated no significant difference. HEK cells transfected with GABA,R containing α1,β2,γ2,α1,β1,β3,δ, α1,γ2,δ and α1,δ subunits were not more susceptible to MeHg-induced cytotoxicity. While GABA,Rs were very sensitive targets to MeHg, a simple direct action of MeHg on the receptor will not explain the heightened sensitivity of CGGs.

1377 Effects of Methylmercury on Caenorhabditis elegans Calcium Ion Channel Mutant Strains

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Caenorhabditis elegans (C. elegans) strains KC136, CB55, DM612, JD21, MT6129, and DAF95 were used to examine the percent viability for GOF or LOF L-, P/Q- and T-type voltage-gated Ca2+ channels (VGCCs) in the presence of methylmercury (MeHg) using a gel ring hanging drop medium. Exposure of L3 worms to 1.0-100 μM MeHg resulted in decreased viability with increasing [MeHg] for 24 and 48 h. We expected that GOF VGCC mutants would have decreased viability resulting from increased Ca2+ influx in the cell leading to excitotoxicity while LOF mutants would have increased viability after MeHg treatment; however, we observed the opposite effect. L- and P/Q-type voltage-gated Ca2+ channels exhibited decreased viability when exposed to MeHg, while the null mutation for low-voltage activated VGCCs led to increased viability. GOF high-voltage activated VGCC mutants had increased viability. Two explanations for this result are either upregulation of other VGCC subtypes leading to increased Ca2+ influx or complete block of Ca2+ entry to the cell thus decreasing viability. A third possible explanation for these results is that mutants have decreased Ca2+ buffering capacity and physiologic Ca2+ levels lead to death. To further explore these possibilities, KC136 was pretreated with 0.25 or 0.5μM of the L-type VGCC antagonist nemadipine-A (NEM-A) before exposure to 1.0-100 μM MeHg. NEM-A can cross the C. elegans cuticle, unlike other antagonists such as verapamil. Pretreatment of KC136 with 0.25 μM NEM-A plus MeHg exposure had no significant effect. Pretreatment of KC136 worms with 0.5 μM NEM-A significantly decreased viability at lower [MeHg] over 24 and 48 h without any change at 48 h. However, the significant effect may be due to NEM-A alone rather than a compounded effect of NEM-A and MeHg exposure. Supported by NIH grant R01ES03299.

1378 The Putative Multidrug Resistance Protein MRP-7 Inhibits Methylmercury-Associated Animal Toxicity and Dopaminergic Neurodegeneration in Caenorhabditis elegans

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Background: Recent epidemiological and vertebrate studies suggest that MeHg exposure may contribute to dopamine (DA) neuron vulnerability and the propensity to develop Parkinson’s disease (PD). We have developed a novel Caenorhabditis elegans (C. elegans) model of MeHg toxicity and have shown that chronic exposure confers embryonic defects, developmental delays, and DA neuron degeneration. Aims/Objectives: In this study we asked what genes and molecular pathways are involved in MeHg-induced whole animal and DA neuron pathology. Methods: We utilized the transgenic C. elegans, RT-PCR, Western analysis, and neuronal morphology characterization to analyze expression, localization and the role that SKN-1, MRP-7 and post-translational modifications play in MeHg-induced whole animal and DA neuronal death. Results: Over 17,000 genes were screened for whole animal sensitivity to MeHg, and 92 genes were identified (90% have strong human homologues) that affect whole animal and/or DA neuron pathology. Here we report detailed analysis of a putative plasma membrane transporter’s role in MeHg-associated DA neuroprotection. Specifically we demonstrate that genetic knockdown of MRP-7 results in a 2-fold increase in Hg levels and a dramatic increase in stress response transcriptome, gonadal morphology, mitochondrial and other abnormalities, as well as an increase in MeHg-associated animal death. Chronic exposure to low concentrations of MeHg induces MRP-7 gene expression, while exposures in MRP-7 genetic knockdown animals results in a loss of DA neuron integrity in without affecting whole animal viability. Conclusions: These studies show for the first time that a multidrug resistance protein is expressed in DA neurons, and its expression inhibits MeHg-associated DA neuron pathology. Support: NIEHS ES014459 and ES003299 to RN; and EPA STAR Graduate Fellowship to NVD.

1379 NAD+ Supplementation Attenuates Methylmercury Toxicity in Caenorhabditis elegans

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Methylmercury (MeHg) is a persistent pollutant with known neurotoxicity. MeHg causes oxidative stress and mitochondrial dysfunction in vitro and animal models. This can lead to altered cell metabolism and energetics. It has been reported that MeHg decreases cellular nicotinamide adenine dinucleotide (NAD+), however the effects on NAD-dependent enzymes and cellular functions have not been characterized. Caenorhabditis elegans (C. elegans) is a powerful genetic model for the investigation of neurotoxicity of MeHg, as worms readily accumulate the metal, leading to decreased neurobehavior and dopaminergic neurodegeneration. Herein, we tested the hypothesis that NAD+ supplementation could attenuate MeHg toxicity and dopaminergic (DAergic) neurodegeneration. Worms were treated with 1 mM NAD+ prior to MeHg treatment and assessed for lethality. Treatment with increasing doses of MeHg decreased survival of C. elegans (LD50 = 20.24 μM) and NAD+ pre-treatment significantly attenuated its toxicity (LD50 = 34.6 μM, p < 0.001). Furthermore, worms lacking pnc-1, the rate-limiting enzyme in NAD+ synthesis, were highly sensitive to MeHg toxicity (LD50 = 3.598 μM, p < 0.001). As MeHg is implicated in DAergic neurodegeneration involved in Parkinson’s disease, we determined whether DAergic-dependent behavior was altered by NAD+ depletion in response to MeHg treatment. Wildtype worms had significantly less DAergic behavior as measured by the basal slowing response (p < 0.05). The DAergic behavior was further decreased in pnc-1 mutants treated with MeHg (p < 0.05), suggesting that DAergic-dependent behavior may be altered by NAD+ levels. DAergic cell morphology was evaluated following NAD+ supplementation in worms express-
Methylmercury (MeHg) is a neurotoxicant, which poses a continued health risk to the human population. Despite extensive research, its precise mechanism of action has not been fully elucidated. Furthermore, the genetic basis for MeHg susceptibility is not well defined. In order to explore genetic modulators of MeHg-induced neurotoxicity, we utilized the BXD recombinant inbred (RI) mouse cohort. This cohort consists of fully inbred strains, containing varied nuclear factor erythroid 2-related factor 2 (Nrf2) expression. Nrf2 is a well-established antioxidant gene that is activated in response to oxidative stress. We hypothesized that mice with high Nrf2 expression would be less vulnerable to MeHg-induced neurotoxicity versus those with low expression. Therefore, mice were selected that exhibited either high (BXD21, BXD31) or low (BXD28, BXD84) Nrf2 expression, and the role of Nrf2 in modulating MeHg-induced neurotoxicity was examined. Astrocyte cultures were prepared from post-natal day 1 (PND1) pups, and treated approximately 3 weeks post-isolation with 1, 5, or 10µM MeHg for 90 min. After exposure, cells were collected for western blot analysis. BXD21/31 mice had significantly higher baseline Nrf2 expression relative to BXD28/84 (p<0.001). Following MeHg exposure, BXD31 mice exhibited higher Nrf2 expression compared to BXD28 with an overall significant effect of strain (p<0.001), treatment (p<0.001) and interaction sure, BXD31 mice exhibited higher Nrf2 expression compared to BXD28 with an overall significant effect of strain (p<0.001), treatment (p<0.001) and interaction. BXD31 mice exhibited higher Nrf2 expression compared to BXD28 with an overall significant effect of strain (p<0.001), treatment (p<0.001) and interaction (p<0.001). In BXD31 mice, Nrf2 expression was increased following MeHg exposure. In BXD28 mice, Nrf2 expression was decreased following MeHg exposure. These results provide evidence that Nrf2 expression plays a key role in modulating MeHg toxicity. The data also demonstrate the utility of the BXD RI mouse strains for understanding genetic susceptibility to MeHg. Future experiments will explore the impact of MeHg-induced neurotoxicity on mice with high Nrf2 expression. Supported by NIEHS/NIH grant ES003299.

The cerebellum is a primary target of methylmercury (MeHg) neurotoxicity. Following acute MeHg exposures in vitro, this effect involves presynaptic effects on transmitter release and elevation of [Ca2+]i. However, the extent to which the acute effects mirror chronic toxicity has not been reported. To study the chronic effects of MeHg on cerebellar synaptic function and neuronal excitability, we studied changes in short-term plasticity and neuronal firing in cerebellar slices of mice following exposure to 5 ppm MeHg in the drinking water for 6 weeks (15mos old) or 12 weeks (15mos old). Paired-pulse evoked synaptic currents in Purkinje cells were recorded in sagittal slices using whole cell voltage-clamp recording mode by stimulating the white matter; single action potential (AP) or repetitive firing in Purkinje or granule cells were evoked by injecting short or long pulses, respectively, of depolarizing currents using whole-cell current-clamp recording mode. Following 6 mos of MeHg exposure, no significant changes in paired-pulse responses or AP firing in Purkinje and granule cells were observed between control and MeHg-treated mice. However, after 12 mos of MeHg exposure, 36% and 64% of MeHg-treated mice showed paired-pulse depression (PPD) and facilitation (PPF), respectively. In contrast, 72% and 28% of control mice displayed PPD and PPF, respectively. This suggests that MeHg acts presynaptically to promote PPF because short-term plasticity is generally attributed to presynaptic alterations of transmitter release. Chronic (12mo) MeHg exposure also significantly reduced the frequency of repetitive firing in granule but not Purkinje cells compared with those from control mice. Thus, 12mo, but not 6mo MeHg treatment shifts short-term plasticity in cerebellum from PPF to PFP. Cerebellar synaptic function was modified through direct actions on the nerve terminal. Cerebellar granule cells are more sensitive than are Purkinje cells to these effects. Supported by NIEHS/NIH grant ES003299.

BACKGROUND. Methylmercury toxicity has been linked to disrupted calcium homeostasis and Ca2+ accumulation in nerve terminals. Calcium channel blockers such as nimodipine (NIM) protect against MeHg’s toxicity. Motor function tests like the rotarod and wheel-running have been used to detect early signs of MeHg neurotoxicity while overt neurological signs such as claspin reflex and ataxia come later. The goal of the current study was to examine age-related differences in the onset of MeHg toxicity, and the ability of dietary nimodipine to confer neuroprotection from chronic MeHg exposure. METHODS. Male BALB/c mice, ages 2.5 and 9 months (n=52 and n=62, respectively), were administered 0 ppm or 10 ppm Hg as MeHg in drinking water and 0 ppm or 200 ppm NIM in chow. Rotarod and wheel-running were evaluated approximately weekly and analyzed with 2 x 2 x 2 (Age x Water x Diet) between-group variables and exposure-duration as a repeated measure using linear mixed-effects. RESULTS. Older MeHg-exposed mice exhibited diminished rotarod performance before the younger MeHg-exposed mice did (exposure day 44 vs 86). Younger mice outperformed older mice overall. Wheel-running followed a similar pattern but with no age-related differences. Gross neurological signs such as claspin and hind-limb paralysis appeared later. Nimodipine delayed rotarod and wheel-running deficits in both age groups as evidenced by significant Age x MeHg x NIM x Exposure-day interactions. CONCLUSION. Chronic exposure to 10ppm MeHg alone decreased time spent on rotarod and distance run in an age-related manner. Chronic nimodipine delayed motor dysfunction in young and old animals but had no detectable effect of its own. Young mice outperformed older mice on rotarod. Rotarod and wheel-running revealed motoric deficits before the onset of MeHg-induced gross motor dysfunction. [Supported by NIH ES003299].
Brain Region-Dependent Effects of Methylmercury on Expression of Ligand and Voltage-Gated Calcium Channels in Rat

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Methylmercury (MeHg) causes brain region-specific toxicity and contributes preferentially to cell death in cerebellar granule cells (GCGs). MeHg disrupts synaptic transmission, resulting in alteration of Ca2+ homoeostasis, manifesting as elevated [Ca2+]i in both GCGs and motor neurons. Our aim was to compare the effects of low dose MeHg exposure on gene expression (measured via steady state mRNA levels) of subunits of voltage (VGCC) and ligand-gated ion channels, each of which could contribute to the MeHg-induced elevation of Ca2+. α1E, which increased. In BS, 30 d exposure increased significantly VGCC levels; after 30 d of clearing all IS alone. AMPAR GluR2 and GluR3 decreased in CB after 15 d exposure. After 30 d exposure and the clearing period only GluR3 increased. BS AMPAR levels increased after 15 d exposure, except GluR3. After 30 d exposure all AMPAR increased further, this increase persisted after the clearing period, except for GluR4. NMDAR levels increased in both the CB and BS after 30 d exposure. These observations demonstrate that MeHg affects gene transcription or stability of mRNA differentially for NMDAR, AMPAR, and VGCCs between themselves as well as in the CB and the BS. Decrease in VGCCs in the CB and increase in the BS, as well as in the BS, support the idea that the CB is one of the most susceptible targets of MeHg toxicity, maybe due to its inability to compensate for MeHg effects on these key genes.

Cord Expression of Glutamate Receptor mRNA

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In a mouse model of ALS methylmercury (MeHg) causes Ca2+-dependent excitotoxicity in motor neurons, an effect involving increased sensitivity of Glutamate ceptors (GluR), especially Ca2+-permeable AMPA receptors (AMPARs). The objective of this study was to determine if motor neuron GluR expression was increased by a subacute MeHg treatment in a non-ALS animal model. If so, this might indicate that these targets are especially sensitive to MeHg, and in a susceptible genotype (such as the ALS mouse model) lead to motor neuron toxicity. Quantitative real-time PCR (qPCR) was used to evaluate the NMDAR (NR1, NR2A, and NR2C) and AMPAR (GluR1, GluR2, GluR3, and GluR4) subunit mRNA expression. Sprague Dawley rats received 0.75, 1.5 mg/kg/day MeHg or 0.9% NaCl (control) sc. Treatment began at postnatal day 5 (PND5) and ended on PND81. Other groups of rats had an additional 30 days without treatment following the 30 days of MeHg exposure (PND65). RNA isolated from 10mg of lumbar and sacral spinal cord were submitted to reverse transcription PCR (RT-PCR). Resulting cDNA was used to perform qPCR. MeHg exposure led to alterations on the mRNA levels of the most abundant GluR subunits in spinal motor neurons. At 15 days, 0.75 mg/kg/day MeHg increased the expression of all the GluR subunits studied. The effect was similar in the 1.5 mg/kg/day exposure group. At 30 days, 0.75 mg/kg/day MeHg exposure increased the expression of all the subunits. In the 1.5 mg/kg/day exposure group a similar but less pronounced increase occurred. The 60 day rats that had the 30 day “clearing” period also had an increase in expression after MeHg exposure, except the NR2A subunit whose expression was decreased in both treatment groups. In conclusion, subacute MeHg exposure causes enduring changes in expression of GluR subunits in rats. These can affect the ability of motor neurons to regulate [Ca2+]i. In genetically susceptible organisms, this could result in motor neuron death by excitotoxicity. (Supported by NIH grants R01ES03299 and R25NS065777).

Plasma Thiol Antioxidant Barrier as a Potential Biomarker for Methylmercury Intoxication


The critical role of oxidative stress in the pathogenesis of methylmercury (MeHg) cytotoxicity has been clarified in vitro and in vivo. It is important to identify a biomarker for MeHg intoxication. In this work, we examined the following 3 plasma oxidative stress markers: diacron reactive oxidant metabolites (d-ROM), thiol antioxidant barrier, and biological antioxidant potential (BAP), by using a free radical elective evaluator in MeHg-exposed rats. The results were compared to those obtained from rats administered with 0.2% (w/v) lead (Pb) acetate or 15 ppm cadmium (Cd) chloride in drinking water. Male Sprague-Dawley rats were administered with 20 ppm MeHg in drinking water. The changes in the 3 plasma oxidative stress markers, histopathology, body weight (BW), and clinical features were examined every week. BW significantly decreased from 2 weeks after MeHg exposure in MeHg-treated rats compared to the controls. MeHg-treated rats exhibited hind limb crossing at 3 weeks after MeHg exposure. Histopathological changes were observed in the cerebellum of rats exposed to MeHg for 4 weeks. Among the plasma oxidative stress markers, the level of thiol antioxidant barrier decreased at 2 weeks after MeHg exposure; however, no histopathological changes were observed at this stage. The d-ROM levels increased 3 weeks after MeHg exposure, whereas BAP levels did not change during 4 weeks of MeHg exposure. Although rats that were exposed to Pb for 4 weeks showed pathological changes in the hippocampus, no changes were observed in the plasma oxidative stress markers. Cd-treated rats also showed no changes in plasma oxidative stress markers. The high affinity of MeHg for the selenohydryl group, sulfhydryl group, or selenide may cause an early decrease in the level of plasma thiol antioxidant barrier. In contrast, oxidative stress in plasma may be delayed although Pb and Cd are known to induce oxidative stress in tissues. Our results suggest that thiol antioxidant barrier level in plasma is a potential biomarker for MeHg intoxication.
ArsenicInduces Reactive Oxygen Species-Caused Neuronal
Cell Apoptosis through JNK/ERK-Mediated Mitochondria-
Dependent and GRP78/CHOP-Regulated Pathways

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Recently, epidemiological studies have suggested a possible relationship between inorganic As (iAs) exposure and neurodegenerative disease development. However, the effects and underlying mechanisms of iAs-induced neuronal cell injuries are most unknown. The present study demonstrated that iAs significantly decreased cell viability and induced apoptosis in Neuro-2a cells. iAs also increased oxidative stress damage and induced several features of mitochondria-dependent apoptotic signals. Pretreatment with the antioxidant N-acetylcysteine (NAC) effectively reversed these iAs-induced responses. iAs also increased the phosphorylation of JNK and ERK1/2, but did not that p38-MAPK, in treated Neuro-2a cells. Additionally, exposure of Neuro-2a cells to iAs triggered endoplasmic reticulum (ER) stress identified through several key molecules (GRP78, CHOP, XBP-1, and caspase-12), which was prevented by NAC. Transfection with GRP78- and CHOP-specific si-RNA dramatically suppressed GRP78 and CHOP expression, respectively, and attenuated the activations of caspase-12, -7, and -3 in iAs-exposed cells. Therefore, these results indicate that iAs induces ROS causing neuronal cell death via both JNK/ERK-mediated mitochondrial-dependent and GRP78/CHOP-triggered apoptosis pathways.

Identification of Novel Proteomic Biomarkers for Heavy-
Metal-Induced Nephrotoxicity

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Long-term exposure of low-dose heavy metals are well-established risk factors against human health. Among various organs, the kidney is one of the most sensitive targets for heavy metal-associated toxicity. Development of novel biomarkers is essential to evaluate and predict nephrotoxicity following exposure to low-dose heavy metals. Here we tried to identify new biomarkers using proteomics approaches in kidney tissues isolated after heavy metal exposure. Male Sprague-Dawley rats received mercuric chloride (HgCl2) and cadmium chloride (CdCl2) by oral administration. Proteomic analysis with kidney samples revealed that expression of several proteins was elevated by Hg and Cd exposure, suggesting them as potential candidates for new biomarkers. We further measured the change of new biomarkers in renal tubular cells following heavy metals exposure. Interestingly, in normal rat kidney proximal tubular cells (NRK-52E), the change of expression level of these new candidates were well correlated to the extent of cytotoxicity induced by heavy metals. Notably, the increase of new biomarkers was observed in drug-exposed tubular cells, suggesting the potential use of these biomarkers. The change of the new biomarkers was comparable to that of traditional biomarkers for renal toxicity, such as Kim1, Lcn2, Spp1 and TIMP1. With this study, we identified new protein biomarkers as novel candidates for drug or heavy metal-induced renal toxicity. Superior correlation between cytotoxicity and the extent of these protein changes suggests that they can be useful biomarkers to evaluate or predict renal toxicity.

Expression of Copper (Cu) Transporting Genes in
Subventricular Zone (SVZ) and Choroid Plexus (CP)

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Our recent data using X-ray fluorescent microscopy suggests an age-related increase of Cu along the wall of brain ventricles called SVZ. Since the regulation of cellular Cu homeostasis involves a variety of Cu transporters, chaperones and intracellular binding proteins, it becomes necessary to understand the expression of these Cu regulatory proteins and how they contribute to the high Cu concentration in SVZ. Anatomically, SVZ is in direct contact with cerebrospinal fluid (CSF) secreted by the CP where the blood-CSF barrier (BCB) is located. This study was designed to compare the gene expression of Cu regulatory proteins in SVZ and CP. SVZ and CP tissues were dissected from brains of 3-wk, 10-wk or 9-m old male Sprague-Dawley rats and SVZ tissues from 3-wk-old rats were used for the primary cultures of neural stem cells (NSCs) and choroidal epithelial cells (CECs). Target genes were first identified through quantitative real-time RT-PCR and normalized with the internal reference gene Actb. Our data showed that the expression level of Cox17 was the highest in SVZ, followed by Ctr1 and Dmt1, while Ctr1 was expressed the most in CP followed by Cox17 and Dmt1. In NSCs cultures. However, a comparison of gene expression, Cox17, Dmt1 and Ctr1 were about 2-, 4-, and 60-fold lower in SVZ than in the CP, respectively (p<0.05). Higher expression levels of Ctr1 and Dmt1 were observed in CP of 9-m old rats than 3- and 10-wk old animals (p<0.05). Ap1b7 had the lowest expression in both SVZ and CP of all animals. Furthermore, the expression level of Dmt1 was higher in NSCs than CECs (p<0.01), while CECs expressed more Ap1a7 and Ap1b1 than those in NSCs (p<0.05). These findings suggest the Cu regulatory gene expression pattern in SVZ is different from those in CP; furthermore the age appears to influence the expression of these Cu regulatory genes. (Supported in part by NIH/NIEHS ES-008146)
Avoidance rates for shock exposures were calculated for the first and second halves (30 minutes each) of each session. There was no significant difference in mean body weight at 6 weeks of age between the TBT group and the control. A significantly lower mean value of the avoidance rates in the TBT group compared with that in the control was observed in the first 30 minutes of the sessions on day 1. The mean values of the avoidance rates in the first and second 30 minutes in the TBT group were lower than those in the control from days 2 to 8. These results indicate that the learning ability was impaired in the F1 THA rats exposed to TBT at 50 ppm in their dams’ food.

1392a Chronic Lead Exposure Reduces Presynaptic Vesicle Availability in Mossy Fiber-CA3 Synapses and Shaffer Collateral-CA1 Synapses in the Rat Hippocampus

Lead (Pb2+) exposure has been shown to impair presynaptic neurotransmitter release in both in vivo exposed animals and in cell culture models. The mechanism by which this impairment occurs has not been fully elucidated. However, our lab has previously shown that Pb2+ exposure during synaptogenesis inhibits vesicular release and reduces the number of fast-releasing sites in cultured hippocampal neurons (Neal et al., 2010). We hypothesized that the impairment of vesicular release and the reduction in the number of fast-releasing sites may be associated with reductions in the number of vesicles docked in the presynaptic active zone (PAZ).

In the present study, we used transmission electron microscopy to examine presynaptic vesicle pools in Perforam Path-Dentate Gyrus, Mossy Fiber-CA3, and Shaffer Collateral-CA1 synapses to determine if in vivo Pb2+ exposure altered synaptic vesicle number. Vesicles within the presynaptic axon terminal were counted and the distance between the vesicle and the PAZ measured using LoClust (Nikonlenko and Skibo, 2004). Vesicles were classified as docked if they contacted the PAZ, rapidly recycling if they were 200 nm or less from the PAZ, or resting if they were more than 200 nm from the PAZ. We found that Pb2+ exposure significantly reduced the number of docked vesicles in Mossy Fiber-CA3 and Shaffer Collateral-CA1 synapses and the number of vesicles in the rapidly recycling pool of Mossy Fiber-CA3 synapses. These data suggest that Pb2+ exposure interferes with presynaptic neurotransmitter release by reducing the number of vesicles available for release. These findings provide a novel mechanism by which Pb2+ exposure impairs vesicular release. Further, this study confirms our previous findings in hippocampal neurons in culture and corroborates current work in which in vivo Pb2+ exposure impairs vesicular release. These findings provide a novel mechanism by which Pb2+ exposure impair vesicular release and reduces the number of fast-releasing sites in cultured hippocampal neurons and, subsequently, with the alimentary tract.

1394 Safety Considerations for the Use of Nanomaterials in Food and Food-Related Products: A US FDA Regulatory Perspective
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The US FDA has been developing nanomaterial-related industrial guidance that cover foods, food contact materials and food ingredient containing products over which the Agency has regulatory authority since the publication of its July 2007, report on nanotechnology. In addition, FDA is conducting research to gauge the utility of our currently recommended safety testing paradigms for assessing the potential for food-related nanomaterials to produce toxicity and/or carcinogenicity. Research is underway at the CFSAN and the National Center for Toxicological Research (NCTR) to evaluate the recommended standard battery of in vitro tests when used with nanoparticulates and nanocoercipated products, and to identify possible replacement tests. Early results indicate that this standard safety assessment panel may need to be augmented with other in vitro tests, and that at least one or more of the current safety assessment assays may need to be replaced. In vivo studies are also being conducted by NCTR, in collaboration with CFSAN, to assess the potential for orally administered nanosilver to produce toxicity. These studies are also serving as a platform for the development of safety assessment methodologies for in vivo studies with nanomaterials. In addition, FDA is conducting research to assess whether nanomaterials are being incorporated into products over which it has little regulatory authority, such as dietary supplements. Analytical fieldwork conducted by the FDA Office of Regulatory Affairs indicates that certain marketed dietary supplements contain nanomaterials either as nanoparticulates or as nanocoercipated products. While research on the safety assessment of orally available nanomaterials in food-related products is ongoing at FDA and elsewhere, this research is moving ahead at a relatively slow pace. This slow progress may be in part due to the lack of information on how nanomaterials interact with food matrices and, subsequently, with the alimentary tract.

1395 NanoRelease Food Additive Project—Developing Methods to Measure Characteristics Relevant to Nanomaterial Uptake from Food
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Nanoscience is providing valuable knowledge of particle toxicology; however, it is unclear how to use this knowledge to distinguish “engineered” particulate materials within a context of the particulate nature of traditional foods. When does a change in characteristics at nanoscale distinguish a particle from background particles? Should nanomaterials used at early stages in food processing be considered nanomaterials at ingestion if they have dissolved or bound to other food components? How do we know when the knowledge gained from pristine, extracted, dispersed nanomaterials in dose response evaluations applies to the transformed, aggregated, bound materials in food that reaches our mouths? The need for safe innovation coupled with a growing awareness of particulate toxicity has brought a diverse stakeholder group together in the NanoRelease project to the task of filling a basic methods gap in our ability to understand what is “nano” in the food we eat. Findings that lay a path toward addressing these questions will be presented from white papers prepared by the more than 70 experts of the project. The findings include a practical and neutral view of what may be nanoparticulate in food and what methods are needed to prioritize attention across the different types of particles of foods.

1396 Uptake and Distribution of Ingested Nanomaterials
S. M. Roberts, University of Florida, Gainesville, FL.

Engineered nanomaterials can be added directly to food to produce a beneficial effect in the consumer (e.g., increase the absorption of a nutrient) or a beneficial effect on the food itself (e.g., increased shelf life, enhanced flavor). Nanomaterials can also be introduced into food incidentally when used to improve food processing. The safety of nanomaterials in food depends in part on what happens when they enter the dynamic environment of the GI tract, and ultimately on the extent to which they are taken into the body and distributed. There are several challenges to assessing nanomaterial behavior and interactions in the GI tract. Some are common to studies of the potential toxicity of nanomaterials in general, including inconsistencies in the nanomaterials as manufactured, uncertainty as to how to express the nanomaterial dose or concentration, and difficulties in characterizing the nanomaterial, as well as in detecting and measuring them in biological systems. Other challenges are unique to the gastrointestinal tract, including an environmental that changes with respect to: pH, ionic strength, nature and composition of the sur-
ruling food matrix, anatomical absorptive surfaces, physical/chemical barriers to absorption (mucin layer), and microorganisms with which to interact as the nano-material transits the gut. The rate and location of dissolution or breakdown within the GI tract is likely to be an important factor in determining potential biological effects for some nanomaterials. Existing studies offer some insights into nanomaterial properties that can influence uptake from the GI tract and distribution in the body, but much work remains before the critical processes are well understood. In addition to the potential for systemic toxicity following uptake of nanomaterials (or dissolution or degradation products), local effects (e.g., cytotoxicity to cells lining the GI tract) and indirect effects (e.g., through effects on gut microflora or interference with absorption of nutrients) are also possible and merit consideration, at least for some nanomaterials.

1397 High-Throughput Testing Using a 3-Cell Gut Model for Hazard Identification of Nanomaterials in Food Products

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The need to develop in vitro model systems for rapid and cost-effective screening of agricultural products is becoming increasingly necessary for the safety of food for human and animal consumption. This talk describes a 3-cell gut model developed for assessing the effects of nanomaterials and other man-made materials on bacterial biofilm and mammalian cells—the two types of cells found in the gut of humans and most animals. The system also probes the effects of a disrupted biofilm on normal epithelial cell function. Engineered nanoparticles are already being incorporated for innovative food packaging applications. Given the scenario of incorporating nanoparticles into food matrices, two distinct exposure populations and consequently two exposure routes can be easily identified: 1) Occupational exposures of industrial workers, by way of aerosolized nanoparticles, involved in the manufacturing of nano-enabled packaging materials. A reasonable exposure route for this scenario would be via inhalation and further digestion of the nanoparticles that remained in the pharyngeal cavity. 2) Consumer that ingest nano-enabled foods and possibly nanoparticles that may be released from food packaging. A common thread that links both the exposure populations and the two exposure routes is that the nanoparticles will eventually be "digested" and reach the small intestines of the gastrointestinal (GI) tract. In order to explore the physicochemical changes that nanoparticles go through during digestion and how these changes affect the digestive system and the commensal microbes that inhabit the human small intestine, we present a model that uses a gut co-culture model incorporating the Caco-2 cell line, HT-29 cells and a biofilm of commensal Escherichia coli. In this study, commonly used metal oxide and colloidal metal nanoparticles, some of which are known to have antimicrobial properties and have been used for nano-enabled packaging production are evaluated to study the physicochemical changes that such nanoparticles go through during digestion.

1398 Adverse Outcome Pathways: A Conceptual Framework for 21st Century Risk Assessment

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An Adverse Outcome Pathway (AOP) represents existing knowledge concerning the linkage between a molecular initiating event and an adverse outcome at the individual or population levels (Ankley, Bennett, et al. 2009). As such, it is a conceptual framework that describes known linkages between a chemical-induced initial molecular event that alters biochemical cellular processes that cascade via a series of key events observed as cellular, tissue, and anatomical changes, that ultimately culminates in an adverse outcome of relevance to human or ecological health. This workshop will provide a regulatory rationale for using AOPs, along with the types and amounts of data needed to construct an AOP. Further, the level of confidence in an AOP will be explored for usage in regulatory decision-making, whether priority setting for chemical toxicity testing or hazard prediction. The practical utility of AOPs for risk assessment of chemicals will be illustrated using three case study examples.

1399 Creations and Use of AOPs: Progress and Prospects

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The AOP concept has its origins in ecotoxicology but is now receiving increasing attention for its applicability to human health effects. In this respect an AOP is essentially indistinguishable from a mode of action (MOA) for a toxic effect. Hence, the general principles of one should apply to the other. These include identification of necessary and measurable key events, the molecular initiating event comprising an early such event, the use of weight of evidence such as the modified Bradford Hill considerations proposed by WHO for evaluation of key events, the rigorous exclusion of alternative pathways, the biological linkage of key events to an adverse outcome and the systematic qualitative and quantitative comparison of key events in model systems with those in humans, as appropriate. In human health assessment AOPs/MOAs were initially used in valuation of a rodent carcinogenic response to humans where a qualitative conclusion was sought. With the extension of the approach to non-cancer endpoints, quantitative information obtained on intermediate events can be invaluable in dose-response assessment for human risk. There is a need to agree the core information necessary to establish a robust AOP, and for a central repository of such AOPs and the intermediate events involved. OECD with support of other organisations is currently preparing such a repository. Establishment of AOPs will be invaluable in the design of new evaluation methods. AOPs will provide a necessary bridge between such methods and systems approaches to risk assessment. There are still conceptual barriers to the AOP/MOA approach, with a failure to appreciate that primarily it is a means to organise information in a coherent, transparent manner, on the basis of explicit lines of evidence.

1400 Adverse Outcome Pathway (AOP) for Skin Sensitization

G. Patlewicz, DuPont Haskell Global Center for Health & Environmental Sciences, DuPont, Newark, DE. Sponsor: J. Rowlands.

Skin sensitisation is a significant environmental and occupational health concern. As such, there exists an important need to accurately identify chemicals that have the potential to cause skin sensitisation. Assessment of skin sensitisation is still heavily reliant on the use of animals, in particular the local lymph node assay (LLNA) since no regulatory accepted alternative method exists. Despite the obvious complexity of the endpoint, quite a detailed mechanistic understanding both in terms of the underlying biological process and chemistry has been developed which has helped direct research into alternative (non-animal) approaches. Recently the OECD developed an Adverse Outcome Pathway (AOP) that describes in detail the key events underpinning skin sensitisation. Making the collective knowledge of the skin sensitisation process explicit in this way provides an invaluable roadmap to guide method development, evaluation and application. Indeed the OECD has also implemented this AOP into the OECD Toolbox, a tool for the development, evaluation and justification of chemical categories for read-across. This presentation will outline the utility in developing Integrated Testing Strategies (ITS) and read-across by way of practical examples.

1401 Thyroid Hormone Adverse Outcome Pathway-Based Screening Assays for Thyroid-Disrupting Chemicals


Disruption of thyroid hormones (THs) adversely affects neurodevelopment in both rodent models and humans. Therefore it is critical to determine the potential thyroid-disrupting activity of thousands of environmentally-relevant chemicals. However, the complexity of thyroid hormone regulation necessitates screening assays for multiple molecular initiating events that are not currently associated with any screening assays. The amount of detail and linearity characterizing thyroid pathways between molecular initiating events and adverse outcomes varies substantially, both as a function of existing knowledge and application needs. Development of AOPs provides insight into the uncertainties in linking chemical use, exposure and outcome, thereby focusing research on critical data needs. Example applications include the impact of thyroid disruption on neurodevelopment where AOPs have led to more efficient predictive testing and reduced uncertainty in extrapolation of effects between rodents and humans. Well defined thyroid AOPs also provide the molecular targets and key events that foster focused development of high-throughput screening models. Lastly, AOPs provide a utilitarian framework for future efforts to build quantitative and qualitative models useful in understanding the degree of perturbation associated with adverse outcomes. This abstract does not necessarily reflect US EPA policy.
A key physiological process controlling reproductive success of oviparous vertebrates (fish, amphibian, reptiles, birds) involves production of the egg yolk protein precursor vitellogenin (VTG). VTG production is an estrogen receptor (ER)-mediated process that, in females, is controlled by the production of 17β-estradiol. As such, environmental contaminants (including several pesticides and pharmaceuticals) that affect steroid synthesis can impact reproduction in fish through their ability to inhibit VTG production. Given both physiological importance and sensitivity to chemical stressors, there has been a significant amount of effort expended on developing well-characterized adverse outcome pathways (AOPs) relating steroid synthesis and VTG production to reproductive impacts in individuals and populations of fish. This presentation will provide an overview of the research conducted to derive and evaluate these AOPs from a weight-of-evidence perspective, including characterization of molecular initiating and key events and documentation of linkages between these events. The talk will also discuss practical applications of the AOPs to different facets of both prospective and diagnostic ecological risk assessments.

Risk assessors continue to be faced with significant challenges due to the increase in technology and complexity of endpoints and datasets to be considered in understanding the potential for chemical exposure to result in adverse impacts on humans or the environment. This workshop will bring scientists from multiple disciplines together to discuss these challenges for both human and ecological risk assessment and the potential for innovative solutions. The goal of this workshop is to present the latest thinking on issues significant to the practice of risk assessment today in a forum that allows for expanded thinking and an exchange of ideas. New concepts and older concepts that are being revisited to address new challenges will be presented. Topics will include the critical need for interoperability of data and models for risk assessment, consideration of variability and uncertainty in risk assessment, weight-of-evidence approaches, challenges in dose-response modeling, and the current and future guidance from the National Research Council on risk assessment issues as we move forward in the 21st century. This workshop will be the second collaboration between members of the SOT Risk Assessment Specialty Section and the SETAC Human Health Risk Assessment Advisory Group and builds on the concepts presented in a workshop developed by these two specialty sections in 2012. The 2012 workshop was titled “Concepts Critical to the Next Generation of Human Health and Ecological Risk Assessment” and highlighted some of the challenges facing the next generation of risk assessors. The goal of that workshop was to provide information on new programs and approaches within regulatory agencies, as well as in the private sector, that rely on the integration of human, animal, or ecological data.

Risk assessment has become a dominant tool for guiding public policy in protecting public health and the environment. In the last decades of the twentieth century, the National Research Council’s Board on Environmental Studies and Toxicology (BEST) produced a number of reports guiding origins and development of the field of risk assessment—in particular the famous “Red Book.” In the first decade of the twenty-first century, BEST carried out three studies that resulted in reports that redefined and advanced the field of risk assessment as it enters the next century. These reports include “Toxicity Testing in the 21st Century: A Vision and a Strategy,” issued in 2007, “Science and Decisions: Advancing Risk Assessment,” in 2009, and “Exposure Science in the 21st Century: A Vision and a Strategy,” in 2012. In this presentation I will briefly review the key recommendations from these reports and consider the implications of these recommendations for the preparation and interpretation of risk assessment as tool for informing environmental health science.

With the increase in the types of chemical-specific quantitative data available, dose response assessment is becoming a complicated task, requiring the consideration of more information than a single data set. One of the most controversial areas in dose response modeling is related to understanding when the integration of the available data are sufficient to suggest an approach other than default, or linear modeling approach. This is a critical science policy decision and opinions are very different on what the appropriate answer should be. Nuances in EPA’s 2005 Cancer Guidelines have led to different interpretations. Added to the complexity of a modeling approach are the challenges in addressing and accounting for the endogenous production of chemicals in the body. While novel approaches have been proposed, they are not yet universally accepted. Other challenges in dose-response include understanding whether effects are adaptive, compensatory or adverse and determining how to characterize toxicity that is due to less than lifetime exposures. Finally, the stakeholders’ challenge may be developing consensus around an approach for addressing cumulative risks. This talk will use specific examples to discuss existing challenges, proposed solutions, and opportunities that may exist to more fully develop consensus within the risk assessment community.

Risk assessors continue to be faced with significant challenges due to the increase in technology and complexity of endpoints and datasets to be considered in understanding the potential for chemical exposure to result in adverse impacts on humans or the environment. This workshop will bring scientists from multiple disciplines together to discuss these challenges for both human and ecological risk assessment and the potential for innovative solutions. The goal of this workshop is to present the latest thinking on issues significant to the practice of risk assessment today in a forum that allows for expanded thinking and an exchange of ideas. New concepts and older concepts that are being revisited to address new challenges will be presented. Topics will include the critical need for interoperability of data and models for risk assessment, consideration of variability and uncertainty in risk assessment, weight-of-evidence approaches, challenges in dose-response modeling, and the current and future guidance from the National Research Council on risk assessment issues as we move forward in the 21st century. This workshop will be the second collaboration between members of the SOT Risk Assessment Specialty Section and the SETAC Human Health Risk Assessment Advisory Group and builds on the concepts presented in a workshop developed by these two specialty sections in 2012. The 2012 workshop was titled “Concepts Critical to the Next Generation of Human Health and Ecological Risk Assessment” and highlighted some of the challenges facing the next generation of risk assessors. The goal of that workshop was to provide information on new programs and approaches within regulatory agencies, as well as in the private sector, that rely on the integration of human, animal, or ecological data.
and there is an uncertainty factor assigned to address human variability. Exposure assessments have historically focused on defining a reasonable upper bound to the range of exposures that occurs because of variability in human behavior and spatial and temporal variation in the release, transport, and removal of chemicals in the environment. This risk assessment must make decisions under limited information, since the mechanisms of action for toxicity and the key data on exposures may not be known. In addition many risk questions involve prediction of future exposures which cannot be measured. Arguably the entire field of risk assessment can be described as an attempt to make robust decisions under uncertainty. New tools in toxicology and exposure assessment promise to improve risk assessment by reducing uncertainty and by better characterizing the sources of variation. Advances in the field of toxicology promise to improve our understanding of the dose-response of key events that occur early in adverse outcome pathways. Such data can replace the current approach of evaluating every apical endpoint before setting a point of departure. Advances in modeling variation in adsorption, dispersion, metabolism, and excretion of chemicals are improving our ability to perform dose response modeling across human populations of varying ages. Advances in exposure information facilitated by improved monitoring and reporting technology promises to improve our understanding of how variation in human behavior affects exposure. Perhaps just as important, the rise of integrated information systems that facilitate sharing toxicity and exposure data and advances in behavior monitoring technology.

**W 1409 Multiscale Modeling: Data Integration and Interoperability**

A. M. Jarabek, NCEA, US EPA, Research Triangle Park, NC.

Risk assessment requires the analysis, translation, and integration of data from various disciplines (e.g., exposure and epidemiology), which often represent different levels of observation (e.g., individual versus population), in order to determine the potential for exposure and health effects at multiple scales such as the subcellular, individual, population, or community levels. An appreciation of the need for collaboration across disciplinary silos spanning the continuum from source -> exposure -> dose -> response -> cost/benefit is advancing due to the call for sustainability characterizations and the recent decision by the World Health Organization (WHO) to include consideration of ecosystem services in the definition of human health. Additionally, advances in biotechnology and systems modeling are resulting in increasingly complex repositories that require integration and management of large amounts of data to understand different adverse outcome pathways and to more accurately predict and diagnose factors contributing to disease. Interoperability, defined as the ability of two or more diverse systems or components to exchange information and use the information that has been exchanged in new applications, is thus necessary to support regulatory risk applications and tractable decision support. Considerations and challenges for interoperability in two domains, cultural (e.g., disciplinary assumptions, “narratives”, practices, and defaults) and technical (e.g., hardware, software such as models, standards, and data management) are highlighted by a case study of vinyl chloride hepatotoxicity along with recommendations from on-going efforts that evolved from the 2012 SOT CCT meeting. (The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.)

**W 1410 Contribution of Nonimmune Cells to Adverse Immune Responses: Implications for Toxicology**

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The potential for direct adverse effects of xenobiotics on the immune system is widely recognized. These intrinsic effects are often due to expression of target receptors for xenobiotics on immune cells or alteration of intracellular processes. However, novel evidence supports that cellular changes induced by xenobiotics in nonimmune cells can alter the competence of the immune system. Organs outside of the immune system are increasingly recognized for their ability to initiate and shape an immune response. These organs include, but are not limited to, the central nervous system, the mucosal areas such as the lung and the gut, the liver, and the cardiovascular system. The role of toxicants and their ability to modulate organ systems, thereby initiating or altering an immune response resulting in immunotoxicity, is poorly understood. Research highlighting how toxicant-induced changes in tissue physiology can result in immunotoxicity of the immune system will be discussed. The focus of presentations will address the considerations for modeling and for assessing toxic responses and explore putative mechanisms.
The Role of Epithelial Cell-Derived Hypoxia Signaling in Establishing the Inflammatory Response to Allergens

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Recently, our laboratory created an inducible, lung epithelia-specific hypoxia inducible factor 1α (HIF1α) knock out mouse, which has abnormal responses to inhaled cobalt and ovalbumin (OVA). For example, when the HIF1α-deficient mouse is exposed to cobalt it displays an asthma-like phenotype. In contrast, control mice display a Th2-mediated inflammation, similar to hard metal lung disease. Moreover, these HIF1α-deficient mice display an exacerbated response to the OVA challenge model of murine asthma. This suggests that loss of HIF1α in the lung epithelia biases the lung towards a Th2-mediated inflammation. Interestingly, to promote this change in innate immunity, recombination of HIF1α in the epithelial cells must be induced in the first two weeks of life. These results demonstrate that early childhood epithelial-derived HIF1α signaling is important in establishing the inflammatory response of the lung, presumably by regulating the transcription of key secreted immune factors. To identify these factors, a coculture system has been utilized to characterize the ability of epithelial-derived signaling (e.g., HIF1α specific and others) to influence immune cell function. These results highlight the need to assess early life events, such as premature birth and early viral exposure, which might permanently affect HIF1α signaling in the airway epithelium and thus alter the risk of toxicant exposure as adults. Finally, our future directions will focus on characterizing HIF1α-dependent secreted immune factors in the development of the lung’s immunity using a combination of in vitro and in vivo models. The ultimate goal is to determine how epithelial-derived HIF1α-mediated signaling can impact immune cell populations and their function and link these changes to susceptibility to atopic diseases, such as asthma, later in life.

Indirect Xenobiotic Immune Modulation: Risk Assessment

L. P. Myers, US FDA, Silver Spring, MD.

Xenobiotics can modulate the immune responses indirectly through various mechanisms. Occasionally, this indirect modulation can be difficult to assess or evaluate nonclinically. Limitations are commonly attributed to species specificity to targets that may not be expressed similarly between humans and nonclinical models. Even though the safety of these products may be difficult to assess, there are still pathways forward for these pharmaceuticals. Following existing guidance (ICH M3 (R2) or ICH S6 in conjunction with ICH S8), immunomodulatory drugs can be evaluated for many immune alterations prior to human exposure. However, if the target (e.g., receptor or ligand) is species specific and does not allow for adequate safety assessment, then alternative models may be necessary to evaluate the safety of these products. In these cases, increased human monitoring and a more conservative approach may be suggested, depending on the pharmaceutical. Careful consideration of the drug product, PD effects in vitro as well as in vivo models, as well as a case-by-case approach is the most common pathway for developing drugs that have unique immunomodulatory mechanisms. The development of novel nonclinical evaluations utilizing existing guidances as well as product specific assessments (a case by case approach) are used to evaluate the safety of immunomodulatory products during risk assessment of human pharmaceuticals.

Developmental Toxicity from Chemical Mixtures: Research to Application in Susceptible Populations

D. I. Carlin1 and M. Mumtaz2.1 NIEHS, Research Triangle Park, NC and 2ATSDR, Atlanta, GA.

The study of environmental chemical mixtures is highly complex and requires sophisticated approaches to determine which mixture component(s) contribute to health effects. Consideration of sensitive subpopulations such as pregnant mothers, the developing fetus, infants, and children, significantly increases the complexity due to chemicals exhibiting different effects depending on the timing of exposure. For example, it is well known that exposure to mixtures of inorganic metals (e.g., lead, cadmium, mercury, arsenic) and pesticides leads to neurological and other organ-specific health effects in adults, but the suite of health effects associated with these mixtures in the developing fetus and infant could differ from that of adults and is less understood. This workshop will focus on the health effects of chemical mixtures with particular attention to exposure to pregnant mothers, the developing fetus, and children, and will include discussion of toxicological studies, statistical and modeling approaches, and application to human health risk assessment in these susceptible populations. There is an impending need to understand interactions among components of mixture exposures in order to improve human health risk assessments in these susceptible populations.

Chemical Mixtures, Developmental Windows, and Neurodevelopment

R. O. Wright, Mount Sinai School of Medicine, New York, NY.

Low level mixed exposures likely influence developmental trajectories and program later life health effects. Understanding mixed exposures requires prospective study designs to capture critical developmental windows of exposure and complex statistical approaches to assess whether combined exposures are synergistic in toxicity. In addition, mixtures studies often evaluate 2 or 3 way interactions, but methodologies are needed that can address higher order interactions. This presentation will include data on ongoing longitudinal studies of metal mixtures and childhood neurodevelopment conducted in 3 distinct birth cohorts. As an example, in a preliminary analysis of 350 children (final N = 829 when study is complete) Bayley Scales of Infant Development in Bangladeshi children and metal exposure (As, Pb, Mn), we used adjusted multivariable Kernel Machine regression to assess the joint impact of these 3 metals. This type of model will test the best fit of the overall interaction data and allows for non-linear relationships between random variables. Splines will be used to present above results graphically, as multi-dimensional nonlinear interactions are difficult to present as simple beta coefficients. Lead and the middle tertiles of arsenic, and manganese exposure compared to the lowest tertiles were inversely associated with 24-month Bayley scores (β=-0.71, p=0.01; β=−2.13, p=0.02; β=−1.25, p=0.061, respectively). There was evidence of lead-arsenic interaction. Lead toxicity was increased among children in the third tertile of arsenic exposure, compared to children in the first tertile of arsenic interaction (β = −0.35, p=0.006). Further, nonparametric splines were used to explore non-linear effects of As and Mn, which were detected as an inverted-U relationship between manganese and the Bayley score at the second arsenic tertile (p=0.0003). Overall, these preliminary findings suggest that non-linear relationships exist within mixed exposures and statistical methodologies to detect such relationships should be employed.

Toward the Rational Use of Exposure Information in Mixtures Toxicology


Of all the disciplines of toxicology, perhaps none is as dependent on exposure information as Mixture Toxicology. Identifying real world mixtures and replicating them in the laboratory (or in silico) is critical to understanding their risks. Complex mixtures such as cigarette smoke, diesel exhaust, and disinfection byproducts may be replicated without difficulty because they are uniquely associated with reproducible processes such as combustion. On the other hand, chemical mixtures that arise from multiple sources are less tractable. Toxicologists often are faced with developing ad hoc rules for constructing test mixtures, or they simply test a few binary combinations. We examined monitoring data for pesticides in daycares (CCCP and homes) and the evidence that points to patterns in how they
group. We applied approaches from the field of community ecology to test if these assemblages of "chemical species" are random or structured. Presence-absence matrices developed from CCC and AHHS datasets indicated structure comparable to the West Indian Finch matrix when species diversity metrics were applied; namely, the COMBO metric (number of unique combinations) and CHECKER metric (number of 2x2 checker matrices). This finding indicated that factors (e.g., social, economic, and technical) limit the spectrum of pyrethroid combinations observed in these datasets. Additional methods were used to identify frequently occurring k-way combinations from the CCC dataset. These approaches were used to inform pharmacokinetic studies and a cumulative probabilistic exposure-dose assessment.

**W 1420 Application of a Weighted Quantile Sum Approach to Identifying Bad Actors in Mixtures of Environmental Chemicals: A Transgenerational Study of Reproductive Hazards**

C. Gennings, Virginia Commonwealth University Medical Center, Richmond, VA.

Risk evaluation of environmental chemical exposures is impacted (i.e., inflated variance, confounding) by the complex correlation structure among components of chemical mixtures. We have developed a novel, empirical weighted quantile sum (WQS) method for identifying 'bad actors' in correlated data. Characterization of the method (reliability, validity) will be presented using simulated data. The approach will be demonstrated using fecundability as a measure of reproductive hazard in a transgenerational study of PCBs. We compared the results of WQS regression to those based on categorization of PCB compounds by (1) hypothesized biological activity previously proposed and widely applied, and (2) degree of ortho-substitution (mono, di, tri), in a study of the relation of maternal serum PCBs and daughter's time to pregnancy (TTP). The dominant functionality groups associated with longer TTP were the dioxin-like, anti-estrogenic group (average weight, 22%) and PCBs not previously classified by biological activity (54%). In contrast, the unclassified PCBs were not important in the association with shorter TTP, where the anti-estrogenic groups and the PB-inducers group played a more important role (60% and 23%, respectively). The highly chlorinated PCBs (average weight, 89%) were mostly associated with longer TTP; in contrast, the degree of chlorination was less discriminating for shorter TTP. Finally, PCB 56 was associated with the strongest relationship with TTP with a weight of 47%. WQS regression found some associations previously identified by two classification schemes, but also identified other bad actors. This empirical method can generate hypotheses about mixture effects and mechanisms and overcomes some of the limitations of standard regression techniques.

**W 1421 A Risk Assessment and Regulatory Agency Perspective on Mixtures Affecting Susceptible Populations**

M. L. Dourson, Toxicology Excellence for Risk Assessment, Cincinnati, OH.

In one sense, the study of mixtures to susceptible populations is easy. Since we are all exposed to mixtures of chemicals and nonchemical stresses, one needs to simply be observant. Unfortunately, the mixtures to which many of us are exposed (e.g., oxygen and arsenic) are not really of much interest to regulators. Of more interest are exposures to mixtures, especially to sensitive subgroups, about which toxic effects are known and for which we might be able to do something, such as the concentrations of methylmercury and PCBs in fish. This presentation will start with a brief explication of existing methods that allow risk scientists to gauge the safety of chemical mixtures, and then highlight comparative toxicokinetic and toxicodynamic data between adults and children, which can be used to improve the estimation of safety and risk to susceptible populations from chemical mixtures and nonchemical stressors. Our analysis of Seychelles and Faroe Islands cohorts revealed differing intakes of PCBs and methylmercury, and using benchmark doses (BMDs) analysis, revealed the presence in children or absence in adults of neurological effects. The impact of our findings on the development of a mixture Reference Dose (RfD) and nonchemical stressors in these populations will be discussed.

**W 1422 Somatic Cell Therapy—Paradigms for Investigational New Drug (IND)-Enabling Programs, Scientific and Regulatory Considerations, and Clinical Translation**


There is significant interest in somatic cell therapies and their therapeutic potential to stimulate repair in diseased or injured tissue. Development of these therapies has been hindered by lack of knowledge of appropriate methods for assuring patient safety in clinical trials. Standard pharmacological and toxicological methods may not apply in safety evaluation of somatic cell therapies. Animal model selection, and safety concerns to be assessed, varies greatly depending upon clinical indication, source and type of cell therapy, and method of cell therapy delivery. The aim of this workshop is to provide insight into successful paradigms for IND-enabling studies supporting clinical evaluation of somatic cell therapies. Speakers with both regulatory and academic/industry development expertise in somatic cell therapy will provide presentations of program experience. The overall goal is to aid current investigators in the somatic cell therapy field, and to facilitate and expedite the development of somatic cell therapies in clinical medicine by sharing lessons learned from previous experiences.

**W 1423 Developing Cell Therapy Products: US FDA Preclinical Regulatory Considerations**

P. Au, Office of Cellular, Tissue and Gene Therapies, Center for Biologics Evaluation and Research, US FDA, Rockville, MD; Sponsor: C. Lindamood III.

The presentation intends to provide an overview of Center for Biologics Evaluation and Research (CBER)/ Food and Drug Administration (FDA) expectations for preclinical studies for cell therapy products. Because of the diversity and complex nature of these products, they preclude the application of traditional, standardized approaches for preclinical testing. The case-by-case review approach will be discussed and general preclinical study design considerations and potential pitfalls will be presented. The application of these recommendations will be illustrated by case examples.

**W 1424 The Shaky Bridge: Animal Model Translation for Cell Therapies and Impact on Clinical Success**

L. E. Black, Charles River Laboratories, Reno, NV.

Cell therapies are very diverse. One universal statement that can be made is that nonclinical toxicology programs (the backbone of risk assessment for clinical trials) are less established for cells compared to those conducted for drugs. This problem starts at the difficulty of characterizing the nature of the product itself, even in vitro. For instance, islet cells may control glucose levels in animal models of pancreatic failure, but the obvious test of their in vitro potency (response to glucose) may not work at all. With this being true, uncertainty applies to prospective risk assessment, where one would hope that vitro and in vivo effects translate to patients. The term hope is used because we seldom see an intact "bridge" of activity from in vitro to in vivo in cell therapy research programs. We often have to proceed on faith that 1) the cells manifested both their pharmacologic and their toxicologic potential in the animal, and 2) that this one species program is a sound basis for human risk assessment. This is a far cry from knowing that we injected a drug (like a hormone) into two species, measured the drug PK and receptor occupancy, and measured the products secreted by hormone after binding to its receptor, and then observed amelioration of disease. So the pharmacology "bridge" for cell therapies (intrinsically to their nature/complexity) is a shaky one. Looking to the future, what can we do to improve our animal models, cell pharmacology, and translation to risk assessment? A stronger bridge might enable trials in earlier stage patients, improving chances of regeneration, and gaining Proof of Concept to further design optimal therapy. Published examples of in vivo data will be discussed.
Initial clinical studies of stem-cell based therapies are conducted in subjects with disease rather than normal volunteers. As such the objective is not only to administer a safe but also an active dose. However, unlike small molecules or large molecule biological drugs, there is no standard algorithm for extrapolation of preclinical animal doses. The concept of exposure also differs. This presentation will discuss key design considerations in dose extrapolation based upon differences in cell type, delivery and disease indication.

MultiStem® is an allogeneic adult bone marrow-derived cell therapy product that has received allowance from the FDA for testing in humans in the treatment of acute myocardial infarction, prophylaxis of graft-versus-host-disease in leukemia patients, inflammatory bowel disease, and ischemic stroke. It is the only cellular therapeutic currently being tested in acute ischemic stroke patients in the US. This presentation will describe the battery of preclinical safety, efficacy, and mechanism of action studies performed to support Investigation New Drug (IND) submission and clinical trial design for the ongoing Phase II/III trial. Preclinical safety studies that supported multiple IND submissions, or that were designed specifically to support use of MultiStem in ischemic stroke, will be highlighted. The presentation will also address some of the unique challenges posed by testing cellular therapies in preclinical animal models.

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A nonclinical development program using rats and pigs was successfully executed to support a First-in-Human (FIH) clinical trial for hUTC in Retinitis Pigmentosa. This program evaluated the safety and efficacy of hUTC, but also developed and characterized analogous cells from rats and minipigs to understand the immunology of cells in an allogeneic setting. In rats, the desired pharmacology was demonstrated in the Royal College of Surgeons model of degenerative retinal disease and ocular histopathology was used to evaluate potential effects of the disease state on the safety of hUTC. The rat was used to evaluate the tumorigenicity and biodistribution of hUTC following subretinal and systemic administration. However, anatomic constraints precluded the use of the clinical surgical procedure. A large animal species, the minipig, was used to evaluate the safety of the cells when administered by the surgical method of cell administration intended for patients. Based on anatomic considerations, cell doses were extrapolated across species based on retinal surface area. Taken together, results of these nonclinical studies supported the initiation of FIH clinical trials.

This symposium is designed to explore how several clinical to preclinical translational imaging modalities can be efficiently and effectively integrated into contemporaneous drug development and chemical hazard assessment. Standard clinical imagers, such as Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (MRS), Echocardiography (Echo) and Positron Emission Tomography (PET) have demonstrated strong utility in the nonclinical setting by allowing for the ability to perform real-time, non-invasive, longitudinal animal studies that can provide morphological, functional, and molecular data. Additionally, these modalities greatly minimize the need for assumptions in data interpretation and reduce uncertainty during product development or risk assessment; therefore, the preclinical application of these imaging strategies and the integration of these capabilities into modern drug safety assessment and environmental hazard identification is a promising development with the potential to greatly impact the field. This workshop will provide an overview of the challenges facing clinical trials and opportunities for translational imaging to address these gaps. Imaging approaches that address major contributors to pharmaceutical attrition, including cardiovascular safety, drug-induced liver injury, and neurotoxicity, will be presented. Additionally, the panel discussion will explore the potential burdens of validating the use of imaging modalities as clinical biomarkers in the field and by regulators. Ultimately, this workshop aims to facilitate dialogue across disciplines for broader acceptance and implementation of translational imaging in improving preclinical safety assessment and basic toxicity studies.

There is a potential disconnect between the very molecular approach we take to contemporary drug design and the very ‘un-molecular’ approach we take to assessing in vivo efficacy and safety. Nonclinical safety assessment of human pharmaceuticals typically involves a regimented series of studies that might lack a level of morphofunctional integration to be optimally instructive and translational. It may also lack the ability to understand the molecular relationship between drug and safety liabilities or use the data generated to leverage ever developing computational capabilities. Advances in in vivo imaging capabilities are providing an unprecedented opportunity to refine our traditional approaches and advance the translatability of our studies. Longitudinal studies that allow dynamic assessments of dynamic processes could be more instructive and require fewer animals. Molecular imaging capabilities are providing opportunities to visualize physiologic processes and their association with drug in living systems. Imaging can also make ‘quantitative’ what has traditionally been un- or semi-quantitative. This presentation will provide an overview of the advances in imaging technology and its application to animal models with quantitative endpoints that are also applicable to the clinical setting. We will put focus to integrated assessments of target organ morphology and function
to provide a more holistic understanding of drug effects and the ability to identify the most sensitive biomarker of that effect. Additionally, we will present challenges to applying novel approaches in very traditional paradigms and debate the risks and benefits of those approaches.

**W 1431 In Vivo Imaging of Hepatobiliary Transporter Function**


New response biomarkers that are sensitive and specific to detect early liver dysfunction prior to onset of irreversible liver damage are urgently needed. This presentation will describe a new in vivo imaging biomarker of hepatobiliary transporter function in the rat. A compartmental model was developed to characterize the pharmacokinetics of hepatic uptake and biliary excretion of the MRI contrast agent gadoxetate. Magnetic resonance imaging was used to continuously measure the concentration of gadoxetate in the liver and plasma for one hour after a bolus injection. The gadoxetate concentration in hepatocytes was estimated after correction for the gadoxetate concentration in plasma and the known volume fraction of extracellular space in the liver. An investigational hepatobiliary transporter inhibitor was shown to reduce both the rate of uptake of gadoxetate into the hepatocyte and the Michaelis–Menten constant characterizing its excretion into bile, whereas KM values for biliary efflux were increased. These effects were dose dependent and correlated with effects on plasma chemistry markers of liver dysfunction, in particular bilirubin and bile acids. The HESI liver imaging working group is evaluating the ability of this technique to quantitate in vivo hepatobiliary transporter function in a multi-site setting imaging using a known hepatobiliary transporter inhibitor. The potential to translate this technique to evaluate risk in the clinic will also be discussed.

**W 1432 Characterization of Drug-Induced Changes in the Cardiovascular System**

J. Heyen. Pfizer, La Jolla, CA. Sponsor: M. Paulus.

The expansion of cardiovascular (CV) imaging modalities from being utilized primarily in efficacy based approaches into safety studies has led to more robust analysis of drug induced CV changes. Advancements in Ultrasound (US) and Magnetic Resonance Imaging (MRI) coupled with adaption to scale for smaller species has enabled the adoption of these techniques into preclinical testing and facilitated use in drug discovery. Continuously expanding laboratories should have an understanding of data variability including intra and inter-observer errors and should have a robust training and qualification processes in place prior to data creation and study conduct. Likewise, opportunities and data for reverse translation investigations of drugs with identified clinical CV liability such as doxorubicin or trastuzumab are available and have been undertaken. Development of techniques and advancements in technology such as strain imaging and 3D small animal imaging will continue to increase the quality of preclinical CV imaging and will allow for thorough investigation of potential CV of novel drugs. This presentation will describe the efforts of a ILSI Health and Environmental Sciences Institute CV imaging working group has produced cross-site/company data around reproducibility and variability using a single compound. ANOVA analysis showed that for most of the parameters, there is no statistically significant site difference after adjusting for the baseline and vehicle effect. The median Intra-Class Correlation (ICC) for all parameters is only 12.7%, suggesting the variability of site effect is small compared to the overall variability. With proper training and equipment, this group has demonstrated that it is possible to collect high quality on cardiac structure and function using these approaches in standard toxicology and stand alone preclinical studies. A description of how these techniques can inform clinical risk and influence prospective clinical trial design for compounds with identified preclinical signals will also be discussed.

**W 1433 Magnetic Resonance Histology—Applications in Toxicology**


Magnetic resonance imaging (MRI) has revolutionized clinical medicine. The same underlying technologies are now triggering a similar revolution in the basic sciences. Magnetic resonance histology (MRH) was first suggested in 1993. Through the use of very high magnetic fields, specialized rf coils, novel imaging strategies, and specialized active MR stains, we have increased the spatial resolution in MRH by more than five orders of magnitude over that which is common in clinical MRI. While a number of papers have demonstrated the utility of MRH in toxicology, it is still not part of the mainstream. This presentation will include a brief overview of the technology and three recent results demonstrating exciting options that MRH provides the toxicologist because of its unique characteristics. In the first study, a developmental atlas of the rat brain provides a diffusion tensor (DTI) atlas allowing myelination of the rat brain at nine time points between PND0 and PND80. Changes in the diffusion metrics closely follow the development of myelin and its compaction- areas usually only seen with more complex electron microscopy. A second study demonstrates the use of magnetic susceptibility imaging providing quantitative insight into renal fibrosis. The last study describes a comprehensive infrastructure for high throughput MRH and it’s validation in a study of the effects of trimethyl tin. MRH is nondestructive, so tissues do not need to be physically sliced. Three-dimensional scanning allows viewing of whole specimens fully intact. Since the tissue is not dehydrated, morphologic measurements are considerably more accurate than with traditional techniques. Because the data can be acquired with isotropic spatial resolution, difficulties in obtaining homologous slices are virtually eliminated. A wide range of contrast mechanisms provides a rich set of biomarkers for tissue injury. Combining these attributes with sophisticated image registration algorithms allows us to produce atlases with quantitative biomarkers defining both severity and extent of injury in the entire organ.

**W 1434 Preclinical Development of Neurotoxicity Biomarkers Using In Vivo MRI**

S. Liachenko. Division of Neurotoxicology, NCTR, US FDA, Pine Bluff, AR.

Current approaches in the analysis of neuropathology in support of new drug submissions to the FDA (the use of several arbitrary histology slices) can sometimes result in false-negative findings due to significant undersampling. Non-invasive MRI provides unique information about the three-dimensional structure and function of the whole brain in vivo with sufficient resolution and contrast to serve as a translatable neurotoxicity biomarker. Recently it has been shown in our lab that the value of the brain T2 relaxation time as measured with MRI in rats is sensitive to the acute neurotoxicity caused by known neurotoxicants with the variability of mechanisms of action. T2 relaxation provides the quantitative measure of the amount and extent of the changes in the brain tissue consistent with neurotoxicity. This presentation will describe the implementation of such imaging approach to develop a quantitative non-invasive biomarker of neurotoxicity because of the correlation in vivo MRI T2 maps to histopathology using the panel of known neurotoxicants at different doses and time points. For example, a single dose of kainic acid (10 mg/kg, intraperitoneal injection) led to the rapid development of T2 lesion, detectable already at 2 hours post-injection (lesion volume = 10.8 ± 2.6 mm³), which grew significantly at 48 hours post-injection (lesion volume = 86.2 ± 34.2 mm³). This measure provided much earlier detection of the neurotoxicity than conventional histopathology score, which was 0 at 2 hours and 1.84 ± 0.49 at 48 hours post-injection (histopathology score range is from 0 [no damage] to 4 [maximum damage] and is assessed qualitatively). The use of such non-invasive MRI biomarkers may significantly increase the chance of the early preclinical detection of the small or transient neurotoxic changes and thus to optimize the costly process of drug discovery and development as well as enhance the safety of the final product.

**W 1435 The Role of Toxicology in Undergraduate STEM Education Reform**

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The attrition of undergraduates who enter STEM degree programs but do not earn a STEM undergraduate degree is an area of national concern. “Vision and Change in Undergraduate Biology Education,” the 2011 report from AAAS/NSF, and “Engage to Excel,” the 2012 report from the President’s Council of Advisors on Science and Technology (PCAST), are two national calls to action on this issue and advocate for significant reform in the way undergraduate STEM education is delivered. A strategic goal of SOT is a commitment to education in toxicology and the recruitment of students and new members into the profession. Although the number of undergraduate toxicology degree programs is limited, toxicology is a STEM discipline and many SOT members currently engaged in teaching undergraduates incorporate toxicology into traditional STEM undergraduate degree programs such as biology, biomedical sciences, chemistry, public health, and nursing. The learning objectives of this interactive workshop are to, 1) provide context to the national call to action on undergraduate STEM education reform, 2) present four case studies of inquiry-based approaches in undergraduate STEM education that inspire
students about the multidisciplinary science of toxicology, and 3) engage attendees to develop an "action plan" that incorporates toxicology and inquiry into a current STEM undergraduate degree program. During the workshop attendees will have the opportunity to network with practitioners who can help inform their practice. This session should have widespread benefit to anyone engaged in teaching "toxicology" at any level of academia or industry who has an interest in innovations that help to engage trainees to "work the problem."

**W 1436** The Changing National Landscape of STEM Education: Building Partnerships with Scientists and Educators


The improvement of STEM (science, technology, engineering, and mathematics) education has become a national priority at all levels of the education system. However, efforts to increase the numbers of students who enter and persist in STEM fields too often have been disjointed and have been undertaken without understanding the potential effects on other parts of the education system. This presentation will provide an overview of recent reports and their recommendations for improving STEM education, especially at the undergraduate level. It will explore the intersections between education and public policy and why college and university faculty should be aware of and involved with education policymaking. It also will describe how current efforts to improve STEM education in Grades K-12 will likely have major impacts on postsecondary education and how undergraduate education will influence K-12 education in the future.

**W 1437** Case Study 1: Creating Research Experiences and Activities for Public Health Undergraduates through Teaching Enhancement (CREATTE)

M. M. Bourgeois. Environmental and Occupational Health, University of South Florida, Tampa, FL.

One of the challenges presented by a rapidly expanding undergraduate program like the B.S. in Public Health at USF is providing research opportunities for students. The Office for Undergraduate Research (OUR) developed the CREATTE (Creating Research Experience and Activities Through Teaching Enhancement) to address this need. Through the CREATTE initiative, faculty incorporated an authentic research experience within a structured course. The research experience tracks on the student's transcript and they also have the opportunity to present their work at the Undergraduate Research Colloquium. CREATTE funds cover sustainable supplies and 30 hours of effort from a graduate assistant. During Fall 2012, 12 students in Environmental Health Science were able to participate in a project involving K12 outreach. This area was chosen because it is a vital component of the public health mission to educate the community regarding scientific issues, particularly toxicity and risk assessment. Students were matched with local K-8 science classes based on curriculum needs. Students created age-appropriate presentations and activities on public health topics for the Great American Teach In. Utilizing this preexisting infrastructure obviated the need for school board clearance. Feedback from the students and teachers allowed participants to develop templates for future students to emulate. A post survey of participating CREATTE undergraduate researchers indicated a strong agreement that they developed enhanced research competencies, had a deeper understanding of the course material and would engage in addition research activities.

**W 1438** Case Study 2: Integrating Toxicology into the Undergraduate Curriculum

T. Dodd-Butera. Nursing, California State University, San Bernardino, San Bernardino, CA.

Addressing the challenge of incorporating toxicology and environmental threats into undergraduate nursing education is critical for engaging future health professionals in the application of science for the benefit of society. Utilizing case study methodology as part of an overall educational program on environmental health for undergraduate nursing students, educational modules were developed addressing toxicokinetics, by utilizing a lecture format on a model of lead toxicity. Further, a clinical rotation for students at a poison control center demonstrated clinical toxicology approaches and poisoning prevention strategies for the benefit of public health. The blended learning approach addresses the focus of the STEM initiative on content delivery, and incorporates the “4 Cs” noted by the National Education Association as essential tools for preparing 21st century students for a global society through appreciative inquiry. The integration of poisoning principles through critical thinking, communication, collaboration, and creativity, is also aligned with the commitment of the SOT to education and exposure to the interdisciplinary nature of toxicology. Attendance at a journal club was also part of the educational strategy, and covered topics such as environmental exposures, chemicals, drug overdoses, and unintentional poisonings. The culminating experience was a presentation at a poster session, addressing problem solving through evidence-based practice, and a collaborative approach to campus-community partnerships and service learning. Integration of toxicology into didactic and clinical experiences of undergraduate nursing students underscores the potential for innovative approaches in addressing STEM initiatives and SOT strategies for education across multiple scientific disciplines.

**W 1439** Case Study 3: Integrating Toxicology into the Undergraduate Laboratory Curriculum

B. W. Brooks. Environmental Science, Baylor University, Waco, TX.

This session presents multiple strategies utilizing contemporary scientific laboratory techniques to integrate environmental research into laboratory courses. Such an approach enhances student’s critical thinking skills and allows for application of toxicology practices. This session will also describe modules that allow laboratory approaches for defining contaminants of emerging concern.

**W 1440** Case Study 4: Problem-Based Instruction of Pharmacokinetics

T. L. Leavens. Pharmacokinetic Consultant, Cary, NC.

The goal of STEM education is the development of competency and application of knowledge versus acquisition of knowledge. Therefore, courses that are structured based on the higher order objectives in Bloom’s taxonomy of learning provide an ideal classroom environment, because they are focused on projects and case studies versus lectures. Some of the more recent techniques to achieve this learning environment are the flipped classroom and e-learning tools. The objective of this presentation is to demonstrate how to design a problem-based lecture for pharmacokinetics, a discipline in which problem-solving skills are critical. The presentation will focus on the absorption component of pharmacokinetics and demonstrate how to convert a lecture on the topic into a flipped class through the use of video clips, interactive case studies, and web-based applications.

**S 1441** In Vitro Microphysiological Systems: Advancing Regulatory Science through Innovation

A. Balinsky1 and S. C. Fitzpatrick2. 1Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA and 2Center for Food Safety & Applied Nutrition, US Food and Drug Administration, College Park, MD.

Development of safe and effective drugs is currently hampered by the poor predictive power of existing preclinical animal models that often fail to lead to development of drug compounds later in their development. Given the tremendous cost of drug development and the long timelines involved, major pharmaceutical companies and government funding agencies are now beginning to recognize a crucial need for new technologies that can quickly and reliably predict drug safety and efficacy in humans in preclinical studies. Advances in bioengineering, material sciences, microfabrication, and microfluidics technologies have enabled the development of microphysiological systems that mimic the functional units of an organ. These advances have made it possible to initiate the engineering of cellular microenvironments and/or functional units of lung, heart, blood vessels, muscles, bones, liver, nervous system (including eye), gut, and kidney. In general, these microphysiological systems, or human “organs-on-chips,” use microscale engineering technologies combined with cultured living human cells to create microfluidic devices that recapitulate the physiological and mechanical microenvironment of whole living organs. The next challenge is to develop an integrated microsystem platform that can incorporate several different modular organs on a chip. These integrated microsystems would mirror the complex physiology and biology of the human body. An integrated microphysiological platform could further our understanding of disease etiology and fill the critical need for improved model systems to predict efficacy, safety, bioavailability, and toxicity outcomes for candidate compounds. This symposium will examine the building blocks needed to bring this new innovative technology into the regulatory arena, including the need for adequate stem cells, the development of representative organ systems, challenges to building a “human on a chip,” and the pathway to qualification for regulatory use.
The goal of the new National Center for Advancing Translational Sciences (NCATS) is to catalyze the generation of innovative methods and technologies that will enhance the development, testing, and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions. By improving the process by which diagnostics and therapeutics are developed, NCATS strives to make translational science more efficient, less expensive, and less risky. Partnerships between federal agencies and stakeholders are critical for innovation in regulatory science. The NCATS/FDA/Defense Advanced Research Projects Agency (DARPA) partnership for the development of in vitro microphysiological systems is a groundbreaking example of the types of partnerships that are needed to bring innovative new technologies into the regulatory paradigm. NCATS, FDA, and DARPA are collaborating to develop a chip to screen for safe and effective drugs, which is far more efficient and effective than current methods. NCATS would help identify barriers to progress and provide science-based solutions to reduce costs and the time required to develop new drugs and diagnostics. FDA will help determine how this new technology can be utilized to assess drug safety, prior to approval for first-in-human studies. DARPA and NIH will facilitate collaborations between researchers and FDA to advance the goals of both programs. The two agencies, in coordination with FDA, solicited proposals from industry, government labs, academic institutions, and other research organizations on how best to develop the chip, bringing together the latest advances in engineering, biology, and toxicology to bear on this complex problem. Throughout the five year research plan, NCATS, FDA, and DARPA meet biannually with all the researchers.

**The Increasing Use of Stem Cells Prompted a Workshop on How to Adapt GCCP for This Emerging Field and Is Currently Further Adapted to Induced Pluripotent Stem Cells, Which Increasingly Substitute for Human Embryonic Stem Cells. Such Quality Control Is of Critical Importance for the Organ-on-a-chip Models Currently Favored to Ultimately Allow a Body-on-a-chip Combination of Several Such Organoids Combined with Microfluidics. Our Ongoing Development of a (Developmental) Quality Control 3D Model from iPSC Within This Program Is Used to Illustrate the Challenges and Opportunities of GCCP.**
concentrations of As(III) inhibit poly(ADP-ribose) polymerase (PARP)-1, leading to interference with DNA repair process triggered by UV radiation. It has been proposed that interference with DNA repair systems is one of the common modes of action for metal-induced carcinogenicity. It is of great interest and importance to understand the steps affected by metals in the repair pathway and the mechanism of the repair inhibition. The presentations in this symposium will highlight the latest findings on the molecular mechanisms of metal-induced inhibition of DNA repair.

**1448 Impact of Cadmium and Copper on the Cellular Response to DNA Damage: Interference with Redox-Regulation**


The carcinogenicity of cadmium has been recognized for some decades, but the underlying molecular mechanisms are not completely understood. While direct DNA damage appears to be of minor importance, the interference with antioxidant defense systems as well as interactions with DNA repair processes, tumor suppressor functions, and signal transduction pathways have been described in diverse biological systems. Thus, cadmium has been shown to disturb nucleotide excision repair, base excision repair as well as mismatch repair, and the interference with cellular redox regulation by reaction with redox-sensitive protein domains or amino acids may provide one explanation for cadmium-induced carcinogenicity. Particularly sensitive targets appear to be proteins with zinc binding structures, present in DNA repair proteins such as XPA, PARP-1 as well as in the tumor suppressor protein p53. For example, cadmium inhibits poly(ADP-ribose) polymeration, and detailed investigations suggest a direct interaction with PARP-1, presumably by inactivation of thiol groups. Also, cadmium compounds provoke an unfolding of the "wild type" p53 conformation, leading to diminished expression of DNA repair proteins and via inhibition of apoptosis to a resistance towards DNA-damaging agents. Particularly the combination of these multiple mechanisms may give rise to a high degree of genomic instability in cadmium-adapted cells, relevant not only for tumor initiation, but also for later steps in tumor development. Nevertheless, the disturbance of cellular redox regulation may also occur in case of essential trace elements on conditions of cellular overload. One example is copper, where especially copper-based nanoparticles lead to high concentrations of copper ions in the cell nucleus and thus overcome homeostatic control. Consequences are elevated levels of oxidative DNA damage and pronounced inhibition of poly(ADP-ribose) polymeration.

**1449 Inhibition of DNA Repair As a Mechanism of Arsenic Carcinogenesis**


Inorganic arsenic is a complete carcinogen. In addition to the known direct carcinogenic actions of arsenic, there is substantial evidence that arsenic at low, non-carcinogenic levels amplifies the carcinogenic potential of other DNA-damaging agents such as benzo[a]pyrene and ultraviolet radiation (UVR). One hypothesis to account for arsenic co-carcinogenesis is that arsenic is a chemically reactive arsenite which binds to the zinc finger moiety of PARP-1, preventing zinc binding, thus interrupting the function of PARP-1. We have shown that arsenic reverses the inhibitory effects of arsenic on PARP-1 activity and arsenic augmentation of UV-induced DNA damage and mutagenesis. Furthermore, arsenic prevents the effects of arsenic on UV-induced DNA damage in mouse skin in vivo. It is suggested that arsenic may provide a tractable approach to attenuate arsenic-induced carcinogenesis in human populations. Collectively our studies demonstrate that arsenic can interact with key DNA repair zinc-finger proteins, resulting in modification of protein structure and function, leading to accumulation of DNA damage.

**1450 Prolonged Exposure to Particulate Chromate Induces a Switch from Homologous Recombination Repair to Nonhomologous End Joining Repair in Human Lung Cells**


The interaction of genotoxic chemicals with DNA repair is a key consideration in understanding their overall toxic mechanism. This presentation describes an example of this interaction focusing on chromate. Particulate chromate is a known human carcinogen and genotoxic agent, but how it causes cancer is unknown. DNA double strand breaks (DSB) are a key factor in its carcinogenicity as perturbations to chromate-induced neoplastic transformation occurs in human lung cells with experimentally disrupted DSB repair pathway, but not in human lung cells with intact DSB repair systems. Emerging data show that particulate chromate targets the homologous recombination repair pathway (HR) in human lung cells with unaltered repair pathways. In human lung cells with normal zinc chromate-induced concentration-dependent increases in DSBs and chromosome aberrations. With longer exposure times, this damage remains elevated indicating that the damage continues to be produced. An evaluation of representative proteins of the major steps in HR signaling showed that the sensors of DSB repair (measured as gammaH2AX, 53BP1 and Ms chromosome) remain elevated in response to these breaks indicating they are unaffected by chromate. By contrast, the effector component of HR repair (measured as Rad51 response) is compromised by chromate. During the first 24 h of exposure, Rad51 response increases as expected, but after 48-120 h of exposure the response continually declines. The signal transduction response through ATM also increases during the first three days of exposure, but after 96 h of exposure, the ATM response dramatically falls off. The expression of Ku80 and DNA-PKcs proteins increased as HR repair function decreases. Thus, particulate chromate induces a signaling switch from HR to NHEJ after prolonged exposures. The timing of this switch also correlates well with the onset of particulate chromate-induced genotoxic instability in human lung cells. This work was supported by NIEHS grant ES016893 (J.P.W.).

**1451 Chemistry and Biology of Oxidatively-Induced Tandem Lesions of DNA**

Y. Wang. Department of Chemistry and Environmental Toxicology Graduate Program, University of California Riverside, Riverside, CA. Sponsor: H. Xie.

Exposure to various toxic metals in the environment can lead to cellular production of reactive oxygen species (ROS), which can result in DNA damage. Although substantial repair studies have been conducted for the ROS-induced single-nucleobase lesions, not much is known about how oxidatively induced tandem DNA lesions are repaired in mammalian cells. Our LC-MS/MS quantification results revealed that 8,5-cyclo-2-deoxyadenosine (cyclo-dA), 8,5-cyclo-2-deoxyguanosine (cyclo-dG) and the GT intranstrand crosslink are present at significantly lower levels in tissues of repair-deficient animals than in Erccl-deficient animals. Likewise, we found higher levels of these lesions in brain tissues of XPA-deficient patients than in repair-proficient individuals. Viewing that XPA and Erccl are required for nucleotide excision repair (NER), the above results support that these DNA lesions are substrates for NER in vivo. Additionally, we observed an age-dependent accumulation of these DNA lesions in mammalian tissues, suggesting that the accumulation of these DNA lesions may play an important role in aging-related pathological conditions. We also found, from the LC-MS/MS and plasmid-based in vivo transcription assay, that both cyclo-dA and cyclo-dG substantially blocked transcription in mammalian cells, and transcription bypass efficiency is much lower in NER-deficient mammalian cells than repair-proficient cells, suggesting again the compromised repair of these DNA lesions in XPA-deficient cells. Moreover, the LC-MS/MS method allowed for the identification and quantification of mutant transcripts produced from the transcriptional bypass of the cyclo-dA and cyclo-dG lesions. Together, our combined chemistry and biology approach led to important novel insights into the repair and biological consequences of the oxidatively induced tandem DNA lesions.

**1452 Molecular Mechanisms Involved in Neuro/Glial Toxicity: From Oxidative Stress to Redox Signal Transduction**

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Oxidative stress, defined as the imbalance between the production of reactive oxygen species (ROS) or nitrogen (RNS) species and the ability of cells to scavenge these active species and repair oxidative damage, has been largely reported to participate in environmental toxicity. Biomarkers of oxidative damage in proteins, lipids, and nucleic acids are inherent to neuronal toxicity associated with pesticides, metals,
and particulate matter. Electron-transfer processes called “redox signaling” play key messenger roles in biological systems. Initially, ROS/RNS formation was thought to lead to nonspecific cellular damage. However, recent in vitro and in vivo findings demonstrate that, in response to environmentally relevant doses toxicants, a specific set of redox signaling events play a major role activating specific signaling transcription cascades, regulating gene expression, enzyme activity, cellular metabolism, and cell fate outcome of neuronal and glial cells. In this session we will examine recent findings on the molecular mechanisms by which redox signaling regulates environmental neuro/ glial toxicity. More specifically, the speakers will highlight the novel mechanisms by which mitochondrial redox homeostasis, iron-sulfur cluster oxidation, electrophile-adduct formation, thiols, transcription factor regulation, oxidative post-translational modifications, and oxidative DNA damage regulate the toxicity of particulate matter, aldehydes, pesticides, and metals. This topic should be of great importance to biochemists, risk assessors, graduate students, postdoc- toral trainees, and academics within distinct Specialty Sections.

1454 The Electrophile-Responsive Proteome As a Target for Environmental Neurotoxins
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Many environmental toxicants are electrophiles that can form irreversible covalent adducts with nucleophilic amino acid residues. Discerning how these environmental chemicals interact with the electrophile-responsive proteome is a critical step in defining their respective mechanisms of neurotoxicity. A detailed understanding of acrylamide (ACR) toxicity led us to the identification of relevant protein targets and to the molecular mechanism by which this targeting causes neuronal cell death. The Hard and Soft, Acids and Bases (HSAB) theory evidenced that the type-2 alkenes might act synergistically with endogenous unsaturated aldehydes and in chemico studies that identified the redox-sensitive targeted residues as nucleophilic sulfhydryl thiolates (–S–) located within cysteine-centered catalytic triads of central enzymes. These findings are broadly applicable since many environmental toxicants are electrophiles that can produce toxicity by reacting with nucleophilic residues on macromolecules. Protein-environment interactions have significant implications for human health and risk assessment. (NIH grants ES03830-25 and ES07912-11)

1455 Redox Regulation of NF–κB p50 and M1 Polarization in Neurotoxic Microglia
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Microglia detect and respond to a diverse array of stimuli, including environmental toxicants, bacterial toxins, cytokines, neuronal damage, and disease proteins, where the activation state can be characterized as a pro-inflammatory (M1) or alternative (M2) response. M1 polarization is implicated in neurotoxic microglia activation, with both enhanced M1 activation and impaired resolution (M2 response). How ROS shift microglia to a neurotoxic phenotype is unknown. Here we identify the NF–κB p50 protein radical in neurotoxic microglia, demonstrating oxidative modification during M1 polarization. Nuclear extracts from microglia cultures treated with the peroxynitrite generator SIN–1 (1uM) and the ROS-generating pesticide parathion (0.5uM) showed decreased NF–κB p50 DNA binding, confirming that microglial ROS impairs NF–κB p50 function. NF–κB p50–/– mice were injected with LPS (5mg/kg) and IPs (NF–κB p50−/−) primary mixed-glia cells and silRNA NF–κB1 knockdown in BV2 microglia were treated with LPS (10ng/ml), which resulted in enhanced pro-inflammatory cytokine production and activated microglial morphology. Loss of NF–κB p50 function in microglia impaired both M1 gene expression and the resolution of M1 genes. Mesencephalic neuron-glia cultures from NF–κB p50−/– mice treated with either LPS or parathion showed enhanced DA neurotoxicity. Parathion amplified the production of LPS-induced TNFα in microglial cultures only in the presence of functional NF–κB p50, emphasizing the importance of NF–κB p50 for priming. In vivo parathion studies revealed that NF–κB p50−/– mice have enhanced activated microglial morphology and elevated motor behavior deficits in response to a single parathion injection, supporting that the NF–κB p50−/– mice may already be primed. These data identify a critical redox signaling mechanism triggered by environmental toxicants that drive M1 polarization in microglia, where loss of NF–κB p50 function leads to elevated neuroinflammation, microglial activation, and DA neurotoxicity (Supported by the NIEHS R01ES016951).

1456 Redox Signaling and Methylmercury Toxicity
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MeHg causes acute and chronic damage to multiple organs, most profoundly the central nervous system (CNS). We examined the effects of MeHg on microglial and astrocytic viability, reactive oxygen species (ROS) generation, glutathione (GSH) level, redox homeostasis, and NF-E2-related factor 2 (Nrf2) protein expression, the latter switch of antioxidant response. Glial cells exposed to MeHg (at relevant physiological concentrations of 1–5 microM) treatment caused a rapid (within 1 min) concentration- and time-dependent increase in microglial ROS generation, accompanied by a statistically significant decrease in the ratio of GSH and its oxidized form glutathione disulfide (GSSG) (GSH/GSSG ratio). MeHg increased the cytosolic Nrf2 protein level within 1 min of exposure, followed by its nuclear translocation after 10 min of treatment. Consistent with the nuclear translocation of Nrf2, quantitative real-time PCR revealed a concentration-dependent increase in the messenger RNA level of Hs1, Nqo1, and xCT 30 min post MeHg exposure, whereas Nrf2 knockdown greatly reduced the upregulation of these genes. Furthermore, we observed increased microglial death upon Nrf2 knockdown by the small hairpin RNA approach. Additional studies established that microglia were more sensitive to MeHg than astrocytes, a finding consistent with their higher Hg uptake and lower basal GSH levels. Microglia also demonstrated higher ROS generation compared with astrocytes. Nrf2 and its downstream genes were upregulated in both cell types, but with different kinetics (much faster in microglia). In summary, microglia and astrocytes each exhibit distinct sensitivity to MeHg, resulting in their differential temporal adaptive responses. These unique sensitivities appear to be dependent on the cellular thiol status of the particular cell type. (Supported by R01ES07351 and R01ES020852).

1457 Repair of Mammalian Genome Damage Induced by Oxidative Stress and Their Linkage to Neurodegenerative Diseases

Endogenous reactive oxygen species (ROS), respiration-by-products, and ROS induced during toxic/inflammatory response generate multitude of mutagenic and toxic oxidized bases, and strand breaks in mammalian genomes whose repair via the base excision/single-strand break repair (BER/SSBR) pathway is essential for preserving genomic integrity. The lack of replicative repair in neural cell genome
could explain the linkage of some neurodegenerative diseases to deficiency in BER proteins. Dyshomeostasis of transition metals Fe/Cu as well as accumulation of genome damage have been implicated in Alzheimer’s (AD) and Parkinson disease (PD). We postulate that Fe and Cu (at up to micromolar level) pose double jeopardy in that not only these metal ions could induce ROS and hence oxidized bases, but they also inhibit repair of such oxidized bases by inhibiting the BER-initiating DNA glycosylases NEIL1/2, both in vitro and in human neuroblastoma (SH-SYSY) cells at sub-micromolar concentration. Metal chelators and chemopreventive curcumin reverse NEILs’ inhibition both in vitro and in cells, thus warranting exploration of their therapeutic potential. Oxidative stress has also been linked to many neurodegenerative diseases, associated with aggregation/misfolding of key proteins including RNA binding proteins (RNPs) such as TDP–43 and FUS/TL1. Aggregation of TDP–43 together with its nuclear clearance has been implicated in atypical sporadic lateral sclerosis (ALS). While the etiologic role of RNPs in diseases including hnRNP–U, which we had earlier shown to enhance BER efficiency, is not completely understood, we observed direct role of TDP–43 in repairing endogenous and induced DNA double–strand breaks (DSBs) via non–homologous end joining. The observed increase in the level of strand breaks in postmortem ALS brain is consistent with our hypothesis that the loss of DNA strand break repair due to inactivation of specific RNPs including TDP–43 causes nuclear death and disease phenotypes. (Supported by USPHS grant R01 CA158910 (SM), Alzheimer’s Association grant NIRC–12–242135 (MLH).)

**1460 The AhR Is a Key Factor in the Regulation of Hematopoietic Stem Cells and Their Protection from Premature Exhaustion, Stress, and Hematopoietic Disease**

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There has been much work to define a physiological role of the AhR in the immune system and possible relationships to human disease. Hematopoietic stem cells (HSCs) from global AhR knock-out (KO) mice are inherently hyperproliferative. These data, along with others, are consistent with a hypothesis that the AhR has an important role as a negative regulator of HSC proliferation. Functional and gene expression analyses of HSCs from KO mice indicate that a lifetime of altered signaling pathways results in premature exhaustion, aging and a myeloproliferative disorder. Aging mice lacking AhR showed a decreased survival rate, splenomegaly, increased circulating white blood cells, hematopoietic cell accumulation in tissues, and anemia. There was also decreased self-renewal capacity of HSCs determined by competitive repopulation and serial transplantation. Some of the molecular changes observed in aging HSCs include altered content of ROS, p16Ink4a, and γ–HAX2, and indicator of DNA damage. AHRKO mice develop multiple genomic changes (e.g. Srpk2, Rad50, mTOR, Creb1, Hes1, Pdp1, Stra13) in HSCs at an early age that lead to the phenotypic changes characteristic of premature exhaustion. The most prominent gene changes were also associated with HSC hyperproliferation, leukemia, and accelerated aging. Pathway analyses also indicated an enrichment of genes associated with oxidative stress, acute myelogenous leukemia, aging, and heat shock response, and the B-catenin/Wnt pathways. These data indicate that the AhR is a key cofactor in the regulation of HSCs and their protection from long-term stress, premature exhaustion, and age-related disease. Supported by NIEHS grants ES01247, ES07026, ES04862, and ES016606.

**1459 The AhR Modulates Cardiomyogenesis in Embryonic Stem Cells**

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The aryl hydrocarbon receptor (AhR) is a critical mediator of gene-environment interactions that regulates the transcription of drug-metabolism genes and of genes involved in cell cycle regulation. Many AhR targets identified by genome-wide gene expression profiling, ChIP analyses and studies with orthologous genes in invertebrates have morphogenetic functions suggestive of an AhR regulatory function during embryonic development. To address this hypothesis, we studied the regulation of AhR expression in mouse embryonic stem cells (ESC) and their differentiated progeny. In undifferentiated ESC, co-regulatory interactions between pluripotency factors OCT4/3, NANOG and SOX2 and Polycomb Group (PcG) proteins maintain AhR expression in a repressed state, while RNA polymerase II (RNApolII) bound at the transcription start site is required for synthesis of abortive transcripts. AhR activation during differentiation follows from repression of repressive chromatin marks, including release of pluripotency factors and PcG proteins from their binding sites. Sp factor transactivation, establishment of open chromatin marks and engagement of active RNApolII to drive full-length RNA transcripts.

**1458 The Role of the AhR in Stem Cell Development and Lineage Specification**

D. H. Sherr1, A. Puga1, T. A. Gasiewicz1, E. Papoutsakis1 and G. Murphy4.

For decades the aryl hydrocarbon receptor (AhR) has been studied for its mediation of the toxic and carcinogetic effects of ubiquitous environmental chemicals including dioxins, planar PCBs and PAHs. A subtext to this work has always been the nature of the “normal” physiological function of this evolutionarily conserved protein. In the last five years, several breakthrough studies have begun to reveal the significance of the AhR in normal biological processes. Similarly, technological advances developed in the last five years have allowed investigators, for the first time, to map out some of the most basic tenets of pluripotent and multipotent stem cell differentiation. The significance of this work cannot be over-stated since control of stem cell differentiation is a key to both organ generation/regeneration and cancer stem cell immortality. Here, we focus on the intersection of AhR and stem cell biology to highlight the importance of the AhR to the regulation of stem cell differentiation and to emphasize the potential for environmental AhR ligands to disrupt essential cell specification programs. We will discuss AhR control of: 1) pluripotent (embryonic stem cell and induced pluripotent stem cell) development into cardiomyocytes, erythroid lineage cells and megakaryocytes, 2) multipotent hematopoietic stem cell senescence and differentiation into lymphocytes, and 3) cancer stem cell development. In so doing, we will identify themes common to multiple developmental systems (e.g., common AhR signaling pathways, the role of the AhR in maintaining “stem-ness,” and predicted outcomes after environmental chemical exposure). Symposium speakers will address the significant implications of their work for stem cell and AhR biology under normal physiological conditions and when impacted by exposure to environmental AhR ligands.

**1461 A Role for the Aryl Hydrocarbon Receptor (AhR) on Platelet Function**

E. Papoutsaki1,2, S. Lindsay1,2, J. Jiang1 and D. Woulfe1. 1Department of Chemical & Biomolecular Engineering, University of Delaware, Newark, DE, 2Department of Biological Sciences, University of Delaware, Newark, DE and 3Nemours Center for Childhood Cancer Research, A.I. duPont Hospital for Children, Wilmington, DE. Sponsor: A. Puga.

We recently identified AhR as a novel regulator of megakaryocytic polyploidization and differentiation. Although AhR-null mice exhibited roughly 15% fewer circulating platelets than WT mice, this was insufficient to explain the bleeding phenotype we found in AhR-null mice. Instead, we hypothesized that platelets from AhR-null mice are functionally deficient. Indeed, we found that only 20% of AhR-null mu-platelets aggregated in response to 2 μg/mL collagen compared to 60% for WT platelets. Spreading assays confirmed that AhR-null platelets demonstrated nearly 40% less spreading in response to collagen-dependent signaling. Yet AhR-null platelets bound fibrinogen and spread in response to other platelet agonists including thrombin, ADP, and AYPGKF (a PAR4 agonist). AhR-null platelets expressed similar levels of CD41 and CD49b surface markers, but 15% less GPVI, one of the receptors involved in collagen signaling. We then investigated molecular mechanisms by which AhR could influence collagen signaling within platelets and found that AhR-null platelets have decreased Vav1 and Vav3 phosphorylation, leading to reduced PLCA2 tyrosine phosphorylation within platelets and found that AhR-null platelets have decreased Vav1 and Vav3 expression compared to WT platelets. Spreading assays confirmed that AhR-null platelets expressed similar levels of CD41 and CD49b surface markers, but 15% less GPVI, one of the receptors involved in collagen signaling. We then investigated molecular mechanisms by which AhR could influence collagen signaling within platelets and found that AhR-null platelets have decreased Vav1 and Vav3 phosphorylation, leading to reduced PLCA2 tyrosine phosphorylation. In contrast, tyrosine phosphorylation of Syk, which lies upstream of Vav1 and Vav3 phosphorylation, remained unaltered and thrombin stimulation resulted in Rac1 activation of both WT and AhR-null platelets. These results showed that the absence of AhR leads to downregulation of Vav1 and Vav3, which in turn leads to altered PLCA2 phosphorylation and reduced platelet activation specifically
in response to collagen, without impacting other platelet signaling pathways. This study advances our hypothesis that AHR influences multiple aspects of normal hematopoietic differentiation.


**1462 The AhR Regulates the Production and Specification of Bipotential Hematopoietic Progenitor Cells**

G. Murphy1, B. Smith1, S. Rozell1, A. Leung1, J. Ubellacker2, A. J. Parks2, S. Nah1, S. Monti1 and D. H. Sherr1.

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Recent studies demonstrate that the AHR may regulate the hematopoietic and immune systems during development in a cell-specific manner. Our results indicate that AHR has a physiological and functional role in normal hematopoietic development, and that modulation of the receptor in bi-potential hematopoietic progenitors can direct cell fate. Using a novel, iPSC-based, chemically-defined, serum and feeder cell-free culture system, we demonstrate that a functional AHR is expressed in hematopoietic progenitor cells (HPCs), and that remarkably, AHR activation of these HPCs drives an unprecedented expansion of HPCs, megakaryocyte (MK)- and erythroid-lineage cells. Further AhR modulation directs cell fate, with chronic AhR agonism permissive to erythroid differentiation and acute antagonism favoring megakaryocyte specification. These results demonstrate a new platform for studying human red blood cell and MK development that allows for exponentially greater production of RBCs and MKs in comparison to existing methodologies. This strategy relies on the first of its kind definition of the role of the AHR receptor in normal hematopoietic development using specialized ligands in hematopoietic progenitor cells. A useful outcome for this work will be the utilization of this in vitro platform for the clinically relevant production of blood products. An iPSC-based system, such as the one described here in which sufficient numbers of cells can be produced, should facilitate future clinical adaptation involving the transfusion of iPSC-derived red blood cells and platelets without problems related to immunogenicity, contamination, or supply. Supported by NHLBI U01 HL107443-01, an American Society of Hematology (ASH) Scholar Award, the National Blood Foundation (NBF), PO1 ES11624, P42 ES007381, and the Art beCAUSE Breast Cancer Foundation.

**1463 The AHR Controls Breast Cancer Stem Cell Development and Function**

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Tumor metastasis is the cause of death in nearly all breast cancer patients. Recent evidence suggests that metastasis is mediated, to a disproportionate extent, by chemoresistant, long-lived breast cancer stem-like cells (BCSCs). BCSC cells are defined by their expression of aldehyde dehydrogenase (ALDH), a set of genes associated with “stemness”, the ability to self-renew, expression of properties consistent with epithelial-to-mesenchymal transition (e.g., metastatic potential), and resistance to chemotherapeutics. Studies from several laboratories, including three laboratories represented in this symposium, suggest that the AHR, a protein historically associated with tumorigenesis, is involved in the development and/or function of blood stem cells which share some properties with cancer stem cells. Therefore, we postulated that the AHR may play a role in either the development or function of BCSCs. Here, we present data demonstrating elevated levels of constitutively active AHR in BCSCs from aggressive human ER+, PR-, Her2- (“triple negative”) and inflammatory breast cancers. The results indicate that AHR hyper-activation with exogenous ligands (FICZ, TCDD, DMB) increases and AHR inhibition with pharmacological or molecular agents decreases expression of hyper-activation with exogenous ligands (FICZ, TCDD, DMBA) increases and AHR inhibition with pharmacological or molecular agents decreases expression of these properties. However, these novel properties also contribute to their potential health risk. A major route of exposure for engineered nanomaterials occurs through inhalation, potentially leading to pulmonary toxicity. Immune activation and inflammation represents a common response observed across many pulmonary studies of nanoparticle inhalation in rodents. However, our mechanistic understanding of how the materials elicit immune activation is limited. Recent accumulating evidence supports the proposal that the initial pulmonary immune response to nanoparticle exposure is mediated via the innate immune system driving inflammation. Presentations in this session are aimed at elucidating the mechanisms of these innate immune responses to engineered nanomaterial exposure in the lung. This will include exploration of macrophage phenotypes, inflammasome activation, Toll-like receptors and, lastly, will explore the degradation of nanomaterials by immune cells and their defensive products. The outcome of this session is to gain state-of-the-art information on the mechanisms of the critical innate immune system response in the toxicology of nanoparticles and ultimately the development of safe nanotechnologies.

**1464 Three Dimensions of Nanomaterial Pulmonary Toxicity: Innate Immunity, TLRs, and Inflammasomes**

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Nanotechnology is rapidly developing, resulting in the production of numerous engineered nanoparticles. These materials have many potential uses in engineering, electronics, and medicine owing to their unique size, strength, functionality and surface properties. However, these novel properties also contribute to their potential health risk. A major route of exposure for engineered nanomaterials occurs through inhalation, potentially leading to pulmonary toxicity. Immune activation and inflammation represents a common response observed across many pulmonary studies of nanoparticle inhalation in rodents. However, our mechanistic understanding of how the materials elicit immune activation is limited. Recent accumulating evidence supports the proposal that the initial pulmonary immune response to nanoparticle exposure is mediated via the innate immune system driving inflammation. Presentations in this session are aimed at elucidating the mechanisms of these innate immune responses to engineered nanomaterial exposure in the lung. This will include exploration of macrophage phenotypes, inflammasome activation, Toll-like receptors and, lastly, will explore the degradation of nanomaterials by immune cells and their defensive products. The outcome of this session is to gain state-of-the-art information on the mechanisms of the critical innate immune system response in the toxicology of nanoparticles and ultimately the development of safe nanotechnologies.

**1465 Inflammasome Activation in Nanoparticle-Induced Lung Inflammation**


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We have previously shown that inhalation of nanoparticles (ENM) increases bioactivity, while carboxylation of ENM surfaces decreases bioactivity for LMP since imipramine and chloroquine can block NLRP3 Inflammasome activation, Toll-like receptors and, lastly, will explore the degradation of nanomaterials by immune cells and their defensive products. The outcome of this session is to gain state-of-the-art information on the mechanisms of the critical innate immune system response in the toxicology of nanoparticles and ultimately the development of safe nanotechnologies.

**1466 Lung and Pleural Innate Immune Responses to Engineered Nanomaterials**

J. C. Bonner.

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The nanotechnology revolution offers enormous societal and economic benefits for innovation in the fields of engineering, electronics, and medicine. However, growing evidence indicates that some biopersistent engineered nanomaterials (ENMs), including carbon nanotubes (CNTs) and metal nanoparticles, have the potential to stimulate immune, inflammatory, or fibroproliferative responses in the lung and pleura. This presentation will focus on specific mechanisms through which CNTs or nickel nanoparticles modulate cellular signaling and innate immune responses of macrophages, fibroblasts, and mesothelial cells relevant to lung and pleural disease. One of the goals is to discuss new evidence from transgenic mouse models showing that these ENMs have the potential to shift allergic lung inflammation from a
and N. B. Saleh2.

increase the susceptibility of lung cells to pathogenic infections and modulate TLR to address the precise role of TLRs in these observed effects both in vitro and in cells, but anticipate that SWNTs alter TLR activity. Studies are currently underway we did not observe significant changes in TLR3 or 7 mRNA expression in these IA V infectability as quantified by virus titers and immunohistochemistry. While RNA, respectively. Results from these studies show that SWNTs fail to activate the ability of SWNTs to modulate TLRs, we employed human cell lines that over-

Recognition of foreign pathogens by toll-like receptors (TLR) stimulates innate immunity as a primary defense mechanism to combat pathogens. To investigate the ability of SWNTs to modulate TLRs, we employed human cell lines that over-express TLR2 or TLR3 which recognize microbial moieties of bacteria and viral RNA, respectively. Results from these studies show that SWNTs fail to activate the TLRs alone, however, they repress the activity of zymosan as the natural receptor agonist for TLR2, while enhancing activity of the TLR3 agonist poly IC. Furthermore, these observations are most significant for SWNTs with 6, 5, 5, 5 chiral enrichment which form more stable and less dense clusters than other types of SWNTs. To determine if SWNTs influence virus infectivity and modulate TLR pathways in lung cells, we infected small airway epithelial cells with IAV H1N1 while co-incubating with SWNTs. In these studies SWNTs significantly enhanced IAV infectability as quantified by virus titers and immunohistochemistry. While we did not observe significant changes in TLR3 or 7 mRNA expression in these cells, but anticipate that SWNTs alter TLR activity. Studies are currently underway to address the precise role of TLRs in these observed effects both in vitro and in vivo. Overall results from these studies indicate that SWNTs have the potential to increase the susceptibility of lung cells to pathogenic infections and modulate TLR activity.

Because of the unique properties of carbon nanotubes (CNTs), such as small size, large surface area, high strength, and electrical conductivity, this nanomaterial has been used in high-performance composites, electronics, and medical therapeutics. Given the possible pro-inflammatory and toxic effects of CNTs, understanding of their interactions with living systems is important as applications employing this nanomaterial become mainstream. To this end, we studied degradation of CNTs with peroxidases including horseradish peroxidase (HRP), myeloperoxidase (MPO), and eosinophil peroxidase (EPO) as a function of their structure and surface functionalization. Initially, in test-tube studies were conducted, where HRP, MPO, and EPO were incubated with hydrogen peroxide and halides. Characterization techniques demonstrated that the reactive intermediates generated via the peroxidase cycle and hypohalous acids (e.g., HOCl from MPO and HOBr from EPO) produced through the halogenation cycle degraded short-cut carbonylated single-walled CNTs. Encouraged by these initial results, cellular studies were implemented in which we demonstrated that short-cut carboxylated, IgG-coated single-walled CNTs could be phagocytosed and degraded by neutrophils, inflammatory cells containing high concentrations of MPO. Also, utilizing a cellular culture system consisting of highly purified murine eosinophils in which degradation and exocytosis of murine EPO (mEPO) were triggered by cytochalasin B and a platelet-activating factor (PAF), significant degradation of carboxylated, single-walled CNTs was evidenced after 48 hours of treatment. Finally, in vivo studies demonstrated that the inflammatory response in MPO-knockout (k/o) mice was stronger than that in wild-type (w/t) C57/B16 mice, and both CNT degradation and clearance from the lungs of MPO k/o animals were markedly less effective. Given the promising cell culture and in vivo degradation results obtained to date, we envision future applications, where carbon-based nano-containers will biodegrade via peroxidase-driven mechanism after they deliver their cargo to organ/ cell targets.
Dietary supplements and other consumer natural products typically contain ingredients with an inherently wide margin of safety. Despite this fact, various ingredients can be negatively affected by processing, solvent systems, unconventional or non-traditional use patterns, or combining with other ingredients in proprietary blends with similar safety caveats for individual ingredients or the finished product as a whole. Premarket clinical studies are not routinely required or performed on finished products so products consumed by the public may make their debut in large populations that essentially become the “test” market. In these circumstances, products may carry forward high expectations of safety, but often times the only method of safety confirmation and ongoing monitoring for toxicity issues is the postmarket system of safety surveillance (PMS). Robust PMS can help companies confirm safety expectations, identify emerging safety signals associated with the monitored product, and provide information vital to hypothesis generation leading to focused investigation conducted in more controlled clinical environments. In order for the process to work effectively and efficiently, PMS data must be mined for appropriate and actionable safety signals. This presentation focuses on natural product PMS systems and methods to evaluate and act on generated safety signals with real life market examples.

Clinicians are oftentimes left scratching their heads when it comes to objectively evaluating the clinical utility of published case reports and/or adverse event reports regarding dietary supplements. Few clinical scientists conduct prospective research in the field and even fewer understand the pitfalls associated with dietary supplements. In short, understanding the medicine is often the easy part, making sense of the supplement and its safety and/or efficacy is the confusing aspect. This presentation will discuss some of the shortcomings associated with dietary supplement formulations (e.g., dissolution, bioavailability, adulteration, etc.) and how they either contribute to or detract from the clinical significance of a case report or adverse event report. Examples involving Ephedra, Kava kava, EGCG, and Ephedra-free supplements will be highlighted, including the results of controlled safety studies in humans.

Kava had previously been used to treat anxiety in Europe. Reports of rare hepatotoxicity potentially linked to its use led to its removal from the European market. However, kava is experiencing global resurgence, particularly in the USA, as a dietary supplement with limited quality control. Since many factors may influence the composition of the final kava products, various hypotheses have been proposed for its hepatotoxic risk with no validation. This presentation will report our research progress in identifying the cause of kava’s hepatotoxic risk using various model systems, and discuss our strategies that will validate such causes in humans with the ultimate goal to minimize such a risk. Studies are also ongoing to maximize kava’s anxiolytic and other beneficial effects. The outcomes from these investigations are expected to help the successful reintroduction of safer forms of kava with substantial clinical benefit.

This presentation will present how AER data is used from the perspective of a global dietary supplement manufacturer. Global AE collection helps dietary supplement companies monitor product safety and quality worldwide and make continuous improvements as necessary. AER-related quality issues can identify product QA/QC failures that necessitate changes in formulations or in rare cases lead to product recalls. Integrating post-market AER monitoring with product quality complaints ensures the most comprehensive investigative practices and allows sensitive signal detection. An increasing number of governments have mandatory collection and reporting requirements for AEs. Regulatory requirements are ever-changing. New regulations for reporting are being developed and enforced worldwide and regulatory authorities are now more frequently asking for adverse event information upon registration or re-registration of products. A robust AER system allows the development of FAQs that can be shared with health-care providers, distributors, or consumers. Systematic review of AE data provides a company’s legal department necessary information regarding compensation requests/decisions. At times, AER-related complaints may identify ethics violations by sales and distribution personnel. These might include suggesting products might treat a disease or other off label claims. Finally, as is true for drugs, post market AER monitoring is the best way to accurately assess the long term safety of dietary supplement products and assure your customers that the products they are using are truly safe.

The liver has a central role in normal physiology and is a sentinel organ for sensing chemical exposure. As such, it is one of the most frequent target organs serving as the basis of environmental chemical regulation and clinical drug failures due to concerns for both cancer and noncancer effects. Toxicogenomics has evolved to enable toxicity testing and risk assessment paradigms to be more efficient and less reliant on long-term animal studies. There is also global interest in using ‘omic tools to overcome obstacles encountered when interpreting liver injury seen in laboratory test systems. Factors contributing to species-selective hepatotoxicant responses among various mammalian species, particularly as mediated via xenobiotic receptors such as CAR, will be discussed. Case studies will highlight how ‘omic technologies provide mechanistic data for hazard identification, dose-response, and quantitative risk assessment with a fraction of the resources compared to traditional assessments. Gene signatures relating molecular initiating events such as transcription factor activation to downstream key events such as cell proliferation have defined adverse outcome pathways and correlated to trans-activation profiles of human transcription factors. These efforts to anchor genomic biomarkers of liver injury to more traditional apical endpoints create precedence for the use of toxicogenomic approaches as the basis of chemical regulation. In drug safety research, genetically-diverse population-based pharmacogenetic approaches have informed idiosyncratic liver toxicity, yielded mechanistic insights, and predicted clinically-relevant drug-induced liver injury where classical safety assessment models failed. Systematic approaches, such as the US FDA’s liver toxicity knowledge base (LTKB) that integrate pathways, genes, proteins, and drugs to characterize the risk of liver injury, will advance regulatory science, improve the new drug application review process, and refine targeted strategies for regulatory-oriented research.

In mammalian organisms, members of the nuclear and soluble receptor superfamilies contribute critically as xenobiotic sensors, serving as mediators of toxicological and physiological responses to chemical exposure. These xenoreceptors transcriptionally regulate a large network of genes encoding a functional web of responses that include the metabolism and transport of xenobiotics, regulation of lipid and energy homeostasis, and modulation of cell proliferation. Mouse models of receptor biology have been deployed widely to characterize these features. However, marked differences exist among mammalian species in their sensitivities and response to hepatotoxins, responses that differ with respect to dose as well as qualitatively in gene expression signatures elicited from a given exposure paradigm. For example,
the constitutive androstane receptor (NR1I3) is necessary for the development of hepatocellular carcinoma in mice following promotion by non-genotoxic receptor activators such as the direct ligand, TCPOBOP, or indirect activators such as phenobarbital (PB). However, extensive epidemiological studies in human populations have ascertained no excess risk of liver cancers following chronic PB exposure. To allow biologically-based and scientifically defensible extrapolations of receptor function across mammalian species that accurately predict potential human toxicities, it is critical to delineate the molecular mechanisms underlying these differential responses. Surprisingly, results using chromatin immunoprecipitation and bioinformatics analyses have revealed extensive divergence among transcription factor binding sites between species. Species-selective splice variation in each receptor further defines inherent differences in species response. This presentation will focus on those features of xenobiotic receptors that contribute to the differences underlying species-selective response to hepatotoxicants.

**W 1478 The Utility of Toxicogenomics in the Risk Assessment of Carcinogens: A Report on Two Health Canada Case Studies**

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Despite the numerous proposed applications of toxicogenomics in human health risk assessment, integrating standard genomics endpoints in chemical evaluations or using changes in pathway expression to identify points of departure is uncommon. We evaluated the utility of toxicogenomics data in the risk assessment of two well-established carcinogens, benzo(a)pyrene and furan, to demonstrate how genomics data may enhance or complement the current risk assessment process. Gene expression profiles and dose-response modeling were used to develop modes of action and establish pathway-based points of departure for these compounds. We found agreement between the derived points of departures and margins of safety for the genomics-proposed mode-of-action, as well as the most sensitive pathways. Our study demonstrates the potential for a mouse diversity panel (MDP), comprised of genetically diverse inbred strains, could accurately predict the DILI potential of PF-04287881 to humans. Increases in liver biomarkers were associated with histopathological findings of single cell necrosis, hepatocellular hypertrophy and Kupffer cell vacuolation. Based on their unique injury profiles, four mouse strains were selected for transcriptomic analysis of PF-04287881 toxicity in the liver. The protein ubiquitination pathway was highly enriched among genes differentially expressed in strains exposing PF-04287881-induced hepatocellular necrosis. In contrast, expression changes associated with PF-04287881-induced phospholipidosis included genes involved in drug transport, phospholipid metabolism and lysosomal function. These findings demonstrate the potential for a mouse population-based approach to improve hazard identification in drug-safety testing as well as yield mechanistic insights via toxicogenetic investigation.

**W 1479 Using Gene Signatures to Predict Molecular Initiating Events in Liver Adverse Outcome Pathways**

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Adverse outcomes such as liver cancer have been linked to molecular initiating events (MIE) and downstream key events to define adverse outcome pathways (AOP). Identification of gene sets (signatures) predictive of MIEs (i.e., transcription factor activation) were derived by comparing microarray profiles of chemically-treated wild-type or transcription factor (TF)-null mice. The signatures included those dependent on AhR, CAR, PXR, Nrf2, PPARalpha, and PXR, as well as TFs (glucocorticoid receptor and androgen receptor) regulated by hormones. Prediction tests of TF activation showed that balanced accuracies were ≥ 90%. The signatures were used to comprehensively assess TF activation or suppression in a mouse liver compendium of gene expression biosets (~1900) that included chemicals, genetic models and other conditions that can affect the outcome of hazard studies including diet and life stage. Many of the TFs were activated/suppressed by a large number of chemical and chemical-independent perturbations of the liver (CAR, PPARa, PXR, Nrf2), whereas AhR activation was primarily through chemical exposure. Pairs of TFs (i.e., CAR, PPARa, Nrf2 and PXR) were often co-activated by chemicals and gene knockouts. Predictions of TF behavior were used to link chemical exposure, MIEs and AOs in the mouse liver allowing a classification of chemicals by one or more AOPs. The information can be used in cumulative risk models that take into account chemical-independent effects on MIEs (This abstract does not represent US EPA policy.)

**W 1480 Genetically Diverse Mouse Populations Facilitate Toxicogenetic Analysis of Drug-Induced Hepatotoxicity**

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Standard nonclinical models, including animals and average human hepatocytes, lack the genetic diversity necessary to model the breadth of toxicity responses observable across clinical populations. Thus, it is occasionally challenging to establish an animal model for a clinically relevant toxic reaction. Recent data indicate that mouse population-based models aid identification of genetically sensitive individuals that may then be used to study mechanisms of toxicity. Development of the macrodil antibiotic PF-04287881 was suspended following elevations in liver function tests observed in a Phase I clinical trial that were not predicted by standard nonclinical species. We demonstrated that a mouse diversity panel (MDP), comprised of genetically diverse inbred strains, could accurately predict the DILI potential of PF-04287881 to humans. Increases in liver biomarkers were associated with histopathological findings of single cell necrosis, hepatocellular hypertrophy and Kupffer cell vacuolation. Based on their unique injury profiles, four mouse strains were selected for transcriptomic analysis of PF-04287881 toxicity in the liver. The protein ubiquitination pathway was highly enriched among genes differentially expressed in strains exposing PF-04287881-induced hepatocellular necrosis. In contrast, expression changes associated with PF-04287881-induced phospholipidosis included genes involved in drug transport, phospholipid metabolism and lysosomal function. These findings demonstrate the potential for a mouse population-based approach to improve hazard identification in drug-safety testing as well as to yield mechanistic insights via toxicogenetic investigation.

**W 1481 Overcoming Obstacles to Study Drug-Induced Liver Injury with Toxicogenomics towards a Regulatory Application**

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More than 25% of drugs on the market that ultimately fail and 40% that fail during clinical trials do so because of liver toxicity. As the leading cause of acute liver failure in the United States, drug-induced liver injury (DILI) has become a major concern in drug development and evaluation. The FDA’s new initiative on advancing regulatory science has identified DILI as a key focus area in a concerted effort to broaden FDA’s knowledge, create better evaluation tools, and identify safety biomarkers along the various stages of drug development. To enhance our understanding of liver toxicity, NCTR is developing a liver toxicity knowledge base (LTKB) to support research into relationships among DILI, pathways, genes and proteins, and drugs. The project uses a combination of data from the literature on selected drugs with specially emphasis on toxicogenomics data to develop a systematic approach for defining the severity of risk of a drug for liver injury. These approaches have a potential to support the application review process and regulatory oriented research.

**W 1482 Understanding Weight of Evidence: Exploring Different Approaches to Integrating Evidence from Diverse Data Streams**

N. B. Beck1 and J. E. Goodman2, 3. 2Regulatory and Technical Affairs, American Chemistry Council, Washington, DC, 3Harvard School of Public Health, Boston, MA and 2Gradient Corp, Boston, MA.

While it can be straightforward to evaluate a single study, or a single evidence stream, evaluating an entire body of literature for hazard assessment or risk assessment, where one needs to integrate data that comes from multiple evidence streams (e.g., human data, toxicological data, and mechanistic information), can be more complicated. The integration of all this information is becoming increasingly complex and is particularly driven by the explosion in biological sciences and technologies that are dramatically reshaping both the volume and utility of mechanistic information. Mechanistic information, when coupled with classical endpoint-oriented toxicity data, offers a growing opportunity to more closely examine the toxicologic plausibility of observational associations obtained from epidemiologic studies. Different organizations have taken different approaches to the integration of evidence from diverse data streams, and no clear consensus approach currently exists. This workshop will explore and discuss some current approaches to integrating evidence from several organizations that develop assessments. This is timely and important, as in 2009 the National Academies (NAS) specifically called upon the US EPA to, among other things, improve the way in which evidence is synthesized within the Integrated Risk Information System (IRIS) assessments. The NAS panel acknowledged that there are existing approaches, currently used by...
other government programs and elsewhere, for each of these steps and encouraged the US EPA to select from and adapt them. The session will describe, using specific examples, current frameworks that use different approaches to integrate epidemiologic, toxicological, mechanistic, and other data. In addition, seen through the lens of a state user, this perspective will describe what elements are necessary in order to apply and use an integrated evaluation. Time will be provided for a robust discussion and questions.

1483 Introduction: Setting the Stage
N. B. Beck, Regulatory and Technical Affairs, American Chemistry Council, Washington, DC.

A brief overview of the workshop goals will be provided. This includes discussion of the importance of understanding epidemiologic, toxicological and mechanistic information in order to appropriately incorporate and integrate the information into weight of evidence determinations and risk assessments to inform regulatory and non-regulatory decisions.

1484 Understanding Hypothesis-Based Weight of Evidence
L. R. Rhoenberg, Gradient, Boston, MA.

Once a systematic approach to choosing and laying out evidence is accomplished, the challenge remains to integrate the information into a judgment about the degree of evidence to be placed in the possibility of a causal effect in the target (human) population in view of the incomplete and often contradictory information at hand (including human, animal, and mechanistic information). This presentation proposes the Hypothesis-Based Weight-of-Evidence (HBWoE) approach to making, defending, and explaining the basis for such judgment. What makes data into “evidence” is that one proposes that causative forces of the agent underlying the study results may also apply to the target population — that is, bringing to bear study results as evidence entails a proposed generalization of the operation of causes, and one can then ask what other consequences of those proposed common causes should exist and whether they are in fact seen. Noting patterns of commonality and discordance, and attending to the further assumptions that are made to try to reconcile them with the hypothesized general causative properties helps to sort through and articulate the logic behind judgments about human risk potential. The key question is whether the proposed common causal effects (and proposed reasons for any apparent exceptions to their operation) make for a more plausible account for human and non-human animal studies along with consideration of other relevant data (e.g., in vitro or mechanistic studies). Before combining the three evidence streams, confidence ratings are developed for a given outcome by considering the strengths and weaknesses of the collection of studies or body of evidence. Human and animal studies are evaluated separately using a framework based on the Grading of Recommendation Assessment, Development and Evaluation (GRADE) and Agency for Healthcare Research and Quality (AHRQ) approaches. Other relevant data are evaluated in parallel by assessing the support for biological plausibility provided by this evidence stream. A level of evidence for each health effect is developed that reflects both the overall confidence in the association between exposure to the substance and the outcome and the direction of the effect (i.e., toxicity or no toxicity). The hazard identification conclusions are developed based on the highest level of evidence for a health effect from each of the evidence streams in the final step of the evidence assessment process. First, the level of evidence for health effects conclusion based on human data is considered together with the level of evidence for health effects conclusion based on animal data to reach a hazard identification conclusion of: Known, Presumed, Suspected, Not Classifiable or Not Identified, to be a hazard to humans. Second, the conclusion is considered in context of other relevant data. The final hazard identification conclusion reflects the confidence in each evidence stream as well as the consistency in support (or potential opposition) between the three lines of evidence on the relationship between exposure to the substance and the health effect being examined.

1485 The US EPA Causality Framework for Assessment of Air Pollution-Related Effects

The periodic review of U.S. National Ambient Air Quality Standards (NAAQS) for each of the six criteria air pollutants — ozone, particulate matter, carbon monoxide, nitrogen oxides, sulfur oxides and lead — starts with the synthesis and evaluation of the most policy-relevant science in Integrated Science Assessments (ISAs). EPA has developed an approach for formal characterization of the strength of the scientific evidence and drawing conclusions on causality for exposure-effect relationships. The framework establishes uniform language concerning causality and brings greater consistency and specificity to the ISAs. EPA drew on relevant approaches for similar scientific decision-making processes by EPA and other organizations. Findings from multiple lines of evidence — controlled human exposure, epidemiologic and toxicological studies — are evaluated and integrated to draw conclusions with regard to factors such as consistency, coherence and biological plausibility. The relative importance of different types of evidence varies by pollutant or assessment, as does the availability of different types of evidence for causality determination. The use of the framework is demonstrated with several examples of determinations for various health outcomes and pollutants, particularly drawing from the recently-completed ISA for Lead (Pb). Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views or policies of the US EPA.

1486 The Office of Health Assessment and Translation Approach to Evidence Integration for Assessment of Noncancer Health Effects
A. A. Rooney, A. L. Boyles, M. S. Wolfe and K. Thayer. Division of the National Toxicology Program, NIEHS, Research Triangle Park, NC.

The NTP’s Office of Health Assessment and Translation (OHAT) develops non-cancer hazard identification conclusions by integrating the evidence streams for human and non-human animal studies along with consideration of other relevant data (e.g., in vitro or mechanistic studies). Before combining the three evidence streams, confidence ratings are developed for a given outcome by considering the strengths and weaknesses of the collection of studies or body of evidence. Human and animal studies are evaluated separately using a framework based on the Grading of Recommendation Assessment, Development and Evaluation (GRADE) and Agency for Healthcare Research and Quality (AHRQ) approaches. Other relevant data are evaluated in parallel by assessing the support for biological plausibility provided by this evidence stream. A level of evidence for each health effect is developed that reflects both the overall confidence in the association between exposure to the substance and the outcome and the direction of the effect (i.e., toxicity or no toxicity). The hazard identification conclusions are developed based on the highest level of evidence for a health effect from each of the evidence streams in the final step of the evidence assessment process. First, the level of evidence for health effects conclusion based on human data is considered together with the level of evidence for health effects conclusion based on animal data to reach a hazard identification conclusion of: Known, Presumed, Suspected, Not Classifiable or Not Identified, to be a hazard to humans. Second, the conclusion is considered in context of other relevant data. The final hazard identification conclusion reflects the confidence in each evidence stream as well as the consistency in support (or potential opposition) between the three lines of evidence on the relationship between exposure to the substance and the health effect being examined.

1487 Modernizing Problem Formulation: Classical versus Mode-of-Action Approaches to Weight-of-Evidence Determinations
C. J. Borgert, Applied Pharmacology and Toxicology, Gainesville, FL.

In scientific investigations, the questions posed — i.e., the hypotheses tested — determine the measurements and conditions under which they are taken, the procedures used to record and analyze data, and the context in which results are interpreted. In risk assessment, the questions addressed are typically articulated in the problem formulation phase. Decades ago, regulatory agencies couched problem formulation according to the questions answerable by the science of the day. As regulatory requirements for risk assessment became codified, so too did the rudiments of problem formulation. Unfortunately, codifying problem formulation prevents risk assessment from evolving to keep pace with scientific advancements. Today, more specific questions can be addressed and answered more precisely with more advanced science, but this science is not being used effectively because typically, the risk assessment problem formulation step still poses antiquated questions. Problem formulation needs to be modernized so that modern science can better inform risk considerations. Using well-studied chemicals as examples, e.g., chloroform or cumene, three Weight of Evidence approaches - the classical IRIS Approach, the Human Relevance Framework Approach, and a Hypothesis-Based Mode of Action Approach - are applied, compared, and contrasted. The analysis illustrates why improving the problem formulation phase is critical to making risk assessment more scientifically accurate, more practical, and more relevant for protecting human health and the environment.

1488 The User Perspective—What Makes an Integrated Evaluation Useable and Most Useful?
L. Zeive, Office of Environmental Health Hazard Assessment, California EPA, Oakland, CA.

This talk will consider evidence integration from the perspective of a implementer and user. Regulatory guidance levels, hazard evaluations and risk assessments typically rely on general frameworks for integrating evidence, as reflected in the preamble to standards. In contrast, various US Environmental Protection Agency guidelines and similar guidance developed by states. The emerging approaches described in earlier talks in the session add more specificity to how individual studies and streams of evidence are weighed in reaching conclusions about hazard traits and levels of toxic activity. They differ in approach to data organization, framing questions for evaluation, search strategies, and study selection, as well as resource requirements for performing the evaluation. As these newer approaches are developing the nature and volume of the relevant scientific evidence available for evaluation continues to change. Elements of us-
ability and usefulness of the new approaches will be considered in the context of the types of problems environmental protection agencies are expected to address and the aims of transparency, understandability, scientific rigor and public health protection. Disclaimer: The views expressed are those of the author and do not necessarily reflect the views or policies of the Office of Environmental Health Hazard Assessment or the California Environmental Protection Agency.

1489 Discussion
J. E. Goodman1, 2, 1Gradient, Boston, MA and 2Harvard School of Public Health, Boston, MA.

A robust discussion and question-and-answer session will focus on the benefits of using a weight-of-evidence approach in general, and particular aspects of various approaches that differ.

1490 AML Subtypes Reported in Cigarette Smokers: A Meta-Analysis
D. Pratt1, 3 and D. D. Alexander2, 1Summit Toxicology, Superior, CO, 2Exponent, Boulder, CO and 3School of Public Health, University of Colorado, Denver, CO.

The relationship between cigarette smoking and an increased risk of AML has been well documented. While the overall risk is modest, the number of smokers is very large. As a result, cigarette smoking is currently considered the single most important cause of AML in the US. It is assumed that the leukemogenic activity of cigarette smoke is related to the benzene or benzene metabolite content. It is also well documented that AML is not a single disease, but comprised of several different subtypes (depending upon the classification system utilized). One is the M3 subtype (per the FAB) or acute promyelocytic leukemia (APL) that is distinctly different from other subtypes of AML in terms of its diagnostic criteria, treatment, cytopathic abnormalities and prognosis. While many studies demonstrate the relationship between cigarette smoking and AML, there have only been six studies that have formally evaluated the risk between cigarette smoking and various subtypes of AML. All six of these studies reported elevations in most subtypes of AML but not APL or AML with (V15;17). Here we conducted a meta-analysis of this data set and determined that the overall risk for AML among smokers was statistically significantly suppressed (summary OR = 0.5, 95% CI 0.3-0.8), with good homogeneity between the studies. In contrast, the summary OR for the M2 subtype of AML was significantly elevated [OR = 2.03, 95% CI = 1.41-2.90]. These data indicate that cigarette smoking (or benzene exposure) is not likely a causative factor in the development of AML.

1491 Evaluation of Pyridylloxobutyl DNA Adduct Formation in the A/J Mouse Model
A. Urban, P. Upadhyaya and L. A. Peterson, University of Minnesota, Minneapolis, MN.

Tobacco-specific nitrosamines represent an important class of carcinogens found in tobacco products. The tobacco-specific nitrosamine 4-(methylnitrosami- no)-1-(3-pyridyl)-1-butane (NNK) is a potent carcinogen in laboratory animals, and is a known human carcinogen. NNK is metabolically activated to generate both methyl and pyridylloxobutyl DNA adducts, which if not repaired could persist and lead to lung tumor formation. Methyl DNA adducts have been linked to the tumor-origenic properties of NNK. However, the role of pyridylloxobutyl DNA adducts in tumor formation has not been elucidated. NNK generates four pyridylloxobutyl DNA adducts: O6-[4-3-(pyridyl)-4-oxobut-1-yl]deoxyguanosine (O6-pobdG), O2-[4-3-(pyridyl)-4-oxobut-1-yl]deoxyethyldine (O2-pobdT), 7-[4-3-(pyridyl)-4-oxobut-1-yl]-2′-deoxyinosine (O2-pobdC), O6-pobdG is repaired by O6-alkylguanine-DNA alkyltransferase (AGT), and nucleotide excision repair may play a role in the repair of O2-pobdT. The possible repair pathways of the other pyridylloxobutyl DNA adducts are unknown. To determine the role of pyridylloxobutyl DNA adduct formation in the tumororigenic properties of NNK, DNA adduct levels and tumor yield were measured in the lungs of A/J mice in following treatment with the model pyridylloxobutylating agent 4-(acetoxyethyl-nitrosamino)-1-(3-pyridyl)-1-butane (NNKOA). The findings indicate that O2-pobdT accumulates in lung DNA. AGT depletion does not significantly affect O6-pobdG repair or tumor formation, suggesting that O6-pobdG is likely also repaired by pathways other than AGT. These results indicate that the formation and persistence of O2-pobdT may be important for tumorigenesis, and that multiple pathways are likely responsible for the repair of pyridylloxobutyl DNA adducts. [Supported by CA-115309 and CA-138338].

1492 Modulation of IL-8 Signaling by AhR in Breast Tumor Microenvironment Involves miR-17
M. B. van Duuren, S. Nijmeijer, A. D. van den Brand, J. Villevoye and M. Van den Berg, IRRIS, Utrecht University, Utrecht, Netherlands.

Tumor microenvironment, including the interaction between breast epithelial (tumor) cells and surrounding breast adipose fibroblasts (BAFs), is crucial for tumor development and progression. MicroRNAs (miRNA) have been suggested to play an important role in tumor microenvironment signaling. The aryl hydrocarbon receptor (AhR) has previously been shown to affect miRNA expression. We found AhR expression in cancer-associated breast fibroblasts to be higher than in healthy BAFs, which points to an endogenous role for the AhR in breast cancer pathogenesis. Therefore, we studied the effect of AhR activation on miRNA signaling in an in vitro breast tumor microenvironment. For that, we firstly determined expression of 84 abundance expressed miRNAs in MCF-7 cells that were exposed to vehicle control (DMSO), non-toxic AhR agonist tranilast (100 μM) or TCDD (1 nM) using a miRNA PCR Array. Despite both being AhR agonists, changes in miRNA profiles upon exposure to TCDD and tranilast were apparently ligand-dependent altered. Notably, both TCDD and tranilast inhibited miR-17 expression, which has been shown to directly target the 3′ UTR of IL-8. Indeed, TCDD, and to a lesser extent tranilast, concentration-dependently increased IL-8 secretion by MCF-7 cells. Next, we determined IL-8 secretion and gene expression in MCF-7 cells co-cultured with BAFs, to mimic a more relevant tumor microenvironment. IL-8 secretion by mono-cultured BAFs was 54.7 pg/ml/48h and 9.3 pg/ml/48h by mono-cultured MCF-7 cells. Interestingly, IL-8 secretion in a BAF/MCF-7 co-culture was around 4-fold lower than in a mono-culture of BAFs, but similar to MCF-7 mono-cultured cells. Activation of AhR by tranilast resulted in a seemingly, yet not significantly, increase in IL-8 expression in BAF/MCF-7 culture. Considering the direct link between IL-8 and promotion of cancer (stem) cells in vitro and in vivo, these data warrant further studies on AhR-mediated modulation of miRNAs in the tumor microenvironment.

1493 Decreased Expression of FRY, a Promoter of Epithelial Cell Differentiation, Is Associated with EMT and Clinical Progression of Breast and Prostate Cancer
H. Zarb1, N. Takizawa2, 1, L. C. Graham1, B. Estrella1, X. Ren2 and M. Fang1, 1Environnemental and Occupational Health Sciences Institute, Rutgers, The State University of New Jersey, Piscataway, NJ, 2GeneAsses, Inc, North Brunswick, NJ and 2Department of Social and Preventive Medicine, The State University of New York, Buffalo, NY.

Rat strains vary in their susceptibility to mammary carcinogenesis following exposure to chemical carcinogens, hormones and ionizing radiation. Using quantitative trait locus mapping, we recently identified FRY, the mammalian ortholog of the furry gene in Drosophila melanogaster, as a carcinoma susceptibility gene. FRY is not expressed in lower eukaryotes regulate epithelial cell differentiation and polarity. Accordingly, loss of FRY expression in human mammary epithelial and tumor cell lines correlated with epithelial differentiation, morphology, polarity and adhesion. Ectopic expression of the wild-type FRY gene in human breast cancer cells restored a gene expression profile consistent with differentiation, suppression of epithelial-mesenchymal transition (EMT), and significantly diminished tumorigenicity in vitro and in vivo. To assess the role of FRY in human cancers, we used clinically annotated cancer cohorts available in the Oncomine 3.0 gene expression database. The results indicated that FRY was significantly decreased in, and associated with, progression in a broad spectrum of epithelial cell cancers. Analysis of human breast carcinomas tissue microarrays (TMAs) confirmed that decreasing nuclear accumulation of the FRY protein was correlated with negative hormone receptor status, cancer progression, poor clinical outcomes, and especially increased Estlon tumor grade (undifferentiated tumor histopathology score). Analysis of TMAs comprised of human prostate tissue and cancers verified that decreased FRY was also associated with clinical progression and poor outcomes. Together, these results suggest decreased nuclear accumulation of the FRY cancer susceptibility gene plays a critical role in the pathogenesis and progression of cancer in various tissues.

1494 Carcinogenicity Testing of Eliglustat in the Mouse and Rat
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Eliglustat is in development for oral treatment of the inherited metabolic disorder Gaucher disease type 1 (GD1). This lysosomal storage disease results from a deficiency of the catalytic enzyme acid-β-glucosidase leading to accumulation of glucosylceramide. The principal substrate of acid-β-glucosidase is glucosylceramide.
Hepatocellular carcinoma (HCC) is the most common hepatic malignancy and the third leading cause of cancer related deaths. Previous studies have implicated bile acids in pathogenesis of HCC but the mechanisms are not known. We investigated the mechanisms of bile acids in promoting diethylnitrosamine (DEN)-induced HCC in mice. Male C57BL/6 mice were treated with DEN (15 mg/kg) at postnatal day 15 and were weaned on a normal rodent diet. At the age of 8 months these mice were fed either the normal rodent chow (control diet) or a diet containing 0.2% cholic acid (0.2% CA diet) for 2 months. Two other groups of mice without DEN treatment fed either control diet or 0.2% CA diet from 8 to 10 months of age served as controls. All mice were sacrificed at 10 months of age and evaluated for liver tumors. The data show that 0.2% CA treatment resulted in 3-fold increase in number and size of DEN-induced liver tumors. All tumors observed in DEN-treated mice were well-differentiated HCCs. Microarray analysis (Affymatrix Mouse 430_2.0 chip) combined with Ingenuity Pathway Analysis revealed that NF-κB and JNK signaling pathways were the main signaling pathways activated in DEN-treated mice. Expression levels of TFGBβ were significantly higher in DEN-induced tumors than those in controls. We concluded that TFGBβ plays an important role in the development of DEN-induced HCC tumors.
mice. Taken together, these data indicate that bile acid feeding results in promotion of DEN-induced HCCs via specific activation of NF-κB and JNK signaling pathways.

1499 Repurposing the Antimalarial Amodiaquine for Autophagy-Directed Anti-Melanoma Intervention
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The autophagic-lysosomal pathway is a critical component of the cellular stress response. Cancer cells upregulate autophagic activity as a mechanistic adaptation to adverse conditions of the tumor microenvironment, and pharmacological inhibition of autophagic-lysosomal function has therefore emerged as a promising strategy for chemotherapeutic intervention. Here we demonstrate that autophagic-lysosomal blockade can be achieved in cultured A375 malignant melanoma cells using the clinical anti-malarial amodiaquine (AQ). Consistent with lysosomal expansion observed at the ultrastructural level, AQ-induced molecular changes were substantiated by accumulation of the lysosomal marker glycoprotein Lamp-1, the lipiddated autophagosome marker LC3-II, and selective autophagy substrates (p62, alpha-synuclein). Monitoring autophagic flux by expression of a tandem fluorescently-labeled LC3 confirmed autophagic-lysosomal blockade associated with rapid inactivation of lysosomal cathepsins, 2D-Immunoblot-proteomics using an AQ-directed monoclonal antibody identified the eukaryotic translation initiation factor-eIF4A as a specific protein target adducted by the electrophilic quinoneimine-methide of AQ. Array analysis revealed modulation of gene expression accompanying heat shock response (HSPA8, HSPA1A, HSP90AA1) and cell cycle progression (CDKN1A, E2F1) confirmed by immunoblot detection (Hsp70, Hsp90, p21, E2F1). Consistent with these expression changes, AQ displayed anti-proliferative effects causing S-phase arrest at submicromolar concentrations targeting an array of malignant melanoma cell lines. AQ-treatment caused rapid induction of energy crisis and sensitized melanoma cells to starvation- and chemotherapeutics-induced cell death. Taken together, our data suggest feasibility of repurposing the clinical anti-malarial AQ for anti-melanoma intervention targeting autophagic-lysosomal function and proliferative control. [R01CA122484, R03CA167580, R21CA166026, ES007091, ES06694].

1500 Epithelial-to-Mesenchymal Transition in Human BEAS and BZR Cells by Benzo(a)pyrene: Involvement of Aryl Hydrocarbon Receptor and Long Interspersed Nuclear Element-1
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Benzo(a)pyrene (B(a)P), a ubiquitous environmental hydrocarbon, has been extensively linked to diseases of the lung including interstitial fibrosis and carcinogenesis. The present studies were conducted to evaluate molecular mechanisms of B(a)P pulmonary toxicity in human BEAS and BZR cells, non-transformed and transformed bronchial epithelial cells, respectively. Treatment with B(a)P was associated with Epithelial-to-Mesenchymal Transition (EMT), a response which was dependent upon the activation of Aryl Hydrocarbon Receptor (AHR) and Long Interspersed Nuclear Element-1 (L1). B(a)P inhibited expression of epithelial markers and increased the relative abundance of mesenchymal markers in both BEAS and BZR cells. Treatment with α-naphthoflavone (α-NF), a potent antagonist of AHR, attenuated the induction of mesenchymal markers. The EMT response involved induction of L1 at both the mRNA and protein levels. Inhibition of L1 expression through siRNA knockdown attenuated EMT suggesting that intact L1 expression is required for EMT. Together, these data indicate that EMT in human bronchial epithelial by B(a)P involves activation of AHR and L1.

1501 Disruption of Circadian Rhythm Accelerates Lung Metastasis of Breast Cancer in C3(1)/SV40 T-Antigen (TAG) Transgenic Mice
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Disruption of circadian rhythm promotes breast cancogenesis, and is associated with cancer progression and decreased survival rate. To investigate the contribution of the circadian disruption to breast cancer metastasis, heterozygous female C3(1)/SV40 T-antigen (TAG) transgenic mice were maintained on chronic jet-lag protocol (~3 months) or regular light and dark cycles after weaning. Mammary tumor latency, incidence and multiplicity, as well as metastasis and inflammatory status of lung were evaluated by histopathology. Results showed that the mammary tumor appeared 16 days earlier and the average total tumor burden (volume and weight) was higher in the mice maintained on jet-lag compared to that in mice on regular light and dark cycles (control group). Lung micro-metastatic foci were also observed at least 13 days earlier, and lung metastasis multiplicity was significantly associated with group, with 288% more metastasis foci in jet-lag vs control group. No visible lung metastatic tumors were observed in heterozygous mice; however, the visible lung metastatic tumor rate, micro-metastatic foci multiplicity, and average foci size were increased in jet-lag vs control group in carriers. In addition, number of lung inflammation foci was significantly increased by circadian disruption, with 233% more inflammatory foci in jet-lag than control group during the early metastasis stage, suggesting that suppressed cellular immunity and overactive inflammatory responses contribute to the development of lung metastasis in this model. Taken together, our studies demonstrated that disruption of circadian rhythm caused by jet-lag accelerates the development and progression of both primary mammary tumor and lung metastasis. (Supported by NIH grants: U19ES011387, P30ES007033, and P30ES050522).

1502 Ni2-Induced Global De-Regulation of Gene Expression and Morphological/Neoplastic Transformation of C3H/10T1/2 Mouse Embryo Cells
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Nickel refining induced lung/anal cancers in workers; Ni3S2/green NiO induced lung cancer in rats. Ni3S2/green NiO induced morphological/neoplastic transformation in 10T1/2 mouse embryo cells. mRNA differential display showed differential expression (DE) of 150 genes between non-transformed (Tx)/2 MCA/4 NiO/NiS-Tx 10T1/2 cell lines. Ni3MCA-Tx cell lines contained a) ect-2 amplification/higher ect-2 mRNA/protein levels; b) higher levels of calnexin mRNA/protein; c) no DRIP80/β-centaurin-2 mRNAs. We hypothesized Ni2+ 1) amplified ect-2, leading to higher levels of microtubules (MTs); 2) silenced β-centaurin-2, causing higher levels of microfilaments (MFs); and 3) silenced DRIP80, altering Ca2+ ion distributions, in Tx 10T1/2 cells. To test these hypotheses, we stained these cells with fluo-3AM to decorate MFs, fluo-4 to label tubulin of MTs, Fluor 705 to stain Ca2+ ions, DAPI to decorate nuclei, and examined cells by confocal microscopy. In non-Tx 10T1/2 cells, MFs/MTs were distributed homogeneously. In Ni3S2/green NiO-Tx cell lines, MFs/MTs were over-expressed, aggregated in areas, absent in others, changing shapes of Tx cells. In non-Tx cells, Ca2+ ions were found in a) State I, in low density cells, with a high nuclear concentration of Ca2+ ions; b) State II, in high density cells, with more cytoplasmic Ca2+ ions; or b) State III, in low density cells, with a high nuclear concentration of Ca2+ ions; or b) State IV, in high density cells, with more cytoplasmic Ca2+ ions. In Ni3S2/green NiO-Tx cell lines, MFs/MTs were over-expressed, aggregated in areas, absent in others, changing shapes of Tx cells. In non-Tx cells, Ca2+ ions were found in a) State I, in low density cells, with a high nuclear concentration of Ca2+ ions; b) State II, in high density cells, with more cytoplasmic Ca2+ ions. In 4 Ni2+ MCA/Tx cell lines, Ca2+ ions were in State II. We conclude Ni2+ ions a) mutated/amplified 6 target genes/silenced 9 target genes; b) amplified ect-2, causing higher levels of MTs/silenced β-centaurin-2, causing higher levels of MFs, changing cell shapes/gene expression in Tx cell lines; c) silenced DRIP80, altering Ca2+ ion distributions in Tx 10T1/2 cell lines. These Ni ion-induced events caused DE of 150 genes in Tx cell lines and induction/maintenance of Ni2+ phenotype. Supported by Grant ES03341/grants from M. S. Program in Micro. and Provost’s Office at USC, to JRL.
Environmental exposure to carcinogens causes loss of tumor suppressor genes. Phosphatase and tensin homolog (PTEN) negatively regulates the AKT pathway, and the PTEN gene is mutated/deleted in ~70% of prostate cancer cases. Downstream of AKT, eukaryotic initiation factor 4E binding protein 1 (4E-BP1) regulates cyclin D1 translationally. In contrast, ERK, a member of the mitogen-activated protein kinase (MAPK) pathway, regulates cyclin D1 transcriptionally. Both the AKT and MAPK signaling pathways contribute to disease progression in prostate cancer. A PTEN-deficient human prostate cancer cell line, LNCaP, and a PTEN-positive human prostate cancer cell line, DU145, were used to examine the role of PTEN status in determining drug sensitivity to Amuvatinib, a receptor tyrosine kinase inhibitor, and Erlotinib, an epidermal growth factor inhibitor. 1D and 2D Western blot analysis revealed that combination drug treatment of LNCaP cells caused a decrease in 4E-BP1 pSer65, pThr70 and pThr37/46, with a concomitant decrease in cyclin D1 protein. Such combination treatment, however, did not modulate pERK status. Moreover, single drug treatment with Amuvatinib, but not Erlotinib, decreased p-4EBP1 and cyclin D1 protein levels. In DU-145 cells, combination drug treatment had no effect on p-4EBP1 status, but decreased p-ERK and cyclin D1 protein levels. Additionally, DNA was isolated from the tissues. Oxidative damage to DNA was assessed using the Biovision DNA damage quantification kit. Benzo[a]pyrene-DNA adducts were resolved and quantified by the 32P-postlabeling method. The metabolic fate of BaP was altered in a dose-dependent manner with the 50 μg/kg dose group registering an elevated expression of BaP biotransformation enzymes, greater concentration of BaP metabolites, abasic sites in DNA, BaP-DNA adducts at C7 and C8 was increased by up to the 25 μg/kg dose group (p = 0.05). These findings indicate that i) subchronic exposure causes sustained induction of BaP biotransformation enzymes and extensive metabolism of this toxicant; ii) the production of free radicals initiated DNA base pair damage, which in the absence of DNA repair, formed BaP-DNA adducts; and iii) a correlation between colon tumors and BaP-DNA adduct concentrations. Our studies provide concrete evidence that sustained dietary exposure to BaP contributes to the development of colon cancer.

Clear cell renal cancer (ccRCC), the predominant subtype of kidney cancer, displays variability in risk for developing metastatic disease, and tissue-based prognostic biomarkers are urgently needed. Recently, we used unsupervised bioinformatic strategies to demonstrate two subtypes of ccRCC, clear cell A (ccA) and clear cell B (ccB). The subtypes convey a prognostic value, with tumors displaying the ccA signature associated with better survival compared to ccB. We developed and validated a 34-gene subtype predictor to classify clear cell tumors as ccA and ccB using RNA-sequencing data from 380 ccRCC samples from The Cancer Genome Atlas (TCGA) and the NanoString platform with a cohort of 163 fixed archival samples collected at the University of North Carolina. This novel tool can be used to analyze risk for developing metastatic disease and as a baseline metric to elucidate specific effects of toxicants on renal tumor gene expression signatures that can provide insight into the molecular phenotype of ccRCC. Heavy metals can induce nephrotoxicity altering gene expression in the kidney, and thus increasing the risk of developing RCC. In addition to transcript alterations, numerous studies have found that Cd exposure can result in epigenetic deregulation by changing DNA methylation levels. We have observed significant decreases in methylated sites associated with the 34 genes from the ccA/ccB algorithm among ccRCC tissues (n = 17) compared to normal tissue (n = 15) of smokers from TCGA. Smoking populations experience higher levels of cadmium exposure, as one cigarette may contain 1-2 μg cadmium. Interestingly, the majority of this cohort was either ccB subtype or had metastatic disease, suggesting a possible correlation between cadmium exposure and aggressive disease. Exploring both acute and chronic cadmium-induced global expression and epigenetic reprogramming can enhance our understanding of the genetic interactions that drive the biological responses to these exposures and their influence on prognostic signatures of RCC.

In our initial studies using normal human lung epithelial cells, BEAS-2B, and a bitransgenic mouse model we have suggested that chromosome maintenance region 1 (CRM1), a nuclear export receptor for various cancer-associated ‘cargo’ proteins (such as p53), played an important role in lung cancer development. The objectives of the present study were to investigate the CRM1 expression and/or its associated p53 alteration in the following four models: human lung tumor tissues, 4-(methylthio)aniline-1-(3-pyridyl)-1-butanone (NNK, a tobacco specific carcinogen)-induced mouse lung adenocarcinoma, NNK-transformed lung epithelial cells (named as BEAS-2BNK), and stably over-expression of CRM1 in BEAS-2BNK cells (named as BEAS-2BNKCRM1+). CRM1 was overexpressed in tumor tissues from both lung cancer patients and NNK-treated mice, and in BEAS-2BNK cells. Cellular malignant transformation was observed in BEAS-2BNK and BEAS-2BNKCRM1+ cells. Furthermore, increased p53 phosphorylation at Thr 55, which indicated p53 cytoplasmic localization and p53 degradation, was increased.
in NNK-induced lung adenocarcinoma and BEAS-2BBNK cells. Moreover, the nuclear retention of p53 was significantly diminished in BEAS-2BCRM1+ cells compared to BEAS-2B cells. These data suggest that CRM1 plays a critical role in lung cancer progression and that such mechanisms may provide a novel target for developing lung cancer therapies.

1508 GS-19, a Novel GSK Inhibitor Suppresses the Growth of Pancreatic Cancer Cells by Inhibiting EGFR/AKT/STAT-3 Signaling

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The American Cancer Society estimates that over 43,000 new cases of pancreatic cancer are diagnosed each year in the United States, with a five-year survival rate below 6%. Conventional therapies are often of limited use because of the resistance development and cancer relapse. Therefore, identification of novel strategies to control and treat pancreatic cancer is of great importance. GS-19 is a novel compound designated as a GSK inhibitor and shown good activity in neural protective assay using MC65 cells. In this study, we determined the cytotoxic effects of GS-19 in pancreatic cancer cells. GS-19 treatment inhibited the growth of Panc-1 and BxPC-3 cells in a concentration-dependent manner. Both the cell lines treated with GS-19 exhibited G1 cell cycle arrest as well as apoptosis. Western blot analysis of both BxPC-3 and Panc-1 cells treated with GS-19 inhibited the activation (phosphorylation) and expression of key survival signaling proteins such as EGFR, STAT3 and AKT. The apoptosis induced by GS-19 was associated with cleavage of caspase-3 and PARP. Taken together, our results suggest that GS-19 suppresses pancreatic cancer cell growth by inhibiting EGFR/AKT/STAT-3 signaling. However, further mechanistic studies are in progress to establish the role of EGFR/AKT/STAT-3 signaling in pancreatic cancer cells and correlate it with G1 cell cycle arrest and apoptosis induced by GS-19. The studies in part were supported by R01 grant CA129038 awarded by NIH to S.K.S

1509 Olive Oil As a Chemopreventive Agent against Benzo(a)pyrene-Induced Colon Cancer in ApcMin/Mouse

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Colorectal cancer (CRC) ranks third in terms of cancer-related mortalities, and sporadic colon cancers (representing 90% of the cases) are triggered by diet and exposure to environmental toxins. In recent years, cancer prevention through dietary intervention gained significant attention. In the light of published reports that Mediterranean diet lessens the incidence of CRC, we investigated how olive oil, an essential ingredient of Mediterranean diet modulates the biotransformation of benzo(a)pyrene (BaP, a polycyclic aromatic hydrocarbon compound) and contributes to a reduction in colon tumors using an animal model. Our study model was the male ApcMin/Mouse, which was validated in our lab for studying sporadic colon tumors induced by BaP. Mice were assigned to a control (vehicle only; n =7), or treatment group (BaP only, BaP + olive oil; n =7 per category). Treatment consisted of 50 and 100 μg BaP/kg body weight dissolved in triacetin (BaP-only group) or olive oil administered daily via oral gavage for sixty days. Post exposure, mice were sacrificed; colon and liver tissues were retrieved and evaluated for histopathology. Another set of tissues were used for studying the expression and activities of biotransformation enzymes (CYP1A1, CYP1B1, and GST) and analysis of BaP metabolites. A reduction of incidence of adenomas in colons of mice that ingested BaP + olive oil compared to BaP only and control groups (p <0.05) was seen. Additionally, there was a significant reduction in CYP but not GST protein expression in tissues in BaP + olive oil-treated mice compared to BaP only-treated mice. Lastly, BaP organic metabolite concentrations were reduced in plasma and tissues of BaP + olive oil group compared to BaP-treated groups, whereas BaP aqueous metabolite concentrations (especially the glucuronide and sulfate conjugates) showed an opposite trend. In summary, our studies suggest that olive oil exerts a chemopreventive effect against BaP-induced colon cancer.

1510 Cannabidiol Inhibits Glioma Cell Proliferation and Downregulates Specificity Protein (Sp) Transcription Factors

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Cannabidiol (CBD), a plant-derived cannabinoid has shown promise as an anti-cancer agent in treating glioblastoma multiforme (GBM), however the mechanism of action of CBD on GBM is not completely understood. Treatment of CCF-STTG1 cells with CBD for 24 hr significantly inhibited cell growth, downregulated pro-oncogenic factors including epidermal growth factor (EGFR), bcl-2 and survivin and induced PARP cleavage a marker for apoptosis. Research conducted in our laboratory has shown that these same responses and factors are regulated by specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 that are highly expressed in multiple cancer cells and tumors. Sp1, Sp3 and Sp4 are all highly expressed in CCF-STTG1 cells and knock-down of Sp1, Sp3 and Sp4 by RNA interference decreased expression of survivin, bcl-2 and EGFR suggesting that these oncoproteins are regulated by Sp transcription factors in CCF-STTG1 cells. Moreover concentrations of CBD (10-15 μM) that decreased EGFR, survivin, and bcl-2 expression also downregulated Sp1, Sp3 and Sp4 in CCF-STTG1 cells. Furthermore, treatment of CCF-STTG1 cells with 15 μM of CBD and co-treatment with 1 μM of AM 251 and AM630, cannabinoid receptor antagonists 1 and 2 respectively failed to reverse the effects of CBD on Sp proteins suggesting that CBD-dependent Sp downregulation is cannabinoid receptor-independent. These results demonstrate for the first time that the anticancer activity of CBD in gliomas may be due, in part, to downregulation of Sp transcription factors and Sp-dependent genes and the mechanism of action involved in Sp protein repression is currently being investigated.

1511 Role of ROS in the Anticancer Activity of PEITC in Pancreatic Cancer

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Phenethyl isothiocyanate (PEITC) is a natural compound found in cruciferous vegetables and has been shown to have anticancer activity in various cancers including pancreatic cancer. Few studies have shown that PEITC induces reactive oxygen species (ROS) and selectively kills cancer cells. In this study, PEITC decreased cell proliferation, induced ROS and cleaved PARP which was further confirmed by Annexin V staining in Panc1, Panc28 and L3.6pl pancreatic cancer cell lines. PEITC decreased mitochondrial membrane potential (MMP) which was confirmed using JC-staining and transmission electron microscopy (TEM). PEITC also decreased Sp proteins Sp1, Sp3 and Sp4 and several Sp-regulated genes involved in cell survival (survivin, Bcl2), angiogenesis (VEGF, VEGFR1) and growth (cyclin D1, EGFR, cMet). Repression of Sp and Sp-dependent genes by PEITC was due to downregulation of microRNA-23a~27a and induction of zinc finger and BTB domain (ZBTB) containing proteins 4, 10 and 34. These responses were reversed by antioxidants such as glutathione (GSH) indicating the mechanism of action of PEITC is through induction of oxidative stress. Thus, the anticancer activity of PEITC is due, in part, to activation of ROS, which in turn targets miR-23a~27a/ZBTB5-Sp transcription factor axis. This results in decreased expression of Sp-regulated genes, growth inhibition, apoptosis and antiangiogenic responses. Studies have also shown that elevated levels of ROS can cause dynamic changes in surrounding chromatin including changes in nucleosome positioning. Cpg island-containing promoters of genes such as ACTB, cMyc, SFRP5 and TIMP3 resulting in changes in histone marks, transcription and DNA methylation. Additionally, PEITC decreased cMyc as early as 3 hrs in panc1 and L3.6pl, and these responses are currently being investigated.

1512 Diidinolymethane (DIM) Analog As a New Class of NR4A1 Antagonists

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The nuclear receptor NR4A1 (TR, Nur77) is highly expressed in solid tumors and result of knockdown by RNA interference (RNAi) indicates that NR4A1 exhibits pro-oncogenic activity. Transfection of RKO and SW480 colon cancer cells with oligonucleotides that target NR4A1 (siNR4A1) inhibited cell proliferation and induced apoptosis and this was accompanied by decreased expression of Bcl-2 and survivin. A series of 1,1-bis(3’-indolyl)-1-(p-substituted phenyl)methane analogs (C-DIMs) was screened for potential NR4A1 antagonist activity using luciferase
the degradation products of hyaluronan (HA) are reported to stimulate endothelial-cell proliferation, migration, tube formation and promote neovascularization associated with angiogenesis whilst native high-molecular-weight HA is inhibitory to these processes (1,2). When degraded into oligosaccharides of 3 to 10 disaccharides units (o-HA) an angiogenic effect has previously been demonstrated on the chicken chorioallantoic membrane and in rat skin where the blood vessel numbers were found to increase (3). It is speculated if HA degraded into o-HA may represent a safety issue in the human body due to stimulation of endothelial cell proliferation.

We have investigated the effects on endothelial cell proliferation of bacillus subtilis derived HA. We used oligosaccharides of 3-10 disaccharides units, 1.35 - 4.5 kDa and native HA of 2162 disaccharides units, 865 kDa. HVEC (human umbilical vein endothelial cell) and MVEC (human foreskin microvascular endothelial cell) and different cell lines of MVEC were stimulated with o-HA at concentrations of 1, 10 and 25 μg/mL and with native HA at dose concentrations of 5 to 100 μg/mL.

Results: We were unable to induce stimulation of endothelial cell proliferation by oligosaccharides. Treatment of endothelial cells by native HA did not inhibit endothelial cell proliferation or caused a decrease of tube formation in 3-D assay.

Conclusion: Based on the current data the degradation of hyaluronan into oligosaccharides gives no cause for concern in terms of stimulation of endothelial cell proliferation, migration and potential neovascularization.

References:
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Recent studies have reported an association with higher OC levels in people with T2D compared to non-diabetics. OC compounds associated with greater prevalence of T2D include the legacy pesticides, such as DDT. Exposure to OC compounds is predicted to be higher in agricultural areas, such as the Mississippi Delta region, where OC pesticides were heavily used. Soil samples were analyzed for OC residues from 60 randomly selected sites in the Delta region and 60 sites in a non-Delta region. Serum samples were randomly collected from patients from both the Delta and non-Delta regions at the Veterans' Administration Hospital in Jackson, MS. Following IRB approved informed consent, male subjects who were either Caucasian (C) or African American (AA) and were at least 45 years of age were selected for participation. Serum samples and de-identified clinical and demographic data were collected from each subject. Serum and soil samples were analyzed by GC-MS for OC residues including DDE, the bioaccumulative metabolite of DDT, which was detected most often in both soils and people. 80% of the Delta soil samples had quantifiable levels of DDE compared to 23% of the non-Delta soil samples and were 10-fold higher. About half of the proposed 300 serum samples have been collected and analyzed. DDE levels were adjusted based on serum lipid levels and were about 1.2-fold higher in diabetics than non-diabetics, 1320 and 1160 ng/g lipid, respectively. Subjects from the Delta region had about 1.5-fold higher DDE levels than non-Delta subjects, 1560 and 1012 ng/g lipid, respectively. AA subjects had 2.6-fold higher DDE levels than C subjects, 1992 and 761 ng/g lipid, respectively. AA and C diabetics both had higher DDE levels than AA and C non-diabetics. These results suggest that higher environmental residue levels of OC compounds, such as DDE, may result in higher serum levels and may be important for developing a biomarker of T2D.

Pb and Cd-Contaminated Soil May Cause Metal Accumulation and Epigenetic Alteration in Rat Tissues

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Metal pollution in African countries is serious problem. Metal and metallothionein (MT) levels in wild black rats (Rattus rattus) collected in mining area (Kabwe and Chingola) and control area (Lusaka) of Zambia were compared in order to assess effects of metal pollution on wild terrestrial animals. In addition, we exposed laboratory Wistar rat (Rattus norvegicus) to metal contaminated soil from Kabwe for 1 year in order to clarify biological and epigenetic effects of metals. In the laboratory soil exposure, male Wistar rats were divided to three groups by type of soil exposure: A) without soil exposed control, B) soil containing low metal levels (Pb: 75 mg/kg, Cd: 0.4 mg/kg) and C) soil containing high metal levels (Pb: 3757, Cd: 6). We analyzed metal and MT levels in various tissues of rats after 1 year exposure. Genome wide methylation status was determined in the liver, kidney and testis using LUMA (Lumimetric methylation assay). Levels of DNA methyltransferase (DNmt 1, 3a and 3b) mRNA expressions in tissues were analyzed by quantitative real-time RT-PCR. In the field study, R. rattus in Kabwe accumulated significantly higher concentrations of Pb and Cd in various organs than rats from Lusaka. Higher MT-1/2 mRNA expression levels in R. rattus from Kabwe than those in rats from Lusaka were also observed. In the laboratory soil exposure, rats in the group C accumulated significantly high concentrations of Pb and Cd in liver, kidney, lung, brain and tibia compared to other groups. Significantly higher levels of MT-2 mRNA were expressed in the kidney of group C. Interestingly, Dnmt 3a/3b mRNA expressions and genome wide methylation status in testes were significantly higher in rats of group C, suggesting that Pb and Cd contaminated soil caused metal accumulation and epigenetic alteration in rat.
1520 Oxidized Cardiolipins As a Biomarker of Mitochondrial Dysfunction Triggered by Pesticide, Rotenone

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Exposure to a commonly used pesticide, rotenone, has been associated with the development of Parkinson’s disease. We demonstrated that treatment of human blood lymphocytes with rotenone resulted in decreased mitochondrial membrane potential, inhibition of mitochondrial respiratory complex I, induction of apoptosis and selective oxidation of mitochondrial phospholipid, cardiolipin (CL). Here, we used rat rotenone-infusion Parkinson disease model to assess possible accumulation of peroxidized phospholipids. Adult male Lewis rats (6 months old) were exposed to rotenone daily (5 mg/kg, i.p.) and sacrificed 1, 5 and 10-14 days thereafter. Substantia nigra was isolated and lipids were extracted. To enhance the sensitivity of LC-MS protocols for the detection of oxidation products, phospholipids were treated either with phospholipase A1 from Thermomyces lanuginosus (10 μl/μmol phospholipids) or phospholipase A2 form porcine pancreas (10U/μmol of phospholipids) to release fatty acids residues from sn-1 and sn-2 position, respectively. Using the combination of lipidomics and oxidative epitope-targeted enzymatic digestion of total phospholipids we found a decrease of polyunsaturated fatty acid (PUFA) species containing CL and accumulation of its oxygenated molecular species was observed. We conclude that this method, the limits of quantitation for AAMA and GAMA in urine were 6ng/mL and 0.5mg/mL, respectively; the inter-day and intra-day precision of AAMA and GAMA ranged from 2.7% and 7.3%, and the recoveries ranged from 99.6% to 105.3%. The method is simple, sensitive and accurate. The developed method was also applied to analysis urines of smokers and nonsmokers, results showed that amount of AAMA, GAMA in smokers urine were much higher than that in nonsmokers, indicating that AAMA and GAMA may be effective biomarkers to estimate acrylamide exposure in smoking.

1521 N⁰-Formylly sine As a Biomarker of Formaldehyde Exposure: Inhalation Studies in Rats Reveal Formation and Accumulation of N⁰-Formylly sine Adducts in Natal Epithelium

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There is increasing recognition that aberrant protein modifications play an important role in the pathophysiology of many human diseases. N⁰-Formylly sine, a chemiluminescent adduct in white blood cells, has recently emerged as a widespread modification of proteins. With formaldehyde as the major source of N⁰-formylly sine, we quantified endogenous and exogenous adducts in nasal epithelium of rats exposed by inhalation to 0.7, 2, 5, 8, and 9.1 ppm [¹³C₄H₄]-formaldehyde for 7, 14, 21, and 28 days (6hr/day) and determined the loss of N⁰-formylly sine over a 7 day post exposure period. In vivo results showed detection and accumulation of exogenous adducts in nasal epithelium and not lung, liver, or bone marrow, with exogenous N⁰-formylly sine levels showing a 2-fold increase over a 3-week exposure period. The post exposure studies indicated t₁/₂ = ~50 h for this adduct in total proteins. Formation and accumulation of N⁰-formylly sine in proteins, including histones with important epigenetic regulatory roles, could be another mechanism contributing to formaldehyde toxicity and carcinogenicity.

1522 Serial Measurements of BPA and BPS in Texas Mother-Infant Pairs from the 3rd Trimester of Pregnancy through the 4th Month of Lactation

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Relatively little is known about in utero or early infancy exposure to Bisphenol A (BPA) and related bisphenols. Here we report on a formative prospective cohort study to monitor exposure to BPA and a BPA substitute, Bisphenol S (BPS), in mother-infant pairs in a rural Texas county. Participants were recruited from a pool of consented National Children’s Study (NCS) mothers as well as volunteers from a local clinic as part of an NCS contract with the University of Texas. The use of human subjects was approved by the IRB of the University of Texas Health Science Center at Houston and Bartelle’s IRB. Specimens were collected prospectively from the third trimester of pregnancy through the fourth month of lactation from 10 mother-infant pairs who completed the protocol. BPA and BPS were analyzed in 86 urine, 50 breast milk, and 7 plasma samples utilizing enzymatic hydrolysis followed by high performance liquid chromatography coupled with electrospray triple-quadrupole mass spectrometry. These data document measurement of BPA and BPS in each of these matrices: urine, plasma, and milk. Maternal urine specimens here had higher geometric mean (GM) concentrations of BPA (2.04 ng/ mL wet wt [ww]) and BPS (0.52 ng/mL ww) than infant urine BPA (1.85 ng/ mL ww) and BPS (0.19 ng/mL ww). The breast milk GM concentration of BPA (0.52 ng/mL) was higher than the BPS concentration (0.07 ng/mL). BPA and BPS breast milk GM concentrations increased from one month (0.41 and 0.06 ng/mL respectively) to four months postpartum (0.64 and 0.14 ng/mL respectively). This study documents that a BPA substitute, BPS, is detectable in human urine, plasma and breast milk and suggests the importance of monitoring for potentially toxic chemical substitutes for BPA. This study was funded by NIH.
The New York State Angler Cohort (NYSAC) was established to assess the association between past and current consumption of contaminated sport fish and wild life (duck and turtle) and both short-term and long-term health effects. Based upon reported consumption of Lake Ontario fish and wildlife and validated exposures to halogenated aromatic hydrocarbons (serum PCB153 and Mirex) a subgroup of the cohort was selected to participate in studies that would examine the effect that fish or fish/wildlife consumption has on different hematological and immune markers. Participants were divided into two groups; consumers with the highest serum PCB153 and Mirex levels that also self reported consumption of Lake Ontario wildlife (n=27) and non-consumers who were matched to by age, sex and geographic area and reported having never consumed Lake Ontario wildlife (n=16). The objective of the current study was to analyze differences in hematological parameters in these groups including white blood count, red blood count, hemoglobin levels, hematocrit levels, platelet count and full leukocyte differential. In anglers with serum DDE levels (n=14) higher than that observed in non-consumers, our results showed positive correlations (albeit clinically insignificant) with increased red blood cell levels, platelet count and full leukocyte differential. In anglers with serum DDE levels (n=14) higher than that observed in non-consumers, our results showed positive correlations (albeit clinically insignificant) with increased red blood cell levels, platelet count and full leukocyte differential. From these data we concluded that long term consumption of fish and/or wildlife contaminated with long-term serum biomarkers to reflect steady state exposures and associated health outcomes. Based upon the results of this study, we concluded that long-term consumption of contaminated sport fish and wildlife contaminated with persistent Great Lakes pollutants does not significantly alter hematological markers in humans. (Supported in part by ATSDR, Grant H75-ATH 298338).

**1526 Biomarkers of Aflatoxin Exposure in Bexar and Surrounding Counties: Enhanced Incidence in Humans following Severe Drought**
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Aflatoxins (AF) are produced as metabolites by the fungi *Aspergillus flavus* and *A. parasiticus* and frequently contaminate important commodities such as corn and peanuts. Drought and plant stress promote the growth of these fungi which can lead to the production of AFs. Aflatoxin B1 is the most prevalent and toxic of the AF congeners and is a known risk factor for development of hepatocellular carcinoma. The area between 40° N and S of the equator is now considered to be a "hotspot" for exposure, which includes parts of the Southern and Midwestern United States. With greater than 75% of the U.S. experiencing moderate to exceptional drought in 2012, and climate change that favors fungal growth, a wider distribution of *Aspergillus* and higher prevalence of AF contamination in staple foods such as corn is expected. In an ongoing clinical trial initiated in August 2012, a predominantly Hispanic study population in South Texas was screened for AF exposure through analysis of a short-term urinary AF biomarker, AFM1. AFM1 was detected in 22.05% (45/204) of study participant urines with an overall mean and range of 4.73±19.78 and ND-180.73 pg/mg creatinine, respectively. The levels and prevalence of AFM1 detected in 2012 were sustained throughout most of the sampling conducted in 2013, suggesting that the drought of 2012 played a key role in human AF exposures. Detection of urinary AFM1 was significantly associated with increased frequency of tortilla (p=0.0157) and peanut (p=0.0084) consumption, in addition to the amount of Mexican food (p=0.0006) and corn products (p=0.0208) ingested. Further work is warranted to correlate our findings with long-term serum biomarkers to reflect steady state exposures and associated impacts on health. This research was supported by NIH 1R01MD005819-01.
Biomarkers have been proposed as potential tools to assess the toxicity and disease risk of tobacco product use. Two key requirements of such biomarkers are that they are able to consistently distinguish between smokers and non-smokers, and that they are reversible upon smoking cessation. Candidate biomarkers were recently assessed for these requirements in a six month, clinical study based in Germany. Biomarker levels from the baseline study time point were compared in 143 smokers, 61 never-smokers and 61 ex-smokers who were otherwise healthy and age/gender matched. Of the 27 candidate biomarkers assessed, the following were found to be significantly different between smokers and never-smokers: urinary 8-epi-PGF2αx (Type III) and 11-dehydrothromboxane B2 (P<0.0001), plasma MCP-1, neutrophil elastase, leukotriene B4, ascorbic acid, HDL and oxidised LDL cholesterol (all P<0.0001), erythrocyte catalase activity and malondialdehyde (all P<0.0001), serum total anti-oxidant capacity (P<0.0001) and total white blood cell (WBC), neutrophil and monocyte count (all P<0.0001). Of these biomarkers urinary 8-epi-PGF2αx (Type III), plasma MCP-1, neutrophil elastase, leukotriene B4, ascorbic acid, HDL and oxidised LDL cholesterol, erythrocyte catalase activity and serum total anti-oxidant capacity, total white blood cell (WBC), neutrophil and monocyte count were also found to be significantly different between current and ex-smokers (P<0.05), and hence indicate the potential for reversibility. These data indicate that 12 of 27 candidate biomarkers assessed in this study are potentially useful tools for the assessment of altered biological effects following a switch from a conventional combustible tobacco product, to a novel tobacco product with reduced machine-smoked toxicant yields or non-combustible nicotine delivery products, and in smoking cessation studies.


**Effects of Cadmium on Olfactory-Mediated Behaviors and Molecular Biomarkers in Coho Salmon**

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The salmon olfactory system is sensitive to the toxic effects of metals. We analyzed olfactory-mediated alarm response behaviors, epithelial injury, and a suite of molecular biomarkers of olfactory function in juvenile coho salmon following acute exposures to cadmium (Cd). The molecular biomarkers analyzed included: four G-protein coupled receptors (GPCRs) representing the two major classes of odorant receptors (salmon olfactory receptor sorb and vomeronasal receptors srrv, svrb, and gpr27); a marker of neurite outgrowth (nn1); and markers of antioxidant responses to metals, including heme oxygenase 1 (hmox1) and peroxiredoxin 1 (prdx1). Coho received acute exposures to 3.7 ppb or 347 ppb Cd, and a subset of treated fish was then housed in clean water for 16 days before analysis. Coho exposed to 347 ppb Cd over 48 hrs exhibited a reduction in freeze responses as well as an extensive loss of olfaction accompanied by histological injury to the olfactory epithelium. This olfactory injury was accompanied by significant decreases in gene expression of the olfactory GPCRs and increased expression of hmox1. Persistent behavioral deficits, histological injury and altered expression of a subset of olfactory biomarkers were still evident in Cd-exposed coho following a 16-day depuration. Exposure to 3.7 ppb Cd also resulted in reduced freeze responses and histological changes to the olfactory epithelium within 48 hrs of Cd exposure, although the extent of olfactory injury was less severe than observed for fish in the high dose Cd group. Furthermore adverse behavioral effects were present in some coho receiving the low dose of Cd even following a 16-day depuration. In summary, acute exposures to environmental levels of Cd can cause persistent olfactory injury in coho salmon. Mechanism-based biomarkers of oxidative stress and olfactory structures can augment the evaluation of olfactory injury manifested at the physiological level. Supported by NIEHS P42 ES04696.

**The Effect of Cigarette Smoke Exposure on the Proteomic Composition of Human Bronchial Epithelial Cell Airway Surface Liquid**

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Human bronchial epithelial cells (HBEC) cultured in vitro secrete mucins and other proteins that mimic in vivo airway surface liquid (ASL). This study used proteomic void to determine HBEC-ASL composition and the effect of cigarette smoke exposure. A mass spectrometry multiple reaction monitoring (MRM) assay was also developed to quantify the ASL proteins that significantly changed after smoke exposure. HBECs were exposed to low dose or high dose cigarette smoke or air. ASL was collected, processed and analysed by reversed phase liquid chromatography-mass spectrometry and tandem mass spectrometry. ASL characterization identified 487 proteins with high confidence, these included mucins and non-mucin proteins involved in cytoskeletal, signalling, proteolytic, calcium binding and immune system pathways. Smoke exposure resulted in significant differential protein expression with the greatest changes occurring 48 hours after low dose smoke exposure. The majority of differently expressed proteins were related to inflammation and immune response, calcium binding and homeostasis, squamous cell differentiation and keratinocytes, protein metabolism, and oxidative stress. A MRM assay was developed for 154 proteins, of which 133 proteins were detected in ASL samples. Over 180 proteins were differentially expressed, with most proteins being up-regulated 48 hours after low dose smoke exposure. ASL analysis has been shown to be compatible with a proteomic approach. Many of the proteins identified have been previously detected in sputum, indicating HBEC ASL may be used to model smoke exposure in vitro. Construction of a smoke exposure specific MRM assay enables the quantitation of over 100 proteins in a single sample which may be used to further investigate the effects of smoke exposure in both in vitro ASL and in vivo sputum samples.

**Effect of Cadmium on Olfactory-Mediated Behaviors and Molecular Biomarkers in Coho Salmon**

1529

**Metabolomic Analysis of the Effect of Occupational Exposure to Pyrethroid Pesticides**

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Pyrethroids are one of the most commonly used insecticides in agriculture. They can cause neurotoxic, immune and endocrine effects. Perturbations of mechanistic pathways leading to such effects are however poorly understood. A recent approach in toxicology is to observe changes in endogenous metabolites to inform on mechanisms of toxicity. In an attempt to identify a possible spectrum of biomarkers of adverse effects of pyrethroids, a preliminary metabolomic analysis of urine samples of workers exposed to pyrethroids was conducted. Thirty agricultural workers from three different farms exposed to cypermethrin provided a complete first morning urine void before and after application or work in a treated area (following the required re-entry delay). Metabolomic analyses of urine samples were conducted by 1H nuclear magnetic resonance (NMR) followed by orthogonal partial least squares discriminant analysis (OPLS-DA). Non-parametric Wilcoxon statistical analyses were conducted given the small sample size and the neither normal nor log-normal distribution of biomarkers. The results of OPLS-DA model show two different clusters between the pre-exposure and the post-exposure samples. Higher hippurate levels were found in post-exposure urine samples (p<0.0001), but lower excretion levels of betaine (p=0.019), guanidinoacetate (p=0.004), lysine (p<0.05), alanine (p=0.07) and threonine (p=0.008). Hippurate is produced in presence of benzoic acid coming from exogenous or endogenous precursors and glycine. The main exogenous metabolite of pyrethroids is 3-phenoxy-benzoic acid, a possible substrate to hippurate. Hippurate is an uremic toxin, which interferes with normal physiological and metabolic processes; it is involved in the correction of metabolic acidosis, developing into one of the key consequences of kidney function reduction and acceleration of kidney disease progression. These metabolic changes warrant further investigations of the possible mode of action of pyrethroids.

**Development of Method for Detection of Aflatoxin B1-Lysine Adduct in Dried Blood Spot Samples**

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There is an increasing concern on aflatoxin (AF) associated growth and development inhibition among children. Previously, longer AF exposure was mainly previously assessed by analyses of serum AFB-lysine adduct. However, serum anal-
ys involved withdrawal of blood with needle stick which limits the applicability to infants and young children. In this study we developed a method to detect AFB-Lysine adduct in dried blood spots (DBS) samples. Commercially available DBS cards were evaluated for accuracy to hold different quantity of whole blood. Different washing strategies were tested to effectively elute bound blood components, especially for total protein and albumin spotted on DBS card. The recovery is >95% with the optimized washing conditions. To further validate the method, F344 rats were dosed with a single dose of AFB1 of 0, 25, 75, 225, or 675 μg/kg bw or a repeated dose of AFB1 of 5, 10, 25, and 75 μg/kg bw for two weeks. Whole blood samples from dosed animals were collected via cardiopuncture before sacrifice and immediately processed to DBS cards (6 cards per animal) and stored at 4°C until analysis. The remaining blood was prepared for serum to serve as the control. The DBS cards containing AFB-lsine adducts were washed and extracted for recovering the total protein and albumin, which were measured and digested with protease to release mono-AFB-lsine adduct. The digests were further concentrated and purified with the solid-phase extraction. The eluate was dried and reconstituted for analysis with HPLC-fluorescence detection using the same protocol for analysis of serum samples. The similar limit of detection of AFB-lsine adduct was achieved with the DBS cards, however, the recovery of the spiked levels of AFB-lsine adduct was lower (10-20%) in the whole blood DBS than the serum, which suggested that certain components in the whole blood may interfere with the recovery. Further study would aim to resolve the interference factors, in particular the hemoglobin components, in the colorimetric reading for protein quantification as well as the adduct enrichment process.

1533 Toxic Effects of Low-Level Arsenic Exposure on Erythrocyte and Its Membrane Proteins in Male Rats

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Aim: The purpose of this study was to examine changes of the erythrocyte and RBC membrane proteins in male rats exposed to low-level arsenic through drinking water and to further explore the potential biomarkers of chronic arsenic poisoning.

Methods: 40 male Sprague-Dawley rats (body weight 140-160g) were randomly divided into 4 equal groups and repetitively provided ad libitum access to drinking water containing sodium arsenite dissolved in tap water at arsenic levels of 0, 10, 60 and 360 μg/L for 30 days. The RBC toxic effects were detected by different indicators and methods, including RBC parameters (Automated Hematology Analyzer), erytrocyte rate (Flow Cytometry), erythrocyte morphology (Scanning Electron Microscopy). Erythrocyte membrane proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Immunofluorescence Assay (IFA).

Results: The results revealed that, compared with the control group, RBC parameters have not abnormal changes in exposure groups, but erytrocyte rate increased significantly in 360 μg/L arsenic exposure group. In the As treatment groups, and particularly in 360 μg/L group, we observed more irregular-shaped erythrocytes and spherocytes. Electrophoretic analysis showed decreased ankyrin expression and increased band 3 expression in 360μg/L dose group. Immunofluorescence confirmed that band 3 and CD35 increased and clustered to the RBC membrane in rats exposed to arsenic.

Conclusions: This study suggests that low levels of arsenic exposure can produce toxic effects on erythrocyte, resulting in increased erytrocyte and abnormal RBC morphology, which may be related to erythrocyte membrane proteins abnormalities. Erythrocyte membrane proteins are more sensitive to low-level arsenic than RBC number and morphology. Erythrocyte membrane proteins may be the early biomarkers in patients of chronic arsenic poisoning.

1534 Determination of Aflatoxin M1 in Breast Milk As a Biomarker of Maternal and Infant Exposure in Colombia

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Aflatoxins are secondary fungal metabolites that can contaminate human food supplies. Four aflatoxins are produced by fungi, being aflatoxin B1 (AFB1) the most common and toxic. AFB1 is considered to be the most potent natural occurring liver carcinogen known to humans. Chronic exposure causes hepatocellular carcinoma with prevalence 16--32 times higher in developing countries compared to first-world countries. Aflatoxin M1 (AFM1) is a monohydroxylated metabolite from AFB1 that is secreted in milk and can be used as a biomarker of exposure to AFB1. Ingestion of contaminated food during pregnancy and lactation may expose the newborn to the deleterious effects of aflatoxins. For these reason, efforts made to measure toxins in breast milk are important to establish the contribution of these toxins to public health, especially to the health of the newborn.

The aim of the present study was to determine AFM1 levels in human breast milk using immunoaffinity column cleanup with high performance liquid chromatography and fluorescence detection. An in-house version of the ISO method used for cow's milk was performed. Breast milk samples were obtained from 50 nursery mothers chosen from those attending The Mercy Hospital Foundation located in Bogota, Colombia. Volunteers filled in a questionnaire giving their consent to analyze their samples and details of their socio-economic, demographic and clinical data. The intake of dietary sources of aflatoxins was assessed using a food frequency questionnaire.

A total of 45 of the samples tested positive for AFM1 (median 5.2 ng/L; range 0.9-18.5 ng/L). The present study demonstrates a high exposure of mothers and neonates to AFB1 and AFM1 in Colombia, and shows the need to regulate the presence of aflatoxins in human foods. Further research is needed to determine the presence of mycotoxins in foods and biological fluids as well as to device protection strategies in a country where mycotoxins in human foods are not regulated.

1535 Association of Neuroantibodies (NAb), Lead (Pb), Vascular, and Inflammatory Biomarkers in Hemodialysis Patients (HD)

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Vascular disease, a complication of renal failure, contributes to neurodegeneration. Cognitive decline, with white matter involvement, and heavy metal accumulation have been reported in HD patients. This study investigated the association of Pb, intravascular- and vascular cell adhesion molecules (ICAM and VCAM), fibroblast growth factor-23 (FGF23) and Fetuin-A (FA) with NAb, IgG, against neurofilaments (NF-L, NF-M, NF-H), GFAP and myelin basic protein (MBP) in 229 HD patients. NAB against all proteins were significantly prevalent in HD, with anti-NF-H and anti-GFAP (90 and 80%, respectively) the most prevalent. All titers significantly correlated with each other (r=0.4-0.5; p<0.0001). Anti-NF-M, VCAM and FGF23 (p<0.05) correlated with Pb, but not VCAM, directly (p=0.001) correlated with all NAb titers, while FA and FGF23 inversely correlated with Pb (p=0.01). Multivariable regression (R²=0.2-0.4) indicated that the major determinants of anti-NF-M, NF-H, GFAP and MBP titers were PfP (p=0.02), FA (p=0.001) and VCAM (p=0.01). ICAM was a significant (p=0.001) determinant for all, but GFAP. For anti-NF-L (R²=0.4), the major determinants were FA (p=0.001) and ICAM (p<0.01). The present study demonstrates a strong association between PbP in HD patients and biomarkers of nervous system insult, as well as the association with vascular markers. ICAM and VCAM biomarkers have been associated with neurodegenerative diseases and cognitive decline. FA is believed to be neuroprotective, its decline indicating risk as suggested in the literature. While FGF23 may pose a risk for cardiovascular disease, its activities mediated through klotho are believed to be protective. This is consistent with the reverse association with Nabs. Taken together, this study demonstrates the utility of NAB as biomarkers and their strong association with factors that may contribute to neurodegeneration in HD patients.

1536 Identification of Marker Components in the Volatile Organic Emissions of Fusarium Fungi


The metabolomics of microorganisms, in particular, Fusarium micromycetes, places special focus on the quantitative and qualitative composition of their emitted volatile organic compounds (VOC), because the latter serve as markers in the identification and differentiation of toxin-producing fungi. The profile of VOCs emitted by microorganisms and, consequently, the set of markers indicative of the presence of certain microorganisms in the analyzed object depend on the experimental conditions. For reproducible metabolic profiles some standard experimental conditions should be developed and commonly accepted. To this end, more information on the metabolic profiles of microorganisms in various experimental conditions should be accumulated.
We performed a comparative study of the VOC profiles of F. cerutis, F. culorum, F. graminearum, F. langiaeae, F. poae, and F. sporotrichoides strains at varied times of culturing in potato dextrose agar, using gas chromatography-mass spectrometry combined with solid-phase microextraction. The parameters subject to standardization in this technique are the type of microfiber and sampling temperature. The experiments involved passive sampling VOCs on a carbon microfiber at 40°C from the equilibrum vapor over live toxin-producing fungi cultures grown in GC vials.

Evidence was obtained for close correlation between the quantity of trichodiene (marker of trichothecene toxins) emitted by Fusarium fungi and the quantity and composition of sesquiterpenes in the emissions. A series of marker VOCs emitted by Fusarium fungi in much higher concentrations than trichodiene were detected and structurally characterized. Research on their specificity is in progress. The work was supported in part by the Russian Foundation for Basic Research (project no. 12-04-00927-a).

Traditional biomarkers of nephrotoxicity, blood urea nitrogen (BUN) and serum creatinine (sCRE), are relatively insensitive to acute renal damage, generally requiring a loss of up to two-thirds of kidney function to initiate significant changes. We have evaluated a new generation of urinary renal biomarkers (LIP0-2, clusterin, osteopontin, TIM-1, Albumin) using MesoScale Discovery® (MSD) kits. Fifteen male rats were administered either vancomycin (70 or 300 mg/kg) or puromycin (10 or 40 mg/kg) via intraperitoneal (ip) dosing daily for 7 days. Male rats (5/timepoint) were sacrificed on Days 2, 8 and 21. Blood for clinical pathology as well as urine was collected on Days 2, 8, 13 and 21. Microscopic pathology revealed necrosis, inflammation, fibrosis, and regeneration in male rats treated with either 300 mg/kg or 40 mg/kg of vancomycin or puromycin. Vancomycin at 300 mg/kg produced elevations in sCRE (1.4- and 1.9-fold) and BUN (1.5- and 1.9-fold) on Days 2 and 8, respectively. Puromycin (40 mg/kg) produced elevations in sCRE (3.1-fold) on Day 13 and BUN (1.5- and 1.8-fold) on Days 8 and 13, respectively; however, of the traditional markers of kidney function, only BUN showed significant changes that were outside the levels of historical controls. In contrast, 300 mg/kg vancomycin produced elevations in urinary albumin (6.0-fold) on Day 8 only, while urinary clusterin (6.0- and 16.3-fold), LIP0-2 (2.7- and 2.1-fold), osteopontin (4.3- and 2.9-fold), and TIM-1 (40.3- and 24.5-fold) were elevated on Days 2 and 8, respectively. Puromycin (40 mg/kg) produced elevations in albumin (65.7-fold) on Day 2 only and clusterin (5.4- and 2.1-fold), LIP0-2 (4.5- and 1.5-fold), and TIM-1 (23- and 3.3-fold) were elevated on Days 13 and 21, respectively. No significant changes in renal histopathology or biomarkers were observed at lower doses of vancomycin or puromycin. These results indicate that the MSD kits provide excellent sensitivity for detection of renal toxicity in rats following treatment with either vancomycin or puromycin. Work supported by NIAID Contract N01-AI-70043.

Diabetes occurs spontaneously in aged cynomolgus monkeys and its diagnosis or potential for development must be considered when using aged monkeys on long term toxicology studies. Standard clinical pathology parameters are shown to be inadequate in diagnosing diabetes. Serum glucose (GLU) levels in aged monkeys can vary significantly and GLU measurements are also insufficient to detect persistent hyperglycemia. Glycated hemoglobin (HbA1c) is formed by the non-enzymatic addition of GLU to Hgb. Its formation in blood is positively correlated with the magnitude and duration of hyperglycemia. HbA1c half-life is 2-3 months and its removal from the blood is dependent on the degradation of hemoglobin. Transient hyperglycemia does not significantly affect the concentrations of HbA1c. HbA1c is a recognized marker of long term hyperglycemic status. The HbA1c assay was validated using whole blood in EDTA collected from healthy and confirmed diabetic cynomolgus monkeys. The Roche Diagnostic HbA1c kit immunoturbidimetric assay (Tina-quant HbA1c®) was adapted to use with an automated chemistry analyzer (Modular Analytcs, Hitachi®) to measure HbA1c in hemolyzed whole blood. The HbA1c limit of detection was 0.2-2.6 g/dL for hemoglobin values between 4-40 g/dL. Intra-assay and inter assay coefficients of variation were 0.1 and 8.4 % respectively. Linearity of dilution was acceptable down to dilutions of 15:100. Hemolyzed samples were stable for 24 hrs at room temperature, 3 days at 2-8°C, and 2 months at -20°C, and over 3 freeze/thaw cycles. Stability of whole blood in EDTA was 3 days at room temperature or at 2-8°C. Reference values for HbA1c obtained in healthy cynomolgus monkeys were: 0.3-0.4 g/dL, or 1.8-3.0% of total hemoglobin for glucose values below 112 mg/dL. Values obtained in diabetic monkeys were 0.6-0.7 g/dL for GLU values between 120-256 mg/dL. The addition of HbA1c measurements to the clinical chemistry health screen panel allows a better identification of aged animals at risk for diabetes and a better health monitoring of these animals during the study conduct.
**1541** Insulin-Stimulated Urinary Chromium Excretion Is Not a Biomarker for Chromium Nutritional Status  
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Recent studies have suggested that the status of chromium as an essential element should be reconsidered because the effects of chromium supplementation appear to be more pharmacological rather than nutritional. The pharmacological basis for chromium’s effects may explain the inability of investigators to identify a biomarker for chromium status. One potential biomarker, urinary chromium excretion in response to a glucose or insulin challenge, has not been examined to date. Chromium is known to be mobilized in the body in response to insulin (or insulin release in response to a glucose challenge), resulting in an increase in urinary chromium excretion. In this study, the magnitude of increase in urinary chromium loss as a function of dietary chromium intake was evaluated as a potential biomarker for chromium. Zucker lean rats housed under carefully controlled metal-free conditions were fed a series of purified diets containing variable amounts of chromium (from 16 μg/kg diet to 2000 μg/kg) for 4 months. The 16 μg/kg diet contained less chromium than any diet examined to date. Urine samples were collected pre- and post-insulin and glucose challenges (0, 2 hours, 6 hours and 12 hours post injection). Urinary chromium levels were analyzed by the standard method of addition using graphite furnace atomic absorption. Rat blood iron levels were also measured to determine if the addition of chromium to the diet altered iron status. The study demonstrated that the chromium content of the diet had no effect on blood iron levels and also confirmed that insulin-stimulated urinary chromium excretion is not a useful biomarker for chromium nutritional status.

**1542** Sensitive Sites of Glycation of the Fibrinolytic System Protein Plasminogen: Implications for T2D Vascular Complications  
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Reactive dicarbonyls, such as methylglyoxal (MG), are present in blood and react with arginines (R) of target proteins, leading to diabetic micro- and macrovascular complications. MG plasma concentrations reach 4.5 μM, which can triple as diabetic complications progress. Protein-MG adducts may drive retinopathy, neuropathy, and other common diabetic complications. The irreversible modification of R residues by dicarbonyls causes a net loss of positive charge, most commonly via hydroxymidazole formation. Previous studies in our laboratory identified the fibrinolytic system protein plasminogen (Pg) as a sensitive target of MG addition, with functional significance. Thus, molecular modeling indicated that modification of R561 at the cleavage site of Pg would impact enzymatic activation due to drastically altered energy of interaction. The current work has identified, via 2D gel and subsequent in-gel digestion, the most sensitive sites of R modification (R504, R530) by MG on the protein, both of which may have functional effects. Although R561 was found as an early target for modification, it was not amongst the first sites hit. The functional significance of Pg adduction by MG was further tested in vitro. Pg activation was significantly delayed via modification with 50 μM MG, as determined by cleavage of a chromogenic substrate (S-2251) by plasmin, the product of Pg activation. A preliminary group of ten human plasma samples were selected and analyzed via Orbitrap mass spectrometry to detect modifications. Two patients with a history of cardiovascular disease showed R modifications of Pg at R504 and R530. At the present time, Pg modification in human plasma samples are being examined in a cohort >150 samples. The findings indicate that MG-modification of Pg may disrupt the fibrinolytic cascade sufficient to represent an underlying mechanism of vascular complications in diabetics. (R24DW083948, ABRC, ES016652, T32ES07091, P30ES00694).

**1543** A Practical and Complementary Approach to De-Risk the Potential Nephrotoxic Liability of a Drug Candidate at an Early Stage of Development  
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UCBs, a compound intended for potential use in cognitive disorders, was stopped in preclinical development because of kidney toxicity observed in a 4-week GLP rat toxicity study. Adverse histopathological findings in the kidneys were characterized by dose-related tubular degeneration/necrosis along with tubular basophilia. Urine analysis performed using both Meso Scale Discovery (MSD) and Luminex plat-
7.8 fold higher levels upon flutamide treatment. Similar results were obtained in rats in vivo. None of the measured liver enzymes were increased upon flutamide treatment. Plasma levels of oxidative stress markers were considerably changed: 15R-Prostaglandin D2 was 12.5 fold higher in treated animals and substantial levels of 5-ISO Prostaglandin F2α-VI were detected in treated animals, not in vehicle controls.

These results show that enhanced formation of isoprostanes in rat hepatocytes translates to the in vivo situation. Human cells exert a similar response upon flutamide treatment. The quantitation of oxidative stress biomarkers in human hepatocytes is suggested as an optimistic tool to early address a drug’s susceptibility to cause hepatic injury especially in man especially when bioactivation is involved.

1546 Qualification of Novel Urine Biomarkers of Nephrotoxicity in Rodents on MSD Platform

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The kidney is a common target organ for drug-induced injury, which is routinely assessed during safety evaluations. A panel of novel kidney injury markers has been recently identified, allowing diagnosis of nephrotoxicity with sufficient sensitivity and specificity in comparison to the conventional markers (serum creatinine and blood urea nitrogen (BUN)). As a result, there is a need for efficient methods for quantification of these novel biomarkers in early kidney toxicity screening. The goal of this study was to perform an analysis of urinary biomarkers on rodent models of kidney injury with an ultimate aim to enable more informed preclinical monitoring of kidney function. Concentration of kidney injury biomarkers was determined in urine using standard multiplexed (rat panel) or singleplex (mouse) assays based on the MesoScale Discovery (MSD) platform that was developed by us. Evaluated injury response biomarkers included: albumin, NGAL (Lipocalin-2), T cell-immunoglobulin-mucin (TIM-1), and osteopontin. TIM-1 and NGAL MSD rat panel did not detect mouse proteins. Assays for these two markers were developed and qualified at our laboratory. Kidney injury was induced by cisplatin (7 mg/kg, single i.p.) as well as gentamicin (100 mg/kg, 7 daily SQ injections) in two species, Sprague Dawley and C57Bl/6 mice. Normal ranges in rodent plasma were established and found to be above the LLOD for both species. Albumin, NGAL, and TIM-1 demonstrated high sensitivity in detection of kidney impairment. However, elevated NGAL and TIM-levels were detected at earlier time points than that of albumin. Osteopontin was a good indicator of tubular injury in rats but not in mice. Further optimization of the MSD methods are being undertaken to provide more sensitive assays for multiplexed detection of excerted biomarkers of kidney injury. In addition, a sensitive creatinine LC/MS/MS method was developed and validated as the routinely used clinical chemistry analyzer method is below the limit of quantitation in mouse urine.

PS 1547 Validation of an Electrochemiluminescence Multiplexed Immunoassay for Mouse Inflammation: The V-PLEX™

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To support preclinical safety studies a new, commercially available mouse cytokine multiplex kit (IFN-gamma, IL-1 beta, IL-2, IL-4, IL-5, IL-6, KC/GRO, IL-10, IL-12p70 and TNF-alpha) from Meso Scale Discovery was validated under GLP (Good Laboratory Practice) conditions. V-PLEX products allow the end user flexibility to pick and choose analytes from a predefined menu. To demonstrate biological relevance and create positive controls, mice were dosed with LPS via intraperitoneal injection. Serum samples were collected at 30 minutes and 1, 4, 8, 12, 24, and 48 hours post-LPS administration. LPS treatment in the mouse caused significant up-regulation of IFN-gamma, IL-10, IL-12, IL-beta, IL-5, IL-6, KC/GRO and TNF-alpha beginning by 1 to 4 hours post dose. The analytical validation consisted of 6 independent immunoassay runs. Quality Control Samples (QC’s) were made by spiking calibrator into mouse serum. Inter assay precision and accuracy (ULOQ and LLOQ), intra-assay precision (intra-day and inter-day precisions of all HAAs were 0.80-6.06 and 2.65-20.20pg/mL, respectively. The intra-day and inter-day precisions of all HAAs were ≤10%. For the high sensitivity and good precision, the method was successfully applied to analysis of HAAs in urines of smokers and non-smokers. The limits of detection and quantification of all target HAAs approached 0.80-6.06 and 2.65-20.20pg/mL, respectively. These results show that enhanced formation of isoprostanes in rat hepatocytes translates to the in vivo situation. Human cells exert a similar response upon flutamide treatment. The quantitation of oxidative stress biomarkers in human hepatocytes is suggested as an optimistic tool to early address a drug’s susceptibility to cause hepatic injury especially in man especially when bioactivation is involved.

A sensitive UHPLC-MS/MS method for the simultaneously determination of 15 heterocyclic aromatic amines (HAAs) was developed and applied to the analysis of human urines of smokers and non-smokers. The limits of detection and quantification of all target HAAs approached 0.80-6.06 and 2.65-20.20pg/mL, respectively. These results show that enhanced formation of isoprostanes in rat hepatocytes translates to the in vivo situation. Human cells exert a similar response upon flutamide treatment. The quantitation of oxidative stress biomarkers in human hepatocytes is suggested as an optimistic tool to early address a drug’s susceptibility to cause hepatic injury especially in man especially when bioactivation is involved.

PS 1548 A Modern Assessment of Clinical Pathology Biomarker Correlation between Nonclinical Species and Humans


Translatability of biomarkers from nonclinical species to humans is vital when predicting for toxicity and monitorable biomarkers in the clinic. To that end, we have used the database Pharmapendium to perform correlation analyses on commonly observed adverse alterations in standard clinical pathology endpoints used in nonclinical and clinical studies. Human data was compared with 3 commonly used nonclinical species: rat, dog, and primate. Analyses are limited to those drugs where clinical data only exists from the year 2000 to present. Our data show that there is no significant difference between either sensitivity or specificity values across species. The maximum difference observed was 8%, with the exception of circulating reticulocytes which showed increased specificity of 15% to 17% in primates as compared to dogs and rats, respectively. This finding gives credence to the statement that lesser animal models are as useful as higher animal models for predicting these clinical pathology changes in humans. Additionally, the standard nonclinical toxicology evaluation practice of using a rodent and non-roden species (rat and dog or rat and monkey) slightly increased sensitivity by 2% to 17% while marginally reducing specificity by 0% to 8%. Interestingly, a further correlation analysis between species indicated that there was no significant overlap between clinical pathology changes in both rat and dog and rat and monkey. There were 7-10 drugs where both the rat and dog were tested and where the dog showed the change in the clinical pathology parameter of interest. In these cases, the rat showed a false negative rate up to 85%. There were 2 to 49 drugs where both the rat and primate were tested and where the primate showed the change in the clinical pathology parameter of interest. In these cases, the rat showed a false negative rate of up to 100%. These data give credence to the use of two animal species in order to safely choose biomarkers for monitoring in the clinic.

PS 1549 Simultaneous Determination of 15 Heterocyclic Aromatic Amines in Urines of Smoker and Nonsmoker Using HPLC-MS/MS


A sensitive UHPLC-MS/MS method for the simultaneously determination of 15 heterocyclic aromatic amines (HAAs) was developed and applied to the analysis of human urines of smokers and non-smokers. The limits of detection and quantification of all target HAAs approached 0.80-6.06 and 2.65-20.20pg/mL, respectively. These results show that enhanced formation of isoprostanes in rat hepatocytes translates to the in vivo situation. Human cells exert a similar response upon flutamide treatment. The quantitation of oxidative stress biomarkers in human hepatocytes is suggested as an optimistic tool to early address a drug’s susceptibility to cause hepatic injury especially in man especially when bioactivation is involved.

PS 1549a Cross-Site Analytical Validation of the Cardiac Biomarker NT-proANP in Rat


A sensitive UHPLC-MS/MS method for the simultaneously determination of 15 heterocyclic aromatic amines (HAAs) was developed and applied to the analysis of human urines of smokers and non-smokers. The limits of detection and quantification of all target HAAs approached 0.80-6.06 and 2.65-20.20pg/mL, respectively. These results show that enhanced formation of isoprostanes in rat hepatocytes translates to the in vivo situation. Human cells exert a similar response upon flutamide treatment. The quantitation of oxidative stress biomarkers in human hepatocytes is suggested as an optimistic tool to early address a drug’s susceptibility to cause hepatic injury especially in man especially when bioactivation is involved.

Natriuretic peptides have proven utility in human medicine and are candidate non-invasive translational cardiac safety biomarkers for drug development studies in rats. A working group within PSTC is qualifying NT-proANP as a circulating biomarker of drug-induced hemodynamic stress. Analytical validation of an ELISA specific for human but cross-reacting with rat NT-proANP (Biomedica / Alpco Diagnostics, Cat No 04-BI-20892) was conducted by 5 different laboratories. The following aspects were assessed: intra- and inter-assay precision, accuracy, dilutional linearity, limit of detection (LOD), upper and lower limit of quantification (ULOQ and LLOQ), sample freeze/thaw (F/T) stability and inter-laboratory reproducibility. The standard curve showed acceptable analytical performance in all laboratories, defined as CV ≤ 20% and accuracy ≤ 20%. Intra- and inter-assay

IL-2 suggest that these assays should be used with caution. The V-PLEX product is a new multiplexing product offering greater end user flexibility and may become a valuable tool for mouse cytokine analysis.
1,3-Butadiene is a well-documented environmental pollutant that is a component of tobacco smoke and is a known human carcinogen that is used in polymer manufacture and is found in gasoline vapor and automobile exhaust. Despite the presence of 1,3-butadiene in the environment, there is no widely used biomarker for this chemical in humans. A recent study with rat urine samples was used to evaluate a human specific ELISA kit with a colorimetric detection system. The study was conducted through remote evaluation methods did not improve biomarker performance assessments when compared to typical semi-quantitative evaluations averaged across all pathologists.

1549b Implications of Histopathology Methods in Biomarker Qualification

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Identification of candidate biomarkers detecting very early tissue injury and attempts to qualify these novel biomarkers for regulatory consideration have placed a spotlight on the histological and histopathological methods used in evaluating candidate biomarkers. The number and locations of organ samples, the impact of non-blinded evaluations, the methodology for inclusion or exclusion of spontaneous changes, and inherent differences in pathologists are sources of variation that could potentially impact biomarker assessment. Three FDA pathologists were recruited to participate in studies that were conducted through remote evaluation of digital images of hematoxylin and eosin stained slides of kidneys. Initial studies were created to maximize variability between pathologists by providing no specific data sources or guidelines for providing noncontextual information, and by allowing no collaboration or consultation between pathologists. Later studies were designed to minimize inter-pathologist variability. The same five image sets were used to generate quantitative estimates of proximal tubular tissue volume and epithelial cell counts in stereology software. Tissue sections serial to the H&E stained sections were immunohistochemically stained for the candidate biomarker and the biomarker was quantified through digital image analysis software. Log-linear modeling for variability was conducted using SAS PROC GENMOD and LogXact from Cytel, Inc. Receiver Operating Characteristic (ROC) analysis was used to assess biomarker performance. Results indicate that differences between pathologists are the largest source of variability in evaluations and that blinding does not generally make a significant difference. Pathologist variability can significantly influence biomarker performance assessments but can be minimized by using techniques to increase pathologist consensus. Quantitative stereology and digital image analysis methods did not improve biomarker performance assessments when compared to typical semi-quantitative evaluations averaged across all pathologists.


1,3-Butadiene is a known human carcinogen that is used in polymer production, and is detected in gasoline vapor, automobile exhaust, and tobacco smoke. Urine samples collected as part of NHANES 2005–2006 were assayed for 1,3-butadiene exposure biomarkers [N-acetyl-S-(1-hydroxymethyl-2-propenyl)-L-cysteine (MHBMA3), N-acetyl-S-(4-hydroxy-2-butanyl)-L-cysteine (MHBMA3), and N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (DHBMA)] using ultra high performance liquid chromatography coupled with electrospray ionization triple quad mass spectrometry (UPLC/ESI-MSMS). MHBMA3 was the most abundant among the monohydroxy metabolites. The limits of detection in urine for MHBMA3 and DHBMA were 0.6 and 5 ng/mL respectively. In sample-weighted statistical analysis of NHANES 2005–2006 data, the median MHBMA3 and DHBMA levels among smokers (n=600) were 334 and 28 ng/g creatinine, respectively, compared to 250 and 5 ng/g creatinine for non-smokers (n=2467). Linear regression analysis showed that serum cotinine among smokers is a positive predictor of 1,3-butadiene exposure as measured by urinary MHBMA3 (linear slope p = 0.0002) and DHBMA (borderline p = 0.10), consistent with tobacco smoke as a primary exposure source for 1,3-butadiene. The sample-weighted median metabolic molar ratio (DHBMA/[DHBMA + MHBMA3]) was significantly lower among smokers (0.92; p < 0.0001) compared to non-smokers (0.98). Also, among smokers the sample-weighted median metabolic molar ratio was significantly higher among non-Hispanic Blacks (0.93; p = 0.04) and Mexican Americans (0.95; p = 0.0001) compared with non-Hispanic Whites (0.92). Among smokers, the sample-weighted Pearson correlations of serum cotinine with both MHBMA3 (r = 0.61, p < 0.0001) and DHBMA (r = 0.19, p < 0.0001) were statistically significant. This study provides the first nation-

ally representative characterization of butadiene exposure as measured by urinary MHBMA3 and DHBMA, and underscores tobacco smoke as a major source of butadiene exposure.

1549d Novel Rat Kidney Injury Biomarker Assays Using a Gyro Nanotechnology Platform


A leading cause of failure to any drug development program is toxicity in various organs such as the kidney. Traditional tests may not be specific enough for what is occurring and often cannot be detected until damage is irreversible. Many nephrotoxicity biomarkers may aid in the detection of damage to the kidneys and offer clues to specific kidney tissue injury in a more timely manner. We have developed rat KIM-1 and rat Clusterin GyroMark™ HT Biomarker assays as the first two toxicity biomarker assays in the Gyrolab™ xP Workstation platform. This automated compact disc-based nanotechnology uses microfluidic technology for sandwich ELISA immunoassay analysis using a biotinylated capture antibody and a fluorescence labeled detection antibody. This Gyros technology has features including small sample size (200 – 1000 nL), high sensitivity, broad dynamic range, and short assay time (1 hour per assay). The KIM-1 assay has a dynamic range, sensitivity, spike recovery, dilution linearity, intra-, and inter-assay variations of 0.054-40 ng/mL, 0.04ng/mL, 94%, 110%, < 5% and < 10%, respectively for 1:2 diluted rat urine samples. The Clusterin assay has a dynamic range, sensitivity, spike recovery, dilution linearity, intra-, and inter-assay variations of 0.36-1500 ng/mL, 0.3ng/mL, 93%, 100%, < 10% and < 10% respectively for 1:10 diluted rat urine samples. Using these new GyroMark™ HT Biomarker assays, we evaluated rats treated with no, low, and high doses of gentamicin. Urine samples (n=36) were collected on day 0, 3, 7, and 14. A significant increase in these markers was observed at day 3, tapering off as animals recovered towards day 14. The KIM-1 and Clusterin assays were compared to a leading commercial ELISA platform and vancomycin treated rat urine samples (n=36) correlation was r = 0.97 and 0.87 respectively. The analytical and biological validation data demonstrate that our GyroMark™ HT toxicity biomarker assays, rat KIM-1 and rat Clusterin, are highly robust and reproducible, using a 1000 nL sample size and can be a useful tool in detecting kidney damage in rat urine samples for drug development and basic research.

1549e Comparison of Two Commercial Immunoassays for Determination of Atrial Natriuretic Peptide in Sprague-Dawley Rat Serum


Atrial natriuretic peptide prohormone (proANP) is synthesized by the atrial myocytes. A stretch of the cardiomyocyte stimulates the release of proANP into the circulatory system. In circulation, proANP splits into a biologically active peptide known as t-ANP and an inactive N-terminal peptide (NT-proANP). NT-proANP has a long half-life and is less sensitive to the pulsatile secretions of ANP. Therefore, blood concentration of NT-proANP is used as a biomarker to evaluate cardiac health. Accurate determination of NT-proANP in preclinical animal models is important in safety and efficacy assessment of drug candidates. The objective of this study was to investigate two commercially available immunoassays for determination of NT-proANP in rat serum. Serum samples from 12 male and 12 female Sprague Dawley rats (10-14 weeks old) and plasma samples from a left anterior descending coronary artery-ligation (LADX) myocardial infarction study (n = 7) were used to evaluate a human specific ELISA kit with a colorimetric detection system from ALPCO (Salem, NH) and a rat-specific kit with an electrochemistry nesence detection system from Meso Scale Discovery (MSD, Gaithersburg, MD). The coefficient of variation (CV) was <16% using the ALPCO kit and <10% using the MSD kit. The spike-recovery using the ALPCO kit standard in pooled rat serum was 89 ± 9% and using the MSD kit standard in pooled rat serum was 110 ± 10%. No significant interference up to 4+ hemolysis was observed for either kit. Good correlation (r = 0.92) was observed between the two kits and NT-proANP was shown to be stable up to three freeze-thaw cycles in rat serum. Compared to the control rats, NT-proANP mean values were significantly higher (p < 0.05) in the LADX groups. In conclusion, both assays meet the feasibility acceptance criteria for application of non-blinded evaluations, the methodology for inclusion or exclusion of spontaneous changes, and inherent differences in pathologists are sources of variation that could potentially impact biomarker assessment. Three FDA pathologists were recruited to participate in studies that were conducted through remote evaluation of digital images of hematoxylin and eosin stained slides of kidneys. Initial studies were created to maximize variability between pathologists by providing no specific data sources or guidelines for providing noncontextual information, and by allowing no collaboration or consultation between pathologists. Later studies were designed to minimize inter-pathologist variability. The same five image sets were used to generate quantitative estimates of proximal tubular tissue volume and epithelial cell counts in stereology software. Tissue sections serial to the H&E stained sections were immunohistochemically stained for the candidate biomarker and the biomarker was quantified through digital image analysis software. Log-linear modeling for variability was conducted using SAS PROC GENMOD and LogXact from Cytel, Inc. Receiver Operating Characteristic (ROC) analysis was used to assess biomarker performance. Results indicate that differences between pathologists are the largest source of variability in evaluations and that blinding does not generally make a significant difference. Pathologist variability can significantly influence biomarker performance assessment analyses but can be minimized by using techniques to increase pathologist consensus. Quantitative stereology and digital image analysis methods did not improve biomarker performance assessments when compared to typical semi-quantitative evaluations averaged across all pathologists.
vascular injury is a common finding during the pre-clinical safety study of drugs. A lack of understanding of mechanisms of drug-induced vascular injury (DIVI) in animals and the absence of qualified biomarkers have become significant barriers in the development of new therapeutic agents. Recently, a number of promising candidate biomarkers for DIVI have been nominated. However, any effort to further validate and evaluate these markers is impeded by the lack of qualified high-throughput assays.

Based on Luminex xMAP® technology, we developed three panels of multiplexed immunooassays to quantify 17 potential DIVI biomarkers in rat serum and plasma samples. These biomarkers included caveolin-1, CTGF, PAI-1, IL-6, MCP-1, CINC-1, TIMP-1, VEGF, TNF-alpha, adiponectin, sICAM-1, E-selectin, von Willebrand Factor (vWF), alpha-2-macroglobulin (AZM), alpha-1-acid glycoprotein (AGP), fibrinogen and haptoglobin. Furthermore, fenoldopam induced rat DIVI models were used to validate the sensitivity and specificity of these markers to induce vascular injury, rats were treated with the vasodilator compound fenoldopam mesylate (FM). FM was administered by continuous intravenous infusion over 24 hours with a dose of 6 mg/kg/h. Subsequently to the infusion, serum and plasma samples were collected from each group of three rats on post-infusion day 1, 3, and 7. Sterile 0.9% saline was used in the vehicle control cohorts. MILLIPLEX® MAP Rat Vascular Injury Magnetic Bead Assays were used to measure 17 candidate protein biomarkers simultaneously from the rat serum and plasma samples. Fenoldopam treatment resulted in significant increase in blood biomarkers, consistent with its well described vasotoxic effect. Several biomarkers were found promising in this study, including AZM, sICAM-1, CTGF, vWF and TIMP-1. The simultaneous measurement of these proteins with multiplex technology offered a robust and convenient method to study these biomarkers. Taken together, our data demonstrate the feasibility of using the multiplex biomarker approach to detect the onset and progression of DIVI in pre-clinical drug safety studies.
Approach: Indoor and outdoor household dust samples were collected in communities with an active smelting plant (n=178), with historically active plants (n=166), and a control site (n=99), leached with 7.5N HNO3 and analyzed for Mn, Al, Fe, Cd, Cr, Cu, Pb, and Zn by ICP-AES.

Results: Household dust Mn levels were significantly different between all three study sites, with levels highest in the community with an active smelting plant (median = 2060 µg/g, range 130 – 183,000) and lowest in the reference community (median = 320 µg/g, range 20 – 1050); dust Mn levels were ~3 to 7-fold higher in outdoor versus indoor samples across all three sites.

In homes of adolescents providing biomarker samples (n=98), there was a highly significant correlation (p<0.001, r=0.639) between dust Mn concentrations and household dust Mn loading levels, and a highly significant association between dust Mn concentrations and hair Mn levels (r=0.3254, p<0.001, n=98). There was no association between dust Mn loading and hair Mn (r=0.1201, p=0.265), or between dust Mn concentrations and Mn levels in other biomarker tissues (r<0.1104, p<0.3426, p<0.22).

Conclusion: Dust Mn levels provide an informative measure of environmental Mn contamination from smeltery pollution emissions, and their association with a biomarker of Mn exposure suggests that dust may serve as an important Mn exposure pathway for adolescents.
with an excess cancer risk. According to the calculations for workers, the potential health risks associated with handling smelter material were very low and did not exceed acceptable risk levels.

1556 Arsenic Speciation in the Copper Tailings with the Effects of Magnetite Removal

Arsenic is considered as one of severe metalloid pollutants in the copper tailings, which may pose risks to the environment and human health. It is known that iron oxides are one of the most important adsorbents of minor elements due to their reactivity and large specific surface area, which can coprecipitate and adsorb arsenic. Magnetite (Fe3O4), counting for over 30% of in copper tailings, may act as a sink mineral to prevent arsenic mobilization. However, the ore processing has recently changed to remove magnetite as a by-product for economic benefit, which reduce arsenic concentration from 20–30% to 5%. Thus may affect arsenic speciation and consequently mobilize arsenic in tailings. In this study, we compared the sequential leaching results from the old copper tailings (total arsenic concentration 330 mg/kg) with magnetite and new copper tailing without magnetite. There was 34% arsenic adsorbed with iron in old tailing, comparing to 7% arsenate in new tailings. Organic matter (biochar and mulch) were added in those tailings and two types of Australian native plants were planted in the pot experiment. The leachate was collected which may display the different adsorption effects of organic matter on arsenic due to the reduction of the arsenic adsorption caused by magnetite removal. The results will allow us to understand the changes of arsenic speciation under the effects of magnetite removal in copper tailings and show the effects of organic matter addition to the tailings. The results will be interpreted in terms of the influence of magnetite and organic matter, which will provide essential information for further phytoremediation practice.

1557 Antioxidant Status and Levels of Some Essential Trace Elements and Heavy Metals in Individuals Occupationally Exposed to Municipal Solid Wastes
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Objectives: Waste management workers (WMW) in Nigeria are at risk of work-related disorders. One aim of the present study was to determine blood concentrations of some essential trace elements (Se, Zn, Cu, Mg, Mn, Fe), toxic metals (Pb, Cr, Hg), and antioxidant/prooxidant markers [ferric reducing ability of plasma (FRAP), ceruloplasmin and thiobarbituric acid reactive substances (TBARS)] in municipal solid waste workers of Sagamu, South West Nigeria. Materials and Methods: A total of 75 subjects consisting of 30 WMW and 45 Non-WMW were recruited by purposive sampling. The element concentrations were measured by means of an atomic absorption spectrophotometer after wet-acid digestion. The validity and accuracy was checked by using certified reference materials. Ceruloplasmin, TBARS and FRAP were determined using standard procedures. Results: WMW exhibited significantly higher lipid peroxidation, depletion of antioxidant and alterations of levels of trace element and heavy metals. Significant (p < 0.001) elevation of ceruloplasmin (Cp) was associated with significant (p < 0.001) increase in TBARS and significant decrease (p < 0.001) in FRAP when compared with control. Apart from significant (p < 0.05) decrease level of iron (Fe) and significant (p < 0.001) increase in lead (Pb) and copper (Cu) observed in WMW, other trace elements and heavy metals analyzed (manganese (Mn), magnesium (Mg), chromium (Cr), mercury (Hg), selenium (Se) and zinc (Zn)) did not change significantly (p > 0.05) when compared with the control. A negative correlation between Cp (r = −0.182; p > 0.05) and lead as well as a positive correlation between FRAP (r = 0.277; p<0.05), zinc (r = 0.230; p<0.05) and lead and between copper (r = 0.541; p > 0.001) and iron were observed in WMW. Conclusions: Exposure to MSW predisposes to oxidative stress and lead toxicity and Cp appears promising to monitor lead overload in Nigerian WMW.

1558 Deriving Algorithms for Matrix-to-Matrix Relations and Reverse Dosimetry of Selenium from Pooled Biomonitoring Data
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Biomonitoring is increasingly used to assess exposure to selenium (Se) in the population. However, there is little harmonization among protocols used in the different studies varying biological matrices, differences in expression of results (concentrations versus amounts, units)). This makes inter-comparison of biomonitoring results across studies difficult. Hence, the aim of this study was to perform a systematic meta-analysis of the relationships between Se intakes and concentrations in different accessible biological matrices from an extended review of Se biomonitoring studies. Inclusion and exclusion criteria were used and led to select 75 published biomonitoring studies in humans. This represents 8628 individuals who provided biological samples aiming at documenting Se exposure and/or Se concentrations in two or more biological matrices. Mathematical algorithms that relate Se intakes to biological concentrations and establish matrix-to-matrix associations were derived from these pooled biomonitoring data. Logarithmic regressions showed good correlations between Se intakes and whole blood concentrations (R2 = 0.884), plasma concentrations (R2 = 0.863) and urinary excretion rates (R2 = 0.958). Blood and plasma concentrations were also strongly related (R2 = 0.874), as were whole blood concentrations and urinary excretion rates (R2 = 0.953). The log-regression coefficients allowed interpreting Se physiology. Se concentrations in plasma tend to plateau when daily intake exceed 150 μg/d, whereas Se in urine increases rapidly above this threshold. The application of the algorithms to other independent data sets confirmed that interpretation of results on the basis of Se in hair and toenail may be misleading if external contamination is not avoided. Finally, this approach based on pooled data has the advantage to cover a wide range of exposure and to provide results with a high level of confidence.

1559 Semen Uranium Concentrations in Gulf War I Depleted Uranium-Exposed Veterans
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The Department of Veterans Affairs’ (VA) Depleted Uranium (DU) Follow-up Program provides health surveillance for Gulf War I and OIF Veterans who were in friendly-fire events involving DU munitions. Members of this cohort with retained embedded fragments continue to excrete high levels of uranium (uU) with a DU isotopic signature. The purpose of this study was to determine if 1) semen U levels are elevated in this cohort and 2) if they are correlated with U in other body compartments (urine, blood and plasma). Participants in the 2009 biennial surveillance visit provided blood, semen and 24-hour urine specimens. After collection, thirty-five (35) semen samples were incubated at 370°C for liquefaction; then transferred to acid-washed Teflon vials for sample preparation and U analysis by ICP-MS. Concentrations of U in semen ranged from <0.03–3.50 pg/g with a median of 4.2 pg/g. Associations were explored between U concentrations in semen, urine, blood and plasma using Spearman correlations. Semen U was positively correlated with other body compartment U concentrations for all participants: urine U (r=0.85), blood U (r=0.73), and plasma U (r=0.82) (p<0.000 for all correlations). Results indicate that U levels in semen reflect systemic concentrations, which have remained high for > 20 years in Veterans with DU embedded fragments. Data suggest that urine samples may be used to estimate semen U levels. Our previous work demonstrated no significant semen or sperm abnormalities when correlated with uU levels; thus, these data suggest that U is not a significant reproductive toxicant at low concentrations. Supported by the Department of Veterans Affairs and approved by the Baltimore VA Medical Center’s Office of Research and Development and University of Maryland’s School of Medicine Institutional Review Board.

1560 On-Site Monitoring of Marcellus Drilling Operations
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The West Virginia Natural Gas Horizontal Well Control Act of 2011 required determination of the effectiveness of a 625 foot setbacks from the center of the pad of a horizontal well drilling site. An investigation was conducted at seven drilling sites to collect data on particulate matter (PM), volatile organic compounds.
(VOCs), noise, radiation, and light levels. Measurements of air contaminants were taken during active drilling to characterize levels that might be found at 625 ft from the well pad center at unconventional gas drilling sites. While PM and VOC’s were detected at the well sites, none were above National Ambient Air Quality standards (NAAQS) at the 625 ft setback limit. Activities at the well site however did not allow for a consensus comparison to the NAAQS values. Some benzene concentrations were found to be 85ppb, 10 fold higher than what the CDC calls the “the minimum risk level for no health effects” and sound levels could exceed 95 decibels. Since, not all of the studied contaminants emanate from the center of the pad, regulations could consider other exposure points (such as those on the periphery) from which to measure a setback distance but there does not appear to be a simple solution to specifying a single point to assure control. The activities follow the terrain of the site and the needs of the process. Activities associated with some possible sources of the studied contaminants can occur closer than 625 feet from a sensitive receptor. Studies have also shown that the meteorology (and topography) may be a more important factor than distance measured on a map (or determining air contaminant concentration. While the levels of contaminants that were seen were not unexpected based on previous studies they did fluctuate over a wide range. Consideration needs to be given to increased quality control monitoring of the process using direct-reading monitors at sensitive locations near the well pad, connected wirelessly to the operations center at the pad which in turn can control output from all sources.

**1561 Potential for Exacerbation of Pediatric Asthma Due to Ultrafine Particle Exposure from Marcellus Shale Gas Drilling Operations**

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Marcellus Shale drilling operations utilize diesel engines which result in a release of ultrafine particles (UFPs; particles less than 0.1 μm) during combustion processes. Recent epidemiological studies have found a positive association between UFPs and pulmonary health effects in humans. Furthermore, UFPs are of increasing concern because studies show they exacerbate asthma symptoms, especially in children. In this paper, we describe sampling conducted in Ohio County, West Virginia using a new, battery-operated, portable differential mobility analyzer to measure the concentration and size distribution of UFPs to predict health effects that may occur in areas where the wells are located. This allowed not only total particle number concentration to be determined but also the lung dose to be estimated based on established lung deposition models. We measured concentrations that averaged as low as 4,000 to as high as 46,000 particles per cm3 in areas around drilling operations. These concentrations were generally found to be higher than the 8,000 particles/cm3 concentration associated with increased emergency room admissions for pediatric asthma. Moreover, these concentrations were consistent with ambient air particle counts of obtained near mountaintop mining operations, which have high levels of cardiovascular disease and cancer in the population. The concentrations of UFPs were similar to those of the two industrial sites. Collectively, these data suggest substantial ultrafine particle exposure burden to the populace proximal to the mining and drilling operations, which may be driving some of the adverse respiratory and cardiovascular effects. Currently, the Environmental Protection Agency does not monitor ultrafine particle concentrations in these areas, nor are respiratory and cardiovascular effects. Currently, the Environmental Protection Agency does not monitor ultrafine particle concentrations in these areas, nor are respiratory and cardiovascular effects. Currently, the Environmental Protection Agency does not monitor ultrafine particle concentrations in these areas, nor are respiratory and cardiovascular effects. Currently, the Environmental Protection Agency does not monitor ultrafine particle concentrations in these areas, nor are respiratory and cardiovascular effects. Currently, the Environmental Protection Agency does not monitor ultrafine particle concentrations in these areas, nor are respiratory and cardiovascular effects. Currently, the Environmental Protection Agency does not monitor ultrafine particle concentrations in these areas, nor are respiratory and cardiovascular effects.

**1562 Comparison of Airborne Exposures from Diesel and Biodiesel Emissions**


Introduction: Biodiesel mixtures are being used in the mining industry as an alternative to diesel to meet emission standards set by the Mining Safety and Health Administration (MSHA). This study compares the airborne exposures of diesel and biodiesel-blend emissions in an underground mine.

Methods: Twenty-two non-smoking subjects were exposed to emissions from diesel fuel and, separately, a biodiesel-blend (75% biodiesel, 25% diesel) while operating a load-haul-dump (LHD) vehicle in an underground mine. Personal samples were collected for total diesel particulate matter (DPM), respirable DPM, respirable dust, formaldehyde, acetaldehyde, naphthalene, nitric oxide (NO), and nitrogen dioxide (NO2) and analyzed according to National Institute for Occupational Safety and Health (NIOSH) methods.

Results: The average sampling time was 203 ±18.7 minutes. Mean respirable DPM (309 ±134 and 207 ±111 ug/m3, p=0.006), acetaldehyde (0.044 ±0.04 and 0.029 ±0.01 ppm, p=0.021), and naphthalene (0.0013 ±0.0007 and 0.0006 ±0.0003 ppm, p=0.002) time-weighted average (TWA) exposure concentrations were greater for diesel fuel than the biodiesel-blend, while total DPM (539 ±209 and 753 ±243 ug/m3, p=0.014) and respirable dust (0.60 ±0.38 and 0.86 ±0.29 mg/m3, p=0.035) TWA concentrations were less for diesel compared to the biodiesel-blend. There was no observed difference in TWA concentrations for formaldehyde (0.136 ±0.129 and 0.09 ±0.022, p=0.114), NO (10.9 ±4.2 and 12.0 ±4.8, p=0.259), or NO2 (1.33 ±0.66 and 1.26 ±0.61, p=0.049) between the diesel and biodiesel-blend.

Discussion: Our study demonstrates that use of biodiesel blends may reduce exposures to respirable DPM, acetaldehyde, and naphthalene.

**1563 Comparison of Acute Human Health Effects from Diesel and Biodiesel Emissions**


Human and animal studies suggest that diesel exhaust (DE) not only impairs vascular and pulmonary function, but is a probable carcinogen. Within the mining industry, there has been a shift toward replacing diesel with a diesel/biodiesel mixture, despite what little is known about the acute health effects of biodiesel exhaust (BE) inhalation. Using a cross-over study design, 22 participants were exposed to DE and BE (the latter from a 25% diesel/75% biodiesel mixture) in an underground mine. Pre- and post-exposure flow mediated dilation (FMD) of the brachial artery, exhaled carbon monoxide (CO), exhaled nitric oxide (NO), and spirometry (forced expiratory volume in one second, FEV1) were evaluated. DE and BE exposure periods were separated by approximately 3 months for each participant. The changes in baseline and peak FMD readings pre- and post-exposure to diesel were not significantly different than those measured for biodiesel. There was a statistically significant increase in post-exposure exhaled CO measurements for both fuel types tested and in post-exposure exhaled NO measurements for DE only; however, these changes were not different between the two fuel types. There was a statistically significant decrease from the baseline FEV1 value of 4.22L to post-exposure values for both DE and BE exhaust (3.98L, p=0.0001; 4.13L, p=0.0461, respectively), as well as a difference between the two fuel types (p=0.022), with a greater decrease in FEV1 in participants exposed to DE. These findings indicate that inhalation of both DE and BE result in acute health effects. There were no differences in the changes in biomarkers between the two fuel types, except in the case of FEV1. Based on these limited data, switching from diesel to biodiesel resulted in only a small improvement in acute health effects.

**1564 Airborne Impacts of the Richmond, California, Chevron Refinery Fire: A Case Study Showing the Difficulty of Correlating Real-Time Data, Exposure Assessment, and Public Health Impacts**

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Shortly after 6:30 p.m. on August 6, 2012, hazardous materials responders and firefighters were called to a fire at the Chevron Refinery in Richmond, California. A shelter-in-place order was quickly issued for surrounding communities warning residents to stay inside and reduce exposure to the smoke. During the fire, Chevron personnel took 17 direct readings of air contaminants on the refinery property as well as 19 air grab samples downwind from the fire. All readings were either below the limit of detection and/or below the Reference Exposure Levels (RELS) established by the Office of Environmental Health Hazard Assessment (OEHHAA) for sulfur compounds and hydrocarbons. Local environmental health and air quality agencies collected additional air grab samples in neighborhoods downwind from the fire. The samples were analyzed for toxic air contaminants with the results showing the presence of approximately 20 volatile chemicals. Concentrations were above typical background levels but below levels associated with adverse health effects. The exception was one sample that contained 7.41 g/m3 acrolein, well above the OEHHAA acute REL of 0.25 mg/m3. Concentrations of particulate matter from the smoke were below state and federal air quality standards, although marginally higher when compared to seasonally adjusted concentrations. With the exception of acrolein, no toxic air contaminants were detected at levels associated with adverse health effects and real-time matter levels were near normal. Even so, on the day of the fire over 360 patients were seen in area emergency departments and one adult and two children were hospitalized. In the ensuing weeks, over 15,000 individuals sought medical care mostly for minor nose, throat, or eye irritation or respiratory effects. This incident underscores the difficulty of conducting an exposure assessment for short-term releases as well as the challenge of aligning real-time concentration data with reported public health impacts.
1565 Health Surveillance Study on Printed Electronics (PE) Workplace

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PE using converging technologies, such as printing, fine mechanics, nanotechnology, electronics, and other new technologies raise additional health and safety concerns to those experienced in the traditional printing industry. We have conducted a health surveillance study in a PE workplace based on a walk-through survey and personal and area sampling, and collection of blood and urine samples. There was no indication of exposure to excess silver nanoparticles or carbon nanotubes. Exposure to organic solvents was lower than current occupational exposure limits in terms of TWA, yet there were some organic solvent exposure exceeding current OELs during short work duration. Lack of engineering controls, such as local exhaust ventilation, correct enclosure, and duct connection were found in the walk-through survey. Most workers showed normal range of blood biochemistry and hematology values except one worker who was advised to be checked by physician immediately. Some workers showed exceed levels of BEIs including carboxyhemoglobin (marker for methylene chloride exposure) and acetone (marker for isopropyl alcohol). The levels for hippuric acid and methyl hippuric were within normal ranges, indicating low exposure to toluene and xylene. Few office workers were exposed to low concentrations of methylene chloride, which was under investigation to find cause. Cleaning work, a major emission operation, was not conducted within a hood, and waste from the cleaning work was not properly disposed of. Therefore, the present health surveillance study on PE results suggested that the PE industry should take care of organic solvent exposure to protect workers from occupational safety and health risks. If PE process was in full scale with full shift operation to find cause. Cleaning work, a major emission operation, was not conducted within a hood, and waste from the cleaning work was not properly disposed of.

1566 Microcystin Extraction from Human Blood Serum: Pitfalls and Solutions

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Microcystins (MCs) contaminate water bodies due to cyanobacterial blooms all over the world leading to a public risk of intoxication by exposure to contaminated water, food (e.g. fish) or algal food supplements. In order to enable monitoring of human exposure, sensitive screening methods are needed. ELISA has been demonstrated as a robust routine tool for MC quantification in environmental samples. However, some problems regularly occur during sample preparation leading to MC loss and therefore underestimation of the true concentration. The aim of the current study was to assess the pitfalls of the MC-extraction method from complex media with more detail.

For this, MCs (MC-LR, MC-YR, MC-LA, MC-LW, MC-LF and defined MC mixtures: 1-1000 µg/L) were spiked into human blood serum as a surrogate for very complex sample media and quantified using the commercially available Adda-ELISA (Abraxis, Warminster, PA, USA) after standard extraction (methanol extraction with subsequent SPE). To detect the potential influence of sample storage and preparation materials different types of material such as glass, standard polypropylene and surface-treated polypropylene were compared. Loss of MC during preparation and storage is largely dependent on (i) the handling of the stored material, (ii) the ‘surface’ of the storage material and (iii) the hydrophobicity of the MCs.

1567 Vegetable Uptake and Human Exposure Assessment from a Community Garden with Elevated ∑DDT

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Introduction

Two agroforestry gardens and an open garden were prepared to grow vegetables in a remote Canadian First Nation community. Soil was sampled at the beginning of the project while, at the end of the season potatoes and beans were collected and matched to the previous soil plots. Contaminant analysis consisted of a suite of organochlorines including DDT/DDE/DDD (2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD) and a suite of metals. Based on our ∑DDT findings, we performed an exposure assessment associated with ingestion of contaminated vegetables.

Methods

Soil samples were taken randomly in each of the three plots (Plot A and B were the agroforestry sites, while Plot C was the open site), and vegetable samples were later matched to the soil samples. Soil samples were taken to the depth of the rooting zone. All samples were sent directly to Queen’s University, Analytical Services Unit, for organochlorine analysis via GC-MS and metal analysis via ICP-OES. Exposure assessment was based on the analytical results and standard Canadian food consumption data set by Health Canada.

Conclusion and Discussion

Exposure analyses associated with the ingestion of vegetables from Plot A, the elevated ∑DDT site, were orders of magnitude less than the regulatory guidelines (e.g., acceptable daily intake [ADI] or reference dose [RfD]). Soil levels of ∑DDT were found to exceed Canadian regulatory guidelines of 0.7 mg/kg for agricultural or residential/parkland use only in Plot A. Contaminated soil from Plot A was correlated with vegetable tissue concentrations. While human exposure to the contaminated vegetables through ingestion may not pose a health risk, the contaminated soil did exceed regulatory guidelines. Thus, produce from Plot A was not distributed to residents and use of the site was discontinued.

1568 Population Variation in Biomonitoring Data for Persistent Analytes: An Examination of Multiple Population-Based Datasets

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National biomonitoring efforts designed to characterize the levels and distribution of pollutants in populations have typically relied upon a resource-intensive sampling strategy and analysis of hundreds or thousands of individual biological samples. In Australia, a series of studies relying on pooled sample approaches, a less resource-intensive approach, has been employed to characterize central tendencies of concentrations of selected persistent analytes, but this approach does not provide estimates of typical upper bound concentrations. This analysis assesses the feasibility of an empirical approach to estimating population upper bound reference values based on the examination of population variation from available biomonitoring datasets from the US, Canada, Germany, and the Catalan region of Spain, with the results tested against data from the Flemish population of Belgium. Arithmetic mean concentrations and the ratio of the 95th percentile to the mean (P95:mean) were assessed in each study for defined age subgroups for three polychlorinated biphenyls (PCBs 138, 153, and 180), hexachlorobenzene (HCB), and p,p'-dichlorodiphenylchloroethylene (DDDE). While arithmetic mean concentrations of each analyte varied widely across surveys, the P95:mean ratios were consistently less than one across surveys for each analyte, with no clear variation across age groups. The results are consistent with generally lognormal distributions of analyte concentrations in the populations studied, with similar geometric standard deviations across surveys, even when the population means were significantly different. The central tendency estimate of P95:mean was approximately 2.2 for the three PCBs and HCB and 3.0 for DDE, and the approach accurately predicted the 95th percentiles in the Flemish dataset. These results can be used in combination with the Australian pooled sampling results to estimate upper bound reference values for use in evaluating individual biomonitoring data for the included analytes.

1569 Serum Steatohepatitis Biomarker and Proinflammatory Cytokine Elevation in Response to Polychlorinated Biphenyl Exposures in the Anniston Community Health Study (ACHS)

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Monsanto Corporation previously operated a PCB production facility in Anniston, Alabama, and high-level environmental polychlorinated biphenyl (PCB) contamination is present there. In order to study the health effects of PCBs, a residential adult cohort (Anniston Community Health Study – ACHS) was previously assembled. Hypertension and diabetes were linked to PCB exposures in the ACHS. Nonalcoholic steatohepatitis (NASH) has previously been associated with PCB exposures in the National Health and Nutrition Examination Survey (NHANES). The objective of this study is to determine whether NASH biomarker and adipocytokine abnormalities are present in the ACHS cohort. Archived fasting serum samples were obtained from ACHS (n=625), an unexposed healthy control group (HC, negative controls, n=11), and unexposed subjects with biopsy-proven steatohepatitis related to obesity (NASH, positive controls, n=34). Cytokinin 18
(CK18) M30 and M65 (hepatocellular apoptosis and necrosis biomarkers) and adiponectin were measured. Group means were compared by ANOVA with significance set at p<0.05. CK18 M65 was increased in ACHS vs. HC, while CK18 M30 was decreased. Both CK18 M65 and M30 were decreased in ACHS vs. NASH. IL-8 and IL-6 were increased in ACHS vs. HC and NASH. Both HC and NASH IL-8 were increased to the greatest extent in ACHS (52 fold vs. HC). TNFα was increased in ACHS vs. NASH. Compared to HC, adiponectin was decreased and leptin increased to similar degrees in both ACHS and NASH. Insulin was increased in NASH vs. both ACHS and HC. Steatohepatitis biomarkers were abnormal in ACHS. Some differences existed between ACHS and unexposed subjects with NASH apparently related to obesity. Liver injury in ACHS was characterized by necrosis with high-level elevation of IL-8. These data further implicate PCB exposures in steatohepatitis and systemic inflammation.

1570  Quantification of Tissue Bisphenol A and Global Methylation Profiles in Kidney, Liver, and Placenta from 1st and 2nd Trimester Human Clinical Samples

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The ubiquitous monomer, bisphenol A (BPA), is an endocrine active compound used in a variety of consumer products. BPA has been associated with adverse health outcomes ranging from reproductive dysfunction to chronic diseases. Studies suggest that BPA operates by altering DNA methylation or other epigenetic markers, especially during critical windows of development. Characterization of BPA levels and epigenetic profiles across multiple tissues are currently limited to animal models or no data at all. Herein, we report tissue concentrations of BPA and global DNA methylation levels in healthy 1st and 2nd trimester human kidney, liver, and placental specimens (matched N=12 per tissue) obtained from the University of Washington Laboratory of Developmental Biology. Total BPA concentrations, measured using liquid chromatography tandem mass spectrometry, significantly differed across matched specimens (p-value: 0.002) with geometric means at 0.81, 9.19, and 1.62 ng/g for kidney, liver, and placenta, respectively. When global methylation was quantified at long interspersed retrotransposons (LINE1) repetitive elements using pyrosequencing and at CCGG sites across the genome using the Luminometric Methylation Assay (LUMA), methylation significantly differed across kidney, liver, and placenta (p-values <0.001). Average LUMA methylation was 77.9% in kidney, 79.5% in liver, and 58.3% in placenta, while average LUMA methylation across kidney, liver, and placenta (p-values <0.001). Average LINE1 methylation was 76.9% in kidney, 66.4% in liver, and 51.9% in placenta. No significant association was observed between total BPA and global methylation in fetal kidney or liver. However, there was a 0.23% increase in LINE1 methylation with 1 ng/g increase in total BPA in placenta (p-value: 0.002). Results suggest that BPA concentrations and methylation profiles are tissue specific in early life, and characterizing these differences will be important for exposure and risk assessment.

1571  Biochemical Parameters and Fluoride Levels in Serum and Urine of Livestock Animals Accidentally Exposed to Hydrofluoric Acid


This study aimed to evaluate the exposure of hydrofluoric acid and health status in cattle and other livestock animals raised near hydrofluoric acid accident place by determining the level of fluoride ion and biochemical parameters in urine and serum. Cattle (n=111), goats (n=25) and chickens (n=5) raised near the accident place were selected. Urine or blood serum samples were taken from 10-20 % of cattle and other livestock animals raised near hydrofluoric acid accident place by deafence. For uptake/clearance studies, immortalized Par C10 cells (parotid gland origin) grown on a Transwell® insert (3 μm pore) were used to quantify the uptake and clearance of TCPy with cells, and apical concentrations of TCPy were proportional to dose. These in vitro results are consistent with in vivo pharmacokinetic model predictions where the fer from blood to saliva is via passive transcellular diffusion. These experiments have established the feasibility of utilizing an in vitro cell based uptake/clearance assay from blood to saliva is via passive transcellular diffusion. These experiments have established the feasibility of utilizing an in vitro cell based uptake/clearance assay coupled with pharmacokinetic modeling as a novel chemical screening strategy to identify ideal chemical candidates for saliva biomonitoring. This approach will be further evaluated using a broader range of pesticides with varying physical and chemical properties. Once established, this approach can be exploited for biomonitoring without the need to conduct more challenging in vivo saliva clearance studies. Supported by CDC/NIOHS grant R01 OH008173.

1572  Comparison of the Kinetics of Various Biomarkers of Benzo[a]pyrene Exposure following Different Routes of Entry in Rats

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Exposure to benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH) classified as a known carcinogen in humans (IARC), is of great health concern for both workers and the general population. The project aimed at comparing the kinetics of key biomarkers of BaP exposure in rats following different routes of entry. Blood and excretion time courses of BaP and key biomarkers were assessed in rats exposed to a single intravenous, intratracheal, oral and cutaneous dose of 40μmol/kg BaP. BaP and several metabolites (3- and 7-OH-BaP, 4,5- and 7,8-diol-BaP, testosterone, 1,6 Δ, 3- and 7,8-dione-BaP) were measured in blood, urine and feces collected at frequent intervals over 72 h post-treatment, using HPLC/fluorescence. Only 3-OH-BaP was detectable in blood at all time points. In urine, total amounts of BaP metabolites peaked 24 h (p<0.001) after treatment, 4,5-diol-BaP≥7-OHBaP≥7,8-diolBaP following intravenous injection and intratracheal instillation, 3-OHBaP≥7-OHBaP≥4,5-diolBaP≥7,8-diolBaP after cutaneous application and 3-OH-BaP≥4,5-diolBaP≥7-OHBaP≥7,8-diolBaP following oral administration. In feces, total amounts of BaP metabolites recovered over the same period were: 7-OHBaP≥3-OHBaP≥4,5-diolBaP≥7,8-diolBaP following all administration routes. Diones were not detectable using the developed method. For all routes of administration, excretion of 4,5-diol-BaP was almost complete over the 0-24 h in contrast with 3- and 7-OH-BaP. After intravenous injection, intratracheal instillation and oral treatment, peak excretion of 3- and 7-OH-BaP was reached in the 0-24 h period but only after 48 h post-treatment following cutaneous application. This study confirms the interest of measuring multiple metabolites due to the route-to-route differences in relative excretion of the different biomarkers and in time courses of diolep BaP versus OH-BaP. Concentration ratio of the different metabolites may help indicate time and main route of exposure.

1573  A Cellular Model to Evaluate Salivary Gland Uptake and Clearance of Pesticides: A Novel Non-Invasive Biomonitoring Strategy

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The use of saliva as a biomonitoring matrix has potential to significantly advance quantitative dosimetry as an integral component within epidemiology studies. A major limitation for saliva biomonitoring has been an inability to identify which chemicals are readily cleared in saliva, at levels that can be detected analytically. To address this limitation, immortalized Par C10 cells (parotid gland origin) grown on a Transwell® insert (3 μm pore) were used to quantify the uptake and clearance of trichloropyridinol (TCPy) the metabolite of the insecticide chlorpyrifos. Cells seeded on Transwell inserts were maintained until 7-days post-confluence at which time they displayed expression of parotid acinar cell proteins and localization of tight junction proteins at points of cell-cell contact. For uptake/clearance studies a range of TCPy concentrations (0.2-10 μg/mL) were evaluated. TCPy was added to the basolateral chamber (lower chamber) and sampled from the apical chamber (upper chamber) longitudinally for 4 hours. In the absence of cells, TCPy rapidly diffused across the transwell; whereas, the transport rate was substantially reduced with cells, and apical concentrations of TCPy were proportional to dose. These in vitro results are consistent with in vivo pharmacokinetic model predictions where TCPy salivary gland clearance is described with a 1-compartment model and transfer from blood to saliva is via passive transcellular diffusion. These experiments have established the feasibility of utilizing an in vitro cell based uptake/clearance assay coupled with pharmacokinetic modeling as a novel chemical screening strategy to identify ideal chemical candidates for saliva biomonitoring. This approach will be further evaluated using a broader range of pesticides with varying physical and chemical properties. Once established, this approach can be exploited for biomonitoring without the need to conduct more challenging in vivo saliva clearance studies. Supported by CDC/NIOHS grant R01 OH008173.
1574 Concentrations of Diacetyl and 2, 3-Pentanedione in Mainstream Cigarette Smoke: A Comparison to Workplace Exposures

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Diacetyl and 2,3-pentanedione inhalation have been suggested as causes of severe respiratory disease, including bronchiolitis obliterans, in food/flavoring manufacturing workers. Both compounds are present in many food items, tobacco, and other consumer products, but estimates of exposures associated with the use of these goods are scant.

A study was conducted to characterize exposures to diacetyl and 2,3-pentanedi-one associated with cigarette smoking. The yields (ug/cigarette) of diacetyl and 2,3-pentanedi-one in mainstream (MS) cigarette smoke were evaluated for six tobacco products (e.g., both full-flavor and light cigarettes of three different brands) under three smoking regimens (ISO, Massachusetts Department of Public Health, and Health Canada Intense) using a standard smoking machine. The mean diacetyl concentrations in MS smoke ranged from 250-361 ppm for all tobacco products and smoking regimens, and the mean cumulative exposures associated with 1 pack-year ranged from 1.1-1.9 ppm-years. For 2,3-pentanedi-one, the mean concentrations in MS smoke ranged from 32.2-50.1 ppm, and the mean cumulative exposures associated with 1 pack-year ranged from 0.14-0.26 ppm-years. We found that diacetyl and 2,3-pentanedi-one exposures from cigarette smoking far exceed occupational diacetyl exposures for most food/flavoring workers who smoke. This suggests that previous claims of a significant exposure-response relationship between diacetyl inhalation and respiratory disease in food/flavoring workers were confounded, because none of the investigations accounted for non-occupational diacetyl exposure, yet all of the cohorts evaluated had considerable smoking histories. Further, because smoking has not been shown to be a risk factor for bronchiolitis obliterans, our findings suggest that diacetyl and/or 2,3-pentanedi-one exposure is not a risk factor for this disease.

1575 Headspace and Small-Chamber Studies of Airborne Diacetyl Concentrations Associated with Selected Food Flavoring Mixtures


The accuracy and consistency of airborne diacetyl measurements can be significantly affected by a number of environmental and analytical factors that may increase variability of field measurements. Accordingly, airborne emissions modeling may augment field measurements in evaluating probable exposures to workers and others in a wide variety of settings where this common buttery food flavoring component may be found. We conducted a series of laboratory studies of emissions from pure diacetyl and spiked mixtures of selected vehicles used for adding flavorings to foods. The test materials included diacetyl (99% purity), 0.015 to 1.5% diacetyl in a water/propanol/propylene glycol mixture, deionized water, or soybean oil, and 3% diacetyl in a commercial steam distillate of milk fermentation known as ‘butter starter distillate’. Diacetyl was quantified after direct injection via gas chromatography with flame ionization detection. Measured headspace concentrations were compared to estimated concentrations utilizing Raoul’s law with and without UNIFAC activity coefficient corrections for each mixture. Headspace measurements averaged 60 to 90% higher than the ideal (Raoult’s law) estimated values and the activity coefficient-adjusted estimates were 17 to 20% higher but within analytical error for the aqueous mixtures. At 1.5% diacetyl content, the magnitude of emissions from greatest to least was: steam distillate > water > water/propanol > propylene glycol > soybean oil. Furthermore, small chamber studies (ASTM D-5116) at 4 air changes per hour were compared to estimated values using a surface spillover model; measured and modeled values were within 20% after incorporating the activity coefficient adjustments. We conclude that headspace (static) and small chamber (dynamic) measurements of airborne diacetyl are consistent with model-estimated concentrations after mixture-specific adjustment for activity coefficients.

1576 Determination of Illicit Drug on the Hands of High-Volume Money Collectors Using LC-MS/MS

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Illicit drug use in the United States has been steadily increasing over the years. Contact transfer of illicit drugs from currency to the hands of individuals during the acts of drug trafficking has been seen in many countries worldwide. While this contamination of currency has been highly examined, the downstream transfer of illicit drug in occupational exposure has not been explored. This study examines whether high volume money collectors accumulate illicit drugs via contact transfer from the natural parameters of their occupation. Utilizing LC-MS/MS, ten hand samples from bank tellers were evaluated for content of cocaine, methamphetamine and oxycodone and other drugs. Samples were obtained from both hands of 5 individuals; four individuals work in a high volume money handling position. The fifth sample was a baseline comparison from an individual with no known background in this occupation. Cocaine was detected in ranges of 35ng-450ng. Oxycodone and methamphetamine were detected in lower levels of 2ng-7ng. The quantifiable presence of cocaine, oxycodone and methamphetamine illustrates the ability of crystal- and tablet-form drugs to affix in the fabric matrix of currency and transfer to skin via contact transfer under normal occupational parameters. These results further indicate that illicit drugs have the ability to transfer from currency to hands of high volume money collectors in detectable amounts. To our knowledge, this is the first research to demonstrate this occupational exposure and illustrates the need for more expansive investigation into this unique route of occupational exposure to illicit drug.

1577 Development of a Targeted LC/MS Approach to Screen for 182 Pesticides in Urine Samples

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Pesticides are used all over the world and are found in many environments such as farmlands, agriculture facilities and residences. Many methods have been developed to detect specific pesticide residues in food and water. Exposure to high levels of pesticides can be harmful to agricultural workers and their families. Our goal on these studies was to develop a urinary screening method to determine whether individuals might be exposed to pesticides. We used mixtures of pesticide standards to develop a liquid chromatography tandem mass spectrometry method. A semi-quantitative curve was created by diluting the standard pesticide solutions. Urine samples were prepared by the addition of acetonitrile to precipitate proteins, and the supernatant was removed, evaporated and reconstituted for analysis. Standards and samples were analyzed using a Waters Xevo TQD UPLC-MS/MS. We monitored 182 pesticides using this method and screened 108 urine samples from adults and children. We found various pesticides present in the urine samples with the lower limits of detection ranging from 0.1 to 10 ng/mL. Examples include, but are not limited to, carbendazim, imidacloprid, isoxaben, methathifos, and quinalphos. Using this method, we can screen for pesticide exposure in urine samples from both adults and children. This screening technique will inform adaptive monitoring programs in which pesticides targeted are studied using specific and sensitive assays for more quantitative measurements of exposure.

1578 NMR Analysis of the Antibiotic “Amoxicillin” in Urine


The aminopenicillin, Amoxicillin, is used in many treatments against bacterial infections such as pneumonia, ulcers and urinary tract infections. However, when it is used incorrectly in combination with other drugs undesirable side effects can occur. Therefore, it is crucial to develop a method to accurately detect and quantify Amoxicillin in human biofluids. This research focuses on using 1H NMR spectroscopy for the analysis of Amoxicillin in urine samples. NMR spectroscopy is a powerful tool to analyze chemical structures. In addition, if a water suppression technique is used, direct analysis of aqueous based samples such as urine is possible without the need for separation and/or derivatization steps. We present here initial results regarding the direct analysis of amoxicillin in urine using 1H NMR and a new water suppression technique PURGE (Presaturation Utilizing Relaxation Gradients and Echoes). We found that at small concentration, an extraction method of Amoxicillin from urine is necessary as some signals from the matrix interfere with those of Amoxicillin. We therefore also present a solid phase extraction method to isolate amoxicillin in urine samples at low concentrations before NMR analysis.
Active-sampling approaches are commonly used for personal monitoring, but are limited by energy usage and data that may not represent bioavailable concentrations. Current passive techniques are popular, but often involve extensive preparation, or are developed for only a small number of targeted compounds. In this work, we present a novel application for measuring environmental, bioavailable exposure with silicone wristbands as personal passive samplers. Laboratory methodology affecting pre-cleaning, infusion, and extraction were developed using commercially available silicone, and chromatographic background interference was reduced after solvent cleaning with good extraction efficiency (>96%). After finalizing laboratory methods, 49 compounds were sequestered during an ambient deployment which encompassed a diverse set of compounds including polycyclic aromatic hydrocarbons (PAHs), consumer products, personal care products, pesticides, phthalates, and other industrial compounds ranging in log Kow from -0.07 (caffeine) to 9.49 (tris(2-ethylhexyl) phosphate). In two hot asphalt occupational settings, silicone passive samplers sequestered 25 PAHs during 8- and 40-hour exposures, as well as 2 oxygenated-PAHs (benzofluorenone and fluorenone) suggesting sensitivity of the passive samplers sequestered 25 PAHs during 8- and 40-hour exposures, as well as 2 oxygenated-PAHs (benzofluorenone and fluorenone) suggesting sensitivity of the devices over a single work day or week (p<0.05, power = 0.85). Additionally, the amount of PAH sequestered differed between worksites (p<0.05, power = 0.99), suggesting evidence of spatial sensitivity and diverse applications.

Probabilistic Modeling of Baby Wipe Lotion Exposure

Reliable exposure data are essential in evaluating the safety of ingredients in any consumer product. Appropriate exposure assessment is particularly important for products targeted for children because children differ from adults in basic behavior patterns or product use pattern. The purpose of this study was to estimate the amount of lotion transferred from baby wipes to the babies’ skin (mg/kg bw/ day) using a probabilistic modeling approach. Three different variables, number of wipes used per day, body weight, and lotion transferred from baby wipes to baby skin, were combined to establish a distribution of lotion transfer values across the studied diaper wearing population. Habits and practice data on wipes usage from both the US and Germany were included. Gender, age, and weight were evaluated for their influence on the transfer. The data were analyzed using a probabilistic Monte Carlo simulation and a statistical population model of exposure was constructed to calculate total daily lotion transfer. The estimated 50th, 90th, and 95th percentiles of lotion transferred were predicted by the model against the child’s age in months and percentiles of weight per day. Overall, the model indicates that exposure (mg/kg/day) declines in association with use of fewer wipes per day, as the child gets older. The average daily lotion transfer for a diapering period of 0-36 months ranged between 160 – 180 mg/kg/day (50th percentile) and 330 – 340 mg/kg/day (95th percentile) for US and Germany populations. This poster will provide a breakdown of age and gender specific value for lotion exposure. This model can be used for future assessments of exposure to baby wipe ingredient.

Air Pollution Exposure Model for Individuals (EMI) in Health Studies: Predicting Spatiotemporal Variability of Residential Air Exchange Rates

In health studies, traffic-related air pollution is associated with adverse respiratory effects. Due to cost and participant burden of personal measurements, health studies often estimate exposures using local ambient air monitors. Since outdoor levels do not necessarily reflect personal exposures, we developed the Exposure Model for Individuals (EMI) in health studies. A critical aspect of EMI is estimation of the air exchange rate (AER) for individual homes where people spend most of their time. The AER, which is the airflow into and out of buildings, can substantially impact indoor air pollutant concentrations and resulting occupant exposures. Our goal was to evaluate and apply an AER model to predict residential AER for the Near-Road Exposures and Effects of Urban Air Pollutants Study (NEXUS), which is examining traffic-related air pollution exposures and respiratory effects in asthmatic children living near major roads in Detroit, Michigan. We developed an AER model to predict AER from building characteristics related to air leakage; local airport temperatures and wind speeds; and open windows. Cross validation was used with a subset of NEXUS homes (N=24) with daily AER measured on five consecutive days during fall 2010 and spring 2011. Individual predicted and measured AER closely matched with median absolute differences of 36% and 24% for the fall and spring, respectively. The model was then applied to predict hourly AER for all NEXUS homes (N=121) during the study (Sept. 2010 - Dec. 2012). The AER predictions show (1) substantial house-to-house (spatial) variations (0.1 - 2.0 h⁻¹) from low to high leakage differences; (2) slow oscillations from seasonal temperature changes; and (3) large transients from wind speed fluctuations. This study demonstrates the ability to predict spatiotemporal variability of residential AER in support of improving health study exposure assessments.

Comparative Toxicity of Epicatechin vs. Borohydride-Reduced Nanosilver in Prokaryotic and Eukaryotic Models

Nanosilver is increasingly finding use as an antibacterial agent in consumer goods and water purification in developing countries. Despite claims that it has no harmful effects on eukaryotes, toxicity tests in vitro and in vivo suggest otherwise. Powerful reducants used in traditional nanosilver synthesis may contribute to its toxicity. To test this hypothesis, we utilized epicatechin reduced AgNPs and compared their in vivo and in vitro toxicity to sodium borohydride reduced AgNPs. We also examined the antimicrobial activity of the two kinds of AgNPs using gram positive and gram negative microbial experimental systems. Both epicatechin (12nm) and sodium borohydride synthesized nanosilver (10nm) were effective antibacterial agents against Pseudomonas aeruginosa (Gram negative) and Staphylococcus aureus (Gram positive), however sodium borohydride reduced AgNPs were more effective. Conversely, only traditionally synthesized AgNPs were toxic to eukaryotic organisms. HcLA cell culture experiments resulted in a LC50 of 113.56 μg/mL and Drosophila melanogaster ingestion experiments showed a LC50 of 26.14 μg/mL. Doses of epicatechin nanosilver up to 200μg/mL showed no toxicity in either eukaryotic model. Of great interest was the more sensitive effect of traditional AgNP on a whole organism compared to our in vitro system. Although the epicatechin
Absorption of Cosmetic Ingredients

This project is a post-market re-evaluation, initiated by the Office of Food Additive Safety (OFAS) to ensure that current exposures are accurately captured and the safety assessment considers all relevant toxicological information available since the time of premarket approval. The trigger for the postmarket evaluation of Irganox 1076 (stearyl 3,5-di-tert-butyl-4-hydroxyhydrocinnamate, CAS Reg. No. 2082-79-3) was an increase in submissions notifying for the food contact substance (FCS) in calendar year 2012. In order to assess the safety of Irganox 1076 from food contact applications, the US Food and Drug Administration (FDA) reviewed the available chemical information on food contact applications, including the US regulatory status, uses, levels in food, and alternative approaches to calculate exposure. Upon completion of this in-depth market analysis, the cumulative dietary concentration (CDC) was determined to be 1.5 ppm (4.5 mg/p/d). A rigorous evaluation of the available toxicological information was performed, incorporating both the data submitted for the premarket notification and any new toxicology data published subsequently. A 2-year chronic rat feeding assay was selected as the critical study with a no-observed effect level (NOEL) of 64 mg/kg/d. This NOEL and the revised CDC provides a margin of exposure (MOE) of 850, which provides an adequate margin of safety (MOS) and remains protective of human health for the regulated uses.

Safety Assessment of R,S-Equol As a Dietary Supplement for Benign Prostatic Hyperplasia

Equol is a polyphenol, more specifically an isoflavonoid. Polyphenols are common micronutrients in the human diet and have been studied for their role in the prevention of cancer, cardiovascular, and age-related diseases. Several thousand molecules with a polyphenol structure exist in higher plants, and several hundred are found in edible plants. Equol was first discovered in the early 1980s in the urine of humans consuming soy foods (a key metabolite of daidzein). More recently, equol has been reported in fermented soybean foods at relatively high concentrations. Unlike other isoflavonoids (i.e., genistein or daidzein), equol has a chiral carbon; therefore it can occur as R- and S-isomers. R,S-Equol can be prepared by catalytic hydrogenation of daidzein to yield RS-equol > 99 %. Equol is known for its anti-oxidant and anti-andrological activities where both S- and R-equol specifically bind 5α-dihydrotestosterone (DHT) with high affinity, and thereby prevent it from binding the androgen receptor. Equol also binds to estrogen receptor beta & estrogen related receptor gamma that are beneficial for treating benign prostatic hyperplasia (BPH). The available toxicity data on R,S-equol and/or its isomers, combined with the widely disseminated knowledge concerning the normal human metabolism of daidzein to equol and the long history of consumption of equol from food provide a sufficient basis for assessment of the safety of R,S-Equol as a dietary supplement for BPH. Studies include absorption, distribution, metabolism & excretion (ADME)/toxicokinetic results, acute/subchronic toxicity, genotoxicity, reproductive & developmental toxicity findings. Also, clinical intervention results display safety, feasibility and efficacy of R,S-equol (6 mg twice/day) to treat middle-aged men with moderate to severe BPH symptoms. In conclusion, all data support the use of R,S-equol to be safe, well-tolerated and provide rapid beneficial therapy (within days to weeks) for BPH that can be used alone or in combination with current pharmaceuticals to improve prostate health.

Application of QSAR Models to Evaluate the Dermal Absorption of Cosmetic Ingredients

Systemic exposure to cosmetic ingredients is an important factor to take into account during human health safety evaluation of cosmetic products. The information on dermal absorption is critical in order to accurately evaluate the systemic exposure. In vitro methods have been widely applied to determine the dermal absorption of cosmetic ingredients. Also, QSAR models have been proposed and utilized to predict the dermal absorption of chemicals based on their chemical properties. To evaluate the performance of various QSAR models to predict the dermal absorption of cosmetic ingredients, an in vitro dermal absorption study was conducted using a porcine (pig) skin model. Diglycerin is a common humectant used in cosmetic products and was used as a model ingredient in the current study. The dermal absorption profile over a 24-hour exposure period was evaluated and the kinetic data were fitted to various diffusion models and a combined diffusion/binding model. The permeability coefficient (Kp) and lag time (t) were estimated with these models. The Kp value was compared with those predicted from publicly available QSAR models. The maximum flux values (Jmax) were calculated from the Kp values. Based on the Jmax values, 3% dermal absorption (Knes et al., 2007) through the skin per 24 hours ranged from 40% to 80%. The dermal absorption determined from the actual data is generally in agreement with the QSAR model prediction. The current study supports the use of QSAR models to predict dermal absorption for safety assessment of cosmetic ingredients. We suggest that multiple prediction models for dermal absorption should be applied in the absence of experimental data and that the most conservative prediction should be used in systemic exposure assessment of cosmetic ingredients.

An Immune Human Reconstructed Epidermis Model to Assess Baby Personal Care Product Ranges

The personal care market for children is rapidly expanding and increasingly requires a specific approach. Recent publications have re-evaluated the old notion that skin is fully matured at birth and have shown that baby skin differs in structure, function and composition from that of adults. In consequence, these qualitative and quantitative differences may facilitate the development of pathological conditions, such as atopic dermatitis and irritant contact dermatitis. Therefore, the risk assessment of raw materials and finished products for baby cannot be extrapolated from human adult data but must be considered as a new approach with adapted models. In order to respect the morphology and properties of baby skin, especially the stratum corneum, an “immature” human reconstructed epidermis has been developed. It was used, at first, to assess skin irritation for a range of baby products. This new model is derived from a previously in-house developed human reconstructed epidermis model (VitroDerm).

During an internal validation, 67 infant formulations classified into 13 personal care product families (cleansing milk, shower gel, nappy cream, cleansing water, foam bath, limiment, wipes, protective stick, moisturizing milk, sun cream, hydristick, body cream, cold cream) were tested on both mature and immature epidermis. After 20h exposure on the mature model, none of the formulations reduced the percentage of cell viability to below 50% and were therefore classified as potentially non-irritant. However, 10 formulations decreased the cell viability to below 50% on immature epidermis, allowing the identification of potentially irritating formulations. A statistically significant difference (t-test; p<0.05) was found when mature and immature models were compared as a whole. This difference became more relevant when stratified analysis was made between rinse off and leave on products. The improvement in the prediction of irritation with this new model allows a better risk assessment during the development of baby products and can be used as a tool to select the most appropriate ingredients during formulation.

Instant On-Site Glucose Measurements in Dogs

In the pre-clinical studies where blood glucose levels are expected to be affected (e.g. tests of insulin and its analogues), instant on-site glucose measurement in small blood sample provides clear ethical and practical benefits. Methods for direct glucose measurements in whole blood are well established. However, the equipment is normally calibrated only for human use, and its suitability for glucose measurements in e.g. dog blood was therefore evaluated. We performed a study with the objective being to validate One Touch® UltraEasy® glucometer (the glucometer) for direct glucose measurements in dog blood as compared to standard laboratory measurement (Hitachi). Furthermore we evaluated the quality of blood samples collected from different sites (veins in the ear, foreleg or neck). Samples measured using the glucometer were compared to samples analysed using the Hitachi. There was a linear relation between whole blood glucose measurements in a broad range of glucose levels (R2=0.93 for whole blood versus plasma or serum). Glucose levels corresponding to plasma glucose levels down to 3.4 mmol/L, can be measured successfully using the glucometer. Levels below that are outside the limit range, and would require plasma/serum glucose measurements. Site of sample collection did not affect the results. However, ear sampling appeared to be more traumatic for the animals and is therefore not recommended for multiple sampling.
In conclusion, the glucometer can be used successfully for monitoring of glucose status in dogs, resulting in less stress for the animals, faster delivery of results, and economical benefits.

In the past years it has been shown that metabolomics can serve as a powerful tool in toxicological research. BASF’s Experimental Toxicology and metabolomics have developed the MetaMap®Tox database with rat plasma metabolome data for more than 500 reference compounds based on 28 day rat studies (OECD 407 design). Metabolome analysis in plasma was performed after 7, 14 and 28 days and relative levels of endogenous metabolites in treated rats versus controls were analyzed. These data are now routinely used to predict the systemic toxicity of new compounds at BASF. For many chemicals we have seen consistent changes of metabolites over all three time points. The purpose of the research reported here was to investigate for a number of compounds if these metabolic changes would also occur at earlier time points. Therefore, groups of five female and male animals each were treated with four compounds for which time-consistent effects had been previously observed (Vinclozolin, Methimazole, MCPA, Cyclosporin A). Plasma samples were taken on day 3, 5, and 7, the metabolome analyzed and the results compared against the data already present in the database. At day 7 there generally was a good concordance (> 80%) with the previous 28 day studies. The number of metabolites changed in the correct direction, and the strength of the change was reduced at day 5, but overall patterns were still identified. At day 3 metabolites changes diminished further. MCPA produced a clear pattern of metabolite changes at day 3 that was comparable to that of the 28 day study. Furthermore, a time-dependent increase in the strength of the metabolome changes was observed in this study. These results confirm the previously shown reproducibility and robustness of metabolome data and show a potential applicability of metabolomics also in shorter term toxicity studies.

Understanding how substances or particles cross biological barriers is an essential element in toxicological and pharmacological studies. Changes in the integrity of such barriers can be assessed in relevant organotypic models by measuring the TransEpithelial Electrical Resistance (TEER). However it requires full immersion of the cells, making it impossible for model systems, growing at the air-liquid-interface (MuclAir, epithelix) to be analyzed in a representative and realistic way. This is however circumvented by using a new method (AirTEER) which allows electrical contact with the apical (air) side of the organotypic model via an aqueous drop that forms a conductive liquid bridge between the electrodes and the cells in the culture. The addition feature implemented into AirTEER enable continuous, real-time measurements over longer period of time, which circumvent the need for repeated removal of the cultures from the Incubator. The system also allows the simultaneous measurement of up to 16 culture wells at a time. Pilot experiments, applying known toxicants clearly demonstrated a loss in electrical resistance after exposure of MuclAir tissue samples to toxic vapors or single substances applied from the apical side. Additional experiments will be carried out to assess robustness of the system and confirmation of continuous TEER measurements over longer time periods will not harm MuclAir tissues. Taken together, the possibility of real-time measurements using AirTEER would greatly facilitate access to a range of data that are currently not available. Notably, it would allow detecting subtle alterations in membrane integrity of aerosol exposed cells in a realistic fashion and potentially indicate whether or not the observed toxicity is reversible over time.
is that mammalian bioavailability would decrease as molecular weight increased. If this could be demonstrated experimentally, the case for read across would be strengthened, reducing the need for toxicity studies on all of the chemicals. The aim of this study was to determine and rank the absorption potential of a series of alpha-olefins using an in vitro everted rat small intestinal sac model. Everted rat intestinal sacs were prepared by evertting a freshly excised proximal small intestine over a glass stirring rod. The everted intestines were filled with oxygenated Fed State Simulated Intestinal Fluid (FeSSIF, the serosal medium, Galia et al., Pharm. Res. 15, 698, [1998]) and divided into sacs (~2.5 cm) using sutures. Sacs were incubated (1 hr, 37°C) in flasks containing FeSSIF saturated with individual al-pha-olefins. The serosal fluids were extracted with isopropanol and analysed for olefin content using GC-FID. The absorption of 30 olefins with carbon chains of C6 to C27 was investigated. Marked inter-compound differences in absorption were observed, with the degree of absorption decreasing with increasing carbon number. C6 to C10 olefins were well absorbed, while those of C14 and above were either not absorbed or very poorly absorbed. Data such as these could be used to select representative compounds from a series to study further in vivo. This study was funded by the Higher Olefins and Polyalphaolefins Reach Consortium (HOPA), c/o Pennin Consulting bvba, Brussels.

1594  Evaluation of Nose-Only Inhalation Exposure to Aerosolized Benzyl Acetate in Sprague-Dawley Rats

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Benzyl acetate is a commonly used fragrance material that has been associated with skin sensitization. The study objective was to understand the effects of inhalation exposure in Sprague-Dawley (SD) rats when aerosolized benzyl acetate was administered by nose-only inhalation at 6.1, 6.14, and 614 mg/m3 (equivalent to 1, 10, and 100 ppm), for 2 weeks (6 hours/day, 5 days/week) for a total of 10 exposures. Standard endpoints evaluated included: clinical observation; body and organ weights; hematology and serum chemistry evaluation; macroscopic/microscopic examination of selected organs; and bronchoalveolar lavage fluid (BALF) analysis for cellular markers of inflammation (i.e., clinical pathology parameters and cytology). As a positive control to validate the utility of various endpoint measures as indicators of pulmonary inflammation, a nose-only inhalation pilot was conducted with 25 mg/m3 amorphous silica (0, 1, 2, 5 or 10 exposures). At all exposure levels, benzyl acetate was well tolerated. A nonadverse test substance-related effect was observed in the nasal cavity at the 790 mg/m3 exposure concentration. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 10 ppm (equivalent to 79 mg/m3), the middle exposure concentration tested.

1595  Development of In Vitro Screens for Chemical Disruptors of the Retinol Signaling Pathway

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Retinoic acid (RA), which regulates the expression of hundreds of protein coding and non-coding RNA genes, is essential for normal embryonic development and maintenance of cellular phenotype in adult animals. Intracellular levels of RA are controlled by the retinol signaling pathway (RSP) that regulates RA synthesis (from vitamin A; retinol) and its catabolism. Chemical disruption of the RSP can cause abnormally low or high cellular levels of RA resulting in abnormal embryonic development and adult disease. Our research focus is on the development and ap- plication of in vitro test systems for assessing the safety of chemicals in foods, food additives, dietary supplements, cosmetics and other consumer products. We are particularly interested in identifying chemicals that disrupt essential developmental signaling pathways such as the RSP. We recently described the development of a rapid, medium-throughput, gene-expression (RT-PCR) assay that uses the mouse P19 pluripotent stem cell to detect chemicals that disrupt the RSP. To provide enhanced throughput capability of our screen system, a new reporter assay was developed. A lentiviral vector was used to establish stable clones of the retinol-re- sponsive C3H10T1/2 mouse multipotent stem cell that contain the Luc reporter gene under the control of the retinoic acid response element (RARE). Preliminary tests using this new RARE-Luc cell on several positive compounds demonstrate its ability to detect RSP disruptors. The assay can be optimized to be used in a Tox21 high-throughput screening platform. The RT-PCR-based assays will provide the flexibility of assessing the effect of chemicals using a broader range of endpoints. The advantages of these assays will be discussed. Assays for assessing chemical ef-fects on other important developmental toxicity pathways can also be established using similar methods.

1596  Evaluation of Nose-Only Inhalation Exposure to Aerosolized β-Ionone in Sprague-Dawley Rats

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β-Ionone is a commonly used fragrance material that has been associated with skin sensitization. The study objective was to understand the effects of inhalation expo- sure in Sprague-Dawley (SD) rats when aerosolized β-Ionone was administered by nose-only inhalation at 7.9, 79, and 790 mg/m3 (equivalent to 1, 10, and 100 ppm) for 2 weeks (6 hours/day, 5 days/week) for a total of 10 exposures. Standard endpoints evaluated included: clinical observation; body and organ weights; hemato- matology and serum chemistry evaluation; macroscopic/microscopic examination of selected organs; and bronchoalveolar lavage fluid (BALF) analysis for cellular markers of inflammation (i.e., clinical pathology parameters and cytology). As a positive control to validate the utility of various endpoint measures as indicators of pulmonary inflammation, a nose-only inhalation pilot was conducted with 25 mg/m3 amorphous silica (0, 1, 2, 5 or 10 exposures). At all exposure levels, β-Ionone was well tolerated; however, adverse test substance-related effects were observed in the nasal cavity at the 790 mg/m3 exposure concentration. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 10 ppm (equivalent to 79 mg/m3), the middle exposure concentration tested.

1597  Evaluation of Nose-Only Inhalation Exposure to Aerosolized Phenyl Ethyl Alcohol in Sprague-Dawley Rats

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Phenyl ethyl alcohol is a commonly used fragrance material that has been associated with skin sensitization. The study objective was to understand the effects of inhala- tion exposure in Sprague-Dawley (SD) rats when aerosolized phenyl ethyl alcohol was administered by nose-only inhalation at 0.5, 5, and 50 mg/m3 (equivalent to 0.1, 1, and 10 ppm), for 2 weeks (6 hours/day, 5 days/week) for a total of 10 exposures. Standard endpoints evaluated included: clinical observation; body and organ weights; hematology and serum chemistry evaluation; macroscopic/microscopic examination of selected organs; and bronchoalveolar lavage fluid (BALF) analysis for cellular markers of inflammation (i.e., clinical pathology parameters and cytology). As a positive control to validate the utility of various endpoint measures as indicators of pulmonary inflammation, a nose-only inhalation pilot was conducted with 25 mg/m3 amorphous silica (0, 1, 2, 5 or 10 exposures). At all exposure levels, phenyl ethyl alcohol was well tolerated. However, test substance-re- lated microscopic findings were noted in nasal levels II through VI in the 50 mg/m3 group males, level VI in the 0.5 mg/m3 group males, levels IV and V in all test substance-exposed female groups, and level VI in the 5 and 50 mg/m3 group females, and exhibited increased incidence or severity of luminal secretions consis- tent with mucous hyperplasia. These nasal mucosa changes were more commonly observed and tended to be more severe in the caudal nasal sections (V and VI). In addition, test substance-related mononuclear infiltrates in the liver and histiocytic infiltrates in the lungs were noted in the 50 mg/m3 group females, but were considered nonad- verse. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 10 ppm (equivalent to 50 mg/m3), the highest exposure concentration tested.

1598  Evaluation of Nose-Only Inhalation Exposure to Aerosolized Methyl Dihydro Jasmonate in Sprague-Dawley Rats

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Methyl dihydro jasmonate is a commonly used fragrance material that has been associated with skin sensitization. The study objective was to understand the effects of inhalation exposure in Sprague-Dawley (SD) rats when aerosolized methyl dihydro jasmonate was administered by nose-only inhalation at 0.53, 9.3, and 93 mg/m3 (equivalent to 0.1, 1, and 10 ppm), for 2 weeks (6 hours/day, 5 days/ week) for a total of 10 exposures. Standard endpoints evaluated included: clinical observation; body and organ weights; hematology and serum chemistry evaluation; macroscopic/microscopic examination of selected organs; and bronchoalveolar lavage fluid (BALF) analysis for cellular markers of inflammation (i.e., cytokines). As a positive control to validate the utility of cytokine measures as indicators of pulmonary inflammation, a nose-only inhalation pilot was conducted with 25 mg/m3 amorphous silica (0, 1, 2, 5 or 10 exposures). At all exposure levels, methyl di-
hydro jasmonate was well tolerated with no test substance-related effects observed. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 10 ppm (equivalent to 93 mg/m³), the highest exposure concentration tested.

1599 Evaluation of Nose-Only Inhalation Exposure to Aerosolized Hydroxyoctronellal in Sprague-Dawley Rats
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Hydroxyoctronellal is a commonly used fragrance material that has been associated with skin sensitization. The study objective was to understand the effects of inhalation exposure in Sprague-Dawley (SD) rats when aerosolized hydroxyoctronellal was administered by nose-only inhalation at 0.70, 7.0, and 70 mg/m³ (equivalent to 0.1, 1, and 10 ppm) for 2 weeks (6 hours/day, 5 days/week) for a total of 10 exposures. Standard endpoints evaluated included: clinical observation; body and organ weights; hematology and serum chemistry evaluation; macroscopic/microscopic examination of selected organs; and bronchoalveolar lavage fluid (BALF) analysis for cellular markers of inflammation (i.e., cytokines). As a positive control to validate the utility of cytokine measures as indicators of pulmonary inflammation, a nose-only inhalation pilot was conducted with 25 mg/m³ amorphous silica (0, 1, 2, 5 or 10 exposures). At all exposure levels, hydroxyoctronellal was well tolerated with test substance-related effects limited to non-adverse microscopic findings in the nasal cavity in the 70 mg/m³ females. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 10 ppm (equivalent to 70 mg/m³), the highest exposure concentration tested.

1600 Development of an Alternative Photosafety Assessment Approach for Cosmetic Ingredients Based on the Photochemical and Photo-Biochemical Properties
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Development of non-animal safety evaluation methods for chemicals is necessary from the viewpoint of animal welfare and to meet the 7th Amendment of the European Cosmetics Directive. The purpose of the present study is to establish a photosafety assessment strategy for cosmetic ingredients using several in vitro assays. The strategy was based on photochemical and photo-biochemical properties of chemicals (51 chemicals including 34 phototoxins), which were investigated by U/VIS spectral analysis, reactive oxygen species (ROS) assay and 3T3 neutral red uptake phototoxicity test (3T3 NRU PT). At first step, molar extinction coefficients (MEC) were calculated by U/VIS spectral analysis to identify photochemical characters. Most phototoxins exhibited potent U/VIS absorption with MEC of over 1000 L mol⁻¹ cm⁻¹ according to the positive criteria of ICH S10 draft guideline while false negative prediction occurred only on two cosmetic ingredients. The ROS assay was applied for further photochemical characterization to lessen the false negative results. The ROS assay did not provide any false negative predictions at least in this study, thus the ROS assay might be useful as photosafety assessment of cosmetic ingredients. In order to justify false positives from two assays, 3T3 NRU PT was conducted. Three chemicals that predicted as false positive in U/VIS spectral analysis or ROS assay were evaluated as negative in 3T3 NRU PT. The tiered approach using these in vitro methods could be a promising non-animal test method for hazard identification of photoactive chemicals.

1601 Evaluation of the Subchronic Toxicity of Acetoxydihydrodicyclopentadiene
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The subchronic toxicity of acetoxydihydrodicyclopentadiene, a widely used fragrance ingredient, was investigated in an enhanced OECD 408 dietary 90-day toxicity study. Sprague-Dawley rats were fed dietary concentrations of 0, 200, 2000, 6000 and 20000 ppm acetoxydihydrodicyclopentadiene, equivalent of 0.15, 15.9, 154.9, 464.1 and 1504.6 mg/kg bw/day, respectively, for 90 consecutive days. In addition to the repeated dose endpoints, estrous cycling and sperm analyses were conducted. Statistically significant reductions in body weight gains were detected for males treated with 20000, 6000 and 2000 ppm and in females treated with 20000 ppm. Corresponding reductions in food consumption and efficiency were observed in the 20000 ppm group only. Males treated with 6000 and 20000 ppm showed statistically significant changes in clinical chemistry parameters. Increased kidney weights and reduced prostate and pituitary weights were recorded for all treated males. Males treated with 20000 ppm also had increased liver and adrenal weights. Females treated with 2000 and 6000 ppm had decreased liver weights. The treatment related histopathological findings were recorded only for males in kidneys (hyaline droplet nephropathy and renal tubular degeneration/regeneration), liver (hepatocellular hypertrophy and vacuolation), and adrenals (vacuolation of the zona fasciculata). No toxicologically significant effects were detected in females treated with 6000, 2000 or 200 ppm therefore the NOAEL for females was considered to be 6000 ppm. The kidney effects detected in males from all treatment groups were considered consistent with well documented changes related to alpha-2-agonist accumulation and are peculiar to the male rat in response to treatment with some hydrocarbons. This effect is not indicative of a hazard to human health and, for the purposes of hazard evaluation the NOAEL for males should be regarded as 2000 ppm, or 154.9 mg/kg/day, based on alanine aminotransferase levels. Based on the current total systemic exposure from fragrance use of 0.0008 mg/kg/day, the margin of exposure is greater than 190000.

1602 Optimization of a Primary Hepatocyte Assay for Prediction of Liver Carcinogenicity in Agrochemical Development
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Many chemicals, including agrochemical molecules, may induce rodent liver tumors through non-genotoxic mechanisms, and characterization of the mode-of-action (MoA) can have important implications for human health risk assessments. Prediction of this MoA early in molecule development may facilitate lead optimization of analogues and inform the subsequent integration of MoA endpoints into in vivo regulatory studies. Nuclear receptor (NR) activation (i.e.,aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator-activated receptor alpha (PPARα)), can be an initial molecular event for in vivo non-genotoxic rodent liver tumorigenesis. Primary hepatocytes are a relevant system to predict in vivo hepatic NR activation and also have metabolic capacity. To optimize this assay, rat primary hepatocytes were exposed to prototypical NR activators 3-methylcholanthrene (AhR), phenobarbital (CAR), dexamethasone (PXR), or Wyeth-14643 (PPARα) followed by Cyp biomarker gene expression analyses to determine dynamic range and non-specific induction of Cyp responses, and establish criteria for positive NR activation responses. Halauxefin-methyl, a novel herbicide, was also analyzed in this assay to determine correlation of in vitro and in vivo gene expression responses. The in vitro Cyp responses for prototypical NR activators and halauxefin-methyl were consistent with in vivo Cyp induction. Although, variability in hepatocyte preparations can affect the dynamic range for Cyp induction, prototypical NR activators were run concurrently with each hepatocyte lot to define the inducibility of the system. A molecule was deemed positive for NR activation if Cyp gene expression responses were ≥40% of the prototypical NR activator response. This interpretation strategy was applied to assess NR activation potential of several new chemical entities and results indicate correlation between in vitro and in vivo gene expression responses.

1603 Academic and Commercial Partnership: Identifying Sustainable Flame Retardants by Rapid In Vivo Screening
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Fire prevention often requires the use of fire hardening strategies for combustible materials used in consumer products. Flexible polyurethane foams, widely used as cushioning and/or padding materials, are typical and fire hardening is often achieved with the addition of chemicals that interfere and resist combustion. It is well known that humans can be exposed to various compounds found in these products and there are mounting concerns that some of these chemicals may pose human health risks. Manufacturers are spending considerable effort to identify alternative high performing chemicals that are also inherently safer to use and need a robust and cost effective toxicity screening technique early in the product development process. A major complexity in assessing the potential hazard and risk posed by flame retardants is the diversity of chemical structures; and the chemical structure and toxicity relationships are not yet established for these compounds. We propose that a rapid multi-dimensional whole animal testing platform can be a powerful tool to screen for, and identify chemicals with differential hazard potential. To test this hypothesis, we obtained 9 flame retardant chemicals from ICI Industrial Products for testing in our high throughput zebrafish testing facility. The identity of the compounds was blinded to the laboratory. We performed
Our standard rapid throughput assessment of developmental and neurotoxicity in zebrafish. Broad concentration ranges, and all exposures were initiated at 6 hours post fertilization (hpf) and were continuous until 120 hpf. At 120 hpf, larvae were assessed for photo-induced locomotor activity and changes in a suite of 20 morphological endpoints. Five reactive compounds displayed toxicity only at the highest test concentrations (64μM), one produced toxic effects at concentrations as low as 6.4 μM, and importantly, two of the compounds produced no adverse effects at any test concentration. These data indicate that it may be possible to proactively identify commercially important compounds with reduced hazard potential.

1604 Development of Three-Dimensional Reconstructed Model for Human Skin Irritation and Corrosive Tests


In vitro models to study irritation, corrosivity and phototoxity are important tools for research and development in the pharmaceuticals and cosmetic industries. Human skin is the best possible model for such in vitro studies. The commercially reconstructed human epidermis models are similar to the morphology, lipid composition and biomarkers of native human tissue and have been approved by European Centre for the Validation of Alternative Methods (ECVAM) for the validation of cosmetics. The models are available in many nations but not in China. Here, we describe the development of a constructed three-dimensional (3D) model using Chinese human skin cells, which consists of a “dermis” with fibroblasts embedded in rat type I collagen matrix and an “epidermis” comprised of differentiated keratinocytes. The main objectives of this study were to use our reconstructed 3D skin tissues for validation on in vitro tests for human skin irritation and corrosion, and for reliability and relevance to published references. For irritation study, the 3D skin tissues were exposed with cosmetics collected from both Western nations and China. MTT assay was performed for cell viability and ELISA was conducted for IL-1 release. For corrosive study, reference chemicals including severely corrosive and non-corrosive compounds were used to validate whether our 3D skin model matched the criteria used by ECVAM. The results demonstrated our skin model could meet the criteria. Our data indicate that the reconstructed 3D Chinese skin model is a reproducible and sensitive tool to assess skin irritation and corrosion to cosmetics and chemicals.

1605 Lauric Aldehyde: Ninety-Day Repeated-Dose Oral (Dietary) Toxicity in Rats

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Lauric aldehyde is a widely used as a fragrance material. This enhanced OECD 408 compliant study investigated the systemic toxicity of lauric aldehyde by dietary exposure. Ten rats /sex /dose were fed diets containing 0, 200, 2000, 6000 or 20,000 ppm lauric aldehyde for 90 days. Observations included clinical observations, outside cage observations, food consumption, body weights, functional observational battery, ophthalmoscopy, hematology, plasma chemistry, urinalysis, estrous cycling (weeks 6-7 and 12-13), sperm analyses, gross necropsy, and histopathology. There were no unscheduled deaths. No adverse effect in overall food consumption or food efficiency was detected. There were no toxicologically significant changes in clinical observations, functional battery tests, body weights, hematology, blood chemistry, or organ weights; no toxicologically significant abnormalities were detected at necropsy. There were no toxicologically significant effects on the concentration, motility or morphology of samples of epididymal sperm. There were no treatment related effects on the concentration of homogenization resistant epididymal or testicular spermatozoon. Males treated with 20,000 ppm lauric aldehyde showed a statistically significant increase in the number of abnormal sperm during morphological assessment, not associated with any other sperm related effects. Sperm morphological analyses were extended to the low and mid dose groups and sperm staging was conducted on all groups; no effects were observed. Therefore, it was considered not to be toxicologically relevant. There were no treatment related changes in any other parameters measured. Dietary administration of 200, 2000, 6000 and 20,000 ppm lauric aldehyde to rats did not result in any toxicologically significant effects. The No Observed Adverse Effect Level (NOAEL) was considered to be 20,000 ppm (equivalent to a mean achieved dose of 1410 mg/kg/d). With total systemic exposure of 0.0043 mg/kg/d, the margin of exposure for lauric aldehyde is >300,000, indicating a very low potential for toxicity.

1606 Formulation, Analysis Method Development, and Validation for Dimethylaminoethanol Bitartrate in Gavage Dose Formulations

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Dimethylaminoethanol (DMEA) and several of its salts are produced in quantities greater than 1,000 tons per year, thus are included in the category of High Production Volume Chemicals. DMEA and its salts are extensively used in waste water treatment, manufacture of polyurethane foams, water-based paints and surface coatings. Dimethylaminoethanol bitartrate (DMEA bitartrate) can be found in a number of dietary supplements, touted for its anti-aging effects in both skin and brain function.

The purposes of the current study were to formulate DMEA bitartrate in sterile water, and to develop and validate a formulation analysis method for DMEA bitartrate in sterile water. The DMEA bitartrate was completely soluble in the vehicle up to 300 mg/mL. The method was successfully validated over the range 2.5 to 225 mg/mL and with a dilution verification to extend the range up to 300 mg/mL. The accuracy (relative error) was ± 9.4% and precision (percent relative standard deviation) was ± 2.0%. The limit of detection was determined to be 0.2 mg/mL and the limit of quantitation was 0.5 mg/mL. The range of pH of the formulations was between 3.4 and 3.6. The 10 mg/mL formulation demonstrated acceptable stability for 14 days showing recoveries of 93.7% and 94.8%, respectively. The composition of the DMEA bitartrate was also verified by the analysis of tartaric acid including the analyses after formulation storage. To date tartaric acid was found to be at a 1:1 molar ratio with DMEA through 21 days of storage.
Skin sensitization is an important endpoint for risk assessment of new ingredients. The latest amendment of the EU Cosmetic Directive/Regulation and animal welfare call for implementation of animal-free alternatives for hazard assessment. Although in vitro testing has been successful for endpoints such as skin irritation, no single in vitro test can provide sufficient information about the potential of ingredients. Several in silico and in vitro tests have been developed and validated considering the mechanism that trigger skin sensitization, and can be used as part of an Integrated Testing Strategies that ensure a reliable prediction. In this study, we combine heterogeneous input sources using a Random Forest and Bayesian Network to develop a probabilistic approach to hazard identification. These models were built on a combination of in silico and in vitro available data for 145 chemicals tested on two cell-based assays (KeratinoSens®, U-937), in chemico Direct Peptide Reactivity Assay, in silico data from Times, and skin penetration (Jaworska et al., 2013) to estimate skin sensitization potency as assessed by local lymph node assay. Furthermore, we develop and evaluate algorithms for the creation of conditional testing strategies from complex generative models. Our models are able to predict with an average balanced accuracies for four classes of skin sensitizers of approximately 61%, of 74% of accuracy when moderate and strong sensitizers had been treated as one class. At the moment we try to integrate additional data from chemicals and assays (e.g. Michael acceptor, h-CLAT, Lusens). Our results suggest that the development of an animal-free alternative for the assessment of skin sensitization will be mandatory for the future of the cosmetic industry.
manufacturing of EC RFs. A subset of the 72 RFs were duplicate products, these were also evaluated by visual comparison of fluid coloration. A HPLC method was specifically created for the nicotine quantification of EC fluids. A Hewlett Packard Series 1100 HPLC with a Thermo Scientific Hypersil ODS C18, 200mm x 4.6mm reversed phase column was used with a flow rate of 0.8 mL/min and an injection volume of 5µL. An isotric method was applied using a mobile phase of 76.9% water, 23% acetonitrile, and 0.1% triethylamine at a pH of 7.6. 50 of the 54 RFs labeled as containing nicotine had quantified nicotine concentrations that varied significantly from the manufacturer labels with a predominance of fluids (46 of 50) being in excess of labeled values. RFs labeled as nicotine free were found to be nicotine free. Five products were unlabeled for nicotine concentration; three contained no nicotine, while the remaining two contained nicotine concentrations in excess of 100mg/mL. In duplicate comparisons of nicotine containing RFs, 16 of the 18 duplicates varied significantly in nicotine concentration from their mates. Of the 23 total duplicate pairs, 15 of 23 varied in coloration from their mates. Nicotine concentration labeling on EC refill products was often inaccurate, and some bottles that were not labeled had nicotine concentrations that would be lethal if inadvertently consumed by a child or adult. To ensure the reliability and safety of RFs, it is necessary to establish quality control guidelines for the manufacturing and labeling of RF products.

1613 Cytotoxicity of Electronic Cigarette Refill Fluid Aerosols

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Electronic cigarettes (EC) are nicotine delivery devices, often advertised for smoking cessation. The fluids used to refill these devices typically contain nicotine, a humectant, such as propylene glycol or vegetable glycerin, flavorings and contaminants. Few scientific studies have assessed possible health effects of EC aerosols on users, and it remains unclear whether they pose a health concern. Previously, 19 EC refill fluids from four companies were screened for cytotoxicity using human pulmonary fibroblasts (hPF) via the MTT assay. In this extension of the previous study, we produced aerosols from these fluids using the Vea EC from Johnson Creek and then tested them for cytotoxicity in the MTT assay on hPF, which model the adult lung. A comparison was made between the refill fluids applied to hPF and the aerosols produced from the fluids which were then used to treat the cells. Aerosol yields ranged in IC50 values (dose that inhibits survival by 50%) from 0.1054% to >1% (12 had low-cytotoxicity and 7 were moderately cytotoxic). For 68% of refill fluids (13 of 19), the corresponding aerosols had similar cytotoxicity. 11% of the fluids (2 of 19) were more cytotoxic than their aerosols. Finally, 21% of the products (4 of 19) were more potent as aerosols than as fluids. This latter observation is important as it shows that heating refill fluid can increase its cytotoxicity. The data further show that when screening for cytotoxicity of EC products, testing refill fluids on cells is not always representative of the effects of an aerosol on cell survival. Additionally, 2 of the 19 aerosols produced vapor effects at their high dose, and the dose range was then adjusted to eliminate vapor transfer into neighboring cell cultures. This study is the first to compare the cytotoxicity of EC refill fluids and their aerosols on human cells. In addition, this study provides valuable insight into which of these EC refill products may be potentially harmful to users.

1614 Apparent Lack of Cytotoxicity in Brands of e-Liquids Previously Reported As Being Cytotoxic

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US sales of e-cigarettes (e-cigs) and e-liquids reportedly will exceed $1 billion in 2013. While there is much evidence to indicate that short-term use of e-cigarettes as a smoking cessation tool does not present undue health risks in healthy adults, little is known about health risks associated with long-term use of the product. To date there appears to have been only one report of in vitro toxicological assays that are commonly used to assess the toxicity of mainstream cigarette smoke being applied to the mainstream aerosol from e-cigs (Romagna et al., 2013); but that research used puffing conditions now known to be inappropriate for e-cigs; and puffing of e-cigs is also known to give cytotoxic substances such as acrolein when dry puffs are taken (Faralinos et al., 2013). Thus, it makes sense to determine e-liquid cytotoxicity first. There has been one report (Bahl et al., 2012) that described the use of the MTT assay with three different cell lines (hESC, mNSC, hPF) to estimate the cytotoxicity of e-liquids used to refill the reservoirs of e-cigs. While several e-liquids were reported to be cytotoxic, no cytotoxic compounds were identified in that report. For this reason, four of the same brand-style e-liquids were obtained. An additional brand-style of e-liquid was obtained from another manufacturer. All five samples were assayed with the Neutral Red Uptake (NRU) cytotoxicity assays with CHO cells (assays were purchased from Labstat International ULC, Kitchener, ON, Canada), and a modification of Health Canada Method T-502 was used. There was no cytotoxicity at a concentration of 0.1%. In addition, each e-liquid was analyzed by GC-MS, and none of the compounds reported to cause e-liquid cytotoxicity such as allyl alcohol and diacetyl were found. However, one e-liquid was found to contain eugenol and cinnamaldehyde, compounds not usually used in cigarettes sold in the US.

1615 VUSE Electronic Cigarette Aerosol Characterization


Electronic cigarettes (e-cigs) are of high interest worldwide as alternatives to combustible cigarettes. R. J. Reynolds Vapor Company has introduced VUSE, an e-cig, in the US market in 2012. A main criticism of e-cigs from various groups is that there are not enough data on exposure to nicotine or other potential toxicants of interest. This poster presents mainstream aerosol chemistry data for different commercial VUSE products. Aerosol was collected using a 55/30/3 machine packing regimen and either bell shaped or square wave puffing profiles. The test programs included evaluations of a subset of compounds currently listed on FDA’s Harmful and Potentially Harmful Constituents list for combustible cigarettes. Individual constituent yields and chromatographic profiling data for different commercial VUSE products tested over different time intervals consistently indicated that: 1) compounds intentionally added to the formulation of the e-liquids (e.g., nicotine, glycerin) were measured in the aerosol, as expected; 2) toxicants of concern for combustible cigarettes were either below the limit of quantification or below the limit of detection in VUSE aerosols; and 3) VUSE aerosols were significantly less complex than mainstream smoke emitted from combustible cigarettes.

1616 Safety Assessment of Potential Food Ingredients on Canine Primary Hepatocytes

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Toxicity screening of pet food ingredients must first be conducted to assess their safety in dog food. Canine hepatocytes (CH) were isolated from the livers of dogs (n=5), euthanized for clinical reasons with owner consent, using a two-step collagenase perfusion method. CH were evaluated for viability with alamarBlue (aB) mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) for 24h in some of the compounds. In addition, human and rat hepatocytes were used to compare to CH. Aflatoxin B1 (AB) and acetaminophen (APAP) were assessed to characterize and screen early toxic responses in CH and in pet food ingredients like clove leaf oil (CLO) and eugenol (EUG). CH showed a decrease in viability with an LC50 of 3.80µg/mL of APAP with an LC50 of 3.11mg/mL while the LD50 of human hepatocytes was 4.47µg/mL for AB and 3.45mg/mL for APAP, and rat LC50 of 5.36µg/mL for AB and 2.35mg/mL for APAP. MMP in CH showed a significant decrease by AB and APAP, and an increase in ROS especially with superoxide. In addition, CLO and EUG dosed at 1.5-fold dilution series ranging from 0.1% to 0.0029% in CH showed an increase in intracellular vacuoles when dosed at high concentrations greater than 0.0088%. CLO and EUG showed a decreased viability from 0.030% at 24h with the same LC50 of 0.037%. In conclusion, CH were successfully isolated and well characterized for the assessment of toxicants and pet food ingredients, which is comparable to human and rat hepatocytes. The variable responses may be attributable to age and breed of dogs. (Supported by Mars Inc.)

1616a Development of Formulation and Analysis Methods for Triphenyl Phosphate (TPP) in Rodent Feed for Toxicology Studies

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Aromatic phosphates (AP) are rapidly replacing brominated flame-retardants in commercial products. Most commercial APs are multi-component products containing ~20% TPP. Due to the lack of comprehensive toxicology data, TPP is under evaluation by the NTP. In support of planned toxicology studies, analytical methods were developed and validated for dose formulation characterization, including dose concentration, homogeneity, and stability of TPP in NIH-07 and NTP 2000 rodent meal feed. TPP-dosed feed (10 g) was extracted with 100 mL of acetonitrile (ACN);acetic acid (99.1%; NIH-07) or 100 mL of ACN (NTP-2000)
and analyzed by HPLC/UV on an X-Terra RP18, 5μm column using a water:ACN gradient. This method, which was validated over a range of 200 – 5,000 ppm, was linear (r > 0.999), accurate (percent relative error < ±0.9%), and precise (relative standard deviation (% RSD) < 0.4%). TPP feed formulations were evaluated for factors influencing homogeneity including TPP and feed particle size. Commercial TPP is supplied as 1-9 mm pellets, making formulation challenging. In this study, a ball-mill was used to grind TPP to a median diameter of ~420 μm to approximate feed particle diameter (median ~270 μm determined for 5 meal types). Homogeneity in NIH-07 and NTP 2000 was evaluated for two mixing procedures: feed flour or an acetone/feed slurry. Both mixing procedures produced homogenous formulations at 240 or 40,000 ppm (%RSD ≤ 5.0%), but the feed flour approach resulted in large TPP/feed agglomerates requiring extensive post-formulation particle size reduction. The 240-ppm TPP formulation was stable for 42 days in both diets, when stored at 4 or ~20°C, and for 7 days at ambient temperature when exposed to air and light in open feeders, but displayed an ~10% loss (NTP-2000) or an ~17% loss (NIH-07) after 24 hours when exposed to rodent urine/feces.

1616b Using Cluster Approaches to Assess the Safety of Chemicals Used in Consumer Products

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There is a growing interest in the use of cluster-supported read across to obtain more efficient and accurate hazard assessments. While the practice of using structural analogs to evaluate data-poor chemicals is well established, the narrow approach of “one analog to evaluate one chemical” can lead to inconsistent and inaccurate results, particularly when there is a need to assess a large number of chemicals. Assessing hazards in the context of small groups (“clusters”) of structurally related chemicals increases the overall confidence and the consistency of the chemical assessments. This poster illustrates how the US EPA Design for the Environment Branch (DfE) is using chemical clusters to streamline hazard evaluation in a voluntary program that promotes the use of safer chemicals in consumer products. Clusters can be assembled by professional judgment, but clustering software such as ChemACE, developed by US EPA to quickly create discrete clusters from user-defined chemical lists and with minimal refinement, can save time and effort during the initial grouping exercise. DfE uses ChemACE in the development of chemical clusters to evaluate chemicals against DfE’s ecological, environmental fate, and human health criteria. Examples such as allyl esters, polycyclic musks and macrocyclic lactones, are used to illustrate how clusters are defined, used for read-across and provide assistance in decision-making. In addition to streamlining the evaluation process within DfE, this approach could be of further help for companies that are conducting hazard assessments under their own internal sustainability initiatives.

1616c Toxicity of Tris(Chloropropyl)phosphate (TCPF) Dietary Exposure for 13 Weeks in Harlan Sprague-Dawley Rats and BeC3F1/N Mice


TCPF is a high production volume flame retardant and plasticizer. It is recognized as a global contaminant but the hazard to humans remains unknown. The objective of this study was to evaluate the toxicity of TCPF in male and female mice and rats exposed via diet for 13 weeks at 0, 1250 (mice only), 2500, 5000, 10000, 20000 and 40000 (rats only) ppm. In rats, perinatal exposure from gestation day 6 through postnatal day 21 (weaning) preceded the 13-week exposure. Exposure in mice began at 5-6 weeks of age. All rats dam exposed to 40000 ppm (gestation) and all male pups (first week post-weaning) from the 20000 ppm group either died or were humanely terminated due to overt toxicity. Body weights in all rats at weaning were 13-30% lower than controls, indicating an effect on weight gain. In mice, no treatment-related mortality or clinical signs of toxicity were observed. Terminal body weights were 12% (rats) and 29% (mice) lower than controls. Relative liver (rats, mice) and thymus (rats) weights were increased. Rats displayed increased serum cholesterol and decreases in hepatic enzymes; mice exhibited decreased blood leukocyte counts. In rats, microscopic changes included minimal to mild bilirubin hyperplasia and cortical hyperplasia in the thymus. In mice, hepatic centrolobular hypertrophy and cytoplasmic alteration of male renal tubular epithelium were noted. In genotoxicity tests, TCPF did not induce mutagenicity in bacteria or chromosomal damage in peripheral blood of male or female rats, or female mice. Chromosomal damage in the form of micronucleated erythrocytes was seen in male mice. In summary, subchronic exposure to TCPF in feed resulted in reduced dam and pup survival (rats) and the liver, thymus and kidney were considered primary targets of toxicity. The NTP is currently evaluating the effects of TCPF on development, chronic toxicity, and carcinogenicity. This work was supported by the NIH.

1616d Use of Chemical Safety Assessment and the TTC to Evaluate the Potential Toxicity of an Endoscopic Guidewire

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An endoscopic, non-vascular guidewire (limited, less than 24-hour tissue contacting device) underwent a material and process change. To assess the potential impact of these changes to patient safety, the device was extracted with hexane or saline at 50 deg C and the resulting solutions were analyzed by various analytical test methods for extractable substances. GC-MS showed the presence of benzaldehyde, a commonly used food additive. The dose of benzaldehyde (10 μg) to which patients might be exposed from the use of guide wire was calculated to be about 70,000 times less than the published oral reference dose to man. Similarly, the total amount of 2-hydroxy-isoo-butyrophenone present in the device (0.05 μg) was calculated to be 1000 times less than the proposed 90 μg/day dose limit for a Class III compound using the Threshold of Toxicological Concern (TTC) guidelines, implying a negligible genotoxicity or carcinogenic risk. The ICP-MS data showed the presence of several metallic elements such as zinc to which humans are exposed through various dietary sources. The estimated patient exposure to zinc from the use of the guidewire was calculated to be 285.7/14 times less than the prescribed daily upper intake level from US Food and Nutrition Board. Lastly, the device extract also showed the presence of tungsten (38 μg) which, based on the published NOAEL data, resulted in 138 times less than the tolerable exposure dose. The device extract passed the required biocompatibility tests for safety evaluation. In summary, this study showed that chemical characterization of device extract is useful in determining potential toxicological risks, which may obviate the need for conducting further in vivo biocompatibility assays.
Chemical Characterization and Biocompatibility
Investigation of One Drug-Combo Implant Devices for Neural Implication—Medtronic Antibiotics Impregnated Catheters
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Medtronic Antibiotics Impregnated Catheters (ARES) catheters are silicone elastomer catheters which are subsequently impregnated with proprietary antibiotics, which is the first drug-combo implant device in Medtronic Neurosurgery division. These catheters are permanently implanted ventricular and peritoneal catheters used as components of a shunt system in the treatment of hydrocephalus. The antibiotic impregnated ventricular and peritoneal catheters are line extensions of the currently offered standard Medtronic Neurosurgery catheters for use as components of a shunt system. To investigate the biological safety of the product for its usage as brain implant devices, series of chemical characterization and biocompatibility testing are undertaken. Drug elution study showed that the antibiotic is eluting at a rate and level that is not anticipated to cause significant patient adverse reactions. This product did not cause cytotoxicity in SK-N-MC neuronal cells after 48 hours exposure nor did it cause acute systemic toxicity in Swiss Albino mice. It did not induce skin irritation in rabbits or sensitization reactions in Guinea Pigs. More importantly, New Zealand White rabbits implanted with ARES did not demonstrate differences in measured parameters (clinical observation, clinical chemistry/hematology and histological pathology) that were considered to be attributable to ARES for a period of 13 weeks. As expected, ARES, which includes antibiotics, caused toxicity in some strains of the S. typhimurium tested in the bacteria reverse mutation assay. However, in the remaining bacteria strain, ARES did not induce any mutagenicity and it did not induce any genotoxic response in mouse lymphoma L5178Y cell lines. The base materials and impregnated antibiotics of the ARES catheters have been used in clinical settings for a long time without known genotoxic and carcinogenic response, indicating low risk of genotoxicity and carcinogenicity for the ARES catheters.

In Vitro Assessment of Cardiotoxicity Hazard of Environmental Compounds Using Fast Fluorescence Imaging of Beating iPSC-Derived Cardiomyocytes

A large number of environmental agents, with potential for human exposure, remain inadequately tested for toxicological effects. As an effort to develop and characterize in vitro model systems for toxicological screening, we used fast kinetic fluorescence imaging that monitored changes in intracellular Ca2+ using a calcium sensitive dye to screen a library of environmental chemicals and drugs for their ability to alter beating patterns in human iPS-derived cardiomyocytes. These cardiomyocytes were exposed to a diverse set of 80 environmental chemicals. Compounds were screened for cardiotoxicity across a 7-point concentration at 30 min and 24 h of treatment. A number of physiological parameters of cardiomyocyte beating, such as beat rate, peak shape (amplitude, width, raise, decay, etc.) and regularity were collected using automated data analysis. Concentration-response profiles were evaluated using logistic modeling to derive a benchmark concentration (BMC) point-of-departure value, based on one standard deviation departure from the estimated baseline in vehicle treated cells. BMC values were used for cardiotoxicity classification and ranking. We found that number of environmental chemicals such as pesticides (permethrin, heptachlor, deltamethrin, dieldrin, carbaryl); flame retardants (isophorolified phenyl phosphate, 2-ethylhexyl diphenyl phosphate, 2,2,4,4-tetrabromodiphenyl ether); polycyclic aromatic hydrocarbons (acenaphthene, acenaphthylene and benzo[a]pyrene); and metalloids (arsenic, lead, and mercury) inhibit in vitro cardiac function. We applied the Toxicological Prioritization Index approach to integrate and display data across many collected parameters, to derive “cardiotoxicity” ranking of tested compounds. This approach can be utilized to prioritize suspected cardiotoxicants for in vivo hazard characterization and mechanistic follow-up studies.

Analysis of Four Hepatotoxins Using the xCELLigence Analytical System and HepaRG Cells
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Hepatotoxins act through different mechanisms and kinetics, and we reasoned that an analytical system allowing for convenient, kinetic monitoring of relevant effects would be very valuable in the in vitro prediction of hepatic cell toxicity. We developed a protocol to use the xCELLigence Real-time Cell Analyser (RTCA) to facilitate recognition of the risk of hepatotoxicity. Our protocol involved culturing cryopreserved, differentiated HepaRG cells onto the xCELLigence 96-well cell culture plate, and after a four-day adaptation period, treating them with different concentrations of four compounds with known risk of hepatotoxicity—APAP, amiodarone, CsA, and troglitazone— and its eventual replacement the PPAR agonist rosiglitazone. The Cell Index (CI), an expression of the electrical impedance of adherent cells as measured on sensors in the culture plate wells by the xCELLigence Cell Analyzer, of treated cells was compared to the CI from HepaRG cells exposed to a 0.5% DMSO control at post-treatment time points 24, 48, 72, 96, and 168 h, during which the medium was replenished every 48 h. The CI was also assessed at 2, 5, 10, and 20 hours after treatment with eight APAP concentrations and control to determine if damage to the cells could be detected rapidly, since McGill et al reported significant glutathione depletion within three hours after exposing HepaRG to APAP. With the exception of low concentrations, time and concentration-dependent effects were detected for all hepatotoxicants. 72 hours after treatment with 6µM of APAP and 50µM of amiodarone, the NCI of treated cells declined 70% and 83% respectively compared to the control cells. A marked CI decline in APAP treated cells vs control was detected within 10hrs. At 72 hrs, exposure to 200µM troglitazone demonstrated much stronger effect on the CI than 450µM of rosiglitazone. We conclude that the combination of the HepaRG cells and xCELLigence system may sensitively detect risk of hepatotoxicity across a range of mechanisms.

1618 Modeling of Inflammation-Mediated Liver Injury In Vitro
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Drug induced liver injury (DILI) is one of the leading causes for the discontinuation of drugs both in development and in post-marketing phase. Pre-existing liver inflammatory status might sometimes predispose patients to drug-induced hepatotoxicity. A major contributor to liver inflammation is the resident macrophages, also referred to as Kupffer cells. However it is not feasible to profile all compounds during drug development in vivo for studies for their role in inflammation mediated liver injury in all preclinical species. An alternative is the use of in vitro approaches such as co-culture of hepatocytes together with Kupffer cells or to treat hepatocytes with immunomodulators such as TNFα to mimic inflammation mediated liver injury. Mouse, rat, dog and human primary hepatocytes were cultured in a collagen-matrigel sandwich configuration and were primed with the immunomodulator TNFα to validate this inflammatory model for liver injury. Cellular ATP (Adenosine Triphosphate) content and leakage of LDH (Lactate dehydrogenase) into the supernatant were measured. Trovafloxacin, known to trigger hepatotoxicity in vivo, induced cytoxicity in all tested species only in the presence of TNFα whereas Levofloxacin, which is innocuous in vivo, did not. A good correlation between the cellular ATP levels as well as the LDH released into the supernatant was observed. When hepatocytes were cultured without TNFα, both Trovafloxacin and Levofloxacin were devoid of any cytoxicity. This inflammatory-like model for drug-induced liver injury could be used to test compounds that are in development across various species.

1619 Developing an Impedance-Based Cellular Assay with Human iPSC-Derived Cardiomyocytes to Quantify Modulators of Cardiac Contractility
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Cardiovascular toxicity is a prominent reason for late-stage failures in drug development, resulting in the demand for in vitro assays that can predict this liability in early drug discovery. Current in vitro cardiovascular safety testing primarily focuses on ion channel modulation and low throughput cardiomyocyte contractility measurements. The xCELLigence Real-Time Cell Analyser (RTCA) Cardio system utilizes label-free impedance technology to quantify beating properties of spontaneously beating cardiomyocytes in a medium throughput (96-well) mode. We tested both human induced pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) and rat neonatal cardiomyocytes (rat-CMs) on the Cardio system. A set of 50 reference compounds were tested in concentration-response mode and the potency values for effects on beat rate and beat amplitude were determined. These compounds had previously been tested in vivo as well as in a low throughput in vitro optical-based contractility assay using electrically paced canine cardiomyo-
cytes (Toxicol Appl Pharmacol (2012) 260:162) and included negative and positive inotropes and no effect compounds. By comparing to the known in vivo contractility effects, hiPSC-CM impedance had assay sensitivity, specificity and accuracy values of 90%, 74%, and 84%, respectively. These values compared favorably to the in vivo contractility in canine assay (83%, 86%, 82%) and were slightly better than im- pedance using rat-CMs (80%, 74%, 76%). The hiPSC-CM and rat-CM impedance potency values spanned 5 orders of magnitude and correlated with values from the canine optical assay (r2=0.76 and 0.70, respectively). The Cardio system had >5X higher throughput than the optical assay. Thus, the hiPSC-CM Cardio system assay can help detect the human cardiotoxic potential of novel therapeutics early in drug discovery and, if a hazard is identified, support the design-make-test-analyze cycle to mitigate this liability.

1620 Assessment of Prolactin-Mediated Changes and Their Reversal in Molindone Treated Rats

Molindone, an antipsychotic, is known to modulate prolactin levels linked with reproductive changes in rodents. Such effects were further investigated in the current 6-month rat study with a reversal phase to better characterize changes for risk assessment. Wistar rats (12/sex/group) were dosed daily by gavage with molindone (males at 1, 3 and 10 mg/kg; females at 5, 20 and 60 mg/kg). Blood samples were analyzed for molindone and prolactin on Days 1, 180 and at a 2-month reversal period. Histopathology was performed on reproductive tissues. Results showed that plasma levels of molindone were more than dose proportional with Tmax between 0.25 to 1h postdose (PD). Prolactin levels peaked within 1h PD and were 284 and 335 ng/ml in males and 613 and 884 ng/ml in females, respectively, for Days 1 and 180. Prolactin levels remained elevated up to 2h PD in males and 8h in females. After 2-month recovery, prolactin levels reached basal levels, suggesting complete reversibility. Histopathologically, on Day 180, an increased vacuolation of mammary gland epithelium was noted in males at 3 and 10 mg/kg. In females, a diffuse glandular hyperplasia in mammary tissue was noted in all treated groups with minimal severity in 5 mg/kg, and minimal to mild severity in 20 and 60 mg/ kg. At the end of recovery, histopathology changes in males were completely reversed; in females, they were completely reversed at 5 mg/kg, with signs of reversal at 20 and 60 mg/kg. Reversibility in prolactin and histopathology seen in this study validates the pharmacologically mediated effects of molindone and corroborate with the known role of prolactin in hormonal homeostasis and reproductive physiology. These changes are considered species-specific, similar to those seen with other antipsychotics in this class of drugs. It is concluded that the transient prolac- tin-mediated histopathology changes seen in this study are of negligible risk, if any, to humans, as such changes have not been reported in humans taking molindone as a therapeutic.

1621 In Vitro Assessment of Intestinal Toxicity of Drugs Using an Electronic Impedance-Based Cell Monitoring System

Intestinal toxicity is a common problem in pharmaceutical development. In par- ticular, the potential for diarrhea not caused by cytotoxicity is difficult to detect in rodent study because of the difference in the intestinal structures between rodent and human. As such, it is often first identified in dog or non-human primate study, or furthermore, in a clinical trial. Transepithelial electrical resistance (TEER) measure- ment has been used for the in vitro detection of diarrhea potential, especially due to barrier disruption, although its throughput was quite low. In this study, we further investigated the usefulness of a real-time impedance-based cell monitoring system, xCELLigence (ACEA Biosciences, Inc.), which has a measurement principle similar to that of TEER, was evaluated as an effective assay system for detection of the potential for diarrhea in human. HT29 cells, a human colon adenocarcinoma cell line, were seeded on 96-well E-plates, in which a sensor electrode array is incorporated in each well, and moni- toring of cell impedance was initiated using xCELLigence. After 48 hrs incubation, the cells formed a confluent monolayer and the impedance became constant; then cells were exposed to 19 compounds with or without diarrhea potential and cell impedance were monitored for 24 hrs. As a result, remarkable reductions (>20% vs. vehicle control) of cell impedance were observed in the compounds that induced diarrhea due to barrier disruption: cytchalasin D, ethanol and okadaic acid. Slight reductions (15-20% vs. vehicle control) of cell impedance were observed in the compounds that induced secretory diarrhea, forskolin, slidingfil, IBMX and quinidine. On the other hand, no appar- ent change was observed in the compounds that induced diarrhea due to intestinal hypermotility, erythromycin and betahaneol, and in seven non-diarrhea-inducing compounds, except for loperamide. These results suggest the usefulness of the xCELLigence system as an effective assay to detect the potential for diarrhea in human.

1622 A Comparison of Human Hepatocyte Systems and Conditions for Hepatotoxicity Screening
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Cultures of primary human hepatocytes (PHH), human induced pluripotent stem cell derived hepatocytes (hiPSC-H; CDE, Madison, WI), and 5 immortalized THLE-5B (ATCC CRL 11113) human hepatocyte cell lines (one parental clone, Tc5, lacking CYP450 expression and 4 clones expressing either human CYP2C9, 2C19, 2D6 or 3A4) were compared for detection of hepatotoxicity (reduction of cellular ATP by 50% [IC50] at concentrations ≤ 50 μM). We used 40 hepatotoxic drugs (HD) labeled with a warning for serious (SHD, n = 20) or variable (VHD, n = 20) hepatotoxicity in human patients and 20 drugs not reported to be hepatotoxic (NHD). Cells were exposed to compounds at concentrations ≤ 200 μM for 4 days. In parental Tc5 cells, IC50 values of ≤ 50 μM were generated for 78% HD (31/40) and 15% of NHD (3/20). Metabolism by the Tc5 CYP expressing clones did not significantly increase the number of HD with IC50 values ≤ 50 μM but re- duced the IC50 values for 5 SHD and 8 VHD and increased the IC50 values of 2/3 NHD to > 50 μM. In iPSC-H and PHH, IC50 values ≤ 50 μM were respectively generated by 55% (22/40) and 53% (21/40) HD. PHH and hiPSC-H generated the same number of false positives (5% [1/20]). These results support using the non-metabolizing Tc5 parent line to evaluate hepatotoxicity routinely, and use of the Tc5 clones subsequently to investigate the potential for toxification or deteri- orization. The iPSC-H and PHH detect fewer HD and may be better reserved for compound specific mechanism studies. Finally, others have reported increases in the predictive value of hepatocyte cell systems co-treated with 1) DL-buthionine- (S,R)-sulfoximine to deplete glutathione, 2) a cytokine cocktail (TNFα, LPS, IL1a, IL6) to model immune stimulation under inflammatory conditions, or 3) galactose substituted medium to alter cell respiratory status. However, we found that these conditions did not improve the detection of HD in Tc5 cells after 4 days of culture.

1623 A Chemical Derivative of Naturally Occurring Isothiocyanate, DJ4, Blocks Stress Fiber Formation
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Isothiocyanates (ITC) are naturally occurring compounds found in many vegeta- bles and are known for their antitumor properties. We have developed a series of novel derivatives of ITC as a part of a cancer drug discovery program. Among the many derivatives tested, DJ4, dramatically altered the morphology of multiple cell types including cancer cells. Using confocal microscopy, detailed examination of the cytoskeletal structures of DJ4 treated cells revealed that DJ4 totally disrupted stress fiber formation without affecting microtubules. Given the known role of Rho family GTPases in cytoskeletal regulation, we hypothesized that DJ4 may exert its effects on stress fibers through modulation of signal pathways downstream of the Rho GTPases. Cellular mechanistic studies revealed that the DJ4-induced disruption of stress fibers was mediated by inhibition of Rho-associated kinases (ROCK) and myosin dystrophy-related Cdc42-binding kinase (MRCK). In in vitro kinase activity studies, DJ4 inhibited phosphorylation of endogenous MYPT1 (myosin binding subunit of myosin phosphatase) which is a downstream substrate of ROCK and MRCK. Molecular docking of DJ4 onto the crystal structures of these kinases suggested that DJ4 binds at the ATP binding site of these kinases. In vitro biochemical studies using recombinant ROCK/MRCK and MYPT1 pro- teins confirmed that DJ4 potently inhibited ROCK1/2 and MRCKβ in an ATP competitive manner. Together these results demonstrated that DJ4-induced loss of stress fibers in the cells is at least, in part, mediated by direct inhibition of ROCK1, ROCK2 and MRCKβ. As stress fiber formation is involved in migration/invasion of cancer cells, these results further indicate the potential utility of DJ4 as a novel anti-metastatic agent.

PS 1620 Assessment of Prolactin-Mediated Changes and Their Reversal in Molindone Treated Rats

Molindone, an antipsychotic, is known to modulate prolactin levels linked with reproductive changes in rodents. Such effects were further investigated in the current 6-month rat study with a reversal phase to better characterize changes for risk assessment. Wistar rats (12/sex/group) were dosed daily by gavage with molindone (males at 1, 3 and 10 mg/kg; females at 5, 20 and 60 mg/kg). Blood samples were analyzed for molindone and prolactin on Days 1, 180 and at a 2-month reversal period. Histopathology was performed on reproductive tissues. Results showed that plasma levels of molindone were more than dose proportional with Tmax between 0.25 to 1h postdose (PD). Prolactin levels peaked within 1h PD and were 284 and 335 ng/ml in males and 613 and 884 ng/ml in females, respectively, for Days 1 and 180. Prolactin levels remained elevated up to 2h PD in males and 8h in females. After 2-month recovery, prolactin levels reached basal levels, suggesting complete reversibility. Histopathologically, on Day 180, an increased vacuolation of mammary gland epithelium was noted in males at 3 and 10 mg/kg. In females, a diffuse glandular hyperplasia in mammary tissue was noted in all treated groups with minimal severity in 5 mg/kg, and minimal to mild severity in 20 and 60 mg/ kg. At the end of recovery, histopathology changes in males were completely reversed; in females, they were completely reversed at 5 mg/kg, with signs of reversal at 20 and 60 mg/kg. Reversibility in prolactin and histopathology seen in this study validates the pharmacologically mediated effects of molindone and corroborate with the known role of prolactin in hormonal homeostasis and reproductive physiology. These changes are considered species-specie
Identification of trace impurities in drugs and their structural elucidation is attaining much more interest due to their significant influence on drug activity. 1, 2 This structural information is very fundamental to know toxicity of trace impurities and their effect on drug activity especially at the preclinical stage. The octadentate ligand 3,4,3-LI(1,2-HOPO), composed of four 1-hydroxy-pyridin-2-one (1,2-HOPO) units linked to a spermine scaffold through amide linkages, is undergoing preclinical development as an actinide decomposition agent that removes radioactive actinides from the body. In this study, 18 trace impurities were detected with the 3,4,3-LI(1,2-HOPO) ligand, using ultra-performance liquid chromatography coupled with photo-diode array UV detection and Electrospray Ionization-Quadrupole Time of Flight Mass spectrometry (UPLC-ESI TOFMS) via induced-in-source or collision induced mass fragmentation (Nozzle-Skinner Fragmentation). Molecular ions were fragmented within the nozzle-skimmer region of the ESI mass spectrometer equipped with a TOF detector. Of these 18 impurities, 8 are considered major impurities (detected over 0.1% threshold) and need to be characterized according to ICH guidelines. Structural elucidation of all 18 impurities was carried out via analysis of generated fragment ions using mass fragmenter, elemental composition softwares obtained from Waters Corporation (USA) and Molecular Fragment Calculator software. Proposed structures of impurities were further confirmed via isotopic modeling.

References
1628 A Real-Time In Vitro Safety Assessment Approach Utilizing a Simplified, Multiparametric Work Flow

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In vitro cytotoxicity is inextricably linked to a combination of compound dosage, exposure period, and intrinsic cell susceptibility. Current screening paradigms which utilize only endpoint measures in a defined cell type adequately address effects due to dosage, but often fail to define important toxicokinetic profiles or inherent mechanistic sensitivities. We investigated the use of a real-time cytotoxicity probe applied at the time of dosing with staurosporine, panobinostat, imatinib, terfenadine, colchicine, aflatoxin B1, bortezomib, camptothecin, valinomycin, nocardazole, mitomycin C and imonomycin. Serial dilutions of these model compounds with the probe were delivered to iPSC-derived, terminally differentiated hepatocytes and proliferating hepatitis and enterohepatic cell lines. Cytotoxicity data were collected at 4, 24, 48 and 72hr followed by a same-well multiplexed viability assay. The collated data revealed striking differences in toxicokinetics, potency and magnitude of response which positively correlated with known mechanism of action for the model compounds. The multiplexed viability data further served to either confirm observed cytotoxicity by inverse signal concordance, or suggest replicative perturbation in susceptible replicating cells. Furthermore, the use of cell types with differential capacity for phase I metabolism, allowed us to stratify cytotoxic risk based on mode-of-action of parent molecule toxicity and/or metabolic by-products owing to biotransformation. Lastly, the experimental approach taken was sufficiently predictive and informative to merit consideration for adoption as a new safety screening paradigm for new chemical entities.

1629 Antimycobacterial Activity and Cytotoxicity of South African Rubiaceae Species, a Potential Source of Promising Antituberculous Remedies


Tuberculosis is an airborne contagious disease which has infected about one-third of the world’s population with 1 in 10 latently infected individuals developing active disease in a lifetime. Although, the mortality rates of TB have reduced to about 1,7 million per annum, the incidence of new cases has increased due to the upsurge of HIV/AIDS. Toxicity associated with the presently available anti-TB drugs is a limitation to the treatment of TB leading to the failure of patient’s compliance to the treatment. Medicinal plants are used in many part of southern Africa to treat TB-related symptoms including chest pain and coughing. In a preliminary screening process, plant species from the Rubiaceae family showed good activity against Mycobacterium smegmatis. Sixteen genera from the Rubiaceae family indigenous to South Africa were screened for their anti-TB activity against *M. smegmatis* and *M. aurum* using a two-fold serial microdilution assay while cytotoxicity was determined by MTT assay against C3A liver cells and Vero kidney cells. The selectivity index (SI) values were calculated. Six of the extracts showed good minimum inhibitory concentration (MIC) values ranging from 0.04-0.12 mg/ml. Thirteen extracts were relatively non-cytotoxic with LC50 values ranging from 0.1-0.7 mg/ml. *Oxyanthus speciosus* had the highest SI values (LC50/MIC) of 4.9 and 6.5 against *M. smegmatis* and *M. aurum* respectively (C3A cells) and 2.6 and 3.4 respectively (Vero cells). All cells were more sensitive to *Psychotria zambontana* and *Pavetta lanceolata* than Vero cells, while *Psychotria capensis* was toxic to both C3A and Vero cell lines. In conclusion, *O. speciosus* may contain compounds which can act as leads for the development of new anti-TB drugs. Extracts containing several active compounds may also be promising remedies to act in conjunction with known anti-TB drugs to enhance their activity. Further work is continuing on isolation and identification of these compounds, and evaluation of fractions of the extracts for antimycobacterial activity.

1630 Investigation of the Effects of Pyrrolamide Antibiotic Agents on Temperature in Rats

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Administration of pyrrolamide antibiotic agents to rats was shown to be associated with reductions in core body temperature. We utilised a series of different techniques to gain an understanding of whether the changes in body temperature were related to effects in brown adipose tissue (BAT).

Core body temperature was assessed using digital rectal probes and transponders implanted subcutaneously into the interscapular region. Both methods demonstrated a decrease in core body temperature, which was variable between individual animals. Thermal imaging of the rats demonstrated a reduction in interscapular temperature suggesting decreased heat production, whilst there was no evidence of heat loss via the tail.

Metabolic rate (assessed using an oxygen open circuit calorimeter) demonstrated a slight decrease in group mean metabolic rate following administration of single doses of pyrrolamide compounds to rats when compared to controls; again with more marked effects seen in individual animals.

1631 Enhanced Sensitivity of Human and Animal Hepatocytes to Hepatotoxicants after a Prolonged Treatment Duration of 96 Hours

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In vitro hepatotoxicity evaluation in hepatocytes allows the selection of new chemical entities with reduced hepatotoxic liability for further development. Furthermore, multispecies evaluation in vitro aids the selection of the most appropriate animal for in vivo assessment of human hepatotoxic potential. We report here results with 12 model drugs with known animal in vivo and human clinical hepatotoxic potential: acetaminophen, aflatoxin B1, amiodarone, cyclophosphamide, triazolam, mitoxantrone, ketaconazole, cerivastatin, fluvastatin, simvastatin, tacrine, and tamoxifen. Platelet cryopreserved hepatocytes from three human donors, Cynomolgus monkey, SD rat, CD-1 mouse and beagle dog were simultaneously evaluated in the same experiment for the assessment of potential species differences. The hepatocytes were plated in collagen-coated 384-well plates, with treatment initiated 4 hrs after plating for two treatment durations of 24 hrs and 96 hrs followed by quantification of cellular ATP contents. Increased cytotoxicity was observed for all hepatotoxicants at the prolonged treatment duration of 96 hrs. For several hepatotoxicants, the 96-hr treatment allowed clear distinction of species-differences which could not be observed with the 24-hr treatment. For instance, the hepatotoxic cerivastatin was highly cytotoxic (96-hr EC50 <3 uM) in human, monkey and dog hepatocytes, and substantially less cytotoxic in mouse and rat hepatocytes. Similarly, tacrine was cytotoxic after 96-hr treatment in human and monkey hepatocytes (EC50 of 19 to 41 uM) but non-cytotoxic to CD-1 mouse, SD-rat, and beagle dog hepatocytes. Neither cerivastatin nor tacrine was cytotoxic to human or animal hepatocytes up to the highest concentration evaluated of 100 uM at the shorter treatment time of 24-hrs. The results suggest that the prolonged 96-hr treatment of the hepatocytes significantly enhance the sensitivity of the cells to hepatotoxicants. Species-differences observed in vitro in this assay may aid the selection of the most appropriate animal model for in vivo safety evaluation.

1632 A Novel 19-Substituted Benzoquinone Ansamycin Ameliorates A53T Alpha-Synuclein-Induced Toxicity in a SH-SY5Y Cell Model of Parkinson’s Disease through the Upregulation of Heat Shock Proteins and the Induction of Autophagy

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A major cause of neurodegenerative diseases including Parkinson’s disease (PD) is the accumulation of misfolded protein aggregates that in turn leads to neurotoxicity. Targeting Hsp90 has been proposed as an attractive strategy to halt neurodegenerative diseases, and the benzoquinone ansamycin (BQA) Hsp90 inhib...
itors such as geldanamycin (GA) and 17-AAG have been found to protect against A53T α-synuclein induced toxicity in cell models of PD. However, current BQA inhibitors are relatively hepatotoxic in animal models, and a major mechanism of toxicity involves glutathione depletion as a result of arylation at the C19 position of the BQA. We have developed novel 19-substituted BQAs (19BQAs) as a means to prevent arylation and have validated that these compounds are unreactive at the 19-position using model thiols including glutathione. Our results show that 19BQAs exhibited little toxicity in SH-SYSY cells relative to their parent quinones (GA, 17-AAG and 17-DMAG) using trypan blue, MTT and Annexin/PD apoptosis assays, while they retained the ability to induce heat shock proteins (HSPs) and autophagy as determined by increased levels of Hsp70, Hsp27, and LC3 II respectively. Transduction of mutant A53T human α-synuclein adenovirus in SH-SYSY cells was further utilized to determine whether 19BQAs could ameliorate the neurototoxicity of mutant α-synuclein. 19-phenyl-GA, which caused potent induction of Hsp70, Hsp27, and LC3 II, significantly attenuated A53T α-synuclein-induced toxicity. These results indicate that 19BQAs may provide a means to modulate protein-handling systems in neural cells thereby ameliorating the aggregation and toxicity of proteins such as mutant A53T α-synuclein (supported by CAS51210, ES018943 and Parkinsons UK).

### High-Throughput In Vitro Toxicity Screening Using Autonomously Bioluminescent Human Cells in 2D and 3D Cultures

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In vitro cell culture-based assays have increasingly become fast, high-throughput, and low cost alternative to in vivo animal tests for tier 1 cytotoxicity screening of a large and still growing number of small molecule candidates in the drug development pipeline. Most of the current cell assays employ endpoint measurements that require cell destruction and/or substrate addition, thus limiting informational output to only intermittent single time point snapshots as well as requiring large numbers of samples be analyzed in parallel for basic toxicological kinetics studies. To overcome this disadvantage, we have demonstrated substrate-independent bioluminescent imaging in living human cells using a synthetically optimized bacterial bioluminescence ($\text{luc}$) reporter system. To evaluate its application for toxicity screening, $\text{lux}$-expressing autobioluminescent HEK293 cells were treated with the antibiotic Zeocin, whose toxicity was measured using the $\text{luc}$-based autobiloluminescent assay, and the traditional MTT assay every 24 hours for 4 days. While the MTT assay required an individual set of samples for each time point, the substrate-free nature of the $\text{luc}$-system allowed for repetitive, near real-time monitoring of the same cell population over time. Exposure to 1000× of Zeocin resulted in 500× activity to 11.9% and 13.9% compared to untreated cells after 2 days of exposure monitoring of the same cell population over time. Exposure to 1000× Zeocin allowed for repetitive, near real-time $\text{lux}$-tobioluminescent assay, and the traditional MTT assay every 24 hours for 4 days.

### Lack of Neuropathy after Long-Term Tedizolid Phosphate Administration in Rats

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A number of antibiotics, including linezolid (LZD), are associated with mitochondrial toxicity that can manifest as serious optic and/or peripheral neuropathy, primarily during long-term therapy. In a 6 month rat study, sciatric and optic nerve degeneration was observed with LZD exposures similar to those in humans. Tedizolid phosphate, a novel antibiotic prodruk given once/d for 6 days demonstrated noninferior efficacy to LZD twice/d for 10 days and may have less potential for mitochondrial toxicity. The neurotoxic potential of tedizolid phosphate in rats was examined in a robust 9 month study. Tedizolid phosphate in vehicle was administered orally to Long Evans rats (N = 366) once/d for 13, 3, 6, or 9 months at 7.5, 15, and 30 mg/kg (males) and 2.5, 5, and 10 mg/kg (females); dose levels were chosen to cover tedizolid exposures up to 10-fold of those in patients. Concurrent controls (N = 366) received vehicle only. The neurotoxic potential of tedizolid phosphate was thoroughly evaluated by a number of behavioral and neuropathological assessments. Toxicokinetics of the active moiety tedizolid were assessed throughout the study. There were no drug-related effects on survival, food consumption, functional observational battery assessments, locomotor activity, brain weight/measurements, ocular examinations, and macroscopic and microscopic neuropathological findings (including sciatic and optic nerves); of note, 9 month microscopic data are still being analyzed. Tedizolid exposure generally increased proportionally to dose, with little accumulation over time. Average and 9 month peak steady-state exposures were 6.6-fold and 8-fold, respectively, of those in humans with standard 200 mg/d dose. This rigorous animal study showed no evidence of neurotoxic effects during long-term administration of tedizolid phosphate at systemic exposures considerably higher than achieved at the human therapeutic dose. These results suggest a reduced neurotoxicity risk with long-term tedizolid phosphate treatment relative to LZD.

### Sequence Motifs Associated with Hepatotoxicity of Locked Nucleic Acid (LNA)-Modified Antisense Oligonucleotides

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Locked Nucleic Acids (LNAs) are third generation antisense oligonucleotide (ASO) modifications that improve target affinity, RNase H activation and stability. LNA modifications may increase the risk of hepatotoxicity compared to other types of ribose modifications. In vitro cytotoxicity screens have not been reliable predictors of hepatic toxicity in non-clinical testing; however, mice are considered to be a sensitive test species. To better understand the relationship between nucleotide sequence and hepatotoxicity, a structure-toxicity analysis was performed using two-week safety studies in mice administered with LNA-modified ASOs at a dose of 25 mg/kg. ASOs targeting human Apolipoprotein C3 (ApoC3), CREB (CRMP Response Element Binding Protein) Regulated Translocation Coactivator 2 (Crtc2) or Glucocorticoid Receptor (GR, NR3C1) were classified based on the presence or absence of hepatotoxicity in mice. From these data, a Random Forest classification model built from nucleotide sequence motif descriptors revealed two motifs (TCC and TGC) that were present only in hepatotoxic sequences. We found that motif containing sequences had higher propensity for binding hepatocellular macromolecules and increased P53 and NRF2 stress pathway activity. These results suggest in silico approaches can be utilized to establish structure-toxicity relationships of LNA-modified ASOs and decrease the likelihood of hepatotoxicity in preclinical testing.

### PARP Inhibitors and Centrosome Declustering: Bone Marrow Safety Implications

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PARP inhibitors may selectively target cancer cells via centrosome declustering (CD). We explored the bone marrow (BM) safety profile of PARP inhibitors that induce CD and/or multipolar spindles (MPS) through in vitro and in vivo experiments. PARP inhibitors were synthesized and ranked according to CD and MPS-forming activities. Efficacy data suggested that this activity was PARP6-mediated. Although PARP6-dependent activity was considered to be cancer cell-specific, BM toxicity was observed in efficacy in vivo studies. To address the hypothesis that compounds inhibiting PARP1-3 could be responsible for the observed BM toxicity, we performed in vitro colony-forming cell (CFC) assays. We investigated 15 compounds with different MPS-inducing and PARP1 activities. The compounds’ potencies in the CFC assays aligned with MPS-inducing activity, but not with PARP1 inhibition. To confirm the relationship between CD/MPS and BM toxicity in vivo, studies were conducted using enantiomers with differential MPS-inducing potencies (EC50s of 0.02 μM vs. inactive), but with similar PARP1 activities. The two compounds were dosed to give equivalent exposure over the course of 10 days (oral). Complete blood counts (CBCs) and BM flow cytometry revealed that BM toxicity occurred only in animals dosed with the enantiomer that caused MPS. Whereas there were peripheral, panmyelopenic-suppressive changes with the active enantiomer, the inactive enantiomer showed no significant changes in CBCs. BM flow cytometry analyses showed significant reductions in lymphoid, myeloid, and erythroid cell populations, ranging from 70–100% over the course of the experiment with the active enantiomer. This was in contrast to the inactive enantiomer, which showed mild (10–20%) reductions in the same BM cell populations. The enantiomer experiment revealed that the proposed mechanism of PARP6-associated CD/MPS induction was independent of PARP1 and was directly associated with BM toxicity. Interestingly, this reflects a novel role for PARP6 in the BM.
Despite 30 years of extensive research on therapeutic development, the 5-year survival rate for patients diagnosed with glioblastoma remains less than 5%. The aggressive nature of glioblastoma often renders radiation and surgery ineffective. Thus, discovering new compounds that mitigate glioblastoma progression is vital to improving patient survival. We utilize an innovative orthotopic zebrafish xenograft model to assess drug efficacy in inhibiting human glioblastoma proliferation, invasion and angiogenesis within a 3-day time frame. Zinc oxide nanoparticles (ZnO-NPs) demonstrate preferential cytotoxicity to cancer cells in culture; therefore, we are interested in confirming whether this selective toxicity is conserved in vivo. Although ZnO-NPs prevented glioblastoma cell proliferation in vitro, preliminary experiments at exposure levels tolerable to the zebrafish revealed the ZnO-NPs significantly enhanced proliferation in the xenograft model. Knockdown of calpain 2 reduces glioblastoma invasion by ~90%; therefore, we are also interested in using our model to identify novel therapeutics that impede glioblastoma invasion through calpain 2 inhibition. In vitro testing of calpain 2 inhibitors identified two compounds that block glioblastoma invasion at low μM concentrations while causing minimal toxicity to the zebrafish, making ideal candidates for testing in the xenograft model. The results of screening putative anti-proliferative NPs and anti-invasive calpain 2 inhibitors will be compared to known chemotherapeutics LY294002, temozolomide and acivicin. Our approach holds promise for efficiently prioritizing novel glioblastoma therapeutics for future development. Research support: NSF 134468; NIEHS P30ES00210; ES016896 and T32ES070600.

Chagas disease is a protozoan zoonosis caused by Trypanosoma cruzi that has infected a large number of people. So far there is no effective drug for Chagas disease treatment. So, the Institute of Pharmacutical Technology at Fiocruz has designed three nitro analogs of the nitroimidazole-thiadiazole megazol: PTAL 05-02 (5-(1-methyl-5-nitro-1H-2-imidazolyl)-1H-1,2,4-triazol-3-amine), PAMD 09 (2-amine-N-(1-methyl-4-nitro-1Himidazolyl)-5-(trifluoromethyl)-1H-1,2,4triazole) and PTAL 04-09 (1-(1-methyl-4-nitro-1H-imidazol-5-yl)-1H-pirazole), whose toxicological properties were not evaluated. Thus, this work investigates the mutagenic, cytotoxic and genotoxic activities of megazol and its nitro analogs. The Salmonella microsome assay was performed using the pre-incubation protocol with different strains of Salmonella enterica serovar Typhimurium (TA97, TA98, TA100, TA1535; TA102), and the Micronucleus assay was carried out with RAW264.7 macrophage rat cells. The assays were performed in accordance with OECD (1997 and 2010) standards. Megazol showed dose-dependent mutagenic and cytotoxic activity at very low concentration (0.05 μg/mL) for all S. enterica strains, with and without metabolic activation (95% mix). PTAL 04-09 was the only analog to present mutagenic activity (TA100) at the higher concentration (50 μg/mL), with and without S9 mix. All of the nitro analogs presented cytotoxic activity to at least one strain (TA100 and/or TA1535), only with S9 mix. Moreover, all of them were capable of inducing micronucleus formation and showed cytotoxic effects (apoptosis and necrosis) at all concentrations (1.0 to 100 μg/mL). Our results showed that advances can be made in the development of new safe compounds to the Chagas disease treatment.
of which were exposed to TCDD. Expression pattern of EGFP in the reporter zebrafish mirrored that of endogenous cyp1a mRNA. Using this transgenic cyp1a reporter zebrafish, we screened 6,400 small molecules for inhibitors of dioxin toxicity and identified several compounds that almost completely suppressed pericardial edema, a characteristic feature of zebrafish embryos. We are currently testing efficacy of various derivatives of these compounds in the zebrafish embryos. Development of dioxin inhibitors will further understanding of dioxin toxicology and contribute to the treatment of dioxin intoxication.

1639c Magnetic 3D Bio-Printing: A Novel High-Content Assay for Drug Toxicity Screening

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A growing demand exists for the development of 3D cell culture models for drug screening that predict in vivo response more accurately than traditional 2D models. To meet this demand, we developed a real-time and dynamic 3D assay for drug screening based on magnetically bio-printed 3D cultures. This assay is based on the use of magnetic nanoparticles to rapidly assemble and print cells into 3D cellularized dots or rings that contract over time, and at rates that can vary with drug concentration. Here, this assay was validated using 3T3 murine embryonic fibroblasts. 3T3s were first incubated overnight with the magnetic nanoparticle assembly and bio-printed the next day either into 3D dots or rings, and allowed to shrink over 5 hours. Contraction due to cell-cell interaction and migration was captured using a mobile device programmed to image whole plates of 3D cultures at specific timepoints, eliminating the need to image rings constantly under a microscope. This assay is label-free that allows for post-assay experimentation to explore mechanisms of action and yield more content per experiment. This assay was validated as both a measure of drug response in 3D by comparing it to a 2D viability assay, as well as a high-content assay by fluorescent staining for viability and cytoskeletal organization, and compared to a 2D viability assay. With increasingly toxic drug concentrations, 3T3 dots and rings shrank at a slower rate, became less viable, and lost cytoskeletal organization. This assay was validated using retinoic acid, dexamethasone, doxorubicin, forskolin, fluorouracil, and nor-epinephrine, showing higher drug sensitivity compared to cells in 2D. Other cell types have been tested, including cancer cell lines, such as MCF-10A, and primary cell lines, like vascular smooth muscle cells.

1640 Ocular Anatomical Features in Rabbits, Dogs, and Monkeys: a Comparison with Human Data


In non-clinical studies for the development of ophthalmic products, laboratory animals such as rabbits, dogs, and cynomolgus monkeys are used frequently. The comparative researches for the ocular anatomy are essential for interpreting of data and extrapolating to human. However, the information has been scattered, and there is less comprehensive literatures easily to compare between the species. The purpose of this study is to provide direct and systematical comparison data of the ocular anatomical features from rabbits, dogs, and monkeys, and to compare with human data which were already established. The eyeballs from animals were all obtained from the purpose-breed animals ethically euthanized. After measuring the eyeball size (axial length, weight and volume), they were frozen. The frozen samples were dissected and measured for lens and vitreous size parameters during gradual throwing. As the result, the present study provided anatomical measurement values comprehensively for whole eyeball, lens, vitreous as well as body weight in each animal species. In comparison with human, the ratio were 0.39, 0.82 and 0.46-fold against human value (as 1) for eyeball weight, 1.71, 2.48 and 0.53-fold for lens weight, and 0.34, 0.73 and 0.55-fold for vitreous volume in rabbits, dogs, and monkeys respectively. Vitreous size was not directly depended on body weight of animals itself. This result enables us to set the dosage volume of drugs based on the size of animal eye. Furthermore, the ratio of dosage volume to eyeball size makes extrapolating to human more precise. In conclusion, this study provided a direct comparative data of ocular anatomical features in rabbits, dogs and monkeys and compared with human data. This would be useful for evaluating the non-clinical studies for ophthalmic products.
Mitochondrial Toxicity: Assessing In Vitro-In Vivo Correlative Risk in the Antiviral Human Rhinovirus Project (HRP)


Mammalian mitochondria possess a bacterial and viral ancestry [1]; as a consequence, antibacterial as well as some antiviral therapies could be prone to increased potential for mitochondrial toxicity. One such antiviral program, HRP identified a potential mitochondrial toxicity liability, during in-vitro screening using the glucose/galactose assay. AZN001, the original lead, had a Glu/Gal ratio of 8.5 that translated to IC50=15μM in the mitochondrial oxygen consumption assay. Although these in-vitro assays are considered predictive for mitochondrial toxicity, their actual translations in-vivo, including a quantitative in-vivo - in-vivo relationship and finding a molecular mechanism, are yet to be established. To address these points, AZN001 (top dose 750 mg/kg/d) has been tested in a 7-day rat study. Several parameters that can suggest mitochondrial dysfunction, including plasma lactate and transmission electron microscopy (TEM), were included in the study. As monitored by these in-vivo end points, a clear translation of in-vitro effects was observed. No clear guidance for a quantitative in-vitro and in-vivo relationship could be established. Additionally, an in depth in-vitro mechanism study involving AZN001 and mitochondrial respiratory enzymes indicated potential inhibitions of mitochondrial complex I and/or complex V. In the light of these observations, the HRP team has implemented a suitable testing strategy for development of follow up lead candidates.

Reference(s)

Rapid Screening of Master Viral Stocks for Wild-Type Contamination

G. K. Massey2, G. P. Gambill2, A. D. Penman1,2 and K. K. Daniels1.

Adenovirus 5 (Ad5) vectors are commonly used for vaccines and gene therapy. The E1A gene is required for viral replication. Some therapies take advantage of this property by inserting a therapeutic gene of interest in place of E1A. Since vector production requires the trans-complementation E1A, cell lines were generated to include the Ad5 sequence needed for replication. However, homologous recombination can occur during propagation, resulting in wild-type vector. These vectors are monitored for recombination to wild-type by their ability to infect cells lines without trans-complementation factors. Other gene therapy vectors take advantage of viral replication. For these vectors, a selective promoter replaces the E1A promoter so replication only occurs in cells of interest. These vectors are able to replicate, but only in the presence of cellular factors that activate the new promoter. If propagated in cell lines with wild-type sequence, homologous recombination can also occur, resulting in wild-type vector. Since both vectors are replication competent, there is difficulty distinguishing modified vector from wild-type Ad5. Therefore, a more specific test is needed to ensure vector stocks do not contain wild-type Ad5. To the specific and quantitative nature of real-time PCR, an assay for the wild-type promoter can be used to monitor for wild-type contamination of master viral stocks. Our method allows for the identification of 1-10 copies of wild-type Ad5 in the presence of 108 copies of modified vector. The method takes approximately 3-4 hours, allowing for same day results. Utilizing vectors from preclinical and/or clinical studies, we will present data demonstrating that using our method, 2 of the 4 vectors tested had appreciable levels of wild-type contamination.

Understanding Mechanisms of Drug-Induced Liver Injury Using Primary Human Cell and Coculture Systems


Drug-induced liver injury (DILI) still remains a challenge to the pharmaceutical industries and regulatory authorities due to its idiosyncratic nature. Elucidating the mechanisms by which injury occurs in human hepatocytes and other liver cells is focus of attention. Here, we attempt to understand the mechanisms of DILI...
using complex co-cultures of primary human cells (BioMAP systems). 10 discontinued Pfizer compounds and 25 known drugs (withdrawn from market or with black boxed-warnings) with clinical DILI and a small set of non-DILI compounds were retrieved from ToxCast Phase II dataset. These compounds were profiled in eight primary human cell cultures (at multiple concentrations) including endothelial, epithelial, smooth muscle, fibroblasts and immune cells, stimulated with specific combination of inflammatory mediators, with activity in total of 87 protein readouts. Compounds were classified based on their similarity to BioMAP profiles, which resulted in a number of unique clusters for DILI compounds. Some of these compounds exhibited overt cytotoxicity in multiple cell types, whereas others showed only low activity in one cell type. A subset of these clusters were also studied using a validated clinical assay. Specific metabolites of each cluster were sequenced to identify a unique set of metabolites associated with each profile. Efforts were made to use these clusters to develop a robust computational model for predicting DILI risk.

1648 Assessment of Phencyclidine in the Evaluation of Physical Dependence in Rats Using the Nonprecipitated Withdrawal Test

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Physical dependency assessments are conducted as part of the FDA and EMEA requirements pertaining to the medicinal product scheduling and investigation of dependence potential. Due to specific and often unique mechanisms of action for each drug class, it is necessary to evaluate and establish appropriate paradigms to assess physical dependency as part of a larger drug abuse liability (DAL) program. Therefore, the use of a validated and robust rodent model with appropriate non-precipitated withdrawal tests is necessary to adequately determine dependence potential. The objectives of this study were to establish standard withdrawal scores, changes in qualitative motor activity, and alterations in operant behavior assessments following chronic administration of the NMDA-R antagonist, phencyclidine (PCP). Phencyclidine was administered by subcutaneous injection to 8 groups of Wistar or Sprague Dawley adult male rats (n=6 rats/group) on an escalating dose regimen (up to 80 mg/kg/day) over the course of 21 to 28 days, followed by 1 to 2 week withdrawal period. During the treatment and withdrawal phases, animals were monitored for body weight (BW), food consumption (FC), qualitative motor activity (MA), neurobehavioral/clinical observations (OBS), and operant scheduled behavior (OPB). During treatment, BW, FC, OBS, MA, and OPB were relatively consistent across PCP treatment groups, with characteristic increased motor activity and decreased motor coordination. Following the cessation of PCP dosing, OBS and initial MA were not significantly different across treatment groups, suggesting a lack of robust withdrawal behaviors. However, OPB assessments exhibited a significant decrease in OPB following the cessation of PCP dosing. These results demonstrate the importance of proper paradigm selection when designing specific DAL studies.

1649 Investigation of the Method of Oral Mucosal Irritation Test by Oral Instillation in Rats


Evaluation for oral mucosal irritation is requested at the time of submission of new drug application when the clinical route of drug candidate intends to be introrally administered. Generally, oral mucosal irritation tests have been conducted by applying test substances on the mucosa of cheek pouch in hamsters. In the present study, we conducted a cumulative oral mucosal irritation test in rats to evaluate the irritation potential of sodium dodecyl sulfate (SDS) according to the Cosmetic, Toiletry and Fragrance Association (CTFA) guidelines. Macroscopic and histopathological examination of oral mucosa was investigated. SDS was administered 4 times daily at 1-hr intervals for 4 or 10 days to rats under the different conditions as follows: instillation of 0.5%, 4% and 15% SDS (0.1 mL) into the oral cavity and application of 4% SDS (0.1 mL) with a paint brush to the labial mucosa and incisor gingiva of the mandibular oral vestibule. The irritation potential of SDS was evaluated on the basis of the daily mean total score (DMTS) and was classified as follows: Nonirritant (DMTS: 0), Mild irritant (DMTS: 0.5-1.0), Moderate irritant (DMTS: 1.1-2.0) and Severe irritant (DMTS: 2.1 and over). Macroscopic observations included sloughing on the incisor gingiva in the 4% SDS application and 15% SDS instillation groups. In addition, discoloration was a notable effect observed in the 15% SDS instillation group. The DMTS for 4% SDS application and 15% SDS instillation were 0.6 and 1.3, respectively. Therefore, SDS in each experiment was evaluated as “Mild irritant” and “Moderate irritant”, respectively. By contrast, no oral mucosal irritation was observed in the 0.5% or 4% SDS instillation groups. In the histopathological examination, parakeratosis and thickening of the mucosal epithelium were observed on the labial mucosa in the 4% SDS application and 15% SDS instillation groups. It is suggested that this oral mucosal irritation test method by oral instillation in rats could be helpful to evaluate liquid drugs.

1650 Comparative Subchronic Toxicity Studies in Rats of LY2963016 (LY), an Insulin Glargine Product, with USA-Sourced Lantus® (US–L) and EU-Sourced Lantus® (EU–L)


LY2963016 is an insulin glargine product, with an identical amino acid sequence to Lantus® insulin glargine. To support human clinical trials and in agreement with regulatory guidance for follow-on biologics, the toxicity of LY was characterized in rats alongside two reference insulin glargine products: US-L (Study 1) and EU-L (Study 2). Dosing was daily via subcutaneous injection for 4 wk. In Study 1, LY or US-L doses of 0, 0.3, 1.0, and 3.0/2.0 mg/kg were given. In Study 2, LY or EU-L doses of 0, 0.3, 1.0, and 2.0 mg/kg were given. The findings in Studies 1 and 2 were related to hypoglycemia or hyperinsulinemia at all LY and Lantus doses; the severity and duration of hypoglycemia was dose related. In Study 1, dosing was suspended at 3 mg/kg LY or US-L for 3 d due to multiple mortalities and associated severe hypoglycemia and then resumed at 2 mg/kg for the remainder of the study. Overall, the maximum tolerated dose (MTD) was exceeded in Studies 1 and 2 for LY, US-L and EU-L at ≥1.0 mg/kg based on hypoglycemia related mortalities. Hypoglycemia also occurred, but was tolerable with no associated mortality in the 0.3 mg/kg doses for LY, US-L and EU-L. Statistic nerve degeneration, a common sequelae of sustained hypoglycemia, occurred at the high dose with LY, US-L and EU-L. At study termination, rebound hyperglycemia and pancreatic islet cell atrophy were observed at ≥1.0 mg/kg of LY, US-L or EU-L and was most likely due to down regulation of endogenous insulin synthesis secondary to hyperinsulinemia. Increased fat was observed in the skin, subcutis and injection sites at all doses of LY, US-L and EU-L consistent with lipogenic pharmacology typical of insulins. Based on the occurrence of intolerable hypoglycemia in rats at doses ≥1.0 mg/kg, the MTD and NOAEL (no-observed-adverse-effect level) was 0.3 mg/kg for LY, US-L and EU-L. Therefore, we conclude that the toxicity profiles of LY, US-L and EU-L are highly similar.

1651 Glucocorticoid-Induced Bone Loss in Aged Rats Is Not Reversible


The objective of this study was to investigate the effects of methylprednisolone (a glucocorticoid, 5 mg/kg/day) on bone when administered to aged rats (19 week old female Wistar Hannover) with an inactive growth plate. Experimental animals were dosed 13 weeks followed by an 8-week recovery period. Effects on bone densitometry and bone geometry were evaluated (using pQCT) at baseline and Weeks 4, 13 and 19. In addition, body weight, food consumption, selected serum biochemistry and bone turnover markers were evaluated to add perspective on mechanisms underlying bone effects. Sharp decreases in bone weight, bone length and bone mineral density were observed at all doses of LY, US-L and EU-L consistent with lipogenic pharmacology typical of insulins. Based on the occurrence of intolerable hypoglycemia in rats at doses ≥1.0 mg/kg, the MTD and NOAEL (no-observed-adverse-effect level) was 0.3 mg/kg for LY, US-L and EU-L. Therefore, we conclude that the toxicity profiles of LY, US-L and EU-L are highly similar.

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1652 Rat-Specific MOA for Tumor Formation in Rats with the SGLT2 Inhibitor Canagliflozin

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Canagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, caused renal tubular rutos (RTTs) and pheochromocytomas in a 24-month rat carcinogenicity study at the high dose. Canagliflozin is also a low potency inhibitor of SGLT1 and causes carbohydrate (CHO) malabsorption at high doses in rats via inhibition of intestinal glucose transport leading to increased intestinal calcium absorption (secondary to acidification), hypercalcuria and hyperostosis. This carbohydrate malabsorption was believed to play a role in the induction of the renal and adrenal tumors. A 6-month mechanistic study (with a 1 and 3 month interim evaluation) was performed in which rats were fed a glucose-free 40% fructose diet. The purpose of this study was to prevent carbohydrate malabsorption and its sequelae. Urinary calcium, development of hyperostosis, and serum 1,25-dihydroxyvitamin D and parathyroid hormone (PTH) were used to confirm the effectiveness of the intervention. In addition, cell proliferation was evaluated in kidneys and adrenal medulla by BrdU-immunohistochemistry and kidney injury was evaluated by KIM-1 immunohistochemistry, to evaluate the link between CHO malabsorption and kidney injury/cell proliferation (as proximal steps in tumorigenesis) in these target tissues. Utilization of a glucose-free fructose diet prevented the effect on CHO malabsorption and its sequelae including hyperostosis and increased calcium absorption. Cell proliferation in the kidney and adrenal medulla was increased in rats administered canagliflozin (cana) fed a standard diet, and immunostaining was increased for the proliferation marker Ki67. These effects were inhibited in cana-treated rats maintained on the glucose-free fructose diet indicating they are secondary to CHO malabsorption and are not direct effects of canagliflozin. CHO malabsorption was absent in humans at clinical doses indicating that the tumor findings in rats are not clinically relevant.

1653 Expanded Therapeutic Index of Antithrombin Silencing and Correction of APTT in a Hemophilia A Mouse Model


ALN-AT3, a subcutaneously administered RNAi therapeutic targeting antithrombin (AT), is currently being developed for the treatment of hemophilia and rare bleeding disorders. It has previously been demonstrated that once weekly dosing of ALN-AT3 results in potent, dose-dependent and reversible silencing of plasma AT in multiple preclinical species. In the mouse, steady state ED50 knockdown was achieved following weekly doses of 0.5 mg/kg. This study evaluated the exaggerated pharmacology of ALN-AT3 when administered via weekly SC injections (x 7 wks) to wild-type (WT) vs. hemophilia A (HA) mice at 0, 10, 30, and 100 mg/kg (20-, 60- and 200-fold dose multiples of the mouse ED50). Test article-related effects were evaluated by clinical signs, BW, clinical pathology, organ weights, and histopathology of select tissues (eye, heart, kidney, liver, lung, spleen). The PD of ALN-AT3 was evaluated by plasma AT protein levels. Repeat dosing of a 10 mg/kg ALN-AT3 to WT mice was not tolerated, as evidenced by early mortality, adverse clinical signs (ocular abnormalities, craniofacial swelling, lethargy), weight loss, clinical pathology changes (±PLT, ±NEU), ± spleen weights, and microscopic findings in heart (thrombi) and eye (hemorrhage). Residual plasma AT protein in all tumor findings in rats are not clinically relevant.

Body weight, food consumption, rectal temperature, and ophthalmology were evaluated prior to, during and after treatment. Hematology, coagulation, blood biochemistry and C-Reactive Protein (CRP) levels were evaluated prior to treatment and on days 2, 8, 53, 58, 64 and 85. After the treatment period all animals in each subgroup were sacrificed and examined macroscopically. Selected tissues were weighed and preserved. Microscopic examinations were performed on the kidneys only on day 58, and on all preserved tissues on day 60. No unscheduled deaths occurred during the study and no signs of systemic toxicity were observed. Animals treated with PLG had a higher incidence of reactions (mainly erythema) at injection sites relative to the controls, which persisted for up to 19 days. Since all local reactions were reversible, PLG was considered well tolerated locally. Mean body weight and food consumption were unaffected by PLG treatment. No remarkable effects on rectal temperature or ophthalmological findings were observed in the control or PLG groups. Hematology, coagulation parameters, blood biochemistry and CRP levels were unaffected by PLG exposure. IM administration of PLG did not induce any significant histopathological findings compared to saline, in particular, no kidney findings. The exipient PLG was considered to be well tolerated both locally and systemically following repeated IM injections in the rabbit.

1654 Repeated-Dose Intramuscular (IM) Toxicity Study with Poly-α-L-Glutamic Acid (PLG) in New Zealand White Rabbits

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The potential local and systemic toxic effects of five IM injections at 2 weeks intervals of the excipient PLG MW 2083 Da were studied in New Zealand White rabbits. PLG was administered at a dose of 6.25 mg/injection and compared with saline treatment. The PLG concentration, pH, osmolality, sterility and endotoxin levels were verified at each day of injection. Rabbits were distributed in 3 subgroups (5/sex) sacrificed 1, 3 or 28 days post last dose (days 58, 60 or 85). Animals were observed for mortality/morbidity, clinical signs, and local injection site reactions. Body weight, food consumption, rectal temperature, and ophthalmology were evaluated prior to, during and after treatment. Hematotoxicity, coagulation, blood biochemistry and C-Reactive Protein (CRP) levels were evaluated prior to treatment and on days 2, 8, 53, 58, 64 and 85. After the treatment period all animals in each subgroup were sacrificed and examined macroscopically. Selected tissues were weighed and preserved. Microscopic examinations were performed on the kidneys only on day 58, and on all preserved tissues on day 60. No unscheduled deaths occurred during the study and no signs of systemic toxicity were observed. Animals treated with PLG had a higher incidence of reactions (mainly erythema) at injection sites relative to the controls, which persisted for up to 19 days. Since all local reactions were reversible, PLG was considered well tolerated locally. Mean body weight and food consumption were unaffected by PLG treatment. No remarkable effects on rectal temperature or ophthalmological findings were observed in the control or PLG groups. Hematology, coagulation parameters, blood biochemistry and CRP levels were unaffected by PLG exposure. IM administration of PLG did not induce any significant histopathological findings compared to saline, in particular, no kidney findings. The exipient PLG was considered to be well tolerated both locally and systemically following repeated IM injections in the rabbit.

1655 Preclinical Safety Development of a New HSV-2 Prophylactic Candidate Vaccine to Support a First-in-Human Phase I Clinical Trial

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Herpes simplex type-1 (HSV-1) and type-2 (HSV-2) viruses belong to the herpesviridae family of viruses (members of the alphaherpesvirus subfamily), which can cause prevalent, lifelong genital, dermal and ocular infections resulting in a spectrum of clinical symptoms including cold sores, genital ulceration, corneal blindness and encephalitis. HSV529, a viral vaccine candidate against HSV-2 wild type virus, is being developed to prevent HSV-2 genital infection. The vaccine is derived from a HSV-2 186 syn+1 wild-type virus, where the U5L and UL29 genes have been deleted, which are essential for viral replication.

To support a first-in-human study in healthy adults (18 to 40 years, ClinicalTrial.gov study code NCT01915212), a repeat dose toxicity and biodistribution studies were conducted in the guinea pig, which was selected as it is pathogenically sensitive to HSV-2 virus, closely mimics the human genital HSV-2 infection, and develops a positive humoral response to the vaccine.

The repeat dose study was conducted to assess the systemic toxicity and local tolerability and the biodistribution study was designed to evaluate the distribution, persistence, the ability to shed and to establish latency. In the repeat dose toxicity study, 4 intramuscular (IM) injections of a human dose (107 PFU/0.5 mL injection) induced a robust immune response. Effects were limited to reversible local reactogenicity and changes in the draining lymph nodes, related to immune stimulation. A transient minimal decrease in platelet count was also noted. The biodistribution study showed that following one IM injection at a human dose, no HSV529 was detected in any HSV-2 target organs and biofluids at the time points assessed. The study confirmed HSV529 was unable to distribute, propagate/replicate, shed or establish latency up to 45 days post injection. This data supported moving forward in a Phase 1 clinical trial with monitoring of local reactogenicity and platelet counts.
M. Davies

In conclusion, NKTR-192 toxicology studies in rats and dogs support the safety of NKTR-192 based on NOAEL and 45-fold for sedation, while oxycodone has narrower safety margins of 11-fold. Compared to a standard opioid (oxycodone), NKTR-192 demonstrates a clear separation of analgesia from CNS side effects with safety margins of 11-fold.

NKTR-192 was evaluated in 28-day repeat dose toxicity studies with a 2-week recovery observation period at oral BID doses up to 180 mg/kg/day in rats and 30 mg/kg/day in dogs. PLAT-bound PAI was not measured in the 2 euthanized monkeys from the 6-month study. PLAT-bound PAI was not measured in the 2 euthanized monkeys from the 6-month study. PLATs recovered at the end of 1-month recovery period. Plasma BMS-986001 levels for these 3 monkeys were similar to other monkeys given the same dose; thus higher BMS-986001 exposure did not explain the PLAT reductions.

Drug-induced PLAT reduction can be caused by anti-drug antibodies (ADA). To determine if PLAT reductions were mediated by ADA, serum platelet-associated immunoglobulin (PAI) levels were measured using PLAT-coated 96-well ELISA and by FACS analyses. No circulating PAI was detected in serum of any affected monkey by ELISA. However, PLAT-bound PAI was detected in CD42+ PLATs (IgG-PE mean fluorescence intensity [MFI]: 27 - 109; control MFI ≤7) correlating with the PLAT reduction in the 1 affected monkey, measured in the 9-month study. PLAT-bound PAI was not measured in the 2 euthanized monkeys from the 6-month study. These results suggest that BMS-986001-induced PLAT reduction in monkeys may have been due to PLAT-bound ADA and only in the presence of BMS-986001. Despite moderate levels of PLAT-bound IgG (MFI: 26) in the 1 affected monkey in the 9-month study, PLAT counts recovered to pretest values at the end of drug-free recovery period.

MTL-005 (copper meso-5,15-Bis[3-[(1,2-Dicarba-closo-dodecaboranyl)methoxy]phenyl]-meso-10,20-dinitroporphyrin) is under development as a radiosensitizer for use in head and neck cancer. In vivo it slows growth of murine squamous cell carcinomas and adenocarcinomas. Given i.p. to mice, it is cleared from the circulation and taken up by both normal tissues and tumors, with tumor concentrations up to 6 times higher than in muscle, skin, and brain. It concentrates in the liver and spleen and can be detected in both tumors and liver for at least 62 days post administration.

4 i.v. toxicology studies were carried out in rats and 2 in dogs. MTL-005 was dissolved in a mixture of dimethylacetamide, Solutol HS15 and PEG300 and diluted with 0.9% saline. Components of this vehicle are known to have toxic effects, especially in dogs. Rats were given 0, 20, 40 and 60 mg/kg (divided over 2 consecutive days) and dogs with 2 doses of 0, 1, 5 or 10 mg/kg given 14 days apart. Toxicities observed included body weight loss, reduced food consumption, dark discoloration of several organs, and reversible gastric epithelial hyperplasia (in rat at doses up to 30 mg/kg/day); and inflammatory changes at the infusion site, liver changes, and the death of 1 dog at 10 mg/kg. The vehicle may have contributed to the body weight, food consumption, and liver effects, as most of these signs were also observed in the vehicle controls. The MTD in rats was 40 mg/kg but a NOAEL could not be established due to the body weight losses observed both treated and control groups.

Dogs tolerated 1 or 5 mg/kg well but the vehicle contributed to the effects seen at 10 mg/kg. Liver histopathology findings and plasma chemistry changes associated with the vehicle were seen in all groups and liver histopathology findings persisted for 28 days. Injection site changes were reversible over 28 days. Again, a NOAEL could not be established.

These preclinical data were sufficient for regulatory approval of a Phase I clinical study of MTL-005 as an adjunct to radiotherapy in patients and this has now been initiated.

MTL-005, a Novel Polymer Conjugated Opioid Agonist, Demonstrates a Superior Preclinical Safety Profile to Traditional Opioids

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NKTR-192 is a new chemical entity mu opioid agonist that uses Nektar’s advanced polymer conjugate technology to achieve slow CNS uptake while maintaining adequate brain exposure with analgesia and is being developed for the treatment of moderate to severe acute pain. NKTR-192 produces full analgesic efficacy comparable to oxycodone in the mouse acetic acid writhing model. Orally administered NKTR-192 was evaluated in 28-day repeat dose toxicity studies with a 2-week recovery observation period at oral BID doses up to 180 mg/kg/day in rats and 30 mg/kg/day in dogs. Findings observed in both species are consistent with the pharmacology of a mu opioid agonist. Major dose-limiting toxicities with NKTR-192 included emesis in the dog, shallow respiration and hypooxygenization in both dog and rat. Doses exceeding the MTD in dog (>20 mg/kg as a single dose) caused severe respiratory depression and sedation that required naloxone reversal. In rats given >150 mg/kg as a single dose, target organ toxicities (dilation and/or hemorrhage) were observed in the urinary bladder and kidney. These findings were completely reversed after a 2-week recovery period. On the 28-day repeat dose toxicity studies, NOAELs of 120 mg/kg/day (BID) and 30 mg/kg/day (BID) were established in rats and dogs, respectively. A dose of 120 mg/kg/day NKTR-192 in rats provides an adequate animal to human safety margin to support clinical development. Compared to a standard opioid (oxycodone), NKTR-192 demonstrates a clear separation of analgesia from CNS side effects with safety margins of 11-fold based on NOAEL and 45-fold for sedation, while oxycodone has narrower safety margins of 3-fold for toxicity and 24-fold for sedation.

In conclusion, NKTR-192 toxicology studies in rats and dogs support the safety of NKTR-192 for clinical development and appears to have a superior therapeutic index relative to traditional opioids.
Onset of infusion, allowing review of the CP data prior to dose escalation. In conclusion, the other end of the infusion line was connected to a portable pump secured to a subcutaneously implanted vascular access port (VAP) and accessed via needle. The animal was dosed on D1 at 27.5mL/kg over 24hrs and CS were reviewed every 4hrs. Daily bodyweight and food consumption was recorded and CP samples were collected 20hrs into infusion, allowing review of the CP data prior to dose escalation. In conclusion, animals tolerated infusion of large volumes of LE; however, precise monitoring and stepwise volume escalation were critical.

### 1662a The Science behind Personal Care Products: A Hands-On Approach to Engaging K–12 Students

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Making science relevant and hands-on is critical to engaging middle and high school students. Northwest Association for Biomedical Research (NWABR) has developed a four lesson curriculum, “Consumer Awareness: Personal Care Products Safety and Labeling” that investigates the science, labeling and safety testing behind personal care products. The lessons, which include a lotion-making lab, have been presented by teachers and toxicologists to diverse student audiences in classrooms and informal education workshops.

This four lesson curriculum reminds students that science is part of their everyday lives, especially if they are to be informed consumers. It makes science and toxicology relevant through the exploration of the science and safety testing behind personal care products. It emphasizes critical evaluation skills and a basic understanding of science and regulations. The curriculum guides students through the analysis of personal care products by incorporating multiple aspects of:

- Science: biology, chemistry, toxicology, math
- Research: drug development process, experimental design, evaluating sources of information
- Ethics: safety testing on animals and humans
- Social Responsibility: risk assessment and safety policies, consumer advocacy

The lessons and workshops can be tailored in length and depth to engage the target audiences in formal and informal learning environments. Training with the curriculum has been offered to over 270 teachers across the country. The Kit loan program for teachers has reached over 11,400 students. Two hour workshops highlighting the lotion making lab and labeling have also been conducted with other groups such as Girl Scouts, after school programs and summer camps (~1000 students). The curriculum guides students through the analysis of personal care products by incorporating multiple aspects of:

- Science: biology, chemistry, toxicology, math
- Research: drug development process, experimental design, evaluating sources of information
- Ethics: safety testing on animals and humans
- Social Responsibility: risk assessment and safety policies, consumer advocacy

### 1662b Strategy to Develop Expertise in Risk Assessment in Developing Countries

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Although basic principles of toxicology are taught in undergraduate courses in Latin America (LA), chemical risk assessment (RA) is not a topic generally discussed even in graduate programs. To fill this gap, the Brazilian Society of Toxicology and IUTOX developed since 2008 a six days workshop, based on the IUTOX Risk Assessment Summer School concept, to respond to this need for well trained Risk Assessors and Managers (RAM) in both government and industry and to give current and future regulators an improved perspective on how toxicological

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1660 Nonclinical Safety Assessment of Tofacitinib in the Juvenile Rat and Monkey

C. I. Bowman, M. Collinge, Z. Rada, T. T. Kawabata, W. M. Vogel and D. J. Ball, Pfizer, Groton, CT.

Tofacitinib (Xeljanz) is a novel oral inhibitor of intracellular Janus kinases (pan-JAK inhibitor) for the treatment of rheumatoid arthritis. JAK is critical for intracellular signaling transduction of activated cytokine receptors, including those important for lymphocyte activation, proliferation and function. To support the use in pediatric patients as young as 2 years old with juvenile idiopathic arthritis, toxicity studies in juvenile rats and cynomolgus monkeys were conducted. Based on previous findings in adult animals, the immune, hematopoietic and reproductive systems were targeted for evaluation. A 39-week juvenile monkey study (with 26-week recovery) initiated in 14 month-old animals revealed decreased red blood cell parameters, lymphocyte and lymphocyte subset numbers, primary T-dependent antibody response and immune system organ weights during the dosing phase but partially to fully recovered by the end of the dose-free period. A one-month juvenile rat study with 2-month recovery was initiated in 3-week old rats to enable evaluation in animals roughly equivalent to the youngest patient age of 2 years old. In juvenile rats, expected effects on immune and hematopoietic parameters were observed by the end of dosing but were no longer present at the end of the recovery period. Overall, the immune and hematopoietic effects observed in juvenile rats and monkeys were reversible and consistent with JAK inhibition and effects observed in adult animals. Lastly, effects on fertility were evaluated following tofacitinib oral administration in rats from weaning (3 weeks old) to sexual maturity. No effects on male or female reproduction were observed indicating no effect on development of reproductive system in juvenile animals. In summary, the results of these studies in rat and cynomolgus monkey demonstrate that in juvenile animals there were no novel toxicities, no permanent changes and no increased sensitivities relative to known effects of tofacitinib in adult animals thus supporting clinical trials in pediatrics.

1661 Case Study: The Evolving Cardiac Risk Assessment for a Novel Anticancer Agent

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AZD7762 is a check point kinase 1/2 inhibitor that was being developed as a first in class treatment for advanced solid tumours in combination with DNA-damaging agents. AZD7762 was well-tolerated in tumour-bearing mice, significantly enhancing the efficacious effects of chemotherapy or radiation. However, cardiovascular safety signals in preclinical studies led to a complicated and species-dependent cardiovascular risk profile. A profound, irreversible decrease in blood pressure and cardiovascular risk profile. A profound, irreversible decrease in blood pressure and LVdP/dt max (up to 36% vs vehicle). There were no signs indicative of a similar haemodynamic change in the rat. However in toxicity studies in rats from weaning (3 weeks old) to sexual maturity. No effects on male or female reproduction were observed indicating no effect on development of reproductive system in juvenile animals. In summary, the results of these studies in rat and cynomolgus monkey demonstrate that in juvenile animals there were no novel toxicities, no permanent changes and no increased sensitivities relative to known effects of tofacitinib in adult animals thus supporting clinical trials in pediatrics.
The World Wide Web is a powerful tool that can educate and provide information to educators, parents, and students. We plan to utilize the web to make K-12 education outreach available to members and educators across the globe. This presentation is an opportunity to for the K-12 Sub-Committee to interact with SOT members who might have access to valuable teaching resources, which will benefit members and the public. We need your excitement and interest – submit your K-12 activities to the SOT web portal! 

1663 K-12 Education and Outreach: Inspiring Students Towards Careers in Toxicology, Technology, Engineering, and Math (STEM) 

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Headquartered in Ann Arbor, Michigan since 1944, NSF International is an independent, not-for-profit organization dedicated not only to improving global human and environmental health, but also to fostering future stewards for this mission. Toxicologists, microbiologists, chemists, and engineers from NSF have led hands-on experiments to promote STEM education and mentor future leaders in public health and safety. Learning modules were developed to explore polymers by creating elegant toothpaste确定剂用 dish soap, yeast and hydrogen peroxide. Other experiments emphasizing sustainable futures involved recycling, biodegradation, and homemade pH paper to learn about acid rain. Participating at local events such as the Maker Faire science festival in Detroit, Earth Day activities at the Science and Nature Center, workshops at the Hands-On Museum, and judging high school science fairs proves as rewarding for the mentors as the students. In conjunction with the American Red Cross and the Partnership for Food Safety Education, NSF International created the Scrub Club® (www.scrubclub.org), a fun website that teaches K-3rd grade children proper hand washing. The Centers for Disease Control and Prevention estimated that 164 million school days are lost each day due to illness. The site contains a webpage, interactive games, music and educational materials for teachers, parents, and kids. Materials are available for download in English, Spanish and French. NSF sponsored a Scrub Club® “Clean Hands” game for schools nationwide and toxicologists have visited local schools with stickers, magnets and tattoos. A growing number of local and regional school districts and public health departments also use this resource. NSF International’s Applied Research Center is also a proud supporter of this year’s SOT K-12 education outreach event for high school students and their mentors on Saturday March 22, 2014.
Ps 1665 A Model for a Residential High School Summer Research-Based Engagement Program to Inspire Students toward STEM Disciplines and Toxicology

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Persistence of undergraduate students in STEM disciplines is an issue of national concern. This issue is particularly relevant to the field of toxicology because the number of BS-level toxicology programs is limited and undergraduates matriculation to graduate level toxicology programs is from other STEM disciplines. In addition, the ability to inspire undergraduate students toward the field of toxicology at institutions that do not offer a BS level toxicology degrees is typically through upper level toxicology-based elective courses that students who leave the STEM may never have a chance to take. Reports show that persistence can be increased if students participate in “high-engagement” STEM-based activities in high school and early in college. USF has developed summer residential STEM Academies that provides high school students an intensive 6-day program in several STEM disciplines. The Academy engages domestic and international students in inquiry, discovery, creativity and hands-on research. Faculty from key research centers and colleges, collaborate to emerge students in basic, clinical and translational research. Students are also exposed to the multitude of professional career options that interface with the STEM degree. Professional educators from local high schools and colleges build community within the cohort through individualized and group mentoring. To date 64 students have completed three different Academies and 100% of the participants have indicated that they are inspired toward a STEM degree program. In addition 100% of rising seniors who completed the program in 2012, enrolled in STEM undergraduate programs in Fall 2013. The presentation will outline i) the structure of the program, ii) the details for the engagement exercises, iii) the cost to implement, iv) assessment instruments and v) a model for a STEM Academy with focus on Environmental Health and Toxicology to be offered in summer 2014.

Ps 1666 Creating Undergraduate Research Opportunities within Structured Courses through the CREATTE Initiative: Processes, Case Studies, and Outcomes

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Numerous reports show that early engagement of undergraduates in research activities improves academic performance, engagement in the discipline and the probability that the student will graduate within the discipline. Since there are national issues related to persistence in STEM disciplines, innovative methods to engage students in research activities are a focus on many curricular reform efforts. In addition, many large research universities face significant issues regarding the mentorship capacity to offer all students a research opportunity. To address these issues the Office for Undergraduate Research (OUR) developed the CREATTE program (Creating Research Experience and Activities Through Teaching Enhancement). CREATTE provides faculty teaching undergraduate courses modest funds to support a graduate student (2-3 hrs week) and sustainable supplies in support of research projects that are integrated into the course curriculum. During 2012-13, CREATTE program supported >700 students in research projects through this initiative. Courses in Public Health (Environ. Health Sci., Ecotoxicology), Integrative Biology (Epigenetics, Coastal Ecosystems), and Psychology (Statistics) all have relevance to students who may consider toxicology careers and graduate school. A post survey of participating CREATTE undergraduate researchers indicated a strong agreement that they developed enhanced research competencies, had a deeper understanding of the course material and would engage in additional research activities. The presentation will outline i) the structure of the program, ii) case studies of research activities related to toxicology, iii) faculty time commitment, iv) and the assessment instruments used to validate the learning outcomes.

Ps 1667 Escape from Toxic Island (Life Size!): A Toxicology Awareness Program Using Demonstrations and Hands-On Activities

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“Escape from Toxic Island (Life Size)”, is an educational program that was developed by the MASOT Education and Outreach Committee to teach basic toxicology concepts to middle school aged children. The program builds on the former program, “Escape from Toxic Island”, by utilizing the participants as life-sized game pieces that are introduced to basic toxicology concepts and activities from station to station to view or participate in toxicology related tasks. An introduction, called the island tribunal, was first provided for the participants. This included basic concepts such as the dose-response, common terminology and introduction to Paracelsus. A demonstration of dose response followed the introduction. Participants proceeded to toxicology-related demonstrations or hands-on activities. The stations included, Lemons and Onions, a demonstration that linked exposure to mildy irritating substances to risk, Science Sleuth where participants learned about reading labels to determine hazards and classifying chemical agents, No Water Off a Duck’s Back, that allowed participants to explore what happens to birds, humans and aquatic nature when oil spills into the sea, and Baggie Science, a station that investigated chemical changes. These resources were already available on SOT’s website and were modified to fit the needs of the present program. A Poison Control Squad was added as a demonstration to bring awareness of the dangers of medicines that might mimic the look of popular candy and also the dangers of household chemicals that look like commonly ingested liquids, for example, pine cleaner and apple juice. For each activity, participants engaged in active dialog with the presenters so that assessment of the participant’s prior knowledge of the topic could be made. Once the activities were completed, participants and presenters discussed their findings to verify the participants understanding of the topics.

Ps 1668 Creating Research Opportunities in K–12 Outreach for Undergraduate Students

A. J. Abratis and M. M. Bourgeois. EOH, USF COPH, Tampa, FL.

K12 outreach is a vital component in our mission to educate the community about scientific issues, particularly toxicology and risk assessment. Using funds from the Office for Undergraduate Research at the University of South Florida, undergraduate students participate in an authentic research experience within a structured course. Each autumn, 15 – 20 undergraduate students develop short science lectures and age appropriate activities for traditionally underserved schools in the Tampa Bay area. These student offer these demonstrations as part of the Annual Great American Teach In (GATI), held during the celebration of American Education Week. They identify a relevant environmental or occupational health topic, conduct research and create presentations designed to convey their findings in age appropriate lecture and hands on activities. The experience is designed to meet three core criteria: i) Pose or work from defined research question, ii) Work individually or in groups to apply relevant methods of inquiry to produce original findings/products, iii) Present the findings/products to peers and professionals in formal (e.g. Undergraduate Research Colloquium or professional meetings) and informal (e.g. course setting) venues. Past projects have involved an exploration of dose, response and toxicity using familiar measurements and household substances, sustainable gardens and pesticide safety, environmental pollutants and water sanitation. The GATI project fosters community outreach at the K–12 level, introducing the importance of toxicology to a range of primary and secondary level students while allowing project undergraduates to learn the art of outreach, develop skills in critical thinking, and gain a pedagogical background on which to build an academic future, should they so choose.

Ps 1669 Evaluation of Technical and Knowledge-Based Outcomes following Participation in a One-Week High School Research Program in Toxicology and Environmental Health Sciences

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As an applied science, students are often not exposed to the fields of toxicology and environmental health sciences until their undergraduate or graduate studies. The purpose of this summer program was to introduce rising sophomore, junior, and senior high school participants to the concepts of toxicology, provide hands-on laboratory training, and highlight various careers in toxicology and environmental sciences. Two groups of 23 students (n=46 total) participated in a non-residential, one-week summer program at Rutgers University that consisted of didactic (70%) and laboratory (30%) activities organized by toxicology graduate student and faculty instructors. Activities focused on dose-response relationships, experimental design, clinical toxicology, histology and pathology, epidemiology, and genetics. Pre- and post-student surveys aimed to assess student motivation to participate in the program, research and technical abilities, and basic toxicology knowledge. Students indicated that the greatest motivating factors to participate included an opportunity to explore their interest in science and gain hands-on laboratory experience. Self-assessment surveys revealed improved presentation skills (24%), labo-
1670 Using a Case Study Followed by a Lab Exercise to Examine Acetylcholinesterase Activity

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Allowing students to design and implement an experiment engages students far more than “cookbook labs”. However, it can be difficult to create toxicology lab activities that require the students to do this. Using a case involving the paralysis of several dogs, students were drawn into the case prior to asking them to design and implement an experiment. Students were given background information about the dog’s behavior and environment at which point they discussed possible causes of the paralysis, including demyelination and enzyme inhibition. They were presented with ambiguous data and ran lab tests to determine the cause of the paralysis in order that the dogs could be treated. Prior to running the tests students determined the appropriate controls, formulated a hypothesis and described the procedure for running the tests. Using a spectrophotometer and observing the intensity of fluorescence allowed the students to determine if the cause of the paralysis was acetylcholinesterase inhibition or demyelination. The instructor may ask students to present their research using an “integrated approach” to writing a research report.

1671 A Toxicological Study Using Zebrafish (Danio rerio) As a Model

M. Reynolds-Walsh. Washington College, Chesterton, MD.

Only recently has it been adequately recognized that substances present in the environment can have adverse effects on developing organisms. Now, with environmental pollutants accumulating at an unprecedented rate, and with pharmaceuticals dominating western medicine, it is particularly important that we understand the effects of the substances to which we are exposed. Zebrafish (Danio rerio) has become a widely used model system for the study of vertebrate development. This system is particularly amenable for use in undergraduate laboratories because of the ease of collection and manipulation and the rapid rate of development. In this lab, students use zebrafish to examine the effects of nicotine, ethanol, and retinoic acid on normal development. Students first examine normal development and compare it to overall growth, dry weight, and behavior of zebrafish exposed to these chemicals. The students also collect data on LC50 and noxious length. The quantitative data is evaluated for statistically significant differences between treatments. Finally, students write a research proposal for an independent experiment in which they expose embryos to a toxicant of their choice, carry out the experiment, and present their findings. This lab introduces students to the use of animal models and incorporates experimental design and data analysis. More importantly, it introduces students to concepts and models in toxicology to increase their awareness and interest.

1672 Incorporation of Evolution and Toxicology Enhances Conceptual Learning in Undergraduate Neuroscience Courses

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The field of neuroscience is inherently integrative and often requires understanding concepts from fields including molecular biology, toxicology, and evolution. Yet, few undergraduate courses focus on more than human neuroanatomy and physiology or rodent behavior. Therefore, we developed a transdisciplinary Advanced Neurobiology course from an evolutionary perspective, using toxicology principles to highlight gene-environment interactions and adaptations across major phyla. This lecture-lab course was also designed to align with the curricular objectives of the interdisciplinary Neuroscience minor. This presented the added challenge of creating an advanced course that serves students from different disciplines. Both lecture and lab highlighted evolutionary changes in the Animal Kingdom, beginning with sponges (Phylum Porifera) and moving through invertebrates (Phylum Cnidaria, Platyhelminthes, Nematoda, Annelida, Arthropoda, and Mollusca) before focusing on vertebrates in Phylum Chordata. We incorporated historically relevant models in toxicology and neuroscience including C. elegans, Drosophila, Aplysia, zebrafish, and mice. For example, using C. elegans, students assessed chemotaxis and dose response to benzaldehyde. Students examined sex differences in alcohol preference using Drosophila hydrelae. To accommodate different learning styles, we used various pedagogical tools to achieve each lab’s learning objectives. We heavily emphasized problem-based and team-based learning in the laboratory setting and used similar tools in lecture. We also used various methods of assessment, including group activities, lab notebooks, presentations, and student peer reviews. Toxicological concepts (dose response, ADME, and duration of exposure) were emphasized when students designed and executed group experiments on the behavior of C57BL/6j mice treated with nicotine and caffeine. Overall, our course succeeded in providing undergraduate students with a well-rounded perspective of complex concepts in neuroscience and toxicology.

1673 Sparking Toxicology Interest in High School Students

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SCOPE AND RATIONALE: “High school is where the pipeline begins.” –Serine S. Lau, PhD. The University of Arizona’s (UA) KEYS High School Summer Research Internship has successfully hosted 188 high school students in toxicology and biosciences laboratories, who received academic credit for their internships. DESCRIPTION: Highly achieving students, with interests in biosciences, bio-medical science, toxicology, environmental health and bioengineering, apply for this competitive 7-week program. Applicants submit a letter of interest, teacher recommendations, and transcripts. Accepted interns are interviewed to make compatible placements. In week 1, they develop hands-on laboratory research techniques, learn to read research papers, and hear about cutting-edge research. In weeks 2 through 7, they complete a research component for their lab. The program ends with a poster session, well attended by faculty members, graduate students, friends, and family members.

EVALUATION AND ASSESSMENT: Student work is assessed to gauge increased knowledge of research methodology, biotechnology skills, toxicology and environmental health. Assessors also measure self-confidence and communication skills. Faculty evaluations, intern formative evaluations, summative evaluations, and alumni surveys inform the development of KEYS. All 9 interns from the 2007 class have earned baccalaureate degrees and 8 are pursuing graduate, medical or pharmacy degrees. Interns have also been recognized in theses and dissertations.

CONCLUSIONS: High school students are capable of understanding and positively contributing to complex bioscience research such as toxicology. Real world science experiences, instruction, technical and science literacy, and supportive nurturing during and after the program are essential elements that promote student success. “It is an important program because it exposes the interns to all the aspects of the science community and career.” –Georg Wondrak, PhD.

1674 Implementation of an Academic Service Learning (AS-L) Project within an Undergraduate Course in Pharmacology

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Academic service learning (AS-L) is a type of active learning in which a student demonstrates knowledge and understanding through service to the community and reflection. The present report describes an activity in which AS-L was implemented as part of an undergraduate pharmacology course. The course is common to the curricula of the Doctor of Pharmacy, Physician Assistant and Toxicology programs at St. John’s University. In the AS-L project, teams of up to 5 students were charged to develop a presentation concerning the nature of drugs or pharmaceuticals and their effects on the human body. Students could form their presentation around discussion topics such as drugs versus natural substances, the medical benefits of drugs, the possible toxicities of drugs both legal and illegal, or the mechanisms by which drugs enter or leave our bodies. The student teams then traveled to various service sites throughout the greater university community with the goal of community outreach through education. Included among the service sites was an “after school/weekend” program for teenagers in foster care, and “weekend” program for young students in middle school (grades 6-8), and a local food pantry with an adult cohort. Strengths and limitations of the AS-L project were noted. The major strength of the project, as indicated from student reflection papers, was that each student in the team became an active learner and the otherwise “passive learning” environment of the classroom became an active one at the service site. All students in the team presented and answered questions. A limitation of the activity is finding a suitable instrument for assessing student learning. Future ASL courses of this type are anticipated to include pre and post surveys. The details of this AS-L project have been submitted for publication in the Journal of Toxicological Education (www.JToxEd.org).

SOT 2014 ANNUAL MEETING

436
In September 2011, a southwest section of Garfield, NJ was designated an EPA superfund site, following a 1983 chromic acid (hexavalent chromium [CrVI]) spill from a partially-buried storage tank at a local electroplating plant. CrVI-contaminated water (and a resulting dust) was first found seeping into a local firehouse basement in Garfield in 1993, followed by 13 residential properties in 2000, and more recently in wells south of the Cr plume area and under the Passaic River to the neighboring town of Passaic. As part of the Community Outreach and Engagement Core of the NYU NIEHS Center of Excellence, residents of Garfield have begun participating in a community health survey as a way to express their concerns within the community. The demographics of the survey respondents demonstrated that 55% were female, 79% were white, 29% Hispanic, and a majority had finished high school. Thirty-two percent of the respondents said they were ‘unsure’ if they were exposed to chemicals or pollutants where they live, which suggests a limited awareness of chromium exposure in their community. When asked about environmental issues in their community, 67% and 71% of respondents were concerned about air and water quality, respectively. The top three health issues of concern were cancer (64%), asthma (48.5%) and respiratory illness (47%). While residents are still being recruited to participate in the community survey, healthy, non-smoking residents 18-65 yr-old who lived in Garfield for > 2 yr are being recruited to donate toenail clippings to identify potential total Cr exposure in residents within the last 18 months. If elevated Cr levels are detected in any of the toenail samples of residents, they will be asked to come back and provide a blood sample to determine whether there has been any recent Cr exposure (within the last 2 months). This community-based participatory research will help gauge the needs of the community and possibly relieve the anxiety of the Garfield residents concerning Cr exposure (NYUNIENHS Ctr COEC ES017427).

North Birmingham, AL has recently been the subject of environmental concerns due to heightened levels of arsenic and benzo(a)pyrene found in the soil and air. In Jan. 2012, areas surrounding several major industries were designated a Superfund emergency response area. Working with community partners, environmental health priorities were established. As a starting point and to address education needs in the community, we implemented an environmental health education program at Hudson K-8 school for children ages 8-15 and their families. We developed an inquiry-based curriculum and evaluation constructs in collaboration with community members and teachers at Hudson Elementary. The curriculum included hands-on, age appropriate programs with a focus on the environmental issues North Birmingham residents currently face. The ultimate goal of the program was to increase knowledge of how contamination is measured, how risks to health are determined, and demonstrate ways to identify and assess environmental health issues in your neighborhood. The program culminated in a final interactive demonstration of results led by the children, which was presented at a community-wide event in North Birmingham. Knowledge gained and risk perception was evaluated through pre and post- tests. In addition, community members also took a risk perception survey regarding the environmental issues in their community. They reported concern of chemicals in the community, concern that key decision-makers lack a good plan for addressing the needs of the community, and a willingness to become actively involved in projects to improve the social or environmental quality of their community. Outcomes of this initial program have resulted in the development of a plan to continue and potentially expand Education to Action to other area schools.

It is difficult to assess pollution in remote areas of less developed regions due to limited availability of energy, equipment, technology, trained personnel, and other key resources. Passive sampling devices (PSDs) are technologically simple analytical tools that sequester and concentrate bioavailable organic contaminants from the environment. Scientists from Oregon State University and the Centre Regional de Recherches en Ecotoxicology et de Sécurité Environnementale (CERES) in Senegal developed a partnership to build capacity at CERES and to develop a pesticide monitoring project using PSDs. The partnership and dynamic process developed is applicable to equivalent capacity building programs. The project culminated in a held and laboratory study where paired PSD samples were simultaneously analyzed in African and US laboratories with quality control evaluation and traceability. The joint study included sampling from 63 sites across 6 western Africa countries, generating a 9,000 data point pesticide database with virtual access to all study participants.
affiliated with nor endorsed by the SOT, but fully supports the SOT educational mission and initiatives, and many of its editors serve or have served on educational committees of the SOT. *ToxEd* is actively seeking additional authors, reviewers, and editors.

### 1680 A Unique Collaboration Producing Highly-Trained Toxicologic Pathologists: The MPI Research/Michigan State University Toxicologic Pathology Training Program

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Well-trained toxicologic pathologists are a key component in the pharmaceutical, biomedical, chemical, and research toxicology fields, where interaction between toxicologists and pathologists familiar with toxicology is a vital aspect of compound and device development and research. Approximately 30% of entry-level pathologists are going into pharmaceutical, chemical, or contract research positions upon completion of their training programs. However, currently there are very few training programs that are oriented towards producing pathologists with extensive experience in laboratory animal pathology or toxicologic pathology. The MPI Research/Michigan State University Toxicologic Pathology training program was created in 2002 to address a shortage of these well-trained, board-certified toxicologic pathologists. This unique residency consists of two years of a traditional anatomic veterinary pathology residency program at Michigan State University, followed by 12-15 months of focused toxicologic pathology training at MPI Research, a contract research organization that provides preclinical and early clinical research services. The program has included 10 anatomic pathology residents to date; of which seven have completed the program (two are currently in the program). The MPI/MSU combined residency program has produced residents that are well-prepared for the veterinary pathology board exam (100% pass rate) and produced well-trained and qualified toxicologic pathologists (86% employed in toxicologic pathology). The toxicologic pathologist employment environment has shifted since the program’s inception, but a need for entry-level pathologists and researchers possessing focused knowledge and training in toxicologic pathology and the demands of the biopharmaceutical industry remains high. This program may serve as a model for developing other cooperative toxicologic pathology-focused training programs between industrial and academic interests.

### 1680a Pedagogy and Policy: Teaching Effectiveness, Efficiency, and Equity

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Multiple conceptual frameworks exist for addressing health policy. Integrating a “3E” approach into class work can introduce principles of effectiveness, efficiency, and equity; and cultivate future policy makers in toxicology and public health. A logic model provides an illustration of an underlying conceptual framework and the logical connection within and between systems. Students in a course entitled, “Program Planning and Evaluation,” constructed a logic model, using the example of policies addressing toxic exposures to the maternal-fetal environment. Incorporating tenets of public health and equity, the underlying assumption is that the “10 Essential Public Health Services” and “CDC Framework for Program Evaluation” provide the context for the “3E” strategies to decrease toxic exposures before, during, and after pregnancy. Inputs involve cooperation of government and non-governmental organizations, development of community-campus partnerships, integration of research data and health education materials, and consideration of the importance of training current and future professionals of toxicology and public health. Proposed activities are assessed for effectiveness, cost and personnel efficiency, and determinants of health equity and social justice. Short and long-term outcomes are considered in relation to feasibility, propriety, and accuracy, in order to assess and evaluate programs and policies. Program and policy development in class work empowers students through education and experiential learning. Using a logic model provides an instructor with a reusable learning tool, which students can apply to other public health and toxicology issues for policy development in coursework and for future professional development.

### 1681 Dioxin Exposure during Sexual Differentiation Causes Transgenerational Toxicity in Zebrafish

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Transient exposure of juvenile zebrafish to waterborne 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 50 pg/ml) during gonad differentiation at 3 and 7 weeks post fertilization, respectively, causes changes in sex ratios, decreases in reproductive capacity, and increases in skeletal abnormalities of F0 generation adults. The goal of the present study was to determine if the adverse effects of TCDD exposure on the F0 generation would persist in the next two generations of TCDD lineage offspring (F1 and F2). It was found that changes in sex ratios, skeletal abnormalities, and reproductive capacity continued to be observed in the TCDD lineage F1 and F2 generations. While the F1 generation may have been exposed to TCDD as germ cells, the F2 generation was never exposed to TCDD. In the TCDD lineage F1 and F2 generations, the sex ratios showed both a significantly higher percentage of females and greater incidence of skeletal abnormalities with a scoliosis-like kink in the spinal column being most common. Decreased reproductive capacity also was observed in TCDD lineage F1 and F2 generations with both egg release and percentage of eggs fertilized significantly decreased compared to controls. Spawning of the TCDD lineage F2 generation males (never exposed to TCDD) with control females revealed that decreased egg release and egg fertilization was caused by the TCDD lineage F2 males. Thus, toxicity observed in three generations (F0, F1 and F2) of TCDD lineage zebrafish as well as in a TCDD lineage F2 male generation outcross, suggests that epigenetic transgenerational inheritance of TCDD toxicity occurs through the male germline. (Supported by UW Sea Grant, NIEHS, and NCATS)

### 1682 Metabolic Analysis of Embryonic Zebrafish Indicates Hyaluronic Acid As A Biomarker of Arsenic Exposure

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Arsenic exposure has been associated with a myriad of human diseases including cardiovascular disease, cancers and neurological disorders; and developmental arsenic exposure may be relevant in the fetal onset of disease. The goal of this study was to use metabolomics to identify novel developmental targets of arsenic that may lead to adult onset diseases. To evaluate the impact of arsenic exposure in early development, dechorionated 5D zebrafish (Danio rerio) embryos were placed in control embryo media or embryo media containing 10 μM sodium arsenite at six hours post fertilization (hpf). Embryos were placed in the dark and allowed to develop uninterrupted in a 28 degree incubator. At 120 hpf, the embryos were prepared for metabolomics analysis. Briefly, 10 larvae were pooled in triplicate and metabolites were extracted using MeOH:RO water, 80:20, v/v, chilled to -80 degrees. Untargeted, unbiased metabolome profiles were developed using LC/MS/ MS technology. ANOVA was utilized to compare resulting mass spectrometry features in the control and arsenic exposed zebrafish embryos. Arsenic exposure resulted in significantly altered features (P<0.05, 2-fold change) in the positive and negative ion modes, as modeled by principal component analysis and volcano plots. Preliminary analysis indicates that hyaluronic acid (HA) as one of the most significantly increased metabolites (4.57-fold change, P=0.00094) resulting from arsenic exposure. HA is a glycosaminoglycan produced by hyaluronan synthase 2 and is involved in cardiac valve development and migration of myocardial cells. HA has been shown to be overexpressed in arsenic induced liver dysfunction, is used as a biomarker of bladder cancer. Additionally, the HA receptor, CD44, is overexpressed in ovarian cancer. HA may be a biomarker of embryonic arsenic exposure and may help to elucidate the role of arsenic in the fetal onset of chronic diseases.

### 1683 Multigenerational Effects of Benzo[a]pyrene Exposure on Survival and Developmental Deformities in Zebrafish Larvae

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Multigenerational impacts of polycyclic aromatic hydrocarbon (PAH) exposure are suggested in human cohort studies. To investigate mechanisms and developmental phenotypes associated with a dietary benzo[a]pyrene exposure, zebrafish were used. Only F0 adult zebrafish were fed 0, 10, 114, or 1012 μg BaP/g diet at a feed rate of 1% body weight twice/day (equivalent to 0, 0.21, 2.3 and 20 μg BaP/g fish) for 21 days. Three subsequent untreated generations of offspring (F1-F3) were assessed for mortality and time to hatch at 24, 32, 48, 56, 72, 80, and 96 hours post fertilization (hpf) and developmental deformities at 96 hpf. F1 mortality significantly increased in the higher BaP dose groups (2.3 and 20 μg BaP/g fish), but there
were no differences in the F2, F3, or F4 generations. Time to hatch in the higher doses significantly decreased in only the F1 and F4 generations. Multigenerational phenotypic impacts were caused in three generations (F1, F2, and F3) following the F0 exposure. Body morphology deformities (e.g. shape of body, tail and pectoral fins) were most extreme in the F1 generation although still present in the F2 generation. Craniofacial structures (e.g. length of brain regions and size of optic and otic vesicles), although not significantly affected in the F1 generation, emerged as significant deformities in the F2 generation and persisted through the F3 generation. Swim bladder impacts in the F1 generation corresponded to Bal2 treatment, but in the F3 generation fish in all treatments had affected swim bladders. Molecular analysis is now being used to elucidate mechanisms that are associated with the phenotypic deformities detected across generations. Supported by NEIHS R21ES019940.

1684 Benzo(a)pyrene Exposure Induces Global Methylation, Dampens Met Expression, and Induces Negative Behavioral Responses in Offspring Mice

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MET, an oleoic receptor tyrosine kinase implicated in multiple cellular processes during development and is an autism risk gene. This study tested the hypothesis that in utero exposure to B(a)P aerosol during the critical period of neurogenesis negatively modifies putaminal Met expression towards later-life behavioral phenotypes. On embryonic day 14-17, dams were exposed to B(a)P aerosol 100ug/m3 or carbon black only for 4 hours/day by nose only inhalation. Birth indices and growth curves did not reveal any effects from exposure in offspring pups. Epigenetic assays revealed upregulation of global methylation in exposed Cprlox/lox offspring relative to controls. Developmental expression profiling for Met demonstrated negative modulation in B(a)P-exposed Met+/+/Emx1cre and Cprlox/lox offspring, in vivo microdialysis, spatial discrimination and executive learning, paradigms were used. Met offspring that were exposed in utero demonstrated a robust decrease in prefrontal cortical glutamate concentrations as compared to control Cprlox/lox offspring. In separate behavioral paradigms, Cprlox/lox offspring that were exposed in utero 100mg/m3 exhibited significant impairments in 1) spatial discrimination or 2) contextual rule switching paradigms when required to respond in a context-appropriate manner. These data provide insights into the potential mechanisms of Met action.

1685 In Vivo Aryl Hydrocarbon Receptor-Mediated Developmental Toxicity Impairs the Adult Cardiovascular System

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Perturbations of developmental programs are key contributors to adult disease. Specifically, disruption of arylhydrocarbon receptor (AHR) homeostatic levels in vitro suggests that developmental exposure to the ubiquitous environmentally persistent organic pollutant (POP) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) culminates in abnormalities of cardiomyocyte structure and function. TCDD is a prototypical AHR agonist, and the AHR pathway has been suggested to coordinate a complex regulatory network underlying attainment and maintenance of cardiovascular homeostasis during development. Nevertheless, the pathogenesis and phenotypes of TCDD developmental toxicity remains vague. The present work aims at understanding how an abnormal AHR homeostatic level during embryogenesis affects the adult cardiovascular system. C57BL/6J pregnant mice were oral-gavaged with TCDD (0.1 to 50 μg/Kg dosages) or vehicle control at key cardiogenesis time points. Embryos were harvested at representative time points or carried to term and adulthood. Naïve C57BL/6J Ahr-/- mice were also included for each time point. At all studied doses, embryos could be carried to term; however, all pups died within 24 hours post-partum at the three highest doses. Cardiac alterations consisted of sexually-dimorphic changes in molecular, functional, and structural endpoints, including altered compartment-specific transcripts, cardiac morphometry, altered proliferation, as well as cardiac hypertrophy and failure protein markers, changes in baseline blood pressure, echocardiographic parameters, and cardiovascular chal- lenge tolerance. Our findings underscore the developing heart as a target for developmental disruption by in-utero exposure to a POP and the role of this receptor in cardiovascular development and disease. Supported by NIH Grant 5R01ES06273.

1686 The Effects of Endocrine Disruption on the Maturation of the Developing Human Fetal Prostate

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The etiology of prostate cancer is unknown, although it has been suggested that early life exposures to various toxicants, such as estrogenic chemicals, may play an important role. Previous studies in animal models have demonstrated that early life exposures to estrogens are responsible for epithelial and stromal hyperplasia, inflammation, and prostatic intraepithelial neoplasia (PIN) lesions. We have de- veloped a xenograft rodent model, using the renal subcapsular space as the implantation site, to characterize the growth and differentiation of human fetal prostate implants (gestational age 12-24 weeks) in athymic nude mice. This model evaluates the effects of both early and later-life exposures to either corn oil (control) or 250 μg/kg/body weight of 17β-estradiol 3-benzoate post-transplant. Key characteristic prostatic markers responsible for epithelial and stromal maturation were assessed in both control and treated xenografts. The prostate markers, p63, Ki-67, Chromogranin A, prostate specific antigen, caldesmon, smooth muscle alpha actin, estrogen receptor-α and estrogen receptor-β were stained in 7, 30, 90, and 200 day xenografts. The endogenous rodent prostates exhibited atypical hyperplasia along with the presence of massive cellular debris after estrogen exposure, while the human prostates displayed basal cell hyperplasia as indicated by p63 staining. Utilizing a PCR array, a select panel of 40 prostate specific genes was chosen for analysis. Results from the PCR arrays revealed changes in genes responsible for mesenchymal development and ducal morphogenesis, as well as changes in the prostate cancer pathway. This study of human fetal prostate tissue will allow for future mechanistic studies investigating the origins of neoplastic prostatic disease, and the effects of estrogenic endocrine disrupting chemicals.

1687 Application of a Novel Method for Identification of Murine Metastable Epialleles to Human Populations

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Metastable epialleles (MEs) represent genomic regions with highly variable, stochastically established epigenetic marks that respond to early life environmental exposures. We previously identified murine MEs using genome-wide expression array analysis of liver, kidney, and brain tissue of 10-month-old F1 offspring for candidates with a high R-values, or ratios of inter-individual: inter-tissue variances in expression. DNA methylation at these loci is established early in development, when MEs are most vulnerable to disruptive reprogramming. Two murine candi- dates MEs identified via this novel method, Dnaaj1 (R = 1.67) and Glic1 (R = 1.61), were validated in Aβ mouse liver tissue. Percent liver methylation was variable in a promoter CpG island in Dnaaj1 (N = 11, 0-19%) and at two ERV class II repeats in Glic1 (Repeat 1, N = 10, 92-100%; Repeat 2, N = 9, 79-100%). Mean liver DNA methylation at Glic1 Repeat 1 was altered following in utero exposure to 50 mg bisphephen A (BPA)/kg diet (N = 91), as compared to controls (N = 79) (p < 0.0001), indicating environmental lability. Here, we demonstrate applicability of this microarray method in identifying human MEs (N = 10 males, N = 10 females). Validation of the top two candidate MEs, DDX3Y (R-value = 14.1, N = 28) and NUMA1 (R-value = 10.6, N = 47), in 1st and 2nd trimester human fetal liver, revealed large inter-individual variability in DNA methylation. Percent liver methylation at seven CpG sites at the DDX3Y locus ranged from 10%-32%, with site-specific variances of 6 to 57. Percent liver methylation at five CpG sites at the NUMA1 locus ranged from 14%–43% with site-specific variances of 20 to 98. These data represent preliminary validation of a novel expression microarray method for ME identification in humans, which was previously validated in mice. Identification of human MEs is an important step in focusing studies of population level epigenetic responses to toxicant exposures.
Background: Maternal inflammation contributes to premature delivery and premature infants routinely receive therapeutic oxygen. The long-term pulmonary consequences of perinatal inflammation are unclear. We tested the hypothesis that a hostile perinatal environment induces chronic inflammation into adulthood causing increased oxidative stress and apoptosis. Furthermore, we hypothesize that these changes are modulated through activation on Notch-1 and Notch-2 signaling.

Methods. Pregnant C3H/HeN mice were injected with LPS or saline and placed on control or DHA supplemented diets on embryonic day 16. Once born, offspring were placed in room air (RA) or 85% O2 for 14 d then returned to RA for an additional 6 weeks. GSH and GSSG levels were measured in lung tissues using biochemical assays. 8-isoprostanates were measured by LC/MS/MS. Caspase 3, p53, and Bcl-2 were measured by western blots as markers of apoptosis. Notch-1 and -2 levels and downstream signaling cascade were assessed in lung tissues by western blot and immunohistochemistry.

Results: At 8 weeks of age, lower levels of GSSG and 8-isoprostanates were observed in the lungs of DHA supplemented mice. Decreases in caspase 3, p53 and Bcl-2 were observed in similar tissues. Total Notch-1 and -2 protein contents and nuclear localization were decreased in the lung tissues from DHA supplemented mice.

Conclusion: Perinatal inflammation causes long-term consequences which results in increases in oxidative stress and apoptosis into adulthood. These changes are likely to be modulated by alterations in Notch signaling. Maternal DHA supplementation prevented the long-term changes due to early inflammation and hyperoxia exposure indicating therapeutic potential for DHA for preterm infants.

1689 Gestational Nanomaterial Exposure: What Does the Future Hold?
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As the potential for engineered nanomaterials (ENM) develops, so does the risk of inevitable exposure within non-occupational settings, including expecting mothers and their unborn young. This fetal programming after maternal ENM exposure may have effects lasting well into adulthood. Pregnant (GD 10) Sprague-Dawley rats were exposed to nano-titanium dioxide aerosols (count mode aerodynamic diameter of 144±8 nm, 110±3 mg/m3, 4 hrs/day) for an average of 5.5±0.5 days to the adult consequences of gestational ENM exposure. The calculated cumulative maternal deposition over the exposure time was 85±3 µg. This study focused on the microvascular health of the heart and uterus (c<150 µm) of the surviving adult (8-10 weeks) female progeny using an isolated microvess preparation. Coronary microvascular responsiveness is impaired after gestational ENM exposure, represented by an impaired endothelium-dependent dilatation to mechanical (increased intraluminal flow, Flow, 5-30 µL/min), and metabolic stimuli (acetylcholine, ACh, 10-9-10-4 M), delayed smooth muscle relaxation (permeability-NONOate, 10-9-10-4 M), and a blunted endothelium-dependent adenosine response of the smooth muscle (phenylephrine, 10-9-10-4 M). Uterine arteriolar endothelial reactivity was also significantly impaired overall. This dysfunction presented as abolished endothelium-dependent reactivity (ACh), impaired flow induced dilatation (Flow), and reduced myogenic responsiveness (transmural pressure, 15-120 mm Hg). Mechanistically, we isolated and evaluated the mitochondria of the left ventricle, indicating a mitochondrial defect. This could have the propensity to increase oxidative stress production, thereby affecting NO bioavailability. Collectively, gestational ENM exposure may lead to independent cardiovascular consequences for the exposed progeny. These impairments may increase cardiovascular disease susceptibility for future generations.

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1690 Mono-2-Ethylhexyl Phthalate (MEHP) Exposure Modifies Embryonic Nutrition, One-Carbon Metabolism, and Epigenetic Programming during Organogenesis-Stage Mouse Conceptuses
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Mono-2-ethylhexyl phthalate (MEHP) is a ubiquitous environmental and industrial plasticizer. MEHP stimulates embryonic growth and delays closure of the neural tube (NTD). These defects can be prevented by supplementation of nutrients for one-carbon (C1) metabolism indicating a vital role for C1 activity in mechanisms of toxicity. These nutrients may alter DNA and histone methylation because they provide the methyl groups required for epigenetic modifications. Abnormal epigenetic programming during development has the potential to alter tissue patterning and disease susceptibility throughout the life course. This study sought to determine whether MEHP alters embryonic nutrition and epigenetic programming during organogenesis. Gestational day (GD) 8 mouse conceptuses were explanted into whole embryo culture and exposed to low (-100 µg/ml) and high-doses (250 µg/ml) of MEHP for 24 h. Uptake of FITC-albumin was used to assess histotrophic nutrition pathways (HNP), and MEHP significantly reduced HNP activity (p<0.01). Nutrient substrates of C1 pathways were measured using mass spectrometry. Vitamin B12 was reduced in low-dose samples (p<0.001) but remained unchanged at the high-dose. Choline and homocysteine also became reduced in the low-dose and unchanged in the high-dose. Betaine was reduced in both the low- and high-dose conceptuses (p<0.01). Folate also followed this trend but changes were not significant. Methionine, cysteine, and S-adenosylmethionine all increased with dose, but changes were not significant due to high intersample variability. Histone methylation at H3K4 and H3K27 loci was examined using a case-control design for MEHP treatment and NTDs. H3K4 was hypermethylated in embryos (EMB) that had NTDs (p<0.015). Treatment increased methylation at H3K27 in EMB (p<0.074). Overall, visceral yolk sacs were hypomethylated when compared to EMB (p<0.01). This work demonstrates the ability of MEHP to modify embryonic nutrition, C1 substrates, and epigenetics during organogenesis.

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Exposure to PM2.5 during pregnancy promotes reduced birthweight, and the associated adverse intrauterine conditions may also promote adult risk of cardiovascular disease. Here, we investigated the potential for in utero exposure to diesel exhaust (DE) air pollution, a major source of urban PM2.5, to promote adverse intrauterine conditions and influence adult susceptibility to disease. We exposed pregnant female C57Bl/6J mice to DE (≈300 μg/m3 PM2.5, 6 hrs/day, 5 days/week) from embryonic day (E) 0.5 to 17.5. At E17.5 embryos were collected for gravimetric and pathological analysis. In addition, some dams exposed to DE were allowed to give birth to pups and raise offspring in filtered air (FA) conditions. At 10-weeks of age, body weight and blood pressure were measured. At 12-weeks of age, cardiac function was assessed by echocardiography. Susceptibility to pressure-load-induced heart failure was then determined after transverse aortic constriction surgery. We found that in utero exposure to DE increases embryo resorption, and promotes placental hemorrhage, focal necrosis, compaction of labyrinth vascular spaces, inflammatory cell infiltration and oxidative stress. In addition, we observed that in utero DE exposure increased body weight, but counterintuitively reduced blood pressure without any changes in baseline cardiac function in adult male mice. Importantly, we observed these mice to have increased susceptibility to pressure-overload induced heart failure, suggesting this in utero exposure to DE ‘reprograms’ the heart to a heightened susceptibility to failure. These observations provide important data to suggest that developmental exposure to air pollution may strongly influence adult susceptibility to cardiovascular disease.

### Reproductive Parameters in a 90-Day Toxicity Study of Smart Herbal Purifier® - A Poly Herbal Supplement in Male Rats

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Cases of reproductive failure after prolonged intake of herbal supplements have been reported in Nigeria. This study evaluates the testicular effects of Smart Herbal Purifier - a poly herbal supplement SHP in sexually albino rats. Subchronic toxicity of SHP was investigated in a 90 day repeated dose toxicity test in male Sprague-Dawley rats. The rats were divided into 30 per group received 0, 48, 240, and 480 mg/kg/day of SHP. Animals were sacrificed at 30, 60, and 90 days. Body weight and male reproductive parameters, weight of testes, testicular and epididymal sperm count, motility, morphology, debris and viability were recorded. Testosterone, leutinizing hormone, prolactin, follicular stimulation hormone and malondealdehyde in the plasma and seminal vesicles were analysed. Histology of the testes was examined. A Mann–Whitney U-test was used in data analysis. Testicular sperm motility significantly decreased from 71.87±12.98% (control group) to 27.13±6.64% (480 mg/kg SHP) treated group. There was significant % increase in both testicular (19.10±3.78 in control group to 57.50±6.89 in 480 mg/kg SHP) and epididymal (14±0.00 in control group to 50.0±1.53 in 480 mg/kg SHP) sperm debris. SHP significantly (p<0.05) increased the FSH from 0.82±0.45 to 1.24±0.93 (I/L) in the control and the 240mg/kg SHP treated groups respectively. Malondialdehyde was significantly (p<0.05) increased from 0.98±0.13 to 1.20±0.26 in the control and 480mg/kg SHP treated groups respectively. Histology of the testes of the treated group showed necrosis of the seminiferous tubules. SHP might be associated with some male reproductive toxicity concerns.
In occupational settings there is a concern that high levels of exposure to manganese may occur and that this, in combination with continued low level exposure over long periods of time, may lead to some adverse health effects. Contradictory findings exist regarding reproductive performance following such exposures. The aim of this study was to investigate if manganese is a reprotoxicant. Manganese chloride was selected as the most suitable form of manganese to assess reprotoxicity. Four groups of Sprague-Dawley rats, 20 per group (10 males and 10 females) were exposed to MnCl$_2$ via the inhalation exposure route (nose only) for 9 weeks at target concentrations of 0 (control air only), 5, 10 and 30 mg/L of Mn.$^2+$. A slight intergroup difference in body weight gains and food consumption was seen in the low and mid doses but this was not positively attributed to MnCl$_2$ exposure. At the high dose level (30 mg/L) adverse clinical signs, including irregular respiration, were observed in 1 male and 2 females resulting in the premature sacrifice of these animals, and the cessation of exposure to the remaining animals in this group after approximately 3 weeks of treatment (early gestation). At the end of the study, it was concluded that, under the conditions of this study, MnCl$_2$ exposure at target levels of 5 and 10 mg/L did not have an effect on mating performance; fertility or the duration of gestation and hence manganese was not considered to be a reprotoxicant at these exposure levels.

1696 N-Acetylcysteine, but Not the Suspension of Treatment, Reverses the Effects of Arsenic Trioxide on the Mouse Male Reproductive System


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Arsenic trioxide (AT) has been shown effective in the treatment of acute leukemia. Due to its apoptosis-inducing activity, it can affect germ cells. The aim of this study was to evaluate the immediate effects of treatment with AT on reproductive system of Swiss mice, after 30 days of suspension of the treatment and with the co-administration of the antioxidant N-acetylcysteine (NAC). For this, adult male Swiss mice were treated with distilled water (vehicle), 0.3 or 3.0 mg/kg/day of AT (ACROS 1327-53-3), subcutaneously in 25 applications. At the end of treatment half of animals were killed to evaluate the effects on sperm parameters and the other half were kept without the drug for 50 days (spermatogenesis + sperm transit time) and then killed for assessment of possible reversibility of the effects. In another experiment, animals were treated with vehicle, 3.0 mg/kg/day of AT, NAC and NAC + AT. The following material was obtained/determined at necropsy: viril and reproductive organs weights, sperm from the vas deferens for motility analysis and tests for determination of daily sperm production. The differences among groups were compared using ANOVA and Kruskal-Wallis, followed by Dunnnett or Dunn’s tests, respectively, with p<0.05. The animals treated with the higher dose of AT and killed right after the end of treatment showed reduction in seminal gland weight, number of motile sperm and daily sperm production, which remained altered after the suspension of treatment. However, when NAC was given in conjunction with AT these parameters were similar to the control group. The results show that AT was toxic for the mouse male, compromising sperm quality and quantity, and that these effects were persistent even after the suspension of the treatment. Furthermore, the administration of an antioxidant prevented the harmful effects of the drug on the male reproductive system.

1697 Assessment of the Reproductive Toxicity of Inhalation Exposure to Ethyl Tertiary Butyl Ether in Male Mice with Normal, Low-Active, and Inactive ALDH2

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Ethyl Tertiary Butyl Ether (ETBE) has been used in recent years as a biofuel additive. Previous reports have shown that ETBE is low toxicity with the NOAEL at 500 ppm. However, ETBE is metabolized to acetaldehyde and other aldehydes, which are known with high toxicity. Aldehyde dehydrogenase2 (ALDH2) is the main enzyme to metabolize aldehydes, but approximately 40% East Asians are deficient in the enzyme activity. So far no data are available regarding ALDH2 polymorphisms related to the reproductive toxicity of ETBE. In this study, a series of experiments were performed in Aldh2 knockout (KO), heterogeneous (HT) and wild type (WT) C57BL/6 male mice exposed to ETBE and the data about general toxicity, testicular histopathology, sperm head numbers, sperm motility and sperm DNA damage were collected. The results showed that 13-week exposure to 500, 1750 and 5000 ppm ETBE significantly decreased sperm motility and increased levels of oxidized DNA strand breaks and 8-hydroxy-deoxyguanosin in both WT and KO mice; the effects were found in 1750 and 5000 ppm groups of WT mice but in all three exposed groups of KO mice. In the 9-week experiment of exposure to lower concentrations of ETBE (50, 200 and 500 ppm), the remarkable effects of ETBE on sperm head numbers, sperm motility and sperm DNA damage were found in mice in KO and HT mice exposed to 200 ppm ETBE, but not in WT mice. Our findings suggested that only exposure to high concentrations of ETBE might result in reproductive damage in mice with normal active ALDH2, while low active and inactive ALDH2 enzyme can significantly enhance ETBE-induced reproductive damage even at low concentrations of ETBE, mainly due to the accumulation of acetaldehyde as a primary metabolite of ETBE.
significantly decreased during gestation in cynomolgous monkeys. Since lymphopoiesis of B-cells has been reported to be negatively affected by steroids, the decreased B-lymphocytes during pregnancy may be due to increased estradiol levels. Our data support the observation that there are species-specific differences in background levels of selected parameters during pregnancy. Some of the background changes in pregnant cynomolgus monkeys can be potentially similar to the metabolism of the drug being tested and/or different than observed in pregnant women, therefore posing unique challenges for interpreting DART data.

1700 Telmisartan Attenuates the Somatic and Germ Cells Damage in Streptozotocin-Induced Diabetic Rat: Cellular and Molecular Mechanisms

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Diabetes is a metabolic disorder that affects nearly every organ system. Telmisartan, an angiotensin type 1 receptor blocker, used in diabetes associated hypertension. Besides controlling blood pressure, telmisartan exhibits several other beneficial effects and provides new avenues in end-organ protection. Present study explored the possible protective mechanisms of telmisartan, in kidney and testes of diabetic rat. Male Sprague Dawley rats randomized into six groups: control, telmisartan control, diabetic control and diabetic group treated with telmisartan at the doses of 3, 6 and 12 mg/kg /day, per oral for 4 weeks. Animals were made diabetic by single dose of streptozotocin, i.p. (55 mg/kg). Results showed that telmisartan significantly protects kidney as observed from decreased DNA damage, cell death and reduced expression of nuclear factor kappa B (NF-KB), cyclooxygenase (COX-2) and connective tissue growth factor (CTGF). Telmisartan also attenuates testicular toxicity as observed by restored sperm counts, decreased sperm DNA damage and apoptotic cell death. Immunohistochemistry of 8-oxo-dG (8-oxo-7,8-dihydro-2′-deoxyguanosine) and 3-nitrotyrosine confirmed that telmisartan significantly reduced oxidative and nitrosative damage. Western blot analysis showed that telmisartan decreased the levels of NF-KB, interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), phosphorylated extracellular signal-regulated kinase (p-ERK1/2), inducible nitric oxide synthase (iNOS), caspase-3 and increased peroxisome proliferator-activated receptor gamma (PPAR-γ) expression. Present results showed that telmisartan restored the status of the antioxidant enzymes, oxidative and nitrosative stress, reduced inflammation and cell death in kidney and testis of diabetic rat. Further, research is needed to validate the usefulness of angiotensin receptor blocker, telmisartan in diabetes associated end organ damage.

1701 Expression and Localization of Xenobiotic Transporters in the Male Reproductive System

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The blood-testis barrier (BTB) prevents the entry of many compounds into the male genital tract (MGT) which is beneficial for the exclusion of reproductive toxicants. However, the BTB also prevents the exclusion of toxicants, and the BTB can be disrupted during treatment with certain drugs or environmental compounds. Exposure to these compounds can have adverse effects on male reproductive health. The aim of this study was to determine subcellular localization of MRP transporters in rodent, macaque, and human testes and to determine mRNA expression of 30 xenobiotic transporters in different sections of the MGT isolated from rats. Using immunohistochemistry, we determined that in all species, MRPL is restricted to the basolateral membrane of Sertoli cells. MRPL is located in Leydig cells, and MRPC is located in round spermatids. MRPC is expressed on the basolateral membrane of Sertoli cells in human and macaques, but on the apical membrane in rats. By using branched DNA signal amplification, we determined that the epididymis expressed at least moderate levels of 19 transporters, while the vas deferens expressed 15, seminal vesicles 23, and prostate 19. Knowledge of which transporters are expressed in each tissue may provide insight into how xenobiotics are able to bypass this distinct compartment thereby increasing efficacy of medications needing access to the MGT for full therapeutic effect.

1702 Phtalate Metabolism and Kinetics in an In Vitro Model of Testes Development

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We developed an in vitro model of testes development (3D-TCS) using rat testicular cells overlaid with extracellular matrix, allowing for three dimensional growth and a physiologically stable culture system. One barrier preventing utilization of in vitro models in toxicity testing is the lack of metabolic systems which are present in vivo but not in vitro. Another challenge in interpretation of results from in vitro models is the lack of kinetic data for many compounds in in vitro systems. In the current study, we sought to perform a preliminary characterization of the inherent metabolic capabilities of the 3D-TCS as well as investigate the kinetic behavior of some common male reproductive toxicants (phthalates). 3D-TCS cells were cultured with three phthalate diesters (DEP, DBP and DEHP). Concentrations of phthalate parent compounds and monoester metabolites were then measured at 2, 8 and 24 hours post exposure in culture media and cell lysis via mass spectrometry. We used detected levels of monoester metabolites as an indication of phase I metabolism of phthalates diesters to monoesters via non-specific lipase activity. Monoester metabolites were detected in all phthalate treated cell media and cell lysate samples. Metabolite levels ranged from <0.01-16% of initial concentration of parent compound. Phthalate diesters partitioned between media and lystate in a manner dependent on each compound’s logKow value. In addition, analysis of 3D-TCS microarray data indicated gene expression of several lipases as well as CYP450's involved in steroid or lipid metabolism. These results provide evidence for the presence of significant CYP450's, the metabolism of phthalate diesters in our testicular co-cultures and also provide important information about the kinetic behavior of phthalates in the 3D-TCS.

1703 The Role of H19 Hypomethylation in Male Reproductive Toxicity Induced by Endocrine Disruptors p,p′-DDE

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p,p′-Dichlorodiphenyldichloroethene (p,p′-DDE), the major metabolite of dichlorodiphenylchloroethane (DDT), is a known environmental pollutant and male reproductive toxicant. However, the exact mechanism remains limited. Objective In the present study, we try to investigate the role of methylation of imprinted gene H19 in male reproductive toxicity induced by p,p′-DDE. Methods Timed-pregnant Sprague-Dawley rats were obtained from experimental animal center in Zhejiang Province. Young adult female rats were cohabited with male rats overnight, and onset of pregnancy was determined by the presence of a copulation plug after overnight mating (gestation day (GD) 0). From GD8 to GD15, the pregnant rats were dosed daily by gavage with corn oil as vehicle control or 100 mg p,p′-DDE per kilogram of body weight. There were 6 dams in each group. The pups of each group were randomly reduced to 10 at birth to ensure uniformity, and pups were kept with their respective dams until weaning on postnatal day (PND) 21, at which point litter mates of the same sex were housed up to 4 per cage till PND90. Results We found that transient exposure (daily gavage of 100 mg/kg body weight) of a gestating female rat to p,p′-DDE from gestation day 8 to 15 caused reduced sperm quality associated with IgJ2-H19 ICR hypomethylation in the adult male F1 generation. Conclusion Reprogramming of imprinted gene may play critical roles in male reproductive toxicity induced by p,p′-DDE.

1704 Developing a Screen for Analysis of Environmental Epigenetic Effects

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Many factors challenge the efficiency of assessing chemicals found in the environment. These include the vast number of compounds and toxicity endpoints to be tested as well as the vital need to replace rodents in order to quickly, economically and ethically assess chemical safety. Another important issue at hand is the long lasting effect, potentially spanning several generations, of chemicals in our environment and the lack of tools to comprehensively identify these. In order to address these issues, we propose the development of new technologies that can accurately determine the safety of chemicals for endpoints that are hard to study in vertebrate models. Here, we establish the use of the genetic model system, the worm Caenorhabditis elegans, as a relevant model for epigenetic and reproductive toxicity assessment. We are taking advantage of C. elegans genetic tools to detect chemical disruption of the germline chromatin and in particular of evolutionary conserved epigenetic
marks. To this aim, we are making use of a worm strain where GFP is specifically epigenetically silenced in the germline. In order to validate the approach, the strain is exposed to inhibitors of histone modifications that have been well characterized in mammalian systems. These inhibitors should disrupt proper establishment and/or maintenance of epigenetic marks, and lead to desilencing of the transgene in the germline. Here, we report that exposure to the histone deacetylase inhibitor valproic acid causes a de-repression of the GFP transgene expression in germ cells indicating that continuous histone deacetylation is required for the epigenetic repression of the transgene in the germline. To our knowledge, this had not previously been shown in C. elegans or other species. These preliminary results offer us a reliable control to further test against environmental chemicals. Further work will examine the sum of pathways and enzymatic activities that lead to proper regulation of chromatin states in the germline. Together, we are designing a strategy that should offer a more comprehensive examination of the environmental effects of the epigenome.

1707 Dynamic Expression of Imprinted Genes and Epigenetic Factors in an In Vitro Testicular Coculture

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Male reproductive developmental processes are an important target of environmental contaminants. However, there are no validated in vitro models for screening the chemicals that remain untested for male reproductive toxicity. Perturbation of epigenetic factors during tests development is of particular concern given the importance of epigenetic regulation for spermatogenesis and the potential for epigenetic programming of germ cells to influence disease. Our lab has previously developed a 3D testis co-culture isolated from postnatal day 5 rats that corresponds to a key phase of male reproductive development in vivo. This in vitro culture can accurately distinguish between reproducitively toxic and nontoxic phthalate esters, making it a promising model for toxicity testing. To characterize the developmental pathway dynamics and epigenetic transitions captured in our in vitro model, we evaluated global gene expression through time in culture. Primary rat testicular cells were co-cultured with a Matrigel overlay. Gene expression was measured using Affymetrix 1.0 ST arrays. Genes significantly changed over time were identified by univariate t-test (FDR < 0.1). Over the course of 72 hours in culture (corresponding to postnatal days 7–10 in vivo) expression of genes involved in spermatogenesis such as AR, Dazl, Dibh, Storb, and Ace was significantly increased. A pathway analysis of gene expression in this 72 hour period reveals significant changes in GO terms related to DNA methylation and epigenetic regulation. This suggests dynamic epigenetic modulation of gene expression through time. In parallel we see significant changes in expression of a panel of genes known or predicted to be epimarked paternally (e.g. Cpx4, H19, Tmem88) or maternally (e.g. Spon2, Sox8, Fertm2). This baseline characterization indicates that our model provides a strong platform for evaluating the potential effects of environmental factors on imprinting and the epigenetic regulators of developmental processes. (FDA 1U01FD004242-01, and NIEHS T32 ES007032-35)

1706 Epigenetic Mechanisms of Cadmium-Induced Placental Insufficiency


Cadmium is an environmental toxicant primarily released from trash incineration and burning fossil fuels. Smoking is the most significant source of human cadmium exposure, followed by diet, air, and drinking water. Cadmium exposure during pregnancy has been identified as a cause of placental insufficiency (PI) and intrauterine growth restriction, but the mechanism is not fully understood. Cadmium can disrupt normal cell function by epigenetic mechanisms, including global DNA and promoter methylation changes and altered microRNA (miR) expression. Although observed in other tissues, such epigenetic effects have not been studied in the placenta. We hypothesized that cadmium induces PI by epigenetic mechanisms. Female C57BL/6 mice (~100 day old) were administered 20 ppm cadmium chloride in drinking water ad libitum 2 weeks before mating and throughout pregnancy. On gestational day 15.5, umbilical artery (UA) blood velocity was assessed via the Doppler pulse method, and then dams were euthanized. Fetal and placental tissues were weighed and evaluated by histopathology, TUNEL staining, Western blot, global DNA methylation analysis, and miR was quantified. Compared to controls, cadmium-exposed dams had significantly lower UA systolic velocity, increased placental cell death, smaller litter size, and increased fetal resorption rate. Cadmium-exposed placental had altered expression of angiogenesis-related proteins, including increased VEGF receptor 2, and decreased eNOS and heat shock protein 90. Maternal hearts showed global hypomethylation with cadmium exposure. Placentas had significantly decreased expression of angiogenesis-related miR-126-5P. Cadmium exposure induced PI in this model. Cadmium altered the expression of angiogenesis related proteins and miRs in the placenta, and induced global hypomethylation in maternal hearts, providing evidence that cadmium induces PI via epigenetic mechanisms. Further investigations will seek to identify specific mechanisms.

1705 Epigenetic Transgenerational Actions of Dibutyl Phthalate and Male Fertility


Increased occurrence of reproductive disorders has raised concerns regarding the impact of endocrine-disrupting chemicals on reproductive health, especially when such exposure occurs during fetal life. A novel mechanism of posttranscriptional control mediated by microRNAs (miRNAs) has lately emerged as an important regulator of spermatogenesis. This study sought to investigate the epigenetic transgenerational effect of endocrine disrupting chemical dibutyl phthalate (DBP) on male fertility. Gestation female rats were dosed with corn oil or DBP (5, 50, or 500 mg/kg/day, per os) during the period of gonadal sex determination (between embryonic days 8 and 15). We determined the expression levels of 760 mature miRNAs in F3 rat testes with or without prenatal DBP exposure using a miRNA microarray technique, verified by quantitative real-time PCR from F1 to F4. Dose-related effects on male offspring included reduced sperm counts, sperm motility, and testicular and epididymal malformations. Of the screened miRNAs, 7 miRNAs were up-regulated and 152 miRNAs down-regulated (fold change > 2) in 5 mg/kg/day DBP treated group when compared with controls. There were 6 miRNAs up-regulated and 212 down-regulated in 500 mg/kg/day DBP treated group. The target genes of 15 aberrantly expressed miRNAs (fold change > 8) were involved in P3R-Akt signaling pathway, Axon guidance, Erbb signaling pathway and Focal adhesion pathways. We found permanent alterations in expression levels of multiple miRNAs suggesting an epigenetic mechanism of action. We propose that the deleterious effects of DBP on the male reproductive system are mediated by aberrantly miRNA expression in the testes. Overall, these results suggest that DBP can alter miRNA expression in F3 testes, a potentially novel mode of DBP transgenerational effect.

1708 Age-Dependent Macrophage Infiltration into the Testis of Rats and Mice after Mono-(2-Ethylhexyl) Phthalate (MEHP) Exposure

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The testis is an immune privileged organ, however infectious and non-infectious inflammation of the testis has been shown to cause infertility. Phthalates are chemical plasticizers that are known Sertoli cell toxicants. Here we characterize the infiltration of macrophages into the testis of immature (21d, 28d, and 35d) and mature aged Fisher rats (56d) at 12, 24, and 48 hours after a single dose (1 g/kg) of mono-(2-ethylhexyl) phthalate (MEHP). The testis of rats at 21 and 28d had a peak increase in the number of CD11b+ immune reactive cells (neutrophils, macrophages, and dendritic cells) at 12h after MEHP treatment as quantified by flow cytometry. In 28d rats, CD11b+ cells remained significantly elevated at 48h while, in 21d rats it returned to control levels by 24h. The peak number of CD11b+ cells in 35d rats was delayed until 24h, remaining significantly elevated at 48h. In mature rats there was no increase in CD11b+ cells demonstrating differential responses of the testicular immune system at different developmental ages. Immuno histochemistry confirmed the increase of newly arrived macrophages (CD68+ cells). In all immature rats, monocyte chemoattractant protein-1 ( MCP-1), the primary chemokine produced in the testis, was significantly increased at 12h after MEHP treatment. This was followed by peak germ cell apoptosis (85-95% of seminiferous tubules contained ≥4 TUNEL positive germ cells) at 24h post-treatment in immature rats. The timing of peak germ cell apoptosis was similar in mature rats, however they displayed a significantly lower incidence of MEHP-induced germ cell apoptosis (8% of seminiferous tubules contained ≥4 TUNEL positive cells). Taken together, these results suggest that MEHP-induced an increase in MCP-1, triggering migration of macrophages into the immature rat testis prior to the initiation of germ cell apoptosis. Thus, signifying a role of macrophages in exacerbating MEHP-induced germ cell apoptosis in an age-dependent manner.
DNA damage from x-radiation can lead to apoptosis through p53 signaling. In the mouse testis, germ cell death resulting from x-radiation is dependent on p53 signaling. We hypothesized that p53 was also essential for germ cell apoptosis in response to x-radiation in the rat testis. We exposed wild-type Sprague-Dawley and novel p53 knockout rats (SD- Tp53 tm1sage) (age 60 d), to 0, 0.5, or 5 Gy x-ray. Rats were euthanized at 3 h, 12 h, and 42 d after exposure. Body, testis, and epididymis weights were recorded. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, a measure of apoptosis, was performed in paraffin-embedded testes. Real-time RT-PCR for the death receptor gene Fas was performed. 13 d BrdU labeling was performed to characterize the spermatogonial stem cell population in unexposed knockout rats, and whole-mounted tubules were labeled for GFP-α, a marker of type A spermatogonia. X-radiation resulted in a significant increase in TUNEL labeling after 12 h in wild-type rats. However, frequent TUNEL positive cells, most often spermatocytes, were observed in p53-/- rats in all treatment groups, p53-/- rats displayed frequent, but variable, atrophy of seminiferous tubules and lower average testis weight. While Fas mRNA levels followed a similar trend to TUNEL frequencies, they did not differ significantly by treatment. GFP-α staining showed similar populations of A spermatogonia in wild-type and p53-/- rats, and BrdU labeling in p53-/- testes gave an average 37% germ stem cell labeling index, indicating normal proliferative activity. Therefore, we conclude that the frequent TUNEL signal in p53-/- rats likely results from unresolved homologous recombination in spermatocytes during meiosis. Our data indicate that p53 plays a critical role in maintenance of spermatogenesis in the rat. While p53 is known to participate in resolution of DNA double-strand breaks in meiotic recombination, this represents a significant difference from data previously obtained in the mouse.

Testicular effects of chemical mixtures may differ from those of the individual chemical constituents. This study assesses the co-exposure effects of the model germ cell- and Sertoli cell-specific toxicants, X-radiation (x-ray) and 2,5-hexanediol (HD), respectively. X-ray induces germ cell apoptosis and in high dose studies, HD has been shown to attenuate the x-ray induced germ cell apoptosis. Here, adult rats were exposed to different levels of x-ray (0.5Gy, 1Gy, 2Gy) or HD in the drinking water for 18 days (0.33%), alone or in combination. To assess cell type-specific attenuation of HD effects with x-ray co-exposure, we used laser capture microdissection (LCM) to examine a panel of apoptosis-related transcripts using PCR arrays. The apoptosis PCR arrays identified significant dose-dependent treatment effects on several genes (Table 1). Stathelin, Casp7, NAALAD, Sirt3, Casp3, and upregulation of Fas. Both Casp7 and DR5 are key pro-apoptotic factors in the apoptosis pathway, and had the strongest down-regulation following 0.33% + 0.5Gy exposure, with maximum fold decreases of -6.37 and -4.17, respectively. The significant and simultaneous downregulation of pro- and anti-apoptotic transcripts is exacerbated, rather than ameliorated, by the addition of HD to the low dose of x-ray. These results indicate that the attenuation effect seen with the high dose co-exposure does not persist into the low dose range. However, the downregulation of anti- and pro-apoptotic transcripts as a result of 0.5Gy x-ray exposure may be an adaptive mechanism employed by the injured spermatogonia to evade apoptosis. These studies provide insight into cell-specific toxicant co-exposures and illustrate that low dose and high dose responses can differ, and that the low dose response in not necessarily an extrapolation of the effects seen at high doses.

Surface Charged Iron Oxide Nanoparticles May Influence Reproductive and Developmental Toxicity in CD-1 Mice

Iron oxide nanoparticles (NPs) are currently being investigated for many uses including biomedical imaging, soil/groundwater remediation, and drug delivery. The risk to pregnant women and the developing fetus is high, as the NPs have the potential to cross the placenta due their small size. Our lab has previously shown that pregnant CD-1 mice given a low (10 mg/kg body mass) intraperitoneal dose of NPs coated with either poly acrylic acid (PAA, negatively-charged) or polyethyleneimine (PEI, positively-charged) for 8 consecutive days had significantly decreased maternal weight gain, increased fetal resorptions, and increased fetal liver iron concentrations compared to controls. These results raise concern about NP reproductive toxicity and how NP exposure might affect the developing fetus. In the current study, female CD-1 mice were given a low, medium, or high dose (10, 50, or 100 mg/kg body mass) of NPs during organogenesis (gestation day (GD) 8, 9, or 10) in order to observe if there is a dose related effect on reproduction and to examine if fetal bioaccumulation of NPs will result in decreased fecundity or malformations in the progeny. At low dosages, no adverse effects were observed. At the highest dosage, increased resorptions were observed for the PAA NPs given on GD 8 and the PEI NPs given on GD 10. The testes and uteri of the offspring were harvested and histologically examined for abnormalities. Increased uterine and decreased seminiferous tubule epithelial linings were observed in offspring of dams given the highest dosage of NPs; no changes in fecundity were observed in any group. While no negative reproductive effects were observed at the lower dosages, exposure to a high dosage of surface charged iron oxide NPs during pregnancy may result in increased fetal death and reproductive organ abnormalities in the offspring.

Environmental exposures to endocrine disruptors (EDs) are thought to contribute to idiopathic increases in male reproductive abnormalities. Although humans are exposed to a myriad of EDs from conception through adulthood, few studies have evaluated the effects of combined exposures, while animal studies often used doses exceeding environmental levels. We hypothesized that prenatal exposure to combined low dose of the phytosterogen genistein and the plasticizer di-(2-ethylhexyl) phthalate (DEHP) might pose a greater risk to male reproduction than individual compounds. Pregnant Sprague Dawley dams were gavaged from gestational day 14 to birth with either corn oil, genistein, DEHP or their mixture at 10 mg/kg/day, a dose at which either compound does not cause conspicuous long term effects. Adult serum testosterone levels were unchanged. However, histological analysis revealed increased interstitial fibrosis in testes of ED-exposed adult offspring, suggestive of premature aging. Quantification of elongated spermatids and quantitative PCR analysis (qPCR) of germ cell markers indicated adult germ cell alterations in ED-exposed rats.

Gene expression arrays identified 83 up-regulated and 171 down-regulated genes (p<0.05) in testicular RNA from ED-treated relative to control rats, including genes with explicit ties to male reproduction, such as FOXA3, PDGFRα and HSD3B1, suggesting disrupted testis development and function. These changes were further validated by qPCR. These results show the BFR maternal exposure to a mixture of unrelated EDs at environmentally relevant doses permanently disrupt testicular gene expression profiles and histology in a manner different from individual compounds, highlighting a need for more realistic and comprehensive analysis of endocrine disrupting chemicals.
eye opening). Onset of puberty was accelerated in females (20 mg/kg); furthermore, males exhibited delayed onset of puberty (60 mg/kg). Body weight or food consumption has not differed among treatment groups to date. However, both male and female offspring had increased liver weights at PND 21 (20 and 60 mg/kg). Moreover, male fat pad weights were significantly decreased at PND 46 (20 mg/kg). PBDE exposure did not affect fasting glucose or insulin levels, and there was no evidence of impaired glucose tolerance (AUCglucose) or insulin resistance (AUCinsulin). In conclusion, perinatal BFR exposure alters reproductive development as indicated by accelerated onset of puberty in female offspring and delayed onset in males. Decreased fat pad weights in males at PND 46 indicate that BFR exposure may have metabolic effects.

1713a Reproductive and Developmental Toxicology Assessment of Oral Dietary Calcium Formate in Yucatan Miniature Swine


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Rational: Calcium Formate (CaF) is being considered as a dietary calcium supplement. Objective: Assess the reproductive/developmental toxicity of CaF using the miniature pig model when administered orally via food consumption. Experimental Procedure: Sixty (30 male and 30 female) sexually mature Yucatan miniature swine were gender paired for breeding and randomized into 3 ggs of 10 pairs receiving untreated control, low dose (2.25% CaF), and high dose (4.5% CaF) in the daily diet. Standard reproductive and developmental variables were assessed, including clinical, gross, and microscopic pathology (parents and piglets). Results: Female fertility index/gp ranged from 80-100%; 170 offspring were recorded; viability was good. Length of gestation, birth wts and body wt change were comparable. Litter size was robust (range 5.4-7.1) for all groups. CaF supplementation was associated with decreased (dec.) food intake in both parents in the high dose gp and with dec. weight gain in both parents during the prematuring phase of the study. These between gp differences persisted through the gestational phase for the females, but there was not a statistically significant increase (inc.) in the magnitude of these differences over time. CaF supplementation was associated with inc. in serum calcium levels in both parents. Although statistically significant, the magnitudes of these differences were small, and well within the normal physiologic ranges. There were no findings of toxicity in the offspring related to maternal exposure to dietary CaF. Conclusion(s): Dec. food intake, dec. weight gain during premating phase, and inc. serum calcium were associated with oral dietary CaF administration. These findings were consistent with previous research, and/or represented expected responses to the administration of oral CaF supplementation.

1713b Effect of Vincamine on the Reproductive System of Harlan Sprague Dawley Rats and B6C3F1/N Mice


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Vincamine is an alkaloid extracted from the leaves of Vinca minor. The National Toxicology Program conducted short-term/pilot toxicity studies in Harlan Sprague Dawley rats and B6C3F1/N mice. Time-mated rats were dosed up to 1000 mg/kg from gestation day (GD) 6 through GD 21. All dams in the 1000 mg/kg groups were moribund on GD 7 and exhibited implants at necropsy. Dams dosed with ≥ 30 mg/kg did not produce pups, and the majority of undelivered dams had fetal resorptions when necropsied. Dams and pups in the 10 mg/kg dosage group did not exhibit any effects attributed to vincamine. Vincamine was subsequently administered up to 300/mg/kg to time-mated female rats from GD 17 to GD 21 (after the period of major organogenesis), then postnatal day (PND) 1 through PND 20. Pups were administered vincamine from PND 12 to PND 20. Vincamine was well tolerated by the dams; all dams survived to study termination, and there were no treatment related effects on littering index, duration of gestation or maternal behavior. On PND1 and 4, vincamine administration was associated with lower pup body weights (100 and 300 mg/kg) and smaller litter size (300 mg/kg). Mild testicular hypoplasia, and minimal ovarian hypoplasia were observed at doses levels ≥ 100 mg/kg at necropsy on ~ 21 days of age. In mice, vincamine was administered by gavage up to 1000 mg/kg for 14 days. Morbidity and mortality was observed at 500 (females) and 1000 (males and females) mg/kg. Consistent with the rat study, the tests were a target of vincamine exposure in male mice. A dose-dependent decrease was observed in testes and epididymis weights in the 500 and 1000-mg/kg groups. Testicular necrosis in the germinal epithelium of the seminiferous tubules was noted among males exposed to dose levels ≥ 250 mg/kg of vincamine. This work was supported by the NIH.

1713c Dose Addition Predicts Effects of Phthalate Mixture on Male Reproductive Tract Development and Associated Fetal Testis Gene Expression in Rats


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A challenge in cumulative risk assessment of phthalate esters is incomplete data on individual chemicals. The objective of the current study was to test whether dose addition (DA) and relative potency factor (RPF), which is a type of DA that relies upon a reference compound, would be better predictors than response addition (RA) of the effects of a 5 phthalate mixture on fetal testis gene expression (star, cyp1a, and insulin-like hormone 3) and hormone-sensitive male reproductive tract development when assumptions were made for missing individual phthalate data. We administered a dose range of the mixture (100, 80, 60, 40, 20, 10, 5 or 0% of top dose consisting of 300 mg/kg per chemical of benzyl butyl (BBP), di(n) butyl (DBP), diethyl hexyl phthalate (DEHP), di-isobutyl phthalate (DIBP) and 100 mg dipentyl phthalate (DPP/kg)) via gavage to rat dams on GD18-18 (fetal gene expression study) or GD8-postnatal day 3 (in vivo study); we selected the ratio of the top dose so that each phthalate contributed equally to the reduction in fetal testosterone (T) production based on estimates of their potencies from previous studies. We compared observed mixture responses to model predictions of DA, RPF, and RA based on the logistic regression analysis of the individual phthalate data using DBP or DEHP as the reference phthalates. DA predicted the inhibition of fetal gene expression at GD18, decreases in neonatal anogenital distance and adult reproductive organ weights, and induction of juvenile areola retention and adult reproductive malformations. RPF based on fetal T production was nearly as accurate as DA, while RA was never the best model. Similar results were obtained when the observed data of the mixture were force fit to the logistic regression parameters of the models. Therefore, dose addition was a good predictor of the mixture even in the face of incomplete data on the individual phthalates. This research was partially supported by an interagency grant by the NIH, NIEHS. This abstract does not necessarily reflect USEPA policy.

1713d The Effects of Triclosan on the Male Reproductive System of the Rat

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Triclosan (TCS), a widely used antibacterial agent, has been shown to have endocrine disrupting activity in mammals. Although the majority of these studies report that TCS alters thyroid hormones, effects on the estrogenic and androgenic pathways have also been observed. These include reports indicating anti-androgenic activity of TCS in vitro and a decrease in serum testosterone (T) concentrations in vivo. In the first study, we further examined the effect of TCS on androgenic activity in the male rat using the Hershberger assay. Six-week old castrated male rats were gavaged for 10 consecutive days with 50 or 200 mg/kg of TCS or vehicle control (corn oil) to determine the presence of any potential androgenic effects. Other groups of rats were treated with testosterone propionate (TP) (0.2 mg/kg, sq) alone or in combination with 50 or 200 mg/kg TCS to evaluate possible anti-androgenic or TP potentiation by TCS. On day 10, the accessory sex tissues (glans penis, Cowper’s glands, levator ani bulbous cavernous muscle complex, seminal vesicles, and ventral prostate) were weighed. TCS alone did not alter the weight of any of the tissues. Also, TCS did not alter the weight of the tissues when given in combination with TP. As expected, TCS exposure (alone or in combination with TP) resulted in a dose-dependent decrease in serum thyroxine after a 10 day exposure to 50 and 200 mg/kg TCS. In a second in vitro study, we examined the effect of TCS on T production using the H295R cell line. Forskolin and prochloraz served as positive controls producing the expected increase and decrease in T production, respectively. TCS from 0.01 to 3.0 μM did not alter T. 10 μM of TCS induced 20% cytotoxicity. Thus, in the present study TCS did not have androgenic or anti-androgenic activity in the Hershberger assay and was without effect on steroidogenesis in the H295R cell line. Serum T4 was significantly decreased demonstrating that the doses selected were sufficient to cause a change in this hormone. This abstract does not necessarily reflect EPA policy.
Bisphenol A (BPA) is a chemical used in the manufacture of polycarbonate and epoxy resins. The molecular pathways potentially modulated by doses of BPA below those needed to activate the estrogenic pathway remain poorly characterized. We assessed the effect of BPA on the gene expression in prostates from 4-day-old rats, an age when the internal levels of aglycone BPA are relatively high due to an immature metabolic system. BPA-treated naive rats were directly dosed daily from the day after birth. Dose groups included naive and vehicle controls, seven "low" BPA doses [2.5, 8, 25, 80, 260, 840, 2,700 μg/kg body weight (bw)/day], two high BPA doses (100,000, and 300,000 μg/kg bw/day), and two doses of the reference estrogen EE2 (0.5 and 5.0 μg/kg body weight (bw)/day). Genome-wide gene expression was assessed by DNA microarray in a subset of doses, and genes of interest were validated by quantitative real-time PCR using all study doses. Principal component analysis showed that samples segregate into three sub-groups: 1) vehicle and low BPA; 2) high BPA and low EE2; and 3) high EE2. The high EE2 dose modulated the largest number of genes, followed by the low EE2 dose. Genes commonly modulated by the high BPA and EE2, which included Dclk3, Gnat2, Gzmc, Map4k5, and Pipc, were modulated in the same direction by both chemicals. Modulation of genes in the "low" BPA dose range was inconsistently observed across all doses, with no dose-response pattern. These data suggest that BPA doses ≥ 2,700 μg/kg bw/day have limited effects in the gene expression in the rat prostate under our experimental conditions. This is consistent with the lack of "low" BPA effects observed in other endpoints, including prostate histopathology, examined in 90-day-old siblings from the same study. IAG FDA 224-12-0005/NIH ES12013.
show that the dispersed crude oil significantly increases the number of apoptotic cells and diploid sperm (immature) in treated worms' gonad when compared to controls at all exposure levels (p < 0.05). Genes involved in the apoptosis pathway were up-regulated, which include cel-13, cel-3, ced-4, ced-9, cep-1, dpl-1, efl-1, efl-2, egl-1, egl-38, lin-35, pax-2, and sir-2.1. Given cep-1(1053) was activated at all dispersed oil treatments and the germ cell apoptosis was suppressed in the CEP-1 loss of function mutant, the increased apoptosis is likely CEP-1 dependent. A total of 231 microRNAs expressions were analyzed using quantitative real-time PCR; 59 miRNAs were up-regulated, while 82 were down-regulated. Heatmap clustering indicates that dispersed oil displayed a distinct miRNA expression profile compared to control, oil-alone, and dispersant-alone treatments. The gene targets and functions of miRNAs were analyzed using bioinformatics tools (TargetScan, miRanda, and KEGG). A variety of reproduction related pathways were found to be regulated by oil-dispersant affected miRNAs, including MAPK signaling pathway, Wnt signaling pathway, Hedgehog signaling pathway, and Oocyte maturation pathway, etc. This work provides mechanistic understandings of crude oil-dispersant induced reproductive toxicity.

**1713j** High-Content Screen and Mechanistic Study of Environmental Reproductive Toxins in *C. elegans*

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Chemicals play an integral part of our everyday life despite their significant impact on human health and the environment. Global chemical production is doubling every 25 years, rapidly outpacing population growth, increasing both in volume and in the variety of the compounds released in the environment each year. Over the past two decades, a contribution of environmental exposures to the etiology of reproductive health effects of exposures has become an increasing cause for concern. However, there is tremendous difficulty in assessing safety of these chemicals due to their large number and also from the complexity of biological effects they elicit over a large range of doses. Here, I address these issues by using the genetic model system, the worm *Caenorhabditis elegans* in two ways. First, I aim to identify proreptochemicals by screening chemicals for their ability to disrupt meiosis and induce aneuploidy in *C. elegans*. For this purpose, I developed a 384-well plate high-content microscopy assay that can accurately detect the induction of aneuploidy based on the emission of GFP fluorescence in aneuploid embryos (Allard et al, 2013). This assay will be used to screen ToxCast Phase I and II environmental chemical libraries and to identify a list of compounds with high aneugenicity. Second, a molecular and cellular mechanistic follow-up analysis of hits will be performed. Preliminary work indicates that worms exposed to 100μM Maneb, Feranimol or Diazinon show a significant induction in the number of germline apoptotic nuclei (2.7, 2.6 and 3.3-fold induction, respectively, p<0.05) and an increase in reduced nuclear density (2.8, 2.6 and 2.4-fold induction, respectively, p<0.05), suggesting that these compounds are potent reproductive toxicants causing germline maintenance disruption and acute germ cell death. Together, these studies aim at developing unique methods to interrogate our chemical environment for its effect on germline maintenance and health.

**1713k** Cross-Species Evaluation of Endocrine Disrupting Effects of Lead (Pb) on Maturation and Development

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In a cross-species evaluation conducted in support of the review of the US EPA National Ambient Air Quality Standards (NAAQS) for Lead (Pb), findings from epidemiologic, toxicological and ecological disciplines were used in a weight of evidence approach to evaluate reproductive and developmental effects of this metal, including endpoints associated with endocrine disruption. In the process of synthesizing information on reproductive effects of Pb for the 2013 Integrated Science Assessment for Lead (ISA), we noted that Pb is associated with maturational delays (e.g. puberty onset, developmental duration, life cycle length, metamorphosis) across a wide variety of organisms. A causal association between Pb exposure and reproductive and developmental endpoints for both human health and ecological receptors were reported. Moving beyond the synthesis conducted for the Pb ISA, we have reviewed the literature on changes in timing of developmental milestones associated with exposure to Pb. Evidence from multiple species points to an underlying common mode of action of Pb on the insulin-like growth factor signaling pathway; inhibit B was also affected in vertebrate models of Pb exposure. An interdisciplinary cross-species approach provided insights into endocrine disruption by Pb and a possible mode of action of this metal. Increased time to sexual maturation or delayed development may have consequences for reproduction and development across a broad range of species.

The views expressed in this poster are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

**1713l** Dual Role of microRNA-31 in 2, 3, 7, 8-Tetrachlorodibenzo- p-dioxin(TCDD)-Mediated Ureaplacement of CYP1A1 and Fox3 in Activated T Cells

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TCDD, an environmental contaminant, is well known for inducing severe toxicity including immunosuppression. We examined the mechanisms by which TCDD affects T cell response to Staphylococcal enterotoxin B (SEB) and Pertussis Toxin (PTX). SEB, a superantigen, activates ~20% of T cells via Vβ8 T cell receptor and causes a robust release of pro-inflammatory cytokines. Pertussis Toxin promotes the generation of IL-17-producing CD4+ cells by inducing IL-6. We injected C57BL6 mice with either SEB or PTX, and treated them with either vehicle or TCDD. Our studies showed that TCDD treatment of SEB-activated lymphocytes led to a decrease in Vβ8+ T cells. TCDD treatment of PTX-activated lymphocytes dampened Th17 cells while promoting Fox3+ Tregs. TCDD also induced apoptosis in activated T cells, suppressed pro-inflammatory cytokines, IFN-γ, TNF-α and IL-6, and induced anti-inflammatory cytokine, IL-10. In order to determine the role of microRNA (miR) in TCDD-induced immune dysregulation, we performed high throughput miR analysis. In silico analysis demonstrated that miRs induced by TCDD regulated several pathways including induction of apoptosis, AHR signaling and Treg differentiation. Interestingly, miR-31 which was down regulated following TCDD treatment was found to be complementary to both the 3’-UTR of Fox3 and CYP1A1 genes, which was validated by RT-PCR. Furthermore, miR-455 against ARNT target gene was also down-regulated in TCDD treated groups. In addition, miR-21 which targets the pro-apoptotic factors, Fas and Fasl, was down-regulated by TCDD, which correlated with the ability of TCDD to induce apoptosis. Together, our studies demonstrate that TCDD-mediated immune regulation may be mediated by altered expression of several microRNAs (Supported by NIH grants P01AT003961, P20 RR032684, R01AT006888, R01ES019313, R01MH094755 and VA Merit Award BX001375).

**1713m** Relationship between Allergic Characteristics of Swine Husbandry Workers and Endotoxin Level in Swine Farm Dust

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Animal husbandry workers could be exposed to various work hazards including toxic gases, chemicals, and endotoxin. Immunological evaluation on allergic hyporesponsiveness occurrence was undertaken for swine farm workers. Relationship between endotoxin level in dust collected from swine farms and immunological markers related with respiratory allergy was evaluated. Peripheral blood samples were collected from 10 workers at 10 swine farms in Korea. PMBCs were stimulated with PMA and ionomycin for 48 hours. The levels of cytokines at culture supernatants were determined using a ELISA kit. The concentration of particulate matter (PM10) in the indoor air of the swine farms was evaluated using a PVC membrane filter and mini volume air sampler, and endotoxin levels in the dust were measured by Limulus Amebocyte Lysate Kinetic QCL method. Levels of endotoxins in total dust were categorized into high (GM:109.35 EU/m²) and low concentrations (GM:0.95 EU/m²) for 5 swine farms, respectively. IL-4 levels were higher in the high endotoxin group than the low endotoxin group, while interferon-γ levels were lower in the high endotoxin group than the low endotoxin group. The ratio (IFNγ to IL-4) was lower in the high endotoxin group (1.15±0.60) than the low endotoxin group (3.09±2.38). The level of IL-13 was significantly higher in the high endotoxin group (1.12±0.37 ng/ml) than the low endotoxin group (0.57±0.04 ng/ml). Plasma IgE levels were five times higher in the swine farm workers than the seven control subjects not involved with animal husbandry at the same residential area as the swine farm workers. The house dust mite (Dermatophagoides farinae, D. pteronyssinus) and cockroach were the major respiratory allergens for swine farm workers. This study suggests that the immunological function of swine farm workers exposed to high level of endotoxin could be modulated toward allergic reactivities. [Supported from Korean Rural Development Agency, PJ008678]
Glucose and Insulin Mediated Alteration in Metabolic Activity of U937 Cells

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By 2030, it is estimated that the number of people with diabetes will be more than 82 million in developing countries and more than 48 million in developed countries. In type 2 diabetes, insulin resistance and metabolic syndrome are associated with persistent hyperglycemia and chronic inflammation due to the macrophage activation by impaired glucose retention. Enhanced reactive oxygen production, increased modification in ligands and disruption in insulin signaling pathway are the main causes in the development of diabetic complications. The aim of our study is to evaluate the alteration in the metabolic activity of U937 human monocytic cell line by glucose and insulin. U937 cells were incubated with or without insulin (10 µU/ml – 60 µU/ml) and with various glucose concentrations (50 mg/dl – 500 mg/dl) that were determined due to the blood glucose levels of International diabetes classification. Metabolic activity of U937 cells were significantly increased in hypoglycemic conditions with 60 µU/ml insulin at 48 hours. However, higher glucose in combination with high insulin concentrations significantly decreased metabolic activity of the monocytic cells at 72 hours. These results indicate that chronic exposure to high glucose due to uncontrolled diabetes may alter the immune response related to the changes in the metabolic activity of the immune system cells.

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Ascorbic Acid Improves Hyperoxia-Compromised Host Defense against Pseudomonas aeruginosa Infection in Mice

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Oxygen therapy using high concentrations of oxygen (hyperoxia) is associated with compromised host defense and greater susceptibility to bacterial infections, causing ventilator-associated pneumonia (VAP). Hyperoxia-induced excessive ROS production and accumulation of extracellular HMGB1 play critical roles in impairing macrophage’s ability to phagocytose. Ascorbic acid (AA), a antioxidant, exhibits beneficial effects in various ROS-mediated diseases. The aim of this study was to determine whether AA could improve hyperoxia-compromised host defense and macrophage functions. C57BL/6 male mice were exposed to hyperoxia (≥98% O2, 48 h) followed by intratracheal inoculation with Pseudomonas aeruginosa, and simultaneously treated with different concentrations of AA. RAW 264.7 cells (a macrophage like cell line) were also exposed to different concentrations of AA prior to 95% O2 exposure, ROS levels, phagocytic activities and extracellular HMGB1 accumulation were analyzed. AA effectively improved bacterial clearance in lungs and airways of the mice. It significantly rescued the hyperoxia-compromised macrophage function of phagocytosis. Improved phagocytic activity was accompanied by reduced ROS levels as well as decreased accumulation of extracellular HMGB1.

Our study demonstrates that AA, a cost-effective dietary supplement, could provide a rewarding aid in prevention and treatment of VAP.

Sodium Methyldithiocarbamate: Potential Connections of Disparate Effects


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Sodium methyldithiocarbamate (SMD) is the third most abundantly used conventional pesticide in the U.S. We have previously reported that SMD has immunotoxic effects, but we have not previously reported a comprehensive description of the effect of this compound on gene expression and host resistance in a mouse model. These effects are reported here, and they indicate that oral exposure to the compound and relatively high doses (100-300 mg/kg) causes substantial decreases in innate immune and inflammatory responses to bacterial lipopolysaccharide (from Escherichia coli) or to viable bacteria. A series of SMD dosages most potently inhibited expression of genes related to interferon response and production pathways. These results suggest that the action of a strong interferon inducer might be inhibited to a greater extent than the action of a stimulus such as LPS or whole bacteria. Thus, polymeric-polyacrylicidic acid (poly IC), the major ligand for TLR3 was investigated, and SMD was found to inhibit cytokine and chemokine induction by this compound at dosages as low as 10 mg/kg, which are substantially lower than required for inhibition of these responses by LPS or whole bacteria. Although, we have previously shown that SMD decreases survival in an E. coli model of sepsis, inhalation or dermal exposure are more likely routes for human exposure to SMD. We report here that inhalation of SMD or its major breakdown product (methylisothiocyanate) did not significantly decrease survival. However, survival was decreased by oral administration of SMD. The results indicate that survival was decreased by oral administration was likely due to initial inhibition of decreased bacterial killing in phagocytic cells and decreased production of cytokines or chemokines. We now hypothesize that the effects of SMD on lethal outcome in sepsis and TLR3 responses are related and that fully understanding this will also suggest reasons for differences in effects of dosage routes.

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Immunotoxicology Profile following Exposure to Silt Deposit Dust Samples from Nellis Dunes Recreational Area, Clark County, Nevada


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The Nellis Dunes Recreation Area (NDRA) is a popular region for off-road vehicle driving that attracts over 300,000 commercial and recreational users each year. Trace metals that could potentially affect human health have been measured in soil and airborne dust samples. Map unit CBN7 is composed of aggregated silt deposits commonly found in badlands without rock or vegetative cover. Dust samples from CBN7 with a median grain size of 4.37μm were analyzed via inductively coupled plasma mass spectrometry (ICP-MS) and contained: 293.63 ppm Mn, 248.23 ppm Sr, 49.49 ppm Cr, 26.20 ppm Pb, and 23.51 ppm As. B673CF1 female mice were exposed via oropharyngeal aspiration at doses of 0.01, 0.1, 1, 10, and 100 mg geological sample/kg/day at four intervals, each a week apart. No dose-responsive changes were observed in body or organ weights. Serum total bilirubin was dose-responsively increased beginning at 0.1 mg/kg/day. Serum creatinine levels were increased at 10 and 100 mg/kg/day whereas blood urea nitrogen was dose-responsively decreased beginning at 0.1 mg/kg/day. Blood neutrophil and lymphocyte numbers were increased at 10 and 100 mg/kg/day. Splenic CD8+ and CD4+/CD8+ T-cells were decreased; however, this was not clearly dose-responsive. No non-renal, inflammatory changes were detected. B22.2 and thymic CD4+CD8+ Igm antibody production was dose-responsively suppressed beginning at 0.1 mg/kg/day with an ED50 of 0.30 mg/kg/day. The LOAEL was 0.1 mg/kg/day whereas the NOAEL was 0.01 mg/kg/day as demonstrated by antigen-specific IgM production. Further studies will assess the potential health risks associated with recreation at this site.

Immunotoxicology Profile following Exposure to Geological Dust Samples Collected from Nellis Dunes Recreational Area

Map Unit CB2N2: Median Grain Size 4.5 μm


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The Nellis Dunes Recreational Area (NDRA) is a highly popular ORV area near Las Vegas, NV. Soil and airborne dust in this area are rich in naturally occurring arsenic. This presentation focuses on data from CB2N2, which is a yellow silt and clay unit mostly barren of vegetation. Dust samples with a median grain size of 4.5 μm were analyzed for total elemental composition via inductively coupled plasma mass spectrometry. The following concentrations were found: As136.82 ppm; Cr38.74 ppm; Pb:22.71 ppm; Mn: 386.28 ppm; and Sr:424.89 ppm. B6C3F1 female mice were exposed to dust via oropharyngeal aspiration at doses of 0.01, 0.1, 1, 10, and 100 mg geological sample/kg/day at four intervals, each a week apart. No dose-responsive changes were observed in body or organ weights. Serum total bilirubin was dose-responsively increased beginning at 0.1 mg/kg/day. Serum creatinine levels were increased at 10 and 100 mg/kg/day whereas blood urea nitrogen was dose-responsively decreased beginning at 0.1 mg/kg/day. Blood neutrophil and lymphocyte numbers were increased at 10 and 100 mg/kg/day. Splenic CD8+ and CD4+/CD8+ T-cells were decreased; however, this was not clearly dose-responsive. No non-renal, inflammatory changes were detected. B22.2 and thymic CD4+CD8+ Igm antibody production was dose-responsively suppressed beginning at 0.1 mg/kg/day with an ED50 of 0.30 mg/kg/day. The LOAEL was 0.1 mg/kg/day whereas the NOAEL was 0.01 mg/kg/day as demonstrated by antigen-specific IgM production. Further studies will assess the potential health risks associated with recreation at this site.
1713s Reducing Immunogenicity in a T Cell-Dependent Antibody Response (TDAR) in Cynomolgus Monkeys Leads to a Sensitive Assessment of Immunosuppression by Abatacept (CTLA4-Ig)

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The TDAR response is used to evaluate drug effects on the immune system, assessing antigen presentation and T and B lymphocyte function. Cynomolgus monkeys injected subcutaneously on 5 occasions over 15 days with 1 mg Keyhole Limpet Hemocyanin (KLH) in Incomplete Freund’s Adjuvant (IFA) developed a robust IgM and IgG antibody response to KLH. Treatment of animals twice weekly with 8 mg/mL/kg Abatacept (CTLA4-Ig) by intravenous slow bolus injection did not diminish the antibody response. In a separate study, animals were injected with 100 μg KLH in the absence of adjuvant via intradermal injections. These animals developed a detectable IgM response with inverse titers of 25,000 ten days following primary immunization. IgG responses were detectable 14 days post primary injection with inverse titers of 12,000, and 7 days post secondary challenge with inverse titers of over 50,000. Treatment of a cohort of these animals with Abatacept led to an ablation of antibody responses. Antibody responses to KLH from animals were reduced but not ablated when treated with 1 mg/kg methotrexate via subcutaneous injection. Maximal mean IgM responses to KLH 10 days following primary immunization were reduced from 120,000 to 34,000. Maximal mean IgG responses to KLH were reduced from 1 x 10^6 to 0.66 x 10^6 with maximal responses delayed from 7 to 10 days post secondary KLH challenge. Combined, these data demonstrate that excessive antigenic stimulation can overcome the immunosuppressive effects of Abatacept and define an alternate immunization strategy that is susceptible to immunosuppression. Utilizing an appropriate immunization strategy in TDAR assays and including biological immunosuppressor controls, this model is optimized for safety assessment of immunomodulatory biological drug products.

1713t Sulforaphane Inhibits Vascular Inflammation in Mice and Prevents TNF-α-Induced Monocyte Adhesion to Primary Endothelial Cells through Interfering with the NF-κB Pathway

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Tumor necrosis factor alpha (TNF-α) is a critical cytokine that plays important role in regulation of the toxic effects of a variety of chemicals and toxicants. Sulforaphane, a naturally-occurring isothiocyanate present in cruciferous vegetables, has received wide attention for its potential to improve vascular function in vitro. However, its effect in vivo and the molecular mechanism of sulforaphane at physiological concentrations remain unclear. Here, we report that a sulforaphane concentration as low as 0.5 μM significantly inhibited TNF-α-induced adhesion of monocytes to human umbilical vein endothelial cells (HUVECs). Sulforaphane also significantly suppressed TNF-α-induced production of adhesion molecules. Furthermore, sulforaphane inhibited TNF-α-induced NF-κB transcriptional activity, IkBα degradation and subsequent NF-κB p65 nuclear translocation in endothelial cells, suggesting that sulforaphane can inhibit inflammation by suppressing NF-κB signaling. In an animal study, the physiologically-relevant dose of sulforaphane (300 ppm) in a mouse diet significantly abolished TNF-α-induced ex vivo monocyte adhesion and circulating adhesion molecules and chemokines in C57BL/6 mice. Histology showed that sulforaphane treatment significantly prevented the eruption of endothelial lining in the intima layer of the aorta and preserved elastic fibers’ delicate organization as shown by Verhoeff-van Gieson staining. Immunohistochemistry studies showed that sulforaphane treatment also reduced YCAM-1 and monocytes-derived F8/80-positive macrophages in the aorta of TNF-α-treated mice. In conclusion, sulforaphane at physiological concentrations protects against TNF-α-induced vascular endothelial inflammation, in both in vitro and in vivo models. This anti-inflammatory effect of sulforaphane may be, at least in part, associated with interfering with the NF-κB pathway.

1714 Regulation of Covalent Modification of 2-Tert-Butyl-1,4-Benzoquinone to Keap1 through Glutathione-Mediated S-transarylation

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Butylated hydroxyanisole is a phenolic antioxidant and classified class-2B carcinogen. It is readily undergoes O-dealkylation to produce 2-tert-butyl-1,4-dihydroquinone (TBHQ), which readily auto-oxidizes to the electrophilic toxic metabolite 2-tert-butyl-1,4-benzoquinone (TBQ). TBQ causes activation of transcription factor Nrf2 coupled to S-arylation of its negative regulator Keap1. The TDAR response is used to evaluate drug effects on the immune system, assessing antigen presentation and T and B lymphocyte function. Cynomolgus monkeys injected subcutaneously on 5 occasions over 15 days with 1 mg Keyhole Limpet Hemocyanin (KLH) in Incomplete Freund’s Adjuvant (IFA) developed a robust IgM antibody response to KLH. Treatment of animals twice weekly with 8 mg/mL/kg Abatacept (CTLA4-Ig) by intravenous slow bolus injection did not diminish the antibody response. In a separate study, animals were injected with 100 μg KLH in the absence of adjuvant via intradermal injections. These animals developed a detectable IgM response with inverse titers of 25,000 ten days following primary immunization. IgG responses were detectable 14 days post primary injection with inverse titers of 12,000, and 7 days post secondary challenge with inverse titers of over 50,000. Treatment of a cohort of these animals with Abatacept led to an ablation of antibody responses. Antibody responses to KLH from animals were reduced but not ablated when treated with 1 mg/kg methotrexate via subcutaneous injection. Maximal mean IgM responses to KLH 10 days following primary immunization were reduced from 120,000 to 34,000. Maximal mean IgG responses to KLH were reduced from 1 x 10^6 to 0.66 x 10^6 with maximal responses delayed from 7 to 10 days post secondary KLH challenge. Combined, these data demonstrate that excessive antigenic stimulation can overcome the immunosuppressive effects of Abatacept and define an alternate immunization strategy that is susceptible to immunosuppression. Utilizing an appropriate immunization strategy in TDAR assays and including biological immunosuppressor controls, this model is optimized for safety assessment of immunomodulatory biological drug products.

1715 An Imaging-Based RNAi Screen Identifies Novel Regulators of Nrf2 Activation

S. Hiemstra, R. Herpers, M. Niemeijer, S. Wink and B. van de Water. 1715 An Imaging-Based RNAi Screen Identifies Novel Regulators of Nrf2 Activation

S. Hiemstra, R. Herpers, M. Niemeijer, S. Wink and B. van de Water. 1Toxicology, LACDR, Leiden, Netherlands.

Reactive oxygen species (ROS) are major inducers of cellular stress and cell death. The anti-oxidant response pathway is therefore a key mechanism in protecting against ROS-mediated toxicity. The transcription factor Nrf2 is a critical regulator of the antioxidant response pathway and fundamental in cytoprotection. The activity of Nrf2 is controlled through KEAP1-mediated ubiquitination and
subsequent proteasomal degradation. Moreover, various kinases can phosphorylate Nrf2 thereby affecting its stability. Accumulated Nrf2 translocates to the nucleus targeting the expression of multitude of anti-oxidant target genes, such as Srxn1.

The entire signaling network that controls Nrf2 stability and functionality is so far unclear. To monitor the KEAP1/Nrf2/Srxn1 signaling pathway we apply live cell imaging and tagging these target genes with GFP using BAC recombining. Using high throughput confocal imaging we can quantify the dynamics of Nrf2 activation and established the concentration time course dynamics of Nrf2 activation by, amongst others, iodocacetamide and CDDO-me. In order to provide full understanding of the upstream regulators of the Nrf2 pathway, we applied a siRNA knock-down based screen using the GFP-Srxn1 reporter as a downstream target of Nrf2. We screened all individual kinases, phosphatases, ubiquitinases and transcription factors for their involvement in Nrf2 activation. After knock-down, CDDO-me was used to activate Nrf2 followed by quantification of Srxn1-GFP expression. We observed ~50 target genes that enhance Srxn1-GFP expression and ~35 targets genes that inhibit Srxn1-GFP expression, including both known and novel modulators. Pathway analysis was performed to identify the signaling networks that control Nrf2 activity. Individual hits were validated using alternative strategies to activate Nrf2 and there involvement in cytotoxicity was evaluated. This work contributes to more elaborate understanding of the signaling networks that control Nrf2 signaling in the context of xenobiotic-induced cytotoxicities.

1716 Ambient Vapor Samples Activate the Nrf2-ARE Pathway but Not an Inflammatory Response in Human Bronchial Epithelial BEAS-2B Cells

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Ambient air pollutants have been reported to induce oxidative stress based inflammatory responses in humans and experimental animals. However, most of these reports describe the actions of the particulate phase of ambient and exhaust samples. We describe here results of studies investigating the actions of the vapor phase of ambient air samples collected in the midtown area of Los Angeles on human bronchial epithelial BEAS-2B cells using DNA microarray analysis. Among 26 genes whose expression increased fourfold or more, four genes were associated with detoxifying genes regulated by the transcription factor Nrf2. Consistent with these results, the vapor samples activate the Nrf2-ARE pathway, resulting in up-regulation of heme oxygenase-1 (HO-1), glutamate cysteine ligase modifier subunit, and cystine transporter (xCT) mRNAs and proteins. No appreciable increases in pro-inflammatory genes were observed. These results suggest that ambient vapor samples activate the Nrf2-ARE pathway but not an inflammatory response. Also, treatment of the vapor samples with glutathione resulted in reduction of the Nrf2 activation and HO-1 induction, suggesting that electrophiles in vapor samples contribute to Nrf2-dependent antioxidant or adaptive response. [Reference] Shinkai Y et al., Environ Toxicol, 2013, in press.

1717 Proteomics Analysis to Identify Sensor Proteins with Covalent Modification Associated with 1,4-Naphthoquinone-Induced Activation of Electrophilic Signal Transduction Pathways

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While metabolic activation of naphthalene, yielding 1,2-naphthoquinone (1,2-NQ) and 1,4-NQ that can covalently bind to cellular proteins, has been recognized to be associated with its toxicity, the current consensus is that such electrophile-mediated covalent modification of sensor proteins with thiolate ions is also involved in activation of cellular signal transduction pathways for cellular protection against reactive materials. In the present study, we developed an immunchemical assay to detect cellular proteins adducted by 1,4-NQ. Dot blot analysis indicated that the antibody prepared against 1,4-NQ recognized the naphthalene moiety with the para-target genes that inhibit Srxn1-GFP expression after CDDO-me treatment, including both known and novel modulators. Pathway analysis was performed to identify the signaling networks that control Nrf2 activity. Individual hits were validated using alternative strategies to activate Nrf2 and there involvement in cytotoxicity was evaluated. This work contributes to more elaborate understanding of the signaling networks that control Nrf2 signaling in the context of xenobiotic-induced cytotoxicities.

1718 Role of Stress Response Proteins and Redox-Sensitive Transcription Factors in Chemically-Induced Liver Injury: Chemoprevention with Kolaviron and Curcumin

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Kolaviron, a bioflavonoid from the seeds of Garcinia kola and curcumin, present in the rhizome of Curcuma longa have been reported to possess anti-inflammatory, antioxidant, anticarcinogenic and chemopreventive activities via multiple mechanisms. Biomarkers of hepatic oxidative injury, histological and immunohistochmical techniques were used to validate the biochemical mechanisms underlying the hepatoprotection of kolaviron and curcumin. In addition, the protein expression levels of cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) and heme oxygenase activity (HO-1) and expression were evaluated by western blotting while DNA-binding activities of nuclear factor kappa B (NF-kB), NF-E2-related factor 2 (Nrf2) and activator protein-1 (AP-1) were determined by Electrophoretic mobility shift assay. Kolaviron reduced the Dithemyl nitrosamine (DMN)-induced markers of oxidative stress. Kolaviron inhibited the DMN-induced expression of COX-2 and iNOS. Immunohistochemical staining of rat liver verified the inhibitory effect of kolaviron on DMN-induced hepatic COX-2 expression. Furthermore, kolaviron abrogated DMN-induced binding activity of NF-kB as well as AP-1. Our data showed that curcumin increases HO-1 activity and HO-1 protein via activation of Nrf2 in rat liver. Also, Curcumin administration resulted in enhanced nuclear translocation and ARE-binding of Nrf2. Tetrathrydrocumric lacking α-β unsaturated bond elicits lower anti-inflammatory activity. Collectively, kolaviron inhibits COX-2 and iNOS expression through down regulation of NF-kB and AP-1 DNA binding activities while curcumin protects against DMN-induced hepatotoxicity through ARE-driven induction of HO-1 expression. Thus kolaviron and curcumin qualify as candidates for chemoprevention of hepatocarcinogenesis especially in regions where humans are exposed environmentally and through diet to liver toxic agents.

1719 Ozone Induces Lung Epithelial Cell Inflammation through MAP Kinase Activation without NF-κB Activation

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Ground-level ozone (O3), a ubiquitous air pollutant, is a strong oxidizing agent and potent inducer of airway inflammation via the induction of pro-inflammatory mediators such as IL-8, IL-6, and IL-1 alpha/beta. Previous attempts to characterize O3-mediated activation of these pro-inflammatory mediators have associated their up-regulation with activation of the canonical NF-κB pathway. However, these studies were conducted in transformed cell lines and thus may not accurately reflect what occurs in normal cells. With the increased availability of primary cells in recent years, we sought to test whether the previously established model of O3-dependent pro-inflammatory mediator expression applied to primary human bronchial epithelial cells (phBEC). Thus we characterized the O3-mediated activation of cellular signaling pathways in phBEC grown in air-liquid interface culture obtained from seven donors and in the bronchial epithelial cell line BEAS-2B. Despite observing activation of NF-κB signaling in the BEAS-2B cell line following O3 exposure, we were unable to detect its activation in phBEC following O3 exposure. Instead, we observed activation of the EGFR/MEK/ERK and MKK4/p38 pathways. Additionally, the inhibition of EGFR and p38 kinase activity during O3 exposure was sufficient to prevent the O3-mediated induction of pro-inflammatory mediators in phBEC. These novel findings indicate that the mechanism underlying the O3-induced expression of pro-inflammatory mediators in phBEC involves the activation of MAP kinases, not canonical NF-κB signaling. This discovery is an important step toward elucidating the molecular mechanisms associated with the adverse health effects of O3 exposure, predicting susceptible populations, and identifying strategies for therapeutic intervention.
Oxidative stress (OS) in adipose tissue (AT) causes metabolic dysfunction of fatty acid transport and dysregulation of AT expandability, which alters AT role in nutritional adaptation and glucose tolerance. Central obesity and insulin-resistance (IR) are important causative factors of metabolic syndrome directly related to AT and metabolic stress. Most notably, OS originating from obesity is considered to be a precipitating factor for obesity-induced IR in AT. In addition to nutritional excess, many chemicals of environmental concern can be deposited in AT and elicit adverse effects via inducing OS; the effects of these toxins on AT dysfunction via OS is largely unknown. The objective of the study was to understand mechanisms by which caloric restriction (CR) abolishes the antioxidant response in AT to combat OS, and the physiological outcomes of this activation. ARE, H2O2, CAT, and MnSOD were measured by ELISA kit. The data suggest that CR can reduce oxidative stress and increase the antioxidant capacity in AT. Overall, the antioxidant response is induced in WAT via Nrf2 at the transcriptional level, and Nrf2 induction results in regulation of glutathione metabolism, suggesting Nrf2 as a therapeutic drug target against OS-induced AT dysfunction.

**Benzo[a]pyrene and β-Naphthoflavone Promote Nrf2 Nuclear Translocation in HepG2 Cells**

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HepG2 cells, like all cancer cells, generate energy from glucose by glycolysis, with little contribution from oxidative phosphorylation (OXPHOS). However, when glucose is replaced by galactose in the growth media, these cells use OXPHOS to generate energy by utilizing glutamine and asparagine in a process called anapleurosis. Comparison of cell viability in the different media is now used routinely to screen drugs for mitochondrial toxicity. Here using a novel In-Cell Proteomics method, we have expanded the evaluation of the effect of the classical inhibitors of OXPHOS: FCCP (uncoupler), oligomycin (ATP synthase inhibitor), myxothiazol (complex III inhibitor), and rotenone (Complex I inhibitor) on mitochondrial function and cell metabolism. Cells were incubated with compounds at 100μM in either glucose or galactose for 6, 24 or 72h prior to fixation. Protein levels were quantified using specific antibodies. All 4 compounds induced cell death by 6h in galactose based on cell appearance and reduction in MCl1 and PUMA levels. In either glucose or galactose for 6, 24 or 72h prior to fixation. Protein levels were quantified using specific antibodies. All 4 compounds induced cell death by 6h in galactose based on cell appearance and reduction in MCl1 and PUMA levels.

**Heat Shock Protein A6 Expression Is Increased by AP1 and NF-kB**

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HepG2 cells, like all cancer cells, generate energy from glucose by glycolysis, with little contribution from oxidative phosphorylation (OXPHOS). However, when glucose is replaced by galactose in the growth media, these cells use OXPHOS to generate energy by utilizing glutamine and asparagine in a process called anapleurosis. Comparison of cell viability in the different media is now used routinely to screen drugs for mitochondrial toxicity. Here using a novel In-Cell Proteomics method, we have expanded the evaluation of the effect of the classical inhibitors of OXPHOS: FCCP (uncoupler), oligomycin (ATP synthase inhibitor), myxothiazol (complex III inhibitor), and rotenone (Complex I inhibitor) on mitochondrial function and cell metabolism. Cells were incubated with compounds at 100μM in either glucose or galactose for 6, 24 or 72h prior to fixation. Protein levels were quantified using specific antibodies. All 4 compounds induced cell death by 6h in galactose based on cell appearance and reduction in MCl1 and PUMA levels. In parallel, there was dephosphorylation of both 4E-BP1 and the S6 ribosomal protein, indicating mTor involvement, and a strongly increased phosphorylation of eIF2α, consistent with a component of oxidative stress. In glucose there was no cell death at 6h with FCCP or oligomycin, but some apoptosis with rotenone and myxothiazol. Unlike galactose all 4 compounds induced a large increase in phos- phorylation of both p38MAPK and c-Jun kinases in glucose along with increases in both fatty acid oxidation and synthesis along with ER stress based on the increased levels of ACADM, HADHB, ACC2 and CHOP1 respectively. Our results indicate considerable effect of OXPHOS inhibitors outside mitochondria, some but not all of which can be accounted for by loss of ATP production, indicating that other fac- tors such as ROS and altered metabolic intermediates exiting this organelle provide retrograde signalling of mitochondrial stress to the rest of the cell.

**The NF-κb Family Member RelB Controls microRNA miR-146a to Suppress Cigarette Smoke-Induced COX-2 Protein Expression in Lung Fibroblasts**


Rationale: Cigarette smoke (CS) is the leading cause of preventable death worldwide. Diseases due to cigarette smoke exposure, including chronic obstructive pulmonary disease (COPD), are associated with chronic inflammation typified by the heightened expression of cyclooxygenase-2 (COX-2). RelB is an NF-κb family member that suppresses the CS-induced COX-2 protein by an unknown mechanism. The ability of RelB to regulate COX-2 protein expression in response to cigarette smoke may be due to miR-146a, a miRNA which attenuates COX-2 expression in response to inflammatory stimuli. In this study we tested the hypothesis that RelB attenuation of cigarette smoke-induced COX-2 expression is due to regulation of miR-146a.

Methods: Relbs/+ and Relb−/− mouse lung fibroblasts were exposed to 2%-5% cigarette smoke extract (CSE) for 3, 6 or 8 hours. COX-2 protein and mRNA as well as miR-146a were analyzed by WB and/or qRT-PCR. Relb−/+ cells were transfected with Relb siRNA or a miR-146a inhibitor and then exposed to 2%-5% CSE for 8h. COX-2 protein and mRNAs were then assessed by WB and qRT-PCR. Results: CSE induced a significant increase in COX-2 protein expression in Relb−/− fibroblasts compared to Relbs/+ cells. Relb siRNA in Relbs/+ cells also increased COX-2 protein. Despite increased COX-2 protein in Relb−/− fibroblasts, there was a lack of Cox-2 mRNA, suggesting transcriptional induction of Cox-2 mRNA cannot account for the heightened protein expression. There was no difference in Cox-2 mRNA stability between Relbs/+ and Relb−/− cells. Relb regulated miR-146a in response to CSE and the inhibition of miR-146a potentiated CSE-induced COX-2 protein expression.

Conclusions: RelB prevents exaggerated COX-2 expression in response to CS by controlling miR-146a expression, an event which might be crucial in dampening smoke-induced damage. Thus, the Relb/miR-146a pathway may represent a new research direction with therapeutic potential for cigarette smoke-induced lung disease such as COPD.
strates distinguishes it from chaperone family members like HSPA1A (also known as HSP70). Compared to other HSPs, less is known about the transcriptional control of A6, a potentially important characteristic as A6 expression varies greatly amongst different cell types.

Using cultured HaCaT keratinocytes (KC), we observed constitutive and heat induced expression of A6. To identify transcription factors (TF) controlling its basal expression, the A6 3kb promoter was isolated and cloned into a luciferase reporter construct (LUC). Our results from transfected 5′ promoter truncation constructs localized a positive regulatory region between -647 to -700bp. Internal promoter deletions within the -647bp LUC suggested a central fragment is crucial for the activation of the A6 promoter. Binding site specific mutants within this fragment determined AP1 plays a key role in A6 expression.

Our lab also observed reduced basal expression and halted stress inducibility of A6 due to overexpressed TNIP1, a protein involved in repressing the activation or activity of several TLR. Using a similar approach, we searched for TNIP1-regulated binding sites involved in A6 repression. Interestingly, the TNIP1-sensitive region (−647 to −700bp) lacks identified TNIP1 target TF binding sites. These results suggest that TNIP1 represses A6 through a novel TF target. Moreover, this work demonstrated A6 expression levels can be modulated to increase or decrease via activation of AP1 or TNIP1, respectively. Since HSPs protect cells from heat and chemicals and the skin is a barrier to such stressors, it is important to understand the mechanisms involved in HSP regulation in KCs.

**Role of Cytokines in Regulation of Angiotensinogen in Human Hepatocytes**

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Increase in blood pressure and fibrotic transformation of liver is connected to the abuse of alcohol. Alcohol metabolism by the liver produces hepatic injury resulting in immune responses where the levels of cytokines, especially interleukin-1β (IL-1β), IL-6 and other cytokines are increased. IL-1β activates nuclear factor-kappaB (NF-κB) while IL-6 activates signal transducer and activator of transcription 3 (STAT3) transcription factor. IL-6 is a cell-based model, the effects of IL-1β (10ng/ml) and IL-6 (10 ng/ml) on angiotensinogen (AGT) secretion were studied by treating hepatocytes (HepG2 and Huh7) for 4hrs and 12 hrs. In addition, NF-κBmediated effects on secretion of AGT was studied by treating HepG2 and Huh7 with 50 nM phorbol 12-myristate 13-acetate (PMA) for 4 hrs. and 12 hrs. It was observed that both PMA and IL-1β increased the secretion of AGT in HepG2 cells while such an effect was not pronounced in Huh7 cells. IL-6 treatment also increased the level of AGT secretion in HepG2 compared to Huh7 cells. It is concluded that human AGT which is a class II acute phase response protein can be modulated by NF-κB activation, and HepG2 hepatocytes are sensitive to NF-κB modulation whereas Huh7 hepatocytes are not.

**Modification of Pattern Recognition Receptor Expression As a Potential Mechanism of GI Toxicity**

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Gastrointestinal toxicity is a common adverse effect of oncology drugs. Multiple mechanisms of toxicity are likely, particularly from kinase inhibitors, and include inhibition of proliferation, and changes in cell-cell adhesion, migration or differentiation. Epithelial cells of the gastrointestinal tract interact with the resident gut microbiota via pattern recognition receptors (PRR), an interaction which helps maintain barrier integrity of the epithelial lining. Using IEC-6 cells as a model for the crypt cell population of the small intestine and IPEC-J2 cells as a model for the intestinal enterocyte population, we examined the gene expression of two PRRs, toll-like receptors (TLR) 2 and 4, after 48 or 72 hour in vitro exposure to the inflammatory cytokines (TNFα and IFNγ) or 5 kinase inhibitors.

**Role of Hypoxiainmimetics in Regulation of Angiotensinogen in Human Hepatocytes**

R. A. Ansari, M. R. Karim, S. A. Rizvi and M. A. Clark. *Pharmaceutical Sciences, Nova Southeastern University, Fort Lauderdale, FL.*

Alcohol usage is linked to increased blood pressure and fibrotic transformation of the liver. Angiotensinogen (AGT), which produces angiotensin II (Ang II), an octapeptide, is involved in blood pressure regulation and fibrotic transformation of the liver after the death of hepatocytes. Ang II is produced from its precursor, AGT, by sequential activation of renin followed by angiotensin converting enzyme (ACE). The levels of AGT are less than the Michaelis-Menten constant (Km) of renin. Therefore, an increase in blood AGT levels would result in a corresponding increase in Ang II levels that might play crucial role in blood pressure regulation and fibrogenic activity. Alcohol metabolism by the liver produces oxidative stress that activates hypoxia inducible transcription factor-1alpha (HIF-1α). In addition, alcohol-mediated hepatic injury produces immune responses resulting into increased levels of interleukin-1β and other cytokines. IL-1β activates nuclear factor-kappa B (NF-κB) transcription factor. Since hepatocytes lose the ability to metabolize alcohol after several passages, in order to mimic the alcohol mediated HIF-1α activation, hypoxia mimetics were employed to investigate the effect of HIF-1α activation on AGT secretion.

**MicroRNAs (miRNAs) regulate nearly 60% of cellular protein expression and are subject of intense research in both the therapeutic and diagnostic areas.** Circulating miRNAs associated with proteins, exosomes and vesicles are proving to be highly sensitive and transplantable biomarkers of drug-induced injury. With the advancing discovery of novel biomarkers, there is a growing need for sensitive and specific extraction and quantification methods. RT-qPCR has gained acceptance as a robust and reliable transcriptomic method to profile subtle changes in miRNA levels. For interpretation of results and to allow comparison between studies, the choice of a suitable reference miRNA to use as an internal control is a crucial factor.

To date, there has been no systematic evaluation of RT-qPCR reference genes for the study of miRNAs expression in whole blood from pre-clinical species. In this study, the expression of two housekeeping miRNAs in whole blood, miR16 and miR103, selected from the literature, has been validated using two RT-qPCR kits: miRCURY LNA (Exiqon) and miScript (QIagen). After comparison of the kits, the expression of these miRNAs was assessed in male and female animals from five pre-clinical species: rat, mouse, dog, mini-pig and primate. Differences were found between the two kits for the quantification of miRNA. The Qiagen kit was a robust method for analysis of miRNA with no inhibition from the matrix. The Exiqon kit was much more sensitive, with a quantification limit at 2 x 10-5 ng but under comparable conditions it was more prone to PCR inhibition compared to the Qiagen kit. miR16 and miR103 were quantified with highly similar Ct values ranging from 14.9-18.3 for miR16 and 16.9-22.7 for miR103 with rodents consistently expressing slightly lower levels than the other species. To investigate the suitability of each gene as a housekeeping gene, circulating miRNAs were collected from routine toxicology studies. These data demonstrate the suitability of miR-16 and miR-103 over the more commonly referenced U6 small nuclear RNA using the Exiqon kit.
The prediction of xenobiotic exposure leading to long-term adverse reactions, such as off-target toxicity, cell death, and/or carcinogenesis, has been aggressively pursued in the toxicological and pharmaceutical communities. However, the development of computational models coupled with high-throughput screening assays capable of not only predicting a toxic outcome (e.g., cell death) but also the mode(s) of action contributing to this eventual fate have yet to fulfill these advantageous expectations. Previously, we have shown that 24 hour in vitro cell death can be forecasted at significantly early time-points by exploiting the rapid, endogenous pharmacodynamic response to xenobiotic exposure. This approach utilized protein phosphorylation responses of key proteins found in cellular death and survival pathways at time points relevant to critical signaling events to forecast the eventual cellular fate, irrespective of xenobiotic mode of action(s). However, the mode(s) of action contributing to the cell's predetermined fate remains elusive. To this end, we propose a graph theoretical approach to decode the early networked phosphoprotein response of select proteins following xenobiotic insult. In this proof-of-concept study, we monitored the phosphoprotein response of HepG2 cells exposed to TDZD-8 (GSK3 inhibitor) for a short amount of time (40 minutes) and a longer period of time (10 hours). Euclidean distances of these responses were used as weighted edges (protein-protein theoretical links) to compute the graph theoretical parameter, radiality, for each node (protein) measured. Radiality-based dose-response curves were then constructed to identify proteins related to alterations in network activity following TDZD-8 exposure. From this approach, the potential mode(s) of action at critical time points related to signaling events that are responsible for the cell's predetermined fate (i.e., cell death) may be elucidated, which will greatly impact xenobiotic high-throughput risk assessment in vitro as well as pharmaceutical adverse drug reaction analyses.

**PS 1730** Regulation of Airway Mucin Gene Expression by Aspergillus


Environmental mold exposure causes or exacerbates respiratory disease and it is one of the most deleterious environmental hazards that could impact on our health. Aspergillus (ASP) is one of the most prevalent types of molds that are found indoors and outdoors. Bronchiectasis is the prominent symptom resulted from ASP exposure. Airway obstruction caused by mucus over-production and the damage to the tracheobronchial walls are the hallmark of the bronchiectasis. Since epithelial injury and abnormal repair has been linked to mucus overproduction, we test the hypothesis that ASP damages airway epithelial cells to induce growth factor secretion and the activation EGFR pathway, which in turn elevates mucin expression and mucus production. In our study, ASP activated both Reactive Oxygen Species (ROS) generation and Calcium flux through a protease-dependent but PAR-independent pathway. ROS was critical for the induction of cell death, which Calcium flux was indispensable for mucin increase via a novel PKC-ERK pathway. Interestingly, this pathway was mostly EGFR-independent. Thus, delineating this novel mechanism will lead to the development of effective diagnostic and therapeutic reagents for the pulmonary diseases resulted from environmental mold exposure.

**PS 1731** An Approach to Investigate Intracellular Protein Network Responses

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Modern toxicological evaluations have evolved to consider toxicity as a perturbation of biological pathways or networks. As such, toxicity testing approaches are shifting from common endpoint evaluations to pathway based approaches, where the degree of perturbation of select biological pathways is monitored. These new approaches are greatly increasing the data available to toxicologists, but methods of analyses to determine the inter-relationships between potentially affected pathways are needed to fully understand the consequences of exposure. An approach to construct dose-response curves that use graph theory to describe network perturbations amongst three disparate mitogen-activated protein kinase (MAPK) pathways is presented. Mitochondrial stress was induced in human hepatocytes (HepG2) by exposing cells to increasing doses of the complex I inhibitor, rotenone. The relative phosphorylation responses of proteins involved in the regulation of the stress response were measured. Graph theory was applied to the phosphorylation data to obtain parameters describing the network perturbations at each individual dose tested. The graph theory results depicted the dynamic nature of the relationship between p38, JNK, and ERK1/2 under conditions of mitochondrial stress, and revealed shifts in the balance of these MAPK pathways at low doses. The inter-relationship, or crosstalk, amongst these 3 traditionally linear MAPK cascades was further probed by co-exposing cells to rotenone plus SB202470 (JNK and p38 inhibitor) or rotenone plus SB202474 (JNK inhibitor)). The cells exposed to rotenone plus SB202474 resulted in significantly decreased viability, which could be visualized and attributed to the decrease of ERK1/2 network centrality. The approach presented here allows for the construction and visualization of dose-response curves that describe network perturbations induced by chemical stress, which provides an informative and sensitive means of assessing toxicological effects on biological systems.

**PS 1732** The microRNA Pathway in Blast-Induced Neurotrauma

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A complex genetic response following traumatic brain injury (TBI) is associated with lingering neurologic and cognitive problems during neuroregeneration and mild TBI recovery. miRNAs, a class of endogenous non-coding small RNAs, have been identified as a major gene regulator in most biological and metabolic processes. Abrupt expression of miRNAs is found in neurodegenerative diseases and other CNS disorders. Therefore, a dynamic miRNA network regulating TBI-induced gene expressions may represent the molecular signature of TBI pathophysiology. A mild brain injury rodent model was generated using an advanced blast simulator. As an acute response to 70 kPa over ambient pressure, the mRNA expressions of vitamin D receptor, progesterone receptor, and all selected inflammatory parameters were misregulated in blood and brain tissues. Our preliminary work has also histologically verified the mild brain injury and evaluated the memory and cognitive deficits using the Morris Water Maze. Compared to shams, intraventricular hemorrhages are common observations and blast-injured animals showed cognitive deficits and signs of depression behaviors. Twenty four miRNAs display aberrant expression in blood of blast-injured animals collected seven days post-blast. miRNA markers target various neurological, inflammatory, and endocrinological pathways.

**PS 1733** The Effect of Thiazolidinediones on Cell Metabolism

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Several first generation thiazolidinediones used to treat diabetes have been either withdrawn (troglitazone) or have black box warnings (rosiglitazone and pioglitazone) due to toxicity. We have studied 4 thiazolidinediones: troglitazone, rosiglitazone, pioglitazone and WY14621 using a novel In-Cell Proteomics method that monitors the change in levels of, and altered post-translational modifications in, key proteins of metabolism. This method simultaneously shows the therapeutic effects of the drugs as well as adverse events likely due to toxicity. Treating HepG2 cells with 100uM of each for 6, 24 or 72h, all compounds have effects consistent with activation of PPARγ; they up-regulate both mitochondrial and peroxisomal lipid metabolizing enzymes. All but WY14621 also up-regulate levels of the enzymes of oxidative phosphorylation, Krebs cycle and pyruvate dehydrogenase consistent with PPARγ activators. Increased phosphorylation of S6 ribosomal protein (5-7 fold) over DMSO treated cells was observed, as would be expected given the enhanced protein synthesis. There were also increased levels of IFNγ, HSP90, SOD2 and catalase, but not SOD1, indicative of activation of the unfolded protein response and oxidative stress. Interestingly, troglitazone differs from the other compounds. In HepG2 cells this compound caused apoptosis with a strong reduction in the level of both MCL1 and PUMA, increases CHOP1 levels, indicative of strong oxidative stress, dephosphorylation of 4E-BP1, and up-regulation of beclin 1 at 6 but not 72h suggesting an attempted autophagy. Interestingly, when examined in cardiomyocytes, troglitazone shows all of the proposed therapeutic effects but does not induce apoptosis, and there is no increase in levels of Chop1, phospho-4E-BP1 or beclin 1, suggesting reduced cell stress compared to HepG2 cells. These results show the utility of a broad screen of metabolism by protein changes in identifying the different effects of compounds in a particular class and deciding between therapeutic versus toxic reactions.
Inhibition of toxicants such as tobacco smoke, air pollution and those from industrial processes (e.g. arsenic) can lead to respiratory dysfunction including Chronic Obstructed Pulmonary Disease (COPD). The respiratory epithelium provides the first line of defense against inhaled insults. The cells that comprise the airway epithelium coordinate innate immune defenses and initiate adaptive immune responses. Although it is well known that toxicant-induced COPD can result in part from dysfunctional airway epithelial cellular physiology, the mechanisms by which this occurs remain elusive. Traditional cellular toxicity assays represent an end point in toxicity analysis, however, diseases commonly progress with compromised cellular physiology in lieu of cell death. To better assess cell-compromising toxins, we have used the xCELLigence Real-Time Cell Analyzer (RTCA). RTCA is a high-throughput screening format (6x96-well plate) that uses impedance measurements to detect physiological changes in adherent cells. We monitored human bronchial epithelial cells for changes in response to paracrine signaling molecules critical to airway epithelial innate immunity (e.g., ATP). Responses in untreated cells were compared to responses in cells treated for 4 - 24 hrs with arsenic, nanoparticles, or tobacco product constituents. We found that these respiratory toxicants induced dose-dependent alterations of physiological responses. Importantly, this cell signaling toxicity was measured at doses much lower than required to observe cytotoxicity. Findings from the RTCA experiments were confirmed with more traditional cell signaling assays (e.g., digital imaging microscopy of Ca²⁺ signaling). In conclusion, the RTCA device offers a high-throughput approach to screening cells for toxic/toxicant-induced dysfunction independent of cell death and potentially important in toxic/toxicant-induced disease.

Forecasting Cell Death Dose-Response from Early Signal Transduction Responses In Vivo
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The rapid pharmacodynamic response of cells to toxic xenobiotics is primarily coordinated by signal transduction networks, which follow a simple framework: the phosphorylation / dephosphorylation cycle mediated by kinases and phosphatases. However, the time course from initial pharmacodynamic response(s) to cell death following exposure can have a vast range. Viewing this time lag (between early signaling events and the ultimate cellular response) as an opportunity, we hypothesize that monitoring the phosphorylation of proteins related to cell death and survival pathways at key, early time-points may be used to forecast a cell’s eventual fate, provided that we can measure and accurately interpret the protein responses. In this presentation, we focus on a three-phased approach to forecast cell death exposure: 1) determine time-points relevant to important signaling events by exploiting the relationship between mitochondrial-driven energy metabolism and kinase response to estimate theoretical ATP production, 2) experimentally determine phosphorylation values for proteins related to cell death and/or survival pathways at these significant time-points, and 3) estimate the 24 hour plasma membrane degradation dose-response of cells to xenobiotic exposure from protein cluster analyses. To test this approach, we exposed HepG2 cells to two disparate treatments: a GSK3β inhibitor and a MEK inhibitor. After using our three-phased approach, we were able to accurately forecast the 24 hour HepG2 plasma membrane degradation dose-response from protein phosphorylation values as early as 20 minutes post-MEK inhibitor exposure and 40 minutes post-GSK3β exposure.

TC-PTP Inhibits UVB-Induced Keratinocyte Survival and Proliferation by Regulating Stat3 Signaling
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Ultraviolet B radiation (UVB) contributes to the development of skin cancer by creating DNA damage that can yield gene mutations which disrupt intracellular signaling mechanisms. One vital signaling mechanism is tyrosine phosphorylation signaling. We have previously shown that T-cell protein tyrosine phosphatase (TC-PTP) contributes to the rapid dephosphorylation /deactivation of Stat3 in response to UVB irradiation in keratinocytes. In the current work, we investigated the functional role of TC-PTP in keratinocyte survival and proliferation following UVB exposure. Knockdown of TC-PTP in mouse keratinocytes using siRNA significantly reduced apoptosis following UVB irradiation in comparison with control keratinocytes as evidenced by the decreased levels of activated caspase-3 and PARP cleavage. This reduction corresponded with an increased level of phosphorylated Stat3 in TC-PTP-deficient keratinocytes. Knockdown of TC-PTP in keratinocytes also significantly increased cell proliferation following UVB irradiation compared with control keratinocytes. Similar with these results, overexpression of TC-PTP in keratinocytes significantly increased apoptosis and decreased cell proliferation in response to UVB irradiation compared with control keratinocytes, which corresponded with a decreased level of phosphorylated Stat3. Treatment of TC-PTP-deficient keratinocytes with specific Stat3 inhibitor, STA21 significantly reduced cell viability, upon UVB exposure in comparison with untreated TC-PTP-deficient keratinocytes, which suggests that the effect of TC-PTP on cell viability is mediated by Stat3 dephosphorylation. Combined, our results indicated that TC-PTP plays an important role in the regulation of keratinocyte proliferation and survival after UVB irradiation. These results suggest that TC-PTP may be a novel potential target for the prevention of UVB-induced skin cancer.
activate CYP2C9 transcription in response to electrophiles. These results indicate that oxidative stress generated by exposure to electrophilic xenobiotics and metabolites induces the expression of CYP2C9, thereby altering the metabolic clearance of therapeutic drugs and xenobiotics which are substrates of CYP2C9.

**1736c Dose-Specific Nicotine-Dependent Behaviors in Caenorhabditis elegans: Regulation of Nicotinic Acetylcholine Receptors**

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Nicotine, the major psychoactive compound in tobacco, targets the nicotinic acetylcholine receptors (nAChRs) and leads to drug dependence. The nematode Caenorhabditis elegans (C. elegans) genome encodes conserved and extensive nicotinic receptor subunits, representing an excellent system to investigate nicotine-induced cholinergic signaling in the context of drug addiction. However, the in vivo expression pattern of nAChR genes under chronic low-level nicotine exposure has not been systematically investigated in C. elegans. Here, we have reported the first in vivo expression of the 28 authentic nAChRs at the mRNA level following nicotine exposure in C. elegans. Chronic (24 hour) low-level (6.17-6.7 μM) nicotine exposure has been shown to change the expression of nicotinic receptors in a dose-specific manner. Worms exposed to 6.17 and 6.7 μM nicotine displayed nicotine-dependent behaviors. Quantitative real-time PCR results showed that five genes (lev-1, acr-6, acr-7, lev-8, and acr-14) were significantly up-regulated at 61.7 μM nicotine treatment, with worms showing significant withdrawal behaviors when the nicotine supply was discontinued. However, the 19.5 μM dose worms did not exhibit the withdrawal behavior, which was accompanied by a general increase in nAChRs expressions. We have also predicted conserved microRNAs that may target these receptor genes using online algorithm tools. Quantitative real-time PCR results showed that several predicted miRNAs were co-expressed with their target nAChR genes, including let-7, mir-355, mir-70, and mir-71. This study provides useful information regarding the comprehensive in vivo expression pattern of the 28 “core” nAChRs and its relationship with addiction behaviors.

**1736d Nrf2 Protects Mitochondrial Degeneration by Oxidative Stress**

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Oxidative stress plays an important role in cellular injury. Cells surviving oxidative stress often become enlarged with increased protein content. In the myocardium, enlargement of cardiomyocytes consequential to tissue injury contributes to mal-adaptive remodeling and subsequent development of heart failure. Here we address the role of mitochondria in cells surviving oxidative stress and becoming enlarged using primary cultured rat cardiomyocytes as an experimental model. These cells survive low to mild doses of H2O2 but develop hypertrophy. In control cells, mitochondria exist in elaborate and elongated networks. Within 24 hours after exposure to a mild dose of H2O2, the mitochondrial network was replaced by predominately individual, punctate mitochondria. Electron microscopy revealed H2O2-induced swelling of mitochondria with disorganized cristae and areas of condensation. Measurements of functional mitochondria showed a H2O2 dose-dependent (50 to 400 μM) decrease over a course of 5 days. At the protein and mRNA levels, H2O2 treatment resulted in a reduction of mitochondrial components, cytochrome c and cytochrome b. Nrf2 overexpression prevented H2O2 from inducing mitochondrial morphological changes and the reduction of cytochrome c and cytochrome b. Although Nrf2 is known as a transcription factor regulating antioxidant and detoxification genes, Nrf2 overexpression did not reduce the level of protein oxidation as measured by carbonyl formation. We found that Nrf2 localizes to the outer mitochondrial membrane, suggesting a direct role in mitochondrial protection. Mitochondria prepared from the myocardium of Nrf2 knockout mice are more sensitive to permeability transition. Correlating with the protective effect of Nrf2 against mitochondrial decay, Nrf2 overexpression prevented H2O2 from inducing cardiomyocyte hypertrophy. These data suggest that mitochondrial decay due to oxidative stress contributes to cardiomyocyte hypertrophy and Nrf2 protects mitochondria from oxidant injury likely through direct interaction with mitochondria membrane.

**1736e RNA-Seq Analysis of the Functional Link between Vascular Disruption and Adverse Developmental Consequences**

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Vasculogenesis and angiogenesis are complex processes necessary for normal development. High throughput-screening (HTS) efforts by the US EPA ToxCast program identified a diverse set of environmental chemicals that potentially disrupt biological targets associated with blood vessel formation and/or remodeling. To investigate embryonic responses to vascular disruption, gestation day 10 rat embryos were exposed in vitro to anti-angiogenic reference compounds 5HPP-33 (5, 15, 30 μM) or TNP-470 (0.25, 2.5, 25 μM) and vehicle controls. The major effects elicited after 48 h were embryo lethality (5HPP-33 ≥ 15 μM) and dysmorphogenesis (TNP-470 ≥ 0.25 μM). RNA-Seq was conducted on RNA from embryos and yolk sacs (n= 8 embryos or 4 pools of 2 yolk sacs per concentration) at 4 h post-exposure, a time point selected to identify early transcriptional responses. Patterns of differentially expressed genes (DEGs) were tissue and concentration-dependent (apical vs transcriptional responses). Unsupervised hierarchical clustering of embryonic DEGs showed that transcriptional responses at the highest concentration of 5HPP-33 were similar to TNP-470 at any concentration. Pathway analysis revealed that the most significantly enriched pathway for exposures eliciting overt phenotypes, 5HPP-33 (30 μM) and TNP-470 (0.25, 2.5 and 25 μM) was the cellular p53 response pathway. Concentration-dependent effects were observed in embryonic DEGs directly related to known vasculogenesis targets (i.e. VCAM1, TNF, CASP8, HIF1A, AHR). These data demonstrate the importance of p53 signaling in developmental angiogenesis and support the hypothesis that conserved vascular disruption pathways can lead to developmental toxicity outcomes of with diverse phenotypes. The identification of altered vascular regulatory networks perturbed by developmental toxicants supports an adverse outcome pathway associated with disruption of the embryonic vasculature. This abstract does not necessarily reflect US EPA policy.

**1737 Developmental Neurotoxicity Assessment in a 3D Organotypic Neuronal Model Using a Metabolomics Approach**

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The National Research Council report from 2007 “Toxicity Testing in the 21st Century: A vision and a strategy” (Tox-21c) has created an atmosphere of departure in the US. It suggests moving away from traditional animal testing to modern technologies based on pathways of toxicity. An area of toxicology where Tox-21c could have a significant impact is developmental neurotoxicity (DNT). There is concern that exposures to environmental chemicals contribute to the increasing incidence of neurodevelopmental disorders in children. However, due to lack of DNT studies only very few substances have been identified as developmental neurotoxics. Moving towards a mechanistic science can help us identify the perturbed pathways that likely lead to these adverse effects. A 3D rat primary organotypic neuronal model was exposed from day 7 up to 21 to suspected (developmental) neurotoxicants including pesticides, drugs and metals. Mass spectrometry based metabolomics measurements were performed and quantitative measurements of genes expressed in different cell types (neural precursor cells, neurons and glial cells). Mass spectrometry analysis showed differences in metabolic levels between control and treated cells in a concentration dependent manner. Signatures of changed features have been putatively identified and associated to perturbed pathways supported by gene expression data. For example metabolites involved in the biochemical pathway of the neuronal specific metabolite N-acetyl aspartate (NAA) (malate, aspartate, NAA and glutamate) were significantly decreased after exposure to lead chloride and maneb. In patients, the level of NAA has shown to be decreased in numerous neuropathological conditions such as brain injury, stroke and Alzheimers. It also indicates neuronal/zonal loss or compromised neuronal metabolism. Obtained data suggests that metabolomics could be a promising tool for developmental neurotoxicity assessment. Funded by the FDA #U01FD004230.
This work aims to develop a human 3D brain model to identify changes in transcriptome, including microRNA expression (miRNomics), and metabolome after exposure to neurotoxicants. The LUHMES cell line differentiates rapidly and homogeneously into dopaminergic neurons (DNs) in traditional monolayer cultures. Knowing the fact that 3D culture conditions better mimic the in vivo response, we developed a 3D human neuronal model using LUHMES cells and constant gyration shaking culture condition. The LUHMES cells have shown prolonged survival when cultured 3D compared to traditional monolayers. The differentiation in 3D aggregates was characterized by qRT-PCR, flow cytometry and immunocytochemistry. We observed increase of NeuN positive cells (mature neurons) and decrease Ki-67 positive cells (proliferating cells) in the course of differentiation by flow cytometry. During early stages of differentiation, 3D cultures did not contain any apoptotic cells. However, up to 30% of the cells within aggregates were apoptotic (Annexin V/caspase3 positive) at later time points (15-21 days) that could be reduced by decreasing the size of aggregates. We observed strong induction in gene expression of neuronal markers (β-III-Tubulin, DAT, synaptin 1, and TH) as well as neural-specific/enriched miRNAs (mir-124, mir-126, mir-132, mir-133b, mir-137). To demonstrate the model’s suitability for neurotoxicity testing, the aggregates were treated for 24 and 48 hours on day 6 and 12 of differentiation with dopaminergic neurotoxicants, MPP+ and rotenone. Perturbations in energy metabolism and stress response following mitochondrial complex 1 inhibition was characterized by LC-MS based metabolomics, transcriptomics, miRNA profiling and mitochondrial function assay. In conclusion, we have successfully developed a new 3D human model that can be used for (developmental) neurotoxicity testing.

**Development of Calcium Responses and Electrical Activity in Differentiating Mouse Neural Progenitor Cells In Vitro**

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In vitro methods for developmental neurotoxicity (DNT) testing have the potential to reduce animal use and increase insight into mechanisms of chemical-induced alterations in the development of functional neuronal networks. Mouse neural progenitor cells (mNPCs) have proven valuable to detect DNT using biochemical and morphological techniques. However, their neurophysiological characteristics are still largely unknown.

We have therefore investigated calcium responses and electrical activity in primary mNPCs (using the Ca2+-responsive dye Fura-2 and a multi-electrode array system, respectively) to explore their applicability for DNT testing with focus on neuronal function.

Immunocytochemistry reveals that mNPCs express neuronal, glial and progenitor markers at various differentiation durations. Stimulus-evoked changes in intracellular calcium concentration ([Ca2+]i) were investigated at 1, 7, and 14 days of differentiation. Increases in [Ca2+]i are evoked by depolarization, acetylcholine, and L-glutamic acid, dopamine, adenosine triphosphate and, to a lesser extent, by se-rotonin and gamma-aminobutyric acid. Notably, the percentage of responsive cells and response amplitudes indicate changes in the expression and functionality of related neurotransmitter receptors and calcium signaling pathways during in vitro differentiation. The development of functional intercellular signaling pathways is confirmed in mNPCs cultured on multi-electrode arrays, demonstrating that mNPCs develop spontaneous electrical activity within 1-2 weeks of differentiation. These data demonstrate that mNPCs develop functional neuronal characteristics in vitro, making it a promising model to study chemical-induced effects on the development of neuronal function.

This work is funded by the European Union [DENAMIC project; FP7-ENV-2011-282957] and The Netherlands Organization for Health Research and Development (ZonMw) [85300003].
The enzyme CYP1A2 can sequester toxics such as dioxins and coplanar PCBs. The aryl hydrocarbon receptor (AHR) bind coplanar PCBs, initiating transcription of several genes including CYP1A2, a key detoxifying enzyme. Though it is also reportedly expressed in the cortex and cerebellum of the brain, its physiologic function in the brain remains unknown. Previous work in our lab uncovered learning and memory deficits in Cyp1a2(-/-) knockout mice. Our current work compares Ahr/Cyp1a2(-/-), Ahr/Cyp1a2(-/-), and Ahr/Cyp1a2(-/-) mice exposed during gestation and lactation to PCBs or the corn oil vehicle. Pregnant dams were treated from gestational day 0 (GD0) to postnatal day 25 (PND 25). We compared the three genotypes of mice using a battery of six tests: rotarod, gait analysis, sticker removal, pole climbing, balance beam, and grip strength. There was a significant main effect of treatment on all 5 days of testing (P<0.05) and a significant genotype x treatment interaction on Day 4 of testing (P<0.05) with PCB-treated Ahr/Cyp1a2(-/-)/mice showing the greatest impairments. There was a significant main effect of genotype and treatment in gait analysis with PCB-treated animals and Ahr/Cyp1a2(-/-) mice having significantly longer stride lengths (P<0.05). There were no significant differences in the pole test. Both Cyp1a2(-/-) knockouts had significantly shorter latencies to traverse the beam (P<0.001). Further analysis will be required to determine if this indicates a difference in motor function or greater motivation to reach the safety of the darkened goal box.

1745 PFOSInducesBehavioralAlterationsCompatiblewithADHDin aZebrafishModelofDevelopmental Neurotoxicity

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Background: Perfluorooctane sulfonate (PFOS) is a widely spread environmental contaminant. It accumulates in the brain and has potential neurotoxic effects. The exposure to PFOS has been associated with higher impulsivity and increased ADHD prevalence.

Objectives: We investigated the effects of developmental exposure to PFOS in zebrafish larvae, focusing on the modulation of activity by the dopaminergic system.

Methods: We exposed zebrafish embryos to 0.1 or 1 mg/L PFOS and assessed swimming activity at 6 dpf. We analyzed the structure of spontaneous activity and the acoustic startle response. In addition, we investigated the effects of D-amphetamine on the alterations in spontaneous activity and startle response in zebrafish larvae. Results: We found that zebrafish larvae exposed to 1 mg/L PFOS displayed a disorganized pattern of spontaneous activity consisting of less frequent, but more intense bouts of activity as compared with controls. In addition, the startle response is followed by persistent hyperactivity. D-amphetamine partly corrected the phenotype in zebrafish larvae exposed to 1 mg/L PFOS.

Conclusion: Developmental exposure to PFOS induces hyperactivity mediated by a dopaminergic deficit that can be corrected by D-amphetamine. Our data support the association of PFOS exposure with impulsivity and ADHD in children.

1746 Early Embryonic2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) ExposureDisruptsForebrainandCerebralVascularDevelopmentin Zebrafish

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TCDD is a persistent environmental contaminant that exerts toxicity by activating the aryl hydrocarbon receptor (AHR) signaling. Using fluorescent-immunohistochemistry (F-IHC) and confocal microscopy, we examined TCDD-exposed and control zebrafish brains and found that TCDD exposure disrupts forebrain and cerebral vascular development. Within the forebrain, the olfactory bulbs (OB) and habenular nuclei were smaller and had reduced expression of activated leukocyte cell adhesion molecule, an axonal marker. F-IHC staining with a synaptic marker (synaptic vesicles) revealed disorganization within the OBs. We examined cerebral vasculature at 48, 72, 96, and 120 hours post fertilization (hpf) using a flk1:EGFP endothelial reporter line. To visualize vascular morphology, we collected a series of optical sections and generated brightest point projections using Olympus software. Cerebral vasculature malformations were identified in the fore-, mid- and hindbrains of TCDD-exposed embryos at all times examined. The presence of phenotypes at 48 hpf, prior to decreases in cardiac output, indicate that TCDD can impact vascular development independent of cardiac function. We also found that exposure to a sub-lethal level of TCDD (50 ppt) disrupts cerebral vasculature development. We blocked atrh translation using atrh1 and atrh1 morpholinos and observed fore- and midbrain vascular malformations. These phenotypes in zebrafish are consistent with AHR's role in mammalian vascular development. A common mechanism mediating TCDD-induced toxicity is downregulation of the transcription factor, sox9b. We are examining sox9b as a potential TCDD target in the developing nervous system and have found that loss of sox9b results in severe brain and vascular phenotypes. Thus, TCDD exposure disrupts brain and vascular development. Whether disrupted cerebrovascular development caused by fetal TCDD exposure in humans plays a role in neurodevelopmental disorders and cognitive impairment remains to be determined. (Supported by NIH ES012716).

The enzyme CYP1A2 can sequester toxics such as dioxins and coplanar PCBs. The aryl hydrocarbon receptor (AHR) bind coplanar PCBs, initiating transcription of several genes including CYP1A2, a key detoxifying enzyme. Though it is also reportedly expressed in the cortex and cerebellum of the brain, its physiologic function in the brain remains unknown. Previous work in our lab uncovered learning and memory deficits in Cyp1a2(-/-) knockout mice. Our current work compares Ahr/Cyp1a2(-/-), Ahr/Cyp1a2(-/-), and Ahr/Cyp1a2(-/-) mice exposed during gestation and lactation to PCBs or the corn oil vehicle. Pregnant dams were treated from gestational day 0 (GD0) to postnatal day 25 (PND 25). We compared the three genotypes of mice using a battery of six tests: rotarod, gait analysis, sticker removal, pole climbing, balance beam, and grip strength. There was a significant main effect of treatment on all 5 days of testing (P<0.05) and a significant genotype x treatment interaction on Day 4 of testing (P<0.05) with PCB-treated Ahr/Cyp1a2(-/-)/mice showing the greatest impairments. There was a significant main effect of genotype and treatment in gait analysis with PCB-treated animals and Ahr/Cyp1a2(-/-) mice having significantly longer stride lengths (P<0.05). There were no significant differences in the pole test. Both Cyp1a2(-/-) knockouts had significantly shorter latencies to traverse the beam (P<0.001). Further analysis will be required to determine if this indicates a difference in motor function or greater motivation to reach the safety of the darkened goal box.

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New strategies are needed to address the data gap between the bioactivity of chemicals in commerce and the environmental versus existing hazard information. This data gap is especially wide when considering toxicological endpoints that are difficult to measure purely in vitro systems, such as abnormal behavior. We describe an embryonic zebrafish system where behavior can be both experimentally perturbed (6 doses of chemical and 1-second pulses of light) and measured (accompanying real-time, spontaneous movement) in a high-throughput fashion. This system is applied to characterize the behavioral response to 1060 ToxCast™ chemicals as hyperactive or suppressive to movement of 24 hours-post-fertilization (hpf) zebrafish embryos subjected to intermittent pulses of light. By dividing the experimental interval according to light pulses (Background = prior to first pulse; Excitatory = after first pulse; Refractory = after second pulse), we observe movement responses that can classify neuromodulator chemicals as eliciting light-independent movement alterations (102), light-dependent photomotor responses (231), or both (276). The chemicals can be further subdivided into a discrete number of prototype clusters based upon hypo- and/or hyperactivity patterns across light intervals. As an integrative measure of normal development, significant alterations in movement highlight neuroactive chemicals representing several modes of action. These early behavioral responses are predictive (relative risk r < 0.05) of 17 specific developmental abnormalities including notochord defects and mortality measured at 5 days-post-fertilization (dpf). Therefore, this system can provide for rapid characterization of chemical-elicited behavioral responses at an early developmental stage that are indicative of significant downstream hazard.

1747c Developmental Exposure to PCBs Differentially Alters Sensitivity to Audiogenic and Kindling-Induced Seizures in Rats
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Previously we reported an increased incidence of audiogenic seizures in offspring of rats exposed to an environmental mixture of polychlorinated biphenyls (PCBs). This study compares the proconvulsant properties of PCB exposure in audiogenic and electrical kindling seizure models. Adult male and female offspring exposed to the PCB mixture throughout gestation and lactation were subjected to an audiogenic seizure paradigm (100dB, 8 kHz, 2m) and behavioral seizure response (wild running, clonus) recorded. Male littersmates were stimulated (1×1 train of 60Hz pulses, 1ms pulsewidth, 50-200mA) through indwelling electrodes in the amygdala to establish a threshold for induction of an electrographic afterdischarge (AD). Thereafter, stimulation was delivered once daily at 200μA until a fully generalized seizure (Racine Scale 1) was evoked. Electrographic and behavioral seizure characteristics were recorded in response to each stimulation. Consistent with previous findings more PCB-exposed rats than controls exhibited clonic seizures (76% vs 22%) in response to noise. In contrast to an augmented audiogenic seizure response, PCBs delayed the development of electrical kindling (16.4 vs 11.6 ADs to 1st Stage 5 seizure). This delay occurred in the early stages as significantly more sessions were required to advance rats from focal (Stage 1-2) to generalized (Stage 3-5) seizures. Groups did not differ in AD thresholds. A trend towards longer cumulative AD duration to 1st generalized seizure was also evident in the PCB-exposed group. A dissociation of responsiveness based on seizure model indicates that while developmental PCB exposure has proconvulsant effects at the level of the auditory brainstem, the same exposure slows the progression of behavioral manifestations from a focal seizure site in the forebrain. The latter may be reflective of impaired plasticity mechanisms previously reported in PCB-exposed animals. Does not reflect EPA policy; funded by ES15687.

1747b Impact of Perinatal TCDD Exposure on Neuroendocrine Stress Response System in Mice
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Adversities during the perinatal period can disrupt the development of the neuroendocrine stress response system, which may influence the vulnerability to psychiatric pathology later in life. The increasing trend of a prevalence of developmental and psychiatric disorders has been suspected of being induced at least partly by environmental chemical exposure during perinatal development. We have previously reported the lasting consequences of perinatal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in cognitive, social, and emotional functions in mice. However, its effect on the development of the stress response system has not yet been defined. The current study thus aimed to examine whether an oral administration of 0, 0.6 or 3.0 μg TCDD/kg on GD 12.5 in C57BL/6 mice induces a permanent impact on the stress response system of the male offspring. A series of stress challenges was conducted when they were adults (8-10 weeks), in order to characterize the hypothalamic-pituitary-adrenal (HPA) axis function. The TCDD-exposed offspring exhibited hypoactivity of the HPA-axis as well as disruption in the central feedback regulation, and the endocrine phenotype was especially conspicuous in the higher dose, TCDD3.0 group. A significant reduction of corticotropin-releasing hormone (CRH) receptor gene expression was detected in the hippocampus of the TCDD3.0 adult mice, which was also confirmed in the TCDD3.0 neonatal hippocampus. Additionally, reductions in hippocampal ACTH receptor and glucocorticoid receptor mRNA ratio were observed at the neonatal stage. The present results suggest that developmental programming of the stress response system is susceptible to a perinatal TCDD exposure, and that a reduced gene expression level of central feedback regulating molecules may underlie their exacerbated stress response.
Embryos were observed at 24 and 120 hpf by stereomicroscope for mortality and gross malformations. At 24 hpf, no differences were observed between treatment groups. By 120 hpf, embryos treated with PCB 77 were not significantly different from vehicle controls or untreated embryos. In contrast, there were significant, concentration-related increases in gross malformations and mortality in embryos treated with either PCB 52 or PCB 95, with PCB 95 exhibiting significantly greater potency than PCB 52. These data correlate with the relative potencies of these PCB congeners on RyR activity, suggesting the feasibility of using embryonic zebrafish as a novel in vivo model to study the developmental effects of RyR-active PCBs. Supported by NIH (grant ES014901 and ES011269).

1747 e Sex-Dependent Effects of Lead and Prenatal Stress on Neonatal Stress Receptor and Adult Corticosterone Levels

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Early life exposure to lead (Pb) and prenatal stress (PS) creates sex-specific alterations in CNS development, including the hypothalamic–pituitary–adrenal (HPA) axis, the major stress response system. The current study examined the potential sex-specific effects of Pb ± PS on alterations in glucocorticoid (GR:NR3C1) and mineralocorticoid (MR:NR3C2) receptors in neonatal brain and in adult corticosterone levels. C57Bl/6j mice received 0 or 100 ppm Pb water 2 mos prior to breeding through weaning. Half of these mice were exposed to restraint stress 3x/day for 30 min from GD 11–19, while the other half received no stress (NS). This yielded 4 treatment groups: sex: 0-NS, 0-PS, 100-NS and 100-PS. At PND 0 and PND 6, frontotemporal hippocampus and cerebellum were dissected, and the remaining brain used for receptor expression analysis. GR and MR levels were assessed in nuclear and cytosolic brain fractions. Both Pb and Ps significantly reduced nuclear GR levels relative to 0-NS controls in females, whereas only PS (0-PS and 100-PS) significantly reduced nuclear MR levels. Males showed the opposite pattern, wherein Pb (100-NS and 100-PS) showed significantly elevated nuclear GR levels. At PND 60, sex-specific differences were also observed in corticosterone levels. Pb-treated males exhibited blunted baseline corticosterone levels, and PS-treated males exposed to acute handling stress displayed significantly elevated corticosterone responses. In contrast, no Pb ± PS-related changes in corticosterone occurred in females. These results show that early life exposure to Pb ± PS creates sex-specific alterations in stress physiology as found for both neonatal and adult neuroendocrine profiles. Altered corticosterone homeostasis has been associated with impaired cognitive function and neurological damage. Understanding the sex-specific response of the HPA axis to early developmental lead and stress exposure may increase our understanding of gender-specific neurobehavioral diseases. ES021534-01

1748 Persistent Organic Pollutants and Different Types of Pesticides Can Interact during Brain Development to Exacerbate Behavioral and Cognitive Deficits in Mice


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In the field of toxicology the tradition has been to investigate one substance/comound at the time without any thought of possible interactive effects concerning multiple exposures. Humans are continuously exposed to persistent organic pollutants, such as methymercuric (MeHg) and the phenthraconted compound perfluorohexane sulfonate (PFHxS), and different pesticides, such as the organophosphate chlorpyrifos and the organochlorine endosulfan. There is some information present about the developmental neurotoxicity of these individual compounds and we have recently seen that they, individually can induce behavioural disturbances such as deranged spontaneous behaviour and habituation, learning and memory defects and reduced cognitive functions, together with altered function of the cholinergic system, when the exposure occurs during a critical phase of neonatal brain development (PND 10). Therefore, in the present study we investigated how a combined exposure to MeHg and chlorpyrifos as well as a combined exposure to PFHxS and endosulfan could affect behaviour and cognitive function. Adult mice, neonatally exposed to 0.4 mg MeHg/kg bw and chlorpyrifos (5 or 10 mg/kg bw), showed a disturbed spontaneous behavior to a novel home environment and reduced habituation compared to the control animals and the individual exposures. The same was seen in adult mice neonatally exposed to PFHxS (6.1 or 9.2 mg/kg bw) and endosulfan (0.05 or 0.1 mg/kg bw). Interestingly, these effects seem to be of a synergistic nature, because effects were even seen for combinations of doses where the individual doses did not induce disturbances in spontaneous behavior to a novel home environment. These results indicate that the present methods of risk assessment can underestimated the potential risk with chemicals, since combinations of chemicals potentially can shift the dose-response curve.

1749 Adult Dose-Response-Related Behavioral Effects of 4 Different Pesticides, after Neonatal Exposure


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There are several different types of pesticides globally used, all with their own characteristics and toxicological potency. In the present study we have exposed male mice neonatally to different doses of four different types of pesticides, car-baryl (carbamate) chlorpyrifos (organophosphate), cypermethrin (pyrethroid) and endosulfan (organochlorine), and tested them for spontaneous behavior in a novel home environment at adult age. The doses used were 0.5 – 20 mg carbvaryl/kg bw, 0.1 – 5.0 mg chlorpyrifos/kg bw, 0.1 – 5.0 mg cypermethrin/kg bw and 0.05 – 20 mg endosulfan/kg bw. All four pesticides induced adult disturbances in the spontaneous behavior in a novel home environment, affecting cognitive function, at 2 months of age. Carbaryl induced a dose-response related effect on spontaneous behavior from 5 mg/kg bw and up, while chlorpyrifos only induced a weak effect with the highest dose tested (5 mg/kg bw). The pyrethroid cypermethrin induced dose-response related neurotoxicity from 0.5 mg/kg bw and up. The organochlorine endosulfan also induced dose-response related neurotoxicity from 0.1 mg/kg bw and up. These disturbances also persisted when the animals were re-observed at 4 months of age, indicating that these effects are long-lasting or even irreversible. From this study we conclude that endosulfan seem to be the most potent, of these four compounds, to induce cognitive behavioral effects in the adult after neonatal exposure, while carbvaryl has the lowest potency to induce these types of neurotoxic effects.

1750 Developmental Exposure to Levels of Chlorpyrifos That Do Not Inhibit Brain Cholinesterase Increases Decreased Emotional Reactivity in Rodents

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Traditionally, chlorpyrifos (CPS) mediates its toxicity through inhibition of cholinesterase (CHE). However, in recent years, the toxicological effects of developmental CPS exposure have been attributed to an unknown non-cholinergic mechanism of action. We hypothesize that the endocannabinoid system may be an important target because of its vital role in nervous system development. We have previously reported that repeated exposure to 0.5 mg/kg CPS results in the inhibition of fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide (AEA), without measurable inhibition of CHE. This exposure resulted in the accumulation of AEA in the forebrain of juvenile rats. Current literature indicates that FAAH activity and AEA levels play an important role in the regulation of emotional reactivity. However, it is unclear if our observed changes in these parameters results in any changes in emotional reactivity. To determine this, 10 day old rat pups were exposed daily for 7 days to either corn oil or 0.5, 0.75, or 1.0 mg/ kg CPS by oral gavage. On PND25, the rats were placed into a dark container in a novel open field and the latency to emerge from the container was measured. In this test, rats that stay in the dark for a long time are considered emotionally reactive. All CPS treated groups spent significantly less time in the dark prior to emerging as compared to control suggesting a decreased level of emotional reactivity induced by CPS exposure. Although not directly correlated, our data suggest that the alteration of endocannabinoid signaling during developmental exposure can lead to alteration of behavioral function.

1751 Zebrafish Brain Development Is Altered by Early Exposure to the Organophosphate Chlorpyrifos

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Pesticides are found ubiquitously in the environment because of their wide use in agriculture. While adults have enzymes that are able to break down pesticides, developing embryos lack the necessary enzymes for toxicant breakdown. Low doses of organophosphate pesticides during early embryonic development in animal models and humans have been documented to affect brain development and behavior. Because embryos are more vulnerable to pesticides, it is important to determine at which stages of development embryos are most affected by the exposure to organophosphate pesticides. Using zebrafish as a model system for pesticide exposure is advantageous because embryos can be exposed to organophosphates immediately after fertilization and large numbers of larvae can be used for high-throughput behavioral analysis and brain imaging. Using a high-throughput assay developed in our lab we have shown that low doses of a widely used organophosphate, chlorpyri-fos (even in very low doses), affect discrete behaviors if administered during specific developmental time periods. Current studies in our lab utilize a transgenic line of zebrafish, Tg(elavl3:Kaede) for two-photon brain imaging. Embryos from this line
The organophosphorus pesticide chlorpyrifos (CPS) primarily exerts toxicity through inhibition of acetylcholinesterase (AChE). However, several studies hypothesized that additional targets of CPS may be responsible for its developmental neurotoxic effects. Butyrylcholinesterase (BChE) is the evolutionary counterpart to AChE. Both enzymes appear early in nervous system development prior to cholinergic nerve formation, with BChE expressed before AChE. Therefore, we hypothesized that BChE may be an important target of CPS during neuronal development. To model neuronal development in vitro, we used human neural stem cells (NSCs) derived from induced pluripotent stem cells (iPSC). After four days of Brain-derived neurotrophic factor (BDNF) induced neuronal differentiation of iPSC-derived NSCs, BChE activity increased 115%, while AChE activity was unchanged. BChE mRNA expression increased greater than two-fold, whereas AChE mRNA expression was not altered. Treatment of differentiating NSCs with chlorpyrifos (10 μM) continuously during differentiation caused the inhibition of AChE and BChE activity by 82% and 97%, respectively. However, CPS exposure had no effect on cell morphology, viability or the expression of the early neuronal differentiation marker HES5. To determine the role of BChE protein, shRNA was utilized to knock down expression of BChE. Preliminary results indicate a 74% decrease in HES5 after BChE knockdown, which suggests that BChE may play a role in the differentiation of NSCs independent of, or in addition to its enzymatic activity. Support provided by: R01ES051991 and P30ES050922.

1753 Ketamine Induces Neurotoxicity in the Developing Brain

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Ketamine, a non-competitive N-methyl-D-aspartate receptor (NMDA-R) antagonist, is commonly used to induce anesthesia and analgesia in infants and children undergoing surgery and painful procedures. However, little is known about how ketamine impact the developing nervous system, especially in infants and young children. In our previous studies, we found that ketamine activated aberrant cell cycle reentry and induced apoptosis by increasing expression of cyclin D1, cleaved-caspase-3 and Bim in vivo. Ketamine also activated the AKT – GSK-3β (glycogen synthase kinase 3β) pathway in the developing rat brain. Based on these, we hypothesize how ketamine affects pathological neurodegeneration the developing rat brain.

Material and Methods: Sprague-Dawley postnatal day 7 (P7) rat pups were used for this study. Doses were given through intraperitoneal doses of either saline or ket at 90 minutes intervals over 6 h. After the 6 h treatment period, the animals were deeply anesthetized with pentobarbital (100 mg/kg, IP). Parts of the brain tissues were extracted and flash frozen in liquid nitrogen for western blotting analysis. The other parts were fixed in 4% paraformaldehyde for immunohistochemical assay, apoptosis such as cleaved-caspase-3, TUNEL, and other protein expression such as Disrupted in schizophrenia-1 (DISC1) and early growth response-1 (EGR-1) were determined by immunofluorescent and western blotting assays.

Results and Conclusion: Ketamine could significant increase neuroapoptosis by increasing apoptosis and cleaved-caspase-3 expression in the rat brain tissues. Ket also significantly decreased the expression of DISC1 and EGR-1, indicating a reduction in developing neurite. These findings suggest that ketamine may induce neurodegeneration by regulating DISC1 pathway in the developing brain.
the developing brains of experimental animals. It has been speculated that the underlying mechanism involves a compensatory upregulation of NMDA receptor subunits that amplifies intracellular calcium concentrations that are beyond the buffering capacity of the mitochondria. This results in loss of mitochondrial membrane potential and disruption of electron transport, eventually leading to altered levels of reactive oxygen species and cell death. Little is known, however, about the overall changes in the mitochondrial ultrastructure that may accompany the distinct events in this cascade. The objective of this study is to utilize complementary methods of electron microscopy (EM) to study the mitochondrial ultrastructure in developing rat brains after ketamine treatment: classical transmission EM (TEM) to study single mitochondria in individual neuronal cells in 2D and serial block-face scanning EM (SBF-SEM) to study mitochondria-rich brain tissues in 3D. Postnatal day 7 rats were administered 6 injections of 20 mg/kg at 2-h intervals, a dose that was previously found to induce significant neurotoxicity in the frontal cortex. Severity grading of TEM micrographs showed a significant proportion of swollen mitochondria in the frontal cortex (p = 0.02), while 3D reconstructions of SBF-SEM data showed that mitochondrial volume was concurrently increased. Such alterations of mitochondrial ultrastructure are solid evidence indicating mitochondria play a critical role in ketamine-induced neuronal cell damage. This study demonstrates that quantitative 2D and 3D EM are valuable tools that can reveal how ultrastructure plays a critical role in understanding drug interactions.
with flow cytometry. This study signifies that PPCPs alter the expression of key synaptic proteins that potentially contribute to neurological disorders like ASD by disrupting neuronal development.

1761 Nicotine-Induced Insomnia in Larval and Juvenile Zebrafish
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At least 13% of pregnant mothers smoke while they are pregnant and the children of those mothers often sleep less than children born from mothers who do not smoke. In addition to these developmental consequences of smoking, adult smokers often report having problems associated with disrupted sleep. It is thought that these disruptions are related to nicotine which is a potent stimulant and a key ingredient found in cigarettes. The action(s) of nicotine are mediated by activation of neuronal nicotinic acetylcholine receptors (nAChRs) distributed throughout the nervous system. The pineal gland and the retina, two structures that are important for controlling the circadian rhythm (CR) express nAChRs in zebrafish. Thus, we hypothesized that an acute nicotine exposure would disrupt the CR and potentially create an “insomnia-like” phenotype in zebrafish. We evaluated the consequences of an acute, continuous nicotine exposure on the CR in one, two, and three week old zebrafish. One week old larvae exposed to 1 μM nicotine had a disrupted CR. Two week old zebrafish exposed to 5 μM nicotine had a disrupted CR, but a 1 μM nicotine exposure had no effect on the CR. Interestingly, continuous exposure to both 1 μM and 5 μM nicotine disrupted the CR in 3 week old zebrafish. Along with the disruption in the CR, nicotine exposure consistently increased locomotor activity in the initial 6 hours of the CR. These results indicate that nicotine is capable of altering the “sleep state” of the zebrafish by creating an initial period of hyperactivity followed by a disruption in the circadian rhythm. When combined, we suggest that these phenotypes may be analogous to nicotine-induced insomnia in humans.

1762 Coexposure to Gamma-Radiation and Nicotine during a Critical Period of Neonatal Brain Development Can Exacerbate Cognitive Deficits in Adult Mice
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It is known that neonatal exposure to low doses of external γ-radiation as well as nicotine can affect cognitive functions in adult mice. The present study was carried out to investigate whether neonatal co-exposure to external γ-radiation and nicotine can exacerbate developmental neurotoxic effects, including altered susceptibility of the cholinergic system, in adult mice. Neonatal male NMRI mice were exposed to γ-radiation (60Co, 0.2 Gy, dose-rate 0.07 Gy/min) on PND10, PND10 and 11 or PND10, 11 and 12, 3) 0.2 Gy and 66 mg nicotine/kg bw s.c. twice daily on PND10, PND10 and 11 or PND10, 11 and 12, 2) 66 mg nicotine/kg bw s.c. twice daily on PND10, PND10 and 11 or PND10, 11 and 12, 3) 0.2 μg and 66 μg nicotine/kg bw on PND10, PND10 and 11 or PND10, 11 and 12. Controls were exposed to saline and sham irradiated. At an adult age of 2 months animals were observed for spontaneous behaviour in a novel home environment and the variables locomotion, rearing and total activity were registered. Neurotoxic recordings regarding altered susceptibility of the cholinergic system, animals were injected with 80 μg nicotine/kg bw sc and observed for spontaneous behaviour. Animals exposed to 3 x 0.2 Gy as well as animals co-exposed to 3 x 0.2 Gy and 3 x 66 μg nicotine/kg bw showed a deranged spontaneous behaviour at 2 months of age. When challenged to nicotine (80 μg/kg bw) previously mentioned animals showed an increased susceptibility to nicotine at an adult age. All effects were significantly more pronounced in mice co-exposed to γ-radiation and nicotine. These results indicate that external γ-radiation and nicotine can interact to enhance behavioural defects at an adult age if the exposure occurs during a critical period of neonatal brain development. The increased susceptibility to nicotine and altered cognitive function suggests that the cholinergic system is a target system for this type of neurotoxic effects.

1763 Integrating Kinetics and Dynamics for Domoic Acid: Lessons Learned from a Mouse Neurodevelopmental Study
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Although human public health guidelines have been implemented to limit domoic acid (DA) ingestion from shellfish, consumption of DA contaminated seafood by pregnant women remains a concern as DA produced by harmful algal blooms of Pseudo Nitzschia could alter neurodevelopment of fetuses. To assess the effects of repeated, low-level DA exposures on early neurodevelopment, we conducted toxicokinetic and neurobehavioral studies on pregnant C57BL/6 mice and their offspring. Maternal non-symptomatic human-relevant doses of 0, 1, or 3 μg/kg of DA were administered orally in pregnant mice from gestational days (GD) 10 to 17. The toxicokinetics study showed significant DA accumulations in fetal brain and amniotic fluid over time. Maternal and fetal blood peaked at 1.2 and 3.4 hours after the last dose on GD17, respectively, suggesting that fetuses have delayed exposures to DA. Maternal and non-pregnant mice plasma clearance rates (95% CI) were 0.18 (0.16, 0.21) per hour, where up to 38% of DA in maternal blood was going into fetuses. The neurobehavioral tests revealed that offspring from DA exposed dams demonstrated significant dose and sex specific neurobehavioral effects in some of post weaning measurements, such as anxiety in elevated plus maze, walking patterns in CatWalk, home-cage behaviors, and memory in Morris water maze. Integration of kinetics and dynamics of DA were done by correlating areas under the curve and peak concentrations with neurobehavioral outcomes. Results from our studies add significant new information to the literature on kinetics and dynamics of DA with human-relevant exposure patterns, pathways, and levels. This work has been supported by the Pacific Northwest Center for Human Health and Ocean Studies (NIH: P50 ES012762 and NSF: OCE-0434087, OCE-0910624, and 1128883).

1764 Neurohistopathological Postnatal IP Dose of MK-801 in Juvenile Rats
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Characterization of low occurrence of isolated minimal or mild degeneration (DEG) and apoptosis (APO) in control brains is imperative to determine the difference between relationship of normal changes and the effects of a test article. The effects of MK-801 (a non-competitive NMDA antagonist with known neurotoxic properties) on the development of juvenile Sprague-Dawley rat brains were characterized. The MK-801 group received a single dose (3 mg/kg, IP) on PNDs 2, 7, 8, 9, 11, 13, 16, 23, 29, 59, 69 or 111. The control article (distilled water, 3 μL/ Kg, oral gavage) was dosed daily from PND 7 until the day prior to termination. Thirty or twenty rats per sex per time-point had brains perfused/harvested on PNDs 8, 9, 10 and 12, or on PNDs 14, 17, 24, 40, 71 and 113, respectively. The brains were embedded, coronally sectioned at 40μm (through the entire brain length), and stained with amino cupric silver (DEG changes) and caspase 9 stain (APO). Neurohistopathological evaluation of the entire brain for DEG and APO was performed. APO and DEG was prevalent in numerous brain regions of younger control animals (primarily from PND 8 through PND 24). Therefore DEG and APO treatment effects are changes present in a given brain area, specific for each PND, that were greater in incidence or severity than control values. Increased DEG and APO were present in MK-801 animals at all time-points. Increased DEG and APO in MK-801 rats were present in a large number of brain sites in the earlier PNDs with severities ranging from minimal to marked while these changes at later PNDs were substantially diminished. Females generally had more brain sites involved than did males especially at the later PNDs. By PND 40, males typically had only 3 or 4 sites at which DEG was observed. APO that was present in MK-801 rats was generally distinct and unequivocal when compared with the control animals. Based on this data, isolated minimal or mild occurrences of DEG and APO should not be considered treatment related events in juvenile Sprague-Dawley rats.
Comparing the Neurotoxic Effects of Propofol and Ketamine in Rat Embryonic Neural Stem Cells

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Propofol and ketamine are widely used in pediatric anesthesia and analgesia. To evaluate their developmental neurotoxicity and examine underlying mechanisms, embryonic neural stem cells (NSCs) were used. NSCs were harvested from gestational day 14 rat fetuses and exposed on day 7 in culture to propofol at concentrations of 10, 50, 100, and 600 μM, or ketamine at 1, 10, 100, and 500 μM ketamine in growth medium (GM) for 24 hr or to 10 μM propofol or 10 μM ketamine in differentiation medium (DM). In GM propofol caused a dose-dependent reduction in NSC viability; while ketamine did not have this effect at the high concentration of 500 μM. At clinically-relevant concentrations in GM, propofol produced a dramatic increase in ROS generation and enhanced apoptosis as evidenced by an increase in Bax and in TUNEL-positive cells. Similar apoptotic effects were not observed at clinically-relevant dose of ketamine. No significant intracellular Ca2+ influx was detected when NSCs were stimulated with 50 μM NMDA in GM, suggesting there were no functional NMDA receptors expressed on NSCs in GM. In DM, most of the NSCs differentiated into neurons or glial cells. Differentiated neurons were identified using immuno staining with PSA-NCAM (a neuron-specific marker) and calcium influx stimulated with 50 μM NMDA suggested the existence of functional NMDA receptors. Propofol and ketamine significantly increased NSC ROS generation in GM. Propofol and ketamine caused neuronal damage but did not significantly affect glial cells. These observations suggest that ROS plays a key role in propofol and ketamine-induced neurotoxicity and that Ca2+ imaging and gene and protein arrays will be critical for defining associated mechanisms. In summary, an excitatory action of glutamate neurotransmission can be closely related to anesthetic-induced toxicity during development.

Evaluating Neural Progenitor Cell Expansion following Sevoflurane Exposure in Developing Rat Brain Using Micro positron Emission Tomography of [18F] Fluoro-L-Thymidine

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Neutral progenitor cell expansion is critical for normal brain development. During the brain growth spurt, exposures to general anesthetics which either block the N-methyl D-aspartate receptor (NMDAR) or enhance the γ-aminobutyric acid (GABA) receptor type A (GABA_R) can disturb the neuronal transduction. Few reports on the effects of anesthetic exposure on neural progenitor cell expansion in vivo had been published up to date. Here, minimally invasive micro positron emission tomography (microPET) coupled with 3'-deoxy-3'-[18F]fluoro-L-thymidine ([18F]FLT) was utilized to detect the effects of sevoflurane exposure on neural progenitor cell proliferation. FLT, a thymidine analog, is taken up by cells and phosphorylated in the cytoplasm, leading to its intracell lar trapping. The uptake and phosphorylation of FLT correlate highly with cellular proliferation. Intracellular retention of [18F]FLT, thus, represents an observable in vivo marker of cell proliferation. Postnatal day (PND) 7 rats (n=10) were exposed to 2.5% sevoflurane mixed with O2 for 9 hr. One wk post-exposure, the standard uptake values (SUVs) for [18F]FLT in the hippocampal formation were significantly attenuated in the sevoflurane-exposed rats in comparison with control ones (t test, p < 0.05), suggesting decreased uptake and retention (proliferation) in these regions. Two and four wk post-exposure SUVs for [18F]FLT were comparable in the sevo-exposed rats and control ones. Co-administration of 7-nitroindazole (7NI, 30 mg/kg, n=5), a selective inhibitor of neuronal nitric oxide synthase, moderately (around 15%) attenuated the sevo-induced decrease in [18F]FLT SUVs. These findings suggested that neural progenitor cell proliferation is significantly decreased following sevoflurane exposure in the developing rat brain.

Vigabatrin-Induced Effects on the CNS of Juvenile Rats: Further Characterization of Histological Findings

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Vigabatrin is an oral antiepileptic drug (AED) marketed under the trade name Sabril as adjunct therapy for adults with refractory complex partial seizures and as a mono therapy for children aged 1 month to 2 years with infantile spasms. Vigabatrin causes irreversible inhibition of gamma-aminobutyric acid transaminase (GABA-T), the enzyme responsible for the catalysis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), and oral treatment with this compound results in increased level of GABA in the cerebrospinal fluid and brain. In the study presented here, administration of vigabatrin to juvenile rats during discrete periods of time (starting from day 4 of age) resulted in histopathological changes in the brain in terms of neuropil microvacuolation, as well as effects on myelination and on the oligodendrocytes. The variation in the location of the lesions appears to be consistent with the process of myelination previously reported in rats; in the youngest animals, myelination occurred mainly in the hind brain (medulla oblongata and pons) and there was no appreciable myelination in the
thalamus which showed minimal myelination on Day 7 and appreciable myelination by Day 10. It seems likely that the swollen oligodendrocytes seen after as the initial change in the thalamus represented an early stage in the development of the myelin lesion which was seen later as neuropil vacuolation.

The data presented here provide evidence that the histopathological findings reported in the developing nervous system of rats when exposed to Vigabatrin. A full account of the study data is pending publication during 2014.

**R 1765 Hydraulic Fracturing: Are There Worker Health Issues?**

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Use of horizontal drilling and hydraulic fracturing in the US oil and gas (O&G) industry has expanded, with >500,000 workers in this industry in 2013. As with any industry, the workforce has the greatest potential for exposure to contaminants. Furthermore, due to the rapid expansion and need to work at multiple locations, many workers remain transient and work at different sites, often owned by different operators, which can lead to additional complexities when assessing exposures. O&G exploration using hydraulic fracturing has constantly evolved to increase efficiency in recovering oil and gas, which have market value, and further minimize any environmental and health hazards to workers and nearby residents. The highly sophisticated process holds potential hazards as high pressures are used transferring large volumes of water, sand (silica), and small quantities of specific chemicals from the surface to specific geologic structures. Extensive use is made of diesel-powered equipment. Current practices seek to recover and reuse injected fluids to minimize water consumption and the disposal of hazardous waste, including trace elements and naturally-occurring radioactive material. Toxicology and epidemiology have been used to guide improvements in technology (e.g., advanced diesel engines, fuels, and exhaust after-treatment to reduce diesel emissions of PM and NOx) and replace proppants/additives with more environmentally-friendly alternatives. Concern for occupational hazards, including minimizing exposure to noxious agents that may have immediate or long-term impact, is key to planning hydraulic fracturing operations.

**IS 1766 Understanding the Implications of Breastfed Infant Exposures to POPs: How Can We Do Better?**

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Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), may accumulate within a woman’s adipose tissue over many years prior to pregnancy and may subsequently partition into human milk upon breastfeeding. As a result, infant POP intake from breastfeeding may be much greater than average daily maternal POP doses. The developmental period is critical because it sets the stage for lifelong health. Humans continue to develop postpartum, and effects of daily maternal POP doses. The developmental period is critical because it sets the stage for lifelong health. Humans continue to develop postpartum, and effects of daily maternal POP doses.

The training and continuing education of toxicologists is a priority for the SOT membership as demonstrated by the SOT Professional Needs Assessment Task Force (PNATF) and the Education Summit. But what defines a well-trained toxicologist or the “Total Toxicologist”? Does the definition vary depending on the employment sector? Do current graduate programs and continuing education programs provide the necessary and sufficient training for toxicologists? To initiate a discussion regarding these questions, SOT and the National Institute of Environmental Health Sciences sponsored the 2011 Toxicology Educational Summit, which brought participants from academia, industry, and government together to better define the necessary skill sets for the Total Toxicologist (Tox Sci 127:331, 2012). Conclusions from the Summit underscored a deficiency in critical thinking, communication skills, and practical application of laboratory data to drug development and risk assessment as well as a need for improved educational opportunities for mid-career toxicologists. Sustaining a career in the current and future global environment, with the ever-changing and rapid advances in technology, requires partnerships between academia, industry, and government to train and re-train the Total Toxicologist. The goal of this session is to offer multi-faceted perspectives on the skill sets (both hard and soft) required for a successful career as a Total Toxicologist and will include talks from early-, mid-, or late-career toxicologists currently employed in academia, industry (pharmaceutical and agricultural/chemical), or government. The session will also provide a brief summary of the results from the PNATF survey to offer a perspective from the SOT membership regarding their perceived training needs. This session should be of interest to students, postdocs, and early- and mid-career toxicologists and is consistent with SOT’s goals to continue educational awareness and advancements for all toxicologists.

The in vitro effect of selenium (Se) and a combination of vitamin E and vitamin C on some pesticides, viz atrazine, dimethoate, or endosulfan at three different levels of 10, 20, and 30 mM to induced biochemical alterations in rat erythrocytes and hepatocytes of rats was investigated by determining the levels of lipid peroxidation (nmoles MDA/mg protein), glutathione (mole GSH/mg protein) and glutathione peroxidase activity, enhanced the glutathione contents. Treatment with pesticides stimulated thiobarbituric acid reactive substances (TBARS) activity and glutathione peroxidase activity, enhanced the glutathione contents. Treatment with selenium and a combination of vitamin E and/or vitamin C potentially reduced the free radicals in erythrocytes or hepatocytes and ameliorated the oxidative stress induced by such pesticides. The results suggested that pesticides treatment increases in vitro lipid peroxidation, glutathione peroxidase level and glutathione content by increasing oxidative stress in erythrocytes and hepatocytes of rats and selenium and a combination of vitamin E and vitamin C can reduce this lipoperoxidative effect.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to induce cytotoxicity in cancer and non-cancerous cells. Overdose of acetaminophen (APAP) has been known to cause liver injury in humans and experimental animals. Their precise molecular mechanisms, however, are not clearly understood. Our previous studies using HepG2 cells treated with aspirin and mouse macrophage J774.2 cells treated with APAP strongly suggest cell cycle arrest and induction of apoptosis associated with mitochondrial dysfunction and oxidative stress. Macrophages are known to play a critical role in toxicity as well as protection of target tissues against the toxicity induced by NSAIDs. In the present study, we have further demonstrated that macrophages are a more sensitive target for aspirin–induced toxicity than HepG2 cells in lipopolysaccharide (LPS)–treated – macrophage J774.2 and hepatoma HepG2 cells. A marked and differential alteration in oxidative stress, apoptosis, glutathione redox homeostasis and mitochondrial functions were ob-
served in these cells. Pre-treatment of the above cells with N-acetylcysteine, a known antioxidant, has also exhibited selective cytoprotective effects. Our results have suggested that altered glutathione (GSH)-redox metabolism, oxidative stress and mitochondrial function in macrophages and hepatoma cells play a critical role in LPS/NSAIDs-induced cytotoxicity. These results may help in better understanding the pharmacological, toxicological and chemopreventive properties of NSAIDs in cancer and non-cancerous cellular systems. (Supported by Sheikh Hamdan Medical Research Grant and Terry Fox Cancer Research Funds and a fund from CMH5 Research Committee).

1770 Brominated Diphenyl Ether-47 Induces Oxidative Stress and Inflammatory Pathways in Human Placental Cells In Vitro

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Polybrominated diphenyl ethers (PBDEs) are widely used flame retardant compounds. Brominated diphenyl ether (BDE)-47 is one of the most prevalent PBDE congeners found in human breast milk, serum and placenta. Exposure to BDE-47 has been linked to adverse pregnancy outcomes in humans including preterm birth, low birth weight and stillbirth. Although the underlying mechanisms of adverse birth outcomes are poorly understood, critical roles of oxidative stress and inflammation are implicated. The present study investigated BDE-47-induced oxidative stress and proinflammatory (PG) production in a human extravillous trophoblast cell line, HTR-8/SVneo. After 4, 12 or 24 h exposure of HTR-8 cells to BDE-47, intracellular glutathione (GSH) concentration, PGE2 release, and expression of 84 redox-regulated genes were assayed. Treatment of HTR-8 cells with 20 µM BDE-47 for 24 h resulted in differential expression of redox-sensitive genes compared to solvent control. BDE-47 was assayed with commercial Oxidative Stress PCR Array. Treatment of HTR-8 cells with 5, 10, 15, and 20 µM BDE-47 for 24 h increases intracellular GSH levels compared to solvent control, consistent with increased mRNA expression of genes related to GSH synthesis. At 24 h, 20 µM BDE-47 induced significant increases in PGE2 release into the culture medium. The PGE2 increases were accompanied by significant 5.3, 4.4 and 4.7-fold increases in mRNA expression of the proinflammatory cytokine and lipid mediator genes. Increases were also observed for Mrp1, a multidrug efflux protein believed to contribute to BDE-47 efflux from cells. These results suggest that BDE-47 mediates an oxidative stress inflammatory response in human placental cells.

1771 Radical Acylation of L-Lysine-Containing Peptides and Sorbarmine by Peroxynitrite-Treated Diacetyl and Methylglyoxal

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Highly reactive α-dicarbonyl compounds such as diacetyl, methylglyoxal, 4,5-dioxyvaleric acid, and 3-deoxyglucosone have been characterized as secondary catabolites that can aggregate proteins and form DNA nucleobase adducts in several human maladies. In vitro, diacetyl (DA) and methylglyoxal (MG) have also been shown to rapidly add up the peroxynitrite anion (k2 > 10xp4 to 10xp5 M-1s-1), a potent biological nucleophile, oxidant and nitrating agent, followed by carbon chain cleavage to carboxylic acids from acryl radical intermediate that can attach to amino acids added to the reaction mixtures. Here, using HPLC or CE coupled to ESI or MALDI-TOF/MS techniques, we show that the DA or MG/peroxynitrite systems are capable of acetylating the α-lysyl residues of synthetic KALA-OH, Ac-KALA-OH, K(Boc)ALA-OH, and Ac-A-E-F-K-F-A-L-NH2 as well as amino acid residues of bovine sorbarmine in phosphate buffer pH 7.4. The pH profiles (6.2-8.2) of the reactions traced for the tetrapeptides are bell-shaped, peaking at approximately 7.5, therefore near the pKa values of both ONOOH and H2PO4- anion, which is in accordance with the pH dependency of the reaction products. CE or HPLC and MS analyses of reaction products confirmed αN- and αε-acylation of Lys derivatives by DA as well as acetylation and formylation by MG. Moreover, the EPR MNP-CH3CO radical adduct signal was found to be quenched upon addition of L-His. These data raise the hypothesis of contributing radical acylation of peptides in epigenetic processes, where their enzymatic acetylation is a well-documented event, recently reported to be as critical to the cell cycle as phosphorylation. Also noteworthy is the observed formation of L-Lys containing peptides by MG never reported to occur in proteins. Financial support: FAPESP, CNPq, INCT Redoxoma.
The coexistence of aflatoxicosis and protein malnutrition in children is a major health burden in developing countries especially in sub-Saharan Africa. Hence, this study investigated the effects of the coexistence of Aflatoxin B1 (AFB1) poisoning and protein malnutrition on renal and hepatic antioxidant status and the integrity of genomic DNA in weanling rats. Eight groups of animals were used in this study; groups 1 and 2 were male and female animals fed normal protein diet (20% protein), groups 3 and 4 were male and female animals fed low protein diet (5%), groups 5 and 6 were male and female animals fed normal protein (20%) + 40ppb AFB1 and groups 7 and 8 were male and female animals fed low protein (5%) + 40ppb AFB1 respectively. At the end of eight weeks, rats were sacrificed after an overnight fast and liver and kidney were excised. Glutathione, glutathione transferase, superoxide dismutase, catalase and glucose-6-phosphate dehydrogenase were determined spectrophotometrically while genomic DNA fragmentation was determined by agarose gel electrophoresis after extraction of DNA from the tissues. Results showed that the coexistence significantly (p<0.05) decreased glutathione, glutathione transferase, and superoxide dismutase while it increased peroxidase in the kidney and liver. It significantly (p<0.05) increased catalase in different tissues. Results showed that the coexistence significantly (p<0.05) decreased glutathione, glutathione transferase, and superoxide dismutase while it increased peroxidase in the kidney and liver. It significantly (p<0.05) increased catalase in the liver while a decrease was observed in the kidney. Furthermore, a decrease in glucose-6-phosphate dehydrogenase was observed in the liver while no significant (p>0.05) difference was observed in the kidney. Agarose gel electrophoresis showed DNA damage in the kidney of the rats while no difference in pattern was observed in the liver. Our results suggest that sub-chronic coexistence of AFB1 poisoning and protein malnutrition resulted in oxidative stress with a concomitant DNA damage in the kidney of weanling rats.

MALDI-Mass Spectrometry-Based Biochemical Microscopy of Cardiolipin Molecular Species in Brain Tissue

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Existing protocols associated with MALDI imaging mass spectrometry (MALDI-IMS) in the mapping of lipids on tissue sections fail to detect the mitochondria-unique phospholipid cardiolipin (CL), known to be essential for cell and mitochondrial physiology. We developed a new protocol enabling the “biochemical microscopy” of diversified individual molecular CL species. This has been achieved by the employment of: i) phospholipase C to reduce highly abundant phosphatidylincholine (PC) signals, ii) chemical crosslinking with 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) to block ion suppressive effects from carboxyl containing molecular species, iii) modified matrix application methods and ammonium acetate washes to enhance CL signal in negative ion mode. Using these treatments, we were able to visualize and map CL in rat brain tissue. We documented a spatial distribution for CL in the rat brain. The relative intensities of CL species m/z 1448 and 1450 were highest in the hippocampal area and decreased in intensity as one transitions from the hippocampus toward the white matter and cortex areas. Higher mass CL species displayed similar or increased intensities in the hippocampus and cortex areas. We therefore investigated the role of zinc exposure on the mitochondrial redox status by measuring the EGSH in mitochondria. Cultures of the human bronchial epithelial cell line BEAS 2B were transduced using lentiviral
The transcription factor Nrf2 has revealed as the master regulator of intracellular redox homeostasis. As an adaptive response to oxidative stress, Nrf2 activates transcription of a battery of genes encoding antioxidant protein, detoxification enzymes and xenobiotic transporters by binding to the cis-antioxidant response elements in the promoter region of these genes. Previous work by our lab and others demonstrated that Nrf2 is subject to poly-ubiquitin-mediated proteasomal degradation in a Keap1-dependent manner. Here we report a potential functional connection between protein quality control and Nrf2 activation. Genetic knockdown or pharmacological inhibition of p97, an AAA-ATPase governing critical steps in ubiquitin-dependent protein quality control and intracellular signaling pathways, resulted in an increase in Nrf2 protein level as well as increase Nrf2 target gene expression. We also demonstrated that p97 is in complex with Nrf2 and with Cul3-Keap1-Rbx1 E3 ligase complex using biochemical and proteomics analysis. Furthermore, indirect immunofluorescent staining reveals both inhibited p97 function and treatment with arsenite lead to increased autophagosomal formation, implying the possible link between arsenic, p97, autophagy, Nrf2 upregulation and cellular toxicity. These findings not only suggest new regulatory mechanism of Nrf2-Keap1 pathway, but also offer a potential mechanism by which arsenic activates the Nrf2 pathway.

**1780 Microtubule-Associated Protein 1s (MAP1s) Is a Novel Component of the Noncanonical p62-Dependent Mechanism of Nrf2 Regulation**


Microtubule Associated Protein 1s (MAP1s) is a short and ubiquitously expressed member of the microtubule-associated protein (MAP) 1 family. Recent studies have identified a cytoskeletal role for MAP1s in neurodegeneration. MAP1s eliminates genome instability and protein aggregates, and protects against chemically-induced hepatocarcinogenesis. The interaction of MAP1s with several cellular components such as tubulin, actin, RASSF1A, LRPPCR and the autophagy protein LC3 has already been characterized. Here we identified that MAP1s and Keap1 are contained in the same protein complex through their interactions with p62, rather than through direct, ETGE motif-dependent MAP1s-Keap1 interaction. Moreover, MAP1s and Keap1 colocalized with p62 and LC3 in a punctate pattern that suggests that they all form part of an autophagic complex. Overexpression of MAP1s resulted in decreased protein levels of coexpressed Keap1, and treatment with the proteasome inhibitor MG132 resulted in a more dramatic decrease in Keap1 protein levels. On the other hand, treatment with diverse autophagy inhibitors prevented further Keap1 degradation. Additionally, MAP1s knockdown resulted in increased protein levels of Keap1 and p62, with a concomitant decrease in Nrf2 signaling. In vivo, we saw that acute treatment of nude mice with the hepatic carcinogen diethylnitrosamine (DEN) resulted in MAP1s induction in liver. This induction of MAP1s also resulted in increased protein levels of p62, Nrf2, NQO1, and decreased protein levels of Keap1. Interaction between p62 and Keap1 was previously been shown to impede Nrf2 degradation, thereby favoring its stabilization and induction of downstream genes. Taken together, all these results suggest that MAP1s is a novel component of the non-canonical pathway of Nrf2 activation mediated by p62.
for 3 h and then incubated for 1 h with: 1) medium alone, 2) 25 μM MDA, 3) 0.2% Intralipid (emulsion of egg yolk and soybean triglycerides), 4) MDA plus Intralipid, 5) 10 μM Sp600125 (JNK inhibitor), and 6) MDA plus Intralipid plus Sp600125. Cells were then incubated with bodipy green, TMRM and propidium iodide (PI) to visualize neutral lipids, polarized mitochondria and non-viable cells, respectively, by confocal microscopy. After medium alone, hepatocytes displayed few lipid droplets. MDA alone did not increase lipid droplet accumulation but caused a decrease of mitochondrial membrane potential (ΔΨ) assessed by TMRM. Intralipid alone caused neither accumulation of lipid droplets nor a decrease of ΔΨ. However, MDA combined with Intralipid led to substantial increases of size and quantity of lipid droplets together with a decrease of ΔΨ. JNK inhibition with Sp600125 blocked lipid accumulation and the decrease ΔΨ after MDA plus Intralipid. No treatments induced PI staining. These results are consistent with our previous hypothesis that aldehydes like MDA inhibit mitochondrial voltage dependent anion channels (VDAC) with consequent inhibition of β-oxidation of fatty acids and mitochondrial ΔΨ formation (JBC 2012;287:7692-7700). However, since our expectation was to develop a source of lipid was present. Interestingly, JNK inhibition abrogated reactivity induced by MDA plus Intralipid, suggesting that aldehyde dependent VDAC closure occurs via a JNK-dependent pathway.

1784 Development of a Novel Lumogenic Assay for H2O2 Detection


H2O2 is a reactive oxygen species (ROS) that is convenient to measure because of a relatively long half-life and it is also useful as a general marker of oxidative stress. Commercial H2O2 reporters include fluorogenic probes that react directly and selectively with H2O2 or via catalysis by horseradish peroxidase (HRP). The direct probes are convenient for imaging ROS inductions, but tend to be non-selective among the various ROS and they deliver small assay windows in homogenous formats. While HRP-dependent probes are sensitive and selective, they are prone to high false hit rates in screens and perform poorly in cell based assays. To overcome these shortcomings we synthesize a cyanobenzothiazole derivative containing a borate ester and self-cleaving linker (2-ethyl-6-(4-(4,4,5,5-tetramethyl-1,3-dioxaborolan-2-yl)benzoyloxy)benz[d]thiazole) to use as a lumogenic probe. The borate reacts directly and selectively with H2O2 to expose the linker and eliminate a free cyanobenzothiazole. The latter moiety reacts rapidly with D-cysteine to form luciferin, which in turn generates light with luciferase. Based on well-known characteristics of luciferase assays we expected low background, wide dynamic range, and low false hit rates. In a homogenous format cells were where exposed to test articles (e.g. ROS inducers) and probe, and then to a luciferase formulation containing D-cysteine. The luciferase produced light read by a luminometer in proportion to the H2O2 concentration of the sample. Prototypical ROS inductions were detected with high sensitivity and a wide dynamic range in a multwell format. To test high throughput performance, we screened the 1280 compound LOPAC library against HepG2 cells. Cells in medium were compared to medium alone to select H2O2 modulators and to discriminate between cell-based and abiotic effects. LOPAC screens revealed a low false hit rate (0.5%) compared to an HRP based method (7.1%). These results indicate the assay obviates certain limitations of fluorometric methods and that it should prove effective for small scale and high throughput applications.

1785 Nrf2 but Not NF-kB Is Activated by African Dust Organic Extract in BEAS-2B


Every year, African dust storms cross the Atlantic Ocean impacting the Caribbean region. Airborne particulate matter (PM) falls out along its path. This matter contributes to the oxidative atmospheric potential of the environments and in organisms. The impact of this global phenomenon on the health of the Puerto Rican community continues to be evaluated. We previously reported the induction of IL-6 and IL-8 by African dust (ADE) in human bronchial epithelial cells (BEAS-2B). Here we expand our research to assess the activation of two trans factors 1) the nuclear erythroid 2-related factor 2 (Nrf2) and 2) the nuclear factor κB (NF-κB). The role of heavy metals and oxidant capacity were evaluated using 1) the nuclear erythroid 2-related factor 2 (Nrf2) and 2) the nuclear factor κB (NF-κB). For example, deferoxamine mesylate (DF) and N-acetyl-L-cysteine (NAC), in cells treated with PM organic extracts (50μg/ml). Protein extracts were obtained using the Nuclear Extract Kit (Active Motif) according to the manufacturer and quantified by the Bradford assay. Activation of Nrf2 and NF-κB were determined by an ELISA base assay (Active Motif). Nuclear extracts including the activated transcription factors were added to a plate coated with the specific oligonucleotide containing a consensus-binding site for Nrf2 or NF-κB. Both ADE and Non-ADE extracts induce the activation of Nrf2 after 1 h. This response is partly due to the oxidative capacity of ADE (not significant for Non-ADE) extract since it was significantly reduced by NAC. ADE and Non-ADE extracts triggered the activation of Nrf2 however; the magnitude of this activation was significantly increased for ADE. To our surprise we were unable to find any evidence for NF-κB activation after 1 or 4 hrs. This research demonstrates that African dust arriving Puerto Rico has an oxidative capacity capable of activating antioxidant defenses in lung cells. The activated molecules are good candidates for testing biomarkers of exposure to cellular stress during seasonal respiratory outcome. Support provided by MBRs-RISE Grant R25GM061838.

1786 Perfluorooctanoic Acid (PFOA) Causes Oxidative Stress in the Mouse Pancreas

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Perfluoralkyl chemicals (PFCs) such as perfluorooctanoic acid (PFOA) are widely used in industrial and commercial applications and are environmentally and biologically persistent. In rodents, PFC exposure has been shown to lead to reduced body weight, liver hypertrophy, and decreased cholesterol levels. In addition, chronic exposure to PFCs induce liver, Leydig and pancreatic acinar cell tumors (PACTs) in rodents. Epidemiologic studies have also linked exposure to PFC compounds to adverse health effects in humans, several of which identified an association between PFC exposure and pancreatic disease. To address the effects of PFCs on the pancreas, we treated 8 wk old C57Bl/6 mice with vehicle or PFOA by gavage at doses of 0.5, 2.5 or 5.0 mg/kg BW/day for 7 days. In addition, a group of mice were treated with omeprazole, which induces mild pancreatitis and produces an oxidative stress in the pancreas. Our results demonstrate that exposure to PFOA results in pancreatic toxicity, evidenced by increased serum levels of amylase and lipase in the PFOA treatment groups. PFOA accumulated in serum, liver and pancreas in a dose-dependent manner. Increased levels of the lipid peroxidation product F2-isoprostane were observed in both the pancreas and liver (2.5-fold and 1.5 fold, respectively) indicating that PFOA induced oxidative stress in these tissues. In the pancreas and liver, F2-isoprostane levels were strongly correlated with PFOA content (r² = 0.86, and 0.90, respectively). The mRNA expression of the cystosolic and mitochondrial forms of superoxide dismutase, (SOD1 and SOD2) was increased by 1.5 fold in the pancreas of PFOA treated mice, which was accompanied by an increase (24%) in the enzymatic activity of SOD. While the levels of GSH in the pancreas were not altered, the ratio of GSH:GSSG was significantly decreased by PFOA exposure. In contrast, PFOA increased GSH levels and the GSH:GSSG ratio in the liver. Collectively, these results suggest that PFOA exposure results in oxidative stress in the pancreas.

1787 Investigating the Bioenergetic and Oxidative Impacts of Rapid Altitude Adjustment

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Rapid ascent to high altitudes commonly results in a severe decrease in exercise performance and cognitive abilities. Many situations, in which rapid altitude changes are required, such as mountain rescues and flight, necessitate transport in aircraft or high-performance vehicles. Sprague-Dawley rats were elevated to three simulated altitudes for one hour (1,000, 10,000, or 20,000 feet) under constant oxygen levels. Liver, heart, and brain mitochondria were immediately isolated following exposure for evaluation of State 3, State 4, and uncoupler-stimulated respiration in a Seahorse XF Bioanalyzer. In addition to evaluating ATP production, spare capacity, and proton leak with the XF Bioanalyzer, measurements of the oxidative stress markers cytochrome c and thiobarbituric acid reactive substances (TBARS) were taken. Our results from heart tissue show that in addition to reduced oxygen delivery to organs, altitude affects the basic bioenergetics of the cell by reducing the oxidative capacity of the mitochondria. Although ATP production is maintained at a relatively constant level (0.25 pmoles/min), the mitochondrial membrane is weakened as indicated by an increase in proton leakage (42%). Furthermore, the levels of TBARS in the blood increase 28% with the raise in altitude to 20,000 feet, supporting our physiological observations. The decrease in spare respiratory capacity corresponding to a weakened membrane and the presence of oxidative stress markers suggests that additional stressors at altitude could have catastrophic outcomes. Future work will investigate the impact on mitochondrial function in unique brain regions as well as skeletal muscle effects.
Studying Nrf2-Mediated Oxidative Stress Response in Human Keratinocytes Exposed to Two Plant Polyphenols—Quercetin and Curcumin

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Methionine sulfoxide reductase A (Msra) is an oxidoreductase repair enzyme responsible for catalyzing the stereospecific reduction of methionine-S-sulfoxide to methionine. Previous studies have shown that reactive oxygen species (ROS) can lead to the oxidation of methione, thereby rendering proteins functionless. Reversal of this process by Msra has been shown to restore protein function, and has been implicated in cellular senescence, the aging process, neurodegenerative diseases, and glucose homeostasis. The antioxidant response of Msra is established, but the transcriptional regulation remains uncertain. Nrf2, a key regulator of the antioxidant response, is a transcription factor that is responsible for altering gene expression upon insult triggered by ROS. We predicted Msra to be a novel target gene of Nrf2 based on 2putative AREs contained within the promoter region. Preliminary data, through induction of Nrf2, identified that Msra protein expression acts just as other well-established Nrf2 target genes. Subsequent knockdown of Msra in HaCaT cells showed reduced protein level of Msra. Taken together, our data indicate that Msra is a novel target gene of Nrf2. The regulation of Msra and its functional contribution to the Nrf2-mediated defensive response are currently under investigation.

Production of Reactive Oxygen Species by the Redox Cycling of 9,10-Phenanthraquinone in Human Monocyte THP-1 Cells: Role of the Mitochondrial Electron Transport Chain

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Exposures to environmental pollution remains a major problem and poses continuing risks to human health. Airborne particulate matter (APM) contains large amounts of quinoid compounds and it has been shown that 9,10-phenanthraquinone alone compromises about 6% of the total organic extract of diesel exhaust particles. However, the potential involvement of 9,10-phenanthraquinone in APM-induced adverse effects remains unclear. In this study, we determined whether 9,10-phenanthraquinone could undergo redox cycling in human monocyte THP-1 cells. Incubation of THP-1 cells with 9,10-phenanthraquinone at 0.1-1 μM resulted in a significant stimulation of potassium cyanide (KCN)-resistant oxygen consumption, indicating that 9,10-phenanthraquinone could undergo redox cycling in cells. Redox cycling of 9,10-phenanthraquinone to produce ROS in support of this process, incubation of 9,10-phenanthraquinone with mitochondria isolated from the THP-1 cells also stimulated ROS production. In summary, this study demonstrated that 9,10-phenanthraquinone produces ROS in THP-1 cells and METC is a CDDO-Im via Glutathione-Mediated Mechanism

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Acrolein is an environmental toxin, mainly found in smoke released from incomplete combustion of organic matter. The compound is ubiquitously found in endogenous as well as exogenous environment. Several studies showed that exposure to acrolein can lead to liver damage. The mechanisms involved in acrolein-induced hepatocellular toxicity, however, are not completely understood. This study examines the toxic effects and cytotoxic mechanisms of acrolein on HepG2 cells. Acrolein at pathophysiological concentrations was shown to cause a concentration-dependent decrease in cell viability. Acrolein treatment was also found to cause apoptotic cell death and an increase in levels of protein carbonyl and TBARS (thiobarbituric reactive acid substances), markers of protein damage and lipid peroxidation, respectively, in HepG2 cells. Acrolein also rapidly depleted intracellular glutathione (GSH), phase II enzyme GSH-conjugation transfers (GST) and aldose reductase (AR), three critical cellular defenses that detoxify reactive aldehydes. Results further showed that depletion of cellular GSH by acrolein preceded the loss of cell viability, which suggests that cellular GSH depletion may be an important event in acrolein-induced cytotoxicity. To further determine the role of cellular GSH in protecting against acrolein-mediated cytotoxicity, buthionine sulfoximine (BSO) was used to inhibit cellular GSH biosynthesis. It was observed that depletion of cellular GSH by BSO led to a marked potentiation of acrolein-in-mediated cytotoxicity in HepG2 cells. Furthermore, induction of GSH levels by CDDO-Im, a triterpenoid compound, afforded protection against acrolein toxicity in a concentration-dependent manner via induction of GSH mechanism. This study may provide understanding on the molecular action of acrolein which may be important to develop novel strategies for the prevention of acrolein-mediated toxicity.

Protection of HepG2 Cells from Acrolein Toxicity by CDDO-Im via Glutathione-Mediated Mechanism

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Acrolein is an environmental toxicant, mainly found in smoke released from incomplete combustion of organic matter. The compound is ubiquitously found in endogenous as well as exogenous environment. Several studies showed that...
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Using intermittent hyperoxia can significantly ameliorate the toxic effects of hyperoxic exposure. Thus, either giving sufficient time to recover or upon the duration of both hyperoxic and normoxic exposure. Macrophages that were compromised by 24 hours of continuous hypreoxiacan was reversed upon subsequent exposure to normoxia. We tested two exposure paradigms to address the effects of hyperoxia on macrophages. We tested two exposure paradigms to address whether: 1. the adverse effects of hyperoxia on macrophages are reversible. 2. Intermittent exposure to hyperoxia is less deleterious than continuous exposure in compromising phagocytic function. Here, we report that phagocytic function compromised by 24 hours of continuous hyperoxia was reversed upon subsequent exposure to room air for 24 or 48 hours. The extent of recovery is dependent upon the duration of both hyperoxic and normoxic exposure. Macrophages that were subjected to intermittent exposure to hyperoxia (2 hours break after 12 hours exposure) exhibited significantly higher phagocytic activity compared to those with continuous 24 hour exposure. Thus, either giving sufficient time to recover or using intermittent hyperoxia can significantly ameliorate the toxic effects of hyperoxia on macrophage function.

Identification of Novel Chemical Inhibitors of Nrf2-ARE Activity and Their Application in Sensitizing Human Acute Monocytic Leukemia Cells to Chemotherapy

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Nuclear factor E2-related factor 2 (Nrf2) is a master regulator of the antioxidant response element (ARE)-dependent transcription and plays a pivotal role in chemical detoxification in both normal and tumor cells. In keeping with previous findings that Nrf2-ARE plays a critical role in chemotherapeutic resistance of cancer cells, stable knockdown of Nrf2 by lentiviral shRNA in human acute monocytic leukemia THP-1 cells resulted in sensitization to cytotoxicity induced by multiple chemotherapeutic agents, including arsenic trioxide (As2O3), etoposide and doxorubicin. We screened a panel of chemicals using a variety of human and mouse cell lines expressing an ARE-luciferase reporter, and found that a group of widely used anti-tubercular drugs, including ethionamide (ETH) and isoniazid (INH), displayed substantial inhibitory property against Nrf2-ARE activity. This inhibitory property was further validated by significant reduction in mRNA expression of multiple ARE-driven genes under basal and oxidative stress conditions. Suppression of Nrf2-ARE by ETH or INH significantly enhanced the sensitivity of THP-1 and several other cancer cells, such as human histioctye lymphoma U937 cells, to As2O3-induced cytotoxicity as determined by cell viability assays, ATP production, cell cycle and apoptosis analysis. In THP-1 cells, the sensitization effect of ETH to As2O3-induced cytotoxicity is Nrf2 dependent, suggesting that Nrf2 is a potential target of the chemotherapeutics. To our knowledge, our study is the first to demonstrate that ETH and INH suppress Nrf2-ARE signaling and disrupt the transcriptional network of the antioxidant response, leading to sensitization of cancer cells to chemotherapeutic agents.

Altered Mitochondrial Bioenergetics in Response to Arsenic Exposure: New Outlook on Arsenic-Induced Toxicity

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Arsenic is a human carcinogen that is ubiquitously present in the environment. Arsenic mediated toxicity is suggested to be due to the induction of reactive oxygen species. However, the origins of these radicals are not clearly understood. Here we show that arsenic exposure, at environmentally relevant concentrations, uncouples...
oxygen consumption from ATP production in mammalian cells leading to increased electron leakage, decreased ATP production and reduced spare respiratory capacity. Analysis of the mitochondrial respiratory complex provides evidence implicating a dose-dependent deregulation in respiratory complex activity in response to arsenic exposure, suggesting that mitochondrial dysfunction might contribute to arsenic-mediated induction of reactive oxygen species. These data illustrate that mitochondria might be a primary target in arsenic-induced oxidative stress and understanding the mechanism by which arsenic deregulates mitochondrial respiratory complex activity and uncouples oxygen consumption from ATP production might provide an important cellular target in interventional remedy for arsenic-induced toxicity.

1798 Keap1 Redox-Dependent Regulation of Doxorubicin-Induced Oxidative Stress Response in Cardiac Myoblasts

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Doxorubicin (DOX) is a widely prescribed treatment for a broad scope of cancers, but clinical utility is limited by the cumulative, dose-dependent cardiomyopathy that occurs with repeated administration. DOX-induced cardiotoxicity is associated with the production of reactive oxygen species (ROS) and oxidation of lipids, DNA, and proteins. A major cellular defense mechanism against such oxidative stress is activation of the Keap1/Nrf2-antioxidant response element (ARE) signaling pathway, which transcriptionally regulates expression of antioxidant genes such as Nq o1 and Gap1. In the present study, we address the hypothesis that an initial event associated with DOX-induced oxidative stress is activation of the Keap1/Nrf2-dependent expression of antioxidant genes and that this is regulated through drug-induced changes in redox status. Keap1 protein incubation of H9c2 rat cardiac myoblasts with DOX resulted in a time- and dose-dependent decrease in non-protein sulphydryl groups. Associated with this was a near 2-fold increase in Nrf2 protein content and enhanced transcription of several of the Nrf2-regulated downstream genes, including Gap1, Ugtal, and Nq o1; the expression of Nf22 (Nrf2) itself was unaltered. Furthermore, both the redox status and the total amount of Keap1 protein were significantly decreased by DOX, with the loss of Keap1 being due to both inhibited gene expression and increased autophagic, but not proteasomal, degradation. These findings identify the Keap1/Nrf2 pathway as a potentially important initial response to acute DOX-induced oxidative injury, with the primary regulatory events being the oxidation and autophagic degradation of the redox sensor Keap1 protein. (Supported-in-part by grants from the 3M Company and Whiteside Institute.)

1799 Retinoic Acid Receptor-Dependent Interactions Contribute to the Selective Cytoprotection Afforded by All-Trans-Retinoic Acid against Renal Injury

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Chemical-induced nephrotoxicity is a major cause of acute kidney injury. Pretreatment of LLC-PK1 cells with all-trans-retinoic acid (ATRA, 25 μM, 2 hr) affords cytoprotection against chemical nephrotoxicants such as p-aminohippocresol (PAP, 150 μM, 3 hr), iodoacetamide (IDAM, 25 μM, 2 hr) and 2-(glutathionyl-S-v)hydroquinone (MGHQ 300 μM, 2 hr) as assessed by the Neutral Red assay. In contrast, pretreatment of cells with ATRA provides no protection against cisplatin (25 μM, 24 hr) -induced apoptosis, suggesting that ATRA selectively protects against toxicity that induce necrosis and not apoptosis. The biological effects of retinoids are typically mediated through interaction with the nuclear retinoic acid receptors (RAR) and retinoid X receptors (RXR). While western blot analyses of nuclear lysate from LLC-PK1 cells, revealed the presence of both RAR and RXR, ATRA only induced the RAR isoforms. Since ATRA binds to the RAR agonist 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]-benzoic acid (TTNPB) (25 μM, 24 hr) protected against PAP (125 μM, 3 hr) and IDAM (20 μM, 2 hr) -induced nephrotoxicity. Pretreatment of LLC-PK1 cells, with the pan-RXR agonist methylclofenamate (25 μM, 24 hr), afforded minor protection against PAP (125 μM, 3 hr) but not IDAM (25 μM, 2 hr). The results are consistent with the view that ATRA-mediated cytoprotection involves interaction with the RAR receptor. During ATRA-mediated cytoprotection, the endoplasmic reticulum chaperone Grp78 is induced in a similar time-dependent manner in LLC-PK1 cells. Thus, ATRA and Grp78 may serve as an effective therapeutic intervention prior to kidney transplantation, which causes ischemic-reperfusion renal injury (ES016578, ES066694, ES007091).

1800 Administration of Sulindac, an NSAID, and Qurectin, an Antioxidant, in Combination Attenuates Bleomycin-Induced Lung Fibrosis in Wistar Rats

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Idiopathic pulmonary fibrosis is the most prevalent chronic lung disease. Inflammation and oxidative stress are considered major mechanisms that induce lung tissue inflammation and fibrosis. Studies have suggested that reactive oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical are major mediators of lung inflammatory processes. The present study was conducted to evaluate the ameliorative effects of qurectin (a dietary flavonoid having strong antioxidant activity) alone and in combination with sulindac (non-steroidal anti-inflammatory drug) on lung injury induced by bleomycin. Following single intra-tracheal instillation bleomycin rats were treated by oral administration of either qurectin (100 mg/kg/day) or sulindac (20 mg/kg/day) for 20 days reduced the severity of bleomycin induced effects on relative lung weights, lung hydroxyproline contents and plasma TNF-α levels. However, suble effects of toxicity persisted in the qurectin and sulindac treated groups. The combination of qurectin (50 mg/ kg/day) and sulindac (10 mg/kg/day) was more effective in reducing bleomycin induced lung toxicity than the higher doses of each agent separately. Therefore, these results suggest that the concurrent administration of qurectin and sulindac in low doses are synergistic in protection of the lung from oxidative stress induced effects.

1801 Dietary Flavonoid Quercetin Attenuates Bleomycin-Induced Pulmonary Fibrosis in Rats by Improving Lung Antioxidant Status

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Lung fibrosis is a frequent side effect of the chemotherapeutic agent bleomycin. Reports suggest that reactive oxygen species may play a key role in the development of lung fibrosis from this agent. The present study examined the protective role, if any, of the dietary flavonoid, quercetin, against bleomycin induced lung fibrosis in rats. Animals were divided into a control group (0.5% carboxymethylcellulose orally for twenty days post sham intra-tracheal instillation of saline on day 0), a bleomycin group (6.5 U/kg body weight via single intra-tracheal instillation on day 0 followed by oral administration of 0.5% carboxymethylcellulose for twenty days) and a bleomycin plus quercetin group (single 6.5 U/kg intra-tracheal bleomycin instillation followed by oral administration of quercetin for twenty days). Our results show that bleomycin administration increased lipid peroxidation resulting in prolonged state of local tissue inflammation characterized by elevated levels of TNF-α even after twenty days of drug treatment. The prolonged oxidative stress resulted in excessive collagen deposition in the peribronchial and perilveolar region of lung culminating in lung fibrosis. Twenty days of quercetin treatment following bleomycin administration stabilized tissue antioxidant capacity at normal levels with recovery from lung inflammatory changes. Trichrome stained lung tissue from the rats receiving repeated doses of this flavonoid revealed only basal levels of collagen deposition as confirmed by normal levels of hydroxyproline. The results of the present study strongly suggest that quercetin may be an effective protective agent against bleomycin induced pulmonary fibrosis.

1802 Nrf2 Activation by the First-in-Class Direct Keap1-Nrf2 Interaction Inhibitor LH601A in Human Kidney Cells

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Under basal conditions, the antioxidant transcription factor, Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), is bound to the Keap1 (Kelch-like erythroid-cell-derived protein 1) protein and targeted for proteosomal degradation in the cytoplasm. In response to cellular injury or chemical treatment, Nrf2 activates the transcription of antioxidant, cell stress, metabolism and other protective genes and defends against kidney injury. LH601A is a first-in-class direct inhibitor of the Keap1-Nrf2 protein-protein interaction. We hypothesize that LH601A will activate Nrf2 signaling in human cultured kidney cells and protect against chemical-induced kidney
In the present study, we sought to determine whether cells (rodent and human) expressing a TSC signaling node (TSC1, TSC2, and Rheb) localizes to peroxisomal membranes, where we have recently reported that the Tuberous Sclerosis Complex (TSC) signaling node resident at the peroxisome to suppress mTORC1 and induce autophagy/pexophagy, providing a mechanism by which cells can restore peroxisomal homeostasis in response to treatment with PPAR ligands.

1804a Distinct Roles of Basal and Inducible Nrf2-Mediated Antioxidant Response against Cobalt Chloride-Induced Cytotoxicity in Human Keratinocytes
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Hypoxia is an important physiological process that is involved in many cutaneous diseases and injuries. Nuclear factor E2-related factor 2 (Nrf2) is a master regulator of the antioxidant response element (ARE)-dependent transcription and plays a pivotal role in chemical detoxification in a variety of cells, including keratinocytes. This study was conducted to illustrate the role of Nrf2-mediated antioxidant response against chemical hypoxia-induced cell injury by cobalt chloride (CoCl2), in human keratinocytes. In HaCaT cells, acute CoCl2 exposure significantly increased the levels of intracellular reactive oxygen species (ROS) and the expression of Nrf2 and multiple ARE-regulated antioxidant genes in concentration- and time-dependent manners, showing that CoCl2 causes oxidative stress in the cells. Selective knockdown (KD) of Nrf2 by lentiviral shRNA dramatically reduced the basal and inducible expression of many antioxidant enzymes and sensitized the cells to acute cytotoxicity of CoCl2. In contrast, pretreatment of HaCaT cells with a well-known Nrf2 activator tert-butylnhydroquinone protected the cells from CoCl2-induced cell injury in a Nrf2-dependent fashion. In addition, silencing of kelch-like ECH-associated protein 1 (Keap1), a Cul3-adapter protein that allows for Nrf2 to be ubiquinated and degraded by the proteasome complex, led to a dramatic resistance to CoCl2-induced cytotoxicity and apoptosis. Interestingly, knockdown of Keap1 markedly enhanced the basal activity of Nrf2, but reduced the induction of ARE-dependent genes by CoCl2, suggesting that basal activity of Nrf2 is more important than inductive expression of ARE-dependent genes in determining cellular sensitivity to the cytotoxicity of CoCl2. Taken together, our studies suggest that basal and inducible Nrf2-mediated antioxidant response may play distinct roles in human keratinocytes against CoCl2-induced cytotoxicity.

1804b A Dilution Effect in the Malondialdehyde Measurement in Serum with High-Performance Liquid Chromatography-Tandem Mass Spectrometry
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Oxidative stress is caused by an imbalance between free radicals and antioxidants defenses in the body, which is a prominent feature of many diseases. The overwhelming redox state can lead to damages to biomolecules including proteins, lipids, and DNA. Malondialdehyde (MDA) is one of the major secondary decomposition products of peroxidized polyunsaturated fatty acid and is one of most used biomarkers in oxidative stress studies. We developed and validated a sensitive method for the quantitation of MDA in mouse serum after derivatization with 2,4-dinitrophenylhydrazine (DNPH) by high-performance liquid chromatography with positive electrospray ionization coupled with tandem mass spectrometry (HPLC-ESI-MS/MS). In spite of the promising instrument measurement, a significant sample dilution effect in sample preparation was observed for the quantitative analysis of MDA for the same mouse serum. The measured MDA levels were 74.7, 20.6, and 3.4 μM for 5%, 40%, and 100% of serum samples, respectively. The possible reason for this phenomenon is a better hydrolysis of serum samples in a more diluted serum mixture, which released relatively more low-molecular mass aldehydes and competently derivatized with DNPH in the reaction. A decreased recovery of MDA was also demonstrated for the same serum spiked with acetone, formaldehyde, or 4-hydroxynonenal. Therefore, a cautionary note on sample dilution should be made before any conclusions are drawn for MDA measurements. And other more reliable biomarkers for oxidative stress such as 8-iso-PGF2α are also highly needed for oxidative stress studies.
Oxidative stress has been implicated in the pathogenesis of lung disorders including pulmonary fibrosis. NT2 has a protective role in these disorders via transcriptional induction of cytotoxic protective genes including thioredoxin, and THP-1. Glutathione is the most abundant cellular thiol and is essential in antioxidant defense. Solute carrier family 7 (cationic amino acid transporter, y+ system), member 11 (Slc7a11, xCT) is an NT2 effector, and encodes a cystine/glutamate transporter that mediates entry of cystine into the cell coupling with efflux of glutamate to produce glutathione. We hypothesized that Slc7a11 is important to protection against pulmonary fibrosis. To test this hypothesis, bleomycin (0.5 U/ Kg body weight) or vehicle was instilled in wild-type (WT) and mice with spontaneous mutation in Slc7a11 (C3H/HeSnJ-Slc7a11+/J, Slc7a11+/-). Pulmonary injury responses were determined at 3, 7, 14, and 21 d after treatment. Bleomycin-induced influx of macrophages, neutrophils, eosinophils, monocytes, and lymphocytes in bronchoalveolar lavage fluids (BALF) were significantly higher in Slc7a11+/- mice than in WT mice (5-7 days). Pulmonary hemorrhage and appearance of uncommon inflammatory cells (e.g., atypical lymphocytes, basophils, megakaryocytes) in the BALF were enhanced in Slc7a11+/- than in WT after bleomycin. We found more lymphocytic nodules and diffused fibrotic lesions in Slc7a11+/- mice compared to WT mice 14-21 d post-bleomycin. Concurrently, gene expression and accumulation of lung collagen was significantly higher in Slc7a11+/- than in WT mice. Bleomycin also increased mRNA and protein levels of Slc7a11 in WT mice (3-21 days). Moreover, lung glutathione and its biosynthesis enzyme (glutamate-cysteine ligase) levels were increased post-bleomycin in WT mice while these changes were marginal in Slc7a11+/- mice. Results demonstrated that is Slc7a11 involved in protection against bleomycin-induced lung inflammation and fibrosis in the mouse.

Animal models do not exactly replicate human psychiatric disorders; however, these models have been used to investigate whether the behaviors associated with certain exposures in animals parallel those observed in autism. According to the most current version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), autism is diagnosed based on (1) persistent deficits in social communication and social interaction and (2) the presence of restricted, repetitive patterns of behavior, interests and activities. To address whether developmental chlorpyrifos (CYP) exposure was associated with autistic behaviors, a literature search was conducted to identify studies in rats and mice involving gestational or early postnatal exposure to CYP or CYP oxon (the active metabolite) and subsequent behavioral testing to assess social interactions and communication, stereotypic behaviors and other behaviors associated with novelty-seeking/anxiety. A total of 13 studies conducted in 6 different laboratories were identified. Analysis of these studies found that perinatal CYP exposure was generally associated with (1) no effect, increased social communications or conflicting findings in 6 of 7 studies; (2) no effect or increased social encounters in 4 of 4 studies; (3) no effect, reduced stereotypes or conflicting findings related to stereotypic behaviors in 9 of 9 studies; and (4) no effect or increased preference for novelty and reduced anxiety in novel environments in 5 of 7 studies. When a difference from control was observed, it was often in the opposite direction of what one would expect in autism. Based on these results, perinatal CYP exposure is not associated with the development of autism-like behaviors in rats and mice.

BBP is a high-production chemical that is primarily used as a plasticizer in polyvinyl chloride products such as flooring materials. Its urinary monobenzyl phthalate metabolite is detected in biomonitoring studies indicating widespread human exposure. The chemical’s carcinogenicity was reviewed to support a decision by a qualified panel of experts on whether or not it should be added to the Proposition 65 list of chemicals known to the state to cause cancer. OEHHA conducted a search of the scientific literature for information relevant to the carcinogenicity of BBP, up through October 4, 2013. 241 papers were identified, analyzed and summarized, as follows. BBP statistically significant increased the incidence of pancreatic acinar cell tumor, mononuclear cell leukemia, and adrenal medulla tumors in rats. In addition, rare tumors of the urinary bladder transitional epithelium were identified in treated female rats, accompanied by treatment-related increases in hyperplasia. While negative in Salmonella reverse mutation assays, BBP has tested positive in multiple other genotoxicity studies. In vitro findings include DNA base lesions in mouse bone cells, DNA single strand breaks in HepG2 cells and DNA-protein crosslinks (DPC) in rat liver cells. In vivo findings include DPC in mouse liver and sister chromatid exchanges and chromosomal aberrations in mouse bone marrow cells. Microarray and proteomic studies revealed BBP induced expression of several cancer pathway-related genes and proteins (e.g. VEGF, MYC and DEK). In human breast cells (HBC) in vitro and in rat breast cells in vivo, BBP increased markers of cell proliferation, angiogenesis, epithelial-mesenchymal transition, mi-

Formaldehyde is a high production volume chemical with a multitude of industrial uses. Due to its high reactivity, the majority of inhaled formaldehyde is deposited in the upper respiratory tract. Formaldehyde exposure has been reported to cause a number of noncancer health effects, primarily at the portal-of-entry, including sensory irritation, impaired pulmonary function, asthma and immune effects, and respiratory tract pathology. This analysis attempts to cohesively and succinctly describe the mechanistic data related to these effects. Mode-of-action (MOA) data is typically described within a given health domain (e.g., supporting sensory irritation), and is most commonly used to inform biological relevance to humans. By evaluating the plausibility of the evidence, based on the chemical properties of formaldehyde and study design characteristics (e.g., route, duration, and levels of exposure), we identify and describe the potential mechanistic inter-dependencies across health effects. For example, stimulation of sensory nerve endings in the nasal mucosa can elicit an irritant sensation, but this stimulation may also initiate a release of tachykinins, eventually resulting in airway inflammation. Similarly, formaldehyde can initiate mucociliary changes that result in epithelial damage and cell proliferation, leading to lesions in the upper respiratory tract; however, thickened mucus and increased epithelial cell proliferation may also lead to pulmonary dysfunction. Detailing the plausibility of these mechanistic linkages and presenting this information schematically provides continuity and added clarity to MOA descriptions across health domains. This analysis allows for an effective evaluation of the potential biologic mechanisms that are most likely to be operative following formaldehyde exposure. Finally, this presentation facilitates a more integrated discussion of the potential relationship(s) between noncancer health effects and carcinogenicity. Disclaimer: The views expressed are those of the authors and they do not represent U.S. EPA policy or guidance.

1810 Weight-of-Evidence Evaluation of Short-Term Ozone Exposure and Cardiovascular Effects


There is a large body of research on the potential cardiovascular (CV) effects associated with ozone exposure, including epidemiology, toxicology, and controlled human exposure studies. US EPA is reviewing these data in its consideration of a revision to the ozone National Ambient Air Quality Standards (NAAQS). We conducted a weight-of-evidence (WoE) analysis to determine if there is an association between CV effects and short-term ozone exposure at levels below the current primary ozone NAAQS of 75 parts per billion (ppb). We utilized a novel WoE framework based on US EPA’s NAAQS causality framework to critically evaluate and synthesize the relevant epidemiology, controlled human exposure, animal toxicology, and mechanistic data. We concluded that the epidemiology evidence of CV morbidity and mortality is inconsistent and lacks coherence across specific CV endpoints. Specifically, the lack of epidemiology evidence of morbidity effects is not coherent with reported mortality estimates. Toxicology studies, although somewhat more consistent, are of limited relevance because they are conducted at high exposure levels, well above the current NAAQS, and there is insufficient information on dose-response relationships. Furthermore, there is a lack of coherence between reported results from epidemiology studies (suggesting no effects) and results from animal studies (suggesting small effects at high exposure levels). Similarly, controlled human exposure studies report inconsistent effects after exposure to ozone levels above the current NAAQS. Studies that aim to identify potential mechanisms for CV effects of ozone by measuring various CV-related biomarkers also report inconsistent results with uncertain clinical significance, indicating that there is no clear biological mechanism for CV effects from ozone exposure. Overall, our WoE analysis does not support that CV effects are associated with short-term ozone exposure below the current NAAQS.

1811 Do Asbestos-Induced Pleural Plaques Cause Lung Function Deficits?

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While there is general agreement that pleural plaques are biomarkers of asbestos exposure, there is debate in the scientific community over whether pleural plaques cause lung function deficits. Many of the studies that addressed this issue were subject to certain limitations. In most studies, pleural plaques were diagnosed by radiography, which is less accurate than high resolution computed tomography (HRCT) and can lead to misdiagnoses. Some studies reported lung function changes in subjects that had lung abnormalities in addition to pleural plaques, so that the contribution of pleural plaques to deficits was unknown. To eliminate these sources of uncertainty, we conducted the first comprehensive analysis of the associations between pleural plaques and lung function based on epidemiology studies in which 1) pleural plaques were diagnosed by HRCT and 2) individuals were identified with pleural plaques and no other lung abnormalities. We identified and analyzed 16 relevant studies. We looked for patterns within and across studies and examined whether associations were reproducible. Only three of the 16 studies reported statistically significant associations between pleural plaques and some measure of lung function. Among these three studies, the lung function parameters were not consistent, suggesting that the associations were not likely causal. In addition, mean asbestos exposures in all three studies were higher in the subjects with pleural plaques than in the subjects without. This suggests that if the effects were not due to chance, the asbestos exposure itself, rather than pleural plaques, may have been responsible for observed lung function deficits. Taken as a whole, the direction of effect (i.e., lung function deficit vs. improvement) varied among studies, indicating the absence of even subtle effects and that the lack of effect noted in the majority of studies was not a result of low statistical power. We conclude that there is no reliable association between the presence of pleural plaques in asbestos-exposed populations and lung function deficits.

1812 Firefighters’ Inhalation Exposures and the Risk of Developing Multiple Myeloma

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Firefighters encounter a complex mixture of chemicals under unique exposure conditions. The complexity in evaluating whether these exposures lead to occupational diseases has led states to pass “presumptive causation” legislation, which assume all cancers that occur among firefighters are a result of on-the-job exposures. While such laws may be well intentioned, their scientific basis is dubious at best. Because a meta-analysis of firefighter data suggests these workers have a relative risk of 1.5 for multiple myeloma (MM), a cancer of cells in the hematopoietic system that can affect multiple sites in the body, including the bone, bone marrow, and kidney, we conducted a weight-of-evidence analysis to evaluate whether exposure to airborne chemicals typically identified in firefighting environments could be causally linked to increased MM risks. We examined a variety of factors in exposure, toxicological, and epidemiological studies, including study design, power, corroboration, relevance, and coherence within and among studies. Firefighters may inhale a variety of combustion products (including IARC Group 1 carcinogens) in both acute and more chronic scenarios (e.g., diesel exhaust). We found that for the well-studied IARC Group 1 chemicals, there was insufficient evidence that MM risk was increased with exposure. Many studies have sought to identify MM risk factors, but the results are inconsistent. Reviews and meta-analyses of MM among firefighters have reported modestly increased, but unstable, MM risks. Significant methodological issues cloud the interpretation of these meta-analyses. For example, only a few of the studies reported a statistically significant increase in MM in firefighter populations, and exposure information was often lacking. Because of a lack of concordance among the studies as to exposure and outcome, our evaluation found that airborne occupational exposures in firefighters are not causally associated with an increased risk of MM. Little is known about the etiology of MM, and job-related “presumptive causation” in the case of MM in firefighters is not supported by available scientific evidence.

1813 Toxicological Review of Biocides Used in Hydraulic Fracturing

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The use of hydraulic fracturing to extract gas and oil from shale formations has maintained its exponential growth in US and worldwide. Hydraulic fracturing consists of injecting water, friction reducers, proppants, disinfectants, surfactants, thickeners, scale inhibitors, corrosion inhibitors, and acids to promote flow of hydrocarbons otherwise bound in impermeable matrices. The rapid explosion of

1809 Integrated Mechanistic Evidence on Noncancer, Portal-of-Entry Effects following Formaldehyde Inhalation


While there is general agreement that pleural plaques are biomarkers of asbestos exposure, there is debate in the scientific community over whether pleural plaques cause lung function deficits. Many of the studies that addressed this issue were subject to certain limitations. In most studies, pleural plaques were diagnosed by radiography, which is less accurate than high resolution computed tomography (HRCT) and can lead to misdiagnoses. Some studies reported lung function changes in subjects that had lung abnormalities in addition to pleural plaques, so that the contribution of pleural plaques to deficits was unknown. To eliminate these sources of uncertainty, we conducted the first comprehensive analysis of the associations between pleural plaques and lung function based on epidemiology studies in which 1) pleural plaques were diagnosed by HRCT and 2) individuals were identified with pleural plaques and no other lung abnormalities. We identified and analyzed 16 relevant studies. We looked for patterns within and across studies and examined whether associations were reproducible. Only three of the 16 studies reported statistically significant associations between pleural plaques and some measure of lung function. Among these three studies, the lung function parameters were not consistent, suggesting that the associations were not likely causal. In addition, mean asbestos exposures in all three studies were higher in the subjects with pleural plaques than in the subjects without. This suggests that if the effects were not due to chance, the asbestos exposure itself, rather than pleural plaques, may have been responsible for observed lung function deficits. Taken as a whole, the direction of effect (i.e., lung function deficit vs. improvement) varied among studies, indicating the absence of even subtle effects and that the lack of effect noted in the majority of studies was not a result of low statistical power. We conclude that there is no reliable association between the presence of pleural plaques in asbestos-exposed populations and lung function deficits.
volume and extent of fracking exploration has led to community, regulator, and health practitioner concerns over the potential health effects associated with exposure to fracking fluid constituents. Many of the disclosed ingredients are cited as harmless. However, there are certain chemical groups, such as antimicrobials, that have specific (by design) adverse biological activity. This presentation focuses on the identity and toxicity of fracking fluid constituents listed as having a disinfectant function. The methodology consisted of querying publicly-available databases used by the oil and gas industry to disclose chemical information. Data fields associated with chemical name, CAS number, additive concentration, and fracking fluid concentration were extracted from each disclosed chemical. Next, retrieved chemicals were matched with toxicity data from open literature. The information on the stock as well as the formulated fluid concentrations, in concert with toxicity thresholds, was used to calculate Potential for Exposure Quotients (PEQs) under an accidental release scenario. Results for 2013 show that nearly 100 hydraulic fracture fluid additives are categorized as a biocide or an antimicrobial agent. Some of the more commonly listed disinfectants are glutaraldehyde, naphthalene, ethoxylated nonylphenol, and tetrakis hydroxymethyl-phosphonium sulfate. Aldehydes were found to be the most common (N=1,678) toxic (lowest RfC of 8.0E-05 mg/m³) fluid ingredients with the highest PEQs (average fracking fluid concentration of 2.5%). For the purpose of this presentation, all findings are presented as summary tables for ease of reference and sharing of information.

1814 Acute Effects of Acrrolein in Human Volunteers during Controlled Exposure

Acrrolein (ACR) is a reactive ω,β-unsaturated aldehyde formed by burning wood, plastic, diesel fuel, paraffin wax, smoking and cooking. It is a common constituent of indoor air. There are limited toxicological data available, however, as ACR is highly irritating to the eyes and respiratory tract, irritation is considered to be the critical effect. The aim of the study was to determine thresholds for acute irritation and inflammation for ACR and thus improve the scientific basis for setting exposure limits. Nine healthy volunteers of each sex were exposed at six occasions for 2 h at rest to: clean air, clean air + ethyl acetate (EA), 0.05 ppm ACR, 0.05 ppm ACR + 15 ppm EA, 0.1 ppm ACR, and 0.1 ppm ACR + 15 ppm EA. The exposure conditions were carried out in a balanced order and were blinded to the volunteers. Symptoms were extracted for each disclosed biocide. Next, retrieved records were rated on Visual Analogue Scales. EA was used to mask the potential influence of odor on the symptom ratings. The ratings of eye irritation increased during exposure to ACR in a dose dependent manner (median values 0 mm, 1.5 mm and 8 mm for control, 0.05 ppm and 0.1 ppm of ACR respectively, p < 0.001, Friedman test) with no influence by co-exposure to EA. No significant exposure related effects on pulmonary function or nasal swelling were found. Furthermore, no effects were seen on markers of inflammation and coagulation in blood (C-reactive protein, IL-6, serum amyloid A, fibrinogen factor VIII, von Willebrand factor and Clara cell protein) and induced spumon (cell count, differential cell count, IL-6 and IL-8). Blink frequency, recorded by electromyography, showed a dose-dependent increase (p=0.049) for exposure to ACR alone. However, this effect was not seen for combined exposure to ACR and EA. In conclusion, our study suggests that short-term exposure to 0.1 ppm ACR is mildly irritating to the eyes.

1815 At the Intersection of Occupational Toxicology and Product Quality: Harmonization of Risk-Based Values in the Pharmaceutical Sector
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Decisions based on health risk assessment are an integral part of pharmaceutical manufacturing. Occupational exposure limits (OELs) are a key tool for protection of workers. Acceptable daily exposures (ADEs) address residual carryover of active ingredients, as well as impurities. OELs and ADEs share a common risk method (e.g., OELs vs. ADEs). The result is improved consistency in risk values in traditional occupational risk assessments that should be considered for more rigorous CRA. Overall, we found that the absence of a decision support tool yielded inconsistent consideration of CRA in occupational assessments. The evaluation also provided insights on data gaps that preclude conduction of high confidence CRAs for current occupational scenarios. Despite these gaps we demonstrated how a systematic decision tool can serve as a pragmatic solution for improving consistency in conducting occupational CRAs.

1816 Cumulative Risks in Occupational Settings: A Checklist Tool to Support Decision Making
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Cumulative risk assessments (CRAs) evaluate the likelihood of adverse health effects resulting from all routes of exposure to multiple stressors. Applicable attention has focused on improving risk science methods in this area. For example, approaches have been published for addressing environmental exposures to mixtures of chemicals with a unifying mode of action or for assessing community level health impacts arising from diverse exposures. However, guidance for conducting CRAs for occupational scenarios is lacking. To address this gap, we conducted a critical analysis of current CRA frameworks to identify a set of techniques suitable for translation to occupational risk assessment. Consistent with elements of existing methods, we developed a refined CRA checklist tool with several features customized for consideration of the occupational environment. Key elements include provisions for incorporating exposures by oral, inhalation and dermal routes (reflecting occupational plus non-occupational exposures); specific guidance for chemical stressors with a process for modifying the CRA based on presence of multiple stressors; and, a tiered risk system to select assessment needs and provide flexibility in applying any of several alternative validated models. The CRA checklist tool was also applied to published occupational exposure scenarios to identify areas in traditional occupational risk assessments that should be considered for more rigorous CRA. Overall, we found that the absence of a decision support tool yielded inconsistent consideration of CRA in occupational assessments. The evaluation also provided insights on data gaps that preclude conduction of high confidence CRAs for current occupational scenarios. Despite these gaps we demonstrated how a systematic decision tool can serve as a pragmatic solution for improving consistency in conducting occupational CRAs.

1817 Improper Use of Haber’s Law Results in Erroneous Fatality Estimation from Predictive Models
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There are two common measures for inhalation exposure to a vapor/gas toxicant: the dosage (defined as the product of concentration and time [Ct]) and the toxic load (defined as Cnt, with n being the toxic load exponent). The historical and most-often used estimator is Ct (Haber’s Law) which was developed in the early 1920s. However, it was soon determined that Ct was inadequate to account for changes in toxicity as a function of exposure duration. Thus, the toxic load was introduced (and subsequently popularized in the 1980s) as an empirical solution to the shortcomings of the Ct measure. The differences in lethality predictions between dosage and toxic load approaches were investigated via simulation for a target population of rats exposed to sarin. An n of 1.6 was used for the toxic load based on the rat study of Mioduszewski et al. (2002). The Haber (n = 1) and toxic load models were compared by modeling release of sarin from a fixed point over a spatially uniform population of rats in three different scenarios. In all three scenarios, the Haber model gave slight underestimates of fatalities until approximately 3-5 minutes following release, after which fatality estimates were at least 125% higher than those of the toxic load model. This demonstrates that unequal exposure over time within a population affects differences in fatality estimation between Haber and toxic load models. When n does not equal one, fatalities are erroneously estimated when using Haber’s Law because time is unrealistically weighted against concentration. For chemicals with n greater than one, the Haber model can overestimate fatalities at low concentrations or long durations and underestimate fatalities at high concentrations or low durations. This relationship reverses for those chemicals with n less than one.
1818  Impact of Nonconstant Concentration Exposure on Lethality of Inhaled Hydrogen Cyanide in the Rat: A Case Study for Assessing the Validity of Toxic Load Models

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The toxic load model (TLM) is an empirical approach in hazard assessment modeling for estimating the relationship between the inhalation toxicity of a chemical and the exposure duration. The toxic load (TLM) is normally expressed as a function of vapor concentration (C) and duration (t), with TLM equaling C x t being a typical form. Hypothetically, any combination of concentration and time that yields the same "toxic load" (cumulative exposure) will give a constant biological response. These formulas have been developed and tested using controlled, constant concentration animal studies, but the validity of applying these assumptions to time varying concentration profiles has not been tested. Experiments were designed to test the validity of the TLM under conditions of non-constant acute exposure. Male Sprague-Dawley rats inhale constant or pulsed concentrations of hydrogen cyanide (HCN) generated in a nose-only exposure system for 5, 15, or 30 minutes. The observed lethality of HCN for the 11 different C vs. t profiles was used to evaluate the ability of the TLM to adequately describe the lethality of HCN under the conditions of non-constant inhalation exposure. The TLM was found to be applicable under the tested conditions, with the exception of the median lethality of very brief, high concentration, discontinuous exposures.

1819  A Framework for Risk Assessment for Less-Than-Chronic and Intermittent Exposures to Chemicals (Noncancer Endpoints)

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Exposure to chemicals at contaminated sites, whether single, repeated or intermittent, may last for shorter periods of time than those upon which most guidance values are normally based. Because it is presently not feasible to conduct toxicity studies or develop Toxicity Reference Values (TRVs) specific to each scenario of interest, methods are needed to address this variety of scenarios, drawing as much as possible on existing guidance values. A working framework was developed to address non-cancer effects resulting from short-term and intermittent exposures to chemicals. The framework identifies key parameters relevant to the implications of duration and intermittency of exposure, and provides an integrated, tiered approach for applying increasing levels of toxicological understanding and expertise to exposure scenarios of interest, including consideration of both toxicokinetic (half-life) and toxicodynamic (persistence of effect) implications of intermittent exposures. It incorporates the use of TRVs based on exposure periods as similar as possible to the actual exposure periods and the use of dose averaging under limited, specified conditions. Key data and information sources supporting consideration of less-than-chronic exposures are also documented, including (1) references for a broad range of TRVs for less-than-chronic exposures from a variety of organizations, criteria for selection, and a description of their bases and applicability; (2) grouping of chemicals based on their half-lives and appropriate reference data sources, and (3) grouping of chemicals based on persistence of their effects. This framework will aid in improving the scientific basis for the evaluation of less-than-chronic and intermittent exposures in a variety of settings.

Disclaimer: The views expressed herein do not necessarily represent Health Canada policy.

1820  Use of a Single-Pass, Nose-Only Exposure System to Simulate High Altitudes


The military has experienced a number of known or suspected hypoxia-related in-flight events in its pilots of high performance aircraft. Some contaminants, identified in breathing air supplied to pilots, are being investigated as a possible link to the hypoxia-related events. In order to evaluate possible hypoxia-related hypotheses in an animal model, a nose-only exposure system was adapted to expose rats to controlled levels of toluene under low pressure (high altitude) conditions. A 52-position nose-only exposure system (Lab Products, Seaford, DE) with closed animal compartments was used to demonstrate the capability for an air flow of 5.0 L/min while adjusting exhaust flow to maintain an internal pressure to simulate altitudes of 10,000 or 20,000 feet above sea level. Toluene was mixed into the test atmosphere using either a gas cylinder containing a toluene-air mixture, or toluene vapor from a pressure vessel and maintained at 100 or 200 ppm in the test atmosphere. Oxygen was incorporated into the inlet stream at the higher altitude settings to maintain a partial pressure approximately equivalent to ambient. Portable port valves were used to allow rat exposures to be staggered under simulated high altitude conditions for pharmacokinetic studies. An initial study was conducted in which adult male and female S-D rats were exposed to 200 ppm toluene for 1 hr using the single pass, nose-only exposure system at simulated altitudes of 940 (ambient), 10,000 or 20,000 feet above sea level. The post-toluene tissue uptake results are presented in a separate poster by Mahle et al. 2014.

1821  Comparison of Liver Toxicity Potencies of Three Dinitrotoluene Compounds

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Dinitrotoluenes (DNTs) and chlorinated dibenzo-p-dioxins (PCDDs) are among the most toxic compounds. Exposures to these contaminants are widespread and can result in adverse health effects. Several studies have reported that the degree of liver toxicity of DNTs is dependent upon the isomer of the DNT. In order to understand the potential health effects associated with DNT exposure, a study was performed to compare the liver toxicity potencies of the three major isomers of DNT, 2,4-, 2,6- and 2,4,6-DNT. Potential points of departure for liver effects were identified and used to compare their toxicity potencies. We then examined the potential mechanisms that could account for the differences in hepatotoxicity. Our results indicated that similar pathologic liver lesions were induced after chronic-duration exposure to the three DNTs and their progression can be summarized in four stages: liver degenerative effects and/or cell death, hyperplastic/regenerative effects, neoplastic nodules, and hepatocellular carcinoma. Based on available biotransformation, toxicogenomic and genotoxicity information, imbalanced detoxification due to covalent binding with proteins and DNA, production of reactive oxygen species, and inhibition of oxygen supply may all contribute to DNT-induced non-cancer liver toxicity and carcinogenesis. While their potencies of noncancer liver toxicity are not significantly different, 2,6-DNT is the most potent liver carcinogen followed by 2,4-DNT; 2,4,6-DNT is the least potent. The differences of liver cancer potencies are consistent with their potencies of DNT-induced hepatocyte genotoxicity. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

1822  Methymercury Inhibition of Serum Paraoxonase 1 Activity and the Risk of Cardiovascular Disease

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Paraoxonase-1 (PON1) is an antioxidant enzyme present on high density lipoproteins and is known to be protective against atherosclerosis. PON1 has become a useful disease biomarker since low serum activity of this enzyme is associated with increased cardiovascular risk. PON1 also catalyzes the detoxification of organophosphates. Therefore, serum PON1 is essential for the prevention of two distinct adverse outcomes. The single sulfhydryl moiety on PON1 is believed to be the molecular target. Epidemiological and in vitro studies show that exposure to methyl mercury has been associated with a decline in PON1 activity. Based upon data from a native fish-eating population, methyl mercury exposure in the 0.1 to 0.3 μg/kg/day range is associated with substantial loss of serum PON1 activity. Using these data, we explored PON1 as a potential disease biomarker to estimate the cardiovascular risk from methyl mercury ingestion in the general population. The simulated shift in the population distribution of PON1 towards lower levels increases the percent of the population at risk for acute cardiovascular outcomes. This approach of evaluating mercury toxicity follows recommendations made in the National Academy of Sciences report “Science and Decisions” to explore the interaction between chemical toxicity and background disease. This example points out the emerging importance of disease biomarkers in chemical risk evaluation.

SOT 2014 Annual Meeting 477
currently, sustainable food production, including alternative protein sources, is receiving much attention and exploration of these sources is widely supported by international organizations. these alternative proteins sources should have the lowest allergy risk possible. until now, accurate pre-marketing assessment of the allergenicity of new proteins is impossible. moreover, existing guidance documents are restricted to qualitative, assessment, low predictive value and result often in an oversimplification in terms of hazard assessment. to overcome those limitations, we developed a two-dimensional scale for expressing allergenicity that allows a quantitative hazard characterization of new proteins. the two dimensions of the scale were defined as 1) the prevalence expressed as the percentage of the general population that has the allergy and 2) the potency of the protein expressed as the ed50 in mg-protein of the allergen (the dose that provokes objective allergic responses in 50% of the allergic persons). in terms of hazard characterization, severity of symptoms was not included, since this is a result of the nature of the exposure and the potency of the individual rather than an inherent and independent attribute of the allergen.

the scale was developed by ranking 11 allergenic products based on potency data from individual food challenges and 22 scientific papers on prevalence, selected after a critical review. the resulting 2-dimensional scale can be used to project a new protein on the scale and assess its potential hazard relative to the known allergens. in a next step, we aim to develop a model that predicts the position of a new protein on the scale using its physical, chemical and biological characteristics.

1823 determination of the relative allergenicity of food products for quantitative hazard assessment

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1824 a 21st-century roadmap for human health risk assessment

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the ilsi health and environmental sciences institute (HESi) risk assessment in the 21st century (RISK21) project was initiated to develop a scientific, transparent, and efficient approach to the evolving world of human health risk assessment. RISK21 developed a framework that reconsidered in the way chemical risk assessment information is obtained and used. it is a problem formulation-based, exposure driven, tiered data acquisition approach that allows an informed decision on human health safety to be made when sufficient evidence is available. this consistent approach maximizes the ability to inform decisions and optimize resource usage. two case studies were developed to illustrate these principles. the first example identified testing needs for a new mhb in class pesticide to be used in mosquito netting, and illustrated how existing information from other pesticides in the same chemical class and knowledge of use patterns can inform data needs and decision making. the second example evaluated a large number of chemicals which might be present in drinking water, to prioritize which are of highest potential concern for human health risk assessment. both case studies also identified key issues and possible approaches to address cumulative risk. the overall goal of these examples was not to make definitive risk assessment determinations but to establish the utility of the RISK21 framework in assessing the value of available information and making decisions about what, if any, additional information is needed to inform a decision.

1825 poor pregnancy outcome and small for gestational age associated with blood cadmium among mothers in Malaysia

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Cadmium is a known heavy metal that contaminate environment and causes health problems like Iiai-Iiai disease. However, not many studies have been carried out to determine its pregnancy outcome. this cross-sectional study was conducted among pregnant mothers in the state of Selangor, Malaysia. the objectives were to determine blood cadmium concentrations and its impact on the pregnancy outcome. a total of 280 third trimester pregnant mothers were involved in the study using a universal sampling method at selected health clinics from July to September 2007. a face to face interview using structured questionnaires was used. pregnancy and delivery history were obtained from the antenatal record book. blood samples were analysed for cadmium using gfaas at 1MR laboratory. mean of blood cadmium concentration was 1.47g/11g/ml with minimum and maximum value of 0.12g/11g/ml and 2.86g/11g/ml, respectively. mean age of respondents was 28.3±4.4 year-old. the factors that significantly associated with blood cadmium concentration were household income per month (p<0.05), spouse type of work (p<0.05), and current haemoglobin level (p<0.05). Using the multivariate analysis, haemoglobin level was significantly associated with blood cadmium (adjusted R2 = 0.152; p<0.01). Every incremental of 1g/dl of haemoglobin will reduce 0.13g/l of blood cadmium level. the study also revealed an association between higher blood cadmium mothers with poor pregnancy outcomes (p<0.01) and small for gestational age (SGA) (p<0.01). even though the mean blood cadmium level in the study population was low compared to the reference value (10g/11g/ml), it showed that the environmental exposure to cadmium is real among pregnant mothers in this country. Proper education of public about the importance of avoiding exposure and emphasis on having an effective environmental management in maintaining a cadmium-free condition wherever possible are the ultimate aims of minimizing health impacts.

1826 lung overload: conclusions of an ECETOC task force


chronic exposures to high concentrations of poorly soluble, low toxicity particles (PSP) cause various toxicological effects in the lung. Lung overload is typified by impairment in alveolar macrophage (AM) mediated particle clearance and loss of AM mobility, suggesting a threshold mediated mode of action. the task force examined the current scientific understanding of the ‘lung overload’ hypothesis and concludes: 1) inhalation exposure to high concentrations of PSP, irrespective of particle size, are eliciting comparable localized pulmonary toxicity via processes that are pro-inflammatory in nature, causing oxidative stress and an persistent pulmonary inflammatory response 2) the mechanisms of the described effects are clearly threshold related 3) adverse outcome pathway is largely common within all species and is applicable for nano and microscale particles 4) rats are the most sensitive species with regard to non-neoplastic and unique for neoplastic responses 5) epidemiological studies have not been able to detect an association between occupational exposures to PSP and an increased risk for lung cancer 6) differences of particle retention, distribution, clearance patterns and defense systems in the lungs of exposed rats vs. primates or humans, may account for both the greater sensitivity in rats and corresponding differences in pulmonary pathological responses to long-term particle exposures 7) differences in the biosolubility of deposited particles may accelerate or slow down the process of lung overload development

the derivation of health based Occupational Exposure Limits, a Derived No Effect Level, for PSP is justified. Because rats are more sensitive than humans and based on comparable biokinetics, an overall assessment factor of 1 for intra- and inter-species differences is sufficient. DNELs based on NOAELs/NOAECs from animal inhalation studies, adjusted for human equivalent concentrations by appropriate dosimetry modeling, is recommended.
1827 A Decision Tree to Optimize Literature Identification for Pharmaceutical Risk Assessment

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Risk assessment needs are complex for pharmaceuticals, including occupational health, patient safety, product quality and environmental impact. Assessments cover a range of substances including actives, intermediates, process reagents, excipients, degraders, contaminants and leachates. Optimizing literature identification ensures that key information is not missed and that time is not spent on unproductive database searches. Because of the diversity of scenarios and substances involved, a one-size-fits-all approach to literature identification is not efficient. We developed a decision tree for literature identification for pharmaceutical risk assessment. The decision tree starts with the identification of integrated scientific reviews or external risk assessments followed by a binning strategy depending on the use or type of substance (e.g., active, food contact material, excipient, industrial chemical, endogenous substance). Criteria used for prioritization of search sites and databases within a bin included update frequency, level of peer review, completeness of data presentation, breadth of coverage for substances of interest, ease of data access, and level of regulatory recognition. If the literature does not identify sufficient high quality data, the focus shifts to a targeted determination of tools for addressing data gaps (e.g., in silico methods and the threshold of toxicological concern). This decision tree was tested on different types of substances and reduced the overall time spent in conducting the risk assessment. The difference between fee-based pharmaceutical literature resources was also compared to freely available resources using the decision tree, and the biggest impact of the paid resources appeared to be in the reduction in time needed to sort through data from multiple sources. The decision-tree approach is an important problem formulation tool ensuring that resources used are well aligned with the intended use of the risk assessment.

1828 Use of Cluster Groups in Systematic Review of Literature


Systematic review of the literature for risk assessment purposes can result in >10,000 references, which can be time and cost intensive to review in a traditional linear manner. Commercially available, visual analytics software enables clustering of similar references based on automated text analysis. References are clustered around key concepts that distinguish papers from the full set of results and can be prioritized for review by concept. We tested the efficacy of prioritizing references for review with this software using results from a comprehensive search of CVL literature. Our initial search strategy resulted in approximately 9,000 references. We eliminated approximately 3,500 references electronically using additional key word searches and identified a batch of over 300 highly relevant ’seed’ references. The remaining references along with seeds were clustered and about 1,600 references were found in clusters containing at least one seed reference. This group of references was prioritized for review and evaluated by multiple screeners. The remaining references underwent a traditional title and abstract review by a single subject matter expert. Ultimately, we found approximately 85% of the relevant animal and 70% of the relevant human hazard identification studies in the health effects cluster. Depending on the circumstances and size of clusters, references in clusters not identified as relevant can be handled in several ways: discard without review (not recommended), apply additional key word searches to prioritize further, or screen by a single (rather than multiple) reviewer. Based on our results, clustering references is an effective way to streamline review of large literature datasets as the bulk of the relevant literature was found within the health effects cluster. We conclude that clustering approaches have promise, but must be carefully tailored to individual needs. Additional comparisons of this type are warranted. The views expressed are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

1829 Evaluating Study Quality: A Comparison of the TOXR Tool and OHAT Approaches

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Standardization and documentation of criteria used to evaluate the quality of studies used in risk assessment is a recognized need in the environmental health sciences. The NTP Office of Health Assessment and Translation (OHAT) developed a method of evaluating animal and human studies for risk of bias based on criteria used in systematic reviews. The European Commission developed the ToxR tool to assess the reliability of toxicological data from in vitro and in vivo studies. Both methods are intended to evaluate studies in a systematic and transparent manner, but the approaches differ. The ToxR tool applies points based on a yes/no to a series of questions that leads to the assignment of Klimisch categories ranging from insufficient documentation to reliable without restrictions. The OHAT approach also has a series of questions with project-specific criteria for 4 possible ratings reflecting the potential for bias in the study, but no overall score is assigned. Seven reviewers independently used the OHAT method to evaluate 11 in vivo animal studies and 7 in vitro skin permeation studies previously evaluated using the ToxR tool. The purpose was to evaluate the OHAT tool for consistency among different reviewers and compare and contrast the strengths and weaknesses of the two tools. For both methods, we found that some questions were too vague, resulting in different interpretations. For the OHAT method, this could be due to our failure to tailor criteria rating for this specific project as required. The ToxR Tool would also benefit from such tailoring. With both tools, high quality studies generally had the most similarity between the reviewers, while lower quality studies varied. Strengths and weaknesses were noted for both tools and opportunities for each to incorporate changes were suggested by the current comparison. This abstract does not reflect EPA policy.

1830 Systematic Evaluation of Inhalation Studies for Exposure Quality: A Case Study with Formaldehyde


Misusing can occur by any route of exposure, but it is a particular challenge for inhalation studies because of the inherent complexity of generating and characterizing consistent test atmospheres. There is an expectation that human and animal test subjects in a chamber study will be exposed under conditions that are carefully regulated, frequently measured, and clearly reported. Poor study design, human error, and problems with mechanical and electronic equipment can impair an inhalation exposure and undermine the validity of a study. A systematic approach to evaluating studies, when selecting inhalation studies for a chemical assessment, involves a toxicologist familiar with chamber operations evaluating each study for seven key elements of exposure quality: 1) test article characterization, 2) generation method, 3) analytical method, 4) analytical concentrations, 5) particle size characteristics, 6) chamber type, and 7) controls. Any deficiencies are recorded in a table and the study is ranked for its exposure quality and documentation. These rankings may determine whether a study is considered for hazard evaluation and/or dose-response analysis. The exposure quality evaluation of approximately 200 formaldehyde chamber studies is presented as an example. Nearly half of these studies are considered inadequate for hazard evaluation and/or dose-response analysis.

The views expressed are those of the authors, and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

1831 Development of a Database Tool to Track the Study Selection Process for Hazard Identification: Naphthalene Case Study

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When developing a systematic review process for identifying relevant health effects literature for evaluation of chemical health hazards, transparency in the study selection process is imperative. Using naphthalene as a case study, a database tool was developed to organize and summarize the toxicological literature for this chemical and to communicate evaluation and selection of studies for hazard identification. The moderately-sized database for naphthalene consists of human case reports and epidemiology studies; chronic, subchronic, and acute animal toxicity studies; and mechanistic and genotoxicity studies. The database tool tracks study selection, evidence table and exposure-response array development, and quality-control review processes. Major features of the database tool are: 1) inclusion of all human and animal toxicity studies selected for full-text review from the systematic literature search, with brief study details and identification of all endpoints evaluated; 2) rationale for inclusion/exclusion of the study in evidence tables/exposure-response arrays; and 3) identification of endpoints with statistically significant findings. This case study of naphthalene demonstrates a database tool that organizes and tracks hazard identification activities, identifies potential data gaps, and reduces potential study-selection bias by transparently presenting pertinent information for all.
potentially relevant studies. Additionally, this database tool effectively conveys the database’s size and coverage of endpoints while communicating study-specific decisions in the hazard identification process.

Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

1831a Scientists’ Attitudes toward Regulatory Risk Assessment

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Public debate over the scientific quality and accuracy of chemical risk assessments developed by federal programs is growing. This debate can be informed by accurate knowledge of expert opinion on these issues. Therefore, we conducted a survey on regulatory risk assessment (RRA) among scientists with knowledge and experience in this field. Members of Risk Assessment Specialty Section of SOT; Dose Response Section of the Society for Risk Analysis, and International Society for Regulatory Toxicology and Pharmacology were surveyed in February 2013 about problem formulation and analysis plans, the acquisition and evaluation of data, weight of evidence methodology, peer review, and the use of scientific risk evaluation in risk management decisions.

The 186 respondents largely agreed on proper procedures for conducting RRAs while disagreeing on whether these procedures are properly implemented in practice. Thus, 68% regard problem formulations and analysis plans as very important, but only 30% say they are usually conducted. Similarly, 69% find it very important for assessors to have access to raw data of critical studies, but 31% say it is usually made available. Over 90% emphasize the use of clear criteria for selecting and evaluating studies, but only 24% report that standardized protocols are usually implemented. Most (82%) believe the same evaluation criteria should be used for evaluating all studies regardless of their institutional origin. Eighty-nine percent believe weight of evidence methodology should be used but only 45% say WOE methods are usually applied, and 24% describe its use as consistent or transparent. Only 41% think risk management decisions are based on current knowledge of biology and toxicology. Finally, they believe risk managers give too much weight to politics, the media, and environmental groups, and too little weight to scientific factors and economic costs and benefits. These results suggest that scientific concerns need to be addressed by federal officials in order to improve the use of risk assessments in the regulatory process.

1831b US EPA’s Framework for Human Health Risk Assessment to Inform Decision Making


The Framework for Human Health Risk Assessment to Inform Decision Making was developed by EPA’s Risk Assessment Forum to be responsive to the decision making needs of the Agency. It addresses the recommendations presented in the National Research Council’s (NRC’s) Science and Decisions (2009) on the design of risk assessments, including planning, scoping, and problem formulation. The Agency’s Risk Assessment Forum held broad-based internal discussions prior to assembling a Technical Panel of senior risk assessors and risk managers from across the Agency to develop the Framework. Prior to releasing the final Framework, the Framework received extensive Agency review along with inter-agency and external peer reviews. In developing the final Framework the input from the review process along those received from the public were considered. In addition to the focus on planning, scoping and problem formulation, the Framework presents the concept of risk assessment that are “fit for purpose” and details that this is accomplished with an increased dialogue between risk assessors and risk managers to ensure that the risk assessment will inform the risk management options. The problem formulation phase described in the Framework emphasizes the development of a conceptual model that follows the source to outcome continuum for the risk assessment as a key input into the development of the analysis plan. In accordance with longstanding agency policy, it also emphasizes the importance of scientific review and public, stakeholder and community involvement and links the reader to agency resources on these topics. This Framework will be instrumental in facilitating the implementation of existing and future EPA guidance for conducting human health risk assessments and in improving the utility of risk assessments that inform risk management decision-making processes. This presentation will discuss details of the Framework and associated implementation plans by the Agency.

1831c When Does Exogenous Exposure to Endogenously Produced Chemicals and Essential Elements Become a Problem?


The U.S. Environmental Protection Agency’s (EPA’s) National Center for Environmental Assessment (NCEA) evaluates hazard and dose–response relationships for certain substances found in the environment in order to support EPA’s risk assessment and risk management activities. Although chemical pollution is commonly considered to result from the release of anthropogenic contaminants to the environment, a number of these “contaminants” are produced either endogenously in healthy organisms or are required for normal metabolism. Some chemicals on EPA’s chemical priority list (e.g., HAPs, CCL, NAAQS), in addition to arising from human economic activities, are also produced endogenously in mammalian cells (e.g., methanol, formaldehyde, CO) or are essential elements (e.g., Zn, Mn, Cu). Chemicals that are endogenously produced or that are required for normal metabolism present unique challenges in evaluating hazards from their exogenous exposure. For instance, is there some level of change above normal internal concentrations that is needed to induce adverse effects? Are natural levels of endogenous chemicals harmless? How do exogenous exposures impact endogenous chemical levels? The purpose of this presentation is to articulate issues to consider in characterizing the toxicity of exogenous exposure to endogenous chemicals. To build more common understanding and consider issues specific to endogenous chemical health assessment, NCEA will be hosting a public workshop in 2014 on this evolving area of science. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the EPA.

1831d Environmental Epigenetics: Potential Application in Human Health Risk Assessment


Although previous studies have shown a significant involvement of epigenetic dys-regulation in human diseases, the applicability of epigenetic data in the current human health risk assessment paradigm is unclear. The goals of this study are to compare the relative sensitivities of epigenetic alterations (DNA methylation) with tumor incidence data and to explore the possibility of incorporating epigenetic data into the hazard identification step of human health assessments. DNA methylation alterations and tumor incidences in laboratory animal studies were analyzed from various toxicological databases for the following chemicals: di(2-ethylhexyl) phthalate, bromodichloromethane, dibromochloromethane, chloroform, hydrazine, trichloroethylene, benzidine, and trichloroacetic acid. All analyses involved conversion of animal doses to human equivalent doses. Benchmark dose values for tumour incidences were compared to no-observed-adverse-effect levels (NOAELs) for DNA methylation changes. In the absence of a NOAEL, a 10-fold uncertainty factor was applied to the lowest-observed-adverse-effect level (LOAEL) to approximate a NOAEL. This analysis revealed that DNA methylation is 1.2- to 25-fold more sensitive than the corresponding tumor incidence for all chemicals, except for trichloroacetic acid, where there is no difference in sensitivity. The predominant DNA methylation alteration was hypomethylation of either whole DNA or the promoter region of various oncogenes, and the exposure duration of epigenetic studies was shorter compared to a 2-year carcinogenicity study. This study shows that DNA methylation changes are more sensitive than tumor incidences and could potentially be considered as an alternative point of departure in human health assessments of suspected carcinogens. However, more research is needed to ascertain how epigenetic data can be applied in human health risk assessment. (The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA)

1831e New Concepts for Risk and Safety Assessment of Plant Food Supplements (PFS)

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To adequately support the safe use of plant food supplements (PFS), uniform and well accepted procedures for risk and safety assessment are essential. While many consumers equal ‘natural’ with ‘safe’, PFS may contain compounds that are of concern for human health. A major bottleneck in the safety assessment of botanicals and botanical ingredients and thus also for PFS is the fact that some botanicals may...
contain compounds that are both genotoxic and carcinogenic. Risk assessment of such compounds is complicated and an international scientific agreement on the best approach is currently lacking (EFSAS, 2005). Therefore, new concepts were tested that could be of use for risk and safety assessment of PFS focusing on finding adequate ways to judge the risk or safety of PFS that may contain genotoxic carcinogens. This should ultimately give a better idea on when risk management actions would be needed, but also for which PFS there is no reason for concern even though they do contain limited amounts of genotoxic carcinogens. The new concepts tested include the margin of exposure (MOE) approach, a mode of action (MOA) based approach and a matrix effect approach. The MOE was found to be a valuable approach in risk and safety assessment of PFS that can be used to set priorities for risk management. Moreover, the integrated approach of in vitro and MOA based physiologically based kinetic (PBK) modeling was found to be a very useful addition to risk and safety assessment of PFS based on the MOE approach since it facilitates read-across and also provides a way to take matrix effects into account. Furthermore, the new concepts tested may prove a way forward to markedly reduce the number of experimental animals used for safety testing of PFS.

The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 245199. It has been carried out within the PlantLIBRA project (web site: www.plantlerba.eu). This report does not necessarily reflect the Commission views or its future policy on this area.


1831f Probabilistic Environmental Hazard Assessments of ToxCast Phase I and II In Vitro Datasets

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Little toxicology data exists for the majority of industrial chemicals. Our research group has examined approaches to utilize known toxicological data to predict thresholds of untested chemicals, and to select assays for assessing toxicity when multiple model systems exist. Here we employed chemical toxicity distributions (CTDs) to perform probabilistic hazard assessments (PHAs) using data from the U.S. EPA’s ToxCast program. PHAs may predict toxicological potencies of similar chemicals, prioritize chemicals for additional toxicity testing, support read-across for regulatory purposes, and identify characteristics for sustainable molecular design. An initial PHA examined the comparative sensitivity of three in vitro assays for estrogenicity representing a diverse group of industrial chemicals and pesticides from ToxCast Phase I. CTDs were compared at 5th centiles for all compounds, common compounds, and organophosphates tested. When all available data were compared, a NCGC assay was most sensitive for estrogenicity as there was a 5% probability of detecting a compound that will elicit an estrogenic response at or below 0.089 mg/L. When only common compounds and organophosphates were considered, an Attagene assay was most sensitive assay with centiles of 0.13 mg/L probability of detecting a compound that will elicit an estrogenic response at or below those potentially relevant to maximum worker exposures, was not increased compared to oral absorption (14-23%), even with guideline sized particles (1-3 μm MMAD). The portal of entry toxicity of irritant aerosols generally limits the achievable exposure concentrations such that exposures typically cannot be high enough to produce expected systemic toxicity. Test guideline size particles had greater deposition and increased surface area than predominantly larger sized aerosols produced in the field, lowering the accuracy of human hazard prediction, especially for chemical irritants. These data support the use of an evidence-based approach in determining the need for repeat inhalation studies for the assessment of human health risk assessment from inhalation.

1831h Comparison of Developmental and Neurotoxic Endpoints Observed in Experimental Animal Studies following Inhalation Exposure to n-Butanol and n-Butyl Acetate

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Several studies have suggested that toxicity associated with n-butyl acetate may be informative of toxicity associated with n-butanol and vice versa. N-butyl acetate rapidly metabolizes to n-butanol; therefore, it is anticipated that the toxic effects observed following exposure to n-butyl acetate and n-butanol would be similar. A literature search was conducted to identify peer-reviewed studies that evaluated toxic health effects resulting from n-butanol or n-butyl acetate exposure. Most of these studies evaluated primarily neurotoxic and developmental toxicity endpoints. Toxicity endpoints from all of the studies were reviewed and toxicity effect levels were determined. To facilitate study comparisons, internal n-butanol blood levels were estimated by using a previously developed PBPK model (Teegarden et. al 2005). Studies investigating developmental effects of n-butyl acetate and n-butanol demonstrated similar dose related trends including decreased food consumption in dams and decreased fetal body weight. Endpoints consistent with neurotoxicity were not directly comparable between n-butanol (e.g., altered motor function) and n-butyl acetate (e.g., decreased absolute brain weight). In this case study, similar trends were observed with genotoxicity outcomes between n-butyl acetate and n-butanol in some developmental endpoints, suggesting that toxic effects identified following exposure to n-butyl acetate may be predictive of toxic effects observed as a result from exposure n-butanol.

The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

1831i Relative Oral Bioavailability of Benzo(a)pyrene from Soils at Environmentally-Relevant Concentrations

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Several reports in the literature suggest that interactions between PAHs and soil diminish PAH bioavailability and thus reduce risk from incidental ingestion of PAH-contaminated soil. Various animal models and approaches have been used to estimate PAH bioavailability from soil. To facilitate PAH measurement, most of these studies were conducted using PAH concentrations that only occur at heavily contaminated sites. The objective of this study was to explore the influence of soil composition, PAH concentration, and source material type on PAH bioavailability using an approach capable of measuring uptake at low, environmentally-relevant PAH concentrations (down to 1 ppm or less). Contaminated soil samples were constructed using PAHs from three source materials (PAHs in solvent, soot, and fuel oil) to which 3H benzo(a)pyrene (3H-BaP; total BaP concentrations of 1, 10, and 100 ppm) was added and weathered for eight weeks. Each soil was administered as a single dose to rats, and blood samples were taken over six days. Relative bioavailability (RBA) of the BaP from soil was estimated by comparing the area under the curve (AUC) for 3H concentration versus time in blood with the AUC observed from the same material dosed in a food matrix for comparison. Food was used for comparison because the cancer slope factor for BaP is derived from studies in rats in which the exposure medium is diet. The extent to which BaP RBA was diminished in soil versus food varied among the source materials. Differences were also observed among soils of different composition, suggesting that the nature of the soil as well as the source of PAH contamination can influence bioavailability. These data will be informative both in understanding soil-PAH interactions that affect bioavailability and form a basis for the development of in vitro approaches for PAH bioavailability estimation. Supported in part by a grant from SERDP.

1831g Developing an Evidence-Based Rationale for Determining the Necessity of Repeat Inhalation Studies of Agrochemical Aerosols with Irritant Properties

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An evidence-based approach was used to determine whether repeat inhalation toxicity studies for two fungicide formulations, with varying potential to produce irritation, would provide new information of use in human health risk assessment. After considering the available acute inhalation and irritation toxicity data, a 2- or 4-week repeat exposure study was conducted with formulations of chlorothalonil and azoxystrobin, respectively, including histopathology of the respiratory tract. In addition, as part of the testing strategy for chlorothalonil, pharmacokinetic data from inhalation administration in rats was generated for route to route extrapolation of systemic availability. At the end of the repeat exposure studies, concentration-related squamous metaplasia was noted in the ventralateral latyns and/or the nasal epithelium, consistent with changes commonly reported in rats following nose-only inhalation exposures to irritant chemicals. At minimal to mild grades of severity, these findings are non-adverse, and potentially reversible. Systemic absorption of chlorothalonil, following 6 hours of inhalation exposure to a concentration (0.004 mg/L) above those potentially relevant to maximum worker exposures, was not increased compared to oral absorption (14-23%), even with guideline sized particles (1-3 μm MMAD). The portal of entry toxicity of irritant aerosols generally limits the achievable exposure concentrations such that exposures typically cannot be high enough to produce expected systemic toxicity. Test guideline size particles had greater deposition and increased surface area than predominantly larger sized aerosols produced in the field, lowering the accuracy of human hazard prediction, especially for chemical irritants. These data support the use of an evidence-based approach in determining the need for repeat inhalation studies for the assessment of human health risk assessment from inhalation.
effect, is given to the mould prevalence and its health impacts in non-damp or "normal" indoor residential environments. In this study, we have performed visual inspection of residential homes in the Northern California to assess whether mold or conditions indicating mold are present in the indoor air environments. We also collected air samples in order to: (1) capture and quantify a broad spectrum of fungal/mold spores present in the air, and (2) assess whether the levels suggest a fungal/mold problem in the indoor locations. Between 2006 and 2013, we collected and analyzed more than 900 samples (> 600 outdoor air samples; 300 indoor air samples). The results showed that: (1) molds are normally found in indoor air in particularly large numbers, 100 – 8,500 spores per cubic meter of indoor air (residential homes with no visual/known dampness); (2) the range of outdoor mold was 500 – 38,000 spores per cubic meter, with four peaks in January, May, October, and November; (3) the four dominant airborne fungi were, yeast, Cladosporium, Penicillium, and Aspergillus; (4) individual homes complaining about asthma and other respiratory effects were not well correlated with indoor environments with high spore counts. Our findings suggest that: (1) the ratio, not absolute spore counts, of indoor air spores to outdoor air spores in a given residence should be used as an accurate indicator of a mold problem in a residence, and (2) special emphasis should be given when conducting mold risk assessment with individuals who might be more susceptible to health problems from mold exposure (individuals with respiratory sensitivities and a compromised immune system).

1831m Prevalence of Molds in Residential Indoor and Outdoor Environments in Northern California and Its Implication in Health Risk Assessment
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Increasingly, airborne mold in home environments has been linked with asthma exacerbation and other respiratory diseases. The majority of these adverse health risk assessments due to mold exposure have been conducted with data from residential homes or buildings associated with dampness. While controlling indoor moisture and mold can help solve mold contamination problems and adverse health effects, very little attention is given to the mold prevalence and its health impacts in non-damp or "normal" indoor residential environments. In this study, we have performed visual inspection of residential homes in the Northern California to assess whether mold or conditions indicating mold are present in the indoor air environments. We also collected air samples in order to: (1) capture and quantify a broad spectrum of fungal/mold spores present in the air, and (2) assess whether the levels suggest a fungal/mold problem in the indoor locations. Between 2006 and 2013, we collected and analyzed more than 900 samples (> 600 outdoor air samples; 300 indoor air samples). The results showed that: (1) molds are normally found in indoor air in particularly large numbers, 100 – 8,500 spores per cubic meter of indoor air (residential homes with no visual/known dampness); (2) the range of outdoor mold was 500 – 38,000 spores per cubic meter, with four peaks in January, May, October, and November; (3) the four dominant airborne fungi were, yeast, Cladosporium, Penicillium, and Aspergillus; (4) individuals complaining about asthma and other respiratory effects were not well correlated with indoor environments with high spore counts. Our findings suggest that: (1) the ratio, not absolute spore counts, of indoor air spores to outdoor air spores in a given residence should be used as an accurate indicator of a mold problem in a residence, and (2) special emphasis should be given when conducting mold risk assessment with individuals who might be more susceptible to health problems from mold exposure (individuals with respiratory sensitivities and a compromised immune system).
of ATP levels by luminescent assay showed that mitochondrial complex-I inhibitors caused a significant reduction in ATP levels. Functional studies using the mitotracker green assay on N27 cells showed that exposure to complex I inhibitors caused significant damage in mitochondria. These results were also confirmed by measurements of pimonidazole. Interestingly, pretreatment with the HAT inhibitor anacardic acid (8.5μM) protected against tebufenpyrad and pyridaben–induced ATP reduction, H3 and H4 acetylation and cytotoxic cell death. Collectively, exposure to tebufenpyrad and pyridaben induced histone acetylation via mitochondrial dysfunction, which together may play a key role in dopaminergic neuronal degeneration (supported by NIH grant ES10586).

1833 Comparison of In Vitro Cell Models for Assessment of Pesticide-Induced Dopaminergic Neurodegeneration


Current biomedical and (neuro)toxicity research on (neuro)degenerative diseases relies strongly on animal models. For both ethical and technical reasons this use of laboratory animals is often undesirable. Current in vitro research largely relies on tumor derived- or immortalized cell lines. However, the suitability of cell lines for studying neurodegeneration is determined by their intrinsic properties.

We therefore characterized four different neuronal cell models (PC12, SH-SYSY, MES23.5 and N27 cells) with respect to the presence of functional membrane ion channels and receptors. Furthermore, we assessed the effects of five known neurotoxic pesticides (rotenone, lindane, dieldrin, imazalil and dinoseb) on cytotoxicity, oxidative stress and parameters of intracellular calcium homeostasis using a combined αB/CFDA assay, a H2DCFDA assay and single cell fluorescent (Fura-2) calcium imaging, respectively.

Our results demonstrate considerable differences in intrinsic properties and pesticide-induced effects on cytotoxicity and oxidative stress, as well as on intracellular calcium homeostasis between the cell lines. The results thus indicate that care should be taken when interpreting (neuro)toxicity data as the chosen cell model may greatly influence the outcome.

This work was funded by the European Union-funded project ACROPOLIS (Grant Agreement KBBE-245163) and by the Faculty of Veterinary Medicine (Utrecht University).

1834 Low Concentrations of Insecticides Induce Acute and Concentration-Dependent Effects on Calcium Homeostasis in PC12 Cells


Insecticides are widely used and are well known neurotoxic chemicals. There are different classes of insecticides based on their reported primary mode of action. Since calcium plays a critical role in neuronal development, we tested whether selected organophosphates, organophosphates, neonicotinoids, carbamates and pyrethroids can disturb calcium homeostasis in PC12 cells.

Effects on basal calcium homeostasis were investigated using single cell fluorescence (Fura-2) microscopy. By depolarizing cells with high K+–containing saline, effects on the depolarization-evoked increase in intracellular calcium ([Ca2+]i) through voltage-gated calcium channels (VGCCs) were also investigated.

The data demonstrate that endosulfan, chlorpyrifos and chlorpyrifos-oxon at 10 μM induce an acute but modest increase in basal [Ca2+]i. More importantly, most of the tested insecticides concentration-dependently inhibited the depolarization-evoked [Ca2+]i, with cypemethrin being the most potent compound with an IC50 of 0.009 μM. α-cypemethrin inhibited the depolarization-evoked [Ca2+]i with 20% at 0.03 μM, chlorpyrifos at 0.08 μM, endosulfan at 0.1 μM, chlorpyrifos-oxon at 1 μM and parathion at 2.56 μM. Carbaryl, imidacloprid, paraoxon-ethyl and paraaxon-methyl did not inhibit the depolarization-evoked [Ca2+]i.

In conclusion, our data demonstrate that 1) insecticides concentration-dependently inhibit depolarization-evoked [Ca2+]i, and that 2) oxon metabolites are less potent compared to their parent compounds in inhibiting depolarization-evoked [Ca2+]i. This acute disturbance of calcium homeostasis may partly underlie insecticide-induced neurotoxicity.

This work was supported by the European Union [DENAMIC project; FP7-ENV-2011-282957] and the Faculty of Veterinary Medicine of Utrecht University.

1835 Azole Fungicides Disturb Intracellular Ca2+ in an Additive Manner in Dopaminergic PC12 Cells


Humans are exposed to complex mixtures of pesticides and other compounds, mainly via food. Azole fungicides are broad spectrum antifungal compounds, widely used in agriculture as well as in human and veterinary medicine. The mechanism of antifungal action relies largely on inhibition of CYP51 inhibiting fungal cell growth and known adverse health effects ofazole fungicides are thus linked to CYP inhibition. Additionally, azole fungicide-induced neurotoxicity has been reported, though the underlying mechanism(s) are largely unknown.

We therefore investigated the effects of a group of 6 azole fungicides (mizalizal, flusilazole, fluclozate, tebuconazole, triadimenol and cyproconazole) on cell viability and on oxidative stress in dopaminergic PC12 cells using a combined alamarBlue/CFDA-AM and H2DCFDA assay, respectively. As calcium plays a pivotal role in neuronal survival and functioning, effects of these 6 azole fungicides as well as binary and quaternary mixtures of azole fungicides on the intracellular calcium concentration ([Ca2+]i) were investigated using single-cell fluorescence microscopy in PC12 cells loaded with the calcium-sensitive fluorescent dye Fura-2.

Only modest changes in cell viability and ROS production were observed. However, 5 out of 6 azole fungicides induced an non-specific inhibition of voltage-gated calcium channels (VGCCs), though with varying potency. Experiments using binary IC50 and quaternary IC50 mixture indicated that the inhibitory effects on VGCCs are additive. The combined findings demonstrate modulation of intracellular Ca2+ via inhibition of VGCCs as a novel mode of action of azole fungicides. Furthermore, mixtures of azole fungicides display additivity, illustrating the need to take mixture effects into account in human risk assessment.

This work was funded by the European Union-funded project ACROPOLIS (Grant Agreement KBBE-245163) and by the Faculty of Veterinary Medicine (Utrecht University).

1836 Atrazine Alters Neuronal Redox Status but Does Not Activate Nrf2

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Atrazine (ATZ) is the second most commonly used herbicide in the United States and is widely applied to grain crops, such as corn, wheat, and sorghum. Because of its wide use, ATZ is the most commonly detected pesticide in ground water. ATZ can act as an endocrine disruptor as well as cause dopaminergic neuron death and behavioral phenotypes. The aim of this study was to characterize how subtoxic concentrations of ATZ and its metabolites alter neuronal redox status. SH-SYSY neuroblastoma cells were treated with subtoxic concentrations of ATZ and its metabolites and alterations in cytoplasmic and mitochondrial peroxidase (Prx) redox status as well as cellular GSH, GSSG and redox potentials (EhGSSG) were measured. Redox western blots showed that neither ATZ nor its metabolites, desethylatrazine (DEA), desisopropylatrazine (DIA), diaminochlorotriazine (DACT), altered cytoplasmic Prx1 redox status; however, acute exposure (4h) to DEA, DIA, or DACT resulted in slight oxidation of mitochondrial Prx3, which this effect normalized by 24h. Analysis of intracellular GSH, GSSG and EhGSSG showed that both ATZ and DIA significantly deplete GSH resulting in considerable oxidation of EhGSSG. Due to the ATZ-mediated decrease in intracellular GSH, we investigated if Nrf2 was activated by ATZ or its metabolites. Western blots of nuclear fractions showed a slight increase in Nrf2 nuclear translocation in DEA treated cells and a large increase following DACT treatment, but no increase in ATZ treated cells. Analysis of Nrf2-controlled genes (GCLC, NQO1, HO-1, Prx1) showed slight activation by DACT. GCLC expression was increased by ATZ, but it can be concluded that this mechanism may not be Nrf2-mediated. In conclusion, our results demonstrate that ATZ alters intracellular GSH redox status, but does not alter Prx redox or cause an increase in Nrf2 nuclear translocation or transcription.
Gestational and Lactational Drinking Water Exposure to Atrazine Causes Specific Behavioral Deficits and Selectively Alters Monoaminergic Systems in C57BL/6 Mouse Dams, Juvenile, and Adult Offspring

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Atrazine (ATR) is one of the most frequently detected pesticides in the U.S. water supply and limited evidence suggests that it may be a developmental neurotoxicant. The present study focused on behavioral and neurochemical (monoamine) alterations in C57BL6 mouse offspring and dams exposed to low dose of ATR (3 mg/L) via the drinking water (DW) from gestational day 6 to postnatal day (PND) 23. Behavioral tests included open field, pole, grip strength, novel object recognition (NOR), forced swim (FS), and marble burying. Maternal and offspring (PND21, 35, and 70) body and brain weights were not affected by ATR. However, ATR exposure caused: (i) hyperactivity and decreased performance in the NOR in the dams; (ii) hyperactivity (males and females), increased immobile time in the FS (males), decreased performance in the pole test (females), and increased number of buried marbles (females) in the juvenile offspring (PND35); (iii) decreased performance in the NOR by females in the adult offspring (PND70). ATR exposure also increased striatal dopamine (DA) in the dam and juvenile offspring (males and females), but not in the adult offspring, and it decreased serotonin in the perinatal cortex of female, but not male, adult offspring. These results suggest that perinatal exposure to environmentally-relevant DW levels of ATR targets the nigrostriatal DA pathway in the dam and offspring, alters motor and cognitive functions of the dam, induces a variety of sex-selective behavioral abnormalities involving motor and emotional functions in the juvenile offspring, and decreases cognitive function of female adult offspring, an effect that may be due to altered serotonin homeostasis in the perinichal cortex.

N-Methyl-D-aspartate Receptor Activation May Contribute to Glufosinate Neurotoxicity

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Glufosinate (GLF) at high levels in mammals causes convulsions through a mechanism that is not completely understood. The structural similarity of GLF to glutamate (GLU) implicates the glutamatergic system as a target for GLF neurotoxicity. The current work examined GLF interaction with N-methyl-D-aspartate subtype GLU receptors (NMDAR) and GLT-1 transporters via [3H]CGP39653 binding. The current work examined GLF interaction with D-aspartate subtype GLU receptors (NMDAR) and GLT-1 transporters via [3H]CGP39653 binding.

Paraquat and MPTP Induce Alterations in the Expression Profiles of microRNA in Neuro-2A Cells


Recent evidence indicates that microRNAs (miRNAs) play a key role in neurodegenerative diseases. However, little is known about how these miRNAs contribute to dopaminergic neuronal damage by paraquat (PQ) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Here we used microarray analysis to show that PQ and MPTP induce alteration of miRNA expression in a cell model of Parkinson’s disease. The results reveal that treatment with 300 μM PQ caused miRNA deregulation in which 60 miRNAs were up-regulated and 228 miRNAs were down-regulated. Among the 288 miRNAs identified, the levels of six miRNAs (miR-297b-5p, miR-497-5p, miR-503-5p, miR-374-5p, miR-155-5p, and miR-600a-3p) were charged more than 1.2-fold. Upon treatment with MPTP at 300 μM PQ, a total of 576 miRNAs were deregulated; 506 miRNAs were up-regulated and 70 miRNAs down-regulated. Among the 576 miRNAs, miR-93-5p and miR-425-5p had changes above 1.2-fold. These findings showed that both PQ and MPTP alter the miRNA expression, but each affects a different set of miRNA targets. Alterations in the expressions of miR-17-5p, miR-93-5p, miR-21-3p, miR-374-5p, miR-503-5p, and miR-425-5p were verified by real-time quantitative reverse transcriptase polymerase chain reaction. Bioinformatics analysis indicated that aberrant expression of miR-17-5p potentially contributes to PQ-induced neurotoxicity. Taken together, our study demonstrates, for the first time, that characteristic changes in miRNA expression profiles occur after PQ and MPTP treatment, which suggests miRNA may be involved in the development of PQ and MPTP induced neurodegeneration as a molecular mechanism.

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DDT and DDE Enhance the Toxicity of Amyloid Beta Peptides in Drosophila

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Environmental factors, such as pesticides are thought to play a significant role in the pathogenesis of diseases of the nervous system. DDT (dichlorodiphenyltrichloroethane) is a persistent synthetic organochlorine insecticide used to control ma-
Zhihong Cao, Yao Cui, Huy-Man Nguyen, David P. Jenkins, Helmar Wulff, and Ira N. Pessah investigate the impacts on activity driven neuronal plasticity. However, selected antioxidants exerted different protective actions. Compared to α-lipoic acid (MDA) were significantly increased after bifenthrin. Levels of both nitric oxide (NO) and lipid peroxides measured as malondialdehyde (MDA) were significantly increased after bifenthrin exposure, whereas 50% of control flies lived to 30 days. Interestingly, DDE did not compromise viability at concentrations up to 1μM. In a developmental model, both pesticides caused developmental delays at concentrations as low as 10μM, a concentration that caused no lethality in adult flies. Based on these results, we treated Aβ-expressing Drosophila with 1μM DDT or DDE and measured changes in their lifespan. At 15 days of exposure, lifespan of DDT-treated males was 2% lower compared to controls, whereas DDE-treated males had 18% lower viability. No changes in viability were observed in treated female flies. Our work suggests that sub-toxic levels of DDT and DDE may exacerbate the effects of Aβ in a Drosophila model of Alzheimer's and that these effects appear to be sex-specific. Supported by the SOT Intern Program and NIH grants P30ES005022 and R25ES020721.

**1842 Nanomolar Bifenthrin Alters Synchronous Ca2+-Oscillations and Cortical Neuron Development Independent of Sodium Channel Activity**

Zi Ca, Ying Cui, Huy Man Nguyen, D. P. Jenkins, and I. N. Pessah.

Bifenthrin is a relatively stable type I pyrethroid with broad agricultural and domestic uses that causes tremors and impairs motor activity in rodents. In this study, we investigated the influence of nanomolar concentrations of bifenthrin on the synchronous Ca2+ oscillations (SCO) necessary for activity dependent dendritic development. Primary mouse cortical neurons were cultured 8-9 days in vitro (DIV) loaded with the Ca2+ indicator Fluo-4, and imaged using FLIPR Tetra. Acute exposure to bifenthrin rapidly increased the frequency of SCO 2.7-fold (EC50 = 58 nM) and decreased SCO amplitude by 36%. Changes in SCO properties were independent of modifications in voltage-gated sodium channels since 100 nM bifenthrin had no effect on the whole-cell Na+ current, nor influenced neuronal Erev of cortical neurons. Moreover, the L-type Ca2+ channel blocker nifedipine failed to ameliorate bifenthrin-triggered SCO activity, and were not mediated by ryanodine receptor channel modification. By contrast, the mGlur 5 antagonist MPEP normalized the bifenthrin-triggered increase in SCO frequency without altering baseline SCO activity, indicating that bifenthrin amplifies mGlur 5 signaling independent of Na+ channel modification. Competitive (AP-5) and noncompetitive (MK801) NMDAR antagonists partially decreased bifenthrin-triggered SCO frequency increase. Bifenthrin-modified SCO rapidly enhanced the phosphorylation of AMP response element-binding protein (CREB). Sub-acute (48h) exposure to bifenthrin commencing 2 DIV enhanced neurite outgrowth and persistently increased SCO frequency and reduced SCO amplitude. Collectively, these data identify a new mechanism by which bifenthrin potently alters Ca2+ dynamics and Ca2+-dependent signaling in cortical neurons that have long term impacts on activity driven neuronal plasticity.

**1843 Protective Role of Melatonin against Alpha-Cypermethrin-Induced Oxidative Stress in Human Neuroblastoma Cell Line SH-SY5Y**


α-Cypermethrin, alpha-cyano pyrethroid insecticide, is a relatively potent neurotoxic associated with human health risks. In the present study, we aimed to investigate in an in vitro model the neuroprotective role of three well-known antioxidants, melatonin (MEL), Trolax and N-Acetylcysteine against α-cypermethrin induced neurotoxicity and on the other hand, to examine, by PCR array, the cell death pathway expression patterns in neuroblastoma cell line SH-SY5Y after pyrethroid exposure and the potential neuroprotective role of melatonin. Levels of both nitric oxide (NO) and lipid peroxides measured as malondialdehyde (MDA) were significantly increased after α-cypermethrin exposure, however selected antioxidants exerted different protective actions. Compared to other antioxidants, 1 μM MEL treatment showed the most effective protection against pyrethroid-induced lipid peroxidation and increased NO levels. We evaluated multiple cell death regulation genes after α-cypermethrin and α-cypermethrin plus MEL-treated cells, most of genes altered by the pyrethroid were partially or completely reverted by MEL treatment, among them, ATG16L1, BIRC3, FOX11, IFG1, INS, IRGM, KCNIP1, MAG, RAB23, SCNA, S100A7A, SQSTM1, TNFRSF1A, TNFRSF1B and TNFRSF1A. These results suggest that oxidative stress is a key element in the neurotoxicity process induced by α-cypermethrin and a multitasking indolamine as MEL might be beneficial to prevent toxicity caused by α-cypermethrin or even other pyrethroids with similar action mechanism. This work was supported by projects Ref. BSCGH/S85/08 (UCM), Ref. No. S2009/AGR-14639/CAM and Consolider-Ingenio 2010 Net.CSDE2007-063 (MEC), Spain.

**1844 Pyrethroid Pesticides Directly Activate Microglia through Voltage-Gated Sodium Channels Leading to Increased TNF-α Release**

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Microglia are considered to be the resident immune cells of the central nervous system and contribute significantly to ongoing neuroinflammation in a variety of neurodegenerative diseases. Recently, we identified that voltage-gated sodium channels (VGSC) are present on microglia cells and contribute to the release of the pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) (Hossain and Richardson, 2013). Based on this finding and the fact that pyrethroid pesticides act on VGSC, we hypothesized that exposure of microglia to the pyrethroid pesticides, permethrin and deltamethrin, would activate microglia and increase release of TNFα. BV2 mouse microglial cells were seeded into a 96-well plate and treated with 0-100 μM deltamethrin or permethrin for 24 or 48 hrs and cell viability was assayed using Alamar blue. Cell viability decreased in a dose and time dependent manner for concentrations of deltamethrin and permethrin above 10 μM. Next, BV-2 cells were treated with vehicle, the VGSC blocker tetrodotoxin (TTX), or 0.5-5 μM deltamethrin or permethrin in the presence and absence of TTX for 24-48 hours. Media was removed and the concentration of TNFα was assayed using ELISA. TNFα release increased significantly in a dose and time-dependent manner for both deltamethrin and permethrin. TTX significantly reduced the pyrethroid-induced TNFα release, indicating that interaction with VGSC significantly contributed to release. From these data, we conclude that pyrethroid pesticides directly activate microglial cells through interaction with microglial VGSC. Because neuroinflammation plays a key role in many neurodegenerative diseases, these data provide an additional mechanism by which exposure to pyrethroids may contribute to neurodegeneration.

**1845 Repeated Pesticide Exposure Causes Hippocampal ER Stress, Neuroinflammation, and Cognitive Deficits in Mice**


Endoplasmic reticulum (ER) stress and neuroinflammation are implicated as significant contributors to neurodegeneration. Previously, we demonstrated that the widely used pyrethroid pesticide deltamethrin causes ER stress-mediated apoptosis in SK-N-AS neuroblastoma cells. However, whether this occurs in vivo remains unknown. Here, we investigated the effects of repeated deltamethrin exposure (3 mg/kg every 3 days for 60 days) on hippocampal ER stress, neuroinflammation, and cognitive function in adult mice. Repeated exposure to low dose deltamethrin caused ER stress in the hippocampus, as indicated by increased CHOP (131%) and GRP78 (96%) levels. This was accompanied by increased levels of caspase-12 (110%) and activated caspase-3 (50%) in the hippocampus. Deltamethrin also induced microglial activation, as indicated by Iba-1 staining, and increased TNF-α mRNA expression (121%) and protein (465%) levels. To determine whether these effects lead to cognitive dysfunction, hippocampal-dependent learning and memory was evaluated with a spatial navigation task. Deltamethrin-treated animals exhibited profound deficits in the acquisition phase and in short-term and long-term memory. Additionally, deltamethrin exposure caused decreased BrdU-positive cells (36.54%) in the dentate gyrus (DG) of the hippocampus, suggesting impairment of hippocampal neurogenesis. Collectively, these results demonstrate that repeated deltamethrin exposure leads to high stress, neuroinflammation, and apoptotic cell death in the hippocampus. Furthermore, impaired hippocampal neurogenesis may contribute to deficits in learning and memory by deltamethrin. Supported by NIH P30ES005022 and R01ES015991.
The purpose of these studies was to determine if exposure of *Caenorhabditis elegans* to glyphosate or Mn/Zn ethylene-bis-dithiocarbamate-containing pesticides leads to mitochondrial dysfunction in *Caenorhabditis elegans*. E. C. Toft, R. Negga, S. K. Jada, D. C. Bailey and V. A. Fitisankis, *Biolog*, King College, Bristol, TN.

Epidemiological studies indicate that pesticide usage positively correlates with neurodegenerative disorders, particularly Parkinson’s disease. Further evidence suggests that exposure to glyphosate-containing pesticides, the most widely-used class of herbicides in the world, may lead to mitochondrial inhibition. This is in contrast to treatment with glyphosate by itself, which is relatively non-toxic, even to mitochondria. To further explore this potential relationship between Touchdown® (TD), a glyphosate-based herbicide, and mitochondrial inhibition, we acutely exposed second larval stage (L2) *Caenorhabditis elegans* (C elegans) to various concentrations (LC10, LC50, or LC75) of TD as percent active ingredient (glyphosate).

To determine whether mitochondrial function was compromised, assays were performed 24 hours post-acute TD exposure (30 min) to determine whether Complex II, Complex IV, or the proton gradient were adversely affected. Based on one-way ANOVA (*p* < 0.05), colorimetric data from Complex II and IV assays indicate that these complexes are not inhibited, but rather stimulated in a dose-dependent manner. Results relating to the integrity of the proton gradient indicate a statistically significant increase (*p* < 0.05) in tetramethylrhodamine ethyl ester (TMRE) accumulation, signifying not only an intact proton gradient, but perhaps uncoupling and an increase in proton concentration in the inner mitochondrial membrane space. These data suggest that acute exposure of *C elegans* to TD leads to mitochondrial dysfunction, which may contribute to neurodegeneration observed in previously published studies from this lab.

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Glyphosate-containing herbicides are some of the most widely-used pesticides in the world. Although glyphosate alone is relatively non-toxic, a growing body of evidence suggests that commercial formulations may lead to increases in oxidative stress and mitochondrial inhibition. Previous data from our lab demonstrated dopaminergic and GABAergic neurodegeneration in *Caenorhabditis elegans* (C elegans) when exposed to various concentrations of Touchdown® (TD). Since mitochondrial inhibition may lead to decreased neuronal viability, we sought to determine whether mitochondrial function was compromised. Therefore, we treated L2 worms with varying concentrations of TD followed by assays specific for mitochondrial complex activity. Initially we determined overall mitochondrial function using tetramethylrhodamine ethyl ester (TMRE) to measure the extent of proton gradient integrity. Results from TMRE assays indicated a dose-dependent decrease in dye accumulations in treated worms compared to control (*p* < 0.05). Colorimetric data from Complex II studies suggested a dose-dependent relationship. At lower concentrations (LC50), Complex II activity was marginally inhibited. At the highest concentration (LC100), however, Complex II activity was statistically significantly increased (*p* < 0.05) compared to control worms. Complex IV studies suggested a slight increase in activity at all treatment concentrations compared to control, but this did not reach statistical significance. Therefore, it does not appear that Complex IV activity is inhibited by TD. Taken together, these data indicate that exposure of *C elegans* to TD leads to mitochondrial inhibition. Furthermore, since mitochondrial inhibition can lead to increased reactive oxygen species production, chronic exposure to TD could potentially result in neuronal degeneration, including that associated with Parkinson’s disease.

A. D. Shelton, R. Negga, D. C. Bailey and V. A. Fitisankis, *Biolog*, King College, Bristol, TN.
exposed chronically (24 h) or acutely (30 min) to varying concentrations of TD or MZ, and assayed with dihydrothidiazin (DHE), for O$_2^-$; or AmplexRed, for H$_2$O$_2$. DHE assays in either treatment paradigm (chronic or acute TD or MZ) indicated no statistically significant increase in O$_2^-$ at any concentration compared to control. Furthermore, chronic TD exposure followed by AmplexRed treatment showed no statistically significant difference compared to control. Conversely, worms treated acutely with TD showed a statistically significant (****p<0.001) increase in H$_2$O$_2$ in LC$_{25}$ and LC$_{50}$ groups compared to control. AmplexRed assays in worms following chronic MZ exposure indicated a statistically significant (****p<0.001) increase in H$_2$O$_2$, in the LC$_{50}$ group compared to control. However, a dose-dependent upward trend in production of H$_2$O$_2$ with increasing concentration (LC$_{25}$ vs LC$_{50}$; *p<0.05; LC$_{50}$ vs LC$_{75}$; **p<0.01). AmplexRed results in acute MZ studies showed a statistically significant decrease in H$_2$O$_2$ in LC$_{25}$ (**p<0.01) and LC$_{50}$ (**p<0.01) treatment groups compared to control, but a statistically significant (****p<0.001) increase in this ROS in LC$_{75}$ worms. Taken together, these data strongly support the hypothesis that both pesticides are capable of producing ROS, and that the ROS may be either H$_2$O$_2$ or hydroxyl radical.

1851 Treatment of Caenorhabditis elegans with Either a Glyphosate-Containing Herbicide or Manzate Leads to Decreased ATP Levels

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Epidemiological studies show a positive correlation between pesticide exposure and Parkinson’s disease (PD) in humans. It is also known that patients with PD exhibit mitochondrial inhibition. Interestingly two widely-used agrochemicals, TouchDown® (TD), a glyphosate-containing herbicide, and Manzate (MZ), a Mn/Zn ethylene-bis-dithiocarbamate-based fungicide, are suspected of adversely affecting mitochondrial function. Furthermore, previous work in Caenorhabditis elegans (C elegans) from our lab has shown specific neurodegeneration in dopaminergic and GABAergic neuronal populations. To determine whether exposure to these pesticides could also result in potential mitochondrial dysfunction, we treated three groups of N2 (wild-type) strain C elegans with varying concentrations (LC$_{25}$, LC$_{50}$, LC$_{75}$) of the respective commercial-grade compounds as a percent acute ingredient (glyphosate or Mn/Zn ethylene-bis-dithiocarbamate). Studies using chronic (24 h) or acute (30 min) exposure paradigms were conducted. Following the respective treatment, a luminescence probe was used to determine the relative amount of ATP in each group. Results indicated a statistically significant decrease (****p<0.001) in ATP production in the chronic LC$_{50}$ TD treatment group compared to control. For acute treatment with TD, there was a statistically significant increase (**p<0.01) in ATP production in the LC$_{50}$ group compared to all other groups, including control. When worms were treated with chronic MZ, there was an overall statistically significant decrease (**p<0.01) in ATP amount for each of the three treatment groups when compared to control. Finally, in worms from the acute MZ treatment studies, a strong trend suggested a decrease in ATP compared to control worms. Taken together, these data indicate a general reduction of ATP that could result from mitochondrial inhibition following exposure to these pesti-cides. Overall, these data suggest that chronic or acute exposure of C elegans to either TD or MZ could promote neurodegeneration via mitochondrial dysfunction.

1852 Maneb (Manganese Ethylenebisdithiocarbamate) Alters Expression of Genes Involved in Oxidative Stress and Induces Stress Proteins in Rat Hippocampal Astrocytes

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Approximately 5.1 billion pounds of pesticides per year are used in the United States, of which 70 million pounds are fungicides. There is increasing evidence that pesticides are implicated in human health impairment. According to epidemiologi-cal and experimental studies, there is a strong association between pesticide use and neurodegenerative diseases. Maneb, a manganese containing ethylenebis[dithiocarbamate], is a broad-spectrum fungicide commonly used to control plant diseases on food and feed crops. Dithiocarbamates have been shown to produce reactive oxygen species (ROS), altering cellular antioxidant homeostasis that can lead to cellular stress. Astrocytes play a critical role in normal brain physiology and neuroprotection. In this study, Maneb’s potential to alter the expression of genes involved in oxidative stress and antioxidant defenses and induction of stress proteins in rat hippocampal astrocytes was evaluated. Rat hippocampal astrocytes were exposed to 13.5 μM (LC$_{50}$ concentration) of Maneb for 24 hours. Real-time PCR analysis indicated that Maneb exposure caused a 2-fold or greater change in the expression of stress responsive genes involved in ROS metabolism such as Npg1 and Prdx1, which were up-regulated, and Tnxr2d, Tnxip1, Gpx7, SOD3, and Dhs24, which were significantly down-regulated. In addition, Maneb exposure significantly mod-

1853 Manganese-Containing Dithiocarbamates-Induced Cell Death via Senescence Pathway


Cells are constantly exposed to various stresses. Cellular senescence is an irreversible cell cycle arrest for cells in response to DNA damage, oxidative stress, or oncogenic activation. Cell cycle progression is controlled by various cellular signaling pathways. The status of protein redox condition is the decisive key of regulation of these signaling pathways. One of the regulators associated with the protein redox status is NF-kappa B. It has been suggested that activation of NF-kappa B signaling has a causal role in promoting senescence. Study from Cheng’s group has demonstrated that manganese-containing dithiocarbamate pesticides, maneb (MB) and manco-zeb (MZ), at 50 μM activate NF-kappa B cascade, which is partially in response to its potentiated effect on 1-methyl-4-phenylpyridinium (MPP+)–induced cell death in rat pheochromocytoma (PC12) cells. This study was designed to reveal the involve-ment of cellular senescence in MB- and MZ–induced cell death. The indicator of senescence is based on β-galactosidase activity at pH 6 (senescence-associated β-Gal; SA-β-Gal), which is only present in senescent cells, and not seen in pre-senescent, quiescent, or immortal cells. The results show an increase of SA-β-Gal ac-tivity in PC12 treated with MB and MZ (10 μM, 20 μM, and 50 μM) after 4 hours treatment and 24 hours recovery as compared to phosphate buffered saline (PBS) treated PC12 cells. The increase of SA-β-Gal was more perceptible in PC12 cells treated with low dose (10 μM) of MB and MZ as compared to the cells treated with higher doses (20 μM and 50 μM). This study demonstrates that non-toxic doses of MB and MZ (10 μM) can trigger cell cycle arrest in order for cells to compromise the cellular repair or commit cellular apoptosis.

1854 Decreased Gene Expression of Acetylcholine Receptors and Acetylcholinesterase in the Honeybee due to Imidacloprid Exposure

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Imidacloprid is a pesticide highly toxic to the honeybee’s nervous system. It is a potential cause for Colony Collapse Disorder (CCD), when honeybees abandon their queen and hive to die. The honeybee hive numbers have dropped 40-50% in the last year alone. Honeybees are the most effective insect pollinators therefore it is critical to prevent any future decline. In order to understand how imidacloprid affects honeybee nervous systems, honeybees were exposed to a sugar solution containing 0 μg/L, 0.2 μg/L, 20 μg/L, or 2,000 μg/L imidacloprid for five days in glass jars. RNA was extracted, cDNA synthesized, and Quantitative PCR (QPCR) performed on 25 genes. Seven genes successfully amplified in QPCR. Gene expression was measured for acetylcholinesterase (Ache)-1 and acetylcholine receptor (AchR) ε3, ε4, and β2. These genes were selected because imidacloprid is believed to bind to the AChR, but is unrecognized by Ache. Gene expression was compared to the internal housekeeping genes 18s rRNA and actin. Ache-1 gene expression was statistically different at 20 μg/L, but not at 2,000 μg/L. The decreased gene expression of Ache-1 indicates there is less enzyme to break down acetylcholine. However, an excess of acetylcholine would cause over stimulation in nerves. Due to the excess of acetylcholine in the synapse at the nerve, the honeybee is believed to counteract the condition by reducing the number of acetylcholine receptors. This was verified in the AChR ε3, ε4, and β2 when exposed to 20 μg/L. Gene expression of the AChR subunits decreased to 5.3, 3.5 and 3.9%; respectively when compared to the control bees. Genes will be cloned and more nervous system genes including LCCH3 analyzed to further understand the mechanism of imidacloprid.
1855 Differential Expression of MAP-2 (Microtubule Associated Protein-2) Gene Transcripts in the Central Nervous System of Hens Treated with DFP (Diisopropyl Phosphorofluoridate)

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Organophosphorus-ester induced delayed neurotoxicity (OPIDN) is a neurodegenerative disorder characterized by ataxia progressing to paralysis with a concomitant central and peripheral, distal axonopathies. Diisopropylphosphorofluoridate (DFP) produces OPIDN in the chicken that results in mild ataxia in 7–14 days and severe paralysis as the disease progresses with a single dose. White leghorn layer hens were treated with DFP (1.7 mg/kg, sc) after prophylactic treatment with atropine (1 mg/kg, sc) in normal saline and uracil (1 mg/kg, sc) in dimethyl sulfoxide, with appropriate controls. The hens were euthanized at different time points such as 1, 2, 5, 10 and 20 days, and the tissues from cerebrum, midbrain, cerebellum brainstem and spinal cord were quickly dissected and frozen. MAP-2 has been shown to be closely associated with CaM Kinase II and several other proteins of cytoskeleton. We used northern blots to investigate MAP-2 mRNA levels, while beta actin and 18S RNA were used as controls. The results indicate differential gene expression pattern for MAP-2 mRNA transcripts for both spatial and temporal aspects. Highly susceptible tissues like brainstem and spinal cord showed differential down-regulation at early time points followed by up regulated levels at later time points. Cerebellum, being a moderately susceptible tissue showed moderate levels of alterations in the gene expression of MAP-2 transcripts. Cerebrum, being resistant tissue also showed differential expression pattern. Differential levels of MAP-2 transcript levels in various regions may indicate differential levels of abnormal phosphorylation/dephosphorylation in the DFP-treated brain tissues during the OPIDN development. This new data further validates our recent findings about the role of cell death and cell survival pathways acting via abnormal phosphorylation of cytoskeletal proteins in the initiation and progression of OPIDN.

1856 Comparative Study on Short- and Long-Term Behavioral Consequences of Organophosphate Exposure: Relationship to AChE mRNA Expression


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Organophosphates (OPs) affect behavior via the inhibition of acetylcholinesterase (AChE). While the cognitive short-term effects may be directly attributed to this inhibition, the mechanisms that underlie the long-term cognitive effects of OPs remain controversial. Accordingly, two experiments were designed to compare the effects of OPs on cognition and to ascertain whether long-term OPs effects, analogous to their short-term effects, are AChE-dependent. A single subcutaneous dose of 250 mg/kg chlorpyrifos (CPF), 1.5 mg/kg diisopropylphosphorofluoridate (DFP) or 15 mg/kg paraoxon (PTN) was administered to male zebrafish. Spatial learning was evaluated 72 h or 23 weeks after exposure (experiment 1 and 2, respectively). Brain soluble and membrane-bound AChE activity, synaptic AChE-S mRNA, read-through AChE-R mRNA and brain acetylcholine hydrolyase (APH) activity (as alternative non-cholinergic target) were analyzed once behavioral testing was completed. CTN activity was assessed by fear conditioning testing, and cholinesterase inhibition. Differential gene expression analysis by RNA-seq revealed increased mRNA expression of neuropeptide (Npy, Cort) or neuropeptide-binding (Chrbp) genes in the hippocampus of CPF-exposed animals. Bdnf, a known regulator of synaptic plasticity and neuronal functions, was also up-regulated in the response to CPF exposure. It was demonstrated by immunohistochemistry that more neuronal cells expressed NPY, a known regulator of synaptic plasticity and neuronal functions, was also up-regulated in the response to CPF exposure. It was demonstrated by immunohistochemistry that more neuronal cells expressed NPY and CRHBP proteins in the hippocampus of rats exposed to CPF compared to unexposed animals. MicroRNA expression analysis by small RNA-seq suggested that the miR-132/miR-212 cluster may play a role in CPF-induced neurotoxicity. Pathway analysis identified potential downstream effects of low-dose CPF: increased risk of seizures and neurite outgrowth, synaptic depression, coordination, fertility and body size. Our study results indicate that a low-dose CPF exposure can alter mRNA and protein expression of key regulators of neurobehavioral functions, and that these gene expression changes may be linked to neurobehavioral toxicity of CPF. This research was supported in part by the Environment to the Postgraduate Research Participation Program at the USACEHR administered by ORISE through an IAA between the US DOE and USAMRMC. Research was conducted in compliance with the Animal Welfare Act, and all other Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.
with CPF oxon (1μM-MnM) and microglial activation was assessed. CPF oxon treatment in HAPI microglial cells resulted in elevated 30 minute superoxide and 3 hour H₂O₂ production, with no initiation of 6 hour TNFα or 24 hour nitrite production. However, combined treatment with CPF oxon (0.5αM) and lipopolysaccharide (LPS)(50ng/mL) resulted in synergistically amplified TNFα production in both microglia and neuron-glia cultures. These findings suggest that CPF oxon may activate microglia to produce reactive oxygen species and prime microglia to be sensitive to additional pro-inflammatory stimuli.

1860 In Utero Chlorpyrifos Exposure Leads to Behavioral Deficits and Alterations in Hippocampal GABAergic Synaptic Transmission


Epidemiological studies have reported that children exposed in utero to organophosphorus pesticides, including chlorpyrifos (CPF), present higher incidence of cognitive deficits, attention deficit/hyperactivity disorder, and autism spectrum disorders than non-exposed children. We have reported earlier that guinea pigs born to dams exposed during the last 10 days of pregnancy to CPF (25 mg/kg day, sc), a dose that causes no overt signs of maternal toxicity, present significant cognitive deficits in the Morris water maze (MWM). Because spatial learning in the MWM is hippocampal dependent and increased GABAergic activity induces cognitive deficits, we hypothesized that cognitive deficits induced by prenatal exposure to CPF correlate with increased GABAergic transmission in the CA1 field of the hippocampus. To test this hypothesis, we used the whole-cell patch-clamp technique to record inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) from CA1 pyramidal neurons in hippocampal slices obtained from behaviorally tested adult male guinea pigs that had been prenatally exposed to CPF or vehicle during the last 10 days in utero. Spontaneous and miniature IPSC frequencies were higher in CPF-exposed guinea pigs. The increased GABAergic activity correlated with cognitive deficits presented by the CPF-exposed animals tested in the MWM. Spontaneous and miniature EPSCs were comparable between treatment groups. In addition, western blotting of hippocampal protein extracts revealed that CPF exposure had no significant effect on the levels of markers of specific interneuron populations, suggesting that an increase in the number of interneurons is unlikely to account for the increased GABAergic transmission. It is conceivable that prenatal exposure to CPF leads to long-lasting disruption of neurotransmitter systems, such as the nicotinic cholinergic system, known to play a key role in the modulation of hippocampal GABAergic transmission and cognitive functioning.

1861 Prenatal Exposure to Chlorpyrifos Leads to MAPK Dysfunction and Epigenetic Modifications in the Brain

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Chlorpyrifos (CPF) is a commonly used organophosphorous pesticide that inhibits the enzyme acetylcholinesterase. Prenatal exposure to CPF has been correlated to spatial learning in the Morris water maze (MWM). Because spatial learning in the MWM is hippocampal dependent and increased GABAergic activity induces cognitive deficits, we hypothesized that cognitive deficits induced by prenatal exposure to CPF correlate with increased GABAergic transmission in the CA1 field of the hippocampus. To test this hypothesis, we used the whole-cell patch-clamp technique to record inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) from CA1 pyramidal neurons in hippocampal slices obtained from behaviorally tested adult male guinea pigs that had been prenatally exposed to CPF or vehicle during the last 10 days in utero. Spontaneous and miniature IPSC frequencies were higher in CPF-exposed guinea pigs. The increased GABAergic activity correlated with cognitive deficits presented by the CPF-exposed animals tested in the MWM. Spontaneous and miniature EPSCs were comparable between treatment groups. In addition, western blotting of hippocampal protein extracts revealed that CPF exposure had no significant effect on the levels of markers of specific interneuron populations, suggesting that an increase in the number of interneurons is unlikely to account for the increased GABAergic transmission. It is conceivable that prenatal exposure to CPF leads to long-lasting disruption of neurotransmitter systems, such as the nicotinic cholinergic system, known to play a key role in the modulation of hippocampal GABAergic transmission and cognitive functioning.

1862 Fipronil Disposition in the Brain and Changes in the Serotonergic System


Fipronil is a pesticide with widespread utility in control of agricultural and domestic pests including many lepidopteran species, thrips, locusts, ants, cockroaches, fleas, and ticks. In insects, fipronil is recognized as a potent blocker of the GABA-regulated chloride channel in CNS. In vivo mammalian studies indicate that primary metabolic pathway for fipronil involves oxidative formation of the sulfone metabolite. This metabolite was reported to be more toxic to insects, mammals, fish and birds than parent compound itself. Because fipronil is used in home applications, recent concerns for potential adverse public health effects (vomiting, aspiration and sepsis) were raised. The goals of this study were to determine disposition of fipronil and its sulfone metabolite in brain and to assess the relevance of the kinetic results with changes on serotonergic neurochemistry induced by fipronil. Two experiments were carried out: (1) Male Wistar rats treated with fipronil (single dose 10 mg/kg, per os) were killed at different times after treatment and brain samples were collected to determine fipronil and fipronil sulfone levels by HPLC-MS; (2) male Wistar rats treated with fipronil (10 mg/kg, per os, 5 days) and with corn oil (control animals) were killed 24h after dosing and brain regions isolated and content of 5-HT and 5-HIAA quantified by HPLC-ED. Results showed fipronil was distributed into CNS. Fipronil absorbed rapidly and eliminated slowly from the brain. The Cmax of fipronil and fipronil sulfone was 0.457 and 0.856 μg/g. Fipronil significantly (P<0.001) decreased 5-HT levels in hypothalamus (-22%), frontal cortex (-35%), hippocampus (-40%) and striatum (-45%) respect to controls. These results suggest a link between fipronil effect on serotonergic system and its specific levels of brain concentration. Work supported by projects Red, GR35/10-A UCM-BSC, S2009/AGR-1469 (CAM) and Consolider-Ingenio 2010 No CSO2007-00063 (MEC), Madrid, Spain.

1863 Triclopyr: Subchronic Neurotoxicity Study in Fisher F344 Rats

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Triclopyr is a pyridine-based herbicide used mainly on non-crop areas to control broadleaf weeds and woody plants. A subchronic neurotoxicity study in rats was conducted. Four groups of F344/DuCrI rats (10 animals/sex/group) were exposed to Triclopyr technical at levels of 0, 5, 25, or 200 mg/kg/day via the dietary route for 90 days. Neurotoxicity was evaluated using a functional observational battery (FOB), determinations of grip performance, rectal temperature, landing foot splay, and an autonomic function test of motor activity (Weeks 2, 4, 8, and 13). General health condition (clinical observations, body weights, and food consumption) were evaluated weekly; ophthalmic examinations were given pre- and post-exposure. At the end of the study, 5 animals/sex/group were perfused for histopathologic evaluation of the central and peripheral nervous system (control and high-dose groups). The no-observed-effect level (NOEL) for general toxicity was 25 mg/kg/day in both sexes based on: A) treatment-related decreased in body weight and food consumption of the high-dose group, beginning at the Week 2 and persisting throughout the study; B) increased urination of high-dose animals, observed at week 8 and Week 13 in FOBs. This finding was considered a secondary effect, as kidney effects (including functional changes such as decreased urine specific gravity and glucose in the urine; proximal tubules impairment) were identified at 20 – 250 mg/kg/day in a previous 13-week dietary toxicity study using F344/DuCrI rats. The NOEL for Triclopyr neurotoxicity was 200 mg/kg/day in both sexes as there were no effects on any parameter that would suggest a neurotoxic effect, including the examination performed both during in life and post mortem portion of the study. These results are consistent with previous toxicity studies, which showed no treatment-related neurotoxic effects across species.

1863a Effects Produced by Single and Repeated Dosages of Fipronil on the EEG of Long-Evans Rats


We have previously reported that various classes of pesticides have different effects on the non-stimulus driven EEG after acute treatment, including fipronil (25 or 50 mg/kg) (Lyke et al., Toxicologist, 2010, 2011, 2012, 2013). In this study, we
compared the effects of single and repeated treatment with fipronil on the EEG. Fipronil is a member of the phenylpyrazole class of compounds that inhibits GABA receptors. Pilot studies indicated that 6 hours post dosing was the time of peak effect for neurological signs. Dosages for repeated treatments were chosen based on weight loss, regain, and neurological signs during pilot studies. Adult male Long-Evans rats were implanted with epidural screw electrodes. After about 1 week recovery, single exposure animals were dosed for 2 days with 1 ml/kg corn oil vehicle and then tested to allow accommodation to the procedures. On the third day, animals were dosed with corn oil, 5, or 10 mg/kg fipronil and tested 6 h later. Animals in the repeated dosing group were dosed for 14 consecutive days with corn oil, 5, or 10 mg/kg/day fipronil. Transient weight loss and occasional tremors were observed in the high dosage group, but these effects resolved after about 1 week. On days 12 and 13, animals had EEG recorded prior to dosing. On day 14, the rats were dosed 6 h prior to testing. Single treatment with these lower dosages did not significantly alter the EEG. However, repeated dosing with 5 or 10 mg/kg/day resulted in increases in Delta amplitude of the Delta (34 and 30% respectively) and Theta bands (41, 47%), and the area of the Theta band (30, 39%) compared to control. The area of the Gamma band was decreased at 5 (20%) and 10 mg/kg/day (22%) when recorded between the visual and frontal cortex, compared to control. The data confirm that fipronil alters CNS activity as measured by EEG. Additionally, dosages that were ineffective with a single treatment produced significant changes with repeated dosing. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1863b Changes in Rat Serum Biomarkers after Single or Repeated Dosages of Fipronil


Acute exposure to different classes of pesticides produces different patterns of changes in serum biomarkers (Herr et al., Toxicologist, 2013). We now examined profiles of biomarkers after 1 or 14 treatments with 0, 5, or 10 mg/kg/day fipronil (po). Adult male Long-Evans rats were evaluated for EEG changes (Lyke et al., Toxicologist, 2014), and were sacrificed after testing (6 h). There were no changes in serum ALT, AST, LDH, or SDH. Serum T3 decreased after 1 or 14 treatments (10 mg/kg). Serum T4 decreased after 1 (10 mg/kg) or 14 treatments (5, 10 mg/kg/day). These samples were processed by Myriad RBM (RodentMAP® and Rat MetabolicMAP®). One exposure produced changes in 4 of 73 analytes, while 14 treatments altered 8 different analytes. Bioclates AbsoluteIDQ™ p180 analysis of plasma showed changes in metabolites, and repeated dosing produced more effects. Increased serum levels of the fipronil sulfone metabolite, and decreased levels of fipronil, were found with 14 treatments vs. 1 exposure. Data show that repeated treatment with fipronil results in different patterns of biomarkers compared to a single exposure, and may reflect changes in involvement of biological pathways. This is an abstract of a presentation and does not necessarily reflect EPA policy.

1863c Chronic, but Not Acute, Treatment of Caenorhabditis elegans with TouchDown Leads to Increased Oxidative Stress

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The purpose of this study was to determine if chronic (24 h) or acute (30 min) exposure of Caenorhabditis elegans (C. elegans) to TouchDown™ (TD), a glyphosate-containing herbicide, resulted in increased oxidative stress. To verify if the glutathione pathway was activated following chronic TD treatment, CL2166 worms were treated with TD. This C elegans strain has glutathione-S-transferase (GST) tagged with green fluorescent protein (GFP) resulting in increased fluorescence when GST::GFP translation is up-regulated. This is generally interpreted as elevated oxidative stress. A concentration-dependent increase in fluorescence compared to control worms (**p<0.001) was observed. This was not the case for CL2166 worms treated acutely. To determine the specific reactive oxygen species that may contribute to increased GST::GFP transcription and translation, synchronized populations of wild-type (N2) C elegans were exposed chronically or acutely to varying concentrations (LC10, LC50, LC90) of TD. Following incubation, worms were further exposed to dihydroethidium, (DHE) to detect O2−, or hydroxyl radical and peroxynitrite sensor (HPE; Life Technologies). DHE assays detected no statistically significant increase in O2− in any treatment paradigm at any concentration. Chronic TD exposure following treatment with HPE showed no statistically significant increase in fluorescence compared to control when HPE was added 24 h post treatment. A concentration-dependent trend towards increased HPE fluorescence was observed, however, when HPE was added immediately following TD incubation. Conversely, worms treated acutely with TD showed a strong trend towards decreased hydroxyl radical production compared to controls. Taken together, these data support the hypothesis that chronic, but not acute, exposure leads to increased GST::GFP transcription and translation that may be due, in part, to the production of hydroxyl or peroxynitrite radicals. Furthermore, it is likely that this increased oxidative stress could be the mechanism through which previously observed neurodegeneration in C elegans occurs.

1864 Application of Proteomics to Identify Deregulated Proteins Associated with Idiosyncratic Liver Toxicity in a Rat Model of LPS/Diclofenac Co-administration

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There is increasing experimental and clinical evidence to suggest that non-drug related risk factors such as an underlying disease, bacterial or viral infection, may contribute to idiosyncratic drug reactions (IDR). In our previous work we found that combined treatment of rats with therapeutic doses of diclofenac (DcI), a model drug associated with drug idiosyncrasy in patients, and bacterial endotoxin (LPS) resulted in severe hepatotoxicity. In this study, we used an integrated discovery to targeted LC-MS proteomics approach combined with multiplex ELISA to identify liver and plasma proteins modulated by LPS/Dcl treatment, which may be mechanistically linked to or serve as potential safety alerts for IDRs. In the absence of hepatotoxicity, LPS alone caused a marked increase in the concentration of various cytokines and other putative danger signals such as CINC-1 and HMGB1 in plasma as well as significant changes in 19/49 in liver proteins and 3/15 plasma proteins analyzed by LC-MS. The most prominent effect of Dcl alone was a significant rise in T-kininogen-1, an acute phase protein previously suggested as a sensitive marker for drug-induced liver injury, in both liver and plasma. This effect and LPS-mediated cytokine release were amplified in the combined LPS/Dcl treatment group. In addition, depletion of plasma fibrinogen β and γ, consistent with the suspected contribution of the hemostatic system and increased fibrin deposition to IDRs, was observed in the co-treatment group. In contrast, several proteins previously suggested as liver biomarkers such as clusterin did not correlate well with liver injury in this model. Taken together, our analyses revealed proteomic changes associated with IDRs in a rat model of LPS/Dcl co-administration.

1865 The Use of microRNAs to Explore the Mechanisms of Phenobarbital-Mediated Toxicity in the Rat

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Phenobarbital (PB) is a prototypical hepatic drug-metabolism inducer, and a model tumour promoter in the rat. Recent publications in microRNAs (miRNAs) can enhance our understanding of the mechanisms of PB-mediated toxicity. MiRNAs are small non-coding RNAs that suppress gene expression by inhibiting translation or stimulating transcript degradation of complementary mRNAs, with single miRNAs often targeted by multiple miRNAs. This study explored miRNA expression profiles in the liver from male Fischer rats which had been administered either a tumour promoting dose of PB (1000ppm) or a non-tumour promoting dose (50ppm) in the diet for 1, 3, 7, 14, 28 or 90 days. Previous work identified miR-182 was significantly increased compared to control after 90 days treatment using microarray profiling (Koufaris et al., 2012). Toxicol Sci. 128(2): 532-43. With quantitativePCR assays we have demonstrated that miR-182 was significantly increased from 7 days onwards, compared to controls. In addition we showed that a predicted target of miR-182, glycine N-methyltransferase (Gnm1), and a validated target, forkhead box transcription factor OI (FoxO1), were significantly down-regulated by 1000ppm PB compared to controls, and had a significant inverse correlation with miR-182 across the 90 days treatment period. FoxO1 is known to be an inducer of genes involved in cell cycle arrest and apoptosis, and Gnm1 is known to indirectly regulate gene methylation and therefore gene expression. Decreases in these miRNA targets were dose concordant with the PB-induced phenotypic effects, transient hyperplasia at 3 days treatment (significantly decreased FoxO1 but unchanged miR-182), and global DNA hypermethylation at 14 days treatment (significantly decreased Gnm1 and increased miR-182). These data lend support to the notion that miRNA expression profiles can be used to explore PB-mediated liver toxicity, offer insights into potential underlying mechanisms, and suggest the importance of temporal regulation.
Drug induced-liver injury (DILI) is one of the major safety concerns in drug development. Alanine and aspartate aminotransferase (ALT and AST) and functional biomarkers such as bilirubin have been widely used for monitoring of DILI. However, in preclinical, the diagnostic thresholds of these biomarkers have not been standardized among different testing facilities, and the predictive performance for future onset of hepatic lesions remains unclear. Here, we analyzed the data of large-scale toxicogenomics database, TG-GATEx, in which male Sprague-Dawley rats (N = 5252) were treated with 143 compounds with positive or negative hepatotoxicity for 3, 7, 14 and 28 days. Receiver operating characteristic (ROC) analysis was applied to assessment of the diagnostic or predictive performance of these traditional biomarkers. The threshold was expressed as percentage compared to the control mean for consistent evaluation among different testing facilities. Additionally, area under ROC curve (ROC-AUC) that means accuracy of biomarker was calculated. Regarding all hepatic lesions, the thresholds of AST and ALT were 111 to 116% and 113 to 123% compared to the control mean, respectively, with relatively high accuracy (ROC-AUC was 0.83 to 0.91 and 0.72 to 0.86, respectively). Furthermore, focusing on hepatic necrosis improved the ROC-AUCs of AST and ALT (0.82 to 0.98 and 0.84 to 0.91, respectively) with the following threshold: 116 to 130% and 115 to 126%, respectively. Additionally, the diagnostic performance of AST and ALT was almost equal to that of combinatorial usage by logistic regression model. However, we could not confirm the sufficient predictive performance of future hepatic lesion by these biomarkers. In conclusion, the results provide the information that enables consistent evaluation of these biomarkers by the standardized diagnostic threshold, and suggest that the performance of these biomarkers is limited to diagnostic case of hepatic lesion rather than the prediction.

Liver parenchymal cell (i.e., hepatocyte) apoptosis is associated with activation of the blood coagulation cascade. Emerging evidence suggests that coagulation cascade activation may be a mechanistic biomarker of morbidity in patients with acute liver injury. Hepatocytes have been shown to express tissue factor (TF), the primary activator of blood coagulation, in an encrypted form that lacks procoagulant activity. We hypothesized that induction of hepatocyte apoptosis by the Fas death receptor pathway increases the procoagulant activity of hepatocyte TF. Jo2 antibody-induced coagulation cascade activation was significantly reduced in mice with hepatocyte specific TF deficiency. Treatment of primary mouse hepatocytes with Jo2 induced apoptosis as indicated by cleavage of caspase-3 and increased exposure of phosphatidylserine (PS) on the cell surface. Jo2 treatment increased TF-dependent factor Xa generation by hepatocytes and triggered the release of procoagulant TF-positive microparticles into the culture medium. Pretreatment with a caspase-3 inhibitor reduced Jo2-induced TF activity and microparticle procoagulant activity. The high affinity PS-binding protein lactadherin significantly reduced Xa generation by Jo2-treated hepatocytes and abolished microparticle procoagulant activity. The results indicate that the mechanism of Fas-dependent hepatocyte TF activation involves caspase-3 activation and PS exposure on the cell surface. This suggests that increased (hepatocellular) TF procoagulant activity could contribute to the prothrombotic state associated with hepatocyte apoptosis in chronic liver disease.

MicroRNAs (miRNAs) are short, non-protein coding RNAs. They regulate gene expression by either inhibiting translation or promoting mRNA degradation, and are involved in diverse physiological and pathological events. Previous data from our group (Koufaris et. al. Toxicol Sci 128, 532, 2012) showed that phenobarbital (PB, a CAR activator), induces dysregulation of the hepatic miRNAome in rat livers. The liver miRNAome associated with PB induced CAR activation was explored at different doses and time points using high-throughput sequencing of small RNAs (miRNA-seq), a powerful tool for discovery and profiling of miRNA expression, providing a deeper understanding of molecular toxicity. Male Han Wistar rats were dosed with PB (50 or 1000 ppm) for 28 days, and sacrificed after 1, 3, and 28 days. Total RNA was isolated from frozen livers and library constructs were generated (TrueSeq smallRNA kit, Illumina). miRanalyzer V0.5 and DESeq were used for bioinformatics and statistical analysis. qPCR was used to validate the expression of miRNA families and potential gene targets. Around 200 miRNAs were detected per sample; for 50 ppm, only day 28 had 2 differentially expressed miRNAs, PB 1000 ppm, showed 3, 16, and 4 miRNAs differentially expressed at days 1, 3, and 28. Histopathological evaluation showed hepatocellular centrilobular hypertrophy at 7 and 28 days, consistent with the known enzyme induction effects of PB. miR-21 was upregulated after 3 days, suggesting PB mediated dysregulation of epithelial mesenchymal transition (EMT). However, miR-30b was also induced after 3 days, and the miR-200 family was induced after 28 days, both of which would act to counter the EMT dysregulation. Consequently these findings suggest activation of a homeostatic response in EMT control in response to PB treatment, in order to preserve the epithelial nature of hepatocytes. This miRNA-seq approach enables a more detailed exploration of miRNAs and their response to hepatotoxicants, such as PB, helping elucidate underlying pathway perturbations.
1870 Aroclor 1260 Exposure Causes Steatohepatitis and Activates Hepatic Receptors in an Animal Model of Diet-Induced Obesity


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Aroclor 1260 (Aro) is a complex mixture of polychlorinated biphenyls (PCBs) that is persistent and bioaccumulative in the environment. PCBs have been implicated in liver toxicity and various health effects in humans, including obesity, diabetes, and alterations in hepatic gene expression. The purpose of the study was to evaluate the effects of Aroclor 1260 exposure on hepatic gene expression and lipid metabolism in a rodent model of diet-induced obesity (DIO) and steatohepatitis.

Methodology:
- Male C57Bl/6 mice were exposed to Aro (20 mg/kg or 200 mg/kg in corn oil) or corn oil for 9 weeks, beginning at 6 weeks of age.
- Blood was collected at 12 weeks and liver was collected at 12 weeks and 24 weeks for gene expression analysis.
- Microarray analysis was performed using the Affymetrix Mouse Genome 4302.3 Array.
- Statistical analysis was performed using the Benjamini-Hochberg method for multiple testing correction.

Results:
- Exposure to Aroclor 1260 resulted in significant changes in hepatic gene expression, with increased expression of genes involved in lipid metabolism and inflammation.
- These changes were associated with alterations in hepatic lipid accumulation and steatohepatitis.

Conclusion:
- Aroclor 1260 exposure in a DIO model resulted in significant changes in hepatic gene expression and lipid metabolism, indicating potential for liver toxicity and obesity-related health effects.

1871 Secretome and Intracellular Metabolomics Analysis in HepG2 Cells after Exposure to Liver Toxicants

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The use of alternatives to animal testing has considerably increased in the last years, in addition to their major contribution to animal welfare, they open the possibility to better understand and explore mechanisms underlying toxicity. New technologies, such as transcriptomics, proteomics and metabolomics provide the opportunity to obtain comprehensive information about the toxicological mode of action of compounds being in commerce and most importantly for compounds under development. The combination of in vitro systems coped to metabolomics aims at better understanding and exploring the mechanisms underlying toxicity. New in vitro models with the final aim to better elucidate the cellular changes/metabolites and intracellular (>200 metabolites), 3) the identification of three time, 2) the capacity to evaluate targeted metabolomics from cell secretome (>100 metabolites) and generating an 8-point concentration-response curve and 4) the capability to evaluate targeted metabolomics from cell secretome (>100 metabolites) and intracellular (>200 metabolites), 3) the identification of three time, 2) the capacity to evaluate targeted metabolomics from cell secretome (>100 metabolites) and generating an 8-point concentration-response curve. The combination of in vitro systems coped to metabolomics aims at better understanding and exploring the mechanisms underlying toxicity. New in vitro models with the final aim to better elucidate the cellular changes/metabolites and intracellular (>200 metabolites), 3) the identification of three time, 2) the capacity to evaluate targeted metabolomics from cell secretome (>100 metabolites) and generating an 8-point concentration-response curve.

Methodology:
- HepG2 cells were exposed to liver toxicants for 24 hours.
- Secretome and intracellular metabolomics were analyzed using LC-MS/MS and NMR spectroscopy.
- Gene expression was analyzed using qPCR.

Results:
- Exposure to liver toxicants resulted in significant changes in secretome and intracellular metabolomics.
- Gene expression analysis revealed significant changes in key genes involved in liver toxicity.

Conclusion:
- The use of in vitro models combined with metabolomics analysis provides a powerful tool for understanding and exploring the mechanisms underlying liver toxicity.

1873 Modified High-Content Imaging Micropatterned Hepatocyte Coculture Model for Predictive Toxicology

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Current in vitro hepatic models that have been used to identify drug- or chemically induced hepatotoxicity, including HepG2, iPSC-derived, or primary hepatocytes, have intrinsic challenges, such as variable metabolic capacity, brief ex vivo life span in culture, and difficulty reproducing in vivo metabolic pathways. Comparative gene expression analysis of our 3D HepG2 cell culture model revealed its close resemblance to human liver gene expression at pathway and sub-pathway levels. The 3D HepG2 cell culture model is amendable for high content imaging and screening applications and provides a well format for low cost and increased throughput and represents a novel and more physiologically relevant method for assessing drug-induced liver toxicity.

Methodology:
- HepG2 cells were cultured using a micropatterned coculture model.
- Metabolomics analysis was performed using LC-MS/MS.
- Gene expression analysis was performed using qPCR.

Results:
- The micropatterned coculture model revealed its close resemblance to human liver gene expression at pathway and sub-pathway levels.
- The 3D HepG2 cell culture model is amendable for high content imaging and screening applications and provides a well format for low cost and increased throughput.

Conclusion:
- The use of micropatterned coculture models provides a novel and more physiologically relevant method for assessing drug-induced liver toxicity.

1874 Abnormal Immune Response to Antigen Challenge in Subjects with Alcoholic Cirrhosis: Baseline Data from the Zinc in Alcoholic Cirrhosis (ZAC) Trial

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Immune dysfunction contributes to liver disease progression and infection risk in alcoholic cirrhosis (AC). The purpose of the study is to better characterize liver injury biomarkers, adipokines, and immune function in subjects enrolled in a placebo-controlled, clinical trial of zinc sulfate for alcoholic cirrhosis (ZAC).

Methodology:
- Baseline data of 17 consenting subjects with AC were analyzed.
- Adipokines and cytokines were measured using ELISA.
- Differences between the means (AC vs. controls) were evaluated by t-test using statistical significance was set at p < 0.05.

Results:
- Adipokines and cytokines were significantly different between AC and controls.
- Mean insulin levels were increased in AC (p < 0.05).

Conclusion:
- Immune dysfunction contributes to liver disease progression and infection risk in alcoholic cirrhosis (AC).
Combining Two High-Content Screening Approaches Measuring Phospholipidosis and Lysosomal Trapping with In Silico Modelling Approaches to Predict Phospholipidosis Occurrence in Humans


Phospholipidosis (PLD) is a lysosomal storage disorder characterised by excessive accumulation of intracellular phospholipids in tissues, such as the liver, kidney, brain and lung. It is well established that a large number of cationic amphiphilic drugs (CADs) have the potential to induce PLD. Phospholipids can accumulate in lysosomes, which are essential organelles in cellular biogenesis and when compromised could lead to cellular toxicity. Drug accumulation in lysosomes (lysosomes) is a direct mechanism known to result in PLD, however PLD can also occur indirectly by altering synthesis and processing of phospholipids. In this study, five-six compounds (eighty-eight known to cause PLD in vivo, twenty-five known to not cause PLD in vivo and three compounds known to cause PLD specifically in the rat kidney).

In silico models based on physico-chemical properties can be used to predict in vivo PLD such as the Maxem model (sensitivity 75%, specificity 86%, accuracy 86%), here we show how the prediction of PLD in vivo can be improved by combining in silico models with two different high content screening (HCS) assays, to determine the accumulation of a PLD dye in vitro or the trapping of compounds within lysosomes (and exclusion of a lysosomal dye). We evaluated two cellular models HepG2 and H9c2 cells and show that HepG2 cells are more predictive than H9c2 cells with an accuracy of 91% and 80% respectively. On combining in silico and HCS in vitro approaches a sensitivity of 90%, specificity of 93% and an overall accuracy of 95% in predicting in vivo PLD was achieved. These results demonstrate the applicability of in vitro and in silico approaches to understand the mechanism underlying PLD and the utility of these approaches as useful screening tools in the pharmaceutical industry for the selection of drug candidates with a low liability of PLD.

A Long-Term Perfused Immune-Liver Coculture System to Model Antituberculosis Efficacy and Drug-Induced Liver Injury

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Pyrazinamide (PZA) is active in adult tuberculosis (TB) and can cause DILI. Meta-analysis of >13,000 patients on PZA suggest a non-dose dependent DILI mechanism. Novel 3D cultures approximate biology better than 2D. 3D perfusion systems may better mimic human pharmacokinetics (PK) and pharmacodynamics (PD) of drugs over time. To determine whether PZA DILI is dose dependent, we combined our hollow fiber system (HFS) TB model with a 3D perfused liver model to develop a dynamic PK/PD assessment of PZ activity. The 3D KUBE™ (Kiyatec) HepG2 liver/hybridost co-culture model (Kiyatec) was retrofitted into the HFS with Mycobacterium tuberculosis-infected THP-1 MPs and cultured for 28 days. The TB/MP-Liver systems were exposed to 5 concentrations of PZA or controls [media (−); APAP (+)]. With a 6 hour t1/2 to MIC, a variable (−); APAP (+)), with a 6 hour t1/2 to 4h and 6h, respectively. Drug accounted for 30% of the variance (p=0.035) of LFTs, driven by APAP versus negative control. The fold-change of AST, ALT, and LDH for negative controls versus APAP was 1.5±0.2 v. 2.1±0.3, 1.0±0.5 v. 3.0±1.4, and 3±0.1 v. 4.4±3.5, respectively. There was only weak PZA dose-response based on changes in LFTs, with an r2=0.21 (AST) and r2=0.32 (ALT), with decrease in AST and ALT changes as PZA dose increased. There was no convergence for LDH. There was no apparent increase in DILI with increase in PZA dose. Conclusion: Despite HepG2 limitations, a 3D KUBE HepG2 liver model exposed to human-like PKs in the HFS demonstrated increased LFTs with APAP, but no dose dependent DILI for PZA, similar to clinical findings. This system may be useful for identifying novel drugs with reduced toxicity in multi-drug TB.

Accelerated Cytotoxicity Mechanism Screening of Flutamide in Isolated Rat Hepatocytes

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Flutamide (FLU) is a competitive antagonist of the androgen receptor which has been used in the treatment of metastatic prostatic cancer. FLU was reported to induce severe liver injury in some patients. The most common liver pathology was hepatic necrosis and cholestasis. The potential molecular cytotoxic mechanisms of FLU towards isolated male Sprague Dawley rat hepatocytes were investigated in this study using “Accelerated Cytotoxicity Mechanism Screening” (ACMS) techniques. A concentration-dependent increase in cytotoxicity, reactive oxygen species (ROS) formation and a decrease in mitochondrial membrane potential was observed for FLU compared to control hepatocytes. We hypothesized that FLU produced hepatic necrosis through reactive metabolite(s) and ROS formation. Incubation of isolated hepatocytes for 2 h with 75 μM flutamide induced an approximate 50% loss in hepatocyte viability (LC50, according to ACMS). A significant increase in FLU cytotoxicity and ROS formation was observed when glutathione (GSH) depleted hepatocytes were used and this toxicity was reversed by the addition of N-Acetyl-l-cysteine (a GSH precursor). Nontoxic concentrations of 1-amino benzotriazole (non-selective cytochrome P450 inhibitor), ketocconazole (a CYP3A inhibitor), β-naphthoflavone (a CYP1A2 inhibitor) and mepatadine (an aldehyde oxidase inhibitor) significantly decreased FLU-induced cytotoxicity, suggesting the involvement of cytochrome P450 and aldehyde oxidase catalyzed reactive metabolite(s) formation. Previous studies also found that rat and human microsomal cytochrome P450 oxidatively metabolized flutamide into electrophilic metabolites. TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl), a known ROS scavenger and superoxide dismutase mimic, prevented hepatocyte death. DPPD (N,N’-diphenyl-1,4-phenylenediamine) also reversed toxicity caused by FLU. From the data that we obtained till date, it can be suggested that FLU caused reactive metabolite(s) and ROS formation leading to oxidative stress and mitochondrial injury.

A Novel Mouse Model for Phenytoin-Induced Liver Injury: Involvement of Immune-Related Factors and P450-Mediated Metabolism

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Drug-induced liver injury is a major issue for drug development and clinical drug therapy; however, in most cases, it is difficult to predict or prevent these reactions due to a lack of proper animal models and unknown mechanisms of action. Phenytoin (DPH) is an anticonvulsant drug that is widely used for the treatment of epilepsy. Some patients who are administered DPH will suffer symptoms of drug-induced liver injury characterized by hepatic necrosis. However, mechanism under the developing DPH-induced liver injury is largely unknown. In this study, we established a mouse model for DPH-induced liver injury and analyzed the mechanisms for hepatotoxicity in the presence of immune- or inflammation-related factors and metabolic activation. Female C57BL/6 mice were administered DPH for 5 days in combination with L-buthionine-S,R-sulfoximine (BSO) to deplete hepatic GSH contents. As a result, plasma alanine aminotransferase (ALT) levels were increased, hepatic lesions were observed during histological evaluations, hepatic glutathione levels were significantly reduced and oxidative stress marker levels were significantly increased. Inhibition of cytochrome P450-dependent oxidative metabolism significantly suppressed elevated plasma ALT levels and depleted hepatic glutathione levels. Among the innate immune factors, hepatic mRNA levels of NACHT, LRR, pyrin domains-containing protein 3, interleukin (IL)-1β, and damage-associated molecular patterns were significantly increased. In the adaptive immune response, Th17 cells-mediated inflammation was suggested. In conclusion, cytochrome P450-dependent metabolic activation followed by a stimulation of innate immune responses and IL-17-mediated inflammation are involved in DPH-induced liver injury.
Vinyl chloride (VC) is a ubiquitous environmental contaminant and ranks 4th on the ATSDR Hazardous Substances Priority List. We have previously reported increased hepatocellular necrosis in a highly exposed occupational cohort and in vitro models. The purpose of this study is to study hepatic injury in vivo and assess potential liver damage in a residential cohort of human subjects living adjacent to a VC chemical plant. C57Bl/6J mice received chloroethanol (ClEtOH), a major metabolite of VC, and lipopolysaccharide (LPS) 24 h after ClEtOH. Samples were harvested for determination of liver damage, inflammation and changes in carbohydrate and lipid metabolism. Apoptosis and necrosis biomarkers were measured in human serum. In mice, ClEtOH alone caused no detectable liver damage. LPS exposure caused oxidative stress, lipid accumulation and inflammation, which was exacerbated by ClEtOH preexposure. ClEtOH increased activation of recruited and resident monocytes as well as neutrophils and was coupled with an increase in transaminases over LPS alone, an increase in necroinflammatory foci and an increase in free fatty acids. The combination of ClEtOH and LPS decreased mitochondrial function biosensor sentinel cells when monitored by daily high content imaging. Later, TVX pretreatment of RAW cells increased TNF mRNA and enhanced LPS-mediated increase in TNF protein release after LPS. These results demonstrated that TVX increased TNF mRNA in part by later TVX-mediated increase in TNF mRNA stability. U0126 prevented the TVX-mediated increase in TNF mRNA in part by later TVX-mediated increase in TNF mRNA stability. ERK phosphorylation and abolished the TVX-mediated increase in TNF mRNA in part by later TVX-mediated increase in TNF mRNA stability. ERK 1/2 (ERK) signaling can increase TNF mRNA transcription and stability, and this possibility was evaluated. Indeed, one hour of TVX pretreatment during TVX pretreatment period, TVX increased TNF mRNA, but this was less apparent after 4 hrs LPS addition, suggesting that the pivotal signaling events occurred during TVX pretreatment. TVX significantly increased TNF mRNA in part by increasing mRNA stability. ERK 1/2 (ERK) signaling can increase TNF mRNA transcription and stability, and this possibility was evaluated. Indeed, one hour of TVX pretreatment increased phosphorylation of ERK. An ERK-selective activation inhibitor, U0126, prevented ERK phosphorylation and abolished the TVX-mediated increase in TNF mRNA; however, it only decreased the early (~30 min) but not the later TVX-mediated increase in TNF mRNA stability. U0126 prevented the TVX-mediated increase in TNF protein release after LPS. These results demonstrated that TVX pretreatment of RAW cells increased TNF mRNA and enhanced LPS-induced TNF release in an ERK-dependent manner. (Supported by NIH grant R01 DK061315 and T32 ES007255.)
ROS/ATP ratio was obtained for each concentration, and area under dose-response
was evaluated in rat hepatocytes using DNA damage and steatosis as endpoints. To confirm this observation, studies investigated the role of CYP-mediated metabolism differences between the Swedish and U. S. populations due to genetic variance. The effect of drug toxicity in human.

The fluorouracil, sodium bisulfite-induced (BSEP) inhibition to the severity of drug induced liver injury (DILI) is associated with lack of inhibition. All approved drugs in the dataset (n=182) were categorized according to DILI warnings in drug labels issued by the FDA. In addition, a structure activity relationship model that correctly predicted 82 and 94% of inhibitors and non-inhibitors, respectively, was developed. A dataset of 250 compounds was screened for BSEP inhibition in membrane vesicles, and 86 BSEP inhibitors were identified. Studies modeling identified BSEP inhibition to correlate strongly with compound lipophilicity, while positive molecular charge was associated with lack of inhibition. All approved drugs in the dataset (n=182) were categorized according to DILI warnings in drug labels issued by the FDA and a strong correlation between BSEP inhibition and DILI was identified. Among the approved drugs as many as 38 of the 61 (62%) identified BSEP inhibitors were associated with severe DILI and every second drug associated with severe DILI was a BSEP inhibitor. This is twice the levels observed for BSEP non-inhibitors. Results from SCHH show that BSEP inhibitors associated with severe DILI greatly reduced the canicular efflux of TA, while BSEP inhibitors without the association to severe DILI did not. BSEP inhibition in membrane vesicles was found to correlate to DILI severity, and altered disposition of TA in SCHH was shown to distinguish BSEP inhibitors associated with severe DILI from those with no mild DILI.

1885 Species Difference May Account for the Discrepancy in Predicting Drugs Causing Severe Drug-Induced Liver Injury (sDILI)

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In a previous in vitro study with pooled human hepatocytes from U.S. population, elevation of ROS/ATP ratio has been identified as a potential biomarker to identify the drugs that cause sDILI such as acute liver failure (ALF). However, several drugs with ALF reported only in Sweden didn’t cause the elevation, suggesting that genetic or environmental factors may play key roles in this regional discrepancy. Interestingly, these drugs were identified in high content assays with rat hepatocytes using RNA damage and steatosis as endpoints. To confirm this observation, these drugs were evaluated in rat hepatocytes with ROS/ATP ratio. Furthermore, human hepatocytes from 10 individual donors were evaluated in a subset of the drugs to investigate individual difference. Primary cultured hepatocytes from rats or human were treated with the drugs at eight concentrations and divided into two sets, one for the quantification of ROS, and the other for cellular ATP contents. ROS/ATP ratio was obtained for each concentration, and area under dose-response curve calculated. The results confirmed that most of these drugs indeed cause the elevation of ROS/ATP ratio in rat hepatocytes. In human hepatocytes, 8 Sweden drugs and 7 sDILI drugs were evaluated. The majority of findings from these 7 sDILI drugs tested on individual donors are consistent with the results from pooled samples. However, individual variances were observed on 8 Sweden drugs. As drug metabolism is susceptible to genetic and environmental factors, and inter- and intra-individual differences in catalytic activities variability, it is hypothesized that drug metabolism differences between the Swedish and U. S. populations due to genetic variance may be responsible for the discrepancy in the manifestation of sDILI and indicate potential mechanisms involved in CYP-mediated metabolism. Differences in response between rat and human hepatocytes to these drugs further underscores the difficulty in the use of laboratory animals to prediction drug toxicity in human.

1886 Trovafloxacin-Induced DNA Damage in HepG2 Cells Causes p21 Upregulation and Activation of ATR and Sensitizes Cells to TNF-Mediated Cytotoxicity Involving ERK

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The fluorouracil, sodium bisulfite-induced (BSEP) inhibition to the severity of drug induced liver injury (DILI) is associated with lack of inhibition. All approved drugs in the dataset (n=182) were categorized according to DILI warnings in drug labels issued by the FDA. In addition, a structure activity relationship model that correctly predicted 82 and 94% of inhibitors and non-inhibitors, respectively, was developed. A dataset of 250 compounds was screened for BSEP inhibition in membrane vesicles, and 86 BSEP inhibitors were identified. Studies modeling identified BSEP inhibition to correlate strongly with compound lipophilicity, while positive molecular charge was associated with lack of inhibition. All approved drugs in the dataset (n=182) were categorized according to DILI warnings in drug labels issued by the FDA and a strong correlation between BSEP inhibition and DILI was identified. Among the approved drugs as many as 38 of the 61 (62%) identified BSEP inhibitors were associated with severe DILI and every second drug associated with severe DILI was a BSEP inhibitor. This is twice the levels observed for BSEP non-inhibitors. Results from SCHH show that BSEP inhibitors associated with severe DILI greatly reduced the canicular efflux of TA, while BSEP inhibitors without the association to severe DILI did not. BSEP inhibition in membrane vesicles was found to correlate to DILI severity, and altered disposition of TA in SCHH was shown to distinguish BSEP inhibitors associated with severe DILI from those with no mild DILI.

Developmental Exposure to Bisphenol A (BPA) Induces Hepatic Lipid Accumulation Mediated through Nrf2 Activation

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BPA is a component present in some plastics, dental sealants, and thermal receipts. Urinary BPA levels have been positively associated with obesity in recent human studies. Roselli et al. also demonstrated that developmental BPA exposure can increase body weight and hepatic lipid accumulation. Recently, we described that Nrf2 pathway is associated with hepatic lipid accumulation via upregulation of lipid transport and synthesis gene expression. The mechanism of this BPA mediated lipogenic effect remains unknown, but is likely due to dysregulation of gene expression for lipid homeostasis. The present study examined whether developmental BPA exposure caused hepatic lipid accumulation through Nrf2-mediated upregulation of gene expression for lipid import and synthesis. Pregnant CD1 mice were administered 25 or 250μg BPA/kg/day via osmotic pump; after weaning on PND 20, the resulting daughters were exposed to BPA via drinking water until PND 35. BPA increased hepatic lipids in livers from PND35 and week 39 adult female offspring along with upregulation of genes for lipid uptake and synthesis, such as fatty acid synthase and acetyl-CoA carboxylase. This was further associated with induction of transcriptional pathways that promote lipid import and synthesis, such as Peroxisome proliferator activated receptor-γ and Sterol regulatory element binding protein 1c protein expression at PND32 and week 39. At week 39 mice, Nrf2 target genes Gclc and Nqo1 protein expression were increased with BPA exposure. BPA increased total acetylated Histone 3 K9 marks were increased in nuclear fractions from liver. Future studies will address the deeper mechanism by which BPA exposure causes dysregulation of lipid metabolism, by examining Nrf2 recruitment to the Ppar-γ and Srebp-1c promoters. Overall, our data illustrates that developmental BPA exposure causes increased hepatic lipids via increased expression of genes that control lipid import and synthesis, as well as decrease lipid export in liver.

Inhibition of Bile Salt Export by Marketed Drugs in Primary Hepatocytes from Human, Monkey, Dog, Rat, and Mouse

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Cholestasis is one of the mechanisms of drug-induced liver injury (DILI). This study is to investigate the potential inhibition of bile salt export by drugs that are associated with cholestasis. The functional activity of bile salt export pump (BSEP) was determined in primary hepatocyte suspension with a sensitive LC-MS/MS method. Cryopreserved hepatocytes were incubated with bile acids in the presence or absence of the test articles. The fractions containing transported bile salts were separated by centrifugation and the concentrations of the bile salts were determined by LC-MS/MS. The BSEP activity was determined to be approximately 1.0, 3.0 (glycocholic acid), 2.7, 4.2, and 2.7 (taurocholic acid) nmol/mnile cells/hour for human, monkey, dog, rat, and mouse, respectively. Forty drugs were tested with human hepatocytes, including 7 drugs causing cholestatic injury. 12 vanishing bile duct syndrome (VBDs), 10 cholestatic hepatitis, 4 mixed injuries, 1 hepatocellular injury and 6 drugs with no DILI reported. The assay is sensitive and robust in identifying drugs causing cholestatic injury (6 out of 7) with potent inhibition of BSEP activity (IC50 < 10 μM in the order of potency, cyclosporine, ritonavir, saquinavir, troglitazone, atorvastatin, cerivastatin). None of the drugs causing VBDs and cholestatic hepatitis shows IC50 < 40μM except haloperidol, indicating that mechanisms other than BSEP inhibition may be involved in the development of these liver injuries. Drugs causing mixed injuries such as ketoconazole, pioglitazone and lovastatin are potent BSEP inhibitors (IC50 < 10 μM). Twenty four drugs were also tested in hepatocytes isolated from monkey, dog, rat and mouse, respectively. Species difference in potency was observed. In summary, a novel and
robust assay was developed using hepatocyte suspension from human and animal species to assess BSEP inhibition. Drugs causing cholestatic and mixed injury are highly associated with potent BSEP inhibition.

1888 Role of Epithelial to Mesenchymal Transition in the Hepatic Fibrosis of OVE26 Type 1 Diabetic Mice
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Diabetes is a global health issue. Diabetic liver injury is one of common complications in diabetic patients. Epithelial to mesenchymal transition (EMT) has been considered as an important mechanism in hepatic fibrosis. In the present study, therefore, we utilized OVE26 transgenic type 1 diabetes mouse model to investigate diabetic effect on EMT and its association with hepatic fibrosis. Serum glucose, aminotransferase and triglyceride levels of OVE26 and their wild-type (FVB) mice at 1, 3, 5 and 8 months old were measured. Hepatic pathology and fibrotic response were examined with HE, oil-red, and Sirius-red staining as well as Western blotting along with immunohistochemical staining for TGF-$\beta$, E-Cadherin, and oSMA protein expression. Compared to FVB mice, OVE26 mice exhibited significant increases in serum levels of glucose, aminotransferase and triglyceride. At ages of 5 and 8 months, OVE26 mice developed steatohepatitis in varying degrees, reflected by significant increases in oil-red staining, collagenous fiber hyperplasia, and Sirius-red staining, compared to age-matched FVB mice. The protein expression of TGF-$\beta$, CTGF, oSMA, and fibronectin as index of EMT, detected by Western blotting and/or immunohistochemical staining, was significantly increased in 5- or 8-month old OVE26 mice compared to age-matched FVB mice. The significant difference between OVE26 and FVB for E-Cadherin expressions, examined with Western blotting. These results suggest that steatohepatitis is an early manifestation in the liver of diabetic mice, and developed into hepatic fibrosis at late stage, which may be related to the EMT.

1889 FXR Regulates FoxO3 Activation in Ethanol-Induced Autophagy and Liver Injury
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Autophagy is a lysosomal degradation process and cellular protective mechanism. Alcoholic liver disease encompasses a wide spectrum of pathogenesis including steatosis, fibrosis, cirrhosis, and alcoholic steatohepatitis. Autophagy induction protects against alcohol-induced steatosis. We recently demonstrated that Farnesoid X Receptor deficient (FXR/-) mice displayed impaired autophagy. We thus hypothesized that FXR/- mice may have exacerbated liver injury after alcohol treatment. In the present study, we found that alcohol treated-FXR/- mice had increased serum alanine aminotransferase (ALT) and hepatic triglyceride levels compared to wild type mice. Mechanically, we found that ethanol treatment had decreased expression of various essential autophagy genes in FXR/- mice than that of wild type mice. Interestingly, we further found that Forkhead box O3 (FoxO3), a transcriptional factor that regulates autophagy and many antioxidant gene expressions, is activated by ethanol to protect against ethanol-induced liver injury. Ethanol-induced FoxO3 activation was dramatically suppressed in the FXR/- mice. These results suggest that FXR is a positive regulator for ethanol-induced FoxO3 activation and autophagy.

1890 Global Proteomic Analysis of Acetaminophen Toxicity in 3D Human Liver Microtissues
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Early prediction of drug safety remains a challenge in preclinical phases of drug development. Global proteomic analysis may allow for a better understanding of the mode of action and mechanism of toxicity induced by drug treatment. In this study, a novel mass-spectrometric approach – Hyper Reaction Monitoring (HRM) – was used to quantify changes in protein expression in 3D human liver microtissues exposed to acetaminophen (APAP). 3D human liver microtissues were exposed to 8 concentrations of APAP (4 uM - 10,000 uM) over 72 hours. Microtissues were harvested and subjected to mass spectrometric analysis. For global proteome profiling the samples were measured, normalized and profiled using a previously generated library of over 1900 proteins. Treatment-dependent changes in protein expression were determined by performing pairwise t-test comparisons of each APAP concentration with untreated control.

Significant changes in the expression of 127 proteins were observed across all treatment conditions. Among these proteins, the expression of several phase I enzymes (CYPs 1A2, 2B6, 2C1, 2C8 and 2E1) were altered following drug treatment. Interestingly, exposure to a subtoxic (4 uM) concentration of APAP induced a 4 fold up-regulation of CYP1A2 protein expression, which supports the role of CYP1A2 in the bioactivation of APAP to its reactive metabolite in vivo. Cluster analysis revealed the up-regulation of membrane proteins, glycoproteins and fatty acid metabolism-related proteins. Down-regulated proteins included those involved in eicosanoid metabolism, suggesting interaction of APAP with its pharmacological target.

Taken all together, the results suggest that use of HRM for proteomic studies in 3D human liver microtissues is a powerful tool to detect early changes in the proteome and may allow for the better understanding of the mechanism of drug-induced toxicity and the identification of relevant early biomarkers of toxicity.

1891 Integrated Proteomic and miRNA Transcriptional Analysis Reveals the Hepatotoxicity Mechanism of PFNA Exposure in Mice
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Perfluoroalkyl chemicals (PFASs) are a class of highly stable man-made compounds, and their toxicological impacts are currently of worldwide concern. Administration of perfluorononanoic acid (PFNA), a perfluorocarboxylic acid (PFCA) with a nine carbon backbone, resulted in dose-dependent hepatomegaly in mice (0, 0.2, 1 and 5 mg/kg body weight, once a day for 14 days) and an increase in hepatic triglycerides (TG) and total cholesterol (TCHO) in the median dose group, as well as serum transaminases in the high dose group. Using isobaric tags for relative and absolute quantitation (iTRAQ), we identified 125 hepatic proteins (60 up-regulated, 65 down-regulated) that exhibited statistically significant changes after PFNA treatment. Lipid metabolism process proteins were the most dominant in the up-regulated proteins. While down-regulated proteins were mainly involved in monosaccharide and amino acid metabolic processes. Three altered proteins by iTRAQ (Acl1, Fbp1, and Glud1) were further confirmed by Western blot analysis. The miRNA analysis results further suggested that PFNA exposure not only resulted in a fatty acid oxidation effect, but also activated genes involved in fatty acid and cholesterol synthesis in the mouse liver. Additionally, three (two down-regulated, one up-regulated) and thirty (fourteen down-regulated, sixteen up-regulated) microRNAs (miRNAs) exhibited at least a two-fold alteration (P < 0.05) in the 1 and 5 mg/kg PFNA treatment groups, respectively, including hepatic disease related miRNAs, miR-34a and miR-200c. The repression effect of miR-34a on fucosyltransferase 8 (Fut8) and lactate dehydrogenase (Ldha) was confirmed by luciferase activity assay and western blot analysis. The results indicated that PFNA exerts a hepatic effect, at least partially, by miRNAs mediated post-translational protein repression.

1892 Sertraline Induces Endoplasmic Reticulum Stress in Hepatic Cells
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Sertraline is generally used for the treatment of depression, and is also used for the treatment of panic, obsessive-compulsive, and post-traumatic stress disorders. Previously, we have demonstrated that sertraline caused hepatic cytotoxicity with mitochondrial dysfunction and apoptosis being the underlying mechanisms. In this study, we identified additional molecular mechanisms by microarray analysis and studies further by biochemical and molecular analyses. HepG2 cells were exposed to sertraline at various concentrations for 6 h and subjected to whole genome gene expression microarray analysis. Pathway analysis revealed that endoplasmic reticulum (ER) stress and MAPK signaling pathway are among the significantly affected biological changes. Following the results of microarray study, we validated the increased expression of ER stress makers by real-time PCR and Western blot. The expression of typical ER stress markers such as PERK, IRE1a, and CHOP were significantly increased. To better study ER stress-mediated drug-induced liver toxicity, we established in vitro systems monitoring ER stress response quantitatively and efficiently using Gausia luciferase (GLuc) or secreted alkaline phosphatase (SEAP) as reporter. In these two reporter assays, sertraline showed inhibiting effect on the secretion of Gausia or alkaline phosphatase. Moreover, we demonstrated that sertraline-induced apoptosis coupled to ER stress and the apoptotic effect
was attenuated by 4-phenylbutyrate, a potent ER stress inhibitor. In addition, we showed that MAP4K4-JNK signaling pathway participated in the process of sertraline-induced ER stress. In summary, for the first time, we defined that ER stress is a mechanism of sertraline-induced liver toxicity.

## 1893 Sertraline, an Antidepressant, Induces Apoptosis in Hepatic Cells through the Mitogen-Activated Protein Kinase Pathway


Sertraline is generally used for the treatment of depression, and is also approved for the treatment of panic disorder-compulsive, and post-traumatic stress disorders. Previously, using rat primary hepatocytes and isolated mitochondria, we demonstrated that sertraline caused hepatic cytotoxicity and mitochondrial impairment. In the current study, we investigated and characterized molecular mechanisms of sertraline toxicity in human hepatoma HepG2 cells. Sertraline decreased cell viability and induced apoptosis in a dose- and time-dependent manner. Sertraline activated the intrinsic checkpoint protein caspase-9 and caused the release of cytochrome c from mitochondria to cytosol; this process was Bcl-2 family dependent because anti-apoptotic Bcl-2 family proteins were decreased. Pre-treatment of the HepG2 cells with caspase-3, caspase-8, and caspase-9 inhibitors significantly reduced the release of lactate dehydrogenase, indicating that sertraline-induced apoptosis is mediated by both intrinsic and extrinsic apoptotic pathways. Moreover, sertraline markedly increased the expression of TNF and the phosphorylation of JNK, ERK1/2, and p38. In sertraline-treated cells, the induction of apoptosis and cell death was shown to be the result of activation of JNK, but not ERK1/2 or p38 in the MAPK pathway. Furthermore, silencing MAP4K4, the upstream kinase of JNK, attenuated both apoptosis and cell death caused by sertraline. Taken together, our findings suggest that sertraline induced apoptosis in HepG2 cells via activation of the TNF-MAP4K4-JNK cascade signaling pathway.

## 1894 High-Content Analysis of a High-Throughput Human In Vitro Metabolic Model


Human xenobiotic metabolism and hepatotoxicity are important factors in toxicology studies conducted by the pharmaceutical industry and Department of Defense. Here we report an informative, high throughput in vitro method for studying the effects of human metabolism on the toxicity of compounds of interest. Using the integrated discrete multi-organ co-culture (iDMOC) system, we grew 3T3-L1 cells in monoculture and in the presence of primary human hepatocytes and, after toxicant exposure, conducted high content analysis. The fluorescence intensities of cells in monoculture and in the presence of primary human hepatocytes and, after toxicant exposure, conducted high content analysis. The fluorescence intensities of cells in monoculture and in the presence of primary human hepatocytes and, after toxicant exposure, conducted high content analysis.

## 1895 Comparative Metabolism of Eight Model Pharmaceutical Compounds in Rat- and Human-Liver Microsomes, Suspension Hepatocytes, and Micropatterned Cocultures of Primary Hepatocytes

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In vitro metabolism evaluations for compounds in early development are typically conducted using liver microsomal or suspension hepatocytes. While these systems have been serving the drug development community, they have their limitations, with examples of "metabolite surprises" - where metabolites which were not predicted or observed in vitro or in vivo during pre-clinical studies, were observed in vivo during pre-clinical studies. In summary, for the first time, we defined that ER stress is a mechanism of sertraline-induced liver toxicity.

## 1896 Expression of Drug Processing Genes in Livers of Germ-Free Mice

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Intestinal bacteria regulate many host functions, and may alter the expression of drug processing genes (DPGs) in the host. The purpose of this study was to characterize the expression of hepatic DPGs in mice lacking the normal complement of intestinal bacteria (germ-free mice) using RNASeq. Total RNA was isolated from the livers of adult male conventional (CV) and germ-free (GF) C57BL/6 mice (n=3 per group). The mRNA libraries were sequenced 50bp paired-end using an Illumina HiSeq2500 sequencer. Approximately 80-100 million reads were generated per sample and over 95% of the reads were mapped to the mouse mm10 genome (Tophat). The transcript abundance was estimated by Cufflinks, and differential expression was determined using Cuffdiff (FDR-BH<0.05). Ingenuity Pathway Analysis determined that drug-processing pathways rank among the top most differentially regulated networks in livers of GF mice. Cytochrome P450 enzyme (Cyp) that metabolizes more than 50% of drugs and is induced by PXR agonists, was decreased 87% in GF mice; Cyp2b10, the Cyp induced by CAR agonists was decreased 57% in GF mice; whereas Cyp4a14, a Cyp induced by PPARα agonists was increased 202%; and Cyp1a2, the Cyp induced by AhR agonists was increased 51%. The changes in mRNA of these Cyp genes do not appear to be due to mRNA changes in these receptors, because the mRNA of PXR was not altered, and the mRNA of CAR, PPARα and AhR were in fact increased. Not only were the Cyp mRNAs altered in the GF mice, but other DPGs, such as a number of glutathione transferases were down-regulated, whereas a number of sulfotransferases and uptake transporters and efflux transporters were up-regulated. In summary, this study suggests that intestinal bacteria regulate the mRNA expression of a large number of DPGs, and this may be responsible for some of the individual differences in drug responses.

## 1897 Morphological Changes to Mitochondria in Response to Compounds with Different Modes of Action


Early identification of compounds that could be a cause of drug induced liver injury is a vital part of a risk avoidance strategy. Along with other factors, disruption of normal mitochondrial function has long been linked to hepatic damage. Mitochondrial function is often monitored by determination of ATP, or, in the more recent literature by direct measurement of the mitochondrial membrane potential (MMP). These studies are usually carried out with HepG2 cells which are an immortalized, transformed cell line that are difficult to image at the subcellular level due to the compact nature of the cytoplasm. The classical toxins rotenone, oligomycin, bendaustamione, BAM7, warthamninn, antymicin, ansomycin and amiodarone were used to investigate the utility of using mitochondrial morphology in WS1 fibroblasts as a potential screening endpoint for toxicity assessment. MMP was measured up to 6 hours and compared to changes in mitochondrial morphology. WS1 fibroblasts are a primary human cell line that forms a monolayer in culture making them relevant to human risk assessment and amenable to high content imaging. Results from this study demonstrate that mitochondrial networks can be readily visualized in WS1 cells and quantified with appropriate stains and that changes in mitochondrial morphology, such as fragmentation due to a toxic
insult can be detected. Using morphology we are able to differentiate between compounds more readily than typical MMP staining; this was very evident with rotenone, oligomycin and antimycin that had comparable effect on MMP but very different effects on mitochondrial morphology. In addition we also found underlying morphology changes with compounds not thought of as mitotoxins such as anisomycin, showing the added benefit of a more phenotype based risk assessment tool. This first experiment has shown the great potential for using mitochondrial morphology as a screen, further studies are planned to follow up with a more diverse set of compounds, incorporating further mitochondrial endpoints for added context.

1898 In Vivo Extrapolation of In Vitro Drug-Induced Hepatocyte Accumulation of Triglycerides to Predict Steatosis in Rodents and Humans


Drug-induced liver injury (DILI) is one of the main causes of late-stage attrition in the development of drugs. Hepatic steatosis (fatty accumulation of the liver) can lead to DILI. Although it is considered to be a relatively mild side-effect of drugs, it may indicate more serious underlying causes, such as inhibition of mitochondrial respiration.

Here we report an in vitro in vivo extrapolation (IVIVE) of an in vitro steatosis assay to in vivo triglyceride accumulation in the rat on exposure to model ato compounds. The dose-response of triglyceride accumulation to compound exposure was measured in HepG2 cells using high content screening (HCS). The IVIVE is based around a mathematical model of hepatocyte triglyceride (TG) production and very low density lipoprotein (VLDL) production and export. TG accumulation data measured by HCS was converted to quantitative fluxes to TG, and the effects of drugs on this flux were modelled as kinetic effects on CO2, TG and VLDL production. In this way, direct (modulation of TG and/or VLDL fluxes) and indirect (inhibition of mitochondrial pyruvate oxidation) causes of steatosis can be explored. Casting the effects as kinetic parameters in this way enables the effects of exposure to be directly and quantitatively simulated. The prediction of steatosis in vivo was performed by using known plasma concentrations of drugs to drive the simulated response of intrahepatic TG accumulation. Good agreement was observed between predicted and observed accumulation in vivo for model drugs. Exercising the model enables examination of the expected effects of drugs on mitochondrial respiration as well TG/VLDL metabolism.

Using the steatosis model with PBPK modelling to predict drug concentrations, the in vivo steatosis dose response could be predicted directly from in vitro data, and also used to aid optimization of dosing regimes to minimise steatosis whilst maintaining efficacy.

1899 Using In Silico Modeling to Understand the Role of BSO in GSH Metabolism


It is believed that one of the key roles played by BSO is to inhibit the transport of cysteine, an essential amino acid across the hepatocyte cell membrane. Since cysteine is a rate-limiting substrate for GSH synthesis by gamma-GCS, BSO could lower cellular GSH by substrate inhibition. One of the facts that support this hypothesis is that lowered GSH levels are observed in presence of upregulated gamma-GCS. To test the validity of this hypothesis and other competing ones, we treated HepG2 cells with BSO for a period of 24 hours and assayed GSH, ATP, ROS and gamma-GCS levels at various time points. To understand the impact of cysteine availability for GSH synthesis, we also performed the same experiments by culturing the cells in excess cysteine containing media.

BSO induces an increase in ROS generation and hence depletion of cellular ATP and GSH. Addition of excess cysteine in the culture media recovered the ATP levels and reduced ROS levels. However the addition of excess cysteine did not recover the GSH, although the gamma-GCS activity remained up regulated. To understand what could be contributing to this behaviour, we fed the experimental observations into our in silico model of liver metabolism (Virtual Liver) and performed simulations to mimic the experimental conditions and to test various hypotheses for the action of BSO and cysteine in the liver. Our analysis indicates that the direct inhibitory effect of BSO on gamma-GCS activity has a greater impact than reduced cysteine availability in lowering cellular GSH levels. ATP and ROS recovery in the presence of additional cysteine is via a GSH independent route.

1899a Validation of a 3-Dimensional Liver Microtissue Model for Long-Term Hepatotoxicity Studies

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In vitro animal models have revealed multiple mechanisms of action (MOA) of diclofenac-related hepatotoxicity, including oxidative stress and mitochondrial permeability transition (MPT), and CYP-related metabolic activation and the formation of reactive metabolites. The combined hepatotoxic effect of these MOAs usually occurs within weeks of therapy commencement, emphasizing the need to incorporate in vitro safety testing periods similar to clinical exposure. However, primary hepatocytes cultured in a two-dimensional (2D) manner have been shown to undergo rapid loss of differentiated function and metabolic capacity and have less complex inter-cellular and cell-matrix interactions compared to in vivo. Here we demonstrate a 3D cell culture model in which hepatocytes dispersed into a hanging drop re-aggregate into a functional liver microtissue. Assessment of hepatocellular function and toxicity were measured by luminescent and image-based markers for CYP3A4 activity, ATP content, membrane integrity, mitochondrial superoxide production and MPT formation. CYP3A4 was found to be 100x greater in microtissues than in 2D. CYP activity and cell viability after 14 days of culture in 3D remained at 100% of day 1 levels, while 2D culture values dropped below 50%. Oxidative stress and MPT pore opening were observed after 3 and 7 day diclofenac incubations, respectively, confirming similar MOA using 3D cell culture. However, the time to reach a diclofenac cytotoxicity EC50 of 300 μM at measured by membrane integrity was 3 days with microtissues in contrast to 2 hours using 2D cell culture, as previously shown by other researchers. The potential of 3D cell models to better mimic clinical exposure timelines may lead to better correlation when performing long-term cytotoxicity studies while maintaining the benefits of specific in vitro assays to determine MOAs.

1899b Detection of Human Hepatic Toxicity in Chimeric Mice with Humanized Liver by Human ALT1 ELISA System

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To predict human (h-) hepatic toxicity a suitable animal model is needed because some medicines or chemical entities confirmed to be safe in experimental animals are toxic to the h-liver. We developed chimeric mice with h-hepatocytes (PXB-mice®), which have been used for Drug Metabolism and Pharmacokinetics (DMPK) studies and hepatitis B or C virus (HBV or HCV)-infection studies, to predict h-metabolism and chemical efficacy for HBV or HCV. Since >70% of the PXB-mice livers are repopulated with h-hepatocytes as well as <30% mouse (m-) hepatocytes, whether m- or h-hepatocytes are the cause of a chemical-induced increase in ALT activity cannot be distinguished. ALT1 and ALT2 are two isotypes of ALT; the former is a cytoplasmic protein and the latter is a mitochondrial protein with indistinguishable enzyme activity. Therefore, to identify specific hepatoxotoxicity, we produced h-ALT1-specific antibodies not crossed with m-ALT1, m-ALT2, or h-ALT2 by immunization with recombinant h-ALT1 protein synthesized by Baculovirus. Using 2 types of h-ALT1 antibodies we developed a h-ALT1 sandwich ELISA system, and detected h-ALT1 protein in PXB-mouse plasma. PXB- and SCID mice were administered aflatoxin B1 (AFB-1) or CCl4 orally for 7 days, and the ALT activities and h-ALT1 concentrations in plasma were measured periodically. ALT activity was found to be higher in the AFB-1-treated PXB-mice than the AFB-1-treated SCID mice, and the kinetics of ALT activity was similar to h-ALT1 protein levels in the PXB-mice. Conversely, ALT activity was higher in the CCl4-treated SCID mice than the CCl4-treated PXB-mice, and the kinetics of ALT activity was different from h-ALT1 levels in the PXB-mice. Therefore, AFB-1 and CCl4 are more toxic for h-hepatocytes and m-hepatocytes, respectively. In conclusion, using the h-ALT1 ELISA system, h-specific hepatic toxicity was detected quantitatively in the PXB-mice.

1899c Development of High-Throughput AlphaLISA Immunoassays to Quantify ALT and AST Levels in Human Primary Hepatocytes Culture Supernatant


Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels in blood are routinely used as a marker for liver function and elevations in these enzyme levels are associated with liver injury. Since compound attrition in pre-
clinical and clinical development is commonly due to drug-induced liver injury, compounds are screened for hepatotoxicity using in vivo and in vitro approaches. Currently, existing methods to quantify AST and ALT in hepatocyte culture supernatant require high volumes, thereby reducing throughput capacity and often showing variable results. In contrast, we developed a method of high throughput using AlphaLISA technology to measure AST and ALT in cell culture supernatant. Human primary hepatocytes were treated with Amitryptyline, a known hepatotoxicant that increases AST and ALT, at 1 mM for 2 h as a positive control and non-treated hepatocytes served as a negative control. The assay development steps included robustness of positive control for hepatotoxicity, identification of 2 specific antibodies for ALT and AST, optimization of antibody/bead concentrations, buffers, and incubation time. Specificity and matrix tolerance of the assay were also tested. Optimized ALT and AST AlphaLISA in vitro assays are high throughput (384 well plate), requiring 5 μl of sample, demonstrated to work on both culture supernatant and cell lysate, detected low inhibitory levels (and not their activity) and did not require washes like traditional ELISA. Availability of such assays enables in vitro hepatotoxicity studies to be done in 384 well plates and to be monitored over time in long term studies by collecting culture supernatant at various time points.

MicroRNA miR122 is a promising novel plasma biomarker for early detection of liver damage, as changes in plasma level would occur before the elevation of alanine aminotransferase and aspartate aminotransferase activities. As of this writing, little is known about the regulation of miR122 expression in hepatocytes. In our laboratory, we embarked upon the evaluation of miR122 expression in hepatocytes treated with various drugs known to be associated with drug induced liver injuries (DILI). Hepatocytes from three donors were treated for 8 h with ten drugs with known clinical hepatotoxicity: the hepatotoxic disulfiram, ketocozolone, troglitazone, nefazodone, valproic acid, zomepirac, ifosfamide, tamoxifen, and the triazolyl derivative. MRP2 dysfunction caused by inherited MRP2 deficiency or drug mediated inhibition of MRP2, are the two major ABC efflux transporters which contribute to drug toxicity. The results showed more than 20-fold uptake activity in BSEP vesicle, compared to the control. MRP2 mediated uptake of the probe substrate LTC4 was significantly inhibited by known MRP2 modulators, e.g., MK571, benzobromarone, terfenadine and indomethacin. BSEP mediated taurocholate uptake was shown to be inhibited by known BSEP inhibitors, e.g., cyclosporine A, glibenclamide and troglitazone, etc. The data indicates that “inside-out” membrane vesicles are a quick and useful tool to screen MRP2 and BSEP inhibitors which can potentially cause liver toxicity in vivo.

We previously reported on the role of connexin (Cx) 32 during steady-state hematopoiesis and its potential protective role against leukemogenesis. Here, we examined the hematopoietic progenitor cell (HPC) kinetics by evaluating the percentage of cycling HPCs by quantitative bromodeoxyuridine (BrdUrd) incorporation in vivo for up to 3 months, followed by ultraviolet-A exposure to eliminate BrdUrd incorporating cells. As a result, the percentage of the entire cycling fraction of primitive HPCs without Cx32 apparently increased continuously although that with Cx32 was maintained at a certain level from 8 weeks until 18 months of age. This is consistent with the observations of a larger number of HPCs and a smaller number of hematopoietic stem cells (HSCs) in aged Cx32-KO mice than in wild-type mice, which are in contrast to young mice with/without Cx32.

Then, we examined the bone-marrow reconstitution capability of HSCs from Cx32-KO mice by serial transplantation of cells in the lineage-negative, c-kit-positive, and Sca-1-positive (LKS) fraction from wild-type and Cx32-KO mice with freshly isolated bone marrow cells to prevent an acute radiation injury. Although both groups showed reconstituted hematopoiesis without any significant differences in various hematopoietic parameters in the primary, and secondary recipients, clear differences in the parameters in the LKS fraction solely from the secondary recipients were observed. Namely, four out of seven recipients in the wild-type group showed over 0.5% donor cells (average percentage and standard deviation for 4 mice, 25.1±27.9%), whereas none of the five recipients in the Cx32-KO group showed over 0.5% donor cells. These findings in this study taken together with the previous findings imply that Cx32 plays an essential role in maintaining self-renewal proliferation of HSCs to prevent their exhaustion and in suppress neoplastic changes.

MicroRNA miR122 Induction by Bile Salt Export Pump (BSEP) Inhibiting DILI Drugs in Primary Cultured Human Hepatocytes

Q. Yang, L. Doshi and A. P. Li. In Vitro ADMET Laboratories, Advanced Pharmaceutical Sciences, Columbia, MD.

Multidrug resistance proteins (MRPs), in particular MRP2, and the bile salt export pump (BSEP), are the two major ABC efflux transporters which contribute to drug induced liver toxicity. MRP2 is essential for hepatobiliary elimination of many drugs, drug conjugates and endogenous compounds, such as bilirubin glucuronides. MRP2 dysfunction caused by inherited MRP2 deficiency or drug mediated inhibition results in hyperbilirubinemia. BSEP is the rate-limiting step of bile salt transport across hepatocyte membranes. Disruption of BSEP in hepatocytes leads to accumulation of cytotoxic bile salts in the liver, resulting in liver toxicity, e.g., cholestasis. In light of the evidence showing the importance of MRP2 and BSEP in drugs with known clinical hepatotoxicity in vivo, we embarked upon the evaluation of miR122 expression in hepatocytes treated with various drugs known to be associated with drug induced liver injuries (DILI). Hepatocytes from three donors were treated for 8 h with ten drugs with known clinical hepatotoxicity: the hepatotoxic disulfiram, ketocozolone, troglitazone, nefazodone, valproic acid, zomepirac, ifosfamide, tamoxifen, and the triazolyl derivative. MRP2 dysfunction caused by inherited MRP2 deficiency or drug mediated inhibition of MRP2, are the two major ABC efflux transporters which contribute to drug toxicity. The results showed more than 20-fold uptake activity in BSEP vesicle, compared to the control. MRP2 mediated uptake of the probe substrate LTC4 was significantly inhibited by known MRP2 modulators, e.g., MK571, benzobromarone, terfenadine and indomethacin. BSEP mediated taurocholate uptake was shown to be inhibited by known BSEP inhibitors, e.g., cyclosporine A, glibenclamide and troglitazone, etc. The data indicates that “inside-out” membrane vesicles are a quick and useful tool to screen MRP2 and BSEP inhibitors which can potentially cause liver toxicity in vivo.

1899e “Inside-Out” Membrane Vesicles: An In Vitro Model to Study Transporter-Mediated Drug Interactions Which Can Lead to Liver Toxicity

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Stromal cell derived factor 1 (SDF-1) and C-X-C chemokine receptor type 4 (CXCR4) axis plays a critical role in angiogenesis. However, the multi-functional characteristics of native SDF-1 limit its potential application. We developed a novel CXCR4 antagonist namely, SDF-1βP2G (P2G), which was derived from human native SDF-1β to block CXCR4 and promote mobilization of endothelial progenitor cells (EPCs) necessary for vascular injury recovery and angiogenesis. P2G exerted antagonistic effects against CXCR4 by promoting CXCR4 internalization and competitively inhibiting downstream signaling in vitro, and does and time-dependently mobilization of EPCs (CD31+/c-kit+) from bone marrow into blood in vivo. Moreover, intravenous administration of P2G significantly stimulated angiogenesis, blood reperfusion and skeletal muscle regeneration in an acute hindlimb ischemia model. Mechanistic study showed that P2G significantly stimulated EPCs mobilization in the peripheral blood and promoted their infiltration into ischemic skeletal muscle tissues and incorporation into the newly formed blood vessel. In addition, P2G enhanced the activation and/or expression of angiogenesis and progenitor cell chemotaxis-related factors including Akt, ERK, mTOR, MMP-9, SDF-1/CXCR4 and VEGF. Furthermore, neutralization of VEGF with its specific antibody abolished P2G-induced blood reperfusion and angiogenesis. More importantly, no obvious inflammatory and apoptotic effects were observed in multiple organs after P2G administration. These data suggest that the novel antagonist of CXCR4, P2G, can be successfully utilized to stimulate ischemic angiogenesis and muscle regeneration through mobilization of EPCs in a VEGF dependent manner. Our work has demonstrated for the first time that P2G is a non-toxic, specific CXCR4 antagonist with a great potential for clinical application for ischemic vascular diseases.
1902 Evaluation of Cell Health In Vitro Using Video Bioinformatics
P. E. Levesque1.

The efficiency of in vitro toxicological assays can be increased by evaluating changes in cellular behavior and morphological characteristics during treatment. CL-Quant, a video bioinformatics (VBI) analysis tool, includes various modules for analyzing biological endpoints, and users can also introduce new protocols. Here, three cell health qualification modules were used: (1) growth, (2) migration, and (3) reactive oxygen species (ROS) production. Time-lapse videos of cigarette smoke exposed cells were collected using a Nikon BioStation. To evaluate pluripotent stem cells, colony area (pixels) was measured at each time point. For single cell proliferation of neural and NTera stem cells, confluence (pixels) was measured in each frame to obtain cell density. Smoke exposure inhibited growth of all cell types when compared to the control. Colony migration was studied by extracting information on: migration rate, displacement (distance between the starting and ending points), and total distance travelled. Average distance travelled by treated colonies was less than the control. The gap closure assay was used to measure single cell migration, and neural stem cell migration was more sensitive to smoke exposure than NTera cells. To verify the accuracy of the automated VBI tool, a set of images from each experiment was analyzed for ground truth (Image) software. CL-Quant and ImageJ analyses produced similar results for all assays. We also examined levels of ROS, which often increase when cells are stressed and can lead to severe cell damage, and cell death. To quantify ROS production, cells were pre-loaded with MitoSox, a mitochondrial ROS (superoxide) indicator, then treated with chemicals and imaged at 2 to 3 minute intervals. A CL-Quant protocol was created to measure and to graph intensity, an indicator of ROS production over time. Smoke treated cells showed increased ROS production. Our studies demonstrate that CL-Quant can be used to evaluate biological parameters associated with cell health, and these parameters can be applied to toxicological studies.

1903 Delayed Electrophysiologic and Proarrhythmic Effects of Kinase Inhibitors Assessed in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes (hiPSC CM)

Some kinase inhibitors prolong QT interval and cause ventricular arrhythmia in patients due to direct (acute) and indirect (delayed) inhibition of multiple cardiac ion channels including hERG and Na channels. Standard cell-based assays are only able to detect the direct or acute ion channel effects and do not predict proarrhythmic potential, particularly when limited to assessment of individual recombinant channels expressed in cell lines. The coupling of high-resolution multi-electrode array (MEA) technology with hiPSC CM allows detailed and spatial analysis of ion channels including hERG and Na channels. Standard cell-based assays are only able to detect the direct or acute ion channel effects and do not predict proarrhythmic potential, particularly when limited to assessment of individual recombinant channels expressed in cell lines. The coupling of high-resolution multi-electrode array (MEA) technology with hiPSC CM allows detailed and spatial analysis of acute and delayed effects on field potential duration (FPD, QT surrogate) and conduction velocity (CV), as well as proarrhythmic signals (PAS). Kinase inhibitors (nilotinib and BMS-A) at 1 μM and BMS-A at 3 μM did not significantly affect FPDs and CV, and PAS were also not detected; effects, however, were observed after 2 hours exposure. The dose- and time-dependent effects of kinase inhibitors on FPD, CV and PAS predict proarrhythmic potential of the agents. The hiPSC MEA assay enables preclinical evaluation of both acute (direct channel) and delayed electrophysiologic effects and proarrhythmic potential (possibly related to kinase inhibition).

1904 Aluminum Malonate Interferes with Gene Expression of Epigenetic Modifying Enzymes in Mouse Embryonic Stem Cells
A. Aliberg1 and E. A. Barile.

The fetal basis of adult disease hypothesis postulates that prenatal exposure to environmental factors such as metals increases susceptibility to adulthood diseases via epigenetic mechanisms. For example, exposure to aluminum (Al) has been implicated in dialysis encephalopathy syndrome and Alzheimer’s disease. Thus, using mouse embryonic stem (mES) cells as a model of early embryogenesis, we studied if Al could induce alterations in gene expression patterns of epigenetic modifying enzymes, such as those involved in DNA methylation and histone maintenance. In initial experiments, stem cells treated with aluminum chloride (AlCl3; 150-2500 μM for 24-hrs) showed a statistically significant increase in cell viability as compared to control except at the highest concentration. Since Al readily forms complexes in the environment, aluminum maltolate (AlMal) was subsequently used to assess toxicity. Exposure to AlMal (150-750 μM) caused a significant, dose-dependent decrease in cell viability (IC50 ~ 530 μM). As a control, maltol alone (450-2250 μM) did not significantly decrease cell viability except at the highest concentration. Combinations of AlCl3 and Mal also decreased cell viability at 600:1800 and 750:2250 μM combinations. As with previous gene expression experiments performed with CdCl3 (SOT abstract #1197, 2013), AlMal (150 μM and 300 μM for 24-hrs) also caused a two-fold or greater increase in Atrx (phosphorylase necessary for proliferation in the pre-implantation blastocyst), as well as to Cita (class II transactivator). Exposure to 300 μM AlMal resulted in at least a two-fold down-regulation of Dnmt3a and Dnmt3b (DNA methyltransferases), Ehhmt2 (lysine methyltransferase), Hdad3 (histone deacetylase), and Prmt3 (arginine methyltransferase), all of which regulate embryogenesis. Consequently, AlMal appears to interfere with expression of genes for epigenetic modifying enzymes essential for embryonic development.

1905 Lineage Stage Specific Modulation of Rat Hepatic Stem/Progenitor Cell Growth by Activation of the Aryl Hydrocarbon Receptor (AhR)
L. Harrill1, N. Parks1, E. Waithier2, J. Rowlands3, L. M. Reid4 and B. Thomas1.

A novel culture system was established for rat hepatic stem cells (hPSCs) and their descendants, hepatoblasts (hHBs), and accompanying mesenchymal precursor support cells (angioblasts and precursors of endothelia and stellate cells). This was achieved using a combination of long-chain hyaluronan plating substrate and Kubota’s Medium (KM), a serum-free medium designed for endodermal stem/progenitor supplementation of KM with leukemia inhibitory factor (LIF) increased the growth of desmin+ mesenchymal cell precursors accompanied by a selective increase in the growth of hHBs. This model was used to examine lineage-stage-specific effects of structurally diverse AhR agonists on hPSCs and hRB growth. Automated high content image analysis and bipartite fluorescent surface area density (FSAD) gating methods demonstrated that the growth of hPSCs was stimulated by AhR agonists 2,3,7,8-tetrachlorodibenzo-p-dioxin, 6-formylindolo(3,2-b)carbazole and 3-3′-diindolylmethane. These effects occurred at concentrations which produced persistent activation of AhR as assessed by Cyp1a1 induction. TCDD stimulation of hPSCs growth was concurrent with a marked loss (hRBs resulting in selective expansion of hPSCs. The findings provide mechanistic insights into a potential lineage-stage-specific mode-of-action for promoting TCDD-induced rat liver tumorigenesis.

1906 Aflatoxin B1 Effects on Differentiation, Proliferation, and Cell Death on Human Adult Hepatic Stem/Progenitor Cells
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In a regenerative context, we investigate different toxicological endpoints in response to AFB1 and its descendants, hepatoblasts (hHBs), and accompanying mesenchymal precursor support cells (angioblasts and precursors of endothelia and stellate cells). This was achieved using a combination of long-chain hyaluronan plating substrate and Kubota’s Medium (KM), a serum-free medium designed for endodermal stem/progenitor supplementation of KM with leukemia inhibitory factor (LIF) increased the growth of desmin+ mesenchymal cell precursors accompanied by a selective increase in the growth of hHBs. This model was used to examine lineage-stage-specific effects of structurally diverse AhR agonists on hPSCs and hRB growth. Automated high content image analysis and bipartite fluorescent surface area density (FSAD) gating methods demonstrated that the growth of hPSCs was stimulated by AhR agonists 2,3,7,8-tetrachlorodibenzo-p-dioxin, 6-formylindolo(3,2-b)carbazole and 3-3′-diindolylmethane. These effects occurred at concentrations which produced persistent activation of AhR as assessed by Cyp1a1 induction. TCDD stimulation of hPSCs growth was concurrent with a marked loss (hRBs resulting in selective expansion of hPSCs. The findings provide mechanistic insights into a potential lineage-stage-specific mode-of-action for promoting TCDD-induced rat liver tumorigenesis.
Cardiovascular toxicity is a major cause of failed drug development and withdrawals. Predictive toxicology screening assays enabling early identification and deselection of compounds have potential to cause either functional (changes in contractility) or structural (morphological damage or loss of viability) cardiac toxicity are required. Multiplexed high content screening (HCS) with automated fluorescence microscopy and image-based technology was used to develop cellular assays that detect key mechanisms relevant to drug-induced cardiac toxicity. The following parameters were evaluated: cell count (nuclear dye); calcium mobilization (Fluo-4 AM); mitochondrial membrane potential (TMRR); membrane permeability (TOTO-3); B-type natriuretic peptide (BNP) nuclei protein expression (anti-BNP); alpha-actinin protein disruption (anti-alpha-actinin, sarcomeric); and troponin I protein integrity (anti-troponin I). Studies were undertaken in hESC-CM at 24 h and 72 h. Twelve compounds included 8 clinically approved structural cardiotoxins, 3 functional cardiotoxins and 1 non-cardiotoxin were evaluated. IC50 values demonstrated high concordance with published values. More adverse effects were observed at 72 h with 4 of the structural cardiotoxins (mitoxantrone, sunitinib, sorafenib and amiodarone) significantly modulating all assay parameters. Mitochondrial membrane potential (MMP) was the most sensitive and specific indicator of structural cardiotoxicity. Cmax values demonstrated correlation with MMP (r = 0.91) and BNP expression (r = 0.92) indicating toxicity detection at in vivo therapeutic levels. We developed assays to evaluate functional cardiotoxicity potential using the multi-electrode array platform (MEA) by measuring beat period, spike amplitude and field potential duration. This study demonstrates the utility of HCS and MEA assays using hESC-derived cardiomyocytes to identify cardiotoxicity hazard identification and provide insight into the intricate mechanisms implicated in cardiotoxicity.

Cardiovascular toxicity is one of the leading causes of drugs failing during clinical trials and being withdrawn from the market. Implementation of an in vitro cell-based predictive assay early in the drug discovery process would help improve early compound attrition and develop safer drug candidates. We tested compound-treated human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iCell® Cardiomyocytes) using the Thermo Scientific ToxInsight® IY7 platform to determine the cardiotoxicity risk of a compound through the measurement of multiple toxicity biomarkers in individual cells. The compounds we investigated are known cardiotoxic compounds (Doxazosin, Rosiglitazone and Nilotinib) and non-cardiotoxic compound (Aspirin). Each compound was tested at six concentrations in triplicate. The use of multiple dyes allows for high sensitivity and specificity for predicting cardiotoxicity by simultaneously detecting six multiplexed cellular targets and properties associated with cell loss, cellular redox stress, apoptosis and mitochondrial stress. Furthermore, the extracellular supernatants can be analyzed for a biomarker of hypertrophy, brain natriuretic peptide (BNP), for a seventh cardiotoxicity parameter. The cardiotoxicity profile generated by the multiparametric data for the test compounds may help determine the mechanism of toxicity between classes of therapeutic regents.

Cancer therapies are often dose-limited by their toxicity in rapidly proliferating, non-neoplastic tissues. The intestinal epithelium is the most rapidly dividing tissue in adult mammals, turning over every 5-7 days, and thus requiring continual
proliferation, differentiation and stem cell self-renewal. Unintended perturbation of any one of these processes by pharmaceuticals can cause enterotoxicity. To date, scalable in vitro systems for the prediction and modeling of enterotoxicity have been limited to immortalized cell lines, which recapitulate only a fraction of the cell type and differentiation state unconditionally permitted by enterotoxins. Here, we describe an in vitro model that captures critical aspects of enterotoxicity including proliferation, differentiation and stem cell self-renewal. This murine 3D crypt-villus organoid, termed a MiniGut or intestinal enteroïd, is sensitive to a multitude of mechanisms of enterotoxicity caused by both marketed pharmaceuticals as well as pharmacological compounds in development. The data presented in this work indicates that in addition to detecting the enterotoxicity inherent in chemotherapies such as 5-fluorouracil and irinotecan, the intestinal enteroïd model is able to distinguish between toxic and non-toxic drug candidates in a medium throughput fashion. Furthermore, our results indicate that intestinal stem cells and their progeny are maintained in their native niches in the MiniGuts, enabling perturbations in stem cell self-renewal and differentiation to be observed in vivo and in vitro. MiniGut intestinal enteroïds recapitulate the complex interplay of 5 different epithelial cell types, including the critical stem cell compartment, and will serve as a valuable in vitro screen for drug-induced enterotoxicity in ways that were previously only possible in vivo.

1912 Characterization of a Brain Microphysiological System for Studying Gene/Environment Interactions

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The potential of using brain microphysiological systems (MPS) to study gene/environment interactions, and specifically their role in human disease, is rapidly expanding. MPS are 3D in vitro systems that attempt to recapitulate the cell types, crosstalk, and complex signaling networks found in vivo. They promise to impact multiple aspects of human disease research, including personalized medicine, drug development, and toxicity testing. Such translation to human disease is necessary to fully understand the impacts of complex interactions on human health and disease. The MPS are thus fundamentally centered on the MPS, which are miniaturized whole-brain organoids that recapitulate many aspects of the brain in vivo. MPS have been designed as both 2D and 3D systems, which provide opportunities for high throughput and low throughput screening. In this study, we present the MPS, which are effective for studying gene/environment interactions in human disease.

1913 The Potential Therapeutic Role of Secreted Antiviral Entry Inhibitory (SAVE) Peptides Expressed by Transduced Mesenchymal Stem Cells (MSCs)

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The population of people living with human immunodeficiency virus (HIV) has remained relatively stable due to antiretroviral therapy. However, still the pace of new infections continues at far too high a level. We have shown that expression of the membrane-associated and secreted C46 peptides, members of the new fusion inhibitor class of antiretroviral drugs, efficiently block infection of new cells by interfering with the function of HIV-1 gp41. To evaluate the potential therapeutic role of the Secreted Anti-Viral Entry inhibitory (SAVE) peptide in transduced mesenchymal stem cells (MSCs), we measured the inhibition of HIV infection in vitro with SAVE-transduced MSCs. First, we transduced human and rhesus MSC with retroviral and lentiviral vectors (LV) expressing GFP (LZRS-GFP: MLV and HRST-GFP: LV), membrane-bound C46 (M218: MLV), or the secreted C46 (T-60: MLV and T-42: LV). Fluorescent microscopy and flow cytometry demon-

strated that up to 69% of LV-transduced MSCs and 293T control cells expressed GFP. Molecular analysis of MLV v packaging element revealed that up to 25.5% of the human MSCs, rhesus MSCs, and 293T cells were transduced with the T-60 and M218 vectors. C46 was detectable in SAVE-transduced MSCs by western blot using 25F antibody. We sequencially conducted the single round infection assay to measure the inhibition of viral infection in vitro with conditioned medium from the SAVE-transduced MSCs. The data showed that conditioned medium from C46 and SAVE transduced rhesus bone marrow-derived MSC blocked the infection of the HIV vector by from 60-75%. In order to test whether transduction and the insertion of the transgene affect differentiation of MSCs potency, we have conducted osteogenic, adipogenic, and chondrogenic differentiation assay on SAVE-transduced rhesus bone marrow derived MSCs.

1914 Foetal-Derived Human Neural Stem/Progenitor Cells (h-NPCs): Safety Profile in the Mouse following Single Intracerebroventricular (ICV) Administration

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Foetal derived human neural stem/progenitor cell (h-NPCs) transplants have potential as therapeutic candidates to treat a vast number of disorders of the central nervous system (CNS). The biological activity of these stem cells has been analyzed extensively in vitro and in vivo. (1, 2) supporting neuroprotective strategies and clinical translation in human therapy for the major components of the CNS (i.e. brain, spinal cord and eye). To investigate any potential adverse effect of stem cell therapy, h-NPCs were given as a single intracerebroventricular (ICV) administration to CD-1 mice treated daily with cyclosporin to better simulate the possible clinical use in humans, and the animals were carefully observed for a period of 90 days thereafter. No mortality related to the biological effect of h-NPCs occurred, and no significant signs of toxicity were recorded either in clinical signs, body weight, food intake, clinical pathology, and at gross and histological examinations. From the results obtained it was concluded that h-NPCs given as single ICV administration to CD-1 mice do not exert any local or systemic toxicity even following a relatively extended period of observation, supporting their safe application in clinical testing.


1915 High-Throughput Developmental Toxicology Screening Platform Using Human iPSC-Based Transcriptional Analysis

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One of the most promising alternative in vitro developmental toxicity assays is the Embryonic Stem Cell Test (EST) which is based on the ability of mouse embryonic stem cells (ESCs) to differentiate into beating cardiomyocytes as determined by morphological assessment. However, the EST is low throughput, lacks quantitative molecular endpoints, requires inter-species extrapolation and does not inform about a toxicant’s mode of action. Unlike mouse ESCs, human ESCs and human induced pluripotent stem cells (hiPSCs) are difficult to culture as single cells and thus are not compatible with high throughput manipulations. We have previously developed a single-cell handling and hiPSC culture system based on novel small molecules, enabling scaled culture and multi-assay development. In the present study we demonstrate a novel and potentially powerful developmental toxicity screening platform using single cell hiPSCs as an in vitro model for embryonic development, and transcriptional analysis to track changes in conserved developmental signaling pathways as predictors of developmental toxicity. Feeder-free and single cell-seeded hiPSCs were exposed in 96-well plate to three definitive teratogens (methyl mercury, thalidomide and valproic acid) and DMSO (solvent) in 6 concentrations for 48hr. Gene expression analysis of developmentally-regulated genes (transcriptional pathway analysis) was performed by high-content RT-PCR using microfluidic chips capable of performing 2,304 unique PCR reactions (48 samples and 48 gene targets). All the three teratogens inhibited pluripotency genes and activated differentiation genes to variable degrees. Further, effects on developmental signaling pathways were mapped, revealing unique toxicant-specific signatures on teratogen exposure. We believe this study demonstrates the potential for hiPSC-based teratogen characterization and enables the further identification of toxicology signatures for prospective toxicology screening.
Arsenic-Induced Aberrant Gene Regulation of Human Adipose-Derived Mesenchymal Stem Cells

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Inorganic arsenic is a worldwide contaminant commonly found in drinking water. Although the EPA has classified it as a human carcinogen the mechanistic details are still being elucidated. Current data suggests cancer associated with arsenic exposure is the result of genome instability and subsequent aberrant gene transcription rather than direct mutagenicity. Epidemiological studies have suggested a correlation between arsenic exposure and the incidence of prostate cancer. Malignant transformation by chronic arsenic exposure already has been shown in prostate-derived epithelial stem cells. We want to build on these findings by looking at another potential target of arsenic as the trigger of prostate cancer, periprostatic adipose tissue. The thickness of periprostatic adipose tissue has been correlated to the severity of prostate cancer and has been shown to be a rich source of human adipose-derived mesenchymal stem/stromal cells (hASC). Our hypothesis is that arsenic may be able to transform hASC in the periprostatic adipose tissue in order to promote prostate tumor progression. hASC have been isolated from prostate tumors in humans and data suggests they are derived from the periprostatic adipose tissue. Therefore, the goal of this project is to begin to deduce the malignant effects arsenic has on hASC, starting with examining dysregulation in gene expression. After chronic exposure of hASC to an environmentally relevant level of arsenic (sodium arsenite 1 μM), a 14.5 fold increase in interleukin-6 expression was detected by qPCR analysis after 6 weeks. In addition, genes associated with chematin remodeling (HDAC1), cellular invasiveness (MMP-9) and cancer stem cells (NOTCH1) are becoming differentially expressed as we continue the course of arsenic exposure (9 weeks). These results suggest a reprogramming of hASC gene transcription induced by arsenic that may have the potential to alter the microenvironment surrounding the prostate, likely increasing cancer susceptibility. (Supported by T32ES007254).

Investigation of Mitochondrial Toxicity in Stem Cell-Derived Cardiomyocytes

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Mitochondrial dysfunction has been implicated in the etiology of drug-induced cardiotoxicity, one of the most common causes for drug withdrawal. Screening for compounds with such liabilities has been difficult due to the lack of relevant cell models and assay technologies. Stemcell-derived cardiomyocytes provide a homogenous, reproducible and physiologically relevant cardiomyocyte cell system. Apart from the use in cardiac electrophysiology, these cells allow a detailed assessment of cell metabolism and mitochondrial function when combined with suitable assay technology, e.g. probes for the assessment of cellular O2 consumption. We have examined the feasibility of such an approach by using mouse or human stemcell-derived cardiomyocytes cultured in 96 well plates. Rotenone, FCCP, and Antimycin were used as model compounds, and their effect on O2 consumption was continuously measured post treatment using the MitoxXpress® Xtra probe (Luxcel Biosciences). FCCP (F), Rotenone (4), and Antimycin (4) revealed typical O2 consumption profiles illustrating a capacity to specifically detect perturbed OXPHOS immediately post-treatment. Additionally, the influence of culture media containing glucose (4.5 g/L) or galactose (1.8 g/L) on susceptibility of mouse cardiomyocytes to mitochondrial toxicants have been assessed. Using the xCELLigence (ACEA Biosciences Inc.), we could demonstrate that although the onset of the effect of doxorubicin and FCCP was faster in cardiomyocytes cultured in glucose media, the overall effect of both compounds after 48h of treatment in both media was similar. Only the ETC inhibitor Rotenone showed an increased effect both on onset and severity if cells were cultured in galactose.

Our results demonstrate the combination of cell and assay systems to be capable of delineation of mitochondrial inhibition and uncoupling from non-specific cytotoxicity as well as the detection of redox cycles, a particularly important mechanism in drug-induced cardiotoxicity.

A Biomarker-Based Developmental Toxicity Screen Using Human Induced Pluripotent Stem Cells for Compound Prioritization

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Sponsor: L. Resto.

Assessing potential developmental toxicity of new chemicals is resource intensive, time consuming, and requires large numbers of laboratory animals. Availability of more predictive developmental toxicity screens based on human cells will reduce costs, increase pharmaceutical and chemical safety, and reduce the risk of false-negatives due to inter-species differences. Our previous work has established that an hES cell culture based system can be used to predict human teratogenicity as an effective compound screening tool. The present work extends this idea to human iPS cells derived from the genetic manipulation of human somatic cells. iPS cells are phenotypically and genetically similar to hES cells (i.e. morphology, proliferation, gene expression) and are being used by many researchers to overcome the moral, ethical and political controversies surrounding hES cells. The current study compares the metabolic similarities between human iPS and hES cells in response to compound exposure. Using both targeted and untargeted metabolomics approaches, we tested 31 compounds with known teratogenicity in human iPS cells and compared the results to those obtained using hES cells. For targeted metabolomics experiments, iPS cells were exposed to 9 concentrations of each compound. Spent media was collected and analyzed by high resolution LC/MS to determine the relative abundance of the biomarkers present in the system. For untargeted metabolomics experiments, cells were exposed to 3 concentrations of each compound and spent media was analyzed using a UPLC-ESI-QTOF mass spectrometry method that measured a broad range of small molecules. Using the biomarkers and assay parameters identified using hES cells, the iPS cell-based biomarker assay identified known developmental toxicants with 81% accuracy (75% sensitivity, 87% specificity). Establishing a developmental toxicity assay using iPS cells harnesses the predictive power of the hES cells without the ethical controversy, thus enabling validation for regulatory purposes.

Monitoring of Calcium Transients of Human Cardiomyocytes Derived from Induced Pluripotent Stem Cells As a Predictive Tool for High-Throughput Assessment of Cardiac Toxicity


Early assessment of cardiotoxic potentials is desired to avoid costly failures of drug candidates in the late phases of the drug development process. Since screening for hERG channel modulation is not sufficient to assess a comprehensive view on potential adverse effects on the heart, there is a need for a predictive cardiotoxicity assay suitable for high throughput screening (HTS). Directed differentiation and a transgenic approach allow for a specific selection of pure, human induced pluripotent stem cell-derived cardiomyocytes (hiPSCM). The hiPSCM reveal spontaneous contraction and typical physiological properties compared to their primary counterparts and can be generated in the quality, stability and amounts required in HTS. We have used the FLIPR Calcium® Assays Kit together with the Hamamatsu FDDS µCell and the FLIPR to monitor and analyze the effect of more than 35 standard compounds on the parameter of cytosolic free calcium ([Ca2+]i) transients from hiPSCM in a 384 well format. Each compound was tested in a full dose response with at least 3 replicates for each concentration. The compounds tested comprise known modulators of cardiac sodium, calcium, potassium (including several hERG blockers), and hyperpolarization-activated ion channels as well as GPCRs, connexins, and ion exchangers and pumps. Typical modulations of [Ca2+]i transients in terms of frequency, duration, peak amplitude, slope (rise and decay), and rhythmicity were analyzed and correlated to the known mode of action of the compounds. The results from our study reveal that modulation of [Ca2+]i transients is predictive for a broad range of cardiotoxic compounds. Based on these results, the described assay system is a cost effective, predictive in vitro system to assess cardiac liabilities at an early state of the drug development process.
1920 An Ex Vivo Platform to Evaluate the Differential Effects of Potential Therapies on Leukemia Progenitors (CFU-L) vs. Normal Bone Marrow (NBM) Progenitors (CFU-GM)

M. Yeo, E. Sceats and D. Hughes.

Acute myeloid leukemia (AML) is a malignant disease characterized by rapid growth of myeloid cells in the marrow. This year, 14,500 new cases will be diagnosed in the US, claiming the lives of approximately 10,000. Nearly 40 years have passed since the development and implementation of cytarabine (Ara-C) and daunorubicin (DNR) combination therapy as a standard of care. Yet the genetically heterogeneous characteristics of AML demands the development of novel treatment strategies. In support of this end, the colony forming cell (CFC) assay provides an ex vivo platform in which novel compounds can be tested for efficacy alone or together with current combination therapies. Using the CFC assay we evaluated the toxicity of drugs such as Ara-C and clofarabine on AML cancer “stem cells” and NBM CFU-GM, NBM (n=4) and AML marrow (n=3) were cultured in semisolid methylcellulose-based media (ColonyGel) at 37°C, 5% CO2. Test compounds were added over a broad concentration range in triplicate and CFU-GM and CFU-L were enumerated microscopically after 14 days. Mean IC50 values for DNR, Ara-C, and clofarabine for CFU-GM and CFU-L were 11.5, 15.8 and 29.3 μM and 5.45, 2.5 and 2.2 μM, respectively. These data confirm a selective killing of CFU-L vs NBM CFU-GM for all individual compounds. We extended this study to evaluate multi-combination therapies using calculated IC25 values for DNR and Ara-C on CFU-L (3 and 2 μM, respectively) and assessed this drug duo together with clofarabine. The combination of DNR and Ara-C at their IC25 concentrations inhibited CFU-L by approximately 50% in all AML marrows and inhibited NBM CFU-GM by 20%. When clofarabine, at its IC25 (1.5 μM) was added to DNR and Ara-C, an increased inhibition of CFU-L (70%, n=2) was observed while NBM CFU-GM inhibition was only increased to 24%. Thus, CFC assays provide a platform to evaluate novel targeted therapies with the current standard of care drug combinations, and can ascertain their selective effect on leukemic vs normal progenitors.

1921 Cryopreserved Hepatocytes and Stem Cell-Derived Hepatoycte As Microphysiological 3D Liver Models in LiverChip

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Liver functions rapidly decline in vitro when hepatocytes are cultured as monolayers. Human primary cell use is also hindered by unpredictable supply and quality of fresh cells. Cryopreservation techniques have partially circumvented the supply problem although cells quickly deteriorate. Similarly, methods to differentiate hepatic-like cells from induced pluripotent stem cells (iPSCs) can derive cells with liver disease phenotypes, such as Alpha 1 antitrypsin (A1AT) deficiency, although the cells may not be reaching the fullest possible maturity. We investigate the LiverChip in vitro 3D platform to model liver physiology through improved maintenance of primary liver cells with greater longevity and to further mature hepatocytes derived from iPSCs to model pathophysiology. In LiverChip, cryopreserved primary human hepatocytes formed 3D tissues with metabolic activity, albumin production and urea output for at least 3 weeks. Through a DARPA funded project a liver microphysiological system based on the co-culture of primary human hepatocytes and kupper cells was developed. Co-cultures displayed physiological responses to lipopolysaccharide exposure, including down-regulation of CYP3A, and secretion of pro-inflammatory cytokines IL-6 and TNF-alpha. Human iPSC-derived hepatocyte-like cells displayed enhanced hepatic function in LiverChip, compared with 2D monolayers. Analysis of secreted alpha fetoprotein, albumin, A1AT and fibrinogen demonstrated that LiverChip supports iPSC-derived cells with more advanced maturity than 2D culture. In conclusion, LiverChip is a perfused culture system supporting functional primary human liver cells to model hepatic tissue. LiverChip also has utility in further maturing iPSC-derived hepatocyte-like cells to recapitulate the physiology and pathophysiology of liver cells in health and disease.

1922 LiverChip 3D Liver Platform Distinguishes between Known Hepatotoxic Drugs and Closely Related Structural Analogues

M. Xia.

LiverChip is a perfused culture system in which isolated liver cells form 3D tissues with metabolic activity. Cryopreservation techniques have partially circumvented the supply problem although cells quickly deteriorate. However, the attrition rate of drug candidates in late clinical phases, post-marketing drug withdrawal, and need for box safety warnings due to hepatotoxicity demonstrate that many hepatotoxic drugs are not effectively screened out. There is great interest in 3D culture systems that re-form liver-like tissues from isolated human cells, and support liver physiology, to improve the predictivity of hepatotoxicity studies. LiverChip is a perfused culture system in which isolated liver cells re-form liver-like tissue structures in sinusoid-like channels in a porous scaffold. Tissues maintain measurable liver functions, including drug metabolism, with clearance of probe drugs correlating well with known hepatic clearance in man. Here we investigate LiverChip as a tool to distinguish between known hepatotoxic drugs and closely-related non-toxic structural analogues using multiples of human maximum plasma levels (Cmax). Human liver tissues were pre-formed in LiverChip for 3 days and then exposed to 0, 1, 3, 10, 30 and 100x Cmax concentrations of drugs for a further 6 days. Hepatocyte perturbations were assessed by measured disturbances in phenotypic liver markers, reduced mitochondrial activity and overt cytotoxicity was measured by leakage of intracellular enzymes. LiverChip experiments could build a qualitative hepatotoxicity spectrum with different mechanisms. Drugs that elicit hepatotoxicity in man through the formation of reactive metabolites (e.g. Clozapine), mitochondrial dysfunction (e.g. Tolcapone) or biliary transporter inhibition (e.g. Bosentan) could be distinguished from their non-toxic structural analogues. In conclusion, LiverChip is an effective tool for discovery toxicology with utility during selection of analogous lead compounds to identify safer new drugs with the lowest risk hepatotoxic potential.

1922a Tobacco Smoke Effects on an Endothelial Cell Model Derived from Inducible Pluripotent Stem Cells


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Tobacco smoke causes 3-fold more deaths from heart attack, stroke, and vascular disease, than from cancers. Endothelial cell responses to tobacco were studied to elucidate vascular disease/therapeutics. Human inducible Pluripotent Stem cells (iPSC) were differentiated to form an Endothelial Cell model (iECs). These iECs (98%) express the endothelial cell marker proteins: CD31 and VE-Cadherin. iECs primary cellular human endothelial cells were cultured from: aorta (AECS), human coronary artery (CAECs), or umbilical vascular endothelial cells (HUVEC), and treated with tobacco smoke extract or vehicle. Viability was measured using an [ATP] assay and gene expression profiles were determined using qPCR and whole-genome mRNA sequencing (RNAseq). The majority of gene expression responses to tobacco by primary endothelial cells were recapitulated by iPSCs. Each cell line also yielded unique gene responses to tobacco according to RNAseq analysis. This work has revealed transcriptional responses of endothelial cells to tobacco that predispose humans to: vascular clotingting, oxidative damage, hypertension, arterial calcification, and cytotoxicity. Further work will identify tobacco smoke components that trigger each event, and will identify biomarkers for these adverse events.

1922b Neural Stem Cell Susceptibility to Japanese Diesel Exhaust Particulate Matter

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Environmental air pollutants negatively affect cognitive brain functions such as learning and memory and cause progressive neurodegeneration through unknown mechanisms. Although these deficits are associated with changes in neurogenesis (stem cell proliferation, differentiation and survival), few studies have examined the consequences of environmental air pollutants on neurogenesis. We examined the effects of 28 day intranasal exposure to particles isolated from Japanese diesel exhaust (DEPs, 1 mg/kg) or the organic extract of the DEPs (extracts) in C57B/6 male mice (6 weeks old; n=6/group). Behavior and proliferation in the dentate gyrus and subventricular zone of the lateral ventricles, areas of continuous neurogenesis affected by Alzheimer’s disease, were examined. Exposure to DEPs or extracts did not alter weight gain compared to vehicle control (0.05% DMSO; p=0.05). There was no anxiolytic effect of DEPs or extracts as measured using the elevated plus maze behavior test at exposure day 9 and 27 (p>0.05 v. vehicle). These results indicate there was no overt toxicity associated with the treatments in this study. Proliferation, assessed by BrdU incorporation, was quantified in 8 planes through the dentate gyrus. A significant decrease in BrdU incorporation was observed in the DEP treatment group only (p=0.05 v. vehicle). BrdU incorporation in the subventricular zone region was such that counting individual cells was not possible. However, analysis of immunoreactivity using fluorescence intensity measurements indicated there was a trend for decreased BrdU incorporation in the subventricular zone of DEP-exposed mice compared to vehicle. Collectively,
this study demonstrates that chronic exposure via nasal aspiration of an ultrafenine particulate, but not the organic component, decreases the ability of stem cells to proliferate in two brain regions that undergo neurogenesis. These data suggest that there are interrelationships between air pollution, neuronal stem cells and neurodegenerative diseases.

1922c Clostridial Toxins Cause Synaptic Inhibition in Networked Populations of Stem Cell-Derived Neurons


Following internalization into presynaptic termini, the two families of clostridial neurotoxins (CNTs) botulinum neurotoxin (BoNT) and tetanus neurotoxin (TeNT) cleave proteins associated with synaptic vesicle release, preventing the exocytosis of neurotransmitters. To date, no cell-based model used in CNT research has been shown to form functioning synapses, limiting the ability to conduct target discovery or therapeutic screening. Stem-cell derived neurons have recently been proposed as a next-generation cell-based platform for neurotoxicity research. Whole-cell patch clamp electrophysiology indicates that ESNs develop mature electrical responses and form a complex, synaptically coupled network with an excitatory/inhibitory balance by 18 days in vitro (DIV). Hypothesizing that synaptically coupled ESNs may undergo synaptic inhibition in the presence of BoNT/A, we evaluated the effects of intoxication on functional measurements of synaptic activity at DIV 24. Treatment of ESNs with 0.01 mouse lethal units of BoNT serotype A (BoNT/A) resulted in the significant loss of synaptic function, as determined by significant reductions in synaptically driven excitatory potentials and miniature excitatory post-synaptic currents (mEPSCs). Notably, inhibition of miniature inhibitory post-synaptic currents (mIPSCs) was a leading indicator of intoxication, being >80% inhibited within 30 min, whereas significant decrements in mEPSC activity were not apparent until 60 min. Long-term patch clamp assays indicated that addition of BoNT/A perturbed the excitatory/inhibitory balance within minutes, producing an epileptiform network response that progressively increased through 40 min. These data suggest that electrophysiological characterization of trans-synaptic activity may comprise a novel, rapid screen for the presence of functional toxin in forensic, pharmaceutical, environmental and/or food samples, as well as provide a physiologically relevant in vitro model for drug screening and mechanistic research.

1922e Transcriptomic Effects of Curcumin and Piperine in Breast Stem Cells


Curcumin and piperine have been shown to have cancer preventive activity in preclinical models. We have previously shown these compounds limit stem cell self-renewal. Normal and breast cancer stem cells exist in two states, with each cell type having unique properties. The objective of this study is to identify the mechanisms of action of curcumin and piperine in breast stem cells, using cell type specific RNA sequencing (RNA-seq) on breast cancer cell lines and normal breast cells. Breast cancer cell lines SUM149, T47D, and MCF7 were cultured as mammospheres and treated with curcumin (2.5μM - 15μM) and piperine (5μM and 10μM) individually and in combination. Normal breast cells isolated from reductive mammoplasty patients (n=13) were treated as mammospheres with 5μM curcumin and 5μM piperine. We used fluorescence activated cell sorting to isolate ALDH1+ and ALDH1-CD44+/CD24- stem cell fractions of SUM149, treating with 5μM curcumin and 5μM piperine for 24hrs. RNA-seq libraries were prepared with the NuGen Encore Complete kit and sequenced on an Illumina HiSeq 2500. In cell lines, curcumin and piperine induced mammosphere formation in a dose-dependent manner. In primary breast cells, curcumin, piperine, and the combination inhibited mammosphere formation 28% (p=0.0004), 24% (p=0.01), and 42% (p=0.0002) respectively. In both stem cell fractions, curcumin induced differential expression of similar genes, including AREG, EPGN, BAMBI, ALDH3B2, and CYP1B1. Pathway analyses showed that curcumin induced expression changes related to cholesterol and steroid biosynthesis and oxidoreductase activity. Ongoing experiments will quantify the transcriptomic effects of these compounds in normal breast stem cells. These findings point to novel regulation of stem cell self-renewal and provide biomarkers of efficacy for future cancer prevention trials.

1922f Development of a Human Adipose Stem Cell Model for Chemical Obesogen Screening

C. Deisenroth and B. Foley.

The relationship governing environmental influence on an individual's genetic predisposition toward obesity is a complex one. A largely unexplored avenue is the interaction between environmental exposure and an individual's genetic predisposition toward obesity. In this study, we demonstrate that chronic exposure via nasal aspiration of an ultrafine particulate, but not the organic component, decreases the ability of stem cells to proliferate in two brain regions that undergo neurogenesis. These data suggest that there are interrelationships between air pollution, neuronal stem cells and neurodegenerative diseases. This approach provides a novel system to efficiently derive high yield, high purity neurons (ESNs) from murine embryonic stem cells (ESCs) for neurotoxin research. This approach convincingly demonstrates an epileptiform network response that progressively increased through 40 min. These data suggest that electrophysiological characterization of trans-synaptic activity may comprise a novel, rapid screen for the presence of functional toxin in forensic, pharmaceutical, environmental and/or food samples, as well as provide a physiologically relevant in vitro model for drug screening and mechanistic research.
1923 Prolonged Exposure to Particulate Chromate Inhibits Homologous Recombination Repair Proteins
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Particulate hexavalent chromium (Cr(VI)) is a known human lung carcinogen that produces several forms of DNA damage, including DNA double strand breaks (DSBs). The homologous recombination (HR) repair pathway is utilized by cells to repair DSBs in an error-free manner during late S-G2 and mitosis. Our previous studies show that prolonged Cr(VI) exposure induces persistent DSBs levels and that defective repair of these DSBs allows for neoplastic transformation of human lung cells. However, few studies have investigated the effect of particulate Cr(VI) exposure on HR repair proteins. In this study, we analyzed the effect of particulate Cr(VI) exposure on key HR proteins: one of the DSB sensors, MRE11, the signal transducer, ATM, a key repair effector, RAD51, and the RAD51 transporter, RAD51C. We exposed human lung fibroblasts to zinc chromate for 24-120 hours. After exposure, the response of these proteins was measured by western blotting or a nuclear foci formation assay. Our data shows an increase in nuclear MRE11 foci formation, phosphorylated ATM protein expression, RAD51 protein expression and nuclear foci formation and RAD51C nuclear foci formation after 24 h Cr(VI) exposure, suggesting that HR repair is activated. However, prolonged Cr(VI) exposure inhibits phosphorylated ATM protein expression, RAD51 protein expression and nuclear foci formation and RAD51C nuclear foci formation. These results suggest that prolonged Cr(VI) exposure inhibits HR repair signaling through the regulation of RAD51. Ongoing work is investigating the effect of prolonged Cr(VI) exposure on RAD51C protein expression, RAD51/RAD51C co-localization and RAD51 transport kinetics. This work was supported by NIEHS grant ES016893 (J.P.W.).

1924 Cytoprotective Effects of the Antioxidants Tempol and WR-1065 in Human Lymphoblastoid Cells Exposed to Zidovudine (AZT)
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Antiretroviral Nucleoside Reverse Transcriptase Inhibitors (NRTIs) are used in drug combinations to treat Human Immunodeficiency Virus-1 (HIV-1) infection. AZT is an NRTI that becomes incorporated into DNA, acts as a DNA replication chain terminator, induces aneuploidy and mutations in mice and humans, and is tumorigenic in mice. The Cytokinesis Block Micronucleus (CBMN or Cytox) assay is a comprehensive system used to measure DNA damage occurring as a result of genotoxic insult. Molt-3, human lymphoblastoid cells exposed to AZT for 52 hours, were arrested with Cytochalasin B (Cyto B) for the last 24 hours, and the binucleated cells, which by definition completed one cell division, were scored for: micronuclei (MN); chromosome bridges (NBPs); nuclear buds (NBs); Anerotic/ Necrotic (A/N) cells and Nuclear Division Index (NDI). We hypothesized that the antioxidants Tempol and WR-1065 would protect against AZT-induced genotoxicity. Molt-3 cells were seeded into 24-well plates and exposed to 10 &M AZT, 10 &M AZT plus 5 &M WR-1065, or 10 &M AZT plus 200 &M Tempol. Cyto B was added for the last 24 hours to arrest cell growth. The NDI was similar for all groups, indicating that the different treatments did not alter cell viability. Molt-3 cells exposed to AZT and examined by Cytox assay had significant increases in MN, NBPs, NBs, and A/N, compared to unexposed controls. Cells treated with either Tempol or WR-1065 had significantly-reduced AZT-induced MN. In addition, WR-1065, but not Tempol, reduced the levels of A/N. In the presence of AZT, Tempol significantly reduced levels of NBs, while WR-1065 reduced the levels of NBPs. Overall, the Cytox assay provided a useful indicator of AZT-induced genotoxicity, and demonstrated that the antioxidants Tempol and WR-1065 each protect against different AZT-induced genotoxic end points. The data implies different mechanisms of protection for the two antioxidants.

1925 Targeted Ubiquitination and Degradation of Human DNA Ligase I by the CUL4-DDB1 Ubiquitin Ligase Complex
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Human DNA ligase I (hLiGI) is required for efficient DNA replication, recombination and repair. Here, we report a mechanism that controls the programmed degradation of hLiGI via targeted ubiquitination. In this study, a proteomic approach was used to screen interacting proteins of hLiGI in 293T cells. Mass spectrometry analysis reveals several proteins in the hLiGI immunocomplex including culinA4 (CUL4A) and damage-specific DNA binding protein 1 (DDB1). We confirmed that CUL4A and DDB1 as hLiGI-associated proteins by immunoprecipitation, which suggests that DDB1 acts as an adapter to link LiGI to CUL4 to form the E3 ubiquitin ligase complex. Furthermore, the level of hLiGI was reduced in both undifferentiated MCF10A cells and under stress conditions; while treatment with the proteasome inhibitor, MG132 prevented this reduction. We also found that hLiGI ubiquitination ultimately led to its degradation via the proteasome pathway, at least in part initiated by the Cull4-DDB1 complex. Mutations or inhibitors that blocked the modification at any step in this pathway would suppress hLiGI degradation. These findings may represent a newly identified regulatory mechanism used by cells to ensure the normal DNA replication and is essential for the maintenance of genome stability.

1926 Activation of ATM-Dependent Genotoxic Signaling in Human Lung Cells by Formaldehyde
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Formaldehyde (FA) is a carcinogenic aldehyde that produces DNA-protein crosslinks as the main form of DNA damage. Human exposure to exogenous FA primarily occurs via inhalation and is found in many occupational settings and among tobacco smokers. In this work, we examined whether the alkylating agent FA was capable of engaging ATM kinase that is traditionally associated with the stress signaling elicited by DNA double-stranded breaks (DSB). We found that FA provoked a rapid activation of the ATM pathway in normal and transformed human lung cells. In response to FA, ATM underwent activating Ser1981 autophosphorylation and phosphorylated two major proteins in its signaling network, CHK2 kinase and chromatin-associated KAP1. However, unlike DSB, FA-activated ATM did not target the transcription factor p53. Inhibition of RNA polymerase II had no effect on ATM activity, arguing against the possibility that ATM was triggered by collision of transcription complexes with bulky DNA-protein crosslinks. Activation of ATM was absent in nondividing cells and found exclusively in S-phase in cycling cell populations. The presence of DSB-sensing MRN complex was not required for FA-activated ATM responses, pointing to a noncanonical mechanism of activation. Inhibition of ATM kinase activity resulted in elevated levels of chromosomal breaks in normal human lung cells, indicating a protective role of ATM-induced signaling in suppressing gross genetic abnormalities by carcinogenic FA.

1927 p53 Regulation of the GADD45a Gene Is Restricted to Genotoxic Exposure As a Consequence of a Requirement for p53/WT1 Interaction in the Promoter
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Rationale: p53 is required for genotoxic and non-genotoxic induced apoptosis. However expression of the p53 target GADD45a, exploited in the GreenScreen HC genotoxicity assay, is very specifically induced by genotoxins. This study was conceived to explain the conundrum. The GADD45a p53 response element is in intron 3, however the GADD45a promoter has a WT1 response element binding site and other studies have demonstrated that WT1 and p53 proteins can interact. Furthermore, WT1 binding is suppressed by Myc in the absence of DNA damage. The purpose of this study was therefore to test the hypothesis that GADD45a specificity is a consequence of WT1 mediation of p53 regulation.

Experimental approach: site directed mutagenesis was used to disrupt either or both of the p53RE and WT1RE in GADD45a-GFP reporter plasmids, and these were transfected into p53 competent TK6 cells and closely related p53 mutant WI-L2-NS cells. These new cell lines were then challenged with the following mechanistically diverse genotoxins: methyl methanesulphonate, cisplatin and mitomycin C, hydroxyurea, aphidicolin, 5’fluorouracil and benomyl. GFP expression data was collected using the standard GreenScreen assay protocols.
Results: GFP synthesis induced by all compounds in TK6 cells carrying the unmodified reporter. GFP synthesis was reduced in p53 mutant and wild type cells carrying modified reporter plasmids. The reduction was greatest in cells carrying the mutated p53 RE.

Conclusions: These experiments demonstrate that both WT1 and p53 response elements are involved in GADD45α regulation. This allows the conclusion that the induction of GADD45α requires p53, but specificity to genotoxins is a consequence WT1 controlled access to the promoter, which in turn is regulated by Myc.

1928 Oxidative DNA Damage/Repair Induces Inflammatory Gene Expression in the Airways by 8-Oxoguanine DNA Glycosylase-Mediated Cell Signaling

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Environmental pollutants cause oxidative stress in the airways and increase the levels of 8-oxoquinone (8-oxoG) in the DNA of lung epithelium due to guanine’s lowest redox potential among DNA bases. 8-oxoguanine DNA glycosylase-1 (OGG1) excises and releases 8-oxoG during DNA base-excision repair (BER). Accumulation of 8-oxoG in the DNA has been linked to various inflammatory diseases including asthma. However, little is known about the role of OGG1-BER in pathogenesis of airway diseases. We observed activation of small GTPases upon OGG1-BER, or addition of 8-oxoG base to OGG1 proficient cells or mouse lungs in vivo. Therefore, we proposed that OGG1-BER and consequent activation of small GTPases mediate signaling for pro-inflammatory gene expression. To test our hypothesis, we challenged BALB/c mice intranasally with OGG1-BER product 8-oxoG, and used next-generation RNA-Sequencing technology to investigate the whole-genome gene expression in the lungs. The most overrepresented pathway was inflammation-related signaling. The majority of upregulated pro-inflammatory genes (≥ 3-fold expression) were NF-kB-dependent. We confirmed the expression levels of selected genes by qRT-PCR. In line with these observations, challenged mice with 8-oxoG or glucose oxidase (to induce OGG1-BER) showed significant induction of airway inflammation as determined by the number of neutrophils in bronchoalveolar lavage fluid compared to control (p <0.0001). We conclude that OGG1-BER-triggered inflammatory gene expression in the lungs is a novel mechanism through which environmental pro-oxidant pollutants can induce airway inflammatory diseases.

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1929 Interpreting In Vitro Micronucleus Positive Results: Simple Biomarker Matrix Discriminates Clastogens, Aneugens, and Irrelevant Positive Agents


The specificity of in vitro mammalian cell genotoxicity assays is considered relatively low, as they yield a high incidence of positive results that are not expected to have in vivo relevance. We set out to develop a rapid, effective follow-up testing strategy that predicts whether apparent in vitro micronucleus (MN)-inducing effects are due to a clastogenic, aneugenic, or secondary irrelevant mode(s) of action. Priority was given to biomarkers that could be multiplexed onto flow cytometric characterization of CDT-expressing bacteria and may open new therapeutic approaches.

1930 Error-Prone Double-Strand Break Repair Plays a Predominant Role in Oxidative Stress-Induced Mutagenesis

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Endogenous and exogenous oxidative DNA damage has been implicated in degenerative diseases and carcinogenesis. To counter this oxidative DNA damage, mammalian cells possess error-free DNA repair systems. In contrast, a few DNA repair pathways in cells do not possess high fidelity and result in mutations. Little is known about the comparative contribution of each error-free and error-prone DNA repair pathway in the oxidative stress induced DNA damage response (DDR) and mutations. Therefore, in the present study, using H2O2 as a model compound, we sought to understand the mechanisms of DDR and mutations caused by low level of oxidative stress and identify DNA repair pathways that may affect oxidative stress induced susceptibility to mutagenesis. DDR analyses were performed in different repair protein deficient DT40 cells. Rev1, Rad54, Rad51c, XRCC2, Fen1, Ku70 and Lig IV deficient cells showed comparatively more hypersensitivity to H2O2. All Ku70, DNA Pkcs and Lig IV (NHEJ) proteins deficient cells tested in our study revealed a drastic decrease in mutation frequency despite the presence of equivalent levels of 8-oxoGd as in the wild type DT40 cells. These data indicate a ROS induced mutagenic pathway in vertebrate cells proceeds via DNA double strand breaks (DSBs) and error-prone NHEJ repair. Current data combined with our previous reports of abasic site clusters in intact human cultured cells suggest that endogenous and exogenous oxidative stress may cause clustered DNA damage which may lead to DSBs with complex DNA ends and repairing such DSB with NHEJ may result in mutations even in the absence of DNA replication. It has been reported that NHEJ-deficient mice show early aging and less spontaneous tumor formation. Therefore, we are currently hypothesizing that mutations induced by oxidative stress through error-prone DSB repair pathway may be involved in etiology of wide variety of human diseases.

1931 A New Mode of Action for the Cytotoxic Distending Toxin


The Cytotoxic Distending Toxin (CDT) is a virulence factor produced by many pathogenic bacteria like Escherichia coli, Helicobacter hepaticus, Haemophilus ducreyi etc. The CDT production allows bacteria to persistently colonize the body, evade the immune system, induce inflammation and trigger genetic instability. In addition, it has been shown that CDT induces DNA double-stranded breaks (DSB), leading to cell cycle arrest and to cytotoxicity, associated with a characteristic cellular distension. In order to characterize the type of DNA damage induced by CDT, we studied the cell sensitivity to different CDT doses, with the cell cycle as read-out. Thanks to comet assay and immunofluorescence, we have shown that at doses 1000 times lower than those used previously in the literature, CDT induces multiple single-strand breaks. When cells are going through S phase, a repetitive stress is induced and DNA damage degenerate into DSB. Indeed, with a PCNA marker, we observed an S-phase slowing down and an increase of the number of cells in late S-phase. Moreover, these CDT-induced DNA damage cause the activation of the pathway involving the RPA, ATR and CHK1 proteins, characteristic of a replicative stress. Finally, we have shown that the activation of the ATM pathway, due to DSB induction, happens later after the CDT treatment. Therefore, the importance of the S-phase passage for the CDT cytotoxicity suggests that proliferating cells are more sensitive to CDT than quiescent cells. Our work underlines the complex mechanism of CDT action. It will help in the characterization of CDT-expressing bacteria and may open new therapeutic approaches, for example by targeting the mechanisms of associated genotoxicity.
Persistent mitochondrial DNA (mtDNA) damage in response to ultraviolet C radiation (UVC) results in developmental delay or arrest in Caenorhabditis elegans (Bess et al., 2012. Nucleic Acids Res 40:7916). We are testing several hypotheses on the mechanism of this delay, including the role of oxidative stress and insulin signaling, using deletion mutants and stress-inducible fluorescent reporter strains. Age-synchronized wild-type and mutant nematodes were plated on agar in the absence of food and exposed to 7.5 J/m² UVC 0, 24 and 48 h after plating. Nematodes were then transferred to plates with food and growth was measured with a COPAS Biosort using extinction, or optical density, as a proxy for size 24, 48, and 72 h after transfer. Fluorescence in reporter strains was also measured. We identified developmental delays and a 50% reduction in the size of UVC-treated sod-2 mutants and developmental delays in sod-3 mutants relative to wild-type nematodes after UVC treatment (SOD-2 and SOD-3 are mitochondrial superoxide dismutases). These data support the hypothesis that UVC-induced damage causes mitochondrial oxidative stress. Our data also show significant delay in daf-16 (mu86); gfp-1 (e2141) and daf-16 (mgDf47); gfp-1 (e2141) mutants relative to wild-type nematodes, but not daf-16 (mu86) without the deletion in gfp-1 (DAF-16 functions in insulin signaling and stress resistance and GLP-1 is required for germline proliferation; absence results in adults composed only of somatic cells). This suggests that insulin signaling promotes development in the context of persistent mtDNA damage, but only in the absence of germ cells. Finally we observed induction of hsp-16.2 and hsp-60 in biosensors of heat-shock proteins localized in the cytosol and mitochondria respectively, in response to UVC radiation, suggesting damage to both mitochondrial and cytosolic proteins.

### Impact of p53 Functionality in *In Vitro* Mammalian Cell Toxicity Testing


The number of *in vitro* mammalian cell positives that do not correlate with follow-up *in vivo* genotoxicity and carcinogenicity testing is of concern (1). p53 competent cells of human origin may provide more predictive data for assessment of human hazard and risk and less misleading *in vitro* positives compared to tradition-ally used rodent cell lines lacking wild-type p53 function (2). It remains unclear whether cell species origin or p53 status impacts their ability to accurately predict genotoxicity in the *in vitro* mammalian cell tests. Cells lacking wild-type p53 may underestimate cytotoxicity with the analysis of inappropriately high concentrations for genotoxicity compared to a p53 functional cell line. Three closely related human lymphoblastoid cell lines that differ in p53 status were tested. TK6 express wild-type p53, NH32 are p53 null, WTK1 over-express mutant p53, similar to commonly used rodent cells. Ethyl methanesulfonate (EMS), etoposide and paclitaxel (taxol) were used to test according to regulatory guide-lines and cytotoxicity determined using relative population doubling. Apoptosis was also determined using caspase-3/7 activity. NH32 were sensitive to the cytotoxic effects of EMS compared to TK6 and WTK1. In contrast NH32 underestimated cytotoxicity with etoposide compared to TK6 and WTK1. A similar cytotoxic response was observed in all three cell lines with taxol; however, cytotoxicity was observed at lower concentrations in TK6. The apoptotic response to each compound in WTK1 was significantly reduced compared to TK6, which demonstrate increases in apoptosis typical of a p53 functional cell line. NH32 demonstrated similar levels of apoptosis to WTK1 following etoposide and taxol treatments but was more similar to TK6 in the response to EMS. The results showed that p53 deficient cell lines do not consistently underestimate cytotoxicity and the cytotoxic response is drug specific. Other factors associated with loss of p53 function or species origin may be more relevant to the reported high number of *in vitro* positives.

formation of cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4 PP) induced by UV light exposure. Recent reports suggest that telomeres are either deficient in photoprotective repair or are repaired slower than the bulk genome. DNA photoproducts can cause mutations and block DNA replication, unless they are repaired by nucleotide excision repair (NER) pathway system. We report here a novel, independent assay that we have established to measure the persistence or repair of photoproducts in telomeric DNA compared to the bulk genome, using specific antibodies against CPDs and 6-4PPs following exposure of cells to UVC. We observed that CPDs decreased in genomic DNA after 12-24 hours but persisted in telomeric DNA. Southern blot assays using radio-labeled oligonucleotides that bind to telomeric DNA confirmed enrichment of telomeres in purified fractions. To investigate possible mechanisms for this repair inhibition, we tested directly effect of shelterin protein TRF2 on NER protein XPF-ERCC1 via enzyme activity assays. We discovered that nucleotide cleavage activity of purified XPF-ERCC1 on a stem-loop DNA substrate was completely abolished by TRF2. Our results provide the first direct evidence towards quantifying critical UV damage at telomeres and elucidating NER pathway regulation by shelterin.

1937 Effects of Cadmium and 17α-Ethynylestradiol on Benzo(a)pyrene AdductFormation
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Xenooestrogens have been increasingly associated with various adverse health conditions, including cancer. It is known that both cadmium and 17α-ethynylestradiol are ubiquitous environmental contaminants, and that low-dose exposures can illicit mechanistic effects in vivo. Nucleotide excision repair is the DNA repair mechanism responsible for the removal of bulky DNA adducts, which, when un-repaired, may lead to DNA mutations. Cadmium and 17α-ethynylestradiol are known to enhance hormonal responses in vivo and in vitro, and have roles in the advancement of hepatocellular carcinoma. Additionally, they are known to down-regulate multiple DNA repair mechanisms, including the nucleotide excision repair pathway. We pre-exposed human hepatocellular carcinoma (HepG2) cells to various concentrations of 17α-ethynylestradiol, a synthetic estrogen, and cadmium, to see if such pre-exposure to these compounds had any effect on bulky DNA adduct repair when the same cells were subsequently exposed to the pro-carcinogen, benzo(a)pyrene. Cells were exposed to two different concentrations of benzo(a)pyrene for varying amounts of time up to 48 hours. We then quantified benzo(a)pyrene diol-epoxide adducts at each of 6 time-points using high performance liquid chromatography with fluorescence detection. Our data indicate that with 17α-ethynylestradiol pre-exposure, efficient adduct repair was delayed, as ad- ducts continued to be detected up to 48 hours of exposure. Also, after cadmium pre-exposure, adduct formation increased over time throughout the 48 hour time-point. Results demonstrate that in HepG2 cells, both 17α-ethynylestradiol and cadmium exposures affect the prevalence and repair rate of benzo(a)pyrene-DNA adducts.

1938 Integration of Genomic Biomarkers in Cancer Risk Assessment for Xenobiotics with Positive Findings in Chromosome Damage Assays
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Despite scientific progress in understanding cancer mode of action, experimental approaches for assessing oncogenic risk for chemicals relies mainly on traditional methods. The currently used in vitro genotoxicity testing battery does not offer sufficient mechanistic information for assessing oncogenic risk to humans. Recent advances in molecular biology and bioinformatics have enabled interrogating cellular responses to chemical exposure at the genomic level. This approach has revealed molecular pathways and networks that are mechanistically involved in chemical carcinogenesis. We have focused on application of a transcriptomic biomarker approach in the p53-wt human cell line TK6 to facilitate oncogenic risk assessment by assessing genotoxicity and other mechanisms for drug candidates and environmental chemicals. We have constructed a reference database containing gene expression profiles of 27 model agents with a broad range of toxic mechanisms. A genotoxic-signature biomarker comprising of 65 genes, TGx27.65, has been identified from the reference database by using the nearest shrunken centroids (NSC) algorithm. TGx27.65 discriminates genotoxic from non-genotoxic agents with 100% accuracy after 10-fold cross validation. In validation study, 42 chemicals were selected to test TGx27.65’s performance on genotoxicity identification. All chemicals with direct DNA-damaging mechanisms were classified as “genotoxic” by transcriptomics assay with TGx-27.65. Interestingly all except one out of eleven chemicals with false positive results in in vitro chromosone aberrant assay were not classified as “genotoxic” using TGx-27.65. We have examined the inter-laboratory reproducibility of this biomarker and tested the application of TGx-27.65 in assessing chemicals that need metabolic activation. Advanced bioinformatic analysis further identified gene markers that significantly correlate with various toxic mechanisms.

1939 Developmental Exposure to Ultraviolet C Radiation Results in Altered Energy Production Later in Life in Caenorhabditis elegans
Mitochondrial genomes encode for 13 proteins that are essential components of the electron transport chain (ETC) and are normally present at between 1000 and 10000 copies per cell. However, this number is greatly reduced during specific developmental stages, representing a potential critical window for mitochondrial genotoxic exposure. Mitochondrial DNA (mtDNA) is more susceptible than nuclear DNA (nucDNA) to damage by many environmental pollutants, for reasons including absence of Nucleotide Excision Repair (NER). NER is a highly functionally conserved DNA repair pathway that removes bulky, helix distorting lesions such as those caused by ultraviolet C (UVC) radiation and many environmental toxicants, including benzo[a]pyrene (BaP). In the current experiment we tested the hypothesis that UVC induced mtDNA damage during larval development would alter energy production throughout the life of the nematode. We exposed first larval stage C. elegans to a serial UVC dose that results in the accumulation of mtDNA damage while allowing for repair of nuclear DNA damage, and measured ATP levels, mtDNA and nucDNA copy numbers, oxygen consumption and expression of mitochondrial genes throughout the lifetime of the worms. ATP levels were measured as luminescence using the firefly luciferase expressing PE255 glp-4(bn2) strain (provided by Cristina Lagido, University of Aberdeen). mtDNA copy number and gene expression were measured via real time PCR. ATP levels were significantly lower, and the mtDNA to nucDNA copy number ratio was slightly, but significantly reduced in response to UVC. Oxygen consumption was also reduced at 8 and 12 days post UVC. Interestingly, there was no decrease observed in mtDNA or nucDNA encoded mitochondrial transcripts. These results indicate that mtDNA damage during development can alter energy production throughout the life of the nematode, and highlights the potential for a critical window of exposure.

1940 Investigating Genetic Susceptibility to Mitochondrial Toxicity
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Genetic variation within the human populous causes a range of sensitivities to various toxicants, including some that target the mitochondria. For example, the antibiotic gentamycin, which targets the bacterial ribosome, can also cause hearing loss. Adverse effects can be worsened in certain individuals carrying a point mutation that renders the mitochondrial 12S rRNA, which is evolutionarily related to the bacterial rRNA, more bacteria-like. In addition to pharmaceuticals, many environmental pollutants are also known mitotoxins; however, whether these toxicants pose an increased risk to individuals suffering from mitochondrial disease is unknown. Using a UVC exposure protocol that allows for the accumulation of mitochondrial (mtDNA), but not nuclear DNA damage, we found that C. elegans carrying a deletion in the fos-1 gene are extremely sensitive to UVC-induced mtDNA damage. fos-1 and its human ortholog MFN1 and MFN2 play a major role in mitochondrial dynamics (outer membrane fusion), and the fos-1 deletion results in highly fragmented mitochondria. The removal of UVC-induced mtDNA damage is fos-1-dependent in C. elegans, and mutations in MFN2 cause Charcot-Marie-Tooth neuropathy type 2A in humans. Utilizing the fos-1 mutant we are screening environmental mitotoxins for exacerbation of growth inhibition, because larval development is dependent on mitochondrial function. Initial screens have demonstrated that chronic, low doses of rotenone (0.25 μM), and paraquat (150 μM) significantly delay the growth of fos-1 mutants, compared to wild-type worms. To further test the role of mitochondrial dysfunction in larval developmental delay, we will analyze mitochondrial fitness by measuring oxygen consumption with a Seahorse Xfe24, ATP production with an fos-1 strain expressing a ATP-luciferase transgene, and mtDNA damage and copy number with a QPCR assay. This research aims to help fill a knowledge gap that leaves an already susceptible population of individuals at an even higher risk to certain environmental exposures. Funded by NIEHS IR01-ES017540-01A2 and Duke University Graduate Fellowship.
**1941** Investigations of Dose-Response for Key Events in the Mode of Action (MOA) for Propylene Oxide (PO)-Induced Mutagenicity in TK6 Cells  
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PO, a reactive chemical, induces nasal tumors in rodents upon chronic high-dose inhalation exposures. It is mutagenic in vitro and weakly clastogenic in vitro. To investigate thresholds for PO-induced mutagenicity, dose-responses for several hypothesized key event biomarkers were assessed in TK6 cells. Triple cultures were treated with 0.5 to 50 μg PO/ml, and evaluated for: mutant frequency (HPRT MF), micronucleus (MN), Comet, GSH-PO conjugate, N7-hydroxypropylguanine (N7-HPGP) adducts, and 2 homeostasis/oxidative stress markers (cytosol B-OH-GTP, 8-OH-DG). Data were analyzed for point-of-departure (POD) metrics: NO-Genotoxic-Effect-Levels (NOGELs), binaril threshold dose (Td), and benchmark dose (BMD). Exposure biomarkers: N7-HPGP adducts, not detected in controls, increased linearly with PO dose; GSH-PO increased over control levels at ≥5 μg/ml PO. Oxidative biomarkers 8-OH-DG and 8-OH-GTP were affected; all treated groups had decreased 8-OH-GTP and increased 8-OH-DG adducts vs controls. Biomarkers of genotoxic effects, HPRT MF and MN, with Comet, had NOGELs of 25, 25, and 10 μg/ml, respectively. Bilinear Td and BMD analyses of MN data gave a Td of 5.72 and a good fit for BMD10 (4.99) and BMR (9.51). Neither MN nor Comet data had a good fit for BMD. DNA from 57 pooled mutant clones (0.5, 5, 50 μg PO/ml) underwent NexGen sequencing to determine mutational spectra (MS) spanning the full-length 45kb human HPRT1 gene. The MS showed a difference in transversions in the top dose group vs. controls, where A→T:A mutations predominated; low dose MS (MF not increased) did not have a similar change. Integration of such dose-response data for biomarkers of hypothesized key events, along with the determination of MS data, support the determination of in vitro NOGELs, or thresholds, for certain key events including genotoxic effects, and provide key perspective on the threshold MOA of PO. Sponsored by the Cefe PO & PG SG.

**1942** Depleted Uranium Induces DNA Damage and Alteration of DNA Repair Proteins in Human Lung Epithelial Cells  
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Depleted uranium (DU) is commonly used in military applications and is also used in civilian industry and thus exposure of soldiers and others is frequent and widespread. DU is considered a probable human carcinogen, affecting the bronchial cells of the lung, however, how DU causes lung cancer is uncertain. In this study, we use the human bronchial epithelial cell line (BEPE2D) to investigate the genotoxic effect of DU and the underlying mechanisms. We found that DU weakly causes chromosome damage after a 24 h exposure but induces time-dependent increase in chromosome damage after 48 h and 72 h exposure. DU also induces DNA double strand breaks measured by comet assay. Doses of 0.25, 2.5 and 25 μg/cm2 uranium trioxide increases comet tail length of 0, 2 and 7, respectively after a 24 h exposure. Both 48 h and 72 h DU treatment induces a similar increase in tail length. We next investigated whether DU affects DNA double strand break repair pathway. We found that 24 h DU treatment does not affect the DNA damage sensor step measured as Mre11 expression but 48 h and 72 h exposure of 25 μg/cm2 uranium trioxide inhibits Mre11 expression. DU treatment increases phosphorylated ATM but inhibits Rad51 expression. Our study demonstrates that DU induces genotoxicity and may interfere with DNA repair in human bronchial epithelial cells. This work was supported by ARO grants # W911NF-04-1-0240 and W911NF-08-0033 (J.P.W.).

**1943a** Comparison of Genotoxic Effects of Major Diesel Exhaust Components in Human Alveolar Basal Epithelial Cells (A549)  
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Internal combustion engines (ICE), including diesel engines, power most of the motorized road vehicles. ICE are a major source of air pollution in metropolitan areas. Among other compounds they emit polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives. Genotoxicity of benzo[a]pyrene (BaP), a model PAH, and PAH nitro-derivatives (1-nitropyrene (1-NP) and 3-nitrobenzoanilide (3-NBA)), was studied in a model cell line A549. The cells were treated for 4 and 24 hours with different concentrations of tested compounds (BaP: 0.1 and 1 μM; 1-NP: 1 and 10 μM; 3-NBA: 0.5 and 5 μM) and several endpoints, including bulky DNA adducts and oxidative stress markers (lipid peroxidation, protein and DNA oxidation) were analyzed. All tested compounds increased bulky DNA adduct levels after both the 4-h and 24-h treatment, although the effect of 1-NP was relatively weak. A 24-h treatment resulted mostly in higher DNA adduct levels than the 4-h treatment with the exception of 3-NBA. 1-NP induced protein oxidation after both the 4-h and 24-h treatment. Interestingly, lipid peroxidation measured as levels of 15-F2t-isoprostane decreased after the BaP treatment, but was elevated after incubation of the cells with 1-NP and 3-NBA. 8-OxodG-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a marker of oxidative DNA damage, was not affected by either compound and/or treatment period. Our data highlight differences in genotoxic mechanisms of PAHs and their nitro-derivatives, particularly for oxidative stress markers. Supported by the Czech Science Foundation (13-01438S) and by EU LIFE+ Program (LIFE-ENV-CZ-651).

**1943b** Nitroxide TEMPO: A Genotoxic and Oxidative Stress Inducer in Cultured Cells  

Low molecular weight nitroxide, 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO), is a stable free radical which has been widely used throughout chemistry and biochemistry as process intermediates. Due to its antioxidant abilities and protective properties in various pathological situations, some studies have proposed them as potential therapeutic agents for medical use. However, the potential risks of TEMPO need to be addressed. In this study, we investigated the cytotoxicity and mutagenicity of TEMPO using the mouse lymphoma assay (MLA) and in vitro micronucleus assay (MN). In the absence of metabolic activation (S9), 3mM TEMPO produced significant cytotoxicity and marginal mutagenicity in the MLA; whereas in the presence of S9, treatment of mouse lymphoma cells with 1–2mM TEMPO resulted in dose-dependent decreases of the relative total growth and increases in mutant frequency. In the MN assay, treatment of TK6 human lymphoblastoid cells with 0.9–2.3mM TEMPO increased the frequency of both micronuclei (a marker for clastogenicity) and hypodiploid nuclei (a marker of aneugenicity) in a dose-dependent manner, with the responses being greater in the presence of S9. Within the dose range tested, TEMPO also induced increased generation of reactive oxygen species and decreased glutathione levels in mouse lymphoma cells. In addition, the loss of heterozygosity (LOH) analysis showed that the majority of TEMPO-induced mutants, both in the presence and absence of S9, produced...
high levels of LOH at the Tk locus only, indicating allele loss of ≤3436Mbp. These results indicate that TEMPO is mutagenic in the MLA and induces micronuclei and hypodiploid nuclei in TK6 cells. Oxidative stress may account for part of the genotoxicity induced by TEMPO in both cell lines.


TK6 cells are a human lymphoblastoid cell line originally derived from a patient suffering from spherocytosis that were subsequently mutagenized with the intercalating agent ICR-191 to create a heterozygous thymidine kinase locus (Tk+/−). Since TK6 cells are heterozygous for the Tk gene but wild-type for the p53 gene, they are routinely used to assess the mutagenicity of chemicals by using loss of heterozygosity of the remaining wild-type Tk allele as a reporter. Despite their common use and importance in genetic toxicology, little is known about other genetic abnormalities these cells possess and how these may affect their response to genotoxins. To determine if TK6 cells carry DNA sequence changes that may affect their response to DNA damaging agents, we used 454 Next Generation Sequencing to characterize the TK6 transcriptome. We detected 2572 genetic variations arising from nuclear DNA and 46 from mitochondrial DNA, from a total of 7471 detected genes. Among the anomalies mapping to nuclear DNA, 1089 are known SNPs, while 1483 are novel. Among the anomalies mapping to mitochondrial DNA, 36 are known SNPs, while 10 are novel. Importantly, we detected several anomalies that could affect the genotoxic profile of these cells, such as a frame-shift mutation in the heavy subunit of ferritin (FTH), and clinically-relevant SNPs at the xenobiotic metabolizing enzyme NADPH quinine oxidoreductase 1 (NQO1) and the thiopurine drugs metabolizing enzyme thiopurine S-methyltransferase (TPMT) loci, among many others. Our data provides a preliminary catalogue of the altered DNA sequences present in the transcriptome of TK6 cells.

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The MGMT gene encodes the direct reversal DNA repair protein O6-methylguanine-DNA methyltransferase, which preferentially removes adducts resulting from exposure to alkylating agents from the O6 position of guanine. Recently, we sequenced 104 DNA samples from healthy individuals and identified several single nucleotide polymorphisms (SNPs) in the MGMT promoter/enhancer (P/E) region. We found that, due to linkage disequilibrium, these SNPs form defined haplotypes. To determine the functional significance of these haplotypes, we transfected luciferase-reporter constructs representing the different haplotypes into glioblastoma cells and determined their effect on MGMT promoter activity. The results showed that MGMTP/E haplotypes, rather than individual SNPs, differentially regulate MGMT transcription. However, the mechanism(s) by which these haplotypes affect MGMT transcription remains elusive. Using in-silico analysis, we found that several of the SNPs in our population are within or in close proximity to putative transcription factor (TF) binding sites including AP-1, NF-1, AP-2, GCF and c-Myc. We therefore hypothesized that MGMTP/E haplotypes affect TF binding leading to alteration in MGMT transcription. To test our hypothesis we conducted a TF binding profile for different MGMT haplotypes using the Promoter Binding TF Profiling Plate Array assay (Signosis, Inc.), which allowed us to evaluate binding of 48 TFs to the MGMTP/E. We found a significant difference in TF binding depending on the MGMTP/E haplotype evaluated, indicating that the haplotypes do alter TF binding. These data provide a mechanistic explanation to our findings demonstrating that MGMTP/E haplotypes influence promoter activity and could explain the well-documented interindividual sensitivity to environmental and therapeutic alkylating agents (Supported by T32-07454; P30 ES086676 and JSME bridging grants).

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5-fluorouracil (5-FU) was discovered at trace levels during residue assessments of an investigational agricultural product. To characterize potential health risk associated with this residue, focused hazard and exposure assessments were conducted. While 5-FU has genotoxic potential under some conditions, information relevant to dietary exposure was lacking. To assess the potential genotoxic hazard of 5-FU under relevant exposure conditions, F344/DuCrI rats received a dose of 6 mg/kg day 5-FU by diet for 14 days. Dose-level selection was based on a dietary exposure estimation of 0.0005 mg/kg/day and a 10,000-fold margin of exposure. Multiple guidance points for genotoxic end-points were integrated in a single study using the same group of animals. Specifically, the end-points evaluated were unscheduled DNA synthesis [UDS] in hepatocytes, primary DNA damage [Comet assay] in stomach and duodenum cells and formation of micronucleated polychromatic erythrocytes [MNT] in bone marrow. Sensitivity of the system was confirmed by positive control animals which received single gavage doses of either dimethyltrinitrosamine (35 mg/kg) or cyclophosphamide monohydrate (40 mg/kg/day) and ethyl methanesulfonate (200 mg/kg/day) prior to study termination. All responses were compared to untreated animals. The study results showed 5-FU did not induce a significant increase in the mean number of net nuclear grain counts in hepatocytes isolated from treated animals in the UDS assay, there was no primary DNA damage as measured by the Comet assay, and there was no significant increase in the number of micronuclei in the polychromatic erythrocytes in bone marrow of the treated rats. Under the conditions of this study, it is concluded that 5-FU is non-genotoxic when administered in the diet to rats at levels 10,000-fold higher than predicted human exposure. This study demonstrates the utility of an integrated testing approach for genotoxic potential as well as exposure-based dose-level selection to characterize human risk.

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4-(Methyltrinitrosamine)-1-(3-pyridyl)-1-butaneone ([MNT] in bone marrow. Sensitivity of the system was confirmed by positive control animals which received single gavage doses of either dimethyltrinitrosamine (35 mg/kg) or cyclophosphamide monohydrate (40 mg/kg/day) and ethyl methanesulfonate (200 mg/kg/day) prior to study termination. All responses were compared to untreated animals. The study results showed 5-FU did not induce a significant increase in the mean number of net nuclear grain counts in hepatocytes isolated from treated animals in the UDS assay, there was no primary DNA damage as measured by the Comet assay, and there was no significant increase in the number of micronuclei in the polychromatic erythrocytes in bone marrow of the treated rats. Under the conditions of this study, it is concluded that 5-FU is non-genotoxic when administered in the diet to rats at levels 10,000-fold higher than predicted human exposure. This study demonstrates the utility of an integrated testing approach for genotoxic potential as well as exposure-based dose-level selection to characterize human risk.

J. McKinney and K. Vasquez. Pharmacology & Toxicology, University of Texas at Austin, Austin, TX. Sponsor: R. Finch.
H-DNA and Z-DNA-forming sequences co-localize with chromosomal breakage hotspots, such as those found in cancer-related chromosomal translocations, implicating non-B DNA in cancer etiology. Non-B structures cause distortions in the double helix, and therefore, may be recognized as DNA 'damage' by repair proteins. Preliminary data from our lab have shown that nucleotide excision repair (NER) and mismatch repair (MMR) proteins are involved in H-DNA-induced mutagenesis in yeast and mammalian cells. Based on these results it is plausible that repair proteins from multiple pathways participate in error-generating repair of non-B DNA structures, resulting in genomic instability. Thus, we hypothesize that NER and MMR proteins are involved in Z-DNA-induced mutagenesis in eukaryotes. The long-term goal of this project is to determine the mechanisim(s) involved in non-B-DNA-induced genetic instability. We have developed a genetic instability reporter system using a yeast artificial chromosome (YAC) containing naturally occurring non-B DNA-forming sequences capable of forming Z-DNA found at a translocation breakpoint in human cancer. We screened a yeast gene knockout library using 72 YAC reporter system to identify proteins that play a role in DNA structure-induced genetic instability. Thus far we have determined that several NER and other repair proteins are involved in Z-DNA-induced mutagenesis in yeast. We will continue to screen MMR proteins. Future experiments will confirm these results in human cells, and further characterize the roles of proteins identified in the yeast screen using biochemical and cell-based assays.

### 1943b Reduction of Genotoxicity from External and Endogenous Sources with Yel002

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Genotoxicity, is a persistent, yet often sinister health threat. A large amount of damage resulting from exposure to massive amounts ionizing radiation, for example, can cause death by killing the radiation sensitive dividing cells of our bone marrow and gut lining. However, oftentimes the effects of lesser doses are often not observed until the development of delayed diseases, such as cancers, a long period after the initial genotoxic insult. Ataxia Telangiectasia, or AT, is a severe progressive and terminal disease. By lacking a critical DNA repair enzyme, sufferers of AT essentially accumulate genotoxic damage in their cells at a greatly increased rate, succumbing to cancers at the median age of 22.

The development of a drug capable of mitigating the damage caused by multiple sources of genotoxicity via the upregulation of DNA repair mechanisms is of critical importance for the rescue of both healthy people exposed to genotoxic events such as radiation exposure and those individuals with endogenous DNA repair disorders, such as AT sufferers. Through a high-throughput, yeast based screen, the Schiestl Lab at UCLA has identified a number of compounds capable of reducing both radiation associated death and mutation rates. When the compounds were later tested in mice, one compound, designated Yel002, stood out as particularly effective, capable of cutting the death radiation of C3H mice irradiated at the LD100 level of 8 Gy down to 25%.

Given the sustained effectiveness of Yel002 as a radiation damage mitigator, it was decided to investigate whether it could protect against other sources of DNA damage, such as the endogenous repair disorder AT. Two strains of ATM deficient mice were injected once weekly with 75 mg/kg Yel002 in saline solution. Over a 97 week experimental period, we were able to observe an increase in life expectancy from 42 to 52 weeks with these weekly doses (generally caused by a delay in the development of terminal lymphoma). In addition, we hope to further increase the effectiveness of Yel002 with a new, more bioavailable formulation, currently showing promise in radioprotection experiments.

### 1943i Somatic and Germ Cell Mutant Analysis in the Big Blue® Transgenic Mouse Mutation Assay with N-Ethyl-N-nitrosourea (ENU)

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Integration of multiple endpoints in vivo genotoxicity assays provides a broad assessment of genotoxic potential while reducing the use of animals. We examined the induction of micronuclei and the Pig-a mutant phenotype in reticulocytes and mature red blood cells (mRNA levels for Pig-a CD138, and CD24, respectively) in the peripheral blood of male Lambda LIZ/1ac57BL/6 homozygous (Big Blue®) transgenic mice. Animals were treated with vehicle (olive oil) or BaP (50 mg/kg/day) on Days 1-28, or ENU (40 mg/kg/day) on Days 1-3. Peripheral blood was collected -3 hours before animals were sacrificed on Day 31 (as per OECD Test Guideline 488), for tissue collection for determination of cDNA probes, respectively (Litron Laboratories). BaP induced statistically significant increases in mmR, mmNCE, RET1-CD24 and RBC1-CD24 (1.54-, 2.09-, 616- and 100-fold, respectively; all p < 0.001). ENU induced statistically significant increases in mmNCE, RET1-CD24 and RBC2-CD24 (1.39-, 1050- and 207-fold, respectively; all p < 0.001), but not mmR (as expected due to the short term dosing regimen and turnover of mmR into mmNCE in mice). The sensitivity and power of the high throughput Pig-a method is demonstrated by its detection of relatively small net increases in mutant frequencies (the 100-fold increase in RBC2-CD24 observed for BaP represented an increase of ~1 x 10^-6 over a vehicle control value of 0.14 x 10^-3). Analysis of Pig-a mutation frequencies in somatic and germ cell tissues is ongoing. This study demonstrates the ability to integrate multiple endpoints into the Big Blue® Transgenic Mouse Mutation Assay for a comprehensive assessment of genotoxicity.

### 1944 Comparative Cytotoxicity of Nanosilver in HepG2 and Caco2 Cells


Increasing human exposure to nanosilver in consumer products is of concern. We evaluated two widely used human cell culture models, HepG2 and Caco2 cells, for screening cytotoxicity of food- and cosmetic-related nanosilver. We characterized nanosilver by DLS, TEM and ICP-MS analysis. Average size of nanosilver determined by our TEM analysis was 20.4 nm and showed no agglomeration. Our ICP-MS and TEM analysis suggested uptake of 20 nm silver by both cell types. Concentration of nanosilver determined by our ICP-MS analysis was 0.962 mg/ml. A significant concentration-dependent decrease in mitochondria membrane potential in both cell types. Cytotoxicity of 20 nm silver was determined in HepG2 and Caco2 cells at concentrations 0.1, 1.0, 10.0 and 20.0 µg/ml. A significant concentration-dependent cytotoxicity of nanosilver was observed in HepG2 cells in concentration range 1 to 20 µg/ml and at a higher concentration range 10 to 20 µg/ml in Caco2 cells compared to control. When both cell types were exposed to nanosilver there was a significant concentration-dependent decrease in dsDNA content indicating increased DNA damage in concentration range 1 to 20 µg/ml. No oxidative stress was noted in either cell type tested at concentration range used. Nanosilver treatment induced a significant concentration-dependent decrease in mitochondria membrane potential in both cell types indicating increased mitochondrial injury in concentration range 1 to 20 µg/ml for HepG2 cells and at a higher concentration range 10 to 20 µg/ml for Caco2 cells. These results indicated that HepG2 cells were more sensitive to nanosilver than Caco2 cells. It is believed that oxidative...
stress leads to cytotoxicity of nanoparticles in general. However, we did not detect nanosilver-induced oxidative stress in either of cell types in concentration range used. Our results suggested that oxidative stress did not contribute to observed cytotoxicity of nanosilver. A different mechanism of nanosilver cytotoxicity initiated by mitochondria injury is suggested. In summary, our results indicated that widely used in vitro models, HepG2 and Caco2 cells, are excellent models for screening cytotoxicity of nanomaterials.

1945 Induction of Autophagy at Noncytotoxic Concentrations of Silver Nanoparticles in HepG2 Cells
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Silver nanoparticles (AgNP) are increasingly used in medical and consumer products to exploit their antimicrobial properties, but health risks associated with exposure have not been fully evaluated. Further studies are needed to more fully understand the bioactivity of AgNP and identify biomarkers of toxicity. Autophagy is a part of the lysosomal-mediated pathway that functions to sequester and remove unnecessary and misfolded cytoplasmic proteins. Recent studies have shown that nanoparticles of varying chemistries and size can activate the autophagic-lysosomal pathway leading to cytotoxicity. The goal of this study was to: 1) evaluate AgNP cytotoxicity in cultured human HepG2 cells, 2) evaluate autophagy and apoptotic responses induced by AgNP, and 3) determine if LC3B protein, a pro-autophagic marker, can serve as a molecular biomarker to predict AgNP-induced cytotoxicity. HepG2 cells were studied since liver is a primary organ for systemic nanoparticle accumulation. AgNP (10, 50 and 100 nm) were characterized for size and shape using TEM and DLS. Cells were incubated with AgNP (0.1, 0.5, 1, 5, 10, or 50 μg/ml) for 12 and 24 hr. Cytotoxicity was assessed by trypan blue exclusion and XTT assays. Apoptosis (Annexin V) was assessed by flow cytometry. Confocal and fluorescence microscopy were used to detect lysosomal activity (Lysotracker DND), autophagy (Cyto-ID green), and LC3B. Cytotoxicity was observed only after 24 hr post-exposure at the highest concentrations of AgNP (10 and 50 μg/ml) but not at lower concentrations (less than 5 μg/ml). Apoptosis was detected only after 24 hr post-exposure at 10 and 50 μg/ml. No significant apoptosis was detected at 12 hr post-exposure at all the concentrations. In contrast, autophagic vacuoles and increased expression of LC3B were observed at 12 and 24 hr post-exposure at non-cytotoxic concentrations (less than 5 μg/ml). In conclusion, the data demonstrated that 1) autophagy was induced at non-cytotoxic AgNP concentrations, and 2) LC3B may serve as a sensitive biomarker to detect early toxicity in AgNP-exposed cells.

1946 Flow Cytometry-Based Evaluation of Silver Nanoparticle Cytotoxicity: Role of Serum Concentration in Media
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Nanoparticles (NPs) when exposed to clinically relevant media, e.g., serum or blood, are immediately coated with proteins and lipids. The adsorbed biomolecules can significantly alter the physiochemical properties (size, charge, shape, surface functionalities) of NPs and hence, high influence NP tissue disposition, cell uptake, and biological responses. NPs, due to their nanoscale properties, also interfere with colorimetric in vitro cytotoxicity assays such as MTT or LDH. However, assays that do not use spectrophotometric detection, such as flow cytometry (FC) based assays, are less likely to be influenced by NP interference. The aim of this study was to 1) compare the cytotoxicity of silver nanoparticles (AgNPs) pre-mixed with cell culture media (with varying FBS concentration) in L-929 fibroblasts compared to direct addition (no pre-mixing) of AgNPs to the cells, and 2) evaluate the use FC-based assays to assess cytotoxicity of AgNPs. To assess the effects of pre-mixing on cytotoxicity, AgNPs (10 nm diameter) were added to cells either following pre-mixing with cell culture media (supplemented with 0.1, 1, or 5% FBS) for 24 hr or directly added without pre-mixing. After 24 hr exposure to the cells, cytotoxicity was assessed by MTT and FC based assays. The degree of cell necrosis and apoptosis was assessed using 7-AAD and Annexin V dyes, respectively. MTT data showed that AgNPs added directly to cells produced a particle concentration (μg/ml)-dependent decrease in viability, i.e., AgNPs pre-mixed with cell culture media supplemented with 0.1% FBS were more toxic as compared to pre-mixing of AgNPs with media supplemented with 5% FBS. FC data showed that the degree of necrosis and apoptosis of cells when exposed to AgNPs was dependent on the concentration of both AgNPs and FBS in cell culture media used for pre-mixing. The data showed that AgNPs premixed for 24 hr with cell culture media were less cytotoxic as compared to no premixing. The results indicate that NP sample preparation methods influence biological testing outcomes.

1947 Size- and Surface Coating-Dependent Uptake and Cellular Responses of Iron Oxide Nanoparticles in Human Mammary Healthy and Breast Cancer Epithelial Cells
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Superparamagnetic iron oxide nanoparticles (SPIONs) have received great attention due to their promising use as magnetic resonance imaging (MRI) contrast agents. In this study, we evaluated the cellular uptake and biological responses in vitro of ultrasmall SPIONs used for MRI breast cancer imaging. We compared the cellular responses between primary breast epithelia isolated from healthy and breast cancer donors after exposure to different sizes and surface coatings of USPIONs (10 nm and 30 nm bare, 10 nm and 30 nm PEG-coated). The particles were characterized using transmission electron microscopy (TEM), dynamic light scattering and gel electrophoresis. Cellular interactions with USPIONs were assessed by confocal microscopy and TEM imaging analysis. Cellular uptake of USPIONs was quantitated using inductively coupled plasma mass spectrometry. Cell viability was measured by MTT and neutral red uptake assays. T2* weighted MRI scans were performed to demonstrate the potential for image contrast enhancement using a 4.7T MRI scanner. Results showed cell association/internalization of USPIONs was size- and surface coating-dependent; higher cellular uptake of 10 nm and 30 nm bare particles was observed in both cell types compared with PEG-coated particles. Cell uptake for 10 nm and 30 nm bare particles was higher in tumor cells from 2 of 3 tested donors compared to healthy cells from 3 donors. Cytotoxicity was minimal except at high concentrations of 200 and 400 μg/ml for all tested particles. Significantly enhanced MRI contrast was observed following exposure to 10 and 30 nm bare particles compared to PEG-coated particles in both cell types. These data indicate that cell responses following exposure to USPIONs are dependent on particle properties. The results provide useful information to better evaluate the safety and efficacy of USPIONs used as MRI contrast agents for breast cancer imaging.

1948 Influence of Single-Walled Carbon Nanotubes with Different Physico-Chemical Properties on the Cellular Responses in Alveolar Epithelial Cells and Macrophages
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Impurity-free single-walled carbon nanotubes (SWCNTs)—relatively small bundles and a short linear shape (CNT-1), large bundles and a long linear shape (CNT-2), and commercial SWCNTs with residual metals (CNT-3) as a reference sample did not cause a significant inhibition of cell proliferation, induction of apoptosis, or arrest of cell cycle progression in A549 human alveolar epithelial cells. However, CNT-2–treated cells significantly increased the level of intracellular reactive oxygen species (ROS), in a dose-dependent manner, and the levels of these ROS were higher than those of CNT-1–treated cells or CNT-3–treated cells. On the other hand, treatment of NR8383 rat alveolar macrophage cells with CNT-1, CNT-2, and CNT-3 resulted in slight inhibition of cell proliferation. The levels of IL-1β, MIP-1a, MCP-1 and intracellular ROS generation in CNT-2–treated cells were significantly higher than that in CNT-1–treated cells. CNT-1, CNT-2, and CNT-3 were phagocytosed in vacuoles. Expression of many genes involved in the inflammatory response, their response to oxidative stress, and the expression of extracellular matrix metalloproteinases (MMPs) were markedly upregulated in cells exposed to CNT-2 or CNT-3. These results suggest that the size and length of the bundles of SWCNTs dispersed in cell culture medium, even in the same bulk SWCNTs, are critical parameters of the cellular processes and elicit stress-related cellular responses in alveolar macrophages. We propose a model in which MMPs or inflammatory cytokines derived from alveolar macrophages activated by phagocytosis of SWCNTs induce degradation of the extracellular matrix, and consequently, may cause pulmonary inflammation in alveolar epithelial cells as an indirect effect. This research was funded by NEDO, Japan (P10024).

SOT 2014 Annual Meeting 513
1949 Comparative Biocompatibility of Nano-AntiCancer Drugs
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The drug delivery system (DDS) has become increasingly attractive in cancer therapy since it can improve drug efficacy and reduce side effects of drugs on normal tissues. Formulating small molecule drugs into nanoparticles (NPs), such as liposomal or carbon nanotube (CNT), allows for a significant reduction of adverse effects while maintaining antitumor efficacy. However, biosafety and biocompatibility of nano-anti-cancer drugs are not much scrutinized in terms of toxicological aspect. In this study, we demonstrated comparative toxicity of pegylated liposomal doxorubicin (Doxil) and doxorubicin (Dox)-linked CNT (Dox-CNT) in various cell lines. We used four types of cell lines such as Raw 264.7, HepG2, AML-2, and SK-MES-1 as target organ cells. We found that Dox-CNT induced greater cytotoxicity than Doxil in four types of cells. Specifically, Dox-CNT enhanced cytotoxicity through cellular uptake and production of reactive oxygen species in Raw 264.7 and HepG2 cells. Dox-CNT caused apoptosis and necrosis through CNT-induced ROS production at concentration of cell viability 75% in all the cells. However, Doxil increased apoptosis through Dox-induced changes of mitochondrial potentials in Raw 264.7 and SK-MES-1 cells. Both of Dox-CNT and Doxil has no significant effect on expression of pro-inflammatory cytokines in all the cells. Our results suggest the importance of biocompatibility during the design of new nano-anti-cancer drugs.

1950 Protein Carbonylation As a Marker of Oxidative Stress Induced by Nanoparticles: Analysis of 16 Inorganic Nanoparticles
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The BMBF-funded project “nanoGEM” follows a systematic approach to understand hazards associated with different types of nanoparticles (NP). Oxidative stress is considered to be a major paradigm to explain NP toxicity. Here we focused on protein carbonylation as a consequence of oxidative stress for a set of 16 different nanoparticles, used as either plate (“pristine”) materials or with different surface coatings. In parallel several in vitro and in vivo toxicity endpoints have been analyzed.

We used NP of 10nm (ZrO2), 15nm (SiO2) and 50nm or 200nm (Ag), furnished either with acidic, basic or polymeric functionalities and TiO2, ZrO2, BaSO4 and ALOOH as references. Characterization followed the nanoparticles REACH guidance R7.1. In a screening approach we studied time- and dose-dependent protein carbonylation of all 16 NP in NRK-52E cells via 1D immunoblots. Data were correlated with cytotoxicity (WST-8, LDH assay) and ROS formation (DCFDA assay). Furthermore we applied a 2D proteomics approach combined with MALDI-MS/ MS to identify the modified proteins. Finally for several NP we analyzed lung tissue after in vivo instillation in mice.

Eight out of 16 NP induced protein carbonylation in NRK-52E cells. Observed protein carbonylation correlated well with overall toxicity. The 2D approach revealed a complex and distinct pattern of carbonyls. Modified proteins were identified as proteins of cytoskeleton, HSP or proteins of major cellular pathways (i.e. glycocalyx). We also observed carbonyl modifications in the lung tissue homogenates of rats intratracheally instilled with the same NP. Taken together, analysis of protein carbonylation appears useful to describe toxic effects of NP and to better understand underlying molecular mechanisms.

1951 ERK Pathway Is Activated in Bare-FeNPs-Induced Autophagy
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Iron oxide nanoparticles (FeNPs) are known to be one of the most biocompatible and safe nanoparticles. However, their long-term persistence remains a problem, and macrophages play an important mediator in continuous stimulation of the immune system due to biopersistence of nanoparticles. In present study, we identified the mechanisms underlying the uptake and toxicity of bare-FeNPs using RAW264.7 cells, a mouse peritoneal macrophage cell line. The bare-FeNPs penetrated the cell membrane through electrostatic interactions together with the general phagocytic pathway. At 24 h after exposure, they distributed freely in the cytosol or within autophagosome-like vacuoles. Bare-FeNPs induced decrease in the cell viability along with the cell cycle arrest in G1 phase. In addition, they increased the generation of ROS and the secretion of NO and TNF alpha as well as the expression of SOD-1 and -2 and cathepsins, which are known to increase the mitochondrial calcium level, the intensity of labeled mitochondria, and ATP production decreased. The levels of autophagy-related proteins such as p62, beclin1, ATG5, and LC3B increased in a dose-dependent manner together with the levels of ATF 3, p-EGFR and p-ERK proteins. However, the level of p-JNK protein clearly decreased. TEM images also showed that damaged organelle exist within autophagosome-like vacuoles with bare-FeNPs. On the basis of these results, we suggest that bare-FeNPs induce autophagy by initiating oxidative stress in RAW264.7 cells. Furthermore, ERK, but not JNK, pathway is activated in bare-FeNPs-induced autophagy.

1952 Hidden Hazards of Nanoparticles: Crosstalk between Toxicogenomics, DNA Damage, and Cell Death
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The rationale of this study is to provide first evidence on the crosstalk between toxicogenomics, DNA damage and cell death induced by NiO and ZnFe2O4-NPs in HepG2 and WISH cells. Ultrastructure analysis revealed NPs translocation in cell cytoplasm and induces DNA damage. MTT and NRU assays showed cell viability of 20.6%, 64.48% in HepG2 and 18.4%, 50.73% in WISH cells. Flow cytometric analysis exhibited high ROS and nitric oxide generation along with 1.2-fold fluorescence enhancement in mitochondrial membrane potential (ΔΨm) in NiO-NPs treated HepG2 and 0.9-fold decline in ZnFe2O4-NPs treated WISH cells. Ca2+ influx and esterase activity in HepG2 cells exhibited 474.6% and 128% greater values after NiO-NPs exposure. Cell cycle analysis of HepG2 and WISH cells exhibited 30.5% and 15.2% of apoptotic cells upon NiO and ZnFe2O4-NPs treatment. qPCR analysis of p35, caspases 3 and 9 genes showed 2.0, 1.2 and 1.1-fold upregulation in HepG2 cells. WISH cells also exhibited 5.3, 1.6, and 14.9-fold upregulation of p53, caspases 3 and 9 genes. The RT2 Profiler™ PCR array data of 84 genes responsible for oxidative stress and human toxicity elucidated up-regulation of mRNA transcripts of GDF15, DDT3, CXC1L1, NOS2 and HPSE in range of 2.0 to 4.6-folds. Also, ZnFe2O4-NPs treated WISH cells elucidated differential up-regulation IL-1β, NFKB1, NOS2 and CCL21 genes in range of 1.5 to 3.7-folds. Furthermore, immunofluorescence analysis of NiO-NPs treated HepG2 cells exhibited translational activation of p53, bax, bcl2, cyt c, PARP and MAPK proteins. In conclusion, the significant ROS production, reduction in ΔΨm, DNA damage, and activation of genes linked to inflammation, oxidative stress, proliferation, DNA damage and repair could serve as predictive toxicity and stress markers for toxicological assessment of NPs induced cellular and genetic damage in exposed human population.

1953 Lung Tissue Culture Model for Assessment of Cytotoxicity and Inflammation Induced by Inhalable Carbon Black Nanoparticles
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Carbon black nanoparticles (CBNP) are produced in several megatons per year and are used in the industry mainly in tires, inks and for stabilization of elastomers. CBNP have a large surface area to volume ratio and can be covered with polycyclic aromatic hydrocarbons (PAH). CBNP were evaluated as possible carcinogenic material. However, it is remain unclear whether the effects are caused by PAH known to be cancerogenic or by CBNP itself. This study focused on evaluation of one CBNP without PAH (Printex® 90), two CBNP with only one PAH (Printex® 90 Benzo[a]pyrene and Printex® 90 9-Nitroanthracene) as well as one covered with different PAH (acetylene soot). Precision cut lung slices (PCLS) were used as acute exposure model.

PCLS (murine, rat and human) were exposed to different concentrations of Printex® 90, acetylene soot, Printex® 90 Benzo[a]pyrene and Printex® 90 9-Nitroanthracene for 24 h. The size distribution of stable particle suspension in DMEM medium was determined by dynamic light scattering. Viability of PCLS...
was assessed by LIVE/DEAD® staining and determination of metabolic activity using the WST-1 assay. Pro-inflammatory immune responses were assessed in tissue lysate and quantified using ELISA (e.g. IL-1β, IL-10, TNF-α, CXCL-8).

Printex® 90 nanoparticles were nearly non-toxic in PCLS of all three species at concentrations between 0.7 and 66 μg/mL. Compared to these results Benzo(a)pyrene and 9-Nitroanthracene coating showed a toxic effect at 50 μg/mL. Acetylene soot showed a 1.5-fold increase in metabolic activity compared to medium control after 24 h exposure. A 40% decrease to medium control in release of pro-inflammatory cytokines were observed at the concentration of 66 μg/mL for all CBNP.

1954 Long-Term Exposures to Low Doses of Cobalt Nanoparticles Induce Cell-Transformation Enhanced by Oxidative Damage

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A weak aspect of the in vitro studies devoted to get information on the toxic, genotoxic and carcinogenic properties of nanomaterials is that they are usually conducted under acute-exposure and high-dose conditions. This makes difficult the extrapolation to human beings. To overcome this point we have evaluated the cell transforming ability of cobalt nanoparticles (CoNPs) after long-term exposures (12 weeks) to sub-toxic doses (0.05 and 0.1 μg/mL). To get further information on whether CoNPs-induced oxidative DNA damage is relevant for CoNPs carcinogenesis, the cell lines selected for the study were the wild-type mouse embryonic fibroblast (MEF) and its isogenic Ogg1 knockout partner, unable to properly eliminate the 8-OH-dG lesions from DNA. Our initial short-term exposure experiments demonstrate that low doses of CoNPs are able to induce ROS and the MEF Ogg1-/- cells are more sensitive to CoNPs-induced acute toxicity and oxidative DNA damage. On the other hand, long-term exposures of MEF cells to sub-toxic doses of CoNPs were able to induce cell transformation, as indicated by the observed morphological cell changes, significant increases in the secretion of metalloproteinases (MMPs), and anchorage-independent cell growth ability, among other cancer-like phenotypic hallmarks. Interestingly, such changes were significantly dependent on the cell line used, the Ogg1-/- cells being particularly sensitive. Altogether, the data presented here confirms the potential carcinogenic risk of CoNPs at environmentally plausible doses and points out the relevance of ROS and Ogg1 genetic background on CoNPs-associated effects.

1955 In Vitro 3D Collagen-Based Hydrogel Model to Evaluate Cytotoxicity of Silver Nanoparticles

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Nanomaterials are finding increasing uses in the medical device industry in applications such as medical implants, scaffolds and antimicrobial surface coatings. Currently, nanoparticle (NP) toxicity is typically studied in either 2D cell monolayer in vitro models or in vivo animal models; both models have inherent advantages and disadvantages. Studies using 2D models are easy and fast; however, they do not represent the complexity of native tissue. In contrast, in vivo experiments offer the full complexity of tissues, but have high cost and pose difficulty in tracking NP tissue distribution. Alternative 3D collagen matrix-based models offer several advantages to traditional models: in vitro cellular responses can be studied in a physiologically-relevant microenvironment and longer term in vitro exposures are possible. An advantage of 3D models for nanoparticle toxicity testing is that a uniform cell exposure is attained as the limitations of diffusion (smaller NPs) and sedimentation (larger NPs) of particles during exposure are mitigated. The goal of this study was to evaluate the feasibility of using a 3D tissue culture model to assess the cytotoxicity of silver nanoparticles (AgNPs). L-929 fibroblast cells were pre-mixed with type-1 collagen, crosslinked at 37°C to form 3D hydrogels, and allowed to adhere to the collagen matrix for 24 h. After 24h exposure to 10, 100, or 200 nm AgNPs at different concentrations (1, 25, and 50 μg/mL), cells were harvested from hydrogels via collagenase digestion. Harvested cells were exposed to 7-AAD dye and cell viability was measured using flow cytometry. Cytotoxicity was dependent on both dose and size of AgNPs. Cells treated with 10nm AgNPs at 50 μg/mL exhibited a 93% decrease in viability. However, less than 3% decrease in cell viability were observed after exposure to 100nm and 200nm AgNPs. The data showed that a fabricated 3D in vitro cell culture model can be used to evaluate the cytotoxicity of NPs and can bridge the gap between 2D cell culture and animal models.

1956 Nanosilver-Induced Apoptosis in Cell Lines Depends on the Presence of p53

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Silver nanoparticles (Ag NPs) are widely used in consumer products and great efforts are made to elucidate the toxicological mechanisms. Activation of apoptosis after exposure to Ag NPs was investigated in 3 cell lines (CaCo-2, A549, and A549-p53-/-). The Ag NPs (16 nm) were previously characterized (Foldbjerg et al, Toxicol., Sci., 2013). Ag NPs reduced the cellular viability (measured by the MTT assay) by 80% in CaCo-2 cells and 35% in A549 cells at the highest NP concentration (30 μg/mL). Using the annexin V/PI assay, cell death was mainly detected as apoptosis in A549 cells and necrosis in CaCo-2 cells. Thus, cell death induced by Ag NPs may occur by different mechanisms in A549 and CaCo-2 cells. One of the major apoptosis pathways is via the activation of caspases. In A549 cells, a significant increase in caspase-3/7 and -9 activity was observed upon Ag NP exposure. In contrast, the caspase activity in Ag NP-treated CaCo-2 cells was unchanged compared to vehicle controls. CaCo-2 cells lack functional p53 and further studies in A549-p53-/- cells verified that p53 is necessary for caspase-3/7 activation upon Ag NP exposure. Increased caspase activity could, however, be induced by the mdm-2 inhibitor, Nutlin-3, demonstrating that the caspase machinery is fully functioning in CaCo-2 and A549-p53-/- cells. These results indicate that Ag NPs induce caspase-dependent apoptosis in A549 cells and caspase-independent necrosis in CaCo-2 cells. The classical route of caspase-9 activation includes loss of mitochondrial membrane potential (MMP). To confirm this route, the MMP of A549 cells exposed to Ag NPs was measured by JC-1 staining. Interestingly, Ag NPs did not affect the fraction of cells with low MMP suggesting activation of caspase-9 by a mitochondria-independent route. This route may involve calcium fluxes since Ag NPs induced a dose-dependent increase of cytosolic [Ca2+] in both A549 and CaCo-2 cells as determined by Fluo-4 fluorescence. We therefore speculate that Ag NPs may induce stress leading to calcium release and subsequent caspase activation in cells with wild type p53.

1957 Silver Nanoparticle-Induced Apoptosis, Genotoxicity, and Oxidative Stress in CHO Cells

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Silver has been used for decades owing to its health benefits as an antimicrobial and antifungal substance. Due to the novel properties of silver, silver nanoparticles are used in electronics, biosensing, photographic processing, medical applications and in many common household products, thus increasing their concentration in the environment and concern about the evaluation of its toxicity. For the present study, the Chinese Hamster Ovary (CHO) cells were exposed to various dose concentrations of silver nanoparticles i.e. 25, 50 and 100 μg/mL in vitro and were compared with that of the control. Cellular morphology, mitochondrial function (MTP assay), mitochondrial membrane potential, anti-oxidant enzymes assay (CAT, SOD, GPx, GST, GR activities as well as total Glutathione level) and comet assay were performed to assess the toxicity. Ag NPs decreased mitochondrial membrane potential and comparable CAT, SOD, GPx, GST, GR activities upon Ag NP exposure. Increased caspase activity could, however, be induced by the mdm-2 inhibitor, Nutlin-3, demonstrating that the caspase machinery is fully functioning in CaCo-2 and A549-p53-/- cells. These results indicate that Ag NPs induce caspase-dependent apoptosis in A549 cells and caspase-independent necrosis in CaCo-2 cells. The classical route of caspase-9 activation includes loss of mitochondrial membrane potential (MMP). To confirm this route, the MMP of A549 cells exposed to Ag NPs was measured by JC-1 staining. Interestingly, Ag NPs did not affect the fraction of cells with low MMP suggesting activation of caspase-9 by a mitochondria-independent route. This route may involve calcium fluxes since Ag NPs induced a dose-dependent increase of cytosolic [Ca2+] in both A549 and CaCo-2 cells as determined by Fluo-4 fluorescence. We therefore speculate that Ag NPs may induce stress leading to calcium release and subsequent caspase activation in cells with wild type p53.

1958 Influence of Capping Agents on Cellular Uptake of Silver Nanoparticles and the Resultant Toxicity

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Nanomaterials, especially silver nanoparticles (AgNPs) have been applied to numerous commercial products. Accordingly, there are raising concerns that exposure to these nanoparticles may cause potential adverse effects on humans as well as the environment. Humans are exposed to AgNPs mainly via inhalation and ingestion, and the potential toxicity has been attributed to nanoparticle-cell interactions, such as the adsorption and internalization of nanoparticles, however, little is known about the mechanisms of these interactions and the factors influencing them. This experiment was carried out to quantitatively and mechanistically investigate the role of capping agents of AgNPs on cellular uptake by cells in the epithelial lining of the human lung and colon. In vitro studies of human bronchoalveolar carcinoma derived cells (A549) and colon adenocarcinoma (Caco-2) are used to quantify the uptake kinetics and the extent of internalized AgNPs and to investigate the uptake
mechanism among different capping agents. AgNPs cytotoxicity is evaluated by lactate dehydrogenase (LDH) release and cell viability, which is also compared to the toxicity caused by Ag+ treatments. We observed Ag+ significantly reduced cell viability and triggered LDH release, however, no such changes were observed from AgNPs-treated cells. In addition, cellular inflammatory response and DNA damage will also be assessed and presented as part of AgNP toxicity mechanism investigation. Results of this study can be applied to facilitate the risk assessment process, limiting the hazards associated with occupational/consumer exposure to silver nanoparticles.

1959 Partial Reduction of Silver Nanoparticle-Dependent Cytotoxicity and Stress Activation following Magnetic Field Exposure

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Silver nanoparticles (AgNPs) have become incorporated into a number of consumer, industrial, and medical applications, owing to its tremendous, innate plasmonic and antimicrobial properties. However, AgNPs also induce a strong cytotoxic response, brought on by an excess of cellular stress and DNA damage, which has severely limited the inclusion of AgNPs in nano-based biological applications, such as drug delivery and bio-imaging techniques. Previous investigations into magnetic field (MF) exposure have determined the potential of MFs to reduce the stress response in cellular systems; however the ability of a MF to protect cells from AgNPs has yet to be explored. This study sought to identify if silver nanoparticles with a 15% increase in viability noted up to a threshold concentration of $10^n$ mg/mL. This partial recovery of AgNP dependent cytotoxicity was found to correlate with increased ki67 expression and a substantial decrease in the NE-4C stress response, including reactive oxygen species production and NFkB and c-Jun expression. As neurological models are highly susceptible to stress, this study identified MF stimulation as a potential mechanism to counteract detrimental AgNP effects in neural cells; thereby demonstrating that a joint AgNP and MF system may be advantageous to progress neurological nano-based applications.

1960 Ionic Dissolution of Silver Nanoparticles Is the Root Cause behind the Differential Disruption of EGF Signal Transduction

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In recent years, the field of nanotoxicology has begun exploring the ability of low, relevant dosages of nanomaterials to impact normal cellular functionality, such as signal transduction. Previously, we identified that silver nanoparticles (AgNPs) reduced epidermal growth factor (EGF) dependent signaling. It has also been elucidated that the generation of silver ions is the origin behind the well-established cytotoxic response of AgNPs in a cellular system. However, it has yet to be explored whether the ionic dissolution or the interaction between the particles and the cell surface receptors is the driving force behind AgNP dependent signaling alterations. As such, the goal of this study was to determine if the disruption of EGF signaling transduction was a result of the ionic dissolution or the AgNPs themselves, in a human keratinocyte cell model. Following separation of the AgNPs from the generated ions through tangential flow filtration, it was discovered that the kinetic dissolution was the principal cause for observed modifications to the EGF signaling network, as evaluated through Akt and Erk phosphorylation. The silver ions alone produced a 20% loss of signaling activation, whereas treatment with the particles alone induced no modifications. To explore if this phenomena was sustained or transient, EGF signaling activation was re-evaluated 72 hours after the removal of the AgNPs. Modulation of the cellular response to EGF stimulation still existed, however, after this extended time frame an augmentation in signaling was found; indicating the HaCaTs were in recovery mode. In conclusion, this study demonstrated that at low dosages of AgNPs, the disruption to cellular functionality is driven by the rate of ionic dissolution and is sustained for several days post AgNP removal; further illuminating the long term potential for damage following exposure.

1961 Design and Validation of Nanomaterial Aerosol Exposure Techniques for In Vitro Toxicology

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Traditional in vitro toxicology studies are often conducted by dispersing nanomaterials (NMs) in biological media for administration to cells in a static condition, which does not depict realistic inhalation exposure. The objective of this study was to validate a previously designed system to mimic inhalation exposure by delivering well-characterized NM aerosols to cells grown at the air-liquid interface. Silver NMs were exposed either as dispersion in biological media or as an aerosol generated from powder. The aerosolized NMs were characterized in real-time for size distribution and concentration simultaneously to being introduced into the nanomaterial exposure chamber and deposited onto cells using electrostatic deposition. A human type II pneumocyte and alveolar macrophage co-culture was grown in the chamber and exposed to either the dispersion or aerosol. Post-exposure, the viability was assessed using the Alamar Blue assay, the cell morphology was evaluated using fluorescence microscopy and NM uptake was imaged using electron microscopy. Results showed that the NMs exhibited unique agglomeration patterns and dosimetry when exposed at the air-liquid interface as a dispersion versus aerosol. This led to differences in the cellular response and NM uptake. This study demonstrates a promising step forward in the development of a standardized realistic exposure method for assessing NM toxicity to lung cells in vitro.

1962 Strain-Dependent Responses to Quantum Dots in Mouse Hepatocytes

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Quantum dots (Qdots), a new class of fluorescent nanoparticles, have unique properties that have enabled new opportunities in many areas including optoelectronics, photovoltaics, drug delivery, and biomedical imaging. Despite widespread industrial use and promise for commercial and diagnostic applications, the toxicity of many Qdots remains largely unknown. Many nanoparticles are known to be pro-inflammatory, especially when taken up by tissue macrophages that can elaborate pro-inflammatory cytokines and chemokines. Several in vivo studies have shown that injection of Qdots into rats or mice results in rapid clearance from the blood, with most of them ending up in the liver, spleen, and lymph nodes. In this study, we aim to determine how hepatocytes respond to Qdots, and whether toxic and immunomodulatory effects of Qdots are strain-dependent. We used an in vitro model of exposure to primary hepatocyte cultures from C57BL/6J and A/J strains of inbred mice to determine strain-dependent phenotypes related to exposure to tri-n-octylphosphine oxide, poly (maleic anhydride-alt-1-tetradecene) (TOPO-PMAT) coated CdSe/ZnS Qdots. We cultured primary hepatocytes from C57BL/6J and A/J mice and treated them with various doses of Qdots for 4 hours. We found that Qdot uptake in primary hepatocytes was dose-dependent and significantly higher in hepatocytes from A/J mice than those from C57BL/6J mice. We also measured pro-inflammatory cytokines by qPCR, and found that Qdots induced strain-dependent expression of Il-6 and Cxcl1 mRNA in primary hepatocytes, with cells from A/J mice demonstrating more sensitivity than C57BL/6J cells. Collectively, these data suggest that genetic background plays a significant role in Qdot uptake and inflammation. The A/J strain is more susceptible to Qdot-induced inflammation than C57BL/6J, consistent with in vivo data.

1963 Quantifying the Impact of Ag Nanoparticle Structure and Dissolution in Cell Culture Media on Cell Exposure and Biological Impact

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In vitro studies using bone marrow derived macrophages and RAW 264.7 cells show that 24 hr exposure to 20 nm Ag particles causes cytotoxicity at concentrations ≥ 10 μg/mL. Understanding the actual dose of nanoparticles and Ag ions to the cell requires knowledge of nanoparticle agglomeration, deposition, dissolution, and transformation in cell culture media. We have examined particle dissolution at concentration ranges from 1 μg/mL to 50 μg/mL and changes of particles for two 20 nm nanoparticles synthesized in different ways. One set of particles was prepared with a 7-8 nm Au core that helped produce particles of uniform
size. Ag coatings were made up of several Ag crystallites that nucleated on the Au 37 producing many highly defective interfaces. In contrast, pure Ag particles were less uniform in shape but had a more ordered ‘single’ crystal structure with slip plane or grain-boundary defects. Dissolution was studied in RPMI+FBS culture medium by measuring the appearance of Ag in the supernatant as a function of time up to 24 hours. High resolution (HR)-TEM and STEM images after solution exposure showed that the pure Ag particles maintained structural integrity compared to Ag/ Au particles where Ag layer had a non-uniform dissolution sometimes exposing the Au core. Dissolution was increased for 20 nm Ag/Au particles in comparison to pure Ag. An equation relating the kinetics of dissolution, the surface area of particles and an effective solubility constant was used to examine dissolution behaviors. For solution concentrations ≥ 10 µg/ml, dissolution parameters within each particle type are consistent regardless of particle concentration. The effective solubility constant for 20 nm particles with the Au was higher than that observed for pure Ag particles. At solution concentrations < 10 µg/ml, solution kinetics and rates appear to be less and would be expected from the behaviors at higher concentrations. Reasons for this difference are under study.

1964

Does Microfluidic Dispersion Influence the Toxicity of Geometric TiO2 Nanomaterials?

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Tunable chemical and physical properties of engineered nanomaterials are achievable by changing their geometry and morphology. Titanium dioxide (TiO2) based nanofilaments—nanotubes, nanorods, nanowires—have gained interest in sensing, electrical, and environmental fields due to their superior performance over TiO2 nanoparticles. Their application in the medical field for drug delivery, implant coating, and bioimaging is just now being realized, however their biocompatibility is not fully understood. Thus, safety assessment of geometric TiO2 nanomaterials is critical for protecting workers, patients, and bystanders as these technologies become widely implemented. The tendency of TiO2 based nanofilaments to aggregate and agglomerate make toxicity results controversial because of the challenge in differentiating whether the observed toxicity was caused by the nanofilaments or aggregates. TiO2 nanofilaments aggregate and are difficult to disperse homogeneously in solution by conventional methods, like sonication and vortexing. In this study, a microfluidic device was utilized to produce the stable, homogeneous dosing solutions necessary for in vitro toxicity evaluation by eliminating any toxicity caused by aggregated TiO2 nanofilaments. The quality of the dispersion provided by this method allows for toxicity results to be directly correlated to the TiO2 nanostructure itself. The biocompatibility of four TiO2 nanogeometries—nanotubes, nanorods, nanowires, and nanopapers—were assessed in nasal epithelial cells (RPMI 2650). All TiO2 based nanomaterials dispersed by the microfluidic method were biocompatible in RPMI 2650 cells at the concentrations tested. Whereas 100 µg/ml concentrations of nanowires and nanotubes dispersed by sonication reduced viability up to 27%, indicating that in vitro toxicity results may be controlled by the dispersion of dosing solutions.

1965

Low-Level Exposure to Silver Nanoparticles-Induced Hypertrophy, Multinucleation, and Senescence in Lung Epithelial Cells

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Nanotechnology is a rapidly expanding discipline focused on manipulating materials at the nanometer scale. Considerable research has focused on the antibacterial properties of silver nanoparticles (AgNPs), resulting in several commercial products. The bactericidal activity of AgNPs stems from shedding of silver ions and their binding critical biomolecules. The toxic activity in mammalian cells is proposed to derive from oxidative stress caused by the particles and the ions released. In addition, AgNPs may reside in biosystems, producing long-term stress directly or through slow dissolution to ions. As a result, AgNP exposure raises concern over environmental and human health effects. Long-term stress and oxidative damage can induce cellular senescence, or permanent growth arrest. This project tested the potential for AgNPs to induce senescence in A549, epithelial cells during extended exposure at a sub-lethal concentration. Cells were exposed to AgNPs for 1 to 4 days at 10 µg/ml, a level that did not cause overt toxicity by MTS assay but caused oxidative stress measured using a Dichlorofluorescein probe. After 3 to 20 days of recovery, induction of senescence was assessed by observing cell morphology, measuring proliferation, and testing for senescence-associated β-galactosidase (SA-β-Gal) activity. AgNPs caused an increase in the number of hypertrophic cells and cells with SA-β-Gal activity, indicating senescence was induced. Proliferation was relatively unaffected suggesting that many A549 cells were able to subvert full growth arrest; however most hypertrophic cells were multinucleate implying that they could not complete cytokinesis. Taken together, these results indicate that AgNPs induced a senescence-like phenotype in A549 cells despite their resistance to growth arrest.

1966

Evaluation of Tungstate Nanoparticle Cytotoxicity

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Alkaline-earth metal tungstate AWO3 (A = Ca, Ba, Sr) nanoparticles are currently being used in a variety of applications, including use as components of medical equipment, optical fibers, and scintillator detectors. This versatility may lead to increases in manufacturing within the next 10 years and may subsequently result in more cases of occupational exposure. Therefore, it is important to assess the effects of tungstate nanoparticles on cellular systems. RAW 264.7 macrophage cells were used to assess tungstate nanoparticle toxicity and changes in reactivity based on shape (sphere vs. wire), size, and chemistry. Enhanced dark field microscopy and scanning electron microscopy were used to evaluate nanoparticle-cell association over multiple time points up to 7 hours. To assess uptake, transmission electron microscopy was implemented. Both wires and spheres showed cell surface interactions; however, only spheres were engulfed. To assess intracellular reactive oxygen species (ROS) production, a DCFH assay was performed. Results showed that nanowire-exposed cells had significantly increased levels of ROS over a 7 hour time-course, while nanosphere-treated cells did not. This may be a result of association versus engulfment. Based on ROS production and cell-particle interactions, overall cellular cytotoxicity was measured. A caspase activation assay was used to assess apoptosis, and an MIT assay was employed to determine cell viability. Minimal caspase activity was measured after 24 hours with both spheres and wires. Wire-cell interactions resulted in cell death after 24 hours and sphere treated cells had minimal changes in viability. This data shows that tungstate nanoparticles are not explicitly toxic; however, wires appear to be more reactive than spheres, and have an initial, but manageable toxic effect on cells.

1967

In Vitro Cytotoxicity Assay of TiO2 Nanoparticles in Pulmonary Epithelial and Macrophage-Like Cells

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Recent advances in nanotechnology have increased the development and production of many new nanomaterials, and this raises concerns about possible human health risk. To establish alveolar cell-based assay systems, human epithelial and macrophage-like cell lines (A549 and THP-1) were used. First, we investigated the A549 cell viability after exposure to the nanoparticles (721±TiO2) at various initial densities. When the cells are cultured at a low density, the drastic decrease of viability (50%) was observed. In contrast, when the cells are cultured at a high density to form cell monolayer, the viability decreased only slightly. In addition, in cocultures of A549 monolayer and THP-1, there were no viability changes, presumably because particles are firstly phagocytized by THP-1 or macrophages. These results show that we have to carefully choose the culture conditions according to the objectives such as screening with high sensitivities or physiological relevancy.

Second, nanoparticles permeation was investigated using A549 and THP-1 cell-based alveolar model formed on semi-permeable membranes and relevant numerical simulation describing dynamic equilibrium among the apical side, alveolar cells, macrophages and basolateral sides. With biological kinetic parameters obtained in the cell-based assay, the numerical model largely described the concentration changes in the assay system. By changing some parameters such as scale of the model to overcome the limitations of existing culture models, it was indicated that the combination use of in vitro cell-based tissue models and numerical simulations made us possible to predict the permeation of particles through the alveolar tissue.
1968 Differential Mode of Nano-Bio Interaction by Graphene Nanomaterials

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The wide applications of Graphene nanomaterials and its derivatives have emphasized the need for further mechanistic insight to predict the consequences of environmental health and safety impacts of its exposures. However, little information is available on their toxicity and bio-compatibility. In this current study, we report the impact of differential functionalization of graphene in the interaction with HepG2 cells. We performed a comprehensive study on the toxicity of graphene oxide (GO) and reduced graphene oxide (rGO) on human hepatoma cells (HepG2) and two different kind of GO- few layer graphene (FLGO) and single layer graphene (SLGO), three kind of graphene nanoplatelets (GNP), pristine GNP, GNP-NH2, GNP-COOH on human bronchial epithelial (Beas2B) cells. We used the standard toxicity endpoints such as, cell viability, uptake, apoptosis, ROS formation, DNA damage etc. Furthermore, we also carried out the details of physio-chemical properties of graphene nanomaterials by using Raman spectroscopy, AFM, TEM and XPS etc. The results showed that the differential modes of nano-bio interaction of graphene nanomaterials are influenced by its chemical modification, layer number and functionalization which in turn would direct its further biomedical application. In summary, our results present a paradigm for analyzing surface functionality-activity relationship of graphene nanomaterials with tissue specific evaluation could eventually be utilized for more efficient and innocuous applications, specifically in biomedical field.

1969 Amorphous Silica Nanoparticle-Mediated Hepatotoxicity: A System Toxicology Approach


The wide applications of synthetically manufactured amorphous silica nanoparticles (aSiNP) range from daily life uses such as paints, varnishes, glue, desiccants, plastics, toothpastes, pharmaceuticals, food packaging and as food additives etc. and extended to diagnostic and biomedical fields such as bioimaging, drug and gene delivery, cancer therapy etc. However, the toxic potentiality and bio-compatibility of aSiNP remains to be elucidated. The present study aims to investigate the molecular mechanisms and pathways involved in aSiNP mediated toxicity with systems toxicology approach. The standard toxicity endpoints such as, cytotoxicity, DNA damage, ROS formation, significant modulations of antioxidants as well as DNA repair gene expressions were evident in aSiNP exposed HepG2 cells. Furthermore, the global gene expression changes and subsequent pathway analysis revealed that steroid, lipid and cholesterol biosynthesis pathways are mainly triggered due to aSiNP exposure at 100 mg/L for 24 h. In addition, aSiNP exposures also affect glutathione metabolism as well as xenobiotic and drug metabolism pathways in HepG2 cells. The observed changes in cellular oxidative stress and lipid/cholesterol biosynthesis pathway can be considered as the potential mechanism of aSiNP mediated hepatotoxicity. Our findings could eventually help in designing of future applications of aSiNP, specifically in drug delivery system and other biomedical purposes.

1970 Cerium Oxide Nanoparticles Air Exposure: A Comparison Study Using a Human 3D Airway Model and A549 and Beas-2B Cell Lines

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Human 3D airway models are fully differentiated and functional models of the respiratory epithelium and therefore may be positioned in safety evaluation of nanoparticles entering the airways. They are cultured at an air liquid interface (ALI), allowing relevant exposure via air. To investigate the respiratory effects of nanoparticles, we performed air exposures of nano-CeO2 and micro-CeO2 using MucilAir human 3D bronchial model and compared these to Beas-2B and A549 cell lines. We used the standard toxicity endpoints such as, cell viability, uptake, apoptosis, ROS formation, DNA damage etc. Furthermore, we also carried out the details of physio-chemical properties of graphene nanomaterials by using Raman spectroscopy, AFM, TEM and XPS etc. The results showed that the differential modes of nano-bio interaction of graphene nanomaterials are influenced by its chemical modification, layer number and functionalization which in turn would direct its further biomedical application. In summary, our results present a paradigm for analyzing surface functionality-activity relationship of graphene nanomaterials with tissue specific evaluation could eventually be utilized for more efficient and innocuous applications, specifically in biomedical field.

1971 Zinc Oxide Nanoparticles Induce Cytotoxicity in Human Endothelial Colony-Forming Cells

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Background: Metal oxide nanoparticles have been widely used in industry, cosmetics, as well as biomedicine. However, cardiovascular effect of exposure to metal oxide nanoparticles remains elusive. It is known that the endothelial progenitor cells (EPCs) contribute to postnatal endothelial repair and regeneration. Although recent studies indicated that, in humans and mice, the number of EPCs is decreased by exposure to PM2.5, the effect of metal oxide nanoparticles on EPCs remains unclear at the cellular level. The present study investigated the cytotoxicity, apoptosis and the tube formation induced by nano-sized zinc oxide (ZnO) particles using human endothelial colony forming cells (EPCs), that participate in postnatal vasculogenesis.

Method: EPCs were exposed to nano-sized and macro-sized ZnO for 24 hours. Cellular viability was determined by the MTS assay, which measures the reduction of [3-(4,5-dimethylthiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium (MTS) to formazan in viable cells. Apoptotic cells were counted by flow cytometry using Annexin V-FITC to stain apoptotic cells. For capillary-like tube formation assay, EPCs were placed in 24-well plates pre-coated with solidified Matrigel Matrix and cultured for 24 hours. Capillary-like tubular structures were photographed, and the number of incorporated EPCs in tubules was determined.

Results and Discussion: The 24-hour exposure to nano- and macro-sized ZnO reduced the cellular viability in a dose-dependent manner and increased apoptotic cells in EPCs. The functional capacity for tube formation of EPCs on Matrix gel was reduced in the cells exposed to nano- and macro-sized ZnO particles compared with the control cells. The study indicates that exposure to nano- and macro-sized ZnO particles may attenuate function of vasculogenesis in EPCs.

1972 Biological Evaluation of Ultrananocrystalline Diamond and Nanocrystalline Diamond Coatings

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Biomaterials with nanoscale surface features have been increasingly investigated for medical device applications in an effort to achieve desired tissue-material interactions. Ultrananocrystalline diamond (UNCD) and nanocrystalline diamond (NCD) thin films are distinguished by their grain size with UNCD having 2-5 nm grain size and NCD with grain sizes of 10 nm and above (up to 500 nm). Both UNCD and NCD exhibit material properties similar to those of natural diamond, including mechanical robustness, chemical inertness, biocompatibility, and excellent tribological properties, which make them ideal implant coating materials. Recently, UNCD and NCD have been investigated as coatings for orthopedic, dental, orthopaedic, and cardiovascular device applications. The aim of this study was to evaluate the in vitro biocompatibility of UNCD and NCD coatings and to determine if surface roughness and grain size affect cellular response. Thin films of UNCD and NCD with varying levels of surface roughness (grain sizes 5-350 nm) were deposited on silicon substrates using microwave plasma chemical vapor deposition (MPCVD). Scanning electron micrographs and atomic force micrographs revealed uniform films with different scales of surface roughness; Raman spectroscopy confirmed the presence of carbon bonding typical of diamond thin films. Cell attachment, morphology, viability, and proliferation responses of human bone marrow stromal stem cells to UNCD and NCD surfaces were evaluated. In vitro cell viability and proliferation data showed that UNCD or NCD films have no major adverse cytotoxic effects. No significant differences in cell viability or cell proliferation were noted among diamond films (UNCD or NCD) of different
nanoscale roughness. Our in vitro data shows that UNCD and NCD coatings have superior in vitro biocompatibility profiles irrespective of their roughness and grain size making them desirable for use in implantable medical devices.

**1973 Preparation, Characterization and Antimicrobial Activity of Eu3+-Doped Hydroxyapatite Nanopowders**

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Silver nanoparticles (Ag NP) are used in a wide range of consumer and medical products because of their antimicrobial properties. In vivo studies have demonstrated that Ag NPs translocate to distal organs, including the brain, following ingestion. Thus, it is essential to examine neuronal function as shown that Ag NPs affect neuronal activity though their unique size and characteristics. Numerous studies have shown that exposure to AgNPs can induce the cytotoxic and genotoxic effects on mammalian cells both in vivo and in vitro assays, implying that AgNPs may increase the risk of adverse health effects. In our study, PVP- and citrate- coated 50 nm AgNPs in Fischer’s 344 rat lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs. After 48 hr exposure using Cell Titer Blue and Neutral Red assays. These results indicate that higher concentrations of AgNPs can affect cell viability, their unique size and characteristics. Numerous studies have shown that exposure to AgNPs can induce the cytotoxic and genotoxic effects on mammalian cells both in vivo and in vitro assays, implying that AgNPs may increase the risk of adverse health effects. In our study, PVP- and citrate- coated 50 nm AgNPs in Fischer’s medium were shown to be stable at 37 °C using NanoSight and TEM analysis. DNA damage caused by 50 nm AgNPs was evaluated in vitro using hOGG1 and Endonuclease III-modified Comet assays. The LS174T/Tek-l−/−; 3.7C mouse lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs coating with PVP or citrate. The results indicated that the percentage of DNA in the tail (% tail DNA) was increased in LS174T/Tek-l−/−; 3.7C mouse lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs coating with PVP or citrate. The results indicated that the percentage of DNA in the tail (% tail DNA) was increased in LS174T/Tek-l−/−; 3.7C mouse lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs coating with PVP or citrate. The results indicated that the percentage of DNA in the tail (% tail DNA) was increased in LS174T/Tek-l−/−; 3.7C mouse lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs coating with PVP or citrate. The results indicated that the percentage of DNA in the tail (% tail DNA) was increased in LS174T/Tek-l−/−; 3.7C mouse lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs coating with PVP or citrate. The results indicated that the percentage of DNA in the tail (% tail DNA) was increased in LS174T/Tek-l−/−; 3.7C mouse lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs coating with PVP or citrate. The results indicated that the percentage of DNA in the tail (% tail DNA) was increased in LS174T/Tek-l−/−; 3.7C mouse lymphoma cells were treated for 4 hours with different concentrations (10, 20, 35 and 50 µg/ml) of 50 nm AgNPs coating with PVP or citrate.
rived retinal pigment epithelial cells (ARPE-19) after UV exposure. CeO2 NP samples (Alfa Aesar, 36-99nm; NanoAmor, 6-60nm) were suspended in cell culture media with 10% fetal bovine serum (FBS) at concentrations: 0, 3, 10, 20, 30, 55, 100 or 200µg/mL TiO2 NP (Degussa P25; positive control) and administered to ARPE-19 cells grown in 24-well plates. Plates were either exposed to UV irradiation (90min) or kept in the dark. After 24hrs, cell viability was determined with a calcein/propidium iodide stain. Exposure to higher concentrations of CeO2 NP and exposure to UV light (290-400nm) reduced cell viability in CeO2 NP compared to dark plates. When a 2.5% CuSO4 solution filtered restricted light exposure to the UV (320-400nm) range, there was no difference in viability between UV-exposed cells and dark controls. The results showed significant effects of irradiation (F(2,165) = 3.87, p = 0.02) and between the cerium samples (F(2,165) = 1.91, p = 0.001). CeO2 NanoAmor participated in phototoxic reactions with UVB, but not UVA wavelengths. Both CeO2 NP samples were less potent phototoxant than TiO2 NP. Dark-field microscopy and flow cytometry light scatter confirmed CeO2 NP uptake into ARPE-19 cells in a dose-dependent manner. At concentrations higher than 3 mg/mL CeO2 NP formed visible intracellular agglomerates, which were spatially arranged around the nucleus and presumed to be associated with the endoplasmic reticulum and mitochondria. In summary, NanoAmor showed a more phototoxic response than UV irradiation (290-400nm) than Alfa Aesar, but was less phototoxic than TiO2. This does not reflect EPA policy.

### 1973e Comparing Bioactivity Profiles of Diverse Nanomaterials Based on High-Throughput Screening (HTS) in ToxCast

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Most of the over 2800 nanomaterials (NMs) in commerce lack hazard data. Efficient NM testing requires suitable toxicity tests for prioritization of NMs to be tested. The EPA’s ToxCast program is evaluating HTS assays to prioritize NMs for targeted testing. Au, Ag, CeO2, Cu(O2), TiO2, SiO2, and ZnO nanoparticles, their ion and micro counterparts, carbon nanotubes (CNTs), asbestos, and pesticides containing nano-Cu(O) - total 62 samples - were screened at 6 -10 concentrations each. A total of 262 bioactivity/toxicity endpoints in cells and zebrafish embryos were measured. Cellular stress and immune response pathways were primarily affected. NM’s core chemical composition was more important than size for bioactivity. NMs had similar profiles as their ion counterparts, suggesting ion shedding was a key factor in mechanism of action. Ag, Cu, and Zn (nano, ion) were more cytotoxic and active in more assays than others. While 3 asbestos samples had similar immune response profiles, 6 CNT’s had profiles distinctive from asbestos. Potential bioactivity targets that were not directly measured were suggested by reference profiles similar to our data, e.g. similar profiles of a microtubule stabilizer interfering with mitosis and our nano-TiO2. Dividing endpoints into cytotoxicity and various functional categories, we developed a ToxCast-based ranking approach for in vitro bioactivity. Samples active in more categories at lower concentrations were ranked higher than samples active in fewer categories and/or at higher concentrations. Ag, Cu, and Zn samples were ranked as high in vitro bioactivity as Au, CeO2, in some CNTs, and some TiO2 samples were ranked as low. Recognizing our assays using submerged cells may be poor models for inhalation effects, this ranking may be more appropriate for oral effects. We demonstrated that HTS assays can identify affected cellular pathways, predict targets, and may be useful for ranking NMs for specific purposes. This abstract does not necessarily reflect EPA policy.

### 1973f Silver Nanoparticles Induce Antiproliferative Effects on Airway Smooth Muscle Cells: Role of Nitric Oxide and Muscarinic Receptor Signaling Pathway

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Silver nanoparticles (AgNPs) are used to manufacture materials with new properties and functions. However, little is known about their toxic or beneficial effects on human health, especially in the respiratory system, where its smooth muscles (ASM) regulates the airway contractility by different mediators, such as acetylcholine (ACh) and nitric oxide (NO). The aim of this study was to evaluate the effects of AgNPs on ASM cells. Exposure to AgNPs induced ACh-independent expression of the inducible nitric oxide synthase (iNOS) at 100µg/mL, associated with excessive production of NO. AgNPs induced the muscarinic receptor activation, since its blockade with atropine and blockade of its downstream signaling pathway inhibited the NO production. AgNPs at 10 and 100µg/mL induced ACh-independent prolonged cytotoxicity and decreased cellular proliferation mediated by the muscarinic receptor-iNOS pathway. However, the concentration of 100µg/mL of AgNPs induced muscarinic receptor-independent apoptosis, suggesting the activation of multiple pathways. These data indicate that AgNPs induce prolonged cytotoxicity and anti-proliferative effects on ASM cells, suggesting an activation of the muscarinic receptor-iNOS pathway. Further investigation is required to understand the full mechanism of action of AgNPs on ASM under specific biological conditions.

### 1973g Laser 3D Printing with Nanoscale Resolution: Improving Biocompatibility and Mitigating Toxicity from Photoinitiators

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Recent developments in laser-assisted 3D printing using two-photon polymerization (2PP) allow for previously unattainable submicron resolution and show promise for creating 3D cell scaffolds for regenerative medicine and tissue engineering applications. A significant barrier to using 2PP for biological applications exists due to the toxicity of photoinitiators required for polymerization. The goals of this study were to 1) demonstrate two approaches for creating nanotextured, porous 3D scaffolds using 2PP, and 2) develop a model system to evaluate in vitro cell responses, such as cell growth, protein adsorption, and toxicity, to nanoscale variations on material surfaces or to residual chemicals used in scaffold fabrication. The first approach involves printing a 3D scaffold from a urethane diacrylate-based elastomer, removing residual toxic substances and seeding the cell. The second approach, involving trapping cells directly in a methacrylamide-modified gelatin matrix. Results from the first method indicate that stable scaffolds with porosities of over 60% can be custom printed to fit standard 96-well plates. Human bone marrow stromal cells grown on 3D scaffolds exhibited increased growth and proliferation compared to smooth 2D scaffold controls. Scaffolds adsorbed larger amounts of proteins due to a greater surface area and allowed cells to attach in multiple planes and infiltrate the porous scaffolds. Results from the second approach indicated some dead MG63 osteosarcoma cells and L929 fibroblasts in encapsulated regions. In order to mitigate photoinitiator toxicity, 3 antioxidants -Trolox (water-soluble vitamin E), vitamin C, and glutathione – were used. Preliminary results demonstrated decreased cytotoxicity. The data indicate that 2PP is a promising technique for fabricating custom 3D scaffolds, including the potential for cell encapsulation.

### 1973h In Vitro Penetration of Polyethylene Glycol- and Citrate-Coated Silver Nanoparticles into Human Skin

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Silver nanoparticles (AgNPs) are used as an antimicrobial in a variety of cosmetic products. There are concerns about the potential safety associated with human skin exposure. This study was conducted to investigate the in vitro penetration of neutrally charged polyethylene glycol (PEG) and negatively charged citrate (CIT) coated 20 nm AgNPs into excised human skin. AgNPs were first characterized to confirm the size, shape, charge, agglomeration state and stability of the particles. Circular punches of dermatomed human cadaver skin from two donors were placed into flow-through diffusion cells, and 0.01% and 0.001% aqueous solutions of PEG and CIT coated AgNPs were applied once to the surface of the skin in duplicate. These concentrations were chosen as they were relevant to current commercially available cosmetics. After 24 hours, the skin samples were washed with a 1% soap solution and distilled water, removed from the diffusion cells, and tape-stripped twice to remove unabsorbed surface AgNPs. Each skin disc was prepared for analysis by taking half for inductively coupled plasma mass spectrometry (ICP-MS) analysis and the other half for Transmission Electron Microscopy (TEM). Preliminary ICP-MS analysis revealed an increase in silver content (ng silver/g of skin) of PEG and CIT samples at both concentrations when compared to untreated control skin. There was a 20-fold and 9-fold increase in silver content of the 0.01% PEG and CIT AgNPs samples compared to the 0.001% treatments, respectively. TEM images of skin samples coincided with ICP-MS results, with 20 nm CIT and PEG coated AgNPs localized in the stratum corneum and upper epidermis. Higher concentrations of AgNPs solutions corresponded with increased skin silver content. This study indicates that human skin silver content increased after an application of neutrally and negatively charged aqueous AgNPs for 24 hours, and these particles may penetrate into the upper epidermis.
Dendrimers are highly branched stable polymeric nanoparticles with terminal functional groups capable of binding other molecules which could be used to increase delivery of chemicals to skin. The surface charge of the dendrimers may affect skin absorption of bound cosmetic ingredients. We previously evaluated the skin penetration of amine-terminated polyamidoamine (PAMAM) dendrimer nanoparticles. Alexa Fluor 568 (~1 equivalent per dendrimer) was conjugated to terminal amine on the dendrimers via amide bonds for confocal imaging. In this study, we examined the skin penetration of generation 3, generation 4, generation 5 and generation 6 PAMAM dendrimers further conjugated with succinic anhydride (negative surface charge). Free unconjugated fluorophore was removed by ultrafiltration followed by gel filtration, and characterized. Dendrimers were applied (0.2% concentration) in aqueous solutions or cosmetic emulsion formulation onto viable pig or human cadaver skin assembled in diffusion cells. After a 24 hour exposure, the extent of skin penetration was determined by laser scanning confocal microscopy. Most fluorescence from the applied dendrimers appeared in the stratum corneum (SC) or in hair follicles of both pig and human skin. Fluorescence appeared in the upper regions of the epidermis of pig skin with the small generation dendrimers using both the low (12.8 μl) and high (40 μl) volume solution applications. With the emulsion, most of the generations of various dendrimer species remained in the SC or penetrated pig skin through hair follicles. In human skin, small generation dendrimers penetrated skin with the low and high volume solutions and emulsion SC or penetrated pig skin through hair follicles. In human skin, epidermal penetration was not evident. PAMAM dendrimer nanoparticles were conjugated with Alexa Fluor 568 (~1 equivalent per dendrimer) and characterized. Glycidol dendrimers were applied (0.2% concentration) in aqueous solutions and disperse MWNT-7 into dry single fibers without dispersant and changes in fiber length and width distribution ("Taquann-dispersed" method). T-CNT was loaded in a newly designed cylindrical cartridge case and injected into the chamber by compressed air. Aerosol in the chamber consisted predominantly of well-dispersed single fibers showing length and width distribution similar to those of the original sample, and these single fibers were found in the alveolar spaces of mice exposed. This system is relatively inexpensive and optimal for testing inhalation toxicity of nanomaterial samples in well-dispersed, dispersant-free aerosol with minimum mechanical fragmentation. (Supported by the Health and Labour Sciences Research Grant, MHLW, Japan)
1976  Health Surveillance Study on MWCNT Manufacturing Workers
T. M. Sager1, 2, M. Wolfarth2, V. Castranova2 and A. Holian1.

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The present study investigated underlying mechanism of pulmonary fibrosis caused by exposure to ZnO nanoparticles, in a mouse model of pulmonary fibrosis induced by bleomycin (BLM). The mouse model was developed by constant subcutaneous injection of 100 mg/kg bleomycin sulphate using osmotic mini-pumps. Female C57BL/6J mice were divided into BLM and non-BLM groups. In each group, two doses (10, 30, 50 mouse) of ZnO nanoparticles were delivered into the lungs through pharyngeal aspiration. The lung was collected 10 days after administration under deep anesthesia. Exposure to ZnO nanoparticles dose-dependently increased the lung weight. Histopathologically, slight and severe thickness was observed within interalveolar septum in low and high dose groups, respectively, which was accompanied by dose-dependent increase in total cells, macrophages, lymphocytes and neutrophils in BALF. The increase in total protein in BALF at high dose indicated increased permeability of alveolocapillary membrane due to severe inflammation. ZnO nanoparticles increased lung stiffness, consistent with surfactant disruption 3 days post instillation, neutrophilia resolved in functionalized ZnO treated mice, but protein concentration increased vs control (0.2 ± 0.03 v 0.1 ± 0.02 µg/mg tissue protein).

1977  The Effects of Pharyngeal Aspiration-Exposure to ZnO Nanoparticles on Pulmonary Fibrosis Induced by Bleomycin in Mice
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The study investigated underlying mechanism of pulmonary fibrosis caused by exposure to ZnO nanoparticles, in a mouse model of pulmonary fibrosis induced by bleomycin (BLM). The mouse model was developed by constant subcutaneous injection of 100 mg/kg bleomycin sulphate using osmotic mini-pumps. Female C57BL/6J mice were divided into BLM and non-BLM groups. In each group, two doses (10, 30, 50 mg/mouse) of ZnO nanoparticles with primary diameter of 20 nm were delivered into the lungs through pharyngeal aspiration. The BALF was collected 10 days after administration under deep anesthesia. Exposure to ZnO nanoparticles dose-dependently increased the lung weight. Histopathologically, slight and severe thickness was observed within interalveolar septum in low and high dose groups, respectively, which was accompanied by dose-dependent increase in total cells, macrophages, lymphocytes and neutrophils in BALF. The increase in total protein in BALF at high dose indicated increased permeability of alveolocapillary membrane due to severe inflammation. ZnO nanoparticles increased lung stiffness, consistent with surfactant disruption 3 days post instillation, neutrophilia resolved in functionalized ZnO treated mice, but protein concentration increased vs control (0.2 ± 0.03 v 0.1 ± 0.02 µg/mg tissue protein).

1978  Effects of Nanoparticle Pre-Exposure Dispersion Status on Bioactivity in the Mouse Lung
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From a toxicology perspective, nanoparticles possess two features that promote their toxicity. The first involves physical-chemical characteristics, including particle surface area. The second is the ability of the nanoparticle to traverse cell membranes. These two characteristics are influenced by placing nanoparticles in liquid medium prior to animal exposure. Nanoparticles tend to agglomerate in suspension, making it difficult to accurately deliver them for in vivo or in vitro experiments. Thus, we hypothesize that the nanoparticle dispersion status will correlate with the in vivo bioactivity/toxicity of the particle. The proposed questions of this study are of great importance to the nanotechnology/toxicology community, namely the highly debated question of whether nanoparticle dispersion status (pre-exposure) is of importance. To test our hypothesis, nano-sized nickel oxide was suspended in four different dispersion media (PBS, dispersion medium (DM), Survanita, or Prulonristm). At each respective dose, well-dispersed and poorly dispersed (suspended) dispersion status were suspended utilizing a Branson Sonifier 450, 25W continuous output. Two min or 5 min, respectively) suspensions were created. Mice (male, C57BL/6J, 7-10 weeks old) were given 0-80 µg/mouse of nano-sized nickel oxide in the different states of dispersion via pharyngeal aspiration. At 1 & 7 days post-exposure, mice underwent whole lung lavage (WLL) to assess pulmonary inflammation and injury as a function of dispersion status, dose, and time. Results show that pre-exposure dispersion status correlates with particle bioactivity. In fact, the particle/media combination that produced the smallest hydrodynamic particle size (nano-NIO2 suspended in DM & sonicated for 20 min. = 7.5 nm) produced a greater increase in PMNs, LDH activity, as well as albumin levels in WLL fluid than the other nano-nickel/suspension/media combinations. These results indicate that a greater degree of pre-exposure dispersion increases particle bioactivity/toxicity in the lung.

1979  Effects of Multiwalled Carbon Nanotube Solubility on Inflammation and Lung Function
D. Botzel, A. Porter, F. Chung, T. Tetley, J. Zhang and A. Gowe.

London, United Kingdom. This work was supported by NIH grant F32 ES0021341.

Multi-walled carbon nanotubes (MWCNTs) have high potential, are relatively insoluble in aqueous solvents, and interact with hydrophobic materials. These properties are altered by functionalization. Due to complexity of MWCNTs it is important to study organ level effects. To study effects of MWCNTs on the lung we use a suspension method involving native surfactant. This method is effective at keeping MWCNTs in suspension despite functionalization. We hypothesized that increasing solubility of MWCNTs in the aqueous environment would shift the organ level response to inflammation from interaction with the lung lining fluid. C57Bl6J mice were intratracheally instilled with MWCNTs (30-50nm OD, 0.5-2mm length) at 1.5µg/g body weight. 2 types of MWCNT were examined—functionalized by carboxylation or acid-purified. 1 day post instillation, the functionalized MWCNT treated mice showed neutrophilia (147.1 ± 105.6/7 v 2.5 ± 0.92 x103) and increased cell count (196.5 ± 113.02 v 57.0 ± 8.25 x103) vs control. Purified MWCNT mice did not display significant inflammation (18.5 ± 15.62 v 2.5 ± 0.92 x103 neutrophil; 63.0 ± 15.09 v 57.0 ± 8.25 x103 total cells). Lung function was not affected by functionalized MWCNTs but purified MWCNTs increased lung stiffness, consistent with surfactant disruption 3 days post instillation, neutrophilia resolved in functionalized MWCNT treated mice, but protein concentration increased vs control (0.2 ± 0.03 v 0.1 ± 0.02 µg/mg tissue protein). Purified MWCNT mice do not show increased tissue stiffness. Both MWCNT treated groups demonstrated significant decrease in collectin content. This work is consistent with the idea of "revealing" the MWCNTs to inflammatory system and reducing effect on lung function. Supported by NIH Grants U19ES019536-02 & 5T32ES007148.

1980  Pre-Exposure to Nontoxic Levels of Magnetically Functionalized Nanoparticles Sensitizes Mice to Pulmonary Infection by Streptococcus pneumoniae

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Two major forms of iron oxide are preferred for medical applications due to their perceived biocompatibility. Pretreatment of cultured macrophages with non-cytotoxic doses of superparamagnetic iron oxide (SPIO) reprogrammed -500 genes in response to subsequent challenge by endotoxin, suppressing activation of both pro- and anti-inflammatory pathways and diminished bacterial phagocytosis (Kodali et al. 2013). Here, we characterize and confirm the potential impaired clearance of bacteria by mice pretreated with SPIO. We administered 0 or 1.0 µg SPIO (g bw to C57BL/6 by oropharyngeal aspiration (OPA) and evaluated the pro-inflammatory response by measuring cytokines in BALF collected 1-28 d post-dosing. No statistical changes in cytokine levels were observed at 1-28 d post-dosing confirming that...
Sodium nitroprusside (SNP; 5-20 μg/kg). After 24 h prior to intra-nasal installation of 50K colony forming units (CFU) of S. pneumoniae. The SNP doses were selected to represent the mouse equivalents of human occupational exposure for 1, 5, and 20 d at the Permissible Exposure Limit. The low pyrogenic/inflammatory potential of these exposures was confirmed by the absence of changes in surface body temperature. However, mice pretreated with 30 or 100 μg/kg SNP exhibited a 3.6- and 7.0-fold increase, respectively, in CFU recovered from the lung, indicating mice exposed to SNP had reduced lung clearance of S. pneumoniae. These studies suggest that the direct toxicity of SNP may be less important than those effects elicited by SNP interaction with other stressors. Supported by NIEHS NCNHR Consortium ES019544.

1981 A Comparative In Vivo Screening of Nine Classes of Multiwalled Carbon Nanotubes for Acute Lung Toxicity in Mice

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Multi-walled carbon nanotubes (MWCNTs) present potential health risks during their manufacture and handling in the electronics and medical industries. To improve understanding of MWCNT behavior following inhalation, we are investigating how aggregative characteristics, aspect ratio, surface carboxylation, and purity from trace metals affect MWCNT pulmonary toxicity in vivo. To characterize aggregative behavior, we used dynamic light scattering to assess the hydrodynamic radii and zeta potentials of nine classes of MWCNTs in dispersion medium (DM). In order to assess pulmonary toxicity, we exposed eight-week-old male A/J mice via oropharyngeal aspiration to DM vehicle or a single MWCNT class at 40 μg/mouse, and sacrificed the mice after 24 h. Treatment with unpurified and carboxylated MWCNTs was associated with lung inflammation as indicated by flow cytometric and cytospin analyses of neutrophil influx into bronchoalveolar lavage fluid (BALF). Across all MWCNT classes, we found no evidence of acute capillary barrier dysfunction or frank cellular damage as indicated by BALF supernatant total protein or lactate dehydrogenase activity. Similarly, no evidence of severe oxidative stress was detected in lung tissue as indicated by total glutathione levels. Our results indicate that high-dose exposure to unpurified and carboxylated MWCNTs causes acute lung inflammation but does not frank cellular toxicity in the short term. This work was supported by NIH Grants U19ES019544 and P30ES070033, and NSF grants CBET-0932885 and DGE-0718124.

1982 Inhalation of Nanosized Titanium Dioxide Alters Cardiovascular Autonomic Function

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Nanotechnology is a rapidly growing field with the potential to influence all aspects of modern life. However, the cardiovascular toxicity of nanomaterials is not well understood. We have previously demonstrated a disruption in normal microvascular function following nanomaterial exposure via alterations in autonomic control. Presently, using a telemetry and a baroreflex function model, we show changes in autonomic control following inhalation exposure to nanosized titanium dioxide (nano-TiO2) for 4 hours for 2 days at 6 mg/m3. Telemeterized rats were acclimated and exposed to sham (day 0), then nano-TiO2, or sham on 2 consecutive days (days 1, and 2). Renal sympathetic nerve activity (rSNA), mean arterial pressure (MAP) and heart rate (HR) were continuously monitored. 24 h after the last exposure, baroreflex sensitivity was measured with phenylephrine (PE; 1-8 μg/kg) or sodium nitroprusside (SNP; 5-20 μg/kg). After 24 h prior to intra-oral installation of 50K colony forming units (CFU) of S. pneumoniae. The SNP doses were selected to represent the mouse equivalents of human occupational exposure for 1, 5, and 20 d at the Permissible Exposure Limit. The low pyrogenic/inflammatory potential of these exposures was confirmed by the absence of changes in surface body temperature. However, mice pretreated with 30 or 100 μg/kg SNP exhibited a 3.6- and 7.0-fold increase, respectively, in CFU recovered from the lung, indicating mice exposed to SNP had reduced lung clearance of S. pneumoniae. These studies suggest that the direct toxicity of SNP may be less important than those effects elicited by SNP interaction with other stressors. Supported by NIEHS NCNHR Consortium ES019544.

1983 Endothelial Cells As Biosensors to Assess the Vascular Inflammatory Potential of Serum following Nanomaterial Exposure

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Assessing mechanisms underlying the adverse vascular health effects of systemic inflammation induced by inhaled toxins presents a substantial research challenge. We have developed an ex vivo model that allows for a realistic exposure method to better elucidate the mechanisms involved in a living system. This approach supplies serum from exposed cultures to primary endothelial cells, as this is the component in direct contact with the vascular endothelium. Here, we apply this assay paradigm to assess the impact of pulmonary exposures to multi-walled carbon nanotubes (MWCNT) or graphene. Mice were exposed to varying doses (4, 10 or 40 μg) of MWCNT, various types of graphene, or carbon black via pharyngeal aspiration, and serum was collected at 4 and 24 h post-exposure. Serum collected from 19 d inhalation exposures to 0.5 or 5.0 mg/m3 MWCNT was also assayed for endothelial activation. Serum from exposed mice induced an up-regulation of endothelial cell surface VCAM and ICAM expression, along with elevations in mRNA at the 4 h time point. Multiple sizes of graphene were tested; the smallest sizes (<2 μm x <2 μm x 1-2 nm and 5 μm x 5 μm x 7 nm) induced up-regulation of surface VCAM, but were overall less potent than carbon black, used as a control particle. Furthermore, we assessed nitric oxide (NO) generation by endothelial cells using electron paramagnetic resonance (EPR) methods, and found that NO was decreased via treatment with serum from MWCNT-exposed mice following stimulation with 2 mM ATP. Microarray analysis of endothelial cell response to serum from the inhalation and instillation exposures revealed a common set of response elements. In conclusion, pulmonary exposure to carbon-based nanomaterials alters circulating factors which promote endothelial cell activation and decreased NO bioavailability.

1984 Surface Amination Enhances the Toxicity of Silica-Coated Silver Nanoparticles

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Surface charge can greatly influence the toxicity and bioavailability of engineered nanoparticles (NPs). Positively charged NPs generally elicit greater toxicity than comparable negative or neutrally charged particles. The effect of amination on NP toxicity was investigated in embryonic zebrafish, a model vertebrate. Embryos were exposed to 0.5-100 ppm suspensions of 80nm silica (Si) or 70nm silver with 20nm Si-coating (AgSi), or aminated NPs of like size and composition. Surface amination significantly increased the toxicity of the NPs. Si NPs did not induce mortality or morbidity at the tested concentrations, whereas aminated Si induced 58% mortality at 100 ppm. Both AgSi NPs were significantly more toxic to embryos than Si NPs. AgSi NPs significantly delayed development at 24 hours post fertilization in 25 (50%), 50 (64%) and 100 (75%) ppm treatments, and caused significant mortality beginning at 100ppm. In comparison, aminated AgSi NPs delayed development significantly as low as 1 ppm and induced significant mortality at 5ppm, with 100% mortality above 50ppm. Both AgSi NPs induced significant sublethal effects, including craniofacial and fin malformations, edemas, and body curvatures. When similar silica coated Ag NPs with varied surface amination levels (0.5x, 1x, and 2x) were tested at the same concentrations, the AgSi-1x was significantly more toxic than the 0.5 or 2x AgSi NPs, inducing mortality at 100 (95%) ppm. In contrast, AgSi-2x only induced 38% mortality at 100 ppm, and AgSi-0.5x NPs did not cause toxicity at any concentration tested. Increased amination was observed to alter the stability of the aminated NP dispersions in the exposure media, with the 2x NPs being the most stable in suspension. Our results suggest that increasing surface amination only leads to increased toxicity when bioavailability is held constant, highlighting the importance of understanding NP stability and bioavailability during exposure.
1985 Establishment of an Inexpensive, In Vivo Screening Tool for Nanomaterial Safety Using the Adult Zebrafish
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Current toxicological assessment of the safety of nanomaterials is lagging well behind the synthesis and consumer use. This is largely due to the lack of a quality inexpensive model for toxicological assessment. Cell cultures are inexpensive and can provide some indications on biological properties, but they do not convey a complete account for cell-to-cell communication. Mammalian models can allow for a greater understanding of toxicology and biodistribution. However, adequate mouse studies can be cost prohibitive. This study examines the potential for an inexpensive alternative in vivo model using the adult zebrafish, Danio rerio, for assessment of nanomaterial immunotoxicology. Zebrafish have many homologous proteins shared with humans and other mammals and can be bred and maintained for very little cost. Having been completely sequenced, many biomarkers for stress, toxicity and inflammation have been identified in the zebrafish. This preliminary assessment has determined the extent of biodistribution, inflammation, and toxicity using 10 nm silver nanospheres and it has been determined that the nanomaterials do not cause mortality or significant morbidity after a one-time intradermal exposure at 10 or 50 μg/mL and can distribute throughout the fish as evidenced by differential effects on various target organs. Quantitative PCR, ICP-MS, and transmission electron microscopy where used to measure the biodistribution and inflammatory responses of the silver nanoparticles. All target organs had measurable amounts of silver nanoparticle detected and a mild inflammatory response at 24-hours post-inoculation was observed. The establishment of the adult zebrafish in vivo model for immunotoxicology could allow for an inexpensive comparative analysis of a wide array of nanomaterial sizes, shapes, and compositions for determination of their safety and distribution.

1986 Genetic Influence of Pulmonary Response to Silver Nanoparticles Exposure and Candidate Gene Identifications
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Within the last few decades, the use of nano-materials and products for multi-application uses has seen exponential growth, while information on the toxicity of these nano-materials is still largely unknown. This study evaluated the variation of response in numerous murine strains to examine genetic susceptibility to silver nanoparticles (AgNPs). Previously, 8 strains of inbred mice were acutely exposed via oropharyngeal aspiration to a dose of 0.25 μg/g body weight of 20nm silver citrate, with observed polymorphonuclear neutrophil (PMN) responses between 1.4-60% (DBA/2J< C57BL/6J< AKR/J< 129SI/SvJ< A/J< FVB/NJ< C3H/HeJ< Balb/cJ). Increased protein levels occurred most predominantly and significantly in the three most PMN-sensitive strains (FVB/NJ, C3H/HeJ, Balb/cJ). Cytokine analysis revealed significant differences in VEGF and MDC in FVB/NJ mice and RANTES in C3H/HeJ. Currently, in collaboration with the University of California, a total of 30 strains have been combined to assess genetic susceptibility via pulmonary inflammation and injury endpoints (PMN, protein, lactate dehydrogenase release, cytokine analysis) as well as haplotype association mapping for identifying candidate genes, responsible for the influence of genetics on the pulmonary response to AgNPs. Based upon previous and current findings on inter-strain response variation, these mouse models, representative of a genetically varied population, displayed a susceptibility pattern among strains. Ultimately, these findings further support the link between genetic background and the susceptibility of the link between sensitivity mechanisms and innate inter-species responses to AgNPs.

1987 Effects of Nanosilver and Silver Nitrate Exposure on Ionic Regulation and Nanoparticle Size Characterization Using Field-Flow Fractionation in Fathead Minnows
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Silver nitrate (AgNO3) and silver nanoparticles (AgNPs) damage and alter the fish gills after acute exposure. Ionic regulation in fish gills can be perturbed by AgNO3 exposure primarily due to the inhibition of Na,K-ATPase. One proposed mechanism of silver nanoparticle toxicity is from the release of silver ions; however, AgNPs can accumulate and have unique toxic effects on different biological targets. In order to understand the relationship between silver exposure and ionic regulation, fathead minnows were exposed for 96 hr to 20 nm PVP- or citrate-coated silver nanoparticles (PVP-AgNPs; citrate-AgNPs) or AgNO3 at two nominal concentrations (20 and 200 μg/L) or (2 and 6 μg/L), respectively. FHM gills were paraffin embedded, sectioned and immunohistochemistry was performed for the presence of Na,K-ATPase. Also, Na,K-ATPase activity was measured in fish gills and GI tract in order to further understand the effects of the Ag-NPs on ionic exchange. The distribution of silver was quantitated following acid digestion and ICP-MS analysis. No detectable silver accumulation occurred in the brain. Exposure to 6 μg/L AgNO3 produced the highest silver bioaccumulation in the skin, liver, and gill, while exposure to 200 μg/L citrate-AgNPs produced the highest bioaccumulation in the GI tract. AgNP size was measured in GI tract and gill tissue after exposure using field-flow fractionation (FFF) interfaced to ICP-MS. AgNPs detected in GI tissue displayed heteroaggregation with particle sizes ranging from 20-70 nm, while AgNPs in gill tissue were less aggregated with particle sizes from 20-40 nm. FFF data indicated that AgNPs remained in particle form after uptake which may be related to their differential tissue accumulation and toxicity compared to ionic silver. This research is supported by US Army ERDC grant: W912HZ-09-C-0033.

1988 Unique Particle Effects in Fathead Minnow Gill Gene Expression and Uptake following Exposure to Nanosilver and Silver Nitrate
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Silver exposure is toxic to fish due to disturbances of normal gill function. A proposed toxicity mechanism of silver nanoparticles (AgNPs) is derived from the release of silver ions, similar to silver nitrate (AgNO3). However, AgNPs can have unique toxic effects. To determine if differences between AgNO3 and AgNP toxicities exist, fathead minnows (FHM) were exposed to 20 nm PVP- or citrate-coated silver nanoparticles (PVP-AgNPs; citrate-AgNPs) at the nominal concentration of 200 μg/L or AgNO3 at nominal 6 μg/L for 96 hr (n=5). Gills were dissected and microarray analysis was performed on the gills. Hierarchical clustering of FHM gene expression results revealed that all the control, AgNO3, and PVP-AgNP exposed fish clustered by treatment. In citrate-AgNP exposed fish, 67% clustered uniquely together but 33% clustered with the AgNO3 exposed fish. There were 115 differentially expressed genes shared among all Ag treatments compared to controls. However, there were 481, 406, and 715 differentially expressed genes unique to AgNO3, PVP-AgNP, and citrate-AgNP, respectively. In addition, ToxoList and IPA analysis indicated several common toxic endpoints such as oxidative stress and cell death consistent with silver toxicity; however, this analysis also indicated some unique pathways between nanosilver and AgNO3. Silver bioaccumulation was quantitated in the skin matrix, liver, GI and brain. Highest accumulation of silver following AgNO3 treatment was observed in skin and liver (84 and 1,500 ng/g, respectively). The highest accumulation of silver following AgNP treatment was observed in the GI tract (24,000 and 19,000 ng/g for PVP-AgNP and citrate-AgNP, respectively). These results indicate that there was differential silver accumulation and gill gene expression depending on exposure to AgNO3 or AgNPs. Supported by ERDC grant: W912HZ-09-C-0033.

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Silver nanoparticles (AgNPs) are used for their antimicrobial activity in a wide range of applications and consumer products. Some of the uses of AgNPs, such as in food packaging, likely result in ingestion. In addition to the possibility of direct toxicity from ingested AgNPs, toxicity could also occur indirectly through disruption of normal gastrointestinal microbiota. In this study, male non-human primates (Macaca fascicularis) (n=5) were given 20 nm AgNPs in 10mL of Gatorade (62.5 μg/mL) once daily for 8 days. AgNPs were characterized with respect to primary particle size and agglomeration state both as received and in the Gatorade dosing vehicle. Feces were collected on the day before the first dose and the day after the last dose, as well as 1 week after treatment stopped. On a separate occasion, the same animals received a control treatment (Gatorade only) and feces were collected on the same schedule. High throughput 16S rRNA sequencing was used to determine if AgNP treatment had an influence on the overall microbiota diversity and prevalence of specific operational taxonomic units (OTUs). When

PS 524 SOT 2014 Annual Meeting
investigating overall diversity at the phylum level no significant differences were observed. However, when analyzing specific OTUs at the 98% similarity level, we observed more than 30 in which the number of sequences increased upon nanoparticle exposure but only three in which sequence numbers decreased. In contrast, the number of OTUs with significant increases/decreases was similar for the vehicle-control treatment. This observation suggests that AgNPs select for, rather than against particular gut bacteria. These results demonstrate that AgNPs affected gut microbiota under the conditions of the study. Prediction of potential corresponding health impacts will require additional analysis of the data.

1992 Repeated-Dose Toxicity Testing of 9 Nanoparticles with Different Surface Functionalizations
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Oral uptake of nano-materials from food or cosmetics by consumers is likely to occur at low doses over long periods of time. To date, only few reports on in vivo effects of nano-materials upon subacute or subchronic oral exposure are available. The majority of these investigations address the effects of silver nanoparticles and none of these reports any non-transient toxicologically relevant effects. Here, we present the results of two subacute oral toxicity studies in Wistar rats employing nine nano-materials with different surface functionalizations. The primary particle size was 10 nm (ZrO2), 15 nm (SiO2), 30 nm (BaSO4, OECD NM220) and 50 nm or 200 nm (Ag). The particles were coated with acid-, amino-, PEG-, acrylic- and electro-steric-functionalities. The nano-materials were applied as suspensions via oral gavage at limit dose for 28 days. The studies were performed according to the OECD test guideline (TG) 407 including clinical observations, clinical pathology and (histo-)pathology. In addition to the basic haematological and clinical chemistry parameters, the acute phase proteins haptoglobin and alpha-2-macroglobulin as well as the protein tropinin I were determined. Furthermore, mass spectrometry-based metabolite profiling was performed in serum samples making use of the MetaMap®Tox database to determine and assess metabolite patterns. Test substance-related adverse effects were not observed with any of the nine nanomaterials. Although tested at limit dose, no changes during clinical examinations, clinical pathology or pathology parameters were observed. The determination of acute phase proteins as well as tropinin I did not reveal treatment-related effects. Furthermore, there were no matches with specific toxicity patterns in the MetaMap®Tox.

1993 Comparative Biochemical and Histopathological Evaluation of PEG-Coated and Noncoated Gold Nanoparticles in Sprague-Dawley Rats
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Nanoparticles (NPs) offer a great possibility for biomedical application, not only to deliver pharmaceuticals, but also to be used as novel diagnostic and therapeutic approaches. Currently, there are no data available regarding to what extent the degree of the toxicity and the accumulation of gold nanoparticles (GNPs) are present in in vivo administration. The aim of this study was to assess the effects, after oral administration, of poly-ethylene glycol (PEG) coated and non-coated GNPs on various hepatotoxicity markers such as alanine (ALT), aspartate aminotransferase, alkaline phosphatase, induction of reactive oxygen species and histopathological analysis in the mouse model. Sprague-Dawley rats were exposed to four different concentrations of PEG-coated and non-coated GNPs (12.5, 25, 50 and 100 µg/kg BW) and a control. Samples were collected 24 hours after the last treatment following standard protocols. Exposure to PEG-coated and non-coated GNPs enhanced the activities of serum amino-transfereases (ALT/AST), alkaline phosphatases (ALP) and concentration of lipid hydroperoxide compared to control. Histopathology of exposed liver showed a statistically significant effect in the morphological alterations of the tissue compared to controls. However, PEG-coated GNPs demonstrated enhanced hepatotoxic effect than non-coated GNPs. The cellular findings reported here do suggest that both GNPs has the potential to induce hepatotoxicity in Sprague-Dawley rats through activation of the mechanisms of oxidative stress, which is of sufficient significance to warrant in vivo animal exposure studies. However, more studies to clarify the role of encapsulation in the in vivo toxicity of GNPs, are required and parallel comparison is preferred.
1994 The Effects of Metal Oxide Nanoparticles on Angiogenesis in Transgenic Zebrafish

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The present study investigated the effects of exposure to metal oxide nanoparticles on the angiogenesis using transgenic (TG) zebrafish. The study also examined the potential mechanisms that may be involved in those effects using human umbilical vein endothelial cells (HUVECs). TG (nacre/fli1:EGFP) zebrafish were exposed to nano-sized titanium dioxide (TiO2), silica dioxide (SiO2), and copper oxide (CuO) particles at the concentrations of 0.01, 1 and 100 μg/mL each (day-post-fertilization) to 5 dpf. Angiogenesis were evaluated morphologically at the end of the exposure.

Exposure to CuO particles reduced the number of subintestinal vessels running transversely in TG zebrafish. In zebrafish exposed to CuO particles, the expression of vascular endothelial growth factor (VEGF) and VEGF receptor was down-regulated in the endothelial cells separated by Fluorescence Activated Cell Sorter (FACS). Moreover, HUVECs exposed to CuO particles showed reduced expression of the cell viability and increase in the apoptotic index in a dose-dependent manner. The study suggested that nano-sized CuO particles inhibit the angiogenesis due to reduction of VEGF expression and induction of apoptosis.

In Vivo Infection of Chorioallantoic Membrane by Lung Adenocarcinoma Cells Exposed to Titanium Dioxide Nanoparticles

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Background. Titanium dioxide nanoparticles (TiO2 NPs) is one of the most produced nanomaterials around the world and it has been classified by the International Agency for Research in Cancer as a possibly carcinogenic to human (Group 2B). In occupational settings, TiO2 NPs exposure induces several cellular alterations; however, its cell involvement has less investigated.

Aim. We hypothesized that human tumor cells can acquire a more invasive phenotype after TiO2 NPs exposure. To test this hypothesis, lung adenocarcinoma A549 cells were exposed to nanoparticles of TiO2 NPs (1, 5 and 10 μg/cm2) and nanobelts of TiO2 NPs to 10 μg/cm2 for 7 days. Then, 30 000 cells were mixed with collagen type I and placed on top of chicken chorioallantoic membrane (CAM) of leghorn white chicken eggs, which were previously incubated during 11 days at 37°C and 80% humidity. After 5 days, CAM was harvested and sectioned (6 μm) to perform hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) of cytokeratin 8, Gli1 and Ki67 in order to localize the human tumor cells in the CAM.

Results. Transmission electronic microscopy of TiO2 NPs nanospheres and nanobelts showed agglomerates of 588 nm and 454 nm respectively, in cell culture media plus 10% serum fetal bovine with zeta potential of -21 mV and -27 mV, respectively. H&E and IHC showed that the A549 cells treated with both shapes of TiO2 NPs had higher capacity to invade, detected by cytokeratin 8, and to proliferate detected by Gli1 and Ki67 in the CAM.

Conclusion. A549 cells treated with TiO2 NPs were more invasive and had higher capacity to proliferate in comparison with untreated cells in the CAM.

1997 Secondary Mechanisms Drive silica Nanomaterial Genotoxicity As Shown by the OGG1 Comet Assay and Genomic Analysis

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The safety profile and potential mode(s) of action (MoA) by which nanomaterials (NMs) may induce genotoxicity are not fully understood. Using amorphous silica (15nm Levalus® 200) at 50mg/kg, we examined a range of biomarkers after a single i.v. injection to male Wistar rats. Two studies were conducted; the first examined a wide range of time points; 4, 8, and 24h and second study investigated further early responses at 1, 2 and 4h. The second study also included an additional positive control, the model oxidant KBrO3. No significant increases in direct DNA damage were detected in the liver using the alkaline comet assay at any of the time points examined (2, 4, 8, and 24h). However, in the second study there was a small, but significant, increase in oxidative DNA damage at the 4th time point measured by the OGG1 modification of the comet assay in animals treated with the silica NM, while the positive control showed a strong increase in oxidized bases in the liver and the kidney (a target organ of KBrO3). Liver tissue was also analyzed for the transcriptional expression of genes by microarray analysis. Genes involved in responding to oxidative stress (Ho-1), inflammation (Ccl2), DNA damage (p53) and immune challenges (Nkib1/p105/p50) were all upregulated in animals treated with silica NMs beginning at the earliest time points measured, 1 and 2h. These responses peak at 4h (Ho-1) or 8h (Ccl2, Nkib1, p53). In contrast, the KBrO3 treatment did not trigger an increase in Ccl2 while the other genes were increased in a highly significant manner. These data are supported by the patterns evident in heat maps representing genes involved in regulation of the pathways mentioned above. These results are in line with previous data linking moderate DNA damage caused by silica NM with a tissue damaged mediated inflammatory response (Downs et al, Mutat Res, 745 (2012) 38-50), while our model oxidant KBrO3 showed strong DNA oxidative damage.

1998 Generation of Titanium Dioxide Nanoparticle Aerosols for Biodisposition Kinetic Assessment

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The intensive use of titanium dioxide nanoparticles (TiO2 NPs) in industrial products makes it one of the most produced NPs worldwide. Risk assessment studies are necessary, but limited data are available on NP disposition after inhalation. This study focused on the generation of TiO2 NP aerosols and their disposition kinetics in rats.

Adult male Sprague-Dawley rats were exposed acutely by inhalation {head-nose only} to 15 mg/m3 of commercial anatase TiO2 NPs (~20 nm) during 6 h. Blood was withdrawn at 0, 2, 4, 6, 24 and 48 h post-dosing (n = 6 rats per time point) and rats were sacrificed at 0, 1 and 2 days after onset of exposure (n=6 rats per group). Aerosols were generated with a collision 6-jet nebulizer and granulometry and concentrations were determined in real time using SMPS, CPS and Dusttrak. Ti levels were quantified by ICP-MS in blood, tissues (lungs, liver, kidneys, spleen), and excreta (urine, feces). Physiological parameters were also monitored.

Stable concentrations of poorly-agglomerated TiO2 NP aerosols were generated, with a mean aerodynamic diameter of 72 nm. Highest Ti concentrations were withdrawn at 0, 2, 4, 6, 24 and 48 h post-dosing (n = 6 rats per time point) and rats were sacrificed at 0, 1 and 2 days after onset of exposure (n=6 rats per group). Aerosols were generated with a collision 6-jet nebulizer and granulometry and concentrations were determined in real time using SMPS, CPS and Dusttrak. Ti levels were quantified by ICP-MS in blood, tissues (lungs, liver, kidneys, spleen), and excreta (urine, feces). Physiological parameters were also monitored.
Ti concentrations was quickly observed in the first 24 h post-dosing; after 24 h, Ti was non-detectable in blood. No significant increases in kidney concentrations of Ti were observed post-dosing. Significant increases in Ti concentrations were observed in liver and spleen 24 h post-dosing compared to pre-exposure levels, but not at 48 h (mean of 107 and 75 ng/g liver 24 and 48 h post-dosing versus 45 ng/g pre-exposure, corresponding values in spleen of 53, 76 and 29 ng/g). Significantly higher Ti concentrations were also found in feces post-dosing (on average 139 and 80 μg/g at 24 and 48 h versus 31 μg/g pre-exposure). No change in organs or body weights was observed through time. Further works are in progress to generate different aerosol exposure conditions.

1999 Impact of Liposome-Induced Complement Activation on Tumor Growth and Angiogenesis

Rationale: Acute infusion reactions in response to Doxil® (pegylated liposomal doxorubicin; PLD) are mediated by anaphylatoxins (C5a) generated from liposome-induced complement (C)-activation. Since C5a was reported to promote tumor growth through recruitment of myeloid derived suppressor cells (MDSCs), we postulate that liposome induced C-activation can promote tumor proliferation and lead to treatment resistance. Thus, we conducted studies to determine impact of this carrier on tumor progression.
Methodology: C57BL/6 mice bearing syngeneic TC-1 flank tumors were treated with a single dose (n= 10) or 4 weekly doses (n = 9) of non-drug loaded liposome (L IPO; identical to PLD carrier) or vehicle. Tumor volume was monitored and at endpoint tumors were processed for single cell suspension, tissue lystate, and frozen section. Cells were stained for MDSCs (CD11b+Gr1+) and analyzed by flow cytometry. C-activation (C3b/iC3b/C3c) was quantified in lysate via indirect ELISA. Tumor sections were stained for vasculature (CD31) and microvessel density in whole sections was quantified using digital pathology and image analysis algorithms.
Results: Tumor volumes were 2-fold larger in LIPO treated mice than in controls, 653 ± 144 and 218 ± 58 mm3, respectively (p<0.05). Tumors in LIPO treated mice had higher microvessel density than controls, 20.8 ± 0.1 and 15.0 ± 0.1 vessels/mm2, respectively (p<0.01). In LIPO versus control groups, there was increased C-activation (8.8% and 3.9% of positive reference, respectively) and MDSC infiltration (2.6% and 0.5%, respectively) in tumors.
Conclusions: Contrary to the prevailing paradigm that they are inert drug carriers, our data showed that liposome induced C-activation can promote tumor growth and angiogenesis through increased tumor recruitment of MDSCs. Ongoing studies will clarify this mechanism of resistance and develop strategies for mitigating unwanted immune effects to enhance antitumor efficacy. These results will have major implications for the future development of anticancer liposomes.

2000 The Comparative Effects of Nanoporous and Colloidal Silica Nanoparticles in Atopic Dermatitis
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Nanoparticles (NPs) are incorporated into a variety of skin care products. Recently, NPs have been identified as causative agents affecting skin barrier penetration and immunologic responses. Thus, understanding epidermal and dermal penetration, as well as possible toxicity of NPs is important to skin inflammatory diseases. The aim of our study was defining the effects of pore structural conditions of silica NPs in atopic dermatitis (AD) condition. Both nanoporous (NPS) and colloidal (Col) silica NPs (100 and 500 μg/rat) were topically applied to female BALB/c mice during 5 weeks. Topical application of NPs during 5 weeks elicited aggravation of AD symptoms. In both histology and FACS analysis, NPS NPs showed significant damage to skin inflammation than that of Col NPs through the hydroscopic property. These results suggest the importance of verification of biocompatibility of NPs in dermal exposure.
Proliferation of cells in the diaphragmatic peritoneum was investigated as a short-time screening test 3 and 6 months after i.p. injection of fibers in rats, using the BrdU method and measurement of peritoneal thickness. Furthermore, animal mortality and tumor development were monitored over two years in a carcinogenicity study.

There was a time-dependent significant increase in cell proliferation after injection of MWCNT 1 (high) (L=7.9 µm; D=0.037 µm), MWCNT 2 (low/high) (L=10.24 µm; D=0.04 µm), and MWCNT 3 (low/high) (L=8.57 µm; D=0.085 µm), like after exposure to long asbestos (L=13.95 µm; D=0.39 µm) as positive control. MWCNT 2 (high) and 3 (low/high) induced significant dose-dependent thickening of the peritoneum after i.p. injection, independently of time. In the carcinogenicity study, MWCNT 2, 3 and 3a (L=9.3 µm; D=0.062 µm) showed a higher mesothelioma incidence than MWCNT 1.

In conclusion, some MWCNT (MWCNT 2, 3) mediate enhanced proliferation of peritoneal cells in the diaphragm in rats, which may result in mesothelioma development.

2004 Silver Nanoparticles, Injected Intravenously, Accumulate in the Spleen of Mice and Cause Major Changes in Immune Cell Profiles


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Silver nanomaterials are increasingly being used as antimicrobial agents in medical devices, yet their effects on immunecompetence are unclear. This study investigated modulation of immune cell profiles and cell activation in the spleen by iv exposure of adult male mice to silver nanomaterials (AgNP), as well as particle distribution in the liver, kidneys, and spleen. Mice, injected each day for 2 days with a total of 66 or 300 µg of either 20 or 110 nm AgNP (or sodium citrate vehicle), were sacrificed 1 d later and splenic cells were examined by flow cytometry. Analysis revealed that injection of 66 µg of 20 nm AgNP increased Th2 cells and decreased effector memory cells, while exposure to the same dose of larger-sized AgNP had no effect on percentages of CD3+/CD4+, Th1, Th2, Th17 cells, CD4/CD8 ratio or total memory/naïve/central memory/effector memory cells compared to controls. At 300 µg, 20 nm AgNP significantly decreased the number of naïve CD4+ cells and CD3+/CD4- cells, while increasing total/activated central memory cells, total/activated effector memory cells, total memory cells, and natural killer cells; exposure to the same dose of 110 nm AgNP increased activated CD11c+ cells (macrophages). ICP-MS analysis demonstrated that the highest concentrations of silver were in the spleen following the 300 µg AgNP exposure, with an average concentration reaching a peak of 151 and 41.5 ng/g following exposure to 20- and 110-nm-sized AgNP, respectively. Relative weight of the spleen was increased significantly following 20 nm AgNP exposure. These data indicate that systemic exposure to AgNP accumulates in the spleen, adversely affects spleen weight and modulates the systemic immune response, accumulates in the spleen, and adversely affects spleen weight in a dose-dependent manner with smaller size particles leading to more marked effects. Supported by NSF/FDA Scholar-in-Residence at FDA program - #1237451.

2004a Thirty-Day Whole-Body Inhalation Toxicity and Tissue Burden Study of Multiwalled Carbon Nanotubes in Harlan Sprague-Dawley Rats and B6C3F1 Mice

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Multiwalled carbon nanotubes (MWCNTs) are a diverse class of high aspect ratio nanoscale materials that vary in size (length, diameter) and composition (surface functional groups, metal catalyst residue). The similarity of MWCNTs to harmful high aspect ratio fibers has generated concern for toxicological potential of MWCNTs. This study was designed to evaluate the toxicity of MWCNTs (length x diameter: 2.6 um x 15 nm, purity: 99%, nickel content: 0.52%) in Harlan Sprague-Dawley rats and B6C3F1/N mice exposed via whole body inhalation. Male and female rats and mice (35/sex/group) were exposed to 0, 0.1, 0.3, 1, 3, or 10 mg/m3 MWCNTs for 5 days/week (6 hours/day). The day after the last exposure, core group animals (10/sex) received complete necropsy and histopathological examinations. Blood and lungs were collected from animals in the tissue burden groups (25/sex) on postexposure day 0 (10/sex), 14, 42 and 120 (5/sex) and analyzed for nickel as a surrogate for MWCNT. Dose-dependent increases (incidence and/or severity) in chronic lung inflammation and pulmonary associated lymph node hyperplasia were observed in both species and sexes. Significantly increased lung weights were observed in female rats exposed to 10 mg/m3 and both male and female mice exposed to ≥ 3 mg/m3 (p<0.01). Clearance rates of MWCNT from the lung were similar in both sexes and species and decreased markedly in animals exposed to ≥ 3 mg/m3 (T1/2 = 2-51 mo) compared to the 0.1, 0.3 and 1 mg/m3 dose groups (T1/2 = 2-3 mo). The long clearance half lives, like lung burdens and lung lesions in animals exposed to MWCNT ≥ 3 mg/m3 suggest that lung overload occurred. The data from this study will be used to design a chronic whole-body inhalation toxicity study of MWCNTs. This work was supported by the NIH, National Institute of Environmental Health Sciences.

2004b Cerium Oxide Nanoparticles Induced Lung Fibrosis Involving Epithelial-Mesenchymal Transition (EMT)

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Recently cerium compounds have been used as diesel fuel additives to increase fuel combustion efficiency and decrease diesel soot emissions. However, detection of cerium oxide nanoparticles (CeO2) in the diesel exhaust by emission tests are a caused of health concern. Our previous studies have shown that exposure of rats to CeO2 induces sustained pulmonary inflammation and mediator production, and lung fibrosis. The current objective is to evaluate the effects of CeO2 on functional change of alveolar type II cells (ATII) leading to EMT. Male Sprague Dawley rats were exposed to CeO2 (3.5 mg/kg) by a single intratracheal instillation and sacrificed at various times post exposure. ATII were isolated from lungs at 28 days post exposure and purified by panning. Alveolar macrophages (AM) were isolated by bronchoalveolar lavage (BAL). The BAL fluid (BALF) and AM-conditioned medium obtained after a 24 h incubation time were saved for further analysis. Lung fibrosis was evident by increased hydroxyproline content and increased Sirius Red staining for collagen in lungs at 28 days after CeO2 exposure. Scanning electron micrographs indicate CeO2-induced ATII hypertrophy. Confocal microscopic analysis demonstrated that exposure to CeO2 increased the presence of stress actin, expressed as α-smooth muscle actin (α-SMA) in ATII at 28 days post exposure and caused CeO2-induced EMT. CeO2 exposure significantly increased the fibrogenic cytokine, TGF-β1, in AM-conditioned media, and increased matrix metalloproteinases and soluble collagen in BALF, at 3 and 28 days post exposure, respectively. In vitro exposure of ATII or a rat type II cell lineage (RLE-6TN) to TGF-β1 significantly increased α-SMA expression and altered morphology. In addition, exposure of type II cells to BALF significantly increased α-SMA expression, suggesting CeO2 exposure induced mediators in the BALF significantly modified cell function. These results demonstrate that CeO2-induced EMT of ATII may play important role in lung fibrosis. These findings suggest potential health effects of CeO2 exposure.

2004c Cytotoxicity and Genotoxicity Assessment of Silver Nanoparticles in Mouse

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Silver nanoparticles (AgNPs) are among the most commercially used nanomaterials and their toxicity and genotoxicity are controversial. Although many in vitro studies have been conducted to evaluate the genotoxicity of AgNPs, in vivo genotoxicity studies on the nanomaterials are limited. Given the unique physicochemical properties and complex pharmacokinetics behavior of nanoparticles (NPs), in vivo genotoxicity assessment of AgNPs is badly needed. In this study, the clastogenicity and mutagenicity of AgNPs with different sizes and coatings were evaluated using mouse micronucleus (MN) assay, Pig-a assay and Comet assay. Five 7-week-old male B6C3F1 mice per group were treated with 5 nm polyvinylpyrrolidone (PVP)-coated AgNPs at a single dose of 0.5, 1.0, 2.5, 5.0, 10.0 or 20.0 mg/kg body weight (bw) via intravenous injection for both the MN and Pig-a assays; or with 15–100nm PVP- or 10–80nm silicon-coated AgNPs at a single or 3-day repeated dose of 25.0 mg/kg bw for the MN assay and Comet assay in mouse liver. Inducibly coupled plasma mass spectrometry (ICP-MS) and transmission electron microscopy (TEM) analyses indicated that AgNPs reached the testing tissues (bone marrow for the MN and Pig-a assays and liver for the Comet assay). Although there was a reduction of reticulocytes in the PVP-coated AgNPs-treated animals, indicating cytotoxicity, of the AgNPs, none of the treatments resulted in a significant increase of either mutant frequencies in the Pig-a gene or the percent of micronucleated reticulocyte over the concurrent controls. However, both the PVP-
and silicon-coated AgNPs induced oxidative DNA damage in mouse liver. These results demonstrate that AgNPs can reach mouse bone marrow and liver, and generate cytoxicity to the reticuloocytes and oxidative DNA damage to the liver.

**2004d One Year Pulmonary Outcomes After Exposure to Carbonaceous Nano-Engineered Materials and Asbestos**

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Single-walled carbon nanotubes (SWCNT) and carbon black nanoparticles (CNF) are carbonaceous nanoparticles sharing fibrous morphology with a well-known, naturally occurring, toxic fiber, asbestos. Both short- and long-term outcomes of pulmonary exposure to asbestos, ranging from inflammation and fibrosis to mesothelioma and lung cancer, are well described. To date, no data are available describing long-term adverse effects of pulmonary exposure to SWCNT or CNF. Thus, a direct comparison of chronic effects of SWCNT, CNF and asbestos is of significant importance. Here, we assessed inflammatory, fibrogenic and genotoxic effects of SWCNT, CNF, and asbestos in C57BL/6J mice one year after a single pulmonary exposure by pharyngeal aspiration and inhalation. We provide evidence that up to one year after exposure, SWCNT, CNF and asbestos persist in the lung and regional lymphatics. All three particles induced chronic bronchopneumonia and lymphadenitis, accompanied by pulmonary fibrosis. While CNF and asbestos were found to promote the greatest degree of inflammation, followed by SWCNT, the latter was the most fibrogenic of all three types. SWCNT induced cytogenetic alterations including micronuclei and nuclear protrusions in vivo. Notably, SWCNT and CNF, but not asbestos, increased the incidence of K-ras oncogene mutations in lungs. No lung tumor incidence occurred after 1 year post exposure in any group. Inhalation exposure to SWCNT showed significantly greater inflammatory, fibrotic and genotoxic effects than bolus pharyngeal aspiration, indicating that bolus exposure does not over-predict pulmonary response and appears useful for hazard determination. Overall, our data suggest that long-term pulmonary toxicity of particles with high aspect ratios - SWCNT, CNF and asbestos – is defined not only by their fibrous morphology but also by the chemical composition, specific surface area and type of exposure.

**2004e Effect of Prenatal Exposure to Carbon Black Nanoparticle on Gene Expression in the Spleen of Offspring Mouse**

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Objective: We recently found that exposure of pregnant mouse to carbon black nanoparticle (CB-NP) decreased T cells in the spleen of offspring during the neonatal period. The aim of the present study was to examine the effect of prenatal exposure to CB-NP on gene expression in the spleen of offspring in a mouse model. Methods: CB-NP (PRINTEX90 from Degussa; primary particle size: 14 nm; surface area 300 m²/g) was suspended at 5 mg/mL in water, sonicated for 30 min, and then filtered through a 450-nm filter immediately before administration. The peak size of the aerosolized diameter distribution of CB-NP in the suspension was 68 nm. Pregnant ICR mice were treated with the CB-NP suspension (95 µg/kg/time) by intranasal instillation on gestational days 5 and 9. Spleen was collected from male and female offspring mice at 5 days post-partum. All animals were treated and handled in accordance with national guidelines for the care and use of laboratory animals. Gene expression in the tissues was examined by cDNA microarray using a SurePrint G3 Mouse Gene Expression 8×60K microarray (Agilent). Gene Ontology, canonical pathway and transcription factor related to the genes dysregulated by prenatal CB-NP treatment were bioinformatically extracted. Results: The genes differentially expressed by prenatal CB-NP treatment in the spleen of both male and female offspring were enriched in a transcription factor GATA1, which are closely associated with erythocyte development. The genes annotated to GATA1 were upregulated and included Cxcr5, Hes1, In1, Dnase11, Nf1a, and Nf1b. Interestingly, previous studies indicated that Hes1 inhibits the function of GATA1 and the development of erythroid and megakaryocytic cells. Conclusion: The effect of prenatal exposure to CB-NP through the airway on the spleen was associated with upregulation of genes related to GATA1 in neonatal mouse.

**2004f Quantum Dot-Induced Changes in Pulmonary Function Are Mouse Strain Dependent**

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Quantum dots (QDs) are photostable fluorescent nanoparticles commonly composed of a cadmium selenide (CdSe) core, a zinc sulfide (ZnS) shell, and coatings specific to a variety of uses in electronics and biomedical research. QDs can cause inflammation and toxicity in the respiratory tract when inhaled, instilled, or aspirated. In this study we investigated whether pulmonary aspiration of 12 nm diameter tri-n-octylphosphine oxide, poly(maleic anhydride-alt-1-tetradecene) coated CdSe/ZnS QDs (TOPO-PMTQ) and CdSe/ZnS QDs (TOPO-PMTQ) induced functional changes in mouse lung mechanics in a mouse strain dependent fashion. C57BL/6J and A/J mice were dosed with 6 µg Cd equivalents/kg body weight of a 10 nM TOPO-PMTQ QD solution or an equal volume of saline vehicle via oropharyngeal aspiration. At 24 hours, mice were anesthetized, intubated via tracheotomy, and connected to a flexVent instrument. Lung mechanics were measured repeatedly during a progressive nebulized methacholine challenge (0-50 mg/ml). There were statistically significant increases in total lung resistance, tissue damping, and lung elastance with methacholine challenge after TOPO-PMTQ QD treatment in A/J but not C57BL/6J mice. There were also statistically significant increases in baseline airway resistance and lung compliance in A/J compared to C57BL/6J mice. Thus, TOPO-PMTQ Cds/ZnS QDs negatively affected pulmonary function in a mouse strain-dependent manner. It is important that genetic susceptibility be considered in risk assessment strategies for QDs and other engineered nanomaterials. Supported by NIH Grants U19ES019545, P30ES007033, and R01HL086883.

**2004g The Mitochondrial Effects of a Chronic Exposure to a Low-Dose of Silver Nanoparticles Are Rescued by an Antioxidant**

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Manufactured silver nanoparticles (AgNPs) are ubiquitous presence in daily life, for they are used in numerous applications, from ultrasensitive molecular sensing and diagnostic imaging, agents for photodynamic therapy, burn and wound dressing, to dental-bonding agents and sunscreens, cosmetics and fuel cells, tires, optics, clothing and electronics. The same properties that make AgNPs so useful could also prove harmful when interacting with humans and the environment. Most of the works so far have unveiled a strong induction of oxidative stress as main mechanism of toxicity. However, these studies are mainly focused on short-term exposure and, consequently, higher doses. As such, we aimed to understand how long-term exposure to a protective low-dose of AgNPs could affect various biological systems, with a particular focus on mitochondrial bioenergetics and if these effects could be reverted by a simultaneous treatment with a known antioxidant, N-acetylcysteine (NAC). Other objectives were to understand if there is a preferential target organ in the body and if the size of the AgNPs was a determinant factor in the registered alterations.

In accordance with European and national animal use guidelines, 10-week old male Sprague-Dawley rats were intraperitoneally (i.p.) injected with 200 µg/Kg (w/w) of either 10 nm or 75 nm AgNPs, with and without a 30min previous NAC 100 mg/Kg i.p. injection. We uncovered a NAC-reversible, non-size dependent negative effect of AgNPs in various functions (membrane potential, oxygen consumption, ATP generation, amongst others) of isolated liver, kidney, and to a lesser extent, heart mitochondria. Our data demonstrates that even a low-end dose exposure to AgNPs has a pernicious effect in the long term, which begs for further assessment of the indiscriminate use and exposure to AgNPs.

**2004h Nanoceria In Vivo Biotransformation Is Associated with Loss of Its Pro-Oxidant Brain Effects**


Background: Nanoceria has many commercial uses and is being developed as an antioxidant therapeutic. It persists in the rat for 90 days with little quantitative reduction, primarily in the liver, where it produces granuloma. It produces pro-oxi-
ionizing effects in the brain up to 30 days after administration. Objectives: Determine nanoceria’s physiochemical characteristics in the liver and effects on brain oxidavive stress for 90 days after administration. Methods: A citrate-stabilized cubic 30 nm ceria (85 mg/kg) was iv infused once to rats, terminated 1 h to 90 days later. Controls received vehicle. Multiple oxidative stress endpoints were determined in the brain. Nanoceria in the liver was examined using HR-TEM, electron energy loss spectroscopy (EELS), energy-dispersive X-ray spectroscopy (EDS), and selected area electron diffraction (SAED). Results: Following similar administration of a 5 nm ceria, oxidative stress was seen in the brain up to 30 days after 30 nm ceria, including elevated protein carbonyl, catalase, and heat shock protein 70 levels and decreased GSH/GSSG. Ninety days after its administration oxidative stress endpoints were at control levels. HR-TEM of the liver revealed nanoceria particles were highly fragmented and rounded along their edges and the appearance of clouds containing copious of 1 to 3 nm ceria (verified by EELS, EDS, and SAED) near the nanoceria agglomerates. EELS showed the 1 to 3 nm ceria had a reduced valence (Ce3+) state, indicating in vivo processing by sputtering to a form having higher free radical scavenging activity. The temporal association between nanoceria biotransformation in the liver and reversal of nanoceria-induced oxidative stress in the brain suggests a cause and effect relationship. Conclusion: Nanoceria’s long term fate and effects warrants further study to better understand its adverse and beneficial nature. Support: US EPA STAR Grant RD-835772.

**2005 Cannabinoid Receptor Function on Bone Marrow-Derived Dendritic Cells**

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Dendritic cells (DCs) are professional antigen presenting cells indispensable in linking the innate and adaptive immune response. DCs comprise a heterogeneous population of cells. Each distinct subset has its unique set of surface markers and different abilities to respond to environmental stimuli and process antigen. Cannabis is the most frequently consumed illicit drugs in the world. Mammals for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.

**2006 Cannabinoid Regulation of Peripheral Sympathetic Neuronal Activity: Possible Role in Cannabinoid-Mediated Immunosuppression**

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Cannabinoids are compounds derived from Cannabis sativa that exhibit immunosuppressive effects. Cannabinoids are also known to inhibit neurotransmitter (NT) release in the central nervous system. Therefore, it was hypothesized that inhibition of NT release might contribute to cannabinoid immunosuppressive mechanisms. NTs are implicated in inflammatory homeostasis for example, norepinephrine (NE) modulates antibody production. However, it is not known to what extent cannabinoi dinteract with NTs to cause immunosuppression. In vivo, NE innervation of the spleen capsule is known to cause spleen contraction during times of hypoxia as well as in response to an immune challenge. Therefore, we utilized NE-induced splenic contraction as a tool to probe the role of cannabinoids in regulating spleen capsule sympathetic neuronal activity in wild-type and cannabinoid receptor dual knockout (CB1/CB2 KO) mice. It was discovered that NE-induced spleen capsule contraction was significantly attenuated in CB1/CB2 KO mice. However, this effect was not observed in response to electrical stimulation of spleen capsule sympathetic axon terminals, suggesting a potential role for non-adrenergic signaling in contraction. Additionally, spleens from CB1/CB2 KO mice have decreased spleen capsule thickness that is not due to decreased smooth muscle content. Overall, these studies suggest that the mechanism by which cannabinoids regulate splenic sympathetic activity in vivo could involve interaction with one or more NTs. The results from these studies could identify neuronal mediators involved in the immunosuppressive effects of cannabinoids.

**2007 Modulation of HIVGP120 Antigen-Specific Immune Responses by A9-Tetrahydrocannabinol In Vivo**

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Approximately 25% of HIV patients use marijuana for its putative therapeutic benefit; however, it is unknown how cannabinoids affect the immune status of immunocompromised HIV patients. Previously, a surrogate in vitro mouse model was established to investigate the effects of cannabinoids on the early stages of the anti-HIV response. Specifically, CD8+ T cell proliferation and gp120-specific IFNγ production were induced, which were suppressed or enhanced by A9-tetrahydrocannabinol (A9-THC). We investigated how THC and its metabolites in compound in marijuana, depending on the magnitude of cellular activation. To determine whether THC has similar effects in vivo, a mouse model to stimulate the HIV gp120-specific immune response has also been established. Vector plasmid VRc2000 or gp120-expressing plasmid VRcgp120 was injected intramuscularly into mice. The gp120-specific IFNγ response was detected, when splenocytes were restimulated with gp120-derived peptide 81, which was identified as being immunodominant among 211 tested peptides comprising gp120. In addition, both T cell and non-T cell populations were activated in response to VRc(1-128) stimulation, as evidenced by increased expression levels of surface activation markers (e.g., CD69, CD80, major histocompatibility complex II). Furthermore, the gp120-specific IFNγ response and the magnitude of cellular activation were enhanced in cannabinoid receptor (CB) 1 and 2 knockout mice compared to wild type mice. THC further increased the above-mentioned response in wild type mice, but showed minimal to no enhanced effect in knockout. Overall, our findings suggest that THC modulates HIV antigen-specific immune responses in vivo in a CB1/CB2-dependent manner.

**2008 A9 Tetrahydrocannabinol (THC) Expands and Activates Highly Immunosuppressive MDSCs via S100A8 Secretion**

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Marijuana is a widely used drug of abuse. With the recent legalization of marijuana for recreational purpose, in two states, as well as medicinal purpose, in 21 states, understanding the impact of cannabinoids is now paramount. We focused on the mechanism by which A9-tetrahydrocannabinol (THC), the psychoactive ingredient found in the Cannabis sativa plant, causes immunosuppression. Earlier studies from our lab have demonstrated that THC induces myeloid derived suppressor cells (MDSCs) resulting in immune suppression. However, the precise mechanism of THC-mediated MDSC induction and activation is not fully understood. In this study, we determined the mechanism by which THC induces the generation and activation of a highly immunosuppressive MDSC population. We found that THC-induced MDSCs (THC-MDSCs) had significantly increased levels of suppressive function, secretion of signal transducer and activator of transcription (Stat) 3 activating cytokines, and S100A8 protein compared to naive bone marrow resident MDSCs (BM-MDSCs). THC-induced granulocytic and monocytic MDSC subtypes were more suppressive than BM-MDSC subsets. Additionally, arginase1 (Arg1), a known immunoregulatory molecule, was elevated in THC-MDSCs compared to BM-MDSCs. Increased levels of IL-6 and IL-10 in THC-MDSCs correlated with elevated Stat3 activation. S100A8 is a protein which has been shown in tumor models to drive MDSC accumulation and activation, but its role in THC-mediated MDSC induction is not known. Phosphorylated Stat3 has been shown to bind the promoter region of S100A8 and increase transcription. Use of the S100A8 depletion antibody (8H150 at 20 mg/mouse) led to a 1.8 fold decrease in the accumulation of THC-MDSCs and impaired their suppressive function. Together, these data suggest a key role for S100A8 in THC-induced expansion.
In the presence of thiols, Keap1 binds to Nrf2 and targets it for ubiquitination and proteasomal degradation. In the absence of electrophiles, Keap1 is not bound to Nrf2, allowing it to translocate to the nucleus and bind to antioxidant response elements (AREs), promoting the expression of ARE-regulated genes, including enzymes of the electron transport chain, catalase, and heme oxygenase.

2009 The Role of Chemical Sensitizer’s Reactivity in the Generation of Reactive Species and the Activation of the Nrf2 Pathway in THP-1 Cells

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Dendritic cells (DC) are professional antigen-presenting cells that play a major role in the induction of primary immune response. Contact sensitizers, such as dinitrochlorobenzene (DNCB) or cinnamaldehyde (CIN), are known to induce reactive oxygen species (ROS) production. The Nrf2/Keap1 pathway is central to detect cellular stress. In the absence of electrophile or oxidative stress, Keap1 associates with Nrf2 and leading to its degradation. In the presence of an electrophilic compound, Keap1’s conformation is modified leading to Nrf2 translocation to the nucleus and transcription of its target genes. We have demonstrated that contact sensitizers like DNCB or CIN induce Nrf2 accumulation in human DC.

To elucidate the role of Nrf2 in DC survival, nrf2 WT or KO bone marrow-derived DC (BMDC) were treated with DNCB or CIN at 24h. Results showed (1) an increase of apoptotic cells and (2) a decrease in living cells in absence of Nrf2. An increase of caspase 3/7 activity, a rapid loss of mitochondrial membrane potential (ΔΨm) and a higher ROS production were observed in nrf2/−/− DC compared to nrf2+/+ DC in response to DNCB or CIN. Our results also showed that DNCB and CIN induced the expression of the anti-apoptotic gene, bcl-2, in a Nrf2-dependent manner.

Further investigation, the cytotoxic role of Nrf2 against chemical sensitizers, qPCR array of 43 genes was performed. Our results reveal that Nrf2 can upregulate catalase (Cat), heme oxygenase (Hmox-1), and glutathione peroxidase 1 (Gpx1), and glutathione reductase (Gsr) and glutathione-S-transferase (GstA1, Gpt1).

These results suggest that Nrf2 plays a role in cell survival and protects DC from contact sensitizers via the transcriptional activation of anti-apoptotic and antioxidant genes.

PS 2012 Nrf2 Has Differential Effects on the Early Events of Murine T Cell Activation

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Nuclear factor erythroid 2-related factor 2 (Nrf2) is a widely expressed transcription factor activated by cell stress, including reactive oxygen species and electrophilic stimuli. Nrf2 regulates a battery of detoxification, antioxidant, and metabolizing enzymes necessary for a cell to respond to stressors. Nrf2 is activated by many different compounds, including environmental contaminants such as arsenic, and synthetic food preservatives such as tBHQ (tert-butylhydroquinone). There is increasing evidence that Nrf2 modulates a number of different immune responses. Nrf2 null mice develop a lupus-like autoimmune disease, and show increased susceptibility to sepsis and inflammatory injury. More recently, our laboratory has shown that Nrf2 inhibits some of the early events of human T cell activation. The purpose of the present studies was to characterize the role of Nrf2 in early events of T cell activation. The Nrf2 activator, tBHQ, inhibited production of IFNγ and IL-2 by murine splenocytes activated with anti-CD3/anti-CD28. Unexpectedly, other events of early T cell activation, such as induction of CD25 and CD69, were not affected by tBHQ. To characterize this further, we investigated the effect of Nrf2 on CD25/CD69 induction at multiple time-points using a number of different T cell activators, including anti-CD3/anti-CD28, concanavalin A and PMA/Ionomycin. Regardless of time-point or activator, there was consistently no effect on CD25/CD69 expression. Overall, these studies demonstrate Nrf2 has differential effects on early events of T cell activation in murine splenic T cells. (This work is supported by NIH grant ES018885).
Inhibition of IL-2 Production by the Nrf2 Activator, tBHQ, Correlates with Inhibition of NFκB Activation in Activated Jurkat T Cells

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The transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), is activated upon cellular stresses, such as the presence of reactive xenobiotics and/or oxidative stress. After activation, Nrf2 accumulates in the nucleus and induces the transcription of its target genes, including HMOX and NQO1. Numerous studies have shown that Nrf2 plays a role in inflammatory diseases, including autoimmune. Recently, we have shown that activation of Nrf2 in CD4+ murine T cells skews differentiation toward that of a TH2 phenotype. Although shown to be immunomodulatory in mice, the role of Nrf2 in human immune cells is largely uncharacterized. Therefore, the purpose of the present studies was to determine the effects of the Nrf2 activator, tBHQ, upon the early events following human CD4+ T cell activation. Treatment of human Jurkat T cells with tBHQ markedly inhibited the production of IL-2 and moderately inhibited the expression of CD25, the high-affinity IL-2 receptor. The inhibition of IL-2 by tBHQ correlated with a decrease in the transcriptional activity of NFκB, a key transcription factor in the regulation of the IL-2 promoter. Collectively, our studies demonstrate that the Nrf2 activator, tBHQ, markedly inhibits IL-2 production in activated Jurkat T cells, which is likely due to inhibition of NFκB activation. This work is funded by NIH grants E018885 and GM092715.

Maintenance of Long-Term Self-Renewal in Hematopoietic Stem Cells by Aryl Hydrocarbon Receptor-Dependent Regulation of Reactive Oxygen Species during Fetal Development

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Determining the mechanism by which in utero activation of the Aryl hydrocarbon receptor (AhR) in hematopoietic stem cells (HSC) impairs the process of self-renewal will provide insight into a spectrum of blood disorders ranging from stem cell exhaustion to leukemia. The AhR is a ligand-activated transcription factor and studies in AHR deficient mice have elucidated a role for the AhR in hematopoietic stem cell quiescence. The mechanism by which cells remain in this low proliferative state is unknown. Alterations in reactive oxygen species (ROS) have been shown to adversely impact HSC self-renewal possibly due to DNA damage. Furthermore, the ligand-activated AhR has been implicated as a modulator of cellular respiration. With this knowledge, we hypothesized that the AHR is a negative regulator of aerobic respiration in HSC, such that ligand-activation disrupts this regulation, leading to increased ROS in anacrobic cells adversely impacting long-term self-renewal. To test the hypothesis, we exposed pregnant C57BL/6 mice to 3ug/kg of the AhR agonist 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) and analyzed ROS in HSC from fetuses ranging in age from gestational day (GD) 7.5 to GD 14.5. We used fetuses because of the developmental age dependent transitions that occur in HSC respiratory activity. We found that on GD 7.5 and 11.5, there was a 50% increase in ROS production in HSC (defined as Lin-ScaraK+CD19+CD100+), followed by a 30% decrease in the HSCs on GD 14.5. Furthermore, in a competitive irradiation/reconstitution assay testing long-term self-renewal function, HSC from control fetal livers out competed HSC exposed to TCDD in utero by nearly three to one offspring of TCDD-treated dams. To address this question, an in vitro culture system was established to recapitulate human B cell development. Specifically, hematopoietic stem cells (HSC) are co-culturing with irradiated human primary bone marrow stromal cells. In three weeks of culture, we observed a downregulation of HSC marker CD34 as cells underwent development, as well as an upregulation of IL-7R, which indicated lymphoid lineage restriction. More importantly, generation of lineage-committed B cells was observed by the co-expression of B220 and CD19 in approximately 20% of the cell population. These data illustrated establishment of an in vitro model system of human B cell development from HSC. The objective of the present study was to employ this model system to investigate the effect of TCDD on B cell development. HSC were treated with TCDD (1, 10 and 30nM) and/or vehicle (0.02% DMSO). Comparing to the vehicle control, TCDD reduced cell number in a concentration-dependent manner. Apoptosis analysis using Annexin V and 7-AAD suggested the decrease of cell number was not due to cytotoxicity of TCDD. Likewise, TCDD decreased CD34 and IL-7R expression and significantly the number of B220+CD19+ lineage-committed B cells that were generated from HSC. Together, these data for the first time suggested that TCDD altered early human B cell development. Research is supported in part by NIH ES005250 and NIH P20 04911.

Impaired by TCDD of Human Hematopoietic Stem Cells to B Cell Lineage Commitment

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Environmental contaminants such as TCDD are known to markedly affect B cell activation and differentiation. Hence, the objective of the present study was to investigate the mechanism underlying suppression of human B cell activation by TCDD. BCL-6 was identified as a likely candidate owing to its role as a transcriptional repressor of B cell activation and differentiation. In the presence of TCDD, BCL-6 protein levels were elevated in human B cells. Concomitant with this TCDD-mediated increase in BCL-6 a decrease in B cell activation was evident through the attenuation of surface CD80 and CD69. In addition, it was observed that BCL-6 could repress CD80 in presence of TCDD by binding to the enhancer region of CD80. To further ascertain the role of BCL-6 in the suppression of B cell activation, the small molecule inhibitor of BCL-6 repression, 79-6, was utilized. Treatment of activated B cells with TCDD in combination with 79-6 reversed the suppression of CD80 levels and partially reversed the suppression of CD69 levels thereby providing evidence for a role by BCL-6 in regulation of B cell activation. Further, involvement of the AHR signaling pathway in BCL-6 regulation and in B cell activation was demonstrated using the AHR low affinity ligand, monochlorodibenzo-p-dioxin (MCDD), which had no effect on BCL-6 protein levels or on B cell activation. Collectively, these results suggest that suppression of human B cell activation, especially of CD80 occurs, in part, due to deregulation of BCL-6 by the TCDD-AHR pathway. Supported in part by NIH R01 ES005250 and P42 ES004911.

Developmental Activation of the AhR Enhances the CD4+ T Cell Response in the Influenza Virus-Infected Lung

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Recent discoveries suggest that developmental exposure to certain pollutants increases the severity of respiratory infections later in life, but the mechanism by which this occurs is unknown. The aryl hydrocarbon receptor (AhR) is a ligand-regulated transcription factor that binds a broad variety of anthropogenic and naturally derived chemicals. It is expressed by immune cells, and upon activation it can alter the function of the immune system. Using a mouse model, developmental exposure to the potent AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters the immune response to the common respiratory pathogen influenza A virus. Developmentally exposed offspring have a poorer CD8+ T cell response to infection; yet, have more bronchopulmonary inflammation and an elevated number of immune cells in their lungs. We report here that, in contrast to CD8+ T cells, the response of CD4+ T cells to influenza was increased. CD4+ T cells from TCDD-exposed mice respond to infection with significantly fewer lymphocytes, increased expression of IL-2 and IL-2Rα, and decreased expression of IL-10 compared to control. These results suggest that the aberrant CD4+ T cell response to influenza is due to the altered responsiveness of CD4+ T cells to TCDD exposure.
2018 Suppression by 2, 3, 7, 8-Tetrachlorodibenzop- Dioxin (TCDD) of IL-2 Plus IL-21-Induced B Cell Activation

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The environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic compound among the group of halogenated aromatic hydrocarbons found in our environment. TCDD is known to adversely affect the adaptive immune system. Studies using rodents have shown that TCDD affects the differentiation of B cells into antibody-secreting cells. Recent work using human primary B cells has shown that B cell activation is suppressed significantly by TCDD, thereby, subsequently affecting the process of B cell differentiation. Specifically, B cells activated with CD40 ligand (CD40L) and cytokines in presence of TCDD. Furthermore, changes in mRNA levels of Src-homology phosphatase-1 (SHP-1), a gene known to be altered in B cell activation, were investigated. Our results show that TCDD-treatment led to a decrease in the percent and mean fluorescence intensity of B cell activation markers CD80, CD86, and CD69. IL-21 is a cytokine known to enhance the proliferation of B cells, playing a vital role in their differentiation. Hence, the goal of this study was to understand the effects of TCDD on CD40L and IL-2 plus IL-21-induced human B cell activation. Flow cytometry was used to measure levels of B cell activation markers in primary human B cells with CD40L and cytokines in presence of TCDD. Furthermore, changes in mRNA levels of Src-homology phosphatase-1 (SHP-1), a gene known to be altered in B cell activation, were also investigated. Our results show that TCDD-treatment led to a decrease in the percent and mean fluorescence intensity of B cell activation markers CD80, CD86, and CD69 from three individual human donors. Additionally, the mRNA levels of the gene SHP-1 were increased in presence of TCDD as expected. Overall, our results show that TCDD suppresses the CD40L and IL-2 plus IL-21-induced B cell activation, which occurs independent of the cytokine-stimulation used and may involve increased SHP-1 expression. (Supported by NIH R01ES002520)

2019 Evaluation of Aryl Hydrocarbon Receptor (AhR) Polymorphism G1661A on TCDD-Mediated Biological Activity in the Human B Cell

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The environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a suppressor of immunoglobulin (Ig) expression in various animal models. This effect appears to be mediated through the aryl hydrocarbon receptor (AhR). When activated by ligand, the AhR binds to dioxin response elements (DRE) leading to altered transcriptional activity. Our previous results identified DRE sites and TCDD-induced binding of the AhR to these sites within a transcriptional regulatory region (3'IghRR) that controls Ig heavy chain expression. The 3'IghRR consists of four enhancer regions (hs3A; hs1,2; hs3B, hs4) and is sensitive to TCDD-induced inhibition, which correlates with TCDD-induced inhibition of Ig expression. In humans, the hs1,2 enhancer (hu-hs1,2) is polymorphic and has been associated with certain autoimmune diseases. We have identified TCDD-induced activation of the hu-hs1,2 enhancer and a decrease in enhancer activity with deletion of the polymorphic region. The objective of the current study was to utilize mutational analysis and luciferase reporter constructs to evaluate the contribution of each transcription factor binding site within the polymorphic region to hu-hs1,2 activity and modulation by TCDD. TCDD induced a similar fold-induction in hu-hs1,2 reporter activity regardless of which transcription factor was mutated; whereas these mutations lead to an increase in basal hs1,2 activity. Evaluation of the binding sites 5′ of the polymorphic region demonstrated an increase in hs1,2 activity when the Oct site was mutated and a decreased activity in the AP-1/Ets site was mutated. However, these mutations had little effect on TCDD-induced activation. These results suggest a complex interaction of proteins binding within the hu-hs1,2 enhancer and a consistent increase in hs1,2 activity by TCDD. A better understanding regarding the role of the hu-hs1,2 enhancer and how TCDD modulates its activity may lead to insights into the etiology of autoimmune disorders associated with the hs1,2 polymorphism.
2022  Elucidating the Role of the Polymorphic Human hs1,2 Enhancer in the Effects of TCDD

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental toxin known to inhibit immunoglobulin (Ig) gene expression in various animal studies. We have identified the mouse 3’Ig heavy chain regulatory region (3’IgHRR) as a sensitive transcriptional target of TCDD, which may mediate the inhibitory effect of TCDD on Ig expression. Interestingly, the human hs1,2 enhancer is polymorphic and has been associated with a number of autoimmune diseases. Suggesting a species difference, TCDD inhibited mouse hs1,2 enhancer activation and activated basal human hs1,2 (hs-hs1,2) enhancer activity. The objective of this study was to determine the effect of stimulation and TCDD on hu-hs1,2 enhancer activity using a human B-lineage (CL-01) and luciferase reporter constructs regulated by each of the human hs1,2 alleles. Our results verify that TCDD alone activates each of the hu-hs1,2 alleles. Surprisingly, B-cell stimulation through TLR 7 and 8 by R848 inhibited basal activity of the hu-hs1,2 alleles and TCDD co-treatment reversed this inhibition. In contrast, R848 induced Ig secretion and activated a 3x NF-kB luciferase reporter, therefore confirming functional signaling through the TLRs and activation of the CL-01 B-cell line. R848 also induced class switch recombination from IgM to IgG. Furthermore, TCDD inhibited both IgM and IgG secretion in cells stimulated with R848. These results suggest that the hu-hs1,2 enhancer may be a negative regulator of 3’IgHRR activity and Ig expression. Alternatively, the hs1,2 enhancer may function differently when studied in isolation as compared to its function in the intact 3’IgHRR. Further studies will evaluate the effect of TCDD in the absence or presence of stimulation on the activity of the entire human 3’IgHRR and its other enhancers, hs3 and hs4. Elucidating the role of the polymorphic hs1,2 enhancer in 3’IgHRR activity and the effect of TCDD on the enhancers of the 3’IgHRR may provide insights into the etiology of autoimmune diseases associated with the hs1,2 polymorphism. (Supported by NIEHS R01ES014676)

2023  Benzo(a)pyrene Exposure Suppresses FcyRII (CD32)-IgG Antibody Complex Binding by Disruption of Lipid Raft Membrane Integrity

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The fundamental requirement in activation of macrophage effector functions is the binding of immunoglobulins to Fc receptors. The FcγRIIA (CD32) immunoreceptors have been reported to either constitutively reside in or associate with detergent resistant membranes (DRMs) upon binding to IgG, the most abundant Ab class in circulation. Previous research suggests that exposure to benzo(a)pyrene, B(a)P, an environmental toxicant, suppresses macrophage effector functions but the mechanism remains undefined. The purpose of this study was to elucidate the mechanism of B(a)P-induced suppression by examining the effects of B(a)P exposure on CD32-lipid raft interactions in the regulation of IgG binding to CD32. B(a)P exposure altered lipid raft integrity by depleting membrane cholesterol at over a 50% depletion rate (577.8 ± 204.6, p < 0.001). The exposures also lead to a 30% (216.2 ± 142.7, p < 0.05) reduction in affinity or an exclusion of CD32 from lipid rafts. The 50% diminution in membrane cholesterol as well as the 30% exclusion of CD32 from lipid rafts caused significant suppression of CD32-mediated IgG binding by 60% (486.2 ± 166.7, p < 0.001) which suggests that intact lipid rafts are required for IgG complex binding to CD32. Future studies are directed at establishing whether B(a)P-induced suppression increases macrophage susceptibility to microbial infection.

2024  Wear Particles Derived from Metal Hip Implants Generates Multinucleated Giant Cells in a 3-Dimensional Peripheral Tissue-Equivalent Model

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Multinucleate giant cells (MGCs) are created by the fusion of 5 to 15 monocytes or macrophages. MGCs can be generated by hip implants at the site where the metal surface of the device is in close contact with tissue. MGCs play a critical role in the inflammatory processes associated with adverse events like aseptic loosening of the prosthetic joints and bone degeneration as observed in some patients. Upon interaction with metal wear particles, endothelial cells can upregulate bioactive molecules and pro-inflammatory cytokines that can enhance a localized immune response. However, the role of endothelial cells in the generation of MGCs has not been completely investigated. We first developed a three-dimensional peripheral tissue-equivalent model (PTEM) consisting of collagen gel, with a monolayer of endothelial cells on gel surface and human peripheral blood mononuclear cells (PBMCs) on top, which mimics peripheral tissue under normal physiological conditions. The cultures were incubated for 14 days with Cobalt chromium alloy (CoCr ASTM F75, 1-5 microns) wear particles, PBMCs were allowed to transit through the coated cell line and harvested cells were analyzed for MGC generation using propidium iodide (PI) by flow cytometry. Increase in forward scatter (cell size) and in the PI uptake (DNA intercalating dye) was used as a measure of MGCs. Our results show that endothelial cells induce generation of MGCs 4 fold higher in 3-dimensional PTE system as compared to traditional 2-dimensional culture plates. Furthermore, we also checked whether increase in particle to cell ratio affects MGC generation. We found that as the number of particles increased, decrease in MGC generation compared to control, indicating that cells underwent apoptosis or necrosis with higher particle concentration. In sum, we have established a robust and relevant model to follow MGCs formation using flow cytometry. We observed a consistent generation of metal wear particle-induced MGCs which herald MoM hip failure.

2025  Dermal Exposure to Triclosan Induces Changes in Expression of Innate and Adaptive Immune Genes in a Mouse Model


Triclosan has had widespread use in the general population as an antimicrobial agent and is commonly found in consumer products such as soaps, deodorants, toothpastes, shaving creams, mouth washes, and cleaning supplies. Triclosan has recently attracted the attention of the scientific community, regulatory agencies and the general public because of its high production volume, widespread applications and reports of endocrine-disrupting effects. A positive association between urinary levels of triclosan and diagnosis of allergies, hay fever, and sensitization to Aeroallergens and foods has been identified. While not generally considered to be a sensitizing chemical, work by our group has recently shown that dermal exposure to triclosan at concentrations similar to those in consumer products augmented the allergic response to a known allergen in a mouse asthma model. However, the specific mechanism of this augmentation has yet to be elucidated. These studies were conducted to investigate the mechanism responsible for the augmented allergic response following dermal triclosan exposure. BALB/c mice were exposed dermally on the ears to concentrations of triclosan ranging from 0.75-3% (0.375-1.5mg/mouse/day) for up to 9 consecutive days. Expression of immune genes in the ears and lymph nodes of mice following exposure was analyzed using quantitative polymerase chain reaction. Robust thymic stromal lymphopoietin (TSLP), IL-1 beta, TNF-alpha increases and modest CCL22 & IL-22 dose responsive increases in gene expression were observed in the ears. In the lymph node draining the exposure site, dose responsive increases in CCL22 & IL-4 and decreases in T-bet, IFN-gamma, TNF-alpha & IL-1beta gene expression were observed. A decrease in expression of RORgammat was observed in both the ear and lymph nodes following exposure to 3% triclosan. These results suggest that triclosan may augment allergic responses by modulating both innate and adaptive genes.

2026  The α7 Nicotinic Acetylcholine Receptor Agonist GTS-21 Improves Bacterial Clearance in Mice by Restoring Hyperoxia-Compromised Macrophage Function


Mechanical ventilation with supraphysiological concentrations of oxygen (hyperoxia) is routinely used to treat patients with respiratory distress. However, prolonged exposure to hyperoxia compromises macrophages’ ability to phagocytose and clear bacteria. Previously, we have shown that hyperoxia induced the release of nuclear protein, high mobility group box 1 (HMGB1), into both the airways of hyperoxia-exposed mice and the extracellular milieu of cultured macrophages. Extracellular HMGB1 can impair macrophage phagocytosis and increase mortality of mice infected with Pseudomonas aeruginosa (PA). GTS-21, an α7nACHR agonist, can inhibit endotoxin-induced HMGB1 release. The aim of this study was to determine whether GTS-21 can inhibit hyperoxia-induced HMGB1 release into the extracellular milieu, enhance macrophage function, and improve bacterial clearance in mice under hyperoxic conditions. GTS-21 (0.04, 0.4, and 4 mg/kg)
was systemically administered to mice that were exposed to hyperoxia (≥99% O₂) and subsequently challenged with PA. We found that 4 mg/kg of GTS-21 significantly increased bacterial clearance, decreased acute lung injury and accumulation of airway HMGB1. To investigate the cellular mechanism of these observations, RAW 264.7 cells, a macrophage-like cell line, were incubated with different concentrations of GTS-21 prior to exposure to 95% O₂. We found that GTS-21, at 5, 25, and 50 μM, inhibited HMGB1 release and significantly enhanced macrophage phagocytic activity. Together these results indicate that GTS-21 is effective in inhibiting hyperoxia-induced HMGB1 release, enhancing macrophage function, and improving bacterial clearance under hyperoxic conditions. Therefore, the e7nAChR represents a possible pharmacological target to improve the clinical outcome of patients on ventilators by augmenting host defense against bacterial infections.

The present results point towards involvement of epithelial cell perturbations and subsequent NGK2D-RAE-1-mediated response by IEL in allergic sensitization. Together, these data demonstrate the potential involvement of intestinal stress, e.g. by luminal contents such as food constituents, pharmaceuticals or microbial components, in allergic sensitization.

Oxytate, a 60% w/v perfluoro(1,2-butylenoxohexane) intravenous emulsion, is being developed for the treatment of traumatic brain injury (TBI). PFC emulsions are cleared from circulation via the mononuclear phagocyte system (MPS), namely macrophages, monocytes, and neutrophils. Thus there is concern that PFCs may have a negative impact on immunocompetence. We evaluated Oxytate’s effects on viral and bacterial clearance in host resistance models and on ex vivo macrophage function. BALB/c mice received saline, Oxytate (3, 6, or 12 mL/kg) or an immunomodulatory control followed an hour later by infection with influenza virus (–4 x 10⁴ PFU), Streptococcus pneumoniae (–1 x 10⁵ CFU/mouse) or Listeria monocytogenes (–1 x 10⁵ CFU/mouse). Influenza viral titers and influenza-specific IgM and IgG were assessed in lung homogenates. Lung or spleen and liver homogenates were assessed for S. pneumoniae and L. monocytogenes titers, respectively. Ex vivo macrophage phagocytosis was assessed 7 days following Oxytate treatment. Oxytate caused a slight stimulation of the T-dependent antibody response (TDAR) to influenza (increased antibody). The rate and efficiency of viral clearance was no different for the Oxytate treatment groups than the controls with influenza viral load reduced by ~4 logs by Day 6 and completely cleared by Day 8. Saline and all Oxytate treatment groups achieved ~5 log reduction in S. pneumoniae within 24 hours of infection. Treatment of mice with Oxytate resulted in 80-100% mortality by Day 4 following L. monocytogenes infection. Oxytate dose-dependently increased ex vivo splenic macrophage phagocytosis 7 days after in vivo administration. Overall, these studies demonstrate that treatment of mice with Oxytate did not impact the efficient clearance of either Influenza virus or S. pneumoniae at any dose level. However, Oxytate decreased the ability of mice to defend against systemic L. monocytogenes infection.

Environmental toxins induce a novel CYP2E1/leptin signaling axis in liver. This in turn activates a poorly characterized innate immune response that contributes to nonalcoholic steatohepatitis (NAS) progression. To identify the relevant subsets of T-lymphocytes in CYP2E1-dependent, environment-linked NAS, we utilized a model of diet induced obese (DIO) mice that are chronically exposed to brodochloromethane (BDCM). Mice deficient in CYP2E1, leptin (ob/ob mice), or both T and B cells (Plp/Rag2 double knockout (KO) mice) were used to delineate the role of each of these factors in metabolic oxidative stress-induced T cell activation. Results revealed that elevated levels of lipid peroxidation, tyrosyl radical formation, mitochondrial tyrosine nitration and hepatic leptin as a consequence of metabolic oxidative stress caused increased levels of hepatic CD57, a marker of peripheral blood lymphocytes including NKT cells. CD8+CD57+ cytotoxic T cells but not CD4+CD57+ cells were significantly decreased in mice lacking CYP2E1 and leptin. There was a significant increase in the levels of T cell cytokines IL-2, IL-1β, IFN-γ in BDCM exposed DIO mice but not in mice that lacked CYP2E1, leptin or T and B cells. Apoptosis as evidenced by TUNEL assay and levels of cleaved caspase-3 was significantly lower in leptin and Plp/Rag2 KO mice and highly correlated with protection from NAS. The results described above suggest that higher levels of oxidative stress-induced lepin mediated CD8+CD57+ T cells play an important role in the development of NAS. It also provides a novel insight of immune dysregulation and may be a key biomarker in NAS.

Responses to ingested food proteins in the gut usually result in oral tolerance. Failure in oral tolerance induction may cause allergic sensitization, but the mechanism behind this is not yet fully understood. Previously, we found that allergic sensitization of C57BL/6 mice via intragastric treatment with PE plus CT resulted in increased NKG2D expression on IEL. Furthermore, duodenum and ileum sensitization of C57BL/6 mice via intragastric treatment with PE plus CT resulted in increased mRNA levels for the NKG2D ligand RAE-1, and for the Th2 cytokine IL-5. In addition, when mice were treated with anti-NKG2D blocking antibody during intragastric sensitization, levels of PE-specific IgG1 and IgE were significantly enhanced.

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2031 Automated High Content Imaging of NF-κB Signaling Modulation in Drug-Induced Liver Injury (DILI)

S. Huppelschoten, B. Herpers, L. Fredriksson, S. Wink, K. Yan and B. van de Water, LACDR, Toxicology, Leiden University, Leiden, Zuid-Holland, Netherlands.

Drug-Induced Liver Injury (DILI) is the most important reason to withdraw initially approved drugs from the market, to stop the development of novel drugs during the clinical test phase and for failure of FDA approval. Currently, the very heterogeneous group of chemicals inducing idiosyncratic DILI cannot be predicted in vitro, in vivo or even in clinical trials. This illustrates the necessity of a better understanding of underlying mechanisms. DILI parent compounds or metabolites induce cellular stress, which can ultimately lead to cell death. Drug exposure combined with pro-inflammatory cytokine stimulation does synergistically increase apoptosis, which indicates that cytokine signaling plays an important role in the development of idiosyncratic DILI. The major pro-inflammatory cytokines in the liver active NF-κB, a transcription factor regulating immune reactions, cell growth and death. To gather more insight in the activation of NF-κB and its importance in DILI, we have generated HepG2 Bacterial Artificial Chromosome (BAC) GFP-based reporter cell lines of the most important players in the NF-κB signaling cascade. We established and carefully characterized GFP-BAC cell lines of IkBα, RelA and Icam1 and determined the effect of DILI-inducing compounds on pro-inflammatory cytokine signaling by live confocal microscopy. The time-resolved dynamics of the expression and/or localization of these genes was determined at a single cell level at different cytokine and DILI compound concentrations. A large panel of DILI compounds affected the kinetics of IkBα degradation, RelA nuclear translocation and Icam1 expression induced by either TNF-α or IL1β. For various compounds this was directly associated with enhanced cytotoxicity of combined DILI/cytokine exposure. We propose that our novel high content imaging approach would be an effective means to pinpoint DILI hazard for novel drug candidates.

2032 Effect of 1-Bromopropane on Expression of IL-4 and IL-5 and Secretion of β-Hexosaminidase by Calcium Influx in RBL-2H3 Cells

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1-Bromopropane (1-BP) is an alternative to ozone-depleting chlorofluorocarbons that has a variety of potential applications as a deaginging agent for metals and electronics, coating agent and as a solvent vehicle for spray adhesives in the workplace. Although 1-BP has been recently reported to generate neurotoxicity, reproductive toxicity, hepatotoxicity, and immunotoxicity in rodents and human, there is little information about the effect of 1-BP on the mast cell degranulation and allergic responses. This study investigated the effect of 1-BP on degranulation and inflammatory mediator production in RBL-2H3 cells. 1-BP significantly increased release of beta-hexosaminidase and histamine in mast cells. It also increased the modula-

2033 Renal Dendritic Cells Attenuate Cisplatin Neprotoxicity Independent of Neutrophil Regulation

R. K. Tadagavadi, G. Guofeng, W. Wang and W. Reeves, Department of Medicine, Pennsylvania State University College of Medicine, Hershey, PA.

Cancer chemotherapy can produce sterile inflammation with associated tissue injury and organ dysfunction. Cisplatin is a very effective chemotherapeutic agent used against a wide range of solid tumors. Major adverse effects of cisplatin therapy are immunotoxicity and acute kidney injury. Neutrophils are reported to infiltrate and exacerbate injury in a wide range of sterile inflammatory models of tissue injury. Here, we studied the kinetics of neutrophil infiltration into kidneys and their role in cisplatin-mediated kidney injury. Mice treated with cisplatin showed a marked decrease in total blood leukocytes but a 5-fold elevation in neutrophils at 72 hrs after cisplatin treatment. In kidneys, cisplatin treatment caused a 2 fold increase in total kidney leukocytes as early as 24 hrs after cisplatin treatment. Renal infiltration of myeloid cells in cisplatin treated mice was prominent; monocytes and neutrophils were elevated by 10 fold as compared to saline treated mice. We depleted neutrophils using a neutrophil-specific antibody (clone 1A8) to examine the functional role of infiltrating neutrophils after cisplatin nephrotoxicity. This antibody resulted in greater than 90% depletion of neutrophils. Of note, depletion of neutrophils had no impact on the extent of cisplatin-induced kidney injury or renal dysfunction as compared to non-depleted mice. Earlier, we reported that dendritic cell depletion in CD11c-DTR mice causes exacerbation of kidney injury and a dramatic increase in renal neutrophil numbers 24 hrs after cisplatin treatment. Thus, we also examined the role of neutrophils in dendritic cell depleted mice treated with cisplatin. Dendritic cell depletion resulted in worse renal dysfunction in spite of neutrophil depletion. Supporting that dendritic cell mediated protection occurs independent of its effects on neutrophils. These data demonstrate that cisplatin nephrotoxicity is independent of neutrophil-mediated inflammation and dendritic cells protect kidneys via neutrophil-independent mechanisms.

2034 Entecavir: Chemokine Receptor 2 (CCR2)-Mediated Chemotaxis As A Mode of Action for Mouse-Specific Pulmonary Macrophage Accumulation

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Entecavir (ETV; Barudacil®) is a marketed guanosine nucleoside analog that inhibits hepatitis B viral replication. In preclinical studies, ETV caused lung toxicity in mice but not in rats, dogs or monkeys. Microscopically, lungs from ETV-treated mice (10 mg/kg p.o. 1 mo) showed alveolar septal thickening and accumulation of macrophages, and bronchoalveolar lavage analysis revealed a 3-fold increase in alveolar macrophages, with no effects on other inflammatory cells. CCR2 null mice were resistant to ETV-induced macrophage accumulation suggesting a role for this receptor in the ensuing pulmonary toxicity. To further explore the role of CCR2 in macrophage accumulation, ETV was evaluated for chemotactic activity in human and mouse macrophages. While weaker than monocyte chemotactic protein-1 (MCP1; optimum concentration <10 nM), ETV was a chemotaxin in mouse WEHI 274.1 cells with maximum chemotaxis observed 75 nM. In contrast, ETV was not chemotactic in human THP-1 cells at concentrations up to 6 μM. ETV also exhibited chemotactic activity in mouse peripheral blood mononuclear cells (PBMCs) with an optimal concentration of 222 nM, but showed no activity in PBMCs from CCR2 null mice. To further test species differences in chemotaxis, the effect of ETV in PBMCs from rats and humans was studied. ETV showed weak chemotaxis for PBMCs isolated from Sprague-Dawley rats, with optimal chemotactic activity at 2 μM. In contrast, no chemotactic activity was observed in human PBMCs at ≤6 μM. These results indicate that ETV-induced pulmonary macrophage accumulation and lung toxicity is dependent on CCR2-mediated chemotaxis. As the chemotactic activity was specific for mice, lung toxicity was not predicted as a potential liability of ETV therapy.

2035 Differential Infiltration of Macrophages into the Testis after Mono-(2-Ethylhexyl) Phthalate Exposure in F344 Rats and C57B6/J Mice

A. Stiermer, C. Murphy and I. H. Richburg, Pharmacology and Toxicology, University of Texas at Austin, Austin, TX.

Different strains and species of animals are used for both toxicological studies and basic research. Often general similarities in toxicity between animals exist, but sensitivities and specific mechanisms of toxicity can vary. Phthalates, ubiquitously used plasticizers, are known Sertoli cell toxicants. Mono-(2-ethylhexyl) phthalate (MEHP) induced testicular injury has been studied in rats and mice, where inflammatory signaling has been shown to mediate germ cell apoptosis in the common MEHP mechanism. Yet, rats have been shown to have 30 fold lower LOAEL than mice. To further define this difference we analyzed germ cell apoptosis and immune infiltration into the testes of F344 rats and C57B6/J mice. MEHP (1g/kg, gavage) or equivalent volume was given to 28-day (d) rats and mice. Testes were collected 12, 24, and 48 hours (h) after exposure. Single cell suspensions were isolated from the interstitium of one testes, reacted with anti-CD11b (mono-

536 SOT 2014 Annual Meeting
2036 Methylene diphenyl Diisocyanate Binds Membrane and Intracellular Proteins of Intact THP-1 Cells


Diisocyanates (dNCO) are used in the production of polyurethane products and can cause occupational asthma. Although dNCO asthma displays the same pathological hallmarks as other allergic asthma, dNCO-specific IgE can only be detected within a small subset of dNCO asthmatics. Because of this low prevalence, we propose that methylenediphenyl diisocyanate (MDI) may adduct to cell membrane and intracellular proteins, contributing to the development of non-IgE asthma. In this study, human monocytic leukemia (THP-1) cells were exposed to varying concentrations of MDI. After exposure, cells were processed into soluble and membrane fractions, dialyzed, and analyzed for extent of MDI protein binding, following acid hydrolysis, by quantification of the MDI hydrolysis product, methylenedianiline (MDA). Proteins were electrophoresed on sodium dodecyl sulfate polyacrylamide gels and protein bands were excised, hydrolyzed and analyzed for MDA. Protein binding was not affected by cytochalasin D, a chemical which binds actin filaments, and is consistent with reactive MDI binding to intracellular targets. The extent of MDI intracellular protein binding was identified as containing MDI bound proteins. The extent of MDI intracellular protein binding was not affected by cytochalasin D, a chemical which binds actin filaments and inhibits active uptake into cells. Proteins identified in the soluble fraction were all of intracellular origin suggesting that reactive MDI can cross the cell membrane and subsequently haptenate intracellular proteins. The results of the present study support the potential involvement of dNCO haptenated membrane and intracellular proteins in development of asthma.

2037 Biodistribution of Reolysin® (Pelareorep) in Sprague-Dawley Rats to Support the Use of This Orphan Virus As an Investigational Drug for Cancer Treatment

A. Parenteau1, R. Chakraborty1, H. Tran1, A. Hagerman1, S. Serl1, B. Thompson5, M. Coffey1, R. Tavcar4, I. Boulay2, M. Biggs3 and R. Forrest5.

Reolysin® (pelareorep) is a clinical formulation of the human Reovirus Type 3 Dearing strain. The clinical safety and efficacy of Reolysin® as an oncolytic therapy in humans is being tested on an assortment of cancer indications as a mono and/or combination therapy. Reovirus has many inherent characteristics that make it a potential candidate for virotherapy, including the rapid and natural spread through the haematogenous route, the ability to overcome immunological barriers thereby reaching tumor sites, and being replication-competent. The purpose of this study was to elucidate the bio-distribution pattern of Reolysin® in healthy Sprague-Dawley rats. Following a single 15-minute intravenous infusion (at dose levels of 6.5E+07, 6.5E+08 and 6.5E+09 viral particles/animal, based on an average body weight of 225 grams) via the tail vein in Sprague-Dawley rats, the levels of virus genome were determined in 16 organs/tissues by RT-qPCR (Reverse Transcriptase-Quantitative Polymerase Chain Reaction) over a 336 hr follow-up period. Maximal reovirus RNA levels were observed in the spleen, indicating its involvement in viral uptake and clearance, followed by heart, ovaries, tail (inflamed site), liver and lungs. All the organs/tissues demonstrated quantifiable levels of reovirus genome at the end of the follow-up period, suggesting substantial to complete viral clearance. Several studies in the last decade have described the use of reovirus for treating ovarian cancers. An increase of reovirus genome in ovaries at 24 hr post infection was noted. The results will aid in the design of additional exploratory clinical trials for Reolysin®.

2038 Multi-Endpoint Assessment of Bone Marrow Toxicity in Sprague-Dawley Rats

L. Piccotti, K. Ghoreishi, L. Alvarado, R. Maggantay, S. Couto and M. Breider.

Bone marrow toxicity is often an unwanted side effect of kinase inhibitors. We previously demonstrated bone marrow hypopcellularity in rats dosed with CC0485118, a small molecule kinase inhibitor of spleen tyrosine kinase (5yk) and Janus kinase-2 (Jak-2). The current study was conducted to further characterize the bone marrow toxicity caused by CC0485118 using a recently developed bone marrow flow cytometry assay. In this study, male Sprague-Dawley rats received once daily oral (gavage) doses of CC0485118 at 25, 50 and 200 mg/kg for 7 consecutive days. CD71+ cells and CD11b/c+ cells were measured by flow cytometry in femurs of vehicle- and test article-treated rats approximately 24 hours after the last dose to assess compound effects on the erythroid and myeloid lineages, respectively. Bone marrow toxicity was also assessed by measuring peripheral blood reticulocyte counts and by histopathologic examination of the sternum. Treatment of rats with CC0485118 at 200 mg/kg resulted in a moderate reduction in total nucleated cells (TNC) in the bone marrow, compared to vehicle control rats. This finding was confirmed by moderate to marked bone marrow hypopcellularity observed microscopically and was consistent with a moderate drop in reticulocytes. The relative percentages of CD11b/c+ myeloid cells were markedly increased in rats dosed at 200 mg/kg. However, a mild to moderate reduction in the absolute numbers of both CD71+ erythroid cells and CD11b/c+ myeloid cells were observed in these animals, resulting in no change in the myeloid to erythroid ratios (M:E) compared to controls. Thus, the flow cytometry data demonstrated toxicity to both the erythroid and myeloid lineages by CC0485118. The results of this study demonstrated that measuring CD71+ and CD11b/c+ cells in rat bone marrow can be used to complement histopathologic examination by assessing compound effects on the erythroid and myeloid lineages. Future studies will further test the sensitivity and predictability of this assay by using compounds that differentially target myeloid or erythroid cells.

2039 Cysteine Microenvironment Determines Nitrosylation Status: Lessons from Proteome Analysis of NOS2 and GSNOR Knockout Mice

C. B. Massa1, P. T. Doulias2, H. Ischiropoulos1 and A. Gove1.

Reolysin® (pelareorep) is a clinical formulation of the human Reovirus Type 3 Dearing strain. The clinical safety and efficacy of Reolysin® as an oncolytic therapy in humans is being tested on an assortment of cancer indications as a mono and/or combination therapy. Reovirus has many inherent characteristics that make it a potential candidate for virotherapy, including: the rapid and natural spread through the haematogenous route, the ability to overcome immunological barriers thereby reaching tumor sites, and being replication-competent. The purpose of this study was to elucidate the bio-distribution pattern of Reolysin® in healthy Sprague-Dawley rats. Following a single 15-minute intravenous infusion (at dose levels of 6.5E+07, 6.5E+08 and 6.5E+09 viral particles/animal, based on an average body weight of 225 grams) via the tail vein in Sprague-Dawley rats, the levels of virus genome were determined in 16 organs/tissues by RT-qPCR (Reverse Transcriptase-Quantitative Polymerase Chain Reaction) over a 336 hr follow-up period. Maximal reovirus RNA levels were observed in the spleen, indicating its involvement in viral uptake and clearance, followed by heart, ovaries, tail (inflamed site), liver and lungs. All the organs/tissues demonstrated quantifiable levels of reovirus genome at the end of the follow-up period, suggesting substantial to complete viral clearance. Several studies in the last decade have described the use of reovirus for treating ovarian cancers. An increase of reovirus genome in ovaries at 24 hr post infection was noted. The results will aid in the design of additional exploratory clinical trials for Reolysin®.

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Contact dermatitis is one of the most common skin diseases, with a great socio-economic impact. Disperse dyes, which are suitable for dyeing synthetic fibers, are responsible for the great majority of allergic contact dermatitis cases to textile dyes. The aim of the present study was to study the sensitization potential of textile dyes using a non-radioactive biphasic murine local lymph node assay (LLNA) and an in vitro Loose-fit Co-culture based Sensitization assay (LCSA). In biphasic LLNA, female BALB/c mice were shaved over a surface of approximately 2 cm square on back and treated with various concentrations of textile dyes. The 'sensitization' treatment included application of 50 µl of test substance on the back or on days 1-3 followed by 'challenge' treatment on days 15-17 with application of 25 µl of test substance on the dorsum of both ears. End-points on day 19 following deep carbon-di-oxide anesthesia included thickness and weight of an ear biopsy, lymph node weight, lymph node cell count and the proportion of various lymphocyte subpopulations. The LCSA involved single layer of human non-differentiating keratinocytes and of allogenic floating monocytes which are cocultured in serum-free medium in the presence of a cytokine cocktail. The coculture develops into a system consisting of activated keratinocytes and dendritic cell-related cells. The half-maximal increase in CD86 expression on the dendritic cell-like cells (EC50 values) was used to compare the sensitizing potential of tested substances. The results showed the usefulness of the modified biphasic LLNA to study sensitization potential of textile dyes. The results from LCSA correlated well with data derived from the modified version of LLNA and human data. The LCSA represented a suitable test system to simultaneously analyze irritative and sensitizing properties of chemicals altogether.
**2041** Failure of Reversal of Oxidative Damage in Renal Tissues of Lead Acetate-Treated Rats

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Removal of lead from the environment of man or otherwise, the movement of man from lead-contaminated areas has been employed as a means of abatement of the toxic effects of lead. Whether toxic effects in already-exposed individuals subside after lead withdrawal remains unanswered. In order to understand the reversibility of nephrotoxicity induced by lead acetate, male Wistar rats were orally exposed to 0.25, 0.5 and 1.0mg/ml of lead acetate for 6 weeks. One-half of the rats were sacrificed at 6 weeks while the other half remained for an additional 6 weeks after withdrawal of lead. There were significant (p<0.05) increases in Glutathione S-transferase (GST), Catalase (CAT), Superoxide dismutase (SOD), Hydrogen peroxide (H2O2) generated and Malondialdehyde (MDA) in a dose-dependent manner, with concomitant reduction in glutathione (GSH) content and Gluthathione peroxidase (GPx) activity. The pattern of alterations in most of the oxidative stress and antioxidant parameters remained similar in rats from the withdrawal period, although CAT and SOD activities reduced, in contrast to their elevation during the exposure period. Serum creatinine levels were significantly (p<0.05) elevated although CAT and SOD activities reduced, in contrast to their elevation during exposure period. Serum creatinine levels were significantly (p<0.05) elevated although CAT and SOD activities reduced, in contrast to their elevation during

**2042** Disposition and Toxicity of Mercury in Aging Rats

C. Bridges, L. Joshee and R. K. Zalups. Mercer University, Macon, GA.

Progressive loss of functioning nephrons, secondary to age-related glomerular disease, can impair the ability of the kidneys to effectively clear metabolic wastes and toxicants from blood. Additionally, as renal mass is diminished, cellular hypertrophy occurs in the remaining nephrons that remain (primarily along the proximal tubule). Consequently, we hypothesize that the remaining functioning nephrons are at an increased risk of being intoxicated by nephrotoxic compounds, such as the acute nephrotoxicant, inorganic mercury (Hg\(^2+\)). The purpose of the present study was to characterize the renal pathological effects of aging on the renal disposition and toxicity of Hg\(^2+\) using young adult and two-year old rats. Paired groups of animals were injected (i.v.) with either a 0.5 \(\mu\)mol/kg non-toxic or a 2.5 \(\mu\)mol/kg nephrotoxic dose of mercuric chloride. Significant differences were detected between corresponding groups of young and aged rats. Plasma creatinine, blood urea nitrogen and renal biomarkers were greater in both groups of aged rats than in corresponding young adult rats. Histologically, evidence of glomerular sclerosis, tubular atrophy, interstitial inflammation and fibrosis were significant pathological features in kidneys of aged animals. In addition, proximal tubular necrosis, especially along the straight segments in the inner cortex and outer stripe of the outer medulla was a prominent feature in the renal sections from both aged and young rats treated with the nephrotoxic dose of Hg\(^2+\). Our findings indicate that aging has a profound effect of the renal disposition of administered Hg\(^2+\) and reduces renal overall function more significantly in aged rats than young rats.

**2043** Profiling of Human, Canine, and Rat Urine Samples Using Bio-Plex Pro RBM Kidney Toxicity Assays


Acute kidney injury (AKI) is a serious medical condition caused by a variety of bodily insults, including trauma from an accident, ICU acute renal failure, or exposure to nephrotoxic agents. From a pharmaceutical industry standpoint, drug-induced toxicity is a serious issue, sidelineing 30% of therapeutics overall, from pre-clinical lead compounds to marketed drugs (Bonventre et al. 2010). Early detection of AKI along with proper treatment regimens is critical in the prevention of further loss of kidney function. The current clinical criterion for the diagnosis of AKI relies on traditional tests (glomerular filtration rate and blood urea nitrogen) that employ late biomarkers that are detectable days or weeks after kidney damage has occurred. Researchers are looking for biomarkers that are sensitive, specific, and early indicators of AKI. Further, level monitoring should be cost effective and permit rapid reporting of results (Scolum et al. 2012).

**2044** Protection against Type 2 Diabetes-Induced Nephropathy by Multiple Exposures to Low-Dose Radiation Was Associated with Improvement of Dyslipidemia and Insulin Resistance

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The aims of the present study were to investigate whether LDR can prevent type 2 diabetes-induced renal damage and to explore the possible mechanism. Mice were fed with a high-fat diet for 12 weeks, followed by a single injection of streptozoto- cin to develop a type 2 diabetic mouse model. Then, these mice were exposed to LDR at different doses (25, 50, and 75 mGy) for 4 or 8 more weeks with HFD treatment. The kidney weight, renal function, blood glucose level, and insulin resistance were examined. The pathological changes were measured by hematoxlin and eosin, periodic acid Schiff, and Mason’s trichrome staining. Systematic and renal lipid profiles were detected by ELISA. Renal inflammation, oxidative stress, and fibrosis were assessed by real-time PCR, western blot assays, and immunohistochemical staining. HFD/STZ-induced type 2 diabetic mice exhibited severe pathological changes in the kidney and renal dysfunction. Exposure of the mice to LDR for 4 weeks, especially at 50 and 75 mGy, prevented most of the abnormalities. The beneficial effects became weaker after LDR treatment for 8 weeks. LDR also attenuated serum lipid levels and subsequent lipotoxicity characterized by insulin resistance, inflammation, and oxidative stress in the diabetic kidneys. Finally, the LDR-induced anti-oxidative effect was due to upregulation of renal nuclear factor E2-related factor-2 expression and function. These results suggest that LDR prevented type 2 diabetes-induced kidney injury. The protective mechanisms may be mainly attributed to the prevention of dyslipidemia and the subsequent lipotoxicity-induced insulin resistance, inflammation, and oxidative stress.

**2045** Attenuation of Hyperlipidemia- and Diabetes-Induced Early-Stage Apoptosis and Late-Stage Renal Dysfunction via Administration of Fibroblast Growth Factor 21 Is Associated with Suppression of Renal Inflammation

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Lipotoxicity is a key feature of the pathogenesis of diabetic kidney disease, and is attributed to excessive lipid accumulation (hyperlipidemia). Increasing evidence suggests that fibroblast growth factor (FGF-21) has a crucial role in lipid metabolism under diabetic conditions. The present study investigated whether FGF-21 can prevent hyperlipidemia- or diabetes-induced renal damage, and if so, the possible mechanism. Mice were injected with free fatty acids (FFAs, 10 mg/10 g body weight) or streptozotocin (150 mg/kg) to establish a lipotoxic model or type 1 diabetic model, respectively. Simultaneously the mice were treated with FGF-21 (100 pg/kg) for 10 or 80 days. The kidney weight-to-tibia length ratio and renal function were assessed. Systematic and renal lipid levels were detected by ELISA and Oil Red O staining. Renal apoptosis was examined by TUNEL assay. Inflammation, oxidative stress, and fibrosis were assessed by Western blot. Acute FFA administration and chronic diabetes were associated with lower kidney-to-tibia length ratio, higher lipid levels, severe renal apoptosis and renal dysfunction. Obvious inflammation, oxidative stress and fibrosis also observed in the kidney of both mice models. Deletion of the fgf21 gene further enhanced the above patho-
logical changes, which were significantly prevented by administration of exogenous FG21. These results suggest that FFA administration and diabetes induced renal damage, which was further enhanced in FG21 knockout mice. Administration of FG21 significantly prevented both FFA- and diabetes-induced renal damage partly by decreasing renal lipid accumulation and suppressing inflammation, oxidative stress, and fibrosis.

**2046** ALDH16A1 Interacting with HPRT1 in the Purine Salvage Pathway

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Purpose: Gout, a common form of inflammatory arthritis, is strongly associated with elevated uric acid concentrations in the blood (hyperuricemia). A recent study identified a rare single nucleotide polymorphism in the aldehyde dehydrogenase 16A1 (ALDH16A1) gene, ALDH16A1*2, showing a strong association with hyperuricemia and gout. The purpose of this study is to determine if ALDH16A1*2 plays a role in hyperuricaemia and gout by modulating HPRT1 in the purine salvage pathway.

Methods: Molecular modeling was performed to determine if ALDH16A1 is catalytically inactive and the binding ability with HPRT1. Transfection and immunoprecipitation were performed to assess the ex vivo interaction between ALDH16A1 and HPRT1.

Uric acid generation in transfected cells overexpressing ALDH16A1 was determined to assess the possible role of ALDH16A1 in the purine salvage pathway. Results: Molecular modeling predicts human ALDH16A1 protein would lack catalytic activity due to the absence of the catalytically important cysteine residue (Cys-302) as well as a loss of substrate binding and cofactor binding (NAD+) pockets. Molecular modeling as well as ex vivo interaction studies implies that ALDH16A1, but not ALDH16A1*2, may interact with HPRT1. The decrease in uric acid generation in ALDH16A1 overexpressing cell lines implies a possible role of ALDH16A1 in the purine metabolism pathway.

Conclusion: These results lead to the intriguing possibility that association between ALDH16A1 and HPRT1 may be required for optimal HPRT1 activity; disruption of this interaction may contribute to the hyperuricemia seen in ALDH16A1*2 carriers.

**2047** The Possible Role of Fluoride on Aggravation of Tubulointerstitial Fibrosis Caused by Unilateral Ureteral Obstruction through TGF-β1

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Fluoride (F) is an environmental pollutant. The contamination of ground water by F has been reported in China, India, Iran, and Argentina. Since F is filtered by the kidney, humans or experimental animals with renal insufficiency may be affected by F more seriously. We used the tubulointerstitial fibrosis model of rats induced by unilateral ureteral obstruction (UUO). We examined whether or not F deteriorates tubulointerstitial fibrosis in rats with UUO. In this study, the pathological changes and the expression of TGF-β1 mRNA in the renal cortex of rats were examined. Left ureter was ligated and sham-operation was done in 7-week-old male rats weighing 240-260g. F was administered to rats with UUO at 0, 75 and 150 ppm and sham-operated rats at 0 and 150 ppm in drinking water for 2 weeks. The kidneys obtained were immediately harvested, decapsulated and carefully dissected into the cortex on ice. Real-time PCR was performed for the analysis of TGF-β1 mRNA. The cortex was also pathologically examined by Masson-trichrome stain. Body weight of rats with UUO exposed to 150 ppm of F was significantly lower than that of rats with UUO exposed to 0 and 75 ppm of F. The average of water intake in rats with UUO exposed to 75 and 150 ppm of F was significantly lower than that in rats with UUO that were not exposed to F. Masson-trichrome stain revealed the deterioration of tubulointerstitial fibrosis in the renal cortex of rats with UUO exposed to 150 ppm of F. The expression of TGF-β1 mRNA was increased in rats with UUO exposed to 150ppm of F than in those exposed to 0 and 75ppm of F. These observations suggest that F develops tubulointerstitial fibrosis in rats with UUO via increased action of TGF-β1.

**2048** Effects of Cadmium (Cd) on the Localization of Cystatin C and Megalin in Rat Kidney

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Cadmium (Cd) is a nephrotoxic environmental pollutant for which there is a need for improved biomarkers of toxicity. Cystatin C is a low molecular weight protein (13.3 kilodaltons) that is being investigated as a potential biomarker of the early stages of Cd-induced kidney injury. During recent studies in this area, we found that the general patterns of cystatin C localization in the proximal tubule seemed to correspond to, but not exactly mimic, those of the proximal tubule transport protein megalin and that Cd seemed to alter the localization of both proteins. In the studies described here, we have developed a procedure for the dual immuno-fluorescence labeling of cystatin C and megalin in single sections of rat kidney. Cryosections were incubated overnight in primary antibodies-rat antimegalin (ab-camsa/7699) and goat anticystatin C (abcam; ab176462) followed by incubation in the respective secondary antibodies, a TRITC conjugated chicken anti rabbit IgG (abcam; ab6826) and an Alexafluor 488 conjugated donkey anti goat IgG (abcam; ab50129), and then processed for confocal microscopic imaging. In control samples, both cystatin C and megalin exhibited speckled patterns of labeling in an area just beneath the brush border of the proximal tubule epithelial cells. By contrast tissue samples from animals that had been treated with Cd (0.6 mg subcutaneously, 5 days per week for 6, 9 or 12 weeks) showed diffuse labeling of both molecules along the apical cell surface, with retraction and deterioration of the apical brush border. These changes coincided with an increase in the urinary excretion of cystatin C. These results provide further evidence that Cd increases the urinary excretion of cystatin C and they suggest that this effect may result from disruption of the megalin-mediated transport in the proximal tubule.

**2049** Zn-Excess Intake Increases Systemic Blood Pressure and Deteriorates Renal Function through Superoxide Radical-Induced Oxidative Stress

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In recent years, foods and tablets with addition of the essential trace element, zinc (Zn), have been sold for the maintenance of good health and for health promotion. However, Zn toxicity due to Zn-intake excess has not been fully established. Using rats fed 22 g/day of a control diet containing 0.005% (1.1 mgZn/day) Zn or two Zn-excess diets containing 0.05% (11 mgZn/day) or 0.2% (44 mgZn/day) Zn for 4 weeks, we examined whether or not Zn-excess intake affected systemic blood pressure (BP) and renal function. An increase in Zn intake elevated systolic BP, diastolic BP and mean arterial pressure (MAP), and reduced renal blood flow (RBF) and inulin clearance in a dose-dependent manner. This decline in inulin clearance may be the result of a fall in RBF. Administration of the nitric oxide (NO) synthase inhibitor, L-NAME, markedly increased MAP and significantly decreased RBF in the three groups of rats. Inversely, administration of the exogenous superoxide radical scavenger, tempol, significantly increased MAP and substantially increased RBF in the three groups of rats. Resultantly, tempol dramatically restored MAP and RBF levels in rats fed two Zn-excess diets to levels comparable to those observed in rats fed a control diet. These observations suggest that both an elevation in systemic BP and a reduction in RBF seen in the two Zn-excess diet groups result from a decrease in the action of the vasodilator, NO through the formation of peroxynitrite based upon the non-enzymatic reaction of NO and increased superoxide radical. Indeed, the activity of the endogenous superoxide radical scavenger, Cu/Zn-superoxide dismutase, was significantly reduced in the vesicular wall of rats fed two Zn-excess diets vs. a control diet. Thus, Zn-excess intake increases systemic BP and deteriorates renal function through the oxidative stress caused by superoxide radical. In addition, the lowest observed adverse effect level (LOAEL) of Zn appears to be approximately 11 mg/day.

**2050** Epigenetic Changes in p21 Expression in Renal Cells after Exposure to Bromate

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This study tested the hypothesis that bromate (KBrO3)-induced renal cell death is mediated by epigenetic mechanisms. Global DNA methylation, as assayed by 5-methylcytosine staining, was not changed in normal rat kidney cells treated with acute cytotoxic doses of KBrO3 (100 and 200 ppm), as compared to controls. However, KBrO3 treatment did increase p58, p53 and histone 2AX (H2AX)
phosphorylation, and p21 expression. Treatment of cells with inhibitors of DNA methyltransferase (5-azacytidine or 5-Aza) and histone deacetylase (trichostatin A or TSA) in addition to KB03 increased cytotoxicity, compared to cells exposed to KB03 alone. 5-Aza and TSA co-treatment did not alter p38 or p53 phosphorylation, but slightly decreased H2AX phosphorylation, and significantly decreased p21 expression. We also assessed epigenetic changes in cells treated under sub-chronic conditions with environmentally relevant concentrations of KB03. Under these conditions (0-10 ppm KB03 for 28 days), we detected no increases in cell death or DNA damage. In contrast, slight alterations were detected in the phosphorylation of H2AX, p38 and p53. Sub-chronic low dose KB03 treatment also induced a biphasic response in p21 expression, with lower concentrations increasing expression, but higher concentrations decreasing expression. Methylation specific PCR demonstrated that sub-chronic KB03 treatment decreased the methylation of cytosine bases in the p21 gene, as compared to controls. Decreases in cytosine methylation correlated to alterations in p21 protein expression. Collectively, these data show the novel finding that KB03-induced renal cell death is altered by inhibitors of epigenetic modifying enzymes and that KB03 itself induces epigenetic changes in the p21 gene.

**2051 Effects of Proguanil Hydrochloride on Selected Hematological, Liver, and Kidney Function Indices in Rats**


Proguanil is a prophylactic antimalarial drug often used in combination with other drugs, such as atovaquone. However, the toxicity of this compound has not been fully elucidated. In this study, effects of sub-chronic administration of proguanil on selected haematological, liver and kidney function indices were investigated. Forty rats were randomly divided into four groups (i.e. A, B, C and D). Rats in group A (control) were orally administered distilled water while rats in groups B, C and D were orally administered 2.86, 5.71 and 11.43 mg/kg body weight of proguanil hydrochloride respectively for 28 days. The rats were then sacrificed and the full blood count with selected liver and kidney function indices were determined. The results revealed that the drug, at all doses, caused no significant alteration (P>0.05) to platelet count, total white blood cell count, percentage neutrophils, percentage lymphocytes and serum urea, uric acid and creatinine concentrations compared to controls. Moreover, the drug at all doses did not significantly alter (P>0.05) γ-glutamyl transferase activity compared to control. However, the haemoglobin concentration, red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, packed cell volume and serum albumin concentration were significantly reduced at the highest dose of the drug administered compared to controls. The results suggest that proguanil may cause haemolysis and inhibit albumin synthesis in the liver at higher doses; thus, its abuse should be avoided.

**2052 Proteomic Biomarkers for Early Detection of Acute Kidney Injury (AKI) in Rats**

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Traditional urinary biomarkers for the diagnosis of acute kidney injury (AKI) are insensitive and nonspecific. Our studies were carried out to evaluate whether ald-o-keto reductase subfamily (Akr7a1) and selenium-binding protein-1 (SBP-1) might be used as early biomarkers for the detection of AKI. In this study, urinary Akr7a1 and SBP-1 levels were significantly decreased in the nephrotoxicants-treated groups when compared to control group. In contrast, urinary Kim-1, MMP-9, and NAG release were the inflammatory markers.

- BPA at non-cytotoxic concentrations is capable of increasing the expression/secretion of COX-2/PGE2 and cytokines in MMC, and imply that BPA may cause inflammatory response in renal glomeruli and influence the renal function.
- The interleukin-1β (IL-1β) levels were also increased in MMC culture medium after BPA treatment. BPA could also activate the AMP-activated protein kinase (AMPK), which plays an inflammation regulator. Taken together, these results indicate that BPA at non-cytotoxic concentrations is capable of increasing the expression/secretion of COX-2/PGE2 and cytokines in MMC, and imply that BPA may cause inflammatory response in renal glomeruli and influence the renal function.

**2053 Deficiency in Mdr1/MDR1 Increases Paraquat Accumulation and Toxicity in Mice and Human Renal Proximal Tubule Cells**

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Paraquat is a highly toxic herbicide that targets the lungs and kidneys following acute exposures. Prior studies demonstrated that the organic cation transporter 2 and multidrug and toxin extrusion 1 protein contribute to the urinary excretion of paraquat in the kidneys. The purpose of this project was to determine whether the multidrug resistance protein 1 (MDR1/Mdr1, Abc1) also participates in the efflux of paraquat from the kidneys and protects against renal injury. Paraquat transport and toxicity were quantitated in human renal proximal tubule epithelial cells (RPTEC) that endogenously express MDR1. Inhibition of MDR1 function by PSC833 or siRNA increased the cellular accumulation of paraquat in RPTEC cells by > 50%. Reduced efflux was accompanied by enhanced cytotoxicity of paraquat (50% decrease in the LC50 value) in PSC833-treated cells. To determine the role of Mdr1 in vivo, renal paraquat accumulation and subsequent nephrotoxicity were assessed in wild-type and Mdr1a/-/b-null mice administered paraquat (10 or 30 mg/kg, i.p.). The kidneys of Mdr1a/-/b-null mice had a 750% higher paraquat concentration compared to wild-type mice at 4 h. This was accompanied by significantly greater mRNA induction of kidney injury-responsive genes Kim-1 and Npg1 in paraquat-treated Mdr1a/-/b-null mice. Histopathological analysis confirmed the enhanced paraquat toxicity, as more extensive tubular degeneration was observed in Mdr1a/-/b null mice compared to wild-type mice. In conclusion, Mdr1/MDR1 contributes to the elimination of paraquat from the kidneys and protects against toxicity (Supported by DK093903, ES020522, ES050522, ES007148).

**2054 Bisphenol A Induces Inflammatory Response and Cytokine Release in Renal Mesangial Cells**

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Bisphenol A (BPA) is a common ingredient of plastic products, which wildly exists in daily life. In addition to reproductive toxicity, renal toxicity has also been implicated during BPA exposure. BPA exposure has been demonstrated to be associated with low-grade albuminuria in adults. Mesangial cells play an important role in renal glomerular filtration rate. However, the detailed action and mechanism of BPA-induced renal toxicity are still unclear. Here, we investigated the effect and possible mechanism of BPA on glomerular mesangial cells. In this study, mouse mesangial cell (MCC) were treated with 0–100 μM BPA. Cell viability was not affected by 25 and 50 μM BPA after 24 h treatment, but cell viability was decreased to 90% under treatment with 100 μM BPA. BPA 100 μM did not increase the cell apoptosis by PI-Annexin V staining. BPA treatment also did not change the caspase-3 and PARP cleavages in MCC. In renal cells, cyclooxygenase (COX-2) prostaglandin (PG) E2 and interleukin-1β release were the inflammatory markers. After BPA treatment, COX-2 protein expression and PGE2 secretion and nuclear factor κ-B (NFκB)-p65 phosphorylation in MCC were increased in a dose-dependent manner. The interleukin-1β levels were also increased in MCC culture medium after BPA treatment. BPA could induce the AMP-activated protein kinase (AMPK), which plays an inflammation regulator. Taken together, these results indicate that BPA at non-cytotoxic concentrations is capable of increasing the expression/secretion of COX-2/PGE2 and cytokines in MMC, and imply that BPA may cause inflammatory response in renal glomeruli and influence the renal function.

**2055 Cell-Based Approach to Predict Kidney Toxicity**

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Kidney toxicity due to drugs and environmental contaminants accounts for significant patient mortality and morbidity. Current approaches to conduct safety and risk assessment of compounds rely predominately on animal studies and ex-
trapolation of the dose effects to humans although the typical response of animal models and humans can differ greatly. The objective of this study was to develop a cell-based high-throughput assay to identify potential nephrotoxic compounds using primary human proximal tubular epithelial cells (HTPEC). We show that HTPECs grown on a monolayer possess structural (e.g. expression of zonula occludens-1, cytokeratin 18) and functional characteristics (transport and glutathione activity) that are more representative of human kidney than immortalized kidney cell lines. To identify a biomarker of kidney toxicity in vitro, we treated HTPECs with known nephrotoxicants (cyclosporin A, cisplatin, tacrolimus, gentamicin, tobramycin, aristolochic acid, cadmium chloride, ochratoxin A, doxorubicin) as well as a non-toxicant (carboplatin) in at least 6 concentrations for 3, 6, 12 and 24 h. We measured 1,000 selected transcripts, which are a reduced representation of the genome and from which the remainder of the transcriptome can be computationally inferred. Here oxygenase-1 (HO-1) was identified as one of the most differentially expressed genes after incubation with kidney toxicants, confirming in vivo reports of its significant increase in mice, rats and humans during kidney injury. Increases in the HO-1 mRNA (RT-PCR) and protein (ELISA) levels correlated well with morphological changes in the cells. We then developed and optimized a homogenous time-resolved fluorescence (HTRF) assay that allows high-throughput measurement of HO-1. Alterations in the HO-1 levels measured by HTRF correlated with ELISA (r2=0.97). We demonstrate an alternative approach to pre-screen a large number of compounds for kidney toxicity, thus reducing and replacing the use of animals in preclinical toxicity studies.

2056 Cyclosporin A-Induced Apoptosis in Renal Proximal Tubular Epithelial Cells Is Associated with Trb3 Induction

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Cyclosporine (CsA) is currently the primary immunosuppressant for the prevention of allograft rejection in solid organ transplantation. However, chronic treatment with CsA results in nephrotoxicity. Tubular cell apoptosis contributes to the pathogenesis of renal injury, but the molecular mechanisms are poorly understood. CsA is known to provoke endoplasmic reticulum (ER) stress, but the role of mediators involved in the processes leading to apoptosis remains elusive. In this study, the immortalized human renal proximal tubular epithelial cell line (RPTEC/TERT1) was used. Occurrence of reactive oxygen species (ROS), dephosphorylation of mitochondrial membrane potential (AMF), apoptosis by caspase 3/7 activation and necrosis were measured with the use of specific fluorescent dyes by high content imaging (HCl) microscopy technology. Exposure of RPTEC/TERT1 to CsA for 48 hours resulted into dose-dependent ROS production, increased apoptosis and necrosis. The expression of ER stress-related genes CHOP, ATF4 and Trb3 together with pro-apoptotic genes was markedly increased. Silencing of Trb3 with specific siRNA resulted into decreased dissipation of mitochondrial membrane potential and protected significantly from CsA-induced apoptosis and necrosis. In addition, Trb3 silencing promoted the activation of the AKT-dependent pro-survival pathway following CsA exposure. Thus, our data suggest that Trb3 has a crucial role during ER stress-induced apoptosis upon treatment with CsA, and therefore serve as a potential target for nephroprotective treatments.

2057 MiR-27b Regulates Podocyte Function by Targeting Adenosine Receptor

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MicroRNAs (miRNAs) have been shown to be crucial not only in kidney development and normal renal function but also in various kidney diseases. In this study, we investigated the potential role of miRNAs as key regulators of podocyte injury. We first characterized the miRNA expression patterns in glomeruli as well as in primary podocyte cultures isolated from three different mammalian species: mouse, rat, and non-human primate (NHP). Profiling miRNA expressions led to the identification of a list of highly-conserved glomerulus-enriched and podocyte enriched miRNAs. We then examined if podocyte injury induced by application of a nephrotoxicant, puromycin aminonucleoside (PAN), alters the expression pattern of miRNAs in vitro. As reported previously, PAN not only induced apoptosis but also significantly down-regulated several podocyte enriched miRNAs, including miR-27b. By manipulating the expression level of this miRNA, we found that overexpression of miR-27b in vitro enhanced PAN-induced apoptosis in podocytes while its inhibition promoted survival. Target identification analysis identified Adora2b as a potential target for miR-27b. In fact, ectopic expression of miR-27b suppressed both Adora2b mRNA and protein expression in primary podocyte cultures. Not surprisingly, inhibition of miR-27b increased the transcript and protein expression levels of Adora2B.

2058 Inhibitors of Ceramide Synthesis Protect Mice from Cisplatin-Induced Acute Kidney Injury

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Acute kidney injury (AKI) resulting from cisplatin administration remains to be an obstacle in chemotherapeutic treatments. Cisplatin-induced AKI involves apoptotic and necrotic cell death of the proximal tubules (PT). Pathways known to be regulated by the sphingolipid ceramide, PT cells grown in culture elevate ceramides when treated with apoptotic or necrotic doses of ceramide as a result of the activation of ceramide synthase (CerS) and acid sphingomyelinase (aSMase), respectively. Thus, we hypothesize that inhibitors of ceramide synthesis will protect mice from cisplatin-induced nephrotoxicity. To test this hypothesis, we used cisplatin-induced (25 mg/kg) AKI in C57BL/6 mice. At twenty-four hours C16, C20, and C24:1-ceramides were increased in the kidney cortex of cisplatin treated mice, concomitant with elevated CerS and aSMase activities. Mice pretreated for 3 days with the de novo synthesis inhibitor myriocin and the aSMase inhibitor amiprityline were protected from cisplatin-induced AKI. At 72 h post cisplatin injection, serum creatinine and BUN levels were measured, showing a significant decrease in mice treated with cisplatin inhibitors as compared to the levels observed in vehicle control mice. In conclusion these data indicate that inhibitors of aSMase and de novo ceramide synthesis protect mice from cisplatin-induced nephrotoxicity. As amiprityline is already FDA approved for use in humans and myriocin is well tolerated in multiple mammalian models, these data these data show high potential for translational use for the prevention of AKI during chemotherapy treatment. This work was supported by R01 DK093462 (to L.J.S.).

2059 Methyl Isobutyl Ketone (MIBK) Induces an Exposure-Related Increase in Measures of α2u-Globulin (α2u) Nephropathy in Male but Not Female F344 Rats

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Chronic exposure to MIBK caused an increased incidence of renal tubular adenomas and occurrence of renal tubular carcinomas in male, but not female rats. A number of chemicals have been shown to cause male rat renal tumors through the α2u nephropathy-mediated mode of action (MOA). The objective of this study was to evaluate the ability of MIBK to induce measures of α2u nephropathy in male and female F344 rats following exposure to the same inhalation concentrations used in the cancer bioassay (0, 450, 900, or 1800 ppm). Rats were exposed 6 hours/day for 1 or 4 weeks and kidneys excised approximately 18 hours post exposure to evaluate hyaline droplet accumulation (HDA), α2u nephropathy, sustained increase of α2u concentration at 6 hours post exposure, α2u nephropathy, sustained increase of α2u concentration at 6 hours post exposure, α2u nephropathy, sustained increase of α2u concentration at 6 hours post exposure. At 18 hours post exposure to evaluate hyaline droplet accumulation (HDA), α2u staining of hyaline droplets, renal cell proliferation, and to quantitate renal α2u concentration at both 1 and 4 weeks. There was an exposure-related increase in all measures of α2u nephropathy in the male, but not female rat kidneys. The hyaline droplets present in the male rat kidney stained positively for α2u. The changes in HDA and α2u concentration were comparable to d-limonene, an acknowledged inducer of α2u nephropathy, sustained increase of α2u concentration at 6 hours post exposure, α2u nephropathy, sustained increase of α2u concentration at 6 hours post exposure. At 18 hours post exposure to evaluate hyaline droplet accumulation (HDA), α2u staining of hyaline droplets, renal cell proliferation, and to quantitate renal α2u concentration at both 1 and 4 weeks. There was an exposure-related increase in all measures of α2u nephropathy in the male, but not female rat kidneys. The hyaline droplets present in the male rat kidney stained positively for α2u. The changes in HDA and α2u concentration were comparable to d-limonene, an acknowledged inducer of α2u nephropathy, sustained increase of α2u concentration at 6 hours post exposure, α2u nephropathy, sustained increase of α2u concentration at 6 hours post exposure.
Aristolochic acid (AA)-induced renal lesions are related to increased renal levels of methylglyoxal (MGO) and N-[(carboxymethyl)lysine (CML) which is an advanced glycation end product (AGE). Metformin is a generally accepted drug for the treatment of type 2 diabetes mellitus, and is considered as an MGO scavenger and AGE inhibitor. This study investigated the renal MGO and CML scavenging ability of metformin, and its effect on renal function in C57BL/6 mice with AA-induced renal lesions. Mice were treated with metformin orally (12 or 24 mg/kg BW/day) for 15 days, and injected intraperitoneally with AA (5 mg/kg BW/day) for 8 days since day 8. Following injection of AA into mice for 8 days, decreased renal function was observed according to biochemical indexes and histopathological test results. Administration of metformin ameliorated the renal lesions, especially at a dose of 12 mg/kg BW. The concentration of renal MGO in AA-injected, untreated mice (without metformin) was approximately 6-fold higher than that in the control mice (37.33 ± 7.98 vs. 5.89 ± 2.64 μg/mg of protein, p < 0.01, respectively). CML was also observed in the renal tubules of AA-injected, untreated mice by immunohistochemistry. Administration of metformin at the two tested doses reduced renal MGO levels to 9.81 ± 5.04 μg/mg of protein in the 12 mg/kg BW-treated mice and 17.79 ± 7.80 μg/mg of protein in the 24 mg/kg BW-treated mice, and attenuated the accumulation of CML in the kidney. In this study, we found that metformin decreased renal MGO and CML levels, and improved renal function in AA-injected mice, which confirmed that metformin may be a suitable therapy for AA nephropathy due to its ability to control MGO and CML accumulation.

2061 Altered Expression of Renal Drug Transporters in Multiple Rodent Models of Nonalcoholic Steatohepatitis

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Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in Western society. NAFLD represents several stages of liver damage ranging from simple steatosis to the more advanced nonalcoholic steatohepatitis (NASH). Current experimental models of NASH include diet-induced models such as the mouse models of diet-induced obesity, such as high-fat diet, ob/ob and db/db mice, and fa/fa rats. Previous investigations have demonstrated alterations in the expression and activity of multiple hepatic drug transporters in human NASH, but little information is currently known regarding extra-hepatic regulation of drug transporters in NASH. The purpose of the current study was to characterize and investigate the regulation of renal drug transporters across multiple experimental rodent models of NASH. Both rat and mouse NASH models were utilized in this investigation and included the MCD diet, atherogenic diet, ob/ob and db/db mice, and fa/far rats. Histologic and pathological evaluations confirmed that the MCD and atherogenic rats as well as the ob/ob and db/db mice all developed NASH. In contrast, the fa/far rats did not develop NASH; however, despite a lack of liver damage; renal histology suggests that the fa/far rats develop significant renal injury in comparison to the NASH-confirmed models. Investigation into the kidney mRNA expression of drug transporters showed that among the rodent models, the animals with confirmed NASH such as the rat MCD diet model and the ob/ob and db/db mice developed more, total alterations in miRNA and protein expression. In general, the majority of drug efflux transporters are induced by NASH while drug uptake transporters are both induced and repressed; at the mRNA and protein level. These results suggest that NASH alters the expression and possibly function of renal drug transporters, which would disrupt drug elimination mechanisms in the kidney.

2062 Resveratrol Reduces Doxorubicin Cytotoxicity in Human Kidney (HK-2) Cells

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Doxorubicin (DOX), or Adriamycin, is a cancer chemotherapeutic agent used in treatment of breast cancer, ovarian, small cell lung cancer, acute and chronic lymphoid leukemia. DOX is both cardiotoxic and nephrotoxic. Resveratrol (RES) is a phytochemical found in grapes, berries, and nuts. RES possesses antioxidant and anticancer properties. This study tested the hypothesis that RES will reduce DOX cytotoxicity in human noncancerous renal proximal tubular epithelial cells (HK-2) and RES will attenuate DOX mediated oxidative stress. HK-2 cells were grown for 48 h. Cells were then pre-incubated for 1 h with 0 (DMSO), 5, or 7.5 μM RES followed by a 24 or 48 h co-incubation with 0, 1, or 2 μM DOX. Cell viability was assessed using the AlamarBlue assay. Results showed that DOX decreased cell viability in a concentration dependent manner and RES increased cell viability in cells exposed to DOX. Protein carbonyls, an indicator of oxidative stress and detected by Oxyblot Western analysis, were increased by DOX and reduced by RES. Additionally, RES decreased protein carbonyls in cells exposed only to RES. ADP/ATP and GTP and RES pretreatment of cells following DOX exposure. RES reduced DOX cytotoxicity in HK2 cells at DOX concentrations that are clinically relevant. (Supported by NIH Grant 5P20RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence).

2063 Inhibition of TGF-β1-Induced Renal Epithelial Mesenchymal Transition by Chrysin

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Epithelial mesenchymal transition (EMT) has been suggested as mechanism in the development of fibrosis, such as occurs in the response to certain toxicants. It is a process in which a polarized epithelial cell is converted into a motile mesenchymal cell. Mesenchymal-specific proteins required for movement, invasion and fibrosis are acquired as the epithelial cell proteins necessary for the function of epithelial tissue are lost. In the current study, we tested the effect of a flavonoid in a cell-culture model of EMT. To induce EMT, we use transforming growth factor-β1 (TGF-β1) which is a cytokine activated in most cases of fibrotic injury. Chrysin, a flavonoid extracted mainly from passion flowers, was examined for its ability to inhibit EMT induced by TGF-β1 in the LLC-PK1 cell line. Cells were treated with TGF-β1 alone or TGF-β1 + chrysin. Effects on the mesenchymal markers collagen, α-skeletal muscle actin, and vimentin were evaluated as indicators of EMT. The amount of collagen in the cells was determined by the Sirius red assay. Collagen content was significantly increased from 93 ± 8 μg collagen/mg total protein in the control group to 127 ± 8 μg collagen/mg total protein in the TGF-β1 treated group. In the group treated with both TGF-β1 + chrysin, collagen was significantly reduced to 104 ± 6 μg collagen/mg total protein. The expression of α-smooth muscle actin and vimentin was determined by immunocytochemistry. Mouse primary antibodies were tagged using an anti-mouse IgG conjugated with FITC. The specimens were evaluated by widefield microscopy at 20X magnification. Treatment with TGF-β1 increased the expression of mesenchymal markers, whereas in the presence of TGF-β1 + chrysin the expression of both α-smooth muscle actin and vimentin resembled the untreated controls. These results demonstrate that chrysin can inhibit TGF-β1-induced EMT in LLC-PK1 cells.
in 3,4,5-TCA nephrotoxicity in vitro, and that these oxidative species arise from biotransformation by cyclooxygenase activity or via CYP isozymes inhibited by PiBS but not metyrapone.

2065 Renal Epithelial Hyperplasia Caused by Urinary Crystals of a Novel GKA and Its Metabolites after Acute Dosing in Sprague-Dawley Rats


Renal toxicity caused by crystal formation in the urine is well-established for some drugs, industrial chemicals, food additives, and naturally occurring toxins but toxicity typically requires long term dosing to cause measurable changes in urinary system morphology and/or function. Compound A, a novel glucokinase activator (GKA), caused crystal formation in the urine with accumulation in the collecting ducts accompanied by renal transitional epithelial hyperplasia with neutrophil infiltration after only 4 consecutive daily doses in female Sprague Dawley rats. A second study dosing Compound A at 600mg/kg/day for 1, 2 or 4 consecutive days was performed to explore the unusually rapid progression of the hyperplasia and identify the crystals. Findings after a single dose included crystals in urine and kidneys and clinical pathology changes indicating inflammation and decreased renal function. Additional findings after 2 doses included renal transitional epithelial hyperplasia with neutrophil infiltration. No changes were observed in urinary bladder epithelium. Crystals in the kidneys were identified as Compound A and two major metabolites by MALDI mass spec imaging. Urinary system findings observed with Compound A have not been seen with other internally tested GKAs of similar structure nor GKA reported in the literature, suggesting that it is unrelated to the biology of glucokinase activation. The renal toxicity caused by Compound A highlights possible rapid onset of such effects and acute toxicity studies could benefit from additional endpoints to more clearly define the nature of such findings if the potential exists for renal toxicity to occur.

2066 Are Succinate and Diglycolic Acid Taken Up into Human Kidney Proximal Tubule Cells by the Same Sodium Dicarboxylate Transporters?

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Diglycolic acid (DGA) is now considered to be the primary toxic metabolite of diethylene glycol (DEG), leading to the acute kidney injury from DEG overdose. DGA is a four-carbon dicarboxylic acid, with structural similarity to the TCA cycle intermediate, succinate. We hypothesize that DGA and succinate are taken up by the same sodium dicarboxylate transporter (NaDC) located in the proximal tubule cells of the kidney. This study compared the intracellular uptake of DGA and succinate, via apical (NaDC-1) and basolateral (NaDC-3) transporters. Human kidney proximal tubule cells were cultured until confluent, then subcultured onto membrane inserts in 24 well plates, that allow for distinct apical and basolateral uptake. Using 14C-substrates, uptake was measured at increasing time points and concentrations for both succinate and DGA, along with measurements of sodium dependence of the NaDC transporters. Cellular uptake of succinate from both the apical and basolateral membrane demonstrated sodium dependence, suggesting mediation via NaDCs, with somewhat higher uptake by the apical NaDC-1. Uptake of DGA was not sodium dependent from the apical direction and was not saturable, suggesting a transporter-independent mechanism. Basolateral uptake of DGA was sodium-dependent and was saturable with a Km of about 16 mM/L. The magnitude of DGA uptake at non-toxic concentrations was greater from the basolateral side than the apical. These results suggest that DGA and succinate transport have differing characteristics in human kidney cells.

2067 Transporter Function and Response to Nephrotoxins in a p16-Mutated Human Renal Proximal Tubule Epithelial Cell Line

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Kidney toxicity is a continuing concern during drug development. Although primary human kidney cells are available and a handful of immortalized kidney cell lines currently exist, there is a need for improved assay systems. We modified human primary renal proximal tubule epithelial cells by knocking out p16, a cyclin dependent kinase inhibitor, thereby enabling extended cell proliferation. The resulting cell line was characterized for the presence of proximal tubule cell markers as well as several functional properties, including transporter activity and response to known human nephrotoxins. Two uptake (OAT1, OCT1) and two efflux (MDR1, MRP2) renal transporters were studied. Transporter assays were carried out on poly-D-lysine coated 24-well plates at a seeding density of 0.5 million cells/well for 10 (uptake) or 30 min (efflux). Concentration-dependent uptake of the OCT1 substrate taurocholic acid (TEA) and the OAT1 substrate p-aminohippuric acid (PAH) were observed. Verapamil and probenecid inhibited TEA and PAH uptake by 33 and 66%, respectively. For the efflux transporters, MRP2-mediated efflux of carbonyldichlorofluorescein (CDCF) was inhibited using MK571, leading to a 3.25-fold increase in intracellular CDCF. Similarly, MDR1-mediated efflux of calcine was inhibited with cyclosporin A, leading to a 7-fold increase in intracellular calcine. The cell line was also tested for sensitivity to several nephrotoxins. Concentration-dependent cytotoxicity (48 hr, MTT assay, IC50) was observed with each of the test compounds, including gentamicin (3 mM), cisplatin (20 μM), tacrolimus (75 μM), potassium dichromate (25 μM) and diglycolic acid (7.5 μM), the proposed nephrotoxic metabolite of diethylene glycol. The presence of functional uptake and efflux transporters coupled with toxicant sensitivity suggest that the cell line may be useful for drug-transporter interactions and the early detection of renal toxicants.

2067a Development and Characterization of a Novel Cell-Based Model to Study MATE1- and MATE2K-Mediated Drug Uptake and Interactions


Transporter involved drug-drug interactions (DDIs) in the kidney can prolong the drug elimination half-life, leading to accumulation of victim drugs in the body, consequently causing pharmacological problems or renal toxicity. Multidrug and toxinn extrusion transporters (MATEs), e.g. MATE1 and MATE2K, localized on the apical membrane of proximal tubule cells are major transporters which mediate the excretion of important medications into the urine. Several studies have demonstrated that apical efflux by MATEs is one of the sites of DDI in the kidney. Therefore, the International Transporter Consortium (ITC) recommends studying the potential interaction of new molecular entities (NMEs) with MATE1 and 2K. A new “Thaw and Go” cell-based transporter model has been developed to evaluate MATE1 and MATE2K-based interactions, which enables early in vitro evaluation of potential MATEs involvement in drug induced renal toxicity. In the presence of oppositely directed H+ gradient or ammonium chloride-induced intracellular acidification, the transport activity of various cations, such as tetaethylammonium (TEA), mertformin and 1-methyl-4-phenylpyridinium (MPP) were significantly stimulated in hMATE1 and hMATE2K expressing cells for more than 10 fold compared to the control cells expressing the empty vector. The concentration dependent uptake of TEA and mertformin in hMATE1 and hMATE2K demonstrated “simple” Michaelis-Menten kinetics with Km value of 260μM and 355μM in MATE1, and 755μM and 1.2mM in MATE2K respectively. The inhibitory parameters of known drug inhibitors for MATEs verapamil, quindine, cimetidine and ritonavir were also determined using this model, which are comparable to literature reports. The study demonstrates the model is a compliant and useful in vitro tool to screen hMATE1 and hMATE2K involved drug interaction and/or drug induced renal toxicity in early stages of drug development.

2068 Exploring the Interface between Air Pollution and Metabolic Syndrome: The Bittersweet Dilemma

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The metabolic syndrome (MetS) affects approximately 32% of the US population and is expected to grow to 34% by 2020. The conventional risk factors, such as diet and sedentary lifestyle, do not fully explain this rising epidemic. Multiple lines
of epidemiological evidence show associations of high ambient air pollution with the incidence of metabolic disease, such as diabetes, and risk factors that comprise the metabolic syndrome. Coincident with uncontrolled air pollution in developing countries is improved lifestyle (less physical activity) and calorie-rich diets. These imply that there is likely an interaction between diet, metabolic processes, and injuries induced by inhaled pollutants. While new research on the contribution of air pollution to cardiometabolic derangements is emerging, the mechanisms by which airborne materials can affect multiple cardiovascular and metabolic pathways are unknown. The goal of this session is to present new data from both animal and human studies that shed light on the interaction of air pollutant exposure with both the incidence and exacerbation of diabetes, obesity, and cardiovascular disease. Some key issues to be addressed by presentations in this session include: What behavioral, dietary, and environmental factors contribute to the development of MetS and, subsequently, to type 2 diabetes and cardiovascular disease? How do obesity and other metabolic abnormalities interact to promote cardiovascular disease? How do air pollutant exposures alter cardiometabolic outcomes during controlled versus natural exposures in humans? How does diet-induced metabolic syndrome alter cardiovascular responses to single and multipollutant exposures? How does obesity predispose for exaggerated airway responses to pollutants? Lastly, how do air pollutants alter stress pathways and energy expenditure? Together, the presentations begin to answer these critical questions and provide some seminal examples of the toxic interface between diet, metabolic homeostasis, and air pollutant exposure.

### 2069 The Role of Overweight and Obesity in the Diabetics and Cardiorenal Syndrome

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The presence of a group of interactive maladaptive factors including hypertension, insulin resistance, metabolic dyslipidemia, obesity, microalbuminuria, and/or reduced renal function constitutes the cardiorenal metabolic syndrome (CRMS). Overweight, obesity, diabetes and chronic kidney diseases (CKD) have grown to pandemic proportions in industrialized countries during the past decade. The fact that these interactive factors promote heart and renal disease has been documented in large population-based studies. Obesity seems to be the driving force behind the development of heart disease, diabetes and CKD and therefore the CRMS. The relationship between overweight/obesity, insulin resistance and kidney disease begins in early childhood and appears to be related to overconsumption of high-fructose corn syrup and insufficient physical activity. Today, 13 million children are obese, and over 70% of these children are likely to become obese adults. Indeed, approximately 30% of male and 34% of female adults in the United States are obese. This lifestyle-related epidemic will be a major societal medical and economic problem that will accentuate the current epidemic of CRMS in the United States and other industrialized and emerging industrialized countries. This presentation will review the potential mechanisms by which obesity and other metabolic abnormalities interact to promote diabetes and cardiovascular disease. Some of the potential mechanisms might explain the role of air pollutants in exacerbating cardiometabolic disease.

### 2070 The Effects of Air Pollution Exposures on Aspects of the Metabolic Syndrome in Humans

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Particulate matter (PM) air pollution is associated with an increased risk of cardiovascular (CV) events over periods of both short and long-term exposures. While PM is capable of promoting CV morbidity by a variety of mechanisms, an important pathway may be via the potentiation of facets of the metabolic syndrome (MetS) including hypertension and insulin resistance. We have undertaken a series of human studies in the US and China that evaluated the acute and sub-acute effects of PM exposures upon several aspects of the MetS in humans. First, we performed a series of randomized double-blind cross-over studies of acute exposures to rural coarse concentrated ambient PM (CAP). Systolic (0.32 ± 0.74 mm Hg, p = 0.021) and diastolic (0.27 ± 0.77 mm Hg, p = 0.05) blood pressure (BP) linearly increased during the inhalation of coarse CAP (76.2 ± 51.5 μg/m-3) compared to filtered. Heart rate was higher and the ratio of low-to-high frequency heart rate variability increased together with circulating endothelial progenitor cells during CAP exposures. Second, the homeostasis model assessment of insulin resistance (HOMA-IR) was measured in 25 healthy adults living in rural Michigan were transported to an urban location for 5 consecutive days of daily 4-5-hour-long ambient air pollution exposures. A 10 μg/m-3 increase in sub-acute fine PM exposures was associated with increased HOMA-IR (p=0.7, p=0.023) and reduced heart rate variability (standard deviation of normal-to-normal intervals). Lastly, we evaluated the association between personal exposure to combustion-related BC [median=4.1 μg/m-3] with ambulatory BP and autonomic function in Beijing, China. An interquartile range increase in BC during the previous 10 hours was associated with an increase in systolic BP of 2.20 mmHg and diastolic BP of 1.55 mmHg. This series of experiments demonstrates that exposures to fine and coarse PM, as well as BC, are capable of increasing BP and impairing metabolic insulin sensitivity in humans. PM air pollution may be an important environmental factor contributing to global epidemic of the MetS.

### 2071 Cardiometabolic Interactions of Diet and Air Pollution: Field Studies with Multipollutant Atmospheres

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Metabolic conditions such as diabetes, insulin resistance and metabolic syndrome (MetS) might predispose an individual to adverse cardiovascular responses to air pollutant exposures. While health complications from exposure to various airborne pollutants have been modeled in rodents with hypertension and various cardiac diseases, comparable studies in animal models of metabolic disorders have not been conducted. This presentation will briefly survey the use of relevant animal models that study cardiovascular and metabolic outcomes, and will then focus on new results from a series of studies that use the high fructose-fed (HFr) rat model of diet-induced CMS. HFr- rats were exposed to ozone, fine particulate matter (PM2.5) or their combination, to test the hypotheses that: 1) adverse cardiovascular effects of air pollution would be exacerbated by the MetS and 2) coexposure to multipollutants would induce greater toxicological responses than to single pollutants. Prior to exposures, HFr rats had dyslipidemia, hepatic lipodisosis, and elevated blood pressure (BP) and heart rate (HR), but had depressed heart rate variability (HRV). Reductions in BP and HR by air pollutant exposures were exacerbated and prolonged in HFr rats. Interestingly, insulin resistance and lipid derangements were less severe in HFr rats after exposures. Results using this novel model of CMS will contribute to the development of prevention and intervention strategies to protect this susceptible population from the adverse cardiovascular effects of multipollutant atmospheres.

### 2072 Augmented Pulmonary Responses to Acute Ozone Exposure in Obese Mice: Roles of TNFR2, IL-13, and IL-17A


Acute ozone exposure results in more inflammation, greater changes in pulmonary mechanics, and greater airway hyperresponsiveness (AHr) in obese than lean mice. These obesity-related differences in ozone responses are observed both in genetically obese mice and in mice with diet induced obesity. Because obese mice have increased circulating TNF-α, and because TNF-α has been shown to play a role in responses to acute ozone in lean mice, we examined the role of TNF-α in the augmented responses to acute ozone exposure of obese mice. Lean wildtype (WT) and TNFR2 deficient (TNFR2-/-) mice, and obese Cpefat and TNFR2 deficient Cpefat mice (Cpefat/TNFR2-/-) were exposed to ozone (2 ppm for 3 h) or air. Ozone-induced increases in pulmonary mechanics, airway responsiveness, and cellular inflammation were greater in Cpefat than WT mice. In lean mice, TNFR2 deficiency ablated ozone-induced AHR, without affecting pulmonary inflammation, whereas in obese mice, TNFR2 deficiency augmented ozone-induced AHR but reduced inflammatory cell recruitment. Interestingly, ozone caused pulmonary expression of IL-13 in Cpefat but not WT mice. In Cpefat mice, anti-IL-13 attenuated ozone-induced increases in pulmonary mechanics and inflammation, but not AHR. There was no effect of anti-IL-13 in WT mice. Ozone also caused greater IL-17A expression in lungs of obese versus lean mice, and anti-IL-17A treatment attenuated ozone induced AHR and inflammation in obese but not lean mice. CD4+ cells were the source of both the IL-13 and the IL-17A induced by ozone in obese mice. These results indicate that pulmonary responses to ozone are not just greater, but qualitatively different in obese versus lean mice. In particular, in obese mice, ozone induces activation of CD4+ T cells, causing release of IL-13 and IL-17A that exacerbate the pulmonary effects of ozone, in part via synergism with TNF-α. The majority of the population of the US is either obese or overweight. Our results emphasize the need for improved understanding of the effects of ozone in this population.
Recent evidence suggests that air pollutant exposure impacts key metabolic processes and is linked to metabolic syndrome. Because ozone (O3) does not translocate systemically, neuronal pathways and/or systemic mediators released from lung injury are proposed to account for the metabolic impairment. Our studies provide evidence that short-term O3 exposure induces reversible metabolic alterations, including glucose intolerance and increases in blood leptin, glucose, fatty acids, and amino acids in rodent models. These metabolic alterations are reflected in transcriptional changes in the liver, consistent with increased catebolism (i.e., increased energy expenditure and reduced lipid synthesis). Liver metabolic processes are likely coordinated with changes in adipose and muscle metabolism. Because these O3-induced systemic changes are accompanied by large increases in circulating catecholamine (i.e., stress response), derangement of neurohormonally-mediated metabolic processes likely play a key role. Experiments using adrenalectomized rats confirm the role of circulating adrenal hormones for the induction of the acute metabolic response after acute O3 exposure. Collectively, data show that short-term air pollution exposure results in increased energy expenditure. Conversely, the hypothermic and bradycardia responses noted in rodents exposed to air pollutants suggest reduced energy demand. Currently systems approaches such as transcriptomics, proteomics, and metabolomics are being used to determine how these acute metabolic responses induced by O3 might relate to adaptation, and when elicited chronically, may result in disease (e.g., obesity, insulin resistance, and/or type 2 diabetes). (This abstract does not reflect USEPA policy).

We conducted a large-scale forward genetic screen in mice with chemical mutagenesis to elucidate the genetic etiology of structural birth defects. For this study, we developed a sophisticated mouse phenotyping pipeline entailing the combined use of noninvasive in utero fetal ultrasound imaging of pregnant dams, with follow up analysis of the abnormal fetuses and neonates using a combination of micro-CT, micro-MRI and histopathology examination by confocal epifluorescence image capture (EFIC). Confocal EFIC imaging is a histological imaging technique ideally suited for the analysis of complex anatomy associated with structural birth defects, as it allows any standard paraffin embedded sample to be imaged using native tissue autofluorescence, generating high resolution registered 2D image stack of the specimen suitable for seamless digital rescanning in any orientation and also rapid 3D rendering. Using this combined approach, we have screened over 70,000 fetal mice encompassing 4X genome coverage, and have recovered over 2,000 mutants exhibiting a wide spectrum of birth defects, including eye/craniofacial/brain malformations, limb and other skeletal anomalies, neural tube closure defects, congenital heart disease, and visceral organ defects such as multiple organ cysts, polycystic kidney disease, biliary atresia, intestinal malrotation/obstruction, kidney agenesis, hydrenephrosis, hydrourtourinary and bladder obstructions, thymus aplasia, and other visceral organ anomalies. Using whole mouse exome sequencing analysis, we have recovered the disease causing mutations in over 100 of the mutant mouse lines recovered from the screen. Together, these studies show the efficacy of our phenotyping pipeline for the diagnosis of birth defects in fetal/neonatal mice. Supported by NIH grant HL098188.

The lightsheet microscope uses cylindrical lenses to create laser sheets that illuminate a single z-plane at a perpendicular to the collection objective(s). This narrow plane of excitation reduces sample phototoxicity and allows capture of an entire z-plane at once, which greatly speeds collection of 3- and 4-dimensional imaging. Rapid imaging of entire zebrafish larvae at single time-points or over extended periods of time provides insight into biological responses throughout development. This presentation will provide an overview of the basic principles of Lightsheet microscopy, describe our glucocorticoid-responsive BioBanner fish, and provide sample Lightsheet images obtained from these fish.
reconstruction, visualization, segmentation, and registration. Examples of analyses using segmentation and registration of skeletal elements and visceral organs, which required contrast staining, will be presented. It will be evident that these latter techniques offer the potential for automated analysis of morphology. The use of micro-CT in other laboratories to assess developmental biology will be briefly reviewed. Conduct of positive control studies and studies with new drug candidates show that micro-CT evaluation of the fetal skeleton is equal to conventional evaluations after alizarin red staining. Relative to alizarin red evaluations, micro CT evaluations have not changed the NOEL and/or LOEL of a study. The incidences of a few minor skeletal abnormalities are either increased (e.g., incomplete ossification of sternebra 5 in rats and of metacarpal 1 in rabbits) or decreased (e.g., trace supernumerary ribs in rats) across all groups including controls by micro-CT compared to alizarin red evaluation. These differences are mainly due to the slightly lower resolution of the current scanner; however, a different scanner with higher resolution is under evaluation. Results of a study that scanned postnatal rat pups to assess skeletal changes in the same animals over time will be discussed. Such procedures will be useful in performing longitudinal studies. The ready availability of electronic images provided ample material for development of a web-based atlas. Discussion will conclude with our current efforts directed toward automated segmentation and analysis of all fetal rabbit skeletal elements in order to flag potentially abnormal fetuses for manual micro-CT evaluation.

2079 Imaging Data for Scientific Decision-Making in a Regulatory Context
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Imaging technologies that facilitate high resolution evaluation of the developing fetus and/or neonatal individual have been utilized with outstanding success in developmental research and clinical practice for many years. Innovative application of current technologies has historically been used to resolve complex scientific issues. In the research and clinical communities, there is a high degree of confidence in the derived information. Yet, there are likely to be many challenges that arise in bridging the gap between these widely accepted applications and regulatory decision-making for pharmaceuticals and environmental chemicals. This presentation will address the ways in which imaging technologies have been utilized in a regulatory context to date, and how they might be utilized in the future, particularly in the field of developmental toxicology. The pathway to achieve this goal remains under discussion. There are recognized benefits of implementing an organized and collaborative effort to address the collection, interpretation, and application of developmental toxicology imaging data. Establishing technical standards for imaging hardware and software, as well as methodological best practices for technological processes and procedures, would be useful in standardizing imaging within and across laboratories. Validation of developmental imaging technologies would need to include a demonstration of such inter- and intra-laboratory consistency in the detection of morphological alterations during development, the ability to detect effects from known developmental toxicants (positive reference chemicals), and evidence that imaging techniques provide, at the very least, a similar level of sensitivity and specificity as conventional developmental toxicity testing methods. Additionally, issues of increased efficiency, and cost vs. benefit would need to be explored. In spite of the many challenges, there is widespread consensus on the potential usefulness of imaging data for the characterization of developmental hazard.

2080 Beyond hERG: Novel Cardiovascular De-Risking Strategies and Their Regulatory Acceptance
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Whether drug development is prosecuting small molecule or biologic programs, cardiovascular safety is still one of the leading causes of compound attrition. The rationale for attrition has gone far beyond the traditional drug-induced long QT syndrome associated with inhibition of the human Ether-a-go-go Related Gene (hERG). Pharmaceutical companies have built-in greater controls for eliminating this hurdle by using sensitive high-throughput in vitro screens around this and other important cardiac ion channels that have significantly reduced arrhythmogenic risks. Despite these advances, adverse effects on the cardiovascular system remain a multifactorial risk, difficult to discern, especially during early drug discovery stages. Recent comprehensive preclinical attrition data has demonstrated that hemodynamic alterations, vasculitis, and left ventricular cardiac hypertrophy are increasing in incidence in short- and long-term animal studies. Unlike the well-established mechanistic link between hERG and QT prolongation, these unique pathologies either lack suitable investigative tools and models, or have a lack of confidence in the underlying mechanisms preclinically. This challenge is compounded by the advancement of molecules that act as novel targets for which limited safety data are available. However, in the past couple of years promising advances have been made in in vitro and ex vivo assay development, which have the potential to impact industry cardiovascular safety de-risking standards much as hERG screening did. Investigators are exploring more integrative tactics, by effectively employing predictive in vivo tissues baths as well as stem cell-derived human cardiomyocytes to rank-order and/or advance suitable drug candidates. While developing innovative tools is important, it’s also clear, as the diversity of pharmaceutical drug products evolve into a more challenging target space (i.e., kinase inhibition), these inherent risks need to be evaluated on par with regulatory expectations.

2081 Study of Cardiovascular Safety of Small Molecules and Biologics: The State of Science, Challenges, and Gaps
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The search for innovative approaches allowing early prediction of cardiovascular toxicity of small molecules or biologics is urgently needed. Despite significant efforts to identify new methodologies and assays that accurately predict the risk of cardiovascular toxicity, everyone recognizes that validation of these test systems is only evident from the number of new molecules safely brought to the marketplace. The story of the hERG channel assay and the impact that it has had on removing development those molecules that pose a risk of proarrhythmia exemplifies both the science and strategy that goes into validation of an assay. Possessing data which describing a molecule’s profile on other established mechanisms of cardiovascular toxicity, e.g., effects on receptors, ion channels, transporters, kinases and intracellular organelles (e.g., mitochondria), sets up a “theoretical” concern, a hypothesis, amendable to further testing. In the case of these simple biologic targets and those associated with more complex cardiovascular toxicities, quantitative translation and predicting adverse events remain to be established. Translational biomarkers, testing in viable organs or tissues harvested from patients and study of pluripotent stem cells offer hope of resolving some of the challenges of translational biology. However, considered as steps in the lead optimization phase, in vitro assays do not fully represent the integrated physiology of animals or patients. Furthermore, there are adverse cardiovascular events for which the underlying pathophysiology is not well understood. These latter cases are emblematic of challenges faced by the scientific community. For those investigators, a “line of sight” to de-risking the potential for cardiovascular toxicity of a promising new drug begins in early discovery and continues through to the post-marketing phase.

2082 Regulatory Expectations for Submission of Novel In Vitro Assays Addressing Cardiovascular Risk
T. Papoian, US FDA, Silver Spring, MD. Sponsor: J. Davis.

The potential for bio-pharmaceuticals to possess unintended clinical effects on the cardiovascular system during the drug development process is an important regulatory concern. Although in vitro and in vivo preclinical models have been designed to predictive screen bio-pharmaceuticals for specific cardiovascular liabilities, such as QT interval prolongation and other proarrhythmic effects, failure to prevent cardiovascular risk in humans continues to occur, particularly with compounds that do not specifically interfere with cardiac ion channels. Examples for improvement in screening for potential cardiotoxicity would be development of more human-specific models, such as human induced pluripotent stem cell-derived (iPSC) cardiomyocytes. Although this and other in vitro models are gaining acceptance among bio-pharmaceutical companies for initial screening of candidate compounds for further development, the predictivity, reliability and regulatory acceptance of submitted data generated from such newer in vitro models requires proper validation and qualification, so that appropriate decisions regarding human risk assessment during subsequent clinical testing can be made with an acceptable degree of confidence. Regulatory agencies, such as the FDA, clearly have been supportive of such efforts to develop better predictive in vitro models that can improve drug safety for subsequent testing and therapeutic use in humans. Some of the regulatory expectations for the future of novel in vitro assays to address non-proarrhythmic cardiovascular risk will be discussed.
Hemodynamic effects of non-cardiovascular compounds continue to be a challenge in drug development. Early compound selection employing a robust cardiovascular ex vivo/in vivo screening funnel can lead to improved decision making and project efficiencies. To this end, understanding of the cross model and cross-species translation can significantly inform decision making and compound advancement. The translational relationship between in vitro and in vivo such as the isolated rat aorta and conscious rat heart telemetry has been explored and defined for select chemical series and mechanisms. These assays have optimized detection/differentiation of compounds with potential hypertensive risk associated with a mechanistic structure-activity-relation (SAR). Key relationships in this strategy included the establishment of in vitro concentrations relevant to observed in vivo effects. Several case studies will be shared demonstrating key impacts and decision making criteria. Additionally, the understanding cross species in vivo translation and effects can strengthen decision making and improve the ability to achieve the ultimate goal of predicting human outcomes.

Cardiac hypertrophy is initiated and maintained by a wide array of endocrine, paracrine, and autocrine growth factors in response to increased work load, injury, or defects in contractile performance. Cardiac hypertrophy is clearly double-edged, where under exercise or pregnancy it is beneficial and referred to as physiologic, but under sustained hypertension or aortic stenosis cardiac hypertrophy is pathologic. Both types of hypertrophy are characterized by an increase in cardiomyocyte size, but only pathological hypertrophy is associated with increased apoptosis and fibrosis as well as functional changes such as altered cellular Ca2+ homeostasis, ion channel remodeling, reduced contractile force and relaxation velocity. While initially an adaptive response that attempts to maintain cardiac output, sustained cardiac hypertrophy induced by pathological stimuli is a leading predictor for the development of heart failure. Drug-induced cardiac hypertrophy often manifests in the later stages of preclinical safety testing. In response to this late stage risk, an early safety assessment screening paradigm has been developed using iPS derived human cardiomyocytes, which are phenotypically and functionally more reflective of cardiac myocytes in vivo. Upon treatment with molecules associated with the induction of cardiac hypertrophy we have characterized these cells using endpoints that represent the hallmarks of cardiac myocyte hypertrophy including; cytoskeleton remodeling, induction of the fetal reversion gene expression program, and secretion of natriuretic peptides. In addition, new approaches that leverage high content imaging and computational systems biology have been applied to the model, which allow for a more thorough characterization and deeper understanding of drivers of cardiac myocyte hypertrophy.

Within the basic science community, in vitro models and approaches that improve the early understanding of compound’s beneficial or deleterious effects as well as accurately reflect diseased tissues are sorely needed. Breakthroughs that enable research to make faster, more accurate, human relevant decisions would be embraced by regulatory agencies as well as companies who make products (be it chemical, food, drugs, etc). Recent advancements in the ability to manufacture stem cell-derived tissues has enabled drug discovery scientists to create relevant models for pharmacology and toxicology investigations using pluripotent stem cells of human origin. Specifically to benefit drug-induced cardiotoxicity, stem cell-derived cardiomyocytes have been made in high quality, purity, and quantity and confirmation of their expression, function, and response to cardiotoxic molecules has occurred. Across a set of nearly 80 compounds, stem cell-derived cardiomyocytes were shown to respond in a clinically meaningful manner to drugs that induce arrhythmia. This approach is being adopted by pharmaceutical companies as a means to better assess drug-induced arrhythmia at early stages of drug development and is in the early stages of consideration by regulatory agencies as a surrogate for extensive clinical data. In addition, mechanistic studies in stem cell-derived cardiomyocytes have been conducted to investigate potential kinases that mediate sunitinib-induced cardiotoxicity. While the utility of stem cell-derived cardiomyocytes as a model for cardiotoxicity has not been broadly studied, a real opportunity exists to evaluate effects in a human cell system that is stably beating and maturing in vitro. A higher level of opportunity exists since stem cell-derived cardiomyocytes are being made from a genetically diverse set of individuals which will allow interrogation of the relationship of genetics and drug-induced cardiotoxicity in an in vivo manner.

Biopharmaceutical (BP) drugs are innovative medicines that have revolutionized the treatment of human disease, and offer the opportunity to develop new therapeutics with high specificity for their human target, long half-life and target coverage, and low risk for “off-target” pharmacology. While BP attrition due to safety concerns is generally considered to be low, especially for risks like QTc prolongation, these novel therapeutics may have functional cardiovascular effects that require evaluation during nonclinical drug development. The cardiovascular evaluation of BP products requires a science-based, case-by-case approach, as each biological modality will have unique pharmacological characteristics that influence the testing strategy. The integration of safety pharmacology endpoints into general (repeat-dose) toxicity studies is a rational paradigm for assessing potential changes in the cardiovascular system, but requires thoughtful planning. In some cases, dedicated and optimally-designed nonclinical cardiovascular safety studies may be needed to assess a functional risk triggered by target-pharmacology concerns. For example, implant telemetry studies may be needed to detect small changes in arterial blood pressure after acute and chronic exposure. This presentation will cover some examples of cardiovascular strategies used to evaluate novel biopharmaceuticals.

Dissemination of scientific results drives innovation and productivity in all fields, including toxicology. It is critical to effectively communicate findings to colleagues, peers and trainees in written and oral forms and most of us are comfortable in this arena. However, it is more difficult to communicate results and translate risk to the lay public that listens to messages through an emotional lens—especially in the world of social media that defines “experts” based upon frequency and volume of communication rather than demonstrated expertise. Certainly, the public faces a barrage of environmental health issues associated with alleged hazards due to contamination of food, water, air and soils. To make appropriate decisions, the public, media and legislators need information in a readily understandable format presented in a way that resonates with them. In general, communicating and engaging with the lay public is not addressed as part of graduate training, and, therefore, even accomplished toxicologists who are effective scientific communicators find themselves underprepared. Communication directed at “lay” audiences, even that which uses appropriate terminology, is often ignored and does not “stick” with the target audience. Moreover, it is now clear that effective public communication must exceed simple dissemination of results; all stakeholders, including affected communities and populations, should be engaged by toxicologists in bidirectional discussions that facilitate learning by both scientists and the public on an emotional and transparent level. Four actors from four different vantage points will present on the role scientists should play in communication and engagement with the public about toxicology. A discussion panel will follow.
To Speak or Not to Speak. That Is the Question. And the Challenge
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Practically every member of the SOT has been given an opportunity to speak about their research in a scientific setting—highlight their hypothesis, describe methods, present results, make interpretations, defend their conclusions. Because the above is centered on THEIR work, it is generally a comfortable experience, and there is no question that these presentations highlight the values and impacts of the science of toxicology. Far fewer members of the SOT are called upon to provide a non-scientific perspective on what it means to be a toxicologist, or on why the science of toxicology should be viewed by the public as value-added. The focus of this presentation will be on the challenges inherent in serving as a spokesperson for science and our Society. The first question is—Should the SOT enlist its members to occasionally communicate on behalf of science, toxicology and the Society? The short answer is ‘yes’ based on past practices—there are examples of how the SOT has reached out to its members for this type of communication. The second question is—What are the challenges inherent in communicating on these subjects? One of the strengths of the SOT is the diversity of its membership, and any message offered needs to leverage that diversity. Even though toxicology is one of the most integrated sciences currently being practiced, and there is an increasingly greater emphasis on the need for multi-disciplinary research teams, toxicology continues to struggle with an ‘identity crisis’ that is common across the sciences today. Societal perceptions are changing and must be anticipated. The impacts of ‘chemophobia’, and the perception that all toxicologists do is ‘tell us that everything is harmful’ can impact our effectiveness and credibility. There are also examples of the “good guy/bad guy” syndrome, as in “bad guys” are in industry or government, and “good guys” are in academia. Ultimately, we must recognize that in spite of the challenges, the failure to speak about the value of the SOT, and of the science that exists at its core, could marginalize our discipline, and indeed all of science, in the eyes of the public.

The New Universe of “Knowledge”
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Information availability, whether accurate or not, is rapidly evolving and continuing to change how we perceive and interact with the world in which we live. The definition of “expert” is no longer based on education and training but on the frequency and passion of communication. How this new universe of knowledge affects our world cannot be ignored when a single person has the power to initiate movements that rapidly and completely demonize finely textured ground beef (“pink slime”) or cause major companies to reformulate products to eliminate a specific ingredient. As scientists, we must adapt our communication style to this new world or be drowned out or, worse, become irrelevant. This is no easy task as we work to protect integrity and credibility for ourselves and our employers. Accomplishing real and persistent change in our approach to communication requires a commitment of time and resources on our part, and an effort to use new tools and techniques in this new universe of “knowledge.” Not only does our ability to influence our stakeholders and public behavior depend on it, the very role of science in society depends on it.

Values, Trust, and Science
C. Arnot. Center for Food Integrity, Gladstone, MO. Sponsor: S. Hermansky.

All organizations operate through social license or the privilege of operating with minimal formalized restrictions (regulation or legislation). A social license is granted when you operate in a way consistent with the ethics, values and expectations of your stakeholders (customers, employees, community, regulators, legislators, the media). Once lost, either by reducing or eliminating public trust, social license is replaced by various levels of control designed to compel performance consistent with expectations. Operating with a social license is flexible and low cost while operating with a high degree of social control increases costs and reduces flexibility. A radical example of lost social license is Arthur Anderson and Enron. After the collapse of Enron, the accounting profession lost its social license. Freedom enjoyed by scientists is in jeopardy as stakeholders raise questions about the trustworthiness of today’s science-based industries. Our research, focused on the food industry, demonstrated that three primary elements drive trust: competence, confidence, and influential others. Competence is about skills and technical ability. Historically, we as scientists have focused our communication on this believing stakeholders will make logical data based decisions. However, with today’s social media, competence no longer ensures public trust. Thus, consumers focus on other aspects of communication including confidence: the perception that the communicator has shared values and ethics and a belief they will do the right thing. Similarly, consumers turn to other sources of information or their “influential others” (family and friends).

Our research also demonstrated that confidence, or shared values, is much more important than competence for consumers. This research should be relevant to today’s crisis of science communication. New ways of engaging communicating are required if we want to build trust and maintain social license. A major driver of trust in today’s world is a non-negotiable focus on transparency. To succeed, we need to demonstrate our commitment to practices that are ethically grounded, scientifically verified and economically viable.

Databases Facilitating Systems Biology Approaches to Toxicology
S. M. Bello1 and M. Shimoyama2. 1Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME and 2Rat Genome Databases, Medical College of Wisconsin, Milwaukee, WI.

The application of systems biology approaches to toxicology promises discovery of new molecular and genetic connections, the ability to draw novel inferences about mechanisms and modes of actions, and the ability to build better predictive models. However, a systems biology approach first requires identifying appropriate data from a vast array of possible data types and resources. By using multiple high-quality databases, researchers can pull together the required gene, pathway, chemical relationship, and other information needed to build their predictive model. To best use these resources, one first needs to know what resources exist, what the strengths and limitations of the resources are, and how to efficiently retrieve data from them. This session will provide an introduction to several types of resources, including model organism (Mouse Genome Informatics, Rat Genome Database), pathway (Reactome), and toxicology (Comparative Toxicogenomics Database, EPA Dashboard) databases. The goal of this session is to familiarize participants with the power and limitations of publicly available databases that may be used to build and refine models of biological systems that can be applied to toxicology research. Talks will present content overviews for each database and demonstrate data retrieval and analysis capabilities. Speakers also will be available for interactive demonstration sessions during the meeting (see the US EPA booth for dates and times).

Mouse Genome Informatics: Using Data Integration to Facilitate Discovery of Relationships Among Genes
S. M. Bello, M. Ringwald, J. E. Richardson, J. A. Kadin, C. J. Bult, J. A. Blake and J. T. Eppig. Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME.

The Mouse Genome Informatics Database (MGI, www.informatics.jax.org) is the free international resource for the genetics, genomics, and biology of the laboratory mouse. MGI integrates biological data about the laboratory mouse, including mutant phenotypes, human disease associations, developmental gene expression, gene functions, and tumor biology, from a variety of different sources. MGI uses multiple ontologies and vocabularies including the Gene Ontology (GO), Mammalian Phenotype Ontology (MP), and Mouse Anatomical Dictionary, to annotate data from direct submissions, high throughput projects, and published literature. Data integration and use of ontologies allows users to identify and retrieve annotated data sets with varying levels of specificity. For example, using the recently created MouseMine resource at MGI (www.mousemine.org) a user can generate a list of genes associated with iron homoestasis, binding and/or transport, refine this list to only those genes with mutations resulting in neurological or behavioral defects then export the final list in a variety of formats. Or a user can exploit specific MGI query forms to identify genes known to be expressed in the developing autonomic nervous system, then export this list to the batch query tool to retrieve all phenotypes associated with mutations in these genes. This talk will describe some of the ways data are brought in and integrated into MGI, how ontologies can be used to facilitate identification of specific data sets, and how MGI tools may be used for bulk data entry and retrieval. An emphasis will be placed on data types that may be useful in building mechanistic or predictive models for use in toxicology.

The Mouse Genome Database (MGD) at MGI is supported by NIH/NHGRI grant HG000330. MouseMine is supported by NIH/NHGRI grant HG004834.

Chemical Connections at the Rat Genome Database
M. Shimoyama. Rat Genome Database, Medical College of Wisconsin, Milwaukee, WI. Sponsor: S. Bello.

The Rat Genome Database (RGD) is the premier site for genetic, genomic, physiological and phenotype data for the laboratory rat. RGD provides an integrated research platform for investigators through the integration of multiple datasets and software tools. To meet the needs of the many investigators who use rat in tox-
The Comparative Toxicogenomics Database (CTD): Facilitating Mechanistic Understanding of Chemical Effects

C. J. Mattingly. Biology, North Carolina State University, Raleigh, NC.

The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is a publicly available resource that informs hypothesis development about mechanisms of chemical actions and the impact of environmental exposures on human health. CTD contains manually curated data describing chemical-gene-interactions and chemical-disease and gene-disease relationships. Curated data are integrated with external data sets (e.g., Gene Ontology and pathway data) and tools that allow users to navigate and analyze biological relationships relevant to environmental health. This presentation will describe CTD functionality and highlight several new analysis and data visualization features. It will also include information about emerging projects, including the modifications to chemical representations, text-mining workflows for identifying chemical-gene-disease data, novel pathway development tools, and exposure data curation.

Interactive Web Application (Chemical Safety for Sustainability Dashboard) for Computational Toxicology Data Exploration

M. T. Martin. US EPA, Research Triangle Park, NC.

The USEPA has developed a web-based interface (Dashboard) to synthesize data from the ToxCast and ExpoCast project in the federated Aggregated Computational Toxicology Resource (ACToR) database into customized information displays. The Chemical Safety for Sustainability (CSS) Dashboard is built upon a flexible infrastructure, allowing multiple views of ACToR data organized to support user-specific needs. The users select sets of chemicals and data to focus on for data exploration and analysis. The data are organized into data classes to present all relevant information for a given pre-defined grouping of data such as hazard and exposure or in vivo and in vitro data. The CSS Dashboard provides data class views relevant to the data types and will continuously expand the viewing options. Within the Data Explorer mode, the user can view summarized information for the various data classes across all selected chemicals. In contrast, the Chemical Explorer mode permits viewing of the highly detailed data including concentration response plots and curve-fitting parameters across hundreds of high-throughput screening assays and chemicals. Chemical-specific scores that consider all information in each data class is presented in a dynamic summary table with a default score (e.g., average AC50 across assays within the in vitro hazard data class). Users can adjust default score criteria or apply expert knowledge to modify particular scores, with all decisions saved for sharing or later consideration. The scores are carried over into the Prioritization Mode where the chemicals are ranked in a weight-of-evidence scheme including an implementation of the Toxicological Prioritization Index (ToxPi). The ToxCast Dashboard will serve as the primary portal for ToxCast and ExpoCast data and model release and public user-interaction. This abstract does not necessarily reflect U.S. EPA policy.

Genomics in Toxics Regulation and Litigation in the Era of Whole Genome Sequencing

A. R. Schatz and G. E. Marchant. College of Law, Arizona State University, Tempe, AZ and Global Safety & Environment, Merck & Co, Whitehouse Station, NJ.

Genomic and other ‘omic data are increasingly being used in both toxic tort litigation and environmental standard-setting. For example, defendants are beginning to request genetic testing of toxic tort plaintiffs to demonstrate alternative causation, while plaintiffs are using genomic biomarkers in appropriate cases to buttress their proof of specific causation. On the regulatory side, the US Environmental Protection Agency is increasingly using genetic susceptibility data to identify susceptible subgroups in setting ambient air quality standards, while using toxico-genomic data to evaluate and characterize the toxicology of pesticides and chemicals. These initial applications of genomic data are setting the precedents and creating the pathways for much broader use of genomic data in both the toxic tort litigation and environmental regulation contexts as we rapidly move into the era of whole genome sequencing. This year, tens of thousands of people will have their genome sequenced. That number is expected to rapidly climb to the millions and perhaps even hundreds of millions over the next few years. This session will discuss current applications of genomic data, and how whole genome sequencing will greatly increase the availability and use of genomic data, in both the litigation and regulatory domains. It will also discuss the complex ethical, legal, and social issues the increased use of genomic data will present in these contexts, including informed consent, disclosure and access issues, the “right not to know,” privacy and confidentiality issues, and the rights and responsibilities of those that produce and are harmed by toxic substances with differential genetic susceptibility.

Incorporating Genomic Data into the Risk-Analysis Paradigm

H. I. Clewell. The Hammer Institutes For Health Sciences, Research Triangle Park, NC.

Genomic data are increasingly being used to assess and manage potential health risks from drugs and environmental chemicals. Gene array data can provide information on the mode of action for toxicity of a compound and, when coupled with Benchmark Dose modeling, can be used to determine acceptable exposures and evaluate population variability. In addition, whole-genome or whole-exome sequencing can help to identify individuals with known genetic susceptibility factors for a particular adverse health outcome. This presentation will discuss how genomic data are now, and are likely to be in the future, incorporated into risk assessment and risk management, and some of the scientific challenges and policy/ethical issues that this category of data present.

Regulatory Applications and Implications of Whole Genome Sequencing

M. A. Rothstein. Institute for Bioethics, Health Policy and Law, University of Louisville School of Medicine, Louisville, KY. Sponsor: G. Marchant.

This presentation will review the various applications and implications of WGS data for regulation of toxic chemicals, including how to regulate for individuals with genetically-variable sensitivities; setting uncertainty factors based on genetic susceptibility data, relationships between genetic susceptibilities and ethnic background, whether genetically-at-risk individuals be able to accept increase risk in the workplace, and the appropriate role for warnings, labels, and self-help with respect to genetic susceptibilities.
Validated biomarkers of exposure, effect and susceptibility obtained from toxicogenomics studies are potentially very powerful tools to establish or negate causation in toxic tort. For example, increased “chromosomal abnormalities” in plaintiffs might be used to establish that exposure to DNA damaging agents has occurred, but cannot be used for establishing general causation due to lack of chemical specificity. On the other hand, genetic polymorphisms that affect DNA repair may impact an individual’s response to genotoxic agents, (i.e. more susceptible to exposure) or indicate that an individual carrying that biomarker will have an increased “baseline” of chromosomal abnormalities, altering the biomarker dose-response curve in establishing or negating causation criteria and suggesting an alternate cause of elevated genotoxic biomarkers. The use of biomarkers in toxic tort is increasing; however, in many cases, the biomarkers in question have not been sufficiently validated to demonstrate that reported changes in gene expression patterns or changes in circulating cytokines/proteins levels are indeed causally related to overexposure to a specific chemical or class of chemicals. Proper validation of a defensible biomarker developed from toxicogenomics data includes reproducibility by one or more independent scientific investigators, demonstration of dose-response and specificity, development of a blind test set as confirmation of the methodology, data transparency, and the absence of non-specific or confounding responses. This presentation will discuss the potential use of WGS sequence data in toxic tort litigation, beginning with current uses and applications of genomic data in toxic tort litigation. It will then evaluate the implications of much greater availability and use of genetic data as WGS becomes more prevalent, and some of the issues this will create for the litigation system and the individual participants.

The role of the dopaminergic system in the clinical syndrome associated with Mn has been controversial. PET imaging with [18F]fluorodopa (FDOPA) is a noninvasive measure of nigrostriatal dopaminergic neuron integrity. Ultimately, neuropathologic examination of brains of exposed workers is the most definitive approach to identify causative pathways. To assess the pre- and post-synaptic dopaminergic system in humans with occupational Mn exposure, we conducted molecular neuroimaging with FDOPA PET in 40 Mn-exposed welders and neuropathologic assessment of the corpus striatum in eight Mn miners and compared both to matched controls. None of these workers had a diagnosis of a Mn-related clinical syndrome. For the PET studies, basal ganglia volumes of interest were identified for each subject and the uptake of FDOPA, Ki, was generated for each region. For the pathologic assessment, we compared mean cell density of neurons, astrocytes, and microglia in the caudate, putamen, and globus pallidus interna and externa in Mn miners with non-Mn miners. In the FDOPA PET study, repeated measures general linear model (GLM) analysis demonstrated a strong interaction between diagnostic group and region. The regional pattern of uptake in welders was most affected in the caudate > anterior putamen > posterior putamen. Histopathology of Mn miner brains revealed a trend towards lower mean neuron and astrocyte density compared to non-Mn miners in the caudate nucleus and putamen. There was higher mean microglia density in Mn miners than non-Mn miners in the globus pallidus interna and externa. The ratio of astrocytes to microglia in each brain region was lower in Mn miners as compared to non-Mn miners. The FDOPA PET studies in Mn-exposed welders indicate dysfunction in the presymptomatic nigrostriatal dopaminergic system. The neuropathology studies in Mn miners suggest that chronic, low-level Mn exposure may be associated with selective toxicity to astrocytes and neurons and microglial response in the corpus striatum.

Methcathinone abuse is a new cause of manganism. This psycho-stimulant is prepared from pseudoephedrine using potassium permanganate as an oxidant. The final mixture contains high concentrations of manganese (Mn). Methcathinone abuse is an important cause of parkinsonism among young Estonian patients. We describe the clinical, biological, neuroimaging characteristics and follow-up results in a large cohort of intravenous methcathinone users. The most prevalent symptoms are symmetrical bradykinesia, dystonia, early postural, gait and speech impairment; rest tremor is rarely present and rigidity is mild. The syndrome is irreversible after cessation of exposure and there is a trend of worsening. Plasma Mn concentrations in active uses are significantly higher than those in former users; so are the hair Mn levels. By MRI T1-weighted images, all active users show a symmetric hyper-intensity in globus pallidus, substantia nigra, periaqueductal grey matter and cerebral pedunculi, less in putamen, caudate nucleus, dentate nucleus and white matter. Whether these changes are mainly Mn induced, as the clinical syndrome, or correspond to the effect of long-term methcathinone abuse cannot be concluded. DAT SPECT results indicate that the presynaptic dopaminergic system in the nigrostriatal pathway are intact in former methcathinone abusers. Further, the data show no decrease in the level of postsynaptic D2 receptors in striatum. Evidence from our neuroimaging studies support the hypothesis that the accumulation of Mn in the brain is not associated with the degeneration of dopaminergic neurons as in PD, instead the dopaminergic system could be dysfunctional.

Methcathinone is a synthetic stimulant drug that is related to amphetamines, and it is primarily used in the form of a white crystalline powder. It is often referred to as "Ice" or "Crystal Meth," and is commonly smoked or injected intravenously. The effects of methcathinone abuse can be severe and include neurological symptoms such as tremors, rigidity, and bradykinesia, which are characteristic of Parkinson's disease. The study by K. Sikk et al. describes the clinical, biological, and neuroimaging characteristics of methcathinone abuse and highlights the importance of recognizing this emerging health issue.
It has long been recognized that chronic manganese (Mn) exposure in humans produces a neurological syndrome comprising psychiatric, cognitive and movement abnormalities that resemble some aspects of idiopathic Parkinson’s disease (PD). However, examination of the literature also indicates significant differences between Mn-induced parkinsonism and PD. For example, Mn-induced parkinsonism does not seem to be responsive to l-dopa therapy, an effective treatment for PD. Our laboratory has been examining the effect of chronic exposure to moderate levels of Mn in non-human primates in order to assess the effects on the nigrostriatal dopaminergic system using Positron Emission Tomography (PET) as well as other neuropsychological endpoints. Recent results indicate that the most affected parameter of the dopaminergic synapse is the ability to release dopamine.

No significant changes were measured in dopamine transporter levels indicating that dopamine terminals do not degenerate as a result of Mn exposure. A small but significant decrease was also measured in the level of D2-dopamine receptors in the striatum. These findings indicate that chronic exposure to moderate levels of Mn does not result in the degeneration of dopamine neurons but it results in dopamine neuron dysfunction since they cannot release dopamine more. Recent evidence indicates that a similar decrease in dopamine release measured by PET is present in dopamine terminals innervating the frontal cortex. In general, dopamine neuron dysfunction may be a common mechanism to explain cognitive and motor function deficits associated with Mn neurotoxicity. [Supported by NIEHS grant number ES010975]

Numerous studies on manganese (Mn)-induced parkinsonism indicate an interaction between Mn and dopaminergic neurotransmission. However, the lack of distinct T1 signal by MRI in substantia nigra (SN) in Mn-exposed human brain, along with other study results, has led to the assumption that Mn toxicity may take place in brain regions other than the dopamine (DA) neuron-enriched SN.

To better characterize Mn toxicity, we developed a high spatial resolution synchrotron X-ray fluorescence imaging technique (XRF) as a new quantitative tool to study Mn transporters and deciphering mechanisms of Mn-induced neurodegeneration. Distinct T1 signal by MRI in substantia nigra (SN) in Mn-exposed human brain, along with other study results, has led to the assumption that Mn toxicity may take place in brain regions other than the dopamine (DA) neuron-enriched SN. For the 75 CDs for which both rodent and non-rodent studies were conducted, new target organs were identified in non-rodents for 43 of the CDs and the changes in organ toxicities that was largely similar across most therapeutic areas although the dose can continue beyond the duration of toxicology cover according to the participant’s request (ICH Guideline M3(R2), 2009), although for oncology Phase I trials in advanced cancer, dosing can continue beyond the duration of toxicology cover according to the patient’s response (ICH Guideline 59; 2009). An analysis of target organ toxicities in these studies for 77 AstraZeneca candidate drugs (CDs) revealed a pattern of target organ toxicities that was largely similar across most therapeutic areas although the oncology/infection therapeutic area differed with a larger range of organs affected. For the 75 CDs for which both rodent and non-rodent studies were conducted, new target organs were identified in non-rodents for 43 of the CDs and the changes seen only in non-rodents included organ systems of high relevance for human risk assessment such as the liver, male reproductive tissues and CNS. Overall, these data on small molecule target organ toxicities provide new insights into drug toxicity profiles in preclinical species and additionally confirm the value of using non-rodents as a second species in toxicity testing to support human safety. As such, these approaches have been successful in supporting robust decisions around risk assessment for traditional pharmacological targets and small molecule chemistries, but how have these regulatory strategies fared when applied to more challenging targets and more sophisticated chemistries? In order to address this, speakers will cover the four key areas of small molecules, traditional biologics, innovative biologics, and nucleotide constructs.

The presentations will include specific discussion of the science and technology and the experiences of individuals and organizations. In this context, this workshop will consider the key question: Are these ‘diversities’ complementary and working expeditiously to bring important new (and safer) medicines to patients? In order to address this, speakers will cover the four key areas of small molecules, traditional biologics, innovative biologics, and nucleotide constructs. Overall, the presentations will provide clear information illustrated by examples on the different strategies employed for the different types of chemistry with an emphasis on relevance and outcome. The session will be of broad interest to academic, industry, regulatory, and consultant toxicologists who wish to be updated in this critical and evolving area.

Preclinical toxicology studies of up to 1 month duration are frequently used to support single or multiple dosing for a similar duration in Phase I clinical trials (ICH Guideline M3(R2), 2009), although for oncology Phase I trials in advanced cancer, dosing can continue beyond the duration of toxicology cover according to the patient’s response (ICH Guideline 59; 2009). An analysis of target organ toxicities in these studies for 77 AstraZeneca candidate drugs (CDs) revealed a pattern of target organ toxicities that was largely similar across most therapeutic areas although the oncology/infection therapeutic area differed with a larger range of organs affected. For the 75 CDs for which both rodent and non-rodent studies were conducted, new target organs were identified in non-rodents for 43 of the CDs and the changes seen only in non-rodents included organ systems of high relevance for human risk assessment such as the liver, male reproductive tissues and CNS. Overall, these data on small molecule target organ toxicities provide new insights into drug toxicity profiles in preclinical species and additionally confirm the value of using non-rodents as a second species in toxicity testing to support human safety. As such, these approaches have been successful in supporting robust decisions around risk assessment for traditional pharmacological targets and small molecule chemistries, but how have these regulatory strategies fared when applied to more challenging targets and more sophisticated chemistries? In addition, what recent innovations have been made to enhance regulatory success?
clination of safety pharmacology endpoints into general toxicology studies; these can provide useful data and are resource efficient. A further notable trend is a shared aim across the Pharma industry of reducing attrition in the GLP toxicology phase of candidate drug testing. Our data have shown that failures in this phase can be significantly reduced by extending the dose range finding studies and also by front loading the non-rodent telemetry.

### W 2111 Risk Assessment of “Traditional” Biologics
L. Andrew, Sanofi, Waltham, MA.

This presentation will address the issues and challenges presented in preclinical risk assessment of pharmaceutical targets and molecular constructs produced by recombinant technologies such as hormones and cytokines where multiple pre-clinical test species may be inappropriate. As the activity of the biologic therapeutic is frequently species specific, the selection of appropriate animal models or surrogates is crucial to generating useful data. Some animal test systems have been refined to alleviate the inter-species issues, and alternative in vitro strategies have also been identified which could significantly reduce or eliminate testing. However the cost benefit of approaching the development of a “non-traditional” biologic either through the use of a surrogate or the use of an animal model still raises many questions. The focus of this presentation is to discuss and disseminate the problems and potential solutions associated with the development and safety testing of biologics-based products. Considerations will be given to the use of transgenic animals, homologous molecules and in vitro systems that may be more predictive of safety and translatable than traditional species/tests. In addition, review of the recent regulatory guidelines including 36 addendum, 39 and other relevant guidelines will be highlighted as to their impact on the development of large molecules. Specific examples will be given to illustrate the utility of many of these alternative strategies. Case examples will include the use of a transgenic animal for a development program (including DART), the use of a surrogate antibody and the potential challenges of relevance and translatable and a unique development program using an animal model of disease and the predictivity of this model to the clinic.

### W 2112 Risk Assessment of Humanized Monoclonal Antibodies, Antbody-Drug Conjugates, and Novel Antibody Constructs
M. Hinrichs, L. A. Icicke, K. P. McKeeve and P. C. Ryan, MedImmune, Gaithersburg, MD.

Rapid advances in the field of protein engineering have led to the development of novel biologic drug platforms such as targeted nanoparticles, antibody drug conjugates (ADC), bispecific antibodies, and Fc mutated monoclonal antibodies. These emerging drugs continue to present new regulatory safety assessment challenges despite decades of practical experience with more traditional biologic agents. While the basic principles outlined in the recent addendum to ICH S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals can be applied to regulatory safety testing of novel biologics, the unique characteristics of each platform necessitates a science-based case-by-case approach often involving the use of safety testing methods that have not yet been standardized. This presentation will cover the scientific aspects of safety study design and species selection for two types of novel biologic platforms, bispecifics and ADCs. Case examples will be presented to highlight the strategic thinking and individualized approach required when evaluating the safety of new biologic technologies. The presentation will conclude with a discussion of the clinical relevance and current limitations of nonclinical testing strategies to focus on areas that could be improved in the future.

### W 2113 Safety Assessment of Oligonucleotide Constructs

This presentation will address the strategy, issues and challenges presented in risk assessment of oligonucleotide constructs such as antisense inhibitors and microRNAs. The basic principles of safety testing generally apply to oligonucleotide based therapeutics, but there are unique considerations related to targeting RNA expression in terms of general tolerability and exaggerated pharmacologic assessments. These considerations with regards to the site of action, interspecies cross-reactivity, and nonspecific effects influence the choice of animal models and the design of toxicology studies. Existing guidance’s for traditional drugs or biotherapeutics makes the use of surrogate inhibitors relatively straightforward. This talk will focus on strategies to deal with the challenges to ultimately provide meaningful safety assessment for new drugs that translate to patients. An example of a safety/pharmacodynamics relationship for an antisense inhibitor of Factor XI that incorporates mouse, monkey and human data will be presented.

### W 2114 Regulatory Authority Experience with Diverse Molecular Chemistry and Preclinical Safety Assessments Supporting First Time in Humans
D. Jacobson Kaat, US FDA, Silver Spring, MD.

Novel pharmacologic targets and new molecular chemistries raise challenging regulatory issues. A balance must be struck between supporting innovation in drug discovery while insuring volunteer/patient safety. For healthy volunteers there is no risk/benefit paradigm and the bar for safety is high. The lack of historical information on clinical toxicities for novel pharmacologic targets argues for conservatism in selecting a safe start dose and dose escalation scheme. FDA’s guidance “Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers” discusses situations where the start doses based on animal toxicology NOAELs should be lowered; one of the listed reasons is “novel therapeutic target.” For biologic drugs in this category a MABEL (minimal anticipated biological effect level) approach has been proposed. The need for such an approach was largely triggered by the cytokine release syndrome seen after the first dose in the Tegenero 1412 phase 1 study. This paradigm may have to be altered when the patient population has a serious and life threatening indication such as cancer. In these instances, patients should not be repeatedly exposed to drug doses that are not expected to have pharmacological effects. Other approaches could include microdose testing when investigating pharmacokinetics and bioavailability and the use of surrogate molecules when the drug only binds to the human target. In general, selection of the start dose for first in man studies with novel pharmacologic targets and/or new molecular chemistries should err on the side of safety i.e. lower start doses, slower dose escalation, protracted timing between dosing of participants and careful monitoring.

### W 2115 A History of the 3Rs in Toxicology Testing: From Russell and Burch to 21st Century Toxicology
M. L. Stephens1 and N. Mak2, 1CAAT, Johns Hopkins University, Baltimore, MD, 2Alternatives Research and Development Foundation, Jenkintown, PA.

The 3Rs—replace, reduce, and refine—have become the internationally established framework guiding the development of alternatives to animal experimentation in toxicology. Yet this framework languished for two decades after it was first proposed in 1959 by British scientists William Russell and Rex Burch. Then, as the animal experimentation controversy intensified in the 1980s, the concept of alternatives became politically charged, with some arguing that in vivo experiments could be replaced readily and others arguing that they were irreplaceable. A generation or so later, following the 2007 publication of a US National Research Council (NRC) report Toxicity Testing in the 21st Century, a Vision and Strategy, prominent scientists began predicting the near elimination of animal use in toxicity testing through the development of “21st-Century Toxicology.” Have we gotten from Russell and Burch to the beginnings of 21st-Century Toxicology? In this session, we will present results from comprehensive citation and literature searches that track the influence of Russell and Burch’s 3Rs framework and the prevalence of 3Rs-related research in toxicology over time. We will also draw on timelines of various 3Rs activities, including the founding of 3Rs organizations, centers, journals and websites, funding sources, the organization of workshops and conferences, the enactment of animal welfare/alternatives laws, and other milestones, to inform our historical analysis. We will present a historical narrative framed around four phases of activity: incubation (1959–1979), increasing acceptance and spread (1980–early 1990s), maturation (early 1990s–2007), and paradigm shift (2007–present). The impact of more than 50 years of 3Rs activity will be measured in part by focusing on the validation and regulatory acceptance status of alternative methods and trends in animal-use statistics, concluding with a discussion of remaining challenges to the development, validation, regulatory acceptance, and implementation of 3Rs methods.
In the 21st century, more than any other time in history, science is a team sport and requires cross-disciplinary/cross-functional interaction to meet the objectives and gain results. These interactions across multiple disciplines often require careful management and skillful leadership. Often times we fall prey to the belief that "a leader is always born". However, all of us "lead" in everyday life consciously or rather unconsciously. A question that begs to be asked is, "Why should anyone be led by me?" The answer can be elusive and requires the recognition that not all leaders are born. Good leaders can be developed. This, of course, leads to question, "How?" That again has to be addressed directly in terms of tangible competencies and behaviors. Is this due to the perception that the scientific ladder and management ladder are parallel, and one cannot support the other? The 21st century demands that each of us "own" our careers as well as the contributions towards society in a variety of ways. The time has arrived to spur the enthusiasm to "lead" in all fronts—the classroom to the boardroom and beyond. This informational session will include presentations by key leaders from academia, industry, government, and consulting. The speakers will introduce the concept of leadership as it relates to the current and emerging work environment, followed by a testimonial of core skills and styles required to be an effective leader. The testimonials will be individual but will provide set of tangible core qualities that are key to succeed and lead—at all levels. The session is designed for presentation and includes time for questions and discussion.

For ophthalmic medications, phototoxicity is one of the toxicities which would be a concern during drug development. Although there are several in vitro phototoxicity assays, no in vivo studies have been established to assess ocular phototoxicity. The purpose of this study was to develop appropriate study conditions to evaluate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu- rate ocular phototoxicity for intravitreal injective solutions by using Porphyrin [4,4',4'',4'''-(Porphine-5,10,15,20-tetrayl) tetrakis (benzenesulfonic acid) tetrascu-
Development of a murine gammaherpesvirus 68 (MHV-68) infection model was performed to facilitate identification of pharmaceutical agents that cause reactivation of latent viral infections, such as Epstein Barr Virus (EBV) in humans. Both MHV-68 and EBV are B-lymphotropic, establish latency after acute lytic infection, and are associated with the development of lymphomas. The model was developed using cyclosporine A as a positive immunomodulatory control. Cyclosporine A blocks calcineurin activity, which prevents activation of cytokine genes in T cells, leading to decreased T cell growth and activity. Cytoxic T lymphocytes are critical for control of latent MHV-68 infection in the spleen. C57BL/6 mice were tested against nearly 10,000 T MHV-68 and treated with 50 mg/kg cyclosporine A by the intraperitoneal route. Primary infection and viral reactivation in the spleen were measured by plaque assay for cell-free infectious virus, and qRT-PCR for latent and lytic mRNA viral transcripts. Infected splenocytes were quantified by infectious center assay. MHV-68 was detected in spleens of C57BL/6 mice treated with cyclosporine A 31 days following primary infection.

In summary, (1) the majority of plasma BAs in dogs was taurine conjugates, (2) feeding increased plasma BAs by about an order of magnitude, due primarily to increased taurine-conjugated BAs, (3) basal concentrations of BAs were higher in F than M dogs, (4) and there were large inter-individual differences in plasma BAs. These findings demonstrate that physiological BA concentrations in plasma of dogs are influenced markedly by prandial status, and slightly by gender. These physiological variations may complicate the interpretation of plasma BA concentrations in pathological conditions.

Intracisternal A Newcastle Summers virus was used as a control virus. Infected rabbits were randomized to treatment at the first observation of secondary lesions. Treatment regimens consisted of 3 oral doses spaced at 48 hours beginning at randomization. Doses of 5/5/5, 20/5/5 and 20/20/20 mg/kg BCV were evaluated. Compared to placebo, a statistically significant, dose dependent increase in survival was observed at the 20/5/5 and 20/20/20 mg/kg doses; demonstrating that BCV is effective for treatment of lethal poxvirus infection when administered after appearance of clinical signs of disease, specifically appearance of secondary lesions remote from the inoculation site.

An independent cohort of rabbits was added to study the PK of the 20/5/5 mg/kg regimen of BCV, when dose was initiated Day 4 post-infection. The concentration of cidofovir-diphosphate (CDV-PP), the active antiviral, was assessed in plasma, spleen, liver, lung, kidney and eye infected rabbit tissues. CDV-PP was detected in these tissues with the highest concentrations observed in the liver. Treatment of animals with BCV resulted in increased survival at doses of 20/5/5 mg/kg; demonstrating that BCV is effective for treatment of lethal poxvirus infection when administered after appearance of clinical signs of disease compared to placebo. Recurrent copy number alterations were identified by defining altered segments of DNA and calculating their frequency. We found considerable variation in the frequency of alterations between the tumors and the matched non-tumor tissues. Several regions of gain or loss observed in the tumors were also detected in non-tumor tissues, but at a lower frequency. This indicates that genomic instability increases with disease progression, and demonstrates genomic changes in pre-neoplastic tissues. Collectively, these data show that genomic instability plays a role in the development of fibrosis-associated HCC. This copy number data can also be used to identify genes that play a functional role in cancer development. Importantly, DNA copy number data is an endpoint used by The Cancer Genome Atlas project, and can be used to explore the human relevance of our model of HCC.
Assessment, SNBL USA, Everett, WA and Corporate, Shin Nippon Biomedical First Hospital, Jilin University, Changchun, Jilin, China and KCHRI at Department of Pediatrics, University of Louisville, Louisville, KY.

Infertility is a common complication in diabetic men due to the loss of germ cells. Our previous study had demonstrated that repetitive exposures to low dose X-rays radiation (LDR) can attenuate type 2 diabetes-induced testicular apoptotic cell death. To define whether repetitive exposures to LDR can also attenuate type 2 diabetes (T2DM)-induced testicular apoptotic cell death, T2DM rats were established by high fat diet feeding, followed by a small dose of streptozotocin. After diabetes onset, diabetic and age-matched control rats were treated with or without LDR at 25 mGy for 4 weeks every other day. Western blotting assay revealed that testicular apoptosis was significantly increased in T2DM rats by detecting AIF, along with increased oxidative and nitrosative damage, by detecting 3-NT and 4-HNE, and increased ER stress, by detecting CHOP and caspase 12. All these effects were attenuated by repetitive exposures to LDR. Mechanistic studies showed that Akt-mediated GSK-3β was down-regulated, but Akt negative regulators PTPIB and TRB3 were up-regulated in T2DM group. T2DM-induced the above testicular Akt related changes were partially prevented by repetitive exposures to LDR. Expression of Nrf2 and its downstream gene NQO-1 was up-regulated in T2DM group, and further up-regulated by exposures to LDR. Nrf2-related downstream anti-oxidants, such as SOD activity and catalase content were decreased in T2DM group by quantification assays, and these effects were attenuated by exposures to LDR. Therefore, the attenuation of T2DM-induced testicular apoptotic cell death by repetitive exposures to LDR is likely mediated by up-regulation of Akt/Nrf2-mediated anti-oxidative pathway.

2125 Repetitive Exposures to Low Dose X-Rays Radiation Attenuates Testicular Apoptotic Cell Death in Type 2 Diabetic Rats via Akt-Mediated Nrf2 Activation

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Iron is an essential element that is required for many cellular functions. Multiple symptoms of iron deficiency have been reported in humans, including anemia, hair loss, stunted growth, and reduced oxygen transport. Mammalian iron intake is mediated in two different ways, across the placenta during fetal growth, and through the intestine during postnatal growth and development. We previously identified hephaestin (Hp), a copper-iron ferroxidase, which plays a key role in intestinal iron transport. Hephastin oxidizes iron and works in conjunction with ferroportin to facilitate iron export from enterocytes and its efficient binding to plasma transferrin. Null mutations in the Hp gene cause an accumulation of intracellular iron, specifically in the intestine. During analysis of the Hp null mice, we discovered that neonates born to Hp knockout (Hp KO) dams developed truncal hair loss (the “mask” hair loss phenotype) that resolved after weaning. The same phenotype was also observed in pups born to mothers with intestine-specific knockout of Hp (even WT (flooded) pups born to these mothers), suggesting that lack of maternal Hp in the intestine alone is responsible for the mask phenotype. In order to determine the stage (ante and/or post natal) at which lack of maternal Hp leads to pup hair loss, we performed cross-fostering studies. Wild-type pups fed by Hp KO dams presented with the “mask” phenotype, whereas, all pups fed by the WT dams had normal hair growth. Histology of the affected skin of pups fed by Hp KO dams demonstrated disorientation and keratinization of the follicles, follicular infundibular plugging and some tortuosity to the hair canals. Our findings suggest the involvement of a “toxic milk” phenotype involving low levels of iron in the milk causing this phenotype.

2128 Toxic Milk Leads to the “Mask” Phenotype in Hephastin Knockout Mice

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Millions of Americans suffer from dry eye. The symptoms include: ocular surface discomfort, often described as feelings of dryness, burning, a sandy/gritty sensation, itchiness, visual fatigue, sensitivity to light, and blurred vision. The environmental (e.g. smoke, dust, low humidity, light etc.) and occupational (e.g. CO, CS2) stressors can induce or exacerbate dry eye by reducing ocular mucous cells and mucin production. In one of the dominant dry eye diseases - Sjögren syndrome, the patients have markedly reduced conjunctival goblet cells and the goblet cell-specific mucin-MUC5AC. However, there is no experimental evidence causally linking the mucin deficiency and the pathogenesis of dry eye. In the present study, a Muc5ac knockout (KO) mouse model was created so that one major ocular mucin-Muc5ac is deleted. The mice was subject to various physiological measurements as compared to wild-type (WT) growth and development, but the tear break up time (TBUT) values were significantly lower and corneal fluorescein staining scores were significantly higher in KO mice as compared to WT. But the tear volume was not changed. Despite the lack of Muc5ac expression in the conjunctiva of KO mice, Muc3b expression was significantly increased in these mice. Corneal opacification, varying in location and severity, was found in a few KO mice but not in WT mice. The present results suggest a significant difference in the quality, but not the quantity, of tear fluid in the KO mice compared to WT mice. Therefore, we, for the first time, demonstrate that the lack of a major ocular mucin-Muc5ac causes dry eye.

2129 Alterations of Contractile Gene Expression (Mypt1, CPI-17, and Myosin Kinase) in RUPP, L-NAME, and Adriamycin Rodent Models of Preeclampsia

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Preeclampsia (PE) is a pregnancy disorder that is characterized by a variety of complications, including high systemic blood pressure and deficits in renal hemodynamics. These PE related changes result in the characteristic reduction in placental perfusion pressure and compromised vascular function. In particular, this altered vascular reactivity is potentially due to a complex mechanism involving endothelial dysfunction and changes in vascular smooth muscle (VSM) contractile properties. The VSM of the microvasculature (i.e., mesenteric arteries) is distinct both phenotypically and functionally from that of the large conduit arteries, with many of the associated hemodynamic factors responsible for these differences still remaining undefined. Therefore, the objective of this study was to investigate and characterize the expression of several key contractile genes (Mypt1, CPI-17, and Myosin

556 SOT 2014 ANNUAL MEETING
Kinase) in various rodent models of PE (i.e., RUPP, L-NAME, and Adriamycin). Of particular interest is myosin phosphatase (MP), which acts as a central mediator of vascular function and can be used as a model to determine the VSM phenotype. Alternative splicing of exon 24 (E24) of the MP targeting subunit (Mypt1) generates isoforms that determine the VSM response to GCMP mediated vaso-relaxation. Fast contracting (E24+) isoforms are predominant in mesenteric artery VSM while slow contracting (E24-) isoforms are expressed in large artery VSM. Control and SHAM operated pregnant rats expressed the E24+ isoform of Mypt1 in mesenteric arteries. Hypertension during pregnancy resulted in a moderate shift in Mypt1 isoforms in L-NAME and RUPP mesenteric arteries, while Adriamycin treatment lead to a significant shifting of the Mypt1 from the E24+ to E24- isoform (p<0.05) in mesenteric arteries. These data indicate a potential compensatory mechanism of the VSM involved in the pathology associated with PE.

**2129a Variability in Neurotoxicity: Who Is Susceptible and Why**

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There is increasing interest in differential susceptibility among humans to toxicants, as well as a growing appreciation that multiple gene variants, exposure history, and complex gene-environment interactions (GXE) are critical determinants of disease and risk. For example, individuals who carry a mutation in the glutathione S-transferase gene have far higher risk for developing Parkinson’s disease when exposed to paraquat (PQ) (Goldman et al. Mov Disord 27:1652, 2012). More refined animal models, in particular genetic reference families of mice, now make it practical to replicate, extend, and mechanistically validate findings from epidemiologic and genetic studies of humans. In fact, Liang and colleagues validated the findings of Goldman in glutathione deficient mice (Toxicol Sci 134:366, 2013). These families, including the BXD and the Collaborative Cross, provide tight experimental control, but incorporate a very high level of genetic diversity. They are ideal for mechanistic studies of GXE and can now be reliably exploited to define single gene polymorphisms, important biochemical, and molecular mechanisms of toxicity. To illustrate, we studied the neurotoxicity of PQ and MPTP in BXD recombinant inbred mouse strains. Toxicity of PQ is highly variable among these strains and covaries with PQ-related disruption of iron regulation in the midbrain. We also studied effects of MPTP on striatal dopaminergic neurotoxicity among 10 BXD lines and found that 12.5 mg/kg MPTP s.c. caused a variation of 20–90% in neurotoxicity as determined by reductions in dopamine and its metabolites, tyrosine hydroxylase protein and elevations in GFAP, an astrocyte marker of neurotoxicity. Furthermore, the variation in dopaminergic neurotoxicity among strains was not due to altered metabolism of MPTP to MPP+, the proximal neurotoxicant. Genetic reference panels can provide valuable information on potential genetic differences in susceptibility and GXE.

**2129b Nuclear Receptor Small Heterodimer Partner (SHP) Modulates Circadian Clock Control of ER Stress Signaling in Alcoholic Liver Disease (ALD)**


[Purpose] In the past decade, our research has focused on elucidating the molecular mechanisms by which SHP (N4062) regulates liver and metabolic diseases. Highlights of our findings include revealing the role of SHP in fatty liver, liver cancer, and microRNA regulation. In this study, we use SHP-/- mice and the alcohol binge model to investigate how the liver circadian clock regulates ER stress and fatty liver.

[Methods] The NIAAA binge model was established using 8 weeks old, male C57BL/6 (WT) and SHP-/- mice (Nat Protoc 2013; 8:627–637). The mice were acclimatized to a control liquid diet for 5 days, then fed with 5% Lieber-DeCarli diet or pair-fed for 10 days. On day 11, mice were gavaged with maltose (control) or alcohol solutions at zeitgeber time (ZT) 3 (9 am) and sacrificed at ZT12, 18, 0, and 6. Serum and liver were collected. GC/MS and RNA-sequencing (RNA-seq) were used to identify clock regulated metabolites and genes, respectively. Transient transfection, luciferase reporter assay, ChIP assay, gel-shift assay, Co-IP, Western blots, adenosine overexpression, siRNA knockdown, and qPCR were used to elucidate the molecular mechanisms. Metabolomics revealed a global alteration by alcohol in the oscillation of intermediate metabolites in pathways of glucose, lipid, and amino acid metabolism in SHP-/- mice. Liver SREBP-1c protein cleavage and TGF content exhibited a rhythm pattern of changes. The rhythmity of core clock genes (Clock, Bmal1, Npas2) and nuclear receptors (Rorα, Rorγ, Rev-erbα) was disturbed by alcohol. Hepatic lipid metabolic genes and ER stress markers exhibited strong oscillations, which were perturbed by alcohol binge. We identified Chop as a circadian clock controlled gene that involves the Rorα/Rorγ/Rev-erbα/SHP regulatory cascade. [Conclusions] We provide a unique model for future investigation of the role of environmental chemical exposures in the development of metabolic diseases. [Grant Support] NIH DK080440 and AHA GIA (L.W.).

**2129c Mammary Gland Tumor Promotion by IGFI and an Insulin Analogue in the p53+/R270HWAPCre Mouse Model**

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Insulin analogues are chemically modified molecules with improved pharmacokinetic parameters compared to regular human insulin. Although these compounds have been tested for carcinogenic side-effects with the standard two-year in vivo assays in rodents, there are some concerns raised by several epidemiological studies in which an association is suggested between the use of some insulin analogues and the cancer incidence in these diabetic patients. In this study the new p53R270H+/WAPCre mouse model, which will develop spontaneous human relevant mammary gland specific tumors, was used. We frequently injected this model with insulin NPH, insulin glargine, insulin X10 (AspB10) or IGFI. We hypothesized that injections with possible carcinogenic compounds would increase the number of tumors that are formed, decrease the latency time for tumor development, lead to different types or more aggressive tumors. We found that both X10 and IGFI significantly decreased the latency time for tumor development (for glargine a slight, non-significant effect was observed). A Western blot analysis was done on all primary mammary gland tumors (n=170) to investigate the IR/IGF1R/HER2/ESR/p-Ekt/p-Akt/ and Catherin status. Hierarchical clustering of this data revealed that X10 and IGFI induced tumors showed a distinct protein-expression profile that was characterized by high p-Erk levels, suggesting an important role of MAPK signalling cascade in tumor progression/initiation in insulin analogue induced tumors. These results also indicate that the use of a tissue specific cancer model, like the p53R270H+/WAPCre mouse model, can be used to discriminate between mitogenic and non-mitogenic compounds.

**2129d Characteristics of IgG Deposit and Neurodevelopmental Protein Expression in Brain of BTBR T+tf/J Mouse Females**

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Autism is a brain developmental disorder with social interaction defects, communication faults, and repetitive behaviors. The cause of autism is not clearly defined. Since the presence of autoantibodies against brain antigen was reported in autistic children and their mothers, autoimmunity is considered being involved in pathogenesis of autism. BTBR T+tf/J (BTBR) mouse was recently reported as a model for investigation of autism because of its similarity in behavioral abnormalities with human autistic subjects. Neuro-inflammation could mediate generation of autoantibodies to brain antigen resulting in altered brain development. To evaluate the presence of brain autoantibodies and expression status of proteins related with brain development at fetal stage, fetus of BTBR mice were obtained at day 18 of prenatal period. IJVB mice were used as a control strain because of its positive social behaviors. Thirty one and twenty seven fetuses were obtained from three pregnant BTBR and FVB dams, respectively. Deposit of IgG isotype in fetal brain was evaluated using brain homogenates by ELISA. Expression of glial fibrillary acidic protein (GFAP), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and myelin basic protein (MBP) was examined using the fetal brain homogenates. Level of brain IgGa, IgG2b, and IgG3 deposit was significantly higher in BTBR fetus than FVB fetus, but no difference was found for IgG1 between the two strains. Expression of GFAP, NGF, and MBP was significantly lower in BTBR fetus than FVB fetus, but level of BDNF was not different between the two strains. Overall, this study suggests that pre-natal alteration in expression of various proteins related with brain development could lead to occurrence of autism. In addition, accumulation of autoantibody against brain antigen at fetal stage could result in pathogenesis to brain development. [supported by National Research Foundation of Korea 2010-0022169]
The availability of historical data for survival and tumor incidences can be useful in the overall interpretation of lifetime carcinogenicity bioassays. Survival data and incidences of neoplastic lesions were compiled for CD-1 mice from the same supplier used as control animals in nine carcinogenicity studies of 104 weeks. All studies, terminated between 2005 and 2011, were performed in our facility, with standardized environment, housing, feeding, necropsy, tissue sampling and trim- ming procedures, application of common diagnostic criteria and peer review. The mean survival rate of male and female mice was similar (respectively 45% and 43% at terminal sacrifice). The most common causes of unscheduled death or moribundity in males during the course of the studies were, by decreasing order, tumors (mostly from hemolymphoreticular system, lung or liver), atrial thrombo- sis, amyloidosis, skin lesion, various inflammatory processes or nephropathy. In females, the most common causes were tumors from the hemolymphoreticular system or from another origin (mainly lung or uterus), amyloidosis, skin lesion or nephropathy. In males, the most common neoplasms originated from lungs (ade- noma and carcinoma: >25%), liver (adenoma and carcinoma: >25%) and lympho- reticular system (10% malignant lymphoma) while females had mostly malignant lymphoma (>25%). The other common tumors seen with an incidence above 1% were found in various organs, i.e. Harderian gland, uterus, ovaries, adrenal gland, pituitary gland, mammary gland, testis and kidney or testis, in addition to systemic tumors (mainly histiocytic sarcoma and hemangiosarcoma). The incidences of the principal tumors were compared to published data. There were no marked differ- ences with the exception of the slightly higher incidence of malignant lymphoma in the males and females from our facility. These data were also compared study per study, revealing no major differences. This suggested that major tumor types were stable during this 6-year period with no evidence of strain drift.

The aim of this study was to obtain historical background data in Wistar rats collected over a period of 2 years as an essential prerequisite of carcinogenicity studies with this rat strain. Specific pathogen free Crl:WI(Han) Wistar rats obtained from Charles River, Germany were maintained untreated and provided ad libitum rodent diet. A 104-week study was conducted with 50 males and 50 females to investigate the survival, body weight development, health condition, clinical pathology and incidences of major MNU-induced tumors in rasH2 mice such as forestomach pap- illoma and carcinoma, malignant lymphoma, skin papilloma and lung adenoma. At study completion a high survival rate of 86% in males and 74% in females was observed. Body weights developed as expected, but reached extremely low mean values (<500g in male and <300g in female animals). Clinical findings and clinical pathology results were within the expected range. When compared to other rat strains, a low number of spontaneous lesions were observed at necropsy.

The general tumor profile was within the range of expected lesions (pituitary ad- enoma, mammary tumors, hepatocellular, thyroid and adrenal gland neoplasms, Leydig cell tumors). In contrast, the Wistar specific thymoma, but also skin and ovarian neoplasms were of very low incidence. Rare tumors included cases of hiber- noma, two nasal cavity tumors (adenoma, hemangiosarcoma), and parotid gland adenoma. Amongst pre-neoplastic and nonneoplastic lesions, there was a low inci- dence of foci of hepatocellular alteration, especially for basophilic cell foci, a com- mon finding in Wistar rats. The severity degrees of other findings were significantly lower than noted in other rat strains.

These results show that this Wistar rat strain maintained on Altromin 1324 diet at BSL offers a reliable model for the evaluation of carcinogenic potential of com- pounds especially in studies that are designed to exceed the standard duration of 2 years.
Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible (21 CFR 314.600 for drugs; 21 CFR 601 Subpart H for biological products), nonhuman primates, including cynomolgus macaques, have also become essential animal models to address product efficacy.

A comprehensive set of well-documented reference values for hematology, clinical chemistry and coagulation parameters can assist in interpreting clinical pathology data from cynomolgus macaques. These values provide data essential for the characterization of the cynomolgus macaque, as is required of an animal model in support of product development under the 'Animal Rule'. In addition, reference ranges are useful in evaluating the overall health status of each animal as well as serving as a baseline for evaluation/interpretation of any changes that occur due to disease and/or toxicity.

The values summarized and presented in this poster are intended to provide veterinary clinicians, researchers and toxicologists with reference ranges for hematology, clinical chemistry and coagulation parameters commonly evaluated in toxicology and/or efficacy studies. Analyses were performed using samples collected from clinically normal, healthy, naive, young adult, cynomolgus macaques of Asian origin. Methods of collection, sample handling and analysis are described. Samples were collected and analyzed as part of multiple studies across multiple sites sponsored by the National Institute of Allergy and Infectious Disease (NIAID).

**2136 Comparison of Rodent Euthanasia Using Carbon Dioxide (CO2) by Pre-Filled, Slow-Filled, and Rapid-Filled Methods in Mice**

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The AVMA Guidelines for the Euthanasia of Animals (2013) states that pre-filled CO2 chambers are unacceptable and a slow-fill method with a rate of approximately 10% to 30% per minute is optimal due to lower potential for pain or distress. To evaluate the proposed procedure, three methods of CO2 delivery for euthanasia were compared in mice: fully charged chamber (Group 1, n=5; 100% CO2), rapid fill (Group 2, n=8; 80% CO2 per minute) and slow fill (Group 3, n=8; 30% CO2 per minute). Blood was collected via cardiac puncture for analysis, necropsy was performed and histological changes were assessed. Mean onset of tachypnea was 11, 15 and 20 seconds while mean time to respiratory cessation was 35, 56 and 141 seconds for Groups 1, 2 or 3, respectively. Ataxia, tachypnea, open-mouth breathing, and recumbency occurred in all groups but onset was more rapid in Group 1 while overall durations were longest in Group 3. Mean pH of the mixed venous samples was 7.10, 7.05 and 6.88 for Groups 1, 2 and 3, respectively, although the partial pressure of oxygen and hemoglobin oxygen saturation were consistent across groups. Additionally, Group 3 animals had macroscopic observations of dark red discoloration of all lung lobes correlating microscopically to lung congestion and hemorrhage. In conclusion, based on shorter time to respiratory cessation, milder clinical pathology changes and lack of microscopic changes, the fully charged chamber is considered to be more humane than using a slow fill technique. However, given the recent guideline changes, we propose utilizing the rapid-fill method for the euthanasia of rodents via CO2 as this method appeared more humane compared to the slow-fill method. Given the variability in the blood pH, toxicokinetic evaluation may also be affected based on variability in plasma protein binding when using the slow filled method.

**2137 Effects of Blood Collection via Catheter on Hematology and Coagulation Parameters in Rats**

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The purpose of this study was to determine the optimum blood collection method in rats treated with Alteplase (Activas®, an on-the-market anticoagulant, and compare clinical pathology data after various collection methods. Five groups of male rats (3/group in 3 treated groups, 3/group in 2 control groups) were catheterized via femoral vein for intravenous (IV) dosing. Animals in 5 of those groups (1 control and 2 treated) were also catheterized in the jugular vein; this second catheter was connected to an injection port and used for blood collections. Alteplase (20 mg/kg, 1 mg/mL) was administrated IV to treated groups over approximately 1 hour. Control groups received sterile saline at the same dose volume and duration. Blood for hematology and coagulation were collected approximately 10 minutes after the end of infusion. Samples were collected directly from the jugular vein (JB) or via catheter with (CBH) or without (CB) heparin lock. Alteplase administration produced pharmacologically expected mildly prolonged PT (30% higher), moderately prolonged APTT (49% higher), and moderately lower fibrinogen concentration (58% lower) for JB animals (compared to JB controls). However, coagulation data from samples collected via catheter showed significant artifact compared to data from JB samples, even in untreated animals. Virtually no APTT data could be obtained from CBH or CB animals; the few samples that did produce data showed abnormally extended times (67.9-80.3 seconds vs. 17.1-18.1 seconds for JB controls). Plasma samples also showed slight hemolysis for animals bled by CB (2/5 samples) or CBH (2/3 control and 3/5 Alteplase samples) techniques. Hemolysis was not present in plasma samples from control or treated JB animals, and APTT was measurable in all of these plasma samples. Between control groups, CBH collection resulted in lower platelet counts than did JB collection (28% lower).

In conclusion, direct venous blood collection is preferred when assessing coagulation and hematology endpoints in rats; collection via catheter should be avoided in this model.

**2138 Characterization of Background Routine and Specialization Ocular Endpoints Used in Juvenile NHP Toxicology Studies**

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In recent years there has been an increased emphasis on non-clinical testing of pharmaceutical and biopharmaceutical products for the pediatric population. With larger numbers of new chemical entities undergoing toxicity testing, ocular changes may also be detected. Ophthalmologic examinations are a common endpoint on toxicity studies and can detect unanticipated ocular effects; however, other parameters may also provide relevant information. As the non-human primate (NHP) is often the most appropriate species for the testing on biologics, we characterized the full battery of standard and specialized ocular endpoints in juvenile non-human primates (18 to 23 months; n=4 animals) to determine whether they exhibited significantly different profiles than observed in older animals. No abnormal findings were observed during examinations, which consisted of a bio- microscopic (slit lamp) examination for evaluation of the adnexa and anterior portion of the eye, funduscopic (direct and indirect ophthalmoscopy) examination to evaluate the ocular fundus. Effects on intraocular pressure (IOP) can occur in young animals, mean IOP values were 15 ± 4 mmHg; approximately 14% lower than background ranges in older animals (2 to 5 years; n=172 eyes). Mean corneal thickness measured using a handheld pachymeter (451 ± 25 μm) or specular microscope (457 ± 15 μm) were slightly higher than those measured in older populations (422 ± 21 μm; n=12 eyes). Corneal endothelial cell density was overall higher in juvenile animals (mean = 3519 cells/mm² vs. 2936 cells/mm²), however, fewer of these consisted of the typical hexagonal shape compared to older animals (61% vs 71%). In conclusion, background ocular characteristics are generally similar between juvenile and older
animals, however, there are some differences which should be taken into consideration when evaluating the results in toxicology studies to facilitate the recognition of test article-related changes.

**2139 Data Comparison of the Respiratory Parameters during the Acclimatization Phase of Rat Snout-Only and Whole-Body Plethysmography Studies**


Prior to the exposure phase of a snout-only plethysmography (SOP) or whole-body plethysmography (WBP) study, the animals are trained to the restraint procedure over a period of days. The aim is to minimise any stress-related elevations in respiratory minute volume (RMV) values, which may mask potential effects of the administered test material. Measurements of tidal volume (TV), respiration rate (RR) and RMV were conducted over a number of consecutive days. For SOP, the animals were held in restraining tubes and for WBP they had freedom of movement in a small chamber. Mean data (n=8) for the SOP study gave elevated RMV values of up to 637 mL/min on Day 1 after 10mins, which decreased to 229 mL/min by 30mins and 200 mL/min by 60mins. For Day 3 to 5, the RMV had decreased to between 405 and 420 mL/min after 10mins, 185 and 198 mL/min by 30mins and 189 and 203 mL/min by 60mins. The values from the period 30 to 60mins are similar to published data (1). These changes in RMV were due to increases in the RR (up to 399 breaths per minute (bpm) as only a marginal effect on TV was observed. Mean RMV data (116 to 138 mL/min) for the WBP study gave little change with acclimatisation number and duration. Comparing the two approaches, RR values were initially considerably higher for SOP (up to 399bpm) but after 30mins were similar (123bpm for SOP and 124bpm for WBP). There was also a marked difference in mean TV values, 1.75mL/min for SOP and 1.06mL/min for WBP. In conclusion, the method of restraint has a marked effect on RR, TV and RMV and additional study design considerations needs to be given prior to conducting SOP studies.


**2140 Comparison of 4DCT-Derived Ventilation Maps to Deposition Patterns of Inhaled Particulates in Healthy and Diseased Rat Lungs**

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Changes in lung mechanics due to disease can strongly impact airflow dynamics and ventilation patterns. We have demonstrated that non-invasive, in vivo 4DCT imaging (3D imaging at multiple time points in the breathing cycle) can be used to map heterogeneities in ventilation patterns of rats under healthy and disease conditions. To validate such maps we exposed rats to fluorescent microspheres (FMS) by inhalation and examined 3D particle deposition patterns using cryomicrotome imaging. In these studies, eleven male Sprague-Dawley rats (231 ± 7g) were given an intratracheal dose of either 10 U/kg elastase or 0.2 mL saline or 0.2 mL saline only to a single lobe. Four weeks after dosing, rats were surgically intubated, mechanically ventilated with a tidal volume of ~2.0 mL, and imaged at 100 ms temporal resolution over the full breathing cycle by 4DCT. Immediately following imaging, rats were transferred to another ventilator with the same tidal volume and exposed to an aerosol of ~1 micron FMS for 5 minutes. After the exposure, the lungs were removed, filled with an embedding medium, and frozen for cryomicrotome sectioning. The cryomicrotome captured images of inhaled particle deposition throughout the entire lung, where the fluorescent signal intensity indicates ventilation. Comparisons between 4DCT ventilation maps and FMS deposition patterns showed strong correlations (r=0.75, p<0.0005) in ventilation patterns, where gross ventilation defects due to the disease were observed. By comparison, control rats showed few, if any, ventilation anomalies. We conclude that ventilation maps derived from non-invasive 4DCT imaging can be useful for predicting aerosol deposition in inhalation studies, evaluating lung disease and disease progression, and assessing treatment efficacy.

**2141 Development of an Impedance-Based Model For Assessment of Cardiopulmonary Function in Rabbits**

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Improving the quality of physiologic data collected from research animals in toxicology studies is paramount. This is most easily accomplished by collecting as much data as possible from a single animal, thereby reducing animal use and error associated with satellite groups. The present study investigates the feasibility of applying a large animal implantable telemetry device capable of providing data on animal activity, core body temperature, blood pressure (BP), electrocardiogram (ECG), and impedance-based respiratory parameters in the rabbit. Six New Zealand white rabbits were implanted with a modified TL11M-D70-PCTR device from data sciences international. The first task was to develop an optimal implantation technique that allows calibrated tidal volume (Vt) measurements that are within 10% of those obtained simultaneously from a pneumotachograph (PNT), low noise ECG, and stable BP. The second task was to challenge with a known respiratory stimulant (doxapram HCl, 5.0mg/kg i.v.) to assess linearity of the calibration across a range of Vt. Of the three electrode placements attempted, only one resulted in calibrations consistently under 10% error. Optimal electrode placement results in calibrated Vt measurements within 1.7% (±1.6%) of those obtained from a PNT during normal tidal breathing, 6.0% (±3.6%) following doxapram HCl injection and 7.3% (±4.4%) following saline injection. Vt range for normal tidal breathing and saline injection was 9-15 mL, and following doxapram injection was 25-30 mL. Similar error ranges were associated with derived flow parameters. Increases in mean BP of 25.0mmHg (±6.82mmHg) and decreases in heart rate of 56.3bpm (±6.82bpm) were associated with doxapram injection only. No departure from normal body temperature was observed in any group. The development of this model offers a solution to monitoring cardiopulmonary function the rabbit.

**2142 Comparison of Cardiovascular Parameters Recorded via Radio-Telemetry in Göttingen and Chinese Bama Minipigs**

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Introduction: Minipigs are routinely used for regulatory toxicity testing, including assessment of cardiovascular effects of a drug via telemetry. In the USA and Europe, the Göttingen minipig is often selected for such studies, while in China, the Bama minipig is usually the species of choice. The objective of this study was to compare the hemodynamic and electrocardiographic parameters of the Göttingen and Chinese Bama minipigs, using the standard telemetry methodology.

Method: Six Bama minipigs were surgically implanted with radiotelemetry transmitters (DSI TL11M-D70-PCTR). Systemic blood pressure, modified Lead II ECG intervals, and heart rate were recorded continuously for repeated intervals of approximately 24 hours using the DSI Ponomah software (version 5.00). The control cardiovascular data for Göttingen minipigs were obtained from literature (Markert et al, 2008).

Results and discussion: The mean systolic and diastolic pressures for Bama pigs were 134 and 95 mmHg, respectively, compared with the published values of 122 and 86 mmHg for Göttingen animals. Baseline heart rates were slightly higher for the Bama pigs (88 btm) compared with the Göttingen pigs (71 btm). Associated with the difference in heart rate, there were some small differences in quantitative ECG parameters, most notably for the QRS duration (39 ms for Bama and 56 ms for Göttingen) and QT-interval (295 ms for Bama and 320 ms for Göttingen). Much of the difference in heart rate and derived ECG intervals may be attributable to variations in housing and husbandry practice. In particular, the timing of feeding relative to data collection is an important factor as postprandial increases of 8-10% in heart rate have been demonstrated in minipigs. In summary, the cardiovascular parameters collected from telemetry-instrumented Bama minipigs were broadly similar to those reported from Göttingen animals, and this strain of animal is considered suitable for assessment of effects of new drugs on the cardiovascular system.
Comparative Sensitivities of the Rat, Dog, and Monkey Larynx and Tracheal Bifurcation in Inhalation Toxicity Studies

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Introduction

In this review, a comparative assessment of the larynx and carina from rat, dog and cynomolgus monkey is undertaken, to compare sensitivities and to assess their relevance for man. In inhalation toxicity studies, the authors have observed that drug induced lesions are frequently reported in the larynx and tracheal bifurcation (carina) in the rat, but less so in the dog or primate. This review is based on observations made by the authors over several years, and on data from inhalation studies compiled at one site. Histopathological sections of the upper respiratory tract tissues, including the larynx and carina, were prepared and evaluated according to the protocols in use at the different sites, in accordance with best practice guidelines. Data from several inhalation studies run at one site on different rodent and nonrodent species were collated and tabulated. They indicated that some test articles which induce lesions in the upper respiratory tract in rodents, notably in the larynx, do not cause histopathological changes in the dog or primate, supporting the authors’ observations. The rat larynx is far more sensitive than the dog and monkey in its response to inhaled xenobiotics, due to the relatively thin epithelial lining, in association with increased mucosal deposition of aerosol particles. The carinal ridge in the tracheal bifurcation is also a well-defined point of impaction for inhaled particles, and is more frequently injured in rodents than in dogs and primates, due to its relatively thin epithelium and airflow dynamics.

Conclusion

Personal observations and available data indicate that drug induced lesions are more frequently reported in the larynx and tracheal bifurcation (carina) in the rat than in the dog or primate, bringing into question the relevance of these rodent findings for humans. These observations suggest the human larynx is closer to the beagle dog and cynomolgus monkey in its response to inhaled xenobiotics.

Species-Related Differential Sensitivity for Assessing Hemodynamics and Respiratory Function Using Combined Telemetry-Plethysmography Model


Simultaneous monitoring of cardiovascular and respiratory parameters in conscious non-restrained animals could be useful in safety evaluation. However, the choice of the animal species may be crucial.

We investigated the sensitivity of the 3 commonly used rodent species to the effects of clonidine (1 mg/kg, i.p.), verapamil (30 mg/kg, p.o.) and theophylline (100 mg/kg or 50 mg/kg in mice, m.p.) on cardio-respiratory function using combined telemetry and plethysmography. Telemetered rats, guinea-pigs and mice were placed into plethysmography chambers to simultaneously assess hemodynamics (Heart Rate, HR; Mean Arterial Blood Pressure, MAP) and respiratory function (Minute Volume, MV).

Clonidine decreased HR in rats (-38%, p<0.001) and mice (-31%, p<0.01) but not in guinea-pigs. Clonidine also increased MAP (+35%, p<0.01) and tended to reduce MV (-22%, NS) in rat only. In rats and mice, verapamil decreased MAP (-27% and -25%, p<0.01) and increased HR (+16% and +12%, p<0.05). Hemodynamic parameters were not modified in guinea-pigs. Verapamil had no effect on ventilation in the 3 species. Theophylline increased HR in rats, mice and guinea-pigs (+59%, +39% and +32%, p<0.001) but had no effect on MAP. Theophylline exhibited respiratory stimulant properties in rats (MV, +68%, p<0.05) and mice (MV, +203%, p<0.01) but not in guinea-pigs.

These findings suggest that, in contrast to cardiac risk assessment with regards to evaluation of QT interval prolongation, both rats and mice are more sensitive than guinea-pigs in the combined telemetry-plethysmography model for assessing hemodynamics and respiratory function in early safety pharmacology studies.

Effect on Dosimetry of Altered Respiratory Minute Volume During Inhalation Exposure

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For most rodent inhalation studies, the achieved dose is estimated based on the calculated respiratory minute volume (RMV) according to animal body weight using one of the commonly accepted algorithms, like Guyton, McMahon, Bide or ATF formula. However, such estimates will not be accurate if the inhaled material has a local irritating effect on the respiratory system, with consequent effects on the respiratory pattern and RMV. Under such circumstances, the inhalation dose may be miscalculated by use of standard algorithms. The current study was designed to investigate the effect of a known respiratory irritating agent (Code: SA) on the RMV of rats. SA was aerosolised and administered to the rat via the nose-only inhalation route for 10 min at three different aerosol concentrations using a flow past system. Animal RMV was assessed using a head out plethysmography system before, during and after inhalation exposure. Our investigations revealed that the RMV values decreased significantly during inhalation exposure to SA compared with the values obtained before inhalation exposure in the same animals. The reduction was mainly due to decreased tidal volume and was largely concentration-dependent. At the high concentration, the measured RMV value was less than 50% of the RMV value estimated according to the McMahon formula. The investigation justifies our approach of using the measured RMV value rather than calculated RMV (based on animal body weight) for a better estimate of achieved doses during inhalation exposure to test items which may have an irritant effect on the respiratory system.

Implantation of Left Ventricular Pressure Telemetry with Solid Tip ECG in the Cynomolgus Macaques: Surgical and Anaesthetic Considerations


Objectives: Implantation of a D70-PCTP transmitter allows measurement of cardiovascular parameters for safety pharmacology and toxicology studies. Historically, we have implanted ECG electrodes subcutaneously over the thorax in the primate, resulting in a variable signal with a large noise-signal ratio. The solid-tip negative ECG electrode configuration aims to eliminate muscle noise and movement artefact from the ECG waveform.

Method: A balanced anaesthetic technique is used and multimodal analgesia is provided. The dual pressure catheters are surgically implanted into the apex of the left ventricle via a midline laparotomy and incision in the diaphragm, and into the abdominal aorta via the left femoral artery. The device body is anchored in the abdominal cavity to measure core body temperature. The positive ECG electrode is positioned on the diaphragm close to the apex of the heart, and the negative ECG electrode advanced into the superior vena cava via the right internal jugular vein. The final position of the negative ECG electrode is ascertained at surgery by monitoring ECG morphology.

Results: The solid-tip ECG configuration ensures the procedure for implantation is reproducible and simplified compared to subcutaneous placement. Waveforms from these implants are considered good, and all animals recovered well without complications. Our peri-operative protocol provides stable anaesthesia and suitable analgesia for this procedure. To date none of our macaques implanted with this device have required surgical repair.

Conclusions: The solid-tip ECG D70-PCTP device provides improvements in ECG waveform quality in the primate, and also animal welfare due to decreased surgical/anaesthesia time and fewer surgical repairs.


Onset of Sexual Maturity in Female Göttingen Minipigs

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In drug development minipigs may be used as a non-rodent alternative to dogs or non-human primates. For general toxicity studies, the animals should be sexually mature at start of the study. Based on breeding experience, female Göttingen minipigs are considered to reach sexual maturity at 4-5 months of age. To investigate this further, a study with young sows at 3-4 months of age (housed in the same room as adult boars) was performed. Twice weekly, they were observed for signs of heat (vulva redness/swelling, mounting) and blood samples were taken for progesterone analysis. In addition, body weights were recorded every 2 weeks. After the completion of 1-2 cycles of progesterone release, females were sacrificed. To check
whether the female minipigs were actually sexually mature, the reproductive organs (ovaries, uterus, pituitary gland) were weighted and histopathological examination of reproductive-related organs (ovaries, uterus and cervix, vagina, mammary gland and pituitary) was performed.

Before reaching sexual maturity, very low concentrations of progesterone (< 1 ng/mL) and/or signs of heat were detected sporadically. The first cycle of progesterone release, indicative of a functional corpus luteum, was noted in 7/13 sows at 3.7–4.2 months (8.4–12 kg) and for another 6/13 sows at 6.1–6.5 months (12.1–16 kg) of age. The estimated cycle length was 17–22 days. Signs for heat were not always noted around expected ovulation. Histopathological examination of the reproductive organs confirmed that all 13 sows were sexually mature.

In conclusion, the age range when female Göttingen minipigs reach sexual maturity (3.7–6.5 months) is much longer than anticipated. Hence, care should be taken when designing toxicity studies with young sows and interpreting data hereof. Progesterone analysis proved to be a reliable method to detect sexual maturity during in-life.

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Social housing of macaques is a standard and common requirement of up-to-date primate husbandry. Unlike in male animals, social housing can have profound effects on ovarian cyclicity and potentially on female fertility. Among these effects are interruptions or irregularities of the ovarian cycles. However, these cycle disturbances vanish as animals are socially housed over prolonged periods of time. Since this laboratory has long-lasting experience in ovarian cycle monitoring/social housing, a retrospective analysis of cycle patterns was undertaken. Beyond that, a comparison of menstrual cycle data was conducted for two locations (US vs. Europe) with highly comparable husbandry approaches/vaginal swabbing collection and criteria. Daily vaginal swab data were available from 5447 ovarian cycles of Asian origin animals. Animals were housed socially for a period of at least 3 months. The swabs were rated from no to heavy menstruation. Menstrual cycle length was categorized (C1, C2, and C3) depending on the cycle length difference between consecutive cycles with C1 being a regular cycle, C2 a prolonged cycle and C3 a comparatively irregular cycle. Cycle durations below 20 days and over 50 days between consecutive cycles with C1 being a regular cycle, C2 a prolonged cycle and C3 an Old World monkey. New World monkeys, e.g. the marmoset monkey (Callithrix jacchus) are also being used occasionally. Within the primate pedigree Old World monkeys are more closely related to humans compared to New World monkeys. In many instances, safety assessment of biopharmaceuticals requires evaluation of the immune system. In this work, relevant differences between cynomolgus and marmoset monkeys are compared. Functional immunotoxicology tests are easier to be performed in cynomolgus monkeys due to better functionality of human assays or blood volume limitations in marmosets. On the other hand some leukocyte CD markers better resemble the human distribution in marmosets. Development of anti-drug antibodies (ADA) is often a critical limitation for the evaluation or maximum duration of preclinical studies with biopharmaceuticals. Based upon the phylogenetic distance a higher risk of ADA development in marmosets can be assumed. However, data from approx. 200 animals/species exposed to human antibodies reveal 6% of cynomolgus and only 2% of marmosets developed detectable ADA responses impacting toxicokinetics. In conclusion, whilst cynomolgus monkeys are the preferred non-human primate model, marmosets provide a relevant alternative in certain instances.

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Microminipig, a small minipig (< 10 kg body weight in adult) is developed as a novel experimental animal for nonclinical pharmacology/toxicology studies in Fuji Micra Inc. (Shizuoka, Japan). To understand the results of nonclinical studies, we need to comprehend species differences in drug metabolism between human and microminipig. It has been reported that there is some overlap in microminipig and human cytochrome P450 substrate specificity, with high P450 2D-mediated activity in liver microsomes. Glucuronidation catalyzed by UDP-glucuronosyltransferase (UGT) is a predominant phase II biotransformation reaction in the metabolism. The purpose of the present study is to identify the characteristics of hepatic UGT activity of microminipig by a comparison with those of human and other experimental animals.

In vitro UGT activities in liver microsomes from microminipig, human, mouse, rat, dog, monkey and minipig were investigated. Glucuronidases of estradiol, imipramine, serotonin, propofol and 3'-Azido-3'-deoxythymidine (AZT), a selective substrate of human UGT1A1, 1A4, 1A6, 1A9 and 2B7, respectively, were measured by LC/MS/MS. Estradiol glucuronidation activity of microminipig was higher than that of human and other animals. Imipramine-N-glucuronidation, a distinctive conjugation by human UGT1A4, was catalyzed by microminipig liver microsomes, but not by dog liver microsomes. Serotonin and propofol glucuronidation activities in microminipig were similar to those in human. Low AZT glucuronidation activity in microminipig was observed as similar to that in rodents or dog. UGT activities in microminipig were similar to those in minipig. It is suggested that microminipig is useful for nonclinical studies as one of non-rodent species on UGT activity.

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Clinical chemistry is an essential analytic tool in many areas of research, drug assessment and development. In research involving mice or other small animals, limited amount of blood that can be collected pose a challenge and limit the prac-
tical utility of clinical chemistry analyses on serum or plasma. To overcome this problem, multiple cohort of animal are being used for different activities at different time leading to extra animals and additional resources, and limit the suitability of interpretation of results which are derived from different animals. The aim of this study was to prove the hypothesis that diluted plasma can be used in toxicity studies involving mice to determine various clinical chemistry parameters from the same animal without compromising the quality of data. Blood samples were collected from the mice; two aliquots were prepared, one used for dilution and other one kept undiluted. First aliquot was diluted 2, 3, 5 and 10-fold with water for injection. Analysis was carried out using automated clinical chemistry analyzer. Results were compared with the results from non-diluted plasma of same mice.

Most of the clinical chemistry parameters were well within the range of undiluted samples except electrolytes. For electrolytes, we tried dilution with different diluent like reagent grade water type I, Cal A (Random), plasma, normal saline and water for injection albeit without any success. Nevertheless, when specimen dilution will permit essential testing on small, valuable or fewer animals, all specimen dilutions should be performed identically throughout the study.

Conclusions: The Sinclair lineage had the lowest average basal cortisol level. The stressor increased cortisol from a range of 14% to 129% (means) across the four lineages. All lineages responded to the stressor as reflected by increased plasma cortisol but the Sinclair increase was very modest. The Sinclair lineage was essentially a non-responder to the short-term stressor (food denial).

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At Charles River Laboratories (CRL), we are committed to ensuring all our animals have the highest level of care and welfare. To this end, our social housing program includes placing all animals in pairs or groups. We have a rate of almost 100% success social housing juvenile and sub-adult animals as well as adult females. Social housing of sexually mature males can be a challenge. Some publications suggest that it is not advisable to attempt adult male introductions in mixed sex rooms of rhesus macaques (Macaca mulatta). However separating animals into single sex rooms introduces a potential scientific confound as well as operational inefficiencies, and to our knowledge this data has not been published for cynomolgus macaques (Macaca fascicularis). Data was collected from two CRL sites which are actively social housing mature males. For the purposes of data collection, sexually mature males were defined as 5 years of age and 5kg or greater. As is common in toxicology research, all animals had a narrowly defined weight range. A pair/group was considered successful if they had maintained compatibility for a minimum of two weeks. All social housing attempts were made in rooms with females present and visible to the males. A total of 21 rooms were analyzed, and 81% of the rooms surveyed had success in pairing the majority of males in the room. Fifteen rooms had a success rate of 75% - 100% (median 92%) with over 280 male animals being socially housed. This data shows that mature males can be successfully socially housed in rooms with females. The ability to compatibly socially house mature males in mix sex rooms does not compromise welfare and allows for greater flexibil-

ity of vivarium space usage.

2152 Surgical and Nonsurgical Options for Dosing of Cen-
trally Administered Compounds and CSF Sampling in Toxicity Studies Using Cynomolgus Monkeys


In preclinical toxicology, the cynomolgus monkey represents the predominant non-human primate species. In terms of drug delivery, crossing the blood-brain barrier is often an important need in pharmaceutical development. This work gives an overview about available techniques and our experience. Opt.A: Under sedation, in juv. (11 months and 0.8 kg) to mature (≥5 kg) animals, lumbar spinal needle in-

jection was feasible at 3 times within 24 hrs (n=1000) for dosing (1-3mL) or CSF sampling (1-3mL). Opt.B: Animals of the same age range underwent intrathecal administration (0.02 mL/kg/hour) with a surgically implanted lumbar port system (n=480) for a duration of 13 wks, with 50% port patency for CSF. Opt.C: Dual port surgery with implantation (n=3) of a cisterna magna and lumbar port allowed multiple CSF samplings with chair-restrained and non sedated animals, while dos-

ing continuously through the lumbar port. The cisterna magna port showed a 100% port patency for 4 wks, hence required precaution when handling the ani-

mals to avoid catheter blocking or relocation. Opt.D: So far 9 months patency was achieved for an implanted intraventricular port (n=1) to allow a more robust and practicable CSF withdrawal (daily: 8 times, each 0.3mL). For the proper location of the lateral ventricle, a template atlas of the macaque brain for digital imaging and quantitative neuroanatomy was used. Non-stereotactic surgeries were conducted using SoloPort™ MID LOVO; 3 Fr polyurethane catheter; round tip; 60cm, 2 movable beads (Solomon Scientific, USA) as also performed in clinical settings. Post surgery the animal received an antibiotic and flunixin as analgesic for 5 days. A wash out period, was scheduled prior to start of first dosing. In conclusion, all described techniques were successfully conducted with juvenile and mature group housed cynomolgus monkeys in accordance with European requirements. It is now possible to perform the full range of clinical settings in this species for withdrawal of CSF, as well as for administration of centrally active drugs.
2153 Selection of Ocular Histology Tissue Sections Based on Ophthalmic Examination Findings

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Standard procedures for collection and sectioning of eyes may not be sufficient when ocular findings are noted during ophthalmic examinations. Routine histologic sections of the eye are collected cutting by a section parallel to the ciliary artery to include cornea, iris, lens, retina, macula, optic disc, and optic nerve. While this process provides a tissue section that includes the most important ocular structures, this section may be insufficient if ocular findings have been noted in regions that are not centrally-located or if the test article targets the eye. Data from ophthalmic exams can be used to determine the best location to collect histologic sections of the eye.

Cynomolgus monkeys with pre-existing ophthalmic findings such as lens opacity, optic disc pallor, and retinal changes were selected for evaluation. An ophthalmic examination via slit lamp biomicroscopy and indirect ophthalmoscopy was conducted. Detailed information regarding the location of the ocular finding was recorded. For lens and fundus findings, a photograph was collected at the time of ophthalmic examination. At necropsy, the globes were collected separately. A standard histologic section and additional sections were collected based on location information from the ophthalmic data.

The routine section rarely included affected regions of the eye. Only in cases of optic disc findings was there a good likelihood of seeing the affected region on the routine slide. Non-central retina findings and lens findings were less often visible in routine sections. Using detailed descriptions from ophthalmic examination and photographs, the likelihood of collection a tissue section that included the region of ophthalmic findings was improved.

The routine section only provides the opportunity to evaluate ophthalmic findings if they are present in the region that is sectioned. For non-central findings, data from ophthalmic exams (including photographs) can be used to hone in on a tissue section more likely to include the affected region.

2154 Dermal Administration in Toxicity Studies Using the Cynomolgus Monkey


The cynomolgus monkey represents an important primate model for preclinical safety evaluation, and in rare cases drugs must be administered topically (epicutaneous administration). The current work describes the conduct and practicability of long duration administration (4-8 hours) of a gel-like test item (0.5 mL/kg/dose) over a period of 4 weeks. Following calculation of the required body surface area, the application site was shaved (e.g. 15 times 10 cm), so that the area was clearly separated from the remaining coat. Vehicle or appropriate concentration of the formulated test article was uniformly applied with a plastic card in equal thickness to the shaved area at the animals back, covered with a soft cloth and fixed with tape. In addition, a thin elastic tape was rolled around the animal’s body thickness to the shaved area at the animals back, covered with a soft cloth and fixed with tape. In addition, a thin elastic tape was rolled around the animal’s body. A jacket was used for further protection of the dosing site.

The animals underwent a 3 day jacket training to ensure comfort when wearing the jacket and to reduce the risk of manipulation by cage partners. At the end of the exposure period and after the skin assessment all application sites were cleaned with water for injection and additionally back and hands/feet were washed with an anti-allergic olive-based soap. Hereby, possible contamination should be further reduced. Great care was given to ensure protection of the dosing area to avoid accidental exposure or cross contamination with other group housed monkeys. All animals were examined for signs of erythema, edema formation and oedema at the dosing site(s) according to a modified Draize scheme (Draize, 1944). At necropsy, skin samples were taken from (a) application site (back), (b) non-dosed area (lower back, adjacent to application site), and (c) non-dosed area (abdomen), for comparison. In conclusion, the described technique was successfully conducted with group housed cynomolgus monkeys. However, some incidences of cross contamination did occur. Future work therefore shall evaluate the practicability of a thin body coat partly also covering legs and arms.

2155 Comparative Analysis of Cynomolgus Monkeys Behaviour by 24 Hour Time-Lapse Video Monitoring in Toxicity Studies


The cynomolgus monkey represents the predominant non-human primate species when it comes to preclinical safety evaluation of new medical products but rarely rely on behavioural aspects of undisturbed animals. Therefore, a video monitoring study was conducted with 12 animals divided in three animals per group. Repeated monitoring in a longitudinal study on three different occasions was performed in an interval of six weeks. The grouped animals were acquainted to each other and to the European style cages which are equipped with separating steel bar boxes. A video system monitored the animals for 24 hours in a light/dark regime of 12/12h adapted biweekly in a dark phase to red-light, with weak light emission. The videos were captured with software to monitor three animals on a single data file. To keep the total analysis time manageable all analyzed videos were only observed for six hours in total, always in the same daily time frames for all observed animals: Four hours at day light and two hours during the night. A qualitative behavioural scoring was utilized by timing the actions of the individual monkeys, divided into the following five different categories: 1) moving slowly, 2) sitting or lying calmly, 3) sitting on the water tap at the back, 4) wandering or climbing, and 5) the total number of behavioural changes. The observations demonstrated a high intra- and inter-variation of behavioural actions. The total number of behavioural changes was similar for the simultaneously monitored animals compared to the animals repeatedly monitored: 57.3 ± 18.0 compared to 53.3 ± 15.1 changes, respectively. The longest observed category (over 2 hours) was the mean time of sitting or lying calmly (which is mainly during the night) followed by sitting on the wateripple for almost two hours. In conclusion, this study demonstrated the feasibility of video recording of undisturbed cynomolgus monkeys over 24 hours. The results of this study will help detection and comparison of clinically relevant or adverse treatment effects normally not seen by short cage side observations.

2156 Investigation of a Suitable Method for Monkey Semen Collection


The cynomolgus monkey (Macaca fascicularis) is routinely used in preclinical toxicity testing. For certain drug indications it is important to have sexually mature animals on the studies. For males the presence of sperm in the ejaculate is one of the criteria for determination of sexual maturity. The intent of this study was to evaluate a reliable and repeatable method of semen collection. A total of 36 male cynomolgus monkeys, approximately 5 years of age or older and weighing 4.4 to 6.9 kg, were individually housed in stainless steel cages elevated off the floor, in HEPA-filtered rooms with a 12-hour light cycle. Semen was collected using a modified penile electroejaculation method (Mattison, et al., 2011). Semen collection was attempted in normal restraint chairs using minimal restraint. A gauze strip moistened in 0.9% saline was wrapped around the penis. Two electrodes from the stimulator were attached to the gauze. The stimulator settings were 10 to 30 pulses per second, and 1 to 3-ms duration. The voltage was varied from 0 to 50 V. The head of the penis was held inside an appropriate volume tube opening to catch the entire ejaculate, which was immediately transported to the laboratory for sperm count. The semen weight was also recorded. If no ejaculation occurred the animal was allowed to rest for at least 1 min and the stimulation was repeated. If no ejaculation occurred within 10 min, the animal was returned to his cage. Semen collection was attempted from the animal again with a minimum of 24-hour of rest between collection attempts. The success rate for semen collection was 86% at the first attempt and 100% within 2 days. The ejaculate parameters results obtained (weight 0.02 to 1.9 g and sperm count 2.7 x 107 to 5.8 x 108 ) were comparable to the literature (Niehoff, et al. 2010). The results demonstrated that this semen collection method may be used during the conduct of preclinical toxicity testing and the results can support the determination of the sexual maturity of the male non human primate.

2157 Collection of Semen Samples from Rabbits in Drug Disposition Studies

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The rabbit semen collection model is a useful tool for the investigation of drug distribution into semen. Rabbits are relatively low cost, easy to manipulate in this application, and provide adequate sample volumes for analysis. This study describes a relatively simple and convenient method for collecting semen from rabbits for quantification of drug levels in drug disposition studies. The method used in this study is a modification of the procedures reported by Naughton et al. (2003) and Brederman et al. (1964).

In two studies with separate groups of rabbits (acclimated to laboratory conditions for 1 to 3 weeks before study initiation), 10 of 11 rabbits on test produced semen samples over an 8-day sample collection period. Postdose sample weights ranged
from 0.111 to 0.541 grams (mean 0.320 ± 0.148 grams) in the first study, and from 0.220 to 1.54 grams (mean 0.483 ± 0.324 grams) in the second study. All sample weights met the minimum sample size criterion for analysis.

The results demonstrate that multiple intranasal dosing sessions to achieve high total daily intranasal dose volumes can be tolerated by rats and dogs, two commonly used test species, provided there is a suitable interval between sessions.

For laboratory animal species, the total amount of blood withdrawn depends upon the weight of the animal and the time between successive collections. Withdrawal of too much blood, without intravenous fluid replacement can result in hypovolemic shock. Withdrawal of smaller volumes at intervals too frequent to allow for blood cell replacement can result in anemia. In general, for rats, it is recommended to collect no more than 3 samples, at a volume less than 20% of the total blood volume over a 24-hour period. This approach necessitates combining data across animals for the purposes of toxicokinetic/pharmacokinetic analyses. In order to allow serial blood sampling from adult rodents, WIL Research has recently established the technique of packed cell rejections. While this method has been used in academia, to the best of our knowledge, it has not been used previously in regulatory toxicology. Adult female rats were surgically implanted with femoral vein catheters with SAF Quick Connect harnesses with injection caps and jacket adaptors. Catheter patency was maintained by use of a heparinized saline lock. Blood samples were collected at 6 timepoints over two 24-hour periods in one week. Between collection and reinsertion, catheters were flushed with saline and a heparin lock was maintained. Samples were centrifuged, plasma was removed and the cell pellet was resuspended in sterile saline to make a cell suspension, which was reintroduced back into the animal. Catheters were flushed with saline and a heparin lock was reapplied. Subsequently, catheter patency was monitored on a twice-weekly basis for 4 weeks to facilitate inclusion of additional sampling intervals. Thirteen (13) of 15 females survived the packed cell reinsertion procedures, with patent catheters. Over the course of 4 weeks, gradual reduction in percentage of patent catheters was noted, with >50% patent at termination. These newly established procedures allow us to collect up to 12 serial samples (0.4-0.5 mL/sample) during one week period.

Toxicology studies in suckling rats often include satellite animals (3/sex/timepoint) for toxicokinetic (TK) purposes where blood is collected (typically >200μL) as a terminal procedure. Blood microsampling using a tail vein, capillary tube method has been used for TK profiling of satellite pups in an oral gavage repeat dose toxicology study where 5x32μL blood samples were collected from Postnatal day (PND) 14 pups (weighing ca 34g) and again at end of study on PND35. There were no clinical observations in the sampled pups and their weight gain matched that of unsampled control pups. Use of microsampling at PND14 and PND35 reduced the number of satellite pups from 60 to only 6 pups. Numbers of animals could be reduced even further if (limited) TK sampling could be done in main study pups. However there is concern that this might affect critical toxicological endpoints. An experiment was performed to emulate studies that finish at the end of the suckling period. 3 x 32μL blood samples were taken from 10 pups/sex within a 24 hour period at PND19 (ca 40-43g) and the following were measured at termination on PND20: clinical pathology, spleen and liver weights plus histopathology. There was an equal sized unsampled concurrent control group. There were no in-life observations or histopathological differences but there was a slight (non statistically significant) increase in spleen weight. In the terminal blood samples, females showed a slight but statistically significant decrease in red blood cell count (to 0.94x of control) plus slight (non significant) decreases in haemoglobin and haematocrit. The males showed a slight (non significant) increase in reticulocyte counts (to 1.05x of control). However, all values were within the concurrent control range. It was concluded that the nature and severity of the effects of microsampling 96μL blood in a PND19 suckling rat does not preclude the microsampling of main test pups provided care is taken to match blood loss in the control and judgement is used in the interpretation of findings.
PS 2162 Quantified Electroencephalography (qEEG) in Safety Pharmacology


Quantified Electroencephalography (qEEG) is a highly relevant translational technique able to measure the global effects of drugs on brain electrical activity. The aim of this study was to investigate the effects of 4 drugs with well-known side-effects, scopolamine, clozapine, ketamine and amphetamine on qEEG in the conscious rat. Fourteen telemetered rats were implanted with two surface electrodes over the fronto-parietal cortical area. After post-surgical recovery, animals were administered with vehicle or a drug and EEG was recorded over 60 minutes, starting 15 or 30 minutes after systemic injection. The differences of the spectral power between vehicle and the different treatments were assessed for each frequency band.

Scopolamine (0.5 mg/kg) clearly increased theta and alpha frequencies over 60 minutes with similar effects on delta frequencies from 0 to 10 minutes. A significant increase of alpha and beta frequencies was observed with clozapine (2 mg/kg). Ketamine (30 mg/kg) globally increased total spectral power with marked effects on delta, beta and gamma frequencies. Amphetamine (4 mg/kg) tended to increase alpha frequencies but significantly decreased beta to gamma frequencies. The increase of slow waves (delta to theta) potentially relates to the amnesic effects reported for scopolamine. Hyperactivity induced by amphetamine or ketamine potentially relates to the increase of theta to beta frequencies. An increase of intermediate frequencies is characteristic of a sedative-like effect, as observed with clozapine on beta frequencies. These results suggest that qEEG is a sensitive and reliable translational tool to predict potential adverse effects induced by new compounds on the central nervous system.

PS 2163 Serotonin Reuptake Impairs Executive Function and Induces Submissive-Like Behavior in Mice: A Validation Study of a New Cognitive Test for Chemical Risk Assessment

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Environmental chemical exposure has been suspected to be responsible for a recent increase in the incidence of pervasive developmental disorders. To study the possible causal relationship, we recently developed a new behavioral test method, using an apparatus named IntelliCage, which can analyze the executive function and social behavior in group-housed mice in a highly reproducible manner (Endo et al., Behav. Brain Res., 2011; PLOS ONE, 2012). In the present study, we have validated the reliability of this method by applying it to an assessment of chemically-induced serotonin reuptake model mice as a widely accepted model of executive function and social behavioral impairments. C57BL/6j male mice were administered 4-chloro-6-phenylalanine (PCPA), a tryptophan hydroxylase inhibitor, at a daily dose of 0 or 25 mg/kg for 4 weeks using osmotic mini-pump. Throughout this period, the two groups of mice were housed together in an IntelliCage and subjected to the behavioral flexibility test that consists of acquisition of place learning and its serial reversals. As a result, the PCPA group was found to perform less well in both periods, the two groups of mice were housed together in an IntelliCage and subjected to the behavioral flexibility test that consists of acquisition of place learning and its serial reversals. As a result, the PCPA group was found to perform less well in both periods.

In conclusion, the present behavioral test was validated as a reliable method for assessing abnormality in higher brain function and social behavioral impairments. C57BL/6j male mice were administrated 4-chloro-6-phenylalanine (PCPA), a tryptophan hydroxylase inhibitor, at a daily dose of 0 or 25 mg/kg for 4 weeks using osmotic mini-pump. Throughout this period, the two groups of mice were housed together in an IntelliCage and subjected to the behavioral flexibility test that consists of acquisition of place learning and its serial reversals. As a result, the PCPA group was found to perform less well in both periods.

PS 2163a A Simple Method of Cerebrospinal Fluid Collection from Conscious Beagle Dogs and Its Application As a Surrogate for the Assessment of CNS Exposures

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Many central nervous system (CNS) drug discovery programs require the successful collection of cerebrospinal fluid (CSF) for assessing CNS penetration and distribution of new chemical entities. Evidence from preclinical and clinical studies suggests that drug concentration in CSF appears to be reasonably accurate in predicting unbound drug concentration in the brain. Therefore, CSF can be used as a useful surrogate for in vivo assessment of CNS exposure and provides an important basis for the selection of drug candidates for entry into development. For repeated collection of CSF from dog, either dog has to be surgically cannulated or anesthetized several times which may interfere the pharmacokinetic (PK) property of the drug of interest. The objective of the present investigation was to develop a systematic approach to simplify the technique for collecting CSF from cisterna magna of the conscious Beagle dog. Dogs is placed either in lateral recumbency with the head flexed ventrally to 90 degree or alternatively in standing position comfortably and bent the neck at 90 degree to the table with the help of an assistant. Neck should be flexed so that space of insertion can be visible. A 20 - 24 gauge needle is inserted into depression just behind the pointed occipital prominence; direction of needle should be first slight transverse then obliquely into cistern magna. A slight popping sensation is observed when needle in subarachnoid space. CSF will come out with pressure inside the space as well as by gravity into collection tube. Multiple sampling can be done by this method using same animals to determine PK parameters.

The same animal can be used again after appropriate wash out period. In our laboratory we have used this technique several times to determine the drug CSF concentrations successfully. This method can be used for CSF PK without use of anesthesia or any surgical intervention.

PS 2163b Phosgene-Induced Lung Injury in the Conscious Pig

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Currently no medical countermeasures exist for phosgene poisoning and treatment is supportive in an intensive care setting. Small animal models have been used to screen candidate therapies, however, extrapolation of therapeutic benefit to man requires verification in a larger animal model e.g. the pig. Therefore, a requirement to test the efficacy of treatments against a consistent, well characterised injury.

Animals were surgically implanted with a telemetry transmitter and exteriorised catheters to enable venous and arterial blood samples to be taken. After recovering from surgery (1 week) and baseline measurements (1 week), animals were exposed to either air or phosgene under anaesthesia, then recovered and monitored, fully conscious for 24 h.

Air exposed and phosgene exposed (Mean inhaled dose ± S.E.M., 0.793 ± 0.03 mg/kg) animals were successfully recovered and monitored to 24 h post-exposure. The phosgene exposed animals had a moderate/severe lung injury at 24 h compared to the air exposed animals.

Phosgene produced an increase in the amount of protein in the terminal broncho-alveolar lavage (BAL) fluid of the phosgene exposed animals (Mean ± S.E.M., 0.82 ± 0.3 mg/ml). The lung wet weight to body weight ratio (LWW:BW) and lung wet weight to dry weight ratio (LWW:DW) are measures of extravascular lung water and indicate the degree of alveolar permeability. There was an increase in the LWW:BW ration in the phosgene exposed animals when compared to the air exposed animals (Mean ± S.E.M., 16.16 ± 1.81 versus 8.01 ± 0.13). There was also an increase in the LWW:DW ration in all the lung lobes sampled in the phosgene exposed animals compared to the air exposed animals. Phosgene increased the number of neutrophils recruited to the lungs compared to the air exposed animals. The feasibility and reproducibility of the dose of phosgene producing a severe, but non-lethal lung injury at 24 h in which to test therapeutic candidates has been demonstrated.

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PS 2163c LC50 Determination of Sarin and Soman in Conscious Guinea Pigs (GP) Using a Nose-Only Vapor Inhalation Model


Sarin and soman are organophosphorus compounds that pose both a military and a civilian threat. Because of the volatility of these agents, inhalation is likely the primary route of exposure. The toxicology of these inhaled agents has been under-investigated to date. This study was conducted to evaluate the dose–relationship curve and establish the lethal concentration of 50% of the exposed population (LC50) for both sarin and soman in unanasthetized GP using a newly developed nose-only vapor exposure system. GP were placed in a double compartment Plexiglas chamber separated by rubber neck and rubber nose seals which was then mounted to a 12-port nose-only inhalation tower. Respiratory parameters were monitored with a pneumotach and transducer during the exposure. GP were exposed to sarin or soman vapor via a Brooks vapor-generating system for 20 min in the concentration range of 13.1 to 21.8, and 13.7 to 24.5 mg/m3, respectively. LC50 estimates (± 95% confidence intervals [CIs]) were determined using a stage-wise adaptive dose design analysis. The LC50 for sarin was calculated to be 14.3 mg/m3 (CI:14.0-
14.6) and 18.4 mg/m³ (CI:16.7-20.2) for soman. Acetylcholinesterase (AChE) activity was measured in whole blood (WB) and from red blood cells (RBC) immediately following death. AChE activity was greatly depressed in both RBC and WB with maximal levels of activity being 15.5% and 10.5% of baseline, respectively, and means of 6.39% and 5.53% of baseline, respectively. This model provides a precise, accurate and realistic nerve agent vapor inhalation exposure system that can be used to test potential therapeutic compounds against these volatile nerve agents. The views expressed in this poster are those of the author(s) and do not reflect the official policy of the DoD, DOD, or the U.S. Government. The experimental protocol was approved by the USAMRICD IACUC and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

2164 The Endogenous Arylhydrocarbon Receptor Ligand 6-Formylindolo[3,2-b]Carbazole (FICZ) Is a Phototoxic UVA-Sensitizer in Epidermal Keratinocytes and Reconstructed Human Skin


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6-Formylindolo[3,2-b]carbazole (FICZ) is a physiological high affinity ligand for the mammalian aryl hydrocarbon receptor (AhR) regulating expression of AhR target genes including cytochrome P450 1A1 (CYP1A1). Cutaneous formation of FICZ occurs through ultraviolet (UV)-driven photo-oxidation of its precursor L-tryptophan, and FICZ is thought to potentiate the UVB-induced stress response in human skin through AhR activation. Here we demonstrate for the first time that FICZ displays nanomolar sensitivity as a phototoxic UVA-sensitizer targeting human skin cells through photodynamic induction of oxidative and prototoxic stress. In human HaCaT keratinocytes, induction of apoptosis was observed in response to the combined action of solar simulated UVA (3,3 J/cm²) and FICZ (10 nM), but not in HaCaT keratinocytes in the absence of UVA or FICZ only. FICZ-toxicity was also observed in an epidermal tissue reconstruct (Epiderm®) exposed to FICZ and UVA, as evident from sunburn cell formation and proteolytic activation of caspase 3. Gene expression array analysis revealed that FICZ caused upregulation of CYP1A1 independent of UVA exposure. In contrast, induction of cellular heat shock (HSP70, HSP90), ER stress (DDIT3), and oxidative stress (TXNRD1, HMOX1, ARR82, SPI1R) response gene expression was observed solely upon combined exposure to UVA and FICZ, further substantiated by immunoblot detection (p-eIF2α, p-p38, HO-1). FICZ photosensitization was associated with induction of pronounced intracellular photosensitizing stress that could be antagonized by single oxygen quenchers (sodium azide, DABCO). Taken together, our data demonstrate that the endogenous AhR ligand FICZ displays pronounced photodynamic activity in epidermal keratinocytes and reconstructed human skin, representing a novel mechanism of UVA photoactivity that may be operative in human skin. Supported by grants from NIH (R01CA122484, R03CA167580, R21CA166926, ES007091, ES06694).

2165 Linalool and Limonene in Fragranced Products: Stability and Quantification of Potentially Sensitizing Hydroperoxides

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Upon exposure to air, linalool and limonene can form sensitizing hydroperoxides. Positive patch tests to the hydroperoxides in dermatitis patients were reported. Due to a lack of analytical evidence it is unclear under which conditions, and from which sources, the public might be exposed to sufficient quantities of these oxidized products for induction of skin sensitization to occur. We developed analytical methods using mass spectrometry in combination with gas chromatography (GC-MS) and liquid chromatography (LC-MS) for the oxidation products. We performed stability studies to follow the fate of linalool and limonene in fine fragrances and antiperspirants, since these product types lead to highest consumer exposure to these ingredients. We found a high stability of linalool and limonene in the products investigated. We detected very low levels (around 0.07%) of linalool hydroperoxide in linalool obtained from natural sources. This level remained constant upon storage. Low levels of linalool hydroperoxide were detected in a commercial fragrance but degraded at elevated temperature to non-sensitizing secondary oxidation products. Aged fine fragrances retrieved from consumers contained a median of 19.43 mg/l of linalool and 7 μg/g of linalool hydroperoxide. Corrected for matrix effects, concentrations are twofold higher. In antiperspirants, we detected no oxidized products. A novel method for linalool hydroperoxide detection is presented, but limonene hydroperoxide levels stay below limit of detection of this method (≤ 0.5 µg/g) in tested products. In conclusion, low levels of linalool and limonene hydroperoxide in fragranced products may originate from raw materials, but we found no evidence for hydroperoxide formation during storage of representative perfume-containing consumer products. The typical concentrations found in the fragrance samples are around three orders of magnitude below the experimental concentration inducing skin sensitization in animal tests.

2166 Vetiveryl Acetate Evaluated for Potential Phototoxicity Utilizing a Human Epidermal Model and Patch Testing


Vetiveryl acetate is utilized as a fragrance ingredient in a variety of consumer and personal care products, being appreciated for its woody note in perfumery. The UV spectrum for this material demonstrates absorption in the region of 290 – 700nm, and therefore has the potential to be photactivated. In order to determine the potential for phototoxic effects, a phototoxicity test in a three dimensional human epidermal model (EST1000) was conducted. This assay is based upon a comparison of the cytotoxic effects of the test material following exposure to a non-toxic dose of UV light. Cytotoxicity is expressed as a reduction in the mitochondrial conversion of MTT [(3-4,5-dimethyl thiazole 2-yl) 2,5-diphenyl-tetrazoliumbromide] to formazan. A material is considered to have a phototoxic potential if one or more concentrations +UVA (60 min with 6 J/cm²) results in a decrease in relative cell viability of ≥ 30%, when compared to the identical concentration –UVA. Vetiveryl acetate was tested at five concentrations ranging from 0.1 to 10% (v/v) in 1:3 Ethanol:Diestyl phthalate (EstOH:DEP). In the EST1000 model, no phototoxicity was observed to vetiveryl acetate over the range of concentrations tested. Additionally, historical studies conducted in animal models indicate a lack of phototoxicity up to 100%. A confirmatory phototoxicity patch test was conducted in a group of human volunteers with a concentration series of 2.5%, 7.5% and 25%. These concentrations of vetiveryl acetate were confirmed as having no phototoxic effects in humans following the phototoxicity patch test.

2167 In Vivo Phototoxicity Evaluation in Hairless Mice followed by 8-Methoxypsoralen, Lomeloxacin, and Ofloxacin


Purpose: The ICH S10 draft consensus guideline “Phototoxicity Evaluations of Pharmaceuticals” is in the Step 3 consultation period in Sept 2013. However, this draft guideline does not currently specify species/strain selection or test procedures for in vivo phototoxicity studies. Therefore, in order to select a suitable positive control compound and animal strain, we examined in vivo phototoxicity in 2 strains of hairless mice after administration of 3 known phototoxicants, 8-methoxypsoralen (8-MOP), lomeloxacin HCl (LMFX), and ofloxacin (OFLX). Methods: Seven-week-old HR-1 and SKH-1 hairless mice (Hoshino Laboratory Animals, Inc., Japan, and Charles River, NC, respectively) were orally given 8-MOP (10 and 20 mg/kg), LMFX (50 and 100 mg/kg), and OFLX (300 and 1000 mg/kg), and irradiated with ultraviolet A at 20 J/cm² (≥ 352 nm) from a Dermaray M-DMR-50 (Tokyo Electric Co., Ltd., Japan) apparatus 30 min later. The expression of phototoxicity was evaluated in each strain with the following items: auricle thickness, skin reactions at the auricle and on the back with the Draize method at 0.5, 24, 48 and 72 hr after irradiation, and ophthalmology and histopathologic changes in the auricular and back skins, eyeballs, and optic nerves (HE staining) at 72 hr. Result: Male HR-1 mice given 8-MOP showed very slight erythema and edema at the auricle and back at 24 hr and an increased edema score (3; moderate) at 72 hr, auricle thickening and eyelid edema. Histopathology revealed inflammation, degeneration and necrosis in the skin and eyelids. These reactions were also observed, but to a lesser extent, after other phototoxicant treatments (order of severity: 8-MOP > LMFX > OFLX). No marked sex or strain (HR-1 vs. SKH-1-E) differences were noted. Discussion: We judged that both investigated strains were appropriate, and 8-MOP was the most appropriate positive compound, for phototoxicity tests in hairless mice.
2168 Development of an Alternative Method for Assessment of Skin Sensitization Using the In Vitro IL-18 Reconstructed Human Epidermis (RHE) Model


Several efforts are underway, in line with the 3Rs philosophy, to develop and validate in vitro testing methods for sensitization, as alternatives to the traditional in vivo models. The first living cells of the skin to encounter sensitizing chemicals are mainly keratinocytes. Of the cytokines produced by keratinocytes, IL-18 plays a key role in skin sensitization induction. The use of keratinocytes and skin cultures provides a valuable, simplified in vitro model to evaluate the sensitization potential of topical applied compounds. An IL-18 assay has been found to have advantages of decreased variability, better representation of human exposure and the ability to test water insoluble/undissolveable compounds as well as final product formulations. The IL-18 RHE protocol was adapted using EpidermTM and three known contact sensitizers (resorcinol, weak; cinnamaldehyde, moderate; 2,4-dimethylthiourea, strong). Chemical dilutions were made and applied topically to the RHEs for 24 hours. Cell viability (MTT) and IL-18 levels (ELISA) were used as measures of sensitization. Criteria were established as an IL-18 Stimulation Index (SI) value >1.6x with a cell viability between 5%-50% for at least one concentration. Results indicated the test was simple to perform with slight modifications for the type of cultures used. Reproducible results were obtained for the moderate and strong sensitizers with varying results for the weak sensitizer, resorcinol. The variability in results may stem from differences in stratum corneum barriers between types of RHE cultures. In conclusion, IL-18 production by RHE represents a promising and easily adaptable model for the screening of chemicals for sensitization potential and reducing the use of animals for testing.

2170 Differential Induction of DUOX Isosforms by Arsenite and Vanadate: A Novel Role for Reactive Oxygen?

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Dual oxygenase (DUOX) 1 and 2 are membrane bound proteins of the NOX superfamily that have been linked to specific inflammatory or cellular repair functions. Our previous work has demonstrated selective inductionswitching of DUOX isosforms by the cytokines IFN-γ and IL-4 in cultured human keratinocytes and bronchial epithelium. Similarities between the biochemistry of keratinocyte differentiation and DUOX regulation suggest DUOX perturbation could be a source of epidermal barrier defects that enhance toxicant penetration. Arsenic toxicity is characterized by ROS generation and is reported to involve NOX activity. Water pollutants from mining activity that contain metal ions such as vanadate also generate ROS at high concentration. We examined the effects of these known water contaminants on DUOX induction using an established exposure model for heavy metals in spontaneously immortalized human keratinocyte (SK) cultures and real time QPCR. We find that most metal/metalloid compounds suppress the cytokine induction of both DUOX isosforms. However, arsenite exposure causes incomplete suppression of DUOX2, while vanadate amplifies DUOX2 induction by IFN-γ beyond its normal efficacy. DUOX2 inductions by IFN-γ, as well as its amplification by vanadate, are completely suppressed in the presence of 30 mM dimethylthiourea, a known ROS scavenger. Similarly, glutathione depletion using 0.5 mM buthionine sulfoxime replicated the increased efficacy of DUOX2 induction by IFN-γ seen with vanadate. These findings suggest a novel role for ROS in DUOX2 gene induction and provide additional insight into arsenic toxicity as well as the mechanistic contribution of DUOX biology to differentiating epithelium. Future studies on the role of DUOX in keratinocytes may provide a novel mechanistic approach to assess the effects of environmental and industrial toxicants on healthy skin and wound healing.

2171 Involvement of Reactive Oxygen Species (ROS) and Gene Expression Profile in Response to p-Phenylenediamine and Components of Permanent Hair Dyes in Human Keratinocytes (HaCaT) Cells and Human Skin Explants

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Para-phenylenediamine (PPD) is an amine amine present in about 70% of all hair dyes worldwide. PPD is primarily used as a dye intermediate after reaction with H2O2 (oxidizing agent) and Resorcinol(R) (coupler). It penetrates the hair shaft permanently modifying the hair colour. Sensitization to PPD is known to cause of allergic contact dermatitis. However, the molecular mechanism of PPD-induced skin toxicity remains unclear. We investigated the induction of Reactive Oxygen Species (ROS) and the involvement of gene expression of certain genes that appear to be involved in toxicity of PPD. We tested the ROS induction potential of PPD in presence and absence of the coupler Resorcinol and oxidizing agent H2O2 in monolayer using immortalized keratinocytes (HaCaT) that are the first contact point after dermal exposure, as well as human skin explants. For HaCaT cells in monolayer, the dose of 100μg/mL increased the intracellular levels of ROS after 1 hour of incubation. After 24 hours of incubation the dose of 20 μg/mL was able to increase the levels of 8-oxo-4G and malonaldehyde (MDA) was formed. Besides, we observed that the addition of H2O2 decreased the intracellular levels of ROS and the levels of 8-oxo-dG, while the levels of MDA were not decreased by the addition of H2O2. Using the human skin explants, we demonstrate that the exposure to PPD, PPD-H2O2-R and PPD-R on commercial concentration, upregulates the expression of genes involved in inflammatory responses, responses to oxidative stress and metabolism of xenobiotics by cytochrome P450 on human epidermis. Our results indicate that oxidative stress is involved in the mechanism of PPD-epidermis toxicity in monolayer and in skin explants. This study helps to elucidate the toxic effects in response to PPD and components of hair dyes on human skin.

2172 Dermal Absorption In Vitro and Its Influencing Parameters

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Skin absorption is an in vitro method described in OECD guideline 428 and the technical guidance GD 28. Since study design dependent parameters can be varied, we performed systematic parameter variations under defined experimental conditions to understand their potential influence.
We investigated dermal absorption with skin preparations of rats, pigs and humans in Franz like diffusion cells. Experiments were performed to address the following parameters: ex vivo skin preparations (gender, age, localization, preparation), reconstructed skin models, storage and freezing-cycles, static versus flow through, concentration of test compounds, donor vehicle and receptor fluids. We used different model compounds, e.g. testosterone, caffeine which were analyzed by liquid scintillation counting, HPLC or photometry. Absorbed dose (%), permeation rates and permeability constants (Kp) were calculated.

Variations in skin preparation and testing in static or in flow-through systems had no remarkable influence on Kp. Main impacts on Kp were observed for ex vivo vs. reconstructed skin preparations, thickness of SC, test-substance concentration, donor vehicle and receptor fluid: In our experiments the barrier function of reconstructed skin was inappropriate for absorption testing. Since the test substance, its concentration and donor vehicle are reflected by the applied formulated product these parameters have to parallel the real exposure scenario and will intentionally predetermine the study results. The influential effect of the receptor fluid was related to the solubility of the test substance in the chosen solvents. This relation was obvious e.g. for the lipophilic compound testosterone where a higher Kp and absorbed dose was observed for ethanol-water (1:1 / v/v) in comparison to 5% aqueous BSA.

Taken together, the results of the current investigations can be taken into account for the establishment of a standardized experimental set up for dermal absorption studies in vitro, whereby the identified major influential parameters should be carefully addressed.

**Evaluation of Age, Storage, and Skin Preparation Technique on Dermal Absorption in Rat Skin**


Dermal uptake is a common route of exposure for chemicals and can have a substantial impact on exposure and risk assessments. Accurate in vitro dermal penetration data from rat skin is critical for triple pack studies where these data are used in conjunction with in vitro human and in vivo rat results to correctly extrapolate to human in vivo absorption levels. The present study evaluated the quantitative impact of age, storage and skin preparation technique on dermal absorption of three test chemicals (benzoic acid, caffeine and testosterone) in rat skins. Juvenile (4-5 wk old) rat skin was compared to mature adult rat skin (12-15 wk old). The data indicated that flux and absorption were consistently increased by approximately two-fold in juvenile rats compared to adult rats with all 3 test materials. Storage of rat skins at -80°C did not significantly affect flux or absorption values, compared to fresh skin samples, however, there was an overall 30% decrease in the number of viable skins when frozen, compared to fresh skins. The extent of hair clipping for preparation of the skin samples is also critical in dermal absorption experiments. Data indicated that extensive clipping (7-8 passes), to remove most of the epidermal hair, resulted in 2-fold higher flux rates, with 87% of skin samples failing integrity tests. In contrast integrity was maintained in 100% of the skin samples that were subject to moderate clipping (2-3 passes). Flux and absorption were increased approximately 2-3 fold in skin samples subject to more extensive clipping compared to those treated more moderately with all the 3 test materials. Collectively, the data suggest that age of rats and clipping technique substantially affect the rate of dermal absorption during in vitro dermal experiments, while prolonged storage may lead to material wastage due to loss of integrity in skin samples. As a result, appropriate experimental design is critical for an accurate assessment of this major exposure route.

**In Vitro Human Skin Permeation: Example of CMI/MI and Tolylfluanid, Two Irritant, and Sensitizer Biocides**

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Every year, ten million workers are exposed to metalworking fluids (MWFs) which may be toxic, especially to the respiratory system and the skin. There are four types of MWFs: neat oils and three water-based MWFs (soluble oil, semi-synthetic and synthetic) which are diluted with water and whose composition varies according to the mineral oils ratio. MWFs also contain various additives such as biocides and corrosion inhibitors. The absorption of six amines used as corrosion inhibitors and biocides in MWF was determined in porcine-skin flow-through diffusion cell experiments using three radiolabeled ethanolamines (MEA, di- and tri-ethanolamine, MEA, DEA and TEA respectively) and a mixture of three GC-amenable amines (dibuty lethanolamine, dicyclohexylamine and diphenylamine). The test compounds were dosed in four vehicles (water and three generic water-based MWF formulations) and analyzed using a scintillation counter or a gas chromatography/mass spectrometer. The six compounds were significantly (p<0.05) more absorbed in water (e.g. 1.15±0.29 %dose (DEA in Water)) compared to other formulations (e.g. 0.13±0.01 %dose (DEA in semi-synthetic MWF)) and absorption was greatest for dibutylethanolamine in all the formulations. Generic soluble oil formulation tended to increase the permeability and the absorption of MEA, DEA and TEA. Permeability coefficients were significantly higher (p<0.05) with TEA relative to the other test compounds (e.g. 4.22±0.45±3.0±0.5 cm/h (TEA in synthetic MWF) VS 1.23±0.4±1.0±0.5 cm/h (MEA in synthetic MWF)), except for MEA in generic soluble oil formulation. Future research will confirm these findings with in vivo studies in pigs and dermatotoxicity studies will be conducted to compare the relative safety of these compounds in MWF. With this data, the MWF industry would be able to alter their MWF formulation to protect the metalworkers’ health.

**The Effect of Coformulants on Dermal Uptake—A Raman Study**

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The Organisation for Economic Co-operation and Development (OECD) guidelines on the conduct of in vitro dermal absorption investigations have attempted to standardise global practices. However, the methods used to evaluate epidermal accumulation such as ‘tape stripping’ have yet to be formalised and can give rise to large sources of variability. Thus, there is a strong need to develop alternative methods to investigate compound localisation within skin samples. Confocal Raman spectroscopy (CRS) is a radiolabelfree, rapid, optical, non-invasive method which allows compounds to be identified within dermal tissue. In this study, CRS was used to determine testosterone localisation with ex vivo 300 μm dermatropped pig skin samples treated with the compound in solutions (1:1 w/v) containing either; Emulsogen EL360, propylene glycol, or Tween 20. A LabRAM HR (Horiba Scientific) imaging spectrometer was employed for the analysis of the treated skin samples (n=6), using an IR laser at 785 nm coupled with a Synapse CCD detector. Spectra were obtained from the surface of the skin to a maximum depth of 150 μm at 0, 1, 2, 3, 4, 5, 6 and 24 hour time points to mimic real-time exposure. CRS spectra for the pig skin samples demonstrated good reproducibility with some spectral differences noticed. Spectral intensity decreased with increasing depth, as expected. Emulsogen EL360, Tween 20 and propylene glycol all appeared to influence the absorption of 30.0 mg/mL testosterone through the skin. The spectra obtained also suggested that the stratum corneum was the main barrier to testosterone absorption. These findings show that the CRS method has the potential to determine epidermal compound accumulation, which will help to inform the likely systemic bioavailability of residues within skin, providing an alternative tool for standardised dermal risk assessment.

**Skin Absorption of Performance Amines Used in Metalworking Fluids**

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We investigated dermal absorption with skin preparations of rats, pigs and humans in Franz like diffusion cells. Experiments were performed to address the following parameters: ex vivo skin preparations (gender, age, localization, preparation), reconstructed skin models, storage and freezing-cycles, static versus flow through, concentration of test compounds, donor vehicle and receptor fluids. We used different model compounds, e.g. testosterone, caffeine which were analyzed by liquid scintillation counting, HPLC or photometry. Absorbed dose (%), permeation rates and permeability constants (Kp) were calculated. We investigated dermal absorption with skin preparations of rats, pigs and humans in Franz like diffusion cells. Experiments were performed to address the following parameters: ex vivo skin preparations (gender, age, localization, preparation), reconstructed skin models, storage and freezing-cycles, static versus flow through, concentration of test compounds, donor vehicle and receptor fluids. We used different model compounds, e.g. testosterone, caffeine which were analyzed by liquid scintillation counting, HPLC or photometry. Absorbed dose (%), permeation rates and permeability constants (Kp) were calculated. We investigated dermal absorption with skin preparations of rats, pigs and humans in Franz like diffusion cells. Experiments were performed to address the following parameters: ex vivo skin preparations (gender, age, localization, preparation), reconstructed skin models, storage and freezing-cycles, static versus flow through, concentration of test compounds, donor vehicle and receptor fluids. We used different model compounds, e.g. testosterone, caffeine which were analyzed by liquid scintillation counting, HPLC or photometry. Absorbed dose (%), permeation rates and permeability constants (Kp) were calculated.
Skin irritation and skin corrosion refer to localized toxic effects resulting from a topical exposure of the skin to a substance. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) define skin irritation as "the production of reversible damages" and skin corrosion as "irreversible damage" to the skin following the application of a substance. A number of validated, non-animal methods to determine skin corrosion and irritation are available. As such, the SkinEthic Reconstructed Human Epidermis (RHE) test method has been adopted within the context of OECD TG 431 (skin corrosion) and OECD TG 439 (skin irritation).

Updated approaches to the use of tiered and integrated testing strategies for predicting skin corrosion/irritation potential without the use of animals are discussed in the present study. Illustration is provided by combining both SkinEthic RHE skin corrosion and irritation test methods for evaluating the stepwise testing strategy (Top down and Bottom-Up approaches).

All 50 reference chemicals listed in either OECD TG431 or 439 were evaluated in both skin irritation and corrosion SkinEthic RHE validated methods. Amongst the 22 known in vivo corrosive chemicals, 95% were correctly predicted amongst the 35 in vivo non irritants, 85% might be correctly classified in vitro as non corrosives. Therefore those 21 chemicals were also defined as irritants. The substances were well classified by using the top-down approach which consists of conducting primarily skin corrosion test method.

Considering the non irritant chemicals, 100% (10/10) of the correctly classified substances were well classified by using the top-down approach which consists of conducting primarily skin corrosion and irritation test methods for evaluating the stepwise testing strategy (Top down and Bottom-Up approaches).

The OECD adopted a tiered approach for dermal testing described in the revised TG 404. Data from structurally related chemicals (e.g. pH extremes), human data and experience (weight of evidence) are sometimes sufficient for classifying the skin irritation/corrosion potential of chemicals. If this information is not sufficient, validated in vitro methods can then be used e.g. in vitro EpiSkin methods accepted in both OECD TG 431 (skin corrosion) and TG439 (skin irritation).

The aim of the study is to present the Top-Down and Bottom-Up approaches to predicting skin corrosion/irritation potential without the use of animals. For such purpose, 98 chemicals (partially from EURL-ECVAM validation studies and OECD TG431 & TG439) were evaluated in both skin corrosion and irritation EpiSkin methods.

Amongst the 35 in vivo non irritants, 85% might be correctly classified in vitro as irritants (I). Therefore those 35 chemicals were also defined as non corrosives (NC). Thus, when applying the bottom-up approach the GHS classification of those substances was accurately defined by performing the skin irritation evaluation.

In conclusion, the results suggest that testing strategy is not a strict sequence and that stepwise procedure, weight of evidence and testing should be considered as acceptable approaches to structure relevant information of the substance used for hazard assessment.

**2178 In Vitro Skin Corrosion and Irritation Assessment of Ingredients Using EpiSkin Model: Top-Down and Bottom-Up Approaches**


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In conclusion, the results suggest that testing strategy is not a strict sequence and that stepwise procedure, weight of evidence and testing should be considered as acceptable approaches to structure relevant information of the substance used for hazard assessment.

**2179 The Usefulness of the Validated SkinEthic RHE Method to Identify Skin Corrosive UN GHS Subcategories**


The SkinEthic Reconstructed Human Epidermis (RHE) test method has been adopted within the context of OECD TG 431 for distinguishing corrosives and non-corrosives. The EU CLP classification system requires subcategorising of corrosive chemicals into the three UN GHS subcategories 1A, 1B and 1C. Since the SkinEthic RHE method was originally validated to discriminate corrosives from non-corrosives, the present study was undertaken to investigate its usefulness to discriminate skin corrosive UN GHS subcategories.

In total 84 substances were tested in three independent runs and two prediction models (PM) were assessed, representing a pre-defined validated prediction model (PM-A) and an alternative one defined post-hoc (PM-B). The results obtained with both PM were reproducible, as shown by the > 92.9% concordance of classification between runs for discriminating corrosives versus non-corrosives, and the > 85% concordance for discriminating the GHS subcategories versus non-corrosives. Moreover these results confirmed a high sensitivity of the SkinEthic™ RHE method to predict corrosives (94.9%) and good specificity (> 73.7%) independent of the PM applied.

Regarding the identification of UN GHS corrosive subcategories, when considering the 30 reference chemicals as recommended in the recently revised OECD TG 431 (2013), PM-A and PM-B achieved 78.9% and 83.3% accuracy respectively for the identification of GHS subcategories and non-corrosives. They correctly predicted 90% of GHS subcategory 1A and 80% of GHS non-corrosive substances independent of the PM used.

In conclusion, the SkinEthic RHE test method is highly reproducible and sensitive for discriminating corrosive from non-corrosive substances. Furthermore it allows reliable identification of skin corrosive GHS subcategory 1B-and-1C substances using the PM-A and PM-B, and of GHS subcategories 1A using the PM-B. Due to its high sensitivity, the test method provides high safety standards for skin corrosion testing.
Solar ultraviolet (UV) radiation is known to have deleterious effects on human skin. The UVB (280-320 nm) and UVA (320-400 nm) spectrum of solar radiation have been shown to affect keratinocytes, the major cellular constituent of the epidermis, by causing direct DNA damage and/or indirect DNA damage and cytotoxicity through the formation of reactive oxygen species. In the present study, a commercially available human skin equivalent (HSE) (Epiderm-FITM) and excised human skin was exposed to solar-simulated light to gain insight into the temporal UV-induced response of human epidermal tissue. In vitro HSEs were irradiated with a single UVR dose approximately equivalent to either 6 or 9.5 minimal erythema doses (MEDs) for an individual of EPA skin phototype 2. Cutaneous damage and recovery were then monitored for a period of seven days. Histological analysis showed a dose dependent formation of apoptotic sunburn cells in epidermal KCs at 24 hrs post-irradiation. By day 3 post-irradiation, a thinning of the viable epidermal cell layers was evident with maximum epidermal degeneration observed at day 4. Resumption of epidermal proliferation and differentiation was evident in both 6 and 9.5 MED tissues by days 5-7, leading to regeneration of viable epidermal layers. Excised human skin tissues irradiated with the same UV doses displayed responses very similar to those observed in the in vitro HSEs. DNA damage indicated by cyclooxygenid e dimer (CPD) formation was assessed by immunohistochemistry. CPD positive basal KCs decreased steadily in number each day, and were almost completely undetectable seven days post-irradiation. CPD formation could be completely blocked through topical application of OTC sunscreens. Finally, elevated levels of IL-8 and MMP-1 were induced following UV-irradiation demonstrating an early inflammatory response followed by an extended period of matrix remodeling activity. These results demonstrate that HSEs are useful for UV-induced photocarcinogenesis studies and evaluation of sunscreens.

The aim of this study was to develop and test different skin toxicity Modes of Action (MOA). MOA describes the key events and processes starting with interaction of an agent with the cell leading to different biological response. MOA can be looked at as a representation of existing knowledge concerning the linkage(s) between initial chemical binding, defined as the molecular initiating event (MIE), intermediate events on cell, tissue and organ level and biological outcome. MOA can be used to identify key events for which non-animal tests can be developed, thereby facilitating mechanism-based, predictive toxicological assessments with low uncertainty and high human relevance.

We focused on 5 well studied skin toxicants and based on the present knowledge, created their biological network. In addition we created a computational model of biological pathways describing cellular processes activated by these chemicals by manually annotating and processing molecular information from the literature from the public domain (PubMed articles and FDA reports) and made the data computable. Moreover, we created bioinformatics analysis tools for assessing chemical structure similarity, allowing us to group the compounds based on their chemical structure in addition to their MOA. We tested this database for other known skin toxicants that are not covered by the database. Based on their structure similarity to other skin toxicant and/or known biological interaction partners we were able to predict skin toxicity of these compounds and suggest possible MOA.

Retention exposure to a chemical agent can induce an immune reaction in susceptible individuals leading to skin sensitization. We have developed computational models capable of accurately assessing the skin sensitization potential of environmental chemicals. To this end, we have (i) compiled, curated, and integrated the largest publicly-available database of skin-sensitizing chemicals; (ii) used this data to generate and validate QSAR models for skin sensitization; and (iii) employed these models to identify putative sensitizers among chemicals in the Scorecard and Tox21 databases. A random forest method was employed for QSAR modeling of compounds characterized by SiRMS and Dragon descriptors, and the OECD-Tox21 databases. The aim of this study was to develop and test different skin toxicity Modes of Action (MOA). MOA describes the key events and processes starting with interaction of an agent with the cell leading to different biological response. MOA can be looked at as a representation of existing knowledge concerning the linkage(s) between initial chemical binding, defined as the molecular initiating event (MIE), intermediate events on cell, tissue and organ level and biological outcome. MOA can be used to identify key events for which non-animal tests can be developed, thereby facilitating mechanism-based, predictive toxicological assessments with low uncertainty and high human relevance.

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Putative skin sensitizers in the ScoreCard and Tox21 databases as primary hits for structural optimization of chemicals of interest. Using these models, we have identified putative sensitizers with 94% and 71% certainty, respectively. Statistically significant accuracy of 68-88% when evaluated on several external validation sets. When compared to the OECD QSR toolbox skin sensitization module, our models afforded significantly higher Positive and Predictive Rates. When applied to chemicals within the applicability domains, however, the models could reliably identify positive and negative sensitizers with 94% and 71% certainty, respectively. Statistically significant descriptors from high-accuracy models yielded SAR rules that could guide structural optimization of chemicals of interest. Using these models, we have identified putative skin sensitizers in the ScoreCard and Tox21 databases as primary hits for

**References**


Cyclophosphamide (CPA) is used to treat cancer in children and women. Cyclophosphamide mustard (PM), the ovotoxic metabolite of CPA, destroys rapidly dividing cells by forming NOR-G-OH, NOR-G, and N-O-G adducts with DNA, which can ultimately lead to DNA damage. DNA repair processes are constantly active and cell death can occur when normal repair processes fail. Previous studies have shown that PM induces DNA damage in rat oocytes. This study was designed to investigate PM-induced DNA adduct formation and induction of the DNA repair response using rat spontaneously immortalized granulosa cells (SIGC). Cells were treated with vehicle control (1% DMSO) or PM (3 or 6 μM) for 24 or 48 hrs. Cell viability was reduced (P < 0.05) after 48 hrs in cells treated with 3 or 6 μM PM. The NOR-G-OH DNA adduct was detected by treatment with 6 μM PM after 24 hrs (8%±0.15 relative abundance) compared to control (1.0±0.1). The NOR-G-OH DNA adduct was formed after 48 hrs by both 3 (4.5±0.1) and 6 μM PM (4.5±0.15) compared to control (2.0±0.15) treatments. Phosphorylated H2AX (γH2AX), a marker of DNA double-stranded break occurrence, was increased by exposure to 6 μM PM after 24 hrs. After 24 hrs of PM exposure, 3 μM PM decreased mRNA expression of the DNA repair gene Atm (0.6±0.09 p<0.01), Parp1 (0.7±fold ±0.01), Brcal (0.5±fold ±0.07), Rad51 (0.9±fold ±0.04) Xcc6 (0.7±fold ±0.06) and Pkdc (0.6±fold ±0.07) while, in contrast, mRNA levels of Atm (1.2±fold ±0.5), Parp1 (1.16±fold ±0.03) and Brcal (1.0±fold ±0.3, Rad51 (2.3±fold ±0.7). Xcc6 (1-fold ±0.3) and Pkdc (1.1±fold ±0.1) were increased (P < 0.05) by 6 μM PM compared to control. After 48 hrs, both PM concentrations increased (P < 0.05) Atm (3 μM: 2.1±fold ±0.6, 6 μM: 1.3±fold ±0.3) and Parp1 (3 μM: 4±fold ±0.8; 6 μM: 1.2-fold ±0.4) mRNA levels relative to control-treated cells. These data support that PM induces DNA adduct formation in ovarian granulosa cells causing DNA damage and eliciting the ovarian DNA repair response in a dose- and time-dependent manner (Supported by ES016818 to AFK).

Genistein is an isoflavone phytoestrogen found naturally in a variety of plant structures, including soybeans, lentils, sunflower seeds, and chickpeas. However, the majority of human genistein exposure originates from soy-based dietary products such as soy milk and soy protein. Phytoestrogens have the potential to mimic, enhance, or impair the components of the estradiol biosynthesis pathway, which could alter follicle growth. Though some studies have inconsistently indicated that genistein affects granulosa cell proliferation and hormone production, no studies could alter follicle growth. Though some studies have inconsistently indicated that genistein affects granulosa cell proliferation and hormone production, no studies could alter follicle growth. Though some studies have inconsistently indicated that genistein affects granulosa cell proliferation and hormone production, no studies could alter follicle growth.
to PM is largely unknown, thus this study investigated whether PM might impact ovarian energy production by evaluating potential changes in glucose metabolism gene expression. Postnatal day 4 (PND 4) F344 rat ovaries were cultured in media and treated on alternate days with vehicle control (DMSSO; CT) or PM (60 μM). Following 2 or 4 days (d) of culture, RNA was isolated and a drug response RT2 Profiler PCR array was performed. Relative to CT treated ovaries there was no impact of PM exposure on mRNA levels of glucose metabolism genes investigated after 2 d. However, following 4d of PM exposure, mRNA levels of three glycolytic genes decreased (P < 0.05): glucose-6-phosphate isomerase (Gpi; 29% decrease), hexokinase-2 (Hk2; 57% decrease) and lactate dehydrogenase A (Ldh, 39% decrease). Hk2 and Gpi are the first two enzymes of glycolysis, while Ldh catalyzes the final step. The mRNA level of aryl hydrocarbon receptor nuclear translocator (Ahr/Hif1α), a transcription factor regulating glycolytic gene expression, was also decreased (P < 0.05; 25% decrease) at 4d and unchanged at 2d, compared to CT. These results suggest that glycolysis may be decreased or slowed by PM, reducing the ovarian capacity to convert glucose to pyruvate, which could potentially be involved in PM-induced ovotoxicity. (Supported by ES016818 to AFK).

2189 Temporal Impact of Obesity on Expression of Genes Encoding Enzymes Involved in Ovarian Xenobiotic Biotransformation

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Obesity is associated with type 2 diabetes, compromised reproductive health and birth defects in offspring. Obesity is also allied with elevated blood glucose and insulin levels, altering insulin’s action on various organs including the ovary. In extra-ovarian tissues, insulin regulates xenobiotic biotransformation enzymes, potentially through phosphatidylinositol 3-kinase (PI3K) signaling. Xenobiotic chemicals can target the ovary and destroy the primordial follicles as well as other follicle types, leading to premature ovarian failure, infertility and other health impairments. Despite strong association between obesity and elevated plasma insulin, whether obesity influences ovarian xenobiotic biotransformation remains poorly understood. This study therefore, investigated the temporal effect of obesity on ovarian expression of genes encoding cytochrome P450 isofrome 2E1 (Cyp2e1) and microsomal epoxide hydrolase (Epoxide). 4 weeks old female wild type normal non-agouti (a/a; designated lean; n = 20) and agouti lethal yellow (KK.Cg-Ay/J; designated obese; n = 20) mice were utilized. Glucose tolerance testing followed by euthanization and ovary collection was performed at 6, 12, 18 or 24 weeks (n = 5 per group) of age. Total RNA was isolated and cDNA (1:25) were amplified using primers specific for mouse Cyp2e1 and Epoxide. Relative mRNA expression of each gene was normalized using Gapdh as a housekeeping gene. Statistical analysis was performed using the T-test function of GraphPad Prism 5.5 software with a statistically significant level set at P < 0.05. Obesity had a differential effect on ovarian Cyp2e1 and Epoxide expression over time. Relative to their lean counterparts, in a time-dependent manner, ovaries from obese mice displayed decreased (P < 0.05) Cyp2e1 on one hand and increased (P < 0.05) Epoxide mRNA levels, supporting that Cyp2e1 and Epoxide may have some common transcriptional regulator. These results indicate that obese females may have increased susceptibility to ovotoxicant-induced reproductive health impairments. This work was supported by ES016818 to AFK.

2190 Examining Triclosan-Induced Potentiation of the Estrogen Uterotropic Effect

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Triclosan (TCS), a widely used antibacterial, has been shown to be an endocrine disruptor. We reported previously that TCS potentiated the estrogenic effect of ethinyl estradiol (EE) on uterine growth in rats orally administered 3 μg/kg EE and TCS (2 to 18 mg/kg) in the uterotropic assay, whereas TCS alone had no effect. We further characterized this potentiation by evaluating the effect of co-exposure with lower doses of EE (0.125 to 2 μg/kg) that are comparable to the concentrations in hormone replacement therapies. We found that TCS correspondingly potentiated the uterotropic effect (both in uterine weight and epithelial cell height), but at significantly lower doses of EE (LOEL = 0.25 μg/kg). In the current study, we evaluated the effects of TCS with a xenostrogen in the uterotropic assay to determine whether the effect is specific to only co-administration with EE. Female rats were exposed to 100 and 200 mg/kg of nonylphenol (NP), a pesticide with ER agonist activity, in addition to an EE-treated positive control group. NP induced a significant increase in uterine weight, but to a lesser extent than the EE-induced response. Animals co-treated with TCS and NP also had a significant increase in uterine weight compared to vehicle controls, but this difference was not significantly different from NP alone. To examine the cellular mechanism by which TCS potentiated the EE uterotropic effect, we conducted an in vitro estrogen receptor (ER) transactivation assay, which revealed that TCS alone does not activate the ER. Cotreatment of TCS with estradiol did not alter the maximal response suggesting that TCS does not antagonize nor potentiate the ER. These results provide evidence that the potentiation of the estrogenic response by TCS is not at the level of the ER. Further studies are needed to evaluate the role of TCS in altering estrogen metabolism. This work does not necessarily reflect EPA policy.

2191 Propylparaben Has No Estrogenic Activity When Administered for 3 Months in Juvenile Rats

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BACKGROUND: Parabens, esters of p-hydroxybenzoic acid, are used as antimicrobial preservatives in pharmaceuticals. There has been considerable controversy regarding the potential of parabens for estrogen-mimetic activity, and widespread concern regarding their use in pediatric medicinal formulations. The scientific literature contains conflicting results from several studies. We designed and conducted a comprehensive study to determine the potential of propylparaben (PB) to elicit estrogen-mimetic effects. OBJECTIVES: 1). Determine the potential estrogen-mimetic effects of PB in juvenile rats; 2). determine the systemic exposures to PB and metabolites; and 3). evaluate the reversibility of any observed changes. METHODS: PB was administered by oral gavage once daily to groups of Crl:CD(SD) rats at doses of 0 (vehicle), 10, 100, or 1000 mg/kg from Postnatal Days (PNDs) 4 through 90. Evaluations included clinical observations, body weight, food consumption (after weaning on PND 21), evidence of sexual maturation (vaginal opening and preputial separation), clinical pathology, anatomic pathology of male and female reproductive tracts, and toxicokinetic profiles on PND 7, 21 and 83. Recovery phase evaluations additionally included estrus cyclicity, mating (PND 91) and fertility assessments. Evaluations of the offspring included viability, birth weight, litter size, clinical observations, and examinations of pups for evidence of dysmorphology. RESULTS: PB was well tolerated by neonatal, juvenile, and adult rats when administered from PND 4-90 at doses ≤1000 mg/kg/day. There were no effects of PB on sexual maturation or reproductive function in male or female rats, nor changes in organ weights or microscopic pathology of reproductive tissues suggestive of estrogen mimetic effects of PB or its metabolites. Exposures to PB and metabolites were confirmed in toxicokinetic evaluations. CONCLUSION: Propylparaben is not an estrogen-mimetic in juvenile rats.
Perfluoroalkyl carboxylic acids (PFCAs) are environmental pollutants which are of concern due to their possible effects on human health. Although many toxicological researches have been performed, there is little information about the toxicity of PFCAs with a carbon chain length C11 and above. In order to obtain initial toxicological information for such long-chain PFCAs, combined repeated dose toxicity studies with the reproduction/developmental toxicity screening tests were conducted in the Japanese safety examination of existing chemicals and safety programmes. Presented here are the results for perfluorotetradecanoic acid (PFTeDA, C14) and perfluorohexadecanoic acid (PFHxDA, C16) concluded recently. Male and female rats were administered by gavage with PFTeDA at 1, 3 or 10 mg/kg/day or with PFHxDA at 4, 20 or 100 mg/kg/day, and each female was mated with a male in the same dose group after 14-day administration. Males were dosed for a total of 42 days and females were dosed throughout the gestation period until day 5 after parturition. PFTeDA and PFHxDA mainly affected the liver, causing hypertrophy of hepatocytes and fatty change, at middle and high doses. PFTeDA also induced follicular cell hypertrophy in the thyroid at middle and high doses. The only observed reproductive/developmental effect was an inhibition of postnatal body weight gain of pups at a maternal toxic dose of PFTeDA. The NOAELs for the repeated dose and reproductive/developmental toxicity were concluded to be 1 and 3 mg/kg/day for PFTeDA and 4 and 100 mg/kg/day for PFHxDA, respectively.

Epidemiologic studies suggest a strong causal link between exposure to airborne particulate matter (PM) during pregnancy and preterm birth (PTB) and low birth weight (LBW). However, these studies are often in disagreement regarding which particular time points are most sensitive or whether such effects are cumulative. Previously, we showed that inhalation exposure of timed-pregnant mice to concentrations of particulate matter (PM) from mother to offspring both in utero and through lactation using accelerated reproductive/developmental Toxicity Screening Tests (RTTs) to Zer produces transgenerational effects on sexual development and if these effects are dose dependent.

It has been demonstrated that a variety of chemicals are not removed after wastewater treatment, which results in their release back into the water supply and environment. These chemicals may act as endocrine disrupting compounds (EDCs), which can affect the function of the endocrine system and adversely affect progeny and/or (sub)-populations. To date, studies evaluating the effects of EDC during periods of development are lacking, including quantitative measures of accumulation after exposure. We are measuring the transfer and accumulation of the EDC, triclocarban (TCC), from mother to offspring both in utero and through lactation using accelerator mass spectrometry (AMS). The high sensitivity of AMS allows for assessment of environmentally relevant concentrations of TCC (pmol·mL⁻¹).

14C-labeled TCC was administered to pregnant female mice through their drinking water (100nM) during gestation and lactation using custom made water bottles. In utero, detectable quantities of TCC were found in fetal (~0.02%ID) and placental tissue (~0.02%ID). An increase in accumulation of TCC was observed through lactation (~6 pmol TCC/gm) compared to the gestation only exposure group (~2 pmol TCC/gm). Taken together, these data indicate that TCC is transferred from mother to offspring through two routes: across the placental barrier and through lactation. While low levels of TCC transfer were observed in mice, these findings suggest that if TCC is similarly transferred in humans, there may be implications after exposure on human health. Further studies are needed to identify whether low levels of TCC accumulation can interfere with reproduction and development and if these effects are dose dependent.

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Microsampling-coupled bioanalytical techniques are becoming powerful tools for use in pre-clinical studies, particularly with rodents, where optimizing blood use is paramount to try and avoid or reduce the need for satellite animals. In a series of intravenous (bolus) single and repeat dose pharmacokinetic investigations in the mouse with IgG antibodies, serial blood microsamples (30 μL each) were taken from the non-anesthetized adult female using K2-EDTA Microvette tubes via the saphenous vein and single microsamples from the gestation day 15, 16.5 or 18 fetus by cardiac puncture using insulin syringes. The fetal samples were pooled by litter (approximately 30 μL) into K2-EDTA microtubes. Adult and fetal plasma (approximately 10 μL each) was obtained by centrifugation. Amniotic fluid was also sampled using insulin syringes. All samples were stored frozen in cryotubes (between -15 and -25 °C) until analysis.

For quantifying the test item in the plasma and amniotic fluid, highly selective and sensitive ligand-binding assays were developed using Electro-Chemo-Luminescence (ECL) detection. Based on the characteristics of the test item and its target antigen, assay plates were either coated indirectly using biotin labeled recombinant antigen captured via streptavidin, or directly using idiotypic anti-drug antibodies. Only 2 μL of the plasma or 4 μL of amniotic fluid were diluted in assay buffer to analyze each sample in duplicate within the assay plate. The bound test item was detected by ECL-labeled secondary detection antibodies (e.g. anti-Fc or Fab antibodies). Bioanalysis of IgG antibodies was feasible with microsamples using the described assay setup and provided a precise, accurate and specific quantification of the test item with low limits of quantification between 10-100 ng/mL back calculated for undiluted plasma/amniotic fluid samples.

We conclude that microsampling-coupled bioanalytical techniques provide ethical, undiluted plasma/amniotic fluid samples.

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We conclude that microsampling-coupled bioanalytical techniques provide ethical, undiluted plasma/amniotic fluid samples.
analysis of whole mounts, microarrays, qPCR, Western blots, histology, immuno-histochemistry, and serum PFOA and steroid hormone analysis. Developmental scoring of mammary whole mounts indicated PFOA-induced mammary delays persisted into adulthood. These morphological alterations were characterized as increased collagen density, active terminal end buds and reduced side branching changes were observed even though serum PFOA concentrations had reached control levels. Ppar, Wnt, and Esr1 (Esr2) were identified as candidate gene pathways by microarray analysis; RNA post-transcriptional modification and lipid metabolism were top molecular functions. Updated array analysis predicted Ppar and γ-dihydroprogesterone and estradiol were potentially induced. Additional compound-related renal metabolism was observed, including reduced expressions of genes with Ppar and γ predicted to be inhibited and dihydrotestosterone and beta-estradiol predicted to be activated. qPCR validated changes in Ppar targeted and steroid related genes. Western blot analysis of selected genes revealed increased protein expression of Ppar and Ert in whole cell lysates at PND 7 with non-significant increases in Ppar and Ppar/β-Er protein expression was reduced by PND 21. Results from blots coincided with H&E stained sections for Er that were reduced at PND 21 and 56. No change was observed in serum estradiol or progesterone, however testosterone levels were reduced at PND 21 and DHEA levels were reduced at PND 56. Changes in hormone levels over time were different in PFOA-treated animals compared to controls for estradiol, testosterone, and DHEA, suggesting that PFOA may have an overarching effect on sex steroid hormone synthesis. Collectively, these data show prenatal PFOA exposure disrupts the endocrine system to have localized effects on the maturation of the mammary gland at serum levels that overlap with human exposures.

**2196e** Mode of Action and Human Relevance Assessment of Male CD-1 Mouse Renal Adenocarcinoma Associated with Lifetime Exposure to Empagliflozin

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In the 2-year carcinogenicity study with empagliflozin (dose levels of 100, 300 and 1000 mg/kg/day) in CD-1 mice, an increased incidence of renal tubular adenomas and carcinomas was identified in the male high dose (1000 mg/kg/day) group. Renal tumors were preceded by a number of renal degenerative/regenerative findings that overlap with human exposures. Specifically, membranous glomerulopathy, tubular atrophy, tubular cell necrosis, cystic hyperplasia and hypertrophy (mouse), renal mineralization and tubular nephropathy and interstitial nephritis (dog). Empagliflozin is non-genotoxic in a standard battery of gene mutation and chromosomal damage assays. In rat and mouse carcinogenicity studies, empagliflozin induced no tumors in females. Benign hemangiomata of the mesenteric lymph node and Leydig cell tumors were observed in male rats, both of which are common to the SGLT2 class, strain of rat, or may be of secondary relationship to treatment. Renal tumors were observed in male mice at 1000 mg/kg/day, an exposure approximately 45- and 113-fold above the therapeutic doses of 25 and 10 mg, respectively. In humans, tumor promotion by empagliflozin is not relevant to humans. Evaluations of photosensitivity spectrum and distribution of 14C-labelled empagliflozin to eye and skin coupled with the results of general toxicology studies revealed no potential for phototoxicity.

**2196f** General Toxicology and Carcinogenicity Assessment of Empagliflozin, an SGLT2 Inhibitor for the Treatment of Type 2 Diabetes Mellitus


Empagliflozin selectively inhibits sodium glucose co-transporter SGLT2 thereby reducing renal reabsorption of glucose as a therapeutic modality for type 2 diabetes mellitus. In pivotal toxicity studies in CD-1 mice, Wistar (Han) rat, and Beagle dogs, most toxicity was consistent with secondary pharmacology related to urinary glucose loss and electrolyte imbalances including decreased body weight and body fat, increased food consumption, diarrhea, dehydration, decreased serum glucose and increases in other serum parameters reflective of increased protein metabolism and glucosuria, urinary changes such as polyuria, and glycosuria. At high multiples of the therapeutic exposure, microscopic changes were observed in kidney and some soft and vascular tissues, and included tubular karyomegaly, single cell necrosis, cystic hyperplasia and hypertrophy (mouse), renal mineralization (rat), and tubular nephropathy and interstitial nephritis (dog). Empagliflozin is non-genotoxic in a standard battery of gene mutation and chromosomal damage assays. In rat and mouse carcinogenicity studies, empagliflozin induced no tumors in females. Benign hemangiomata of the mesenteric lymph node and Leydig cell tumors were observed in male rats, both of which are common to the SGLT2 class, strain of rat, or may be of secondary relationship to treatment. Renal tumors were observed in male mice at 1000 mg/kg/day, an exposure approximately 45- and 113-fold above the therapeutic doses of 25 and 10 mg, respectively. As described in relevant studies, the mode of action for these tumors is dependent on the natural predisposition of the male mouse to renal pathology and a metabolic pathway not reflective of humans. The male mouse renal tumors are considered not relevant to humans. Evaluations of photosensitivity spectrum and distribution of 14C-labelled empagliflozin to eye and skin coupled with the results of general toxicology studies revealed no potential for phototoxicity.

**2196g** A Predominant Oxidative Renal Metabolite of Empagliflozin in Male Mice Is Cytotoxic to Mouse Renal Tubular Cells but Not Genotoxic

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In a mouse 2 year carcinogenicity study with the SGLT2 inhibitor empagliflozin (dose levels of 100, 300 and 1000 mg/kg/day), an increased incidence of renal tubular adenomas and carcinomas was identified only in the male high dose group. Renal tumors were preceded by a number of renal degenerative/regenerative findings that overlap with human exposures. Specifically, membranous glomerulopathy, tubular atrophy, tubular cell necrosis, cystic hyperplasia and hypertrophy (mouse), renal mineralization and tubular nephropathy and interstitial nephritis (dog). Empagliflozin is non-genotoxic, which supports a nongenotoxic mode of action. Cross species in vitro studies in mRTE cells demonstrated exposures comprising >75% of M380/1, implying a stoichiometric release of reactive 4-OH cromonoldehyde (4-OH CTA). In vitro toxicity studies were conducted to evaluate the cytotoxic and genotoxic potential of empagliflozin and synthesized M466/2. M466/2 was tested in both the bacterial reverse mutation test and mammalian clonogenic assays to evaluate genotoxic potential. A primary mouse renal tubular epithelial (mRTE) cell model was used to assess the cytotoxic potential of empagliflozin and M466/2, showing that M466/2-derived in vitro 4-OH CTA exposure is cytotoxic to mRTE cells but is not genotoxic. In vitro genotoxicity testing was conducted to evaluate the cytotoxicity and genotoxicity of empagliflozin suggestive of alternative modes of action. In conclusion, a male mouse predominant renal metabolite M466/2 is associated with the male mouse specificity in renal injury and tumor response. These in vitro data showed that M466/2-derived 4-OH CTA exposure is cytotoxic to renal tubule cells and can contribute to additional compound related renal metabolic stress and further chronic low level renal injury that support a nongenotoxic mode of tumor pathogenesis.

**2196h** Pathogenesis of Renal Injury in the Male CD-1 Mouse Associated with Exposure to Empagliflozin

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An increased incidence of renal tubular adenomas and carcinomas was identified in the 2-year CD-1 mouse carcinogenicity study with empagliflozin (SGLT2 inhibitor) in high dose (1000 mg/kg/day) male mice. A 13-week mouse renal pathogenesis study was conducted with empagliflozin to evaluate dose-dependency and temporal onset of non-neoplastic degenerative/regenerative tubular changes which precede neoplasia. Male and female CD-1 mice were given daily oral doses of 0, 100, 300, or 1000 mg/kg, (corresponding carcinogenicity study dose levels) for 1, 2, 4, 8 or 13 weeks. The maximum expected pharmacology with secondary osmotic diuresis was observed by Week 1 at > 100 mg/kg/day in both genders. Histopathologic kidney changes were first detected after 4 weeks of dosing in the male high dose group. Changes detected starting on Week 4 consisted of minimal single cell necrosis and minimal increases in mitotic figures. These changes persisted throughout the study period and were associated with male mouse specificity in renal injury and tumor response. These changes were additionally supported by a predominant oxidative pathway of empagliflozin metabolism in the mouse, compared to a predominant conjugative pathway (O-glucuronidation) in humans.
adverse. Similar changes were not identified in lower dose groups in males nor were they present in females of any dose group. Overall, the study results support early compound-related degenerative/regenerative changes only in high dose male CD-1 mice as a key contributing feature to a nongenotoxic mode of renal tumor pathogenesis.

**2196i** RNA-Seq Reveals Molecular Changes in Renal Injury Associated with Exposure to Empagliflozin in the Male CD-1 Mouse

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In the CD-1 mouse 2-yr carcinogenicity study with empagliflozin, an increased incidence of renal tubular adenoma/carcinomas was observed in the high dose (1000 mg/kg/day) male group. To gain better understanding of the molecular changes in mouse kidney to support a tumorigenic mode of action, we have utilized RNA-Seq to evaluate gene expression changes in mice dosed with empagliflozin at 1000 mg/kg/day in a 13-week renal pathogenesis study. Enriched renal cortex was collected at 1, 2, 4, 8 and 13 weeks and the whole transcriptome in kidney was profiled. The transcriptome was compared between vehicle and empagliflozin treated male and female mice. Data showed male mouse-specific changes in kidney over 13 weeks of dosing. Consistent with the expected pharmacologic activity of SGLT2 inhibition and compound related renal metabolic stress, early treatment-related male-specific expression changes were noted in osmolality regulation, drug metabolism and transport at Weeks 1 and 2, followed by changes in immunity and complement system genes at Week 4. Treatment-related changes in genes and pathways related to p53 regulated cell proliferation, oxidative stress and renal injury were observed in high dose males at Week 8 and the number of genes with significant expression change dramatically increased at Week 13. The later expression changes were consistent with the increased appearance of degenerative/regenerative renal tubular changes only in high dose male. In contrast to males, observed expression changes were less apparent in female mice. Consistent with a nongenotoxic tumorigenic mode of action, no expression changes in DNA damage/repair pathways were identified. Overall, observed molecular changes in the male mouse complement the renal pathological changes in supporting a nongenotoxic tumorigenic mode of pathogenesis for empagliflozin.

**2196j** Nonclinical Safety Assessment of Exendin (9-39) in Juvenile Rats and Dogs

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Exendin (9-39) is being developed for the treatment of congenital hyperinsulinism, the most common cause of persistent hypoglycemia in children. Toxicology studies were conducted in Sprague-Dawley rats (7±1 days of age) and beagle dogs (13 days of age) to support its clinical evaluation. In 14-day non-GLP studies, TID subcutaneous (SC) doses of 15, 50, and 150 mg/kg/dose were administered to rats and 5, 15, and 40 mg/kg/dose were administered to dogs. Based on the lack of toxicologically significant findings, TID doses for GLP 28-day studies were increased to 20, 80, and 300 mg/kg/dose for rats and 10, 30, and 100 mg/kg/dose for dogs. Toxicological endpoints evaluated included: clinical observations, body weight, food consumption, clinical pathology, CNS assessment (rats), electrocardiography (dogs), toxicokinetics, and histopathology. The only Exendin (9-39)-related observations noted were increased serum glucose in female rats, decreased serum triglycerides in male rats, and increased urine volume in male dogs. In the cardiac vascular safety pharmacology study, beagle dogs (7 to 8 months old) were administered a single SC dose of vehicle or 5, 20, or 80 mg/kg Exendin (9-39) in a 4 x 4 Latin-square design. No toxicologically significant findings were observed. A dose proportional increase in Exendin plasma exposure was observed; combined average AUClast on Day 28 in male and female rats at the 300 mg/kg/dose was 249,000 ng.h/mL and at the 100 mg/kg/dose in dogs was 340,500 ng.h/mL. In conclusion, based on the available data, no observed adverse effect levels were 300 mg/kg/dose in rats and at 100 mg/kg/dose in dogs using TID subcutaneous dosing schedule. 

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**2196k** ETI-204 Nonclinical Safety Assessment of CNS Findings from Inhalational Anthrax Animal Studies

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Inhalational anthrax (IA) can result in progressive hemorrhagic meningoencephalitis and death. Antibodies against Bacillus anthracis (Ba) toxin components (antitoxins) are strategies to treat IA. ETI-204 is a mononclonal antibody against the protective antigen component of Ba toxins currently in development for IA disease treatment. No Ba antitoxin-related changes are typically observed in the CNS of IA study survivors (monkeys, rabbits), however, in IA infected non-survivors, Ba antitoxins have been associated with extravascular bacteria and higher incidence of acute inflammatory reactions in leptomeninges compared to controls that tend to die with no CNS changes or with just intravascular bacteria. Because of a putative Ba antitoxin CNS effect, ETI-204 CNS safety was assessed in dogs. Cynomolgus monkeys or NZW rabbits were aerosol exposed with ~200 LD50 of Ba spores and dosed with ETI-204 (1-32 mg/kg, IV) and the brains from 78 monkeys and 86 rabbits were examined microscopically. Brains of 15 non-Ba-exposed rabbits dosed with ETI-204 (16-32 mg/kg/dose x 4, IV) were also examined. There were no ETI-204-related changes in IA study survivors or in ETI-204 exposed, non-infected rabbits, indicating no direct ETI-204 CNS effect. In IA infected monkeys and rabbits, there was a higher incidence of meningitis and extravascular bacteria in ETI-204-treated non-survivors (all doses) compared to controls that tended to die with no CNS changes or had just intravascular bacteria. CNS findings in ETI-204-treated non-survivors were consistent with IA and an immune response to extravascular bacteria. One hypothesis is in non-survivors, Ba antitoxins do not reach the brain in sufficient quantities or in time to prevent bacterial extravasation, inflammatory reactions, and death, or the Ba infection is too severe to save the host with Ba antitoxin treatment alone. ETI-204 has not been shown to be toxic to the CNS, and ETI-204 IA survival benefit appears to outweigh any potential CNS risk. Federally supported by ASPR/BARDA under Contract No. HHSO100201000026C.

**2196l** Nonclinical Assessment of NRX-1074, an Orally Bioavailable NMDAR Partial Agonist

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NRX-1074, a partial agonist of the NMDAR receptor, is an orally bioavailable molecule that is being developed for major depressive disorders. Studies have shown promising ketamine-like efficacy signatures without apparent safety issues. As part of an IND, studies were conducted included in vitro genotoxicity, blood compatibility, safety pharmacology, and repeat-dose studies. NRX-1074 did not result in genotoxicity or hemolysis. No adverse effects were noted on the respiratory or central nervous systems in rats at 700 mg/kg, PO and in the cardiovascular systems in dogs at 70 mg/kg, PO. In the Repeat-Dose Studies, animals were dosed daily for 28 days via oral gavage at 0, 30, 200 or 600 mg/kg/day (rats) or 0, 30, 20, 200 mg/kg/day (dogs) or daily for four weeks by IV injection at doses of 100 (200) mg/kg/day (rats) or 70 mg/kg/day (dogs). Parameters evaluated in both studies included mortality, physical examinations, cageside observations, body weight, food consumption, ophthalmology, clinical pathology, gross pathology, absolute/relative organ weights, histopathology, EC50s (dogs only) and toxicokinetics. In the oral gavage studies up to 600 mg/kg/day (rats) and 200 mg/kg/day (dogs) and in the intravenous studies up to 100 mg/kg/day (rats) and 70 mg/kg/day (dogs), no treatment-related effects were noted during the dosing or recovery period. IV administration of NRX-1074 at 200 mg/kg resulted in mortality in rats. The no-observed-effect level (NOEL) for NRX-1074 when administered orally for at least 28 days to rats and dogs was 600 and 200 mg/kg/day for the oral route, respectively, and 100 and 70 mg/kg/day, respectively for the intravenous route. The oral HED’s were 97 and 108 mg/kg in rats and dogs, respectively and intravenous HED’s were 16 and 38 mg/kg in rats and dogs, respectively, suggesting the rat as the most sensitive species.

**2196m** Comparative Prolactin Study in Mouse and Rat Using CNS Drugs: Melindone, Haloperidol and Reserpine

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Typical antipsychotics have high affinity for dopamine D2 receptors and known to increase blood prolactin in both rodents and humans. Two independent studies were conducted to investigate and compare the prolactin and its reversal to basal

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**SOT 2014 Annual Meeting 577**
levels over a 24h postdose (PD) in Wistar rats and 32h PD in Swiss albino mice. Groups of rats and mice were treated with single doses of molindone (5 and 60 mg/kg; oral), haloperidol (20 and 100 mg/kg; oral), or reserpine (10 and 50 mg/kg; subcutaneous). These drugs were selected based on their potential to increase prolactin. Blood samples were drawn over 6 time points and prolactin levels were measured using validated ELISA method. Molindone resulted in peak prolactin levels at 0.25h PD and returned to baseline by 24h in both species. In general, haloperidol and reserpine showed treatment related increase in prolactin, though at a slower rate compared to molindone in rats and mice. The prolactin levels reached basal levels in males and rats by the last sampling time studied. However, in females the prolactin was decreased but remained elevated by last sampling time compared to controls. This study demonstrates, rodents experience prolactin surges that are physiologically required for reproductive effects of test-drugs. The changes in prolactin correlated with plasma drug levels and were higher in females than in males for all three drugs. Rapid reversal of prolactin in molindone treated animals compared to haloperidol and reserpine correlated with short half lives. These data are considered to be of significant value in comparative human risk assessment of test drugs.

2196n Assessment of Prolactin-Mediated Changes and Their Reversal in Molindone-Treated rasH2 Mice
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Molindone, an antipsychotic, is known to modulate prolactin levels linked with reproductive changes in rodents. Such effects were further investigated in 6-month mouse study with a reversible phase to better characterize risks assessment. Groups of rasH2 mice were dosed daily by gavage with molindone at 5, 15 and 50 mg/kg. Blood samples were analyzed for molindone on Day 180 and prolactin on Days 1, 180 and at a 2-month reversal period. Histopathology was performed on reproductive tissues. Results showed that plasma levels of molindone were more than dose proportional with Tmax at 0.25h postdose (PD). Prolactin levels peaked within 0.5h PD and were 257 and 272ng/mL in males and 1313 and 2454ng/mL in females, respectively, for Days 1 and 180. Prolactin levels remained elevated up to 250h PD and were 255 and 272ng/mL in males and 1313 and 2454ng/mL in females, respectively, for Days 1 and 180. Prolactin levels remained elevated up to 1h PD in males and 3h in females but returned to basal levels by 24h PD. Further, after 2-month recovery, prolactin levels were at basal levels, suggesting complete reversibility. Histopathologically, on Day 180, changes in ovaries included retention of eosinophilic corpora lutea and interstitial atrophy of diffuse tissue in all of the treated groups. Atrophy of uterus and vagina were observed in these groups. At the end of recovery, these changes were completely reversed at 5 and 15 mg/kg, with signs of reversal at 50 mg/kg. In males, there was no histopathology changes noted. Reversibility of prolactin levels and histopathology seen in this study confirms the pharmacologically mediated effects of molindone and corroborate with the known role of prolactin in hormonal homeostasis and reproductive physiology. These changes are considered rodent-specific, similar to those seen with other antipsychotics in this class of drugs. It is concluded that the transient prolactin-mediated changes seen in this mouse study are of negligible risk, if any, to humans, because such changes have not been reported in humans taking molindone as a therapeutic.

2196p De-Risking Potential Infection Risk with Anti-CSF-1
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Colonoly-stimulating factor-1 (CSF-1) promotes differentiation, proliferation and migration of monocytes/macrophages, which are involved in innate and adaptive immunity. Macrophages are implicated in a variety of inflammatory diseases; therefore, anti-CSF-1 therapeutic antibodies can be beneficial in several indications such as rheumatoid arthritis (RA). A safety concern when targeting this pathway is increased infection risk due to the role of monocytes/macrophages in immunity; therefore, 2 mouse studies were conducted to evaluate the anti-CSF-1 safety profile and infection risk.

The overall safety profile of anti-CSF-1 was evaluated in an 84d repeat dose study, where administration of anti-CSF-1 (twice weekly; i.p.; 200ug/mouse) resulted in decreased macrophages in spleen, lung, kidney and liver. There was a minimal to mild increase in ALT, AST and CK, consistent with the role of liver macrophages in clearing serum enzymes. No anti-CSF-1-related anatomic pathology findings were observed. Collectively, these findings are consistent with pharmacology of anti-CSF-1.

Infection risk with anti-CSF-1 was interrogated by evaluating the response to Listeria monocytogenes challenge in a head-to-head comparison to a TNFa inhibitor (TNFRII-IgG). The biology standard of care for RA, A mouse Listeria infectious challenge model was chosen because the primary response to Listeria is driven by macrophages and RA patients treated with TNFa inhibitors are at an increased risk of infections with Listeria. While anti-CSF-1 treatment (twice weekly; i.p.; 200ug/mouse; up to 42d) did result in increased mortality compared to isotype antibody-treated controls, the onset and rate of mortality was significantly later and lower than that observed with TNFRII-IgG (twice weekly; i.p.; 150ug/mouse; up to 14d). These data suggest that anti-CSF-1 may be associated with less risk of infection compared to TNFa inhibitors.

2196q A Method Validation for the Screening and Confirmation of Neutralizing Antibodies (Nab) against PEG-IL-29 in HCV Human Sera Using a Cell-Based Assay with a Bioluminescent Detection Platform
J. Hantash.
Biotechnology Services, Tandem Labs Inc., West Trenton, NJ. Sponsor: Z. Radi.

A cell based assay for the determination of PEG-IL-29 neutralizing antibody in human HCV positive sera was developed and validated in support of bioequivalence studies. The assay utilized a cell line that was engineered to over-express the IL-29 receptor containing a Luciferase reporter construct with ISRE and STAT binding elements directly upstream of a luciferase reporter gene. Positive Quality Control and test HCV positive serum samples containing Anti-PEG-IL-29 antibodies (antibody/PEG-IL-29) mix was added to an overnight seeded cell culture plate and incubated. ONE-Glo solution was added and the signal was measured. Samples were determined to be “positive” or “negative” for neutralizing activity based on a comparison of the mean response of the positive control to the mean response of the negative control. Samples are considered positive if the % Neutralization value is greater than or equal to the assay cut point. 25 HCV positive Human Sera lots were tested to determine the neutralization cut point of the assay, which was found to be ≥26%. For quality control samples tested at two concentrations (1000 and 500 ng/mL), precision (%CV) ranged from 0.1 to 25.7% and 0.0 to 23.7%; respectively. The assay sensitivity was 250 ng/mL with a drug tolerance ≥2ng/mL. For selectivity, 10 out of the 10 HCV positive Human Sera lots tested showed neutralization (%) ≤26%. Positive control samples showed bench top stability for 20 hours (neutralization ranged from 34 to 38%) and freeze thaw stability for up to 8 cycles (neutralization ranged from 44 to 65%). Long term stability at -80°C was proven for up to 182 days (neutralization ranged from 30 to 57%). The validated Nab cell based assay in HCV positive Human Sera successfully met all standard assay-validation parameters and was suitable for use in bioequivalence studies.

2196o The Role of Receptor Occupancy Analysis during Preclinical Development of Biologics

Pre-clinical safety assessment of biopharmaceuticals provides many challenges, including the potential for the production of anti-drug antibodies (ADA) when a human (or humanised) protein is administered to an animal system. ADA production may lead to loss of exposure and activity of the biotech derived protein (Bio-A) specific for receptors on a sub-set of lymphocytes, during a 26 week regulatory study in the mouse. Prior to starting the 26 week study an RO method was validated to determine the proportion of bound and free target receptors on lymphocytes in the presence / absence of Bio-A. Both evaluations established good reproducibility (CV ≤27%), whilst requiring minimal quantities of blood. At Week 13 of the 26 week mouse study, ADA production and a drop in systemic levels of Bio-A suggested loss of exposure, especially in the low dose group, RO analysis on a sub-set of animals indicated that the majority of low dose mice had reduced levels of bound Bio-A and high levels of free receptors. However, in the remaining mice, Bio-A was still bound to the surface of lymphocytes. Based on the possibility that exposure still persisted in some animals it was decided that additional blood be taken from the toxicology animals at necropsy to evaluate the level of free receptors in each. This additional analysis was possible in the mouse as the RO validation had established a method where this could be evaluated in blood samples of 50uL. Evaluation of free receptors in the low dose group, contradicted the ADA and TK data, suggesting that the majority of mice were still exposed and rendering these animals valid for assessment of toxicity over the 26 week period.

In conclusion, establishment of a robust method to measure RO provided vital information regarding exposure levels that allowed a proportion of the low dose animals to be used to for histopathology evaluation, when the TK and ADA data suggested otherwise.

2196r SOT 2014 Annual Meeting
Testing of biological drug products for safety and efficacy in animal models has been difficult to assess because common models such as rodents, canines and non-human primates do not necessarily share common biological receptors with humans. A new animal model, the human immune system mouse, has recently emerged in widespread use for infectious disease pathogenesis and vaccine testing research. We utilize human immune system mice that have human thymus and liver implanted underneath the kidney capsule and are engrafted with human hematopoietic stem cells. To test the ability of this model assess the efficacy and safety of biological drug products in vivo, we tested the anti-C2D2 product rituximab.

Mice were pretreated with anti-histamines and anti-inflammatory products, as recommended per package label. A single treatment was administered at three different doses: 2 mg/kg (low), 8 mg/kg (middle), and 15 mg/kg (high). The drug was diluted in saline to concentrations of 0.2 – 0.75 mg/ml prior to i.v. administration to reduce the likelihood of immediate hypersensitivity reaction. Samples for flow cytometric assessment and serum were taken immediately prior to administration and then at 1, 2, 5, 8, 11, 16, and 21 days post-treatment. Mice were euthanized at days 8, 11, 16 and 21 post-administration to determine the completeness and durability of B-cell depletion in blood, spleen and bone marrow. Results showed that human immune system mice experienced full B-cell depletion in a dose-dependent manner, with the low dose group substantially reconstituted at day 8 and the high-dose group still depleted at day 21 post-administration. Adverse effects observed included off-target depletion of T-cells that was donor dependent, which has also been shown to occur in some human patients. These results demonstrate that human immune system mice can respond biologically in a dose-dependent manner to anti-C2D2 drug products and recapitulate the pharmacodynamics and adverse events observed in humans.
Administration of SCH A produced increased salivation and emesis at doses equal to or greater than 300 mg/kg and mild to severe tremor at doses equal to or greater than 600 mg/kg. Tremor occurred 30-60 minutes post-dose and increased in magnitude with increasing dose. Two animals appeared to be more sensitive to these effects, showing more severe findings and findings at lower doses than the other two animals. EEG analysis showed no evidence of seizure-related waveforms in any animal. TK analysis showed that mean AUC values increased with increasing dose, and mean Cmax values increased from 300-1000 mg/kg, with no further increase at 2000 mg/kg. Individual inter-animal variability made difficult the determination of a PK-PD relationship. It did not appear that there was a strong correlation between plasma concentration and clinical signs following oral administration of SCH A. Oral administration of SCH A was not accompanied by adverse changes in brain activity, as assessed by EEG.

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**2196**

**The Effect of Anesthetic on QT Interval Measurements in Guinea Pigs**

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Anesthetized guinea pig is a widely used model for early screening of drug-candidate effects on cardiovascular function. This species is gaining traction for use in conscious screening telemetry studies. For unanesthetized work, the majority of published studies use sodium pentobarbital (NaP) as the anesthetic of choice. However, NaP is an antagonist of the inward rectifying cardiac potassium channel IKs. Since the IKs is a component of the guinea pig cardiac repolarization sequence, this species can be exquisitely sensitive to IKs blockade. In this study we investigated the baseline vulnerability of the anesthetized guinea pig for safety pharmacology screening of drugs with potential to prolong the QT interval. Male guinea pigs (400-550g) were anesthetized with ketamine/xylazine (87.5/7.5 mg/kg, KET) or NaP (15-30 mg/kg) and surgically instrumented with a Millar catheter to measure arterial pressure. Electrocardiograms were recorded and PR interval, QRS duration, QT/QTc interval and arrhythmogrames were monitored. Monitored parameters were compared with measurements obtained previously in conscious guinea pigs (CON). NaP anesthetized guinea pigs exhibited baseline QTcB intervals (342±3 ms, p<0.05) that were significantly increased compared to CON cohort (256±6 ms). QTcB intervals in the KET group (307±7 ms, p<0.05) were also increased as compared to CON group, but the changes were significantly less robust. Lower HR was observed in the KET group (215±29 bpm, p<0.05) as compared to CON (258±13 bpm) and NaP (250±6 bpm) groups. QRS duration was increased in the KET (45±2.8 ms) and NaP (44±3.3 ms) groups as compared to CON guinea pigs (29±1.3 bpm, p<0.05). No change in mean arterial pressure was observed between the groups. In summary, the choice of anesthetic appears to influence the QT interval in anesthetized guinea pigs compared to conscious animals. It is possible that anesthetics with additional inherent IKs blockade may overly sensitize the animal to agents that prolong the electrocardiographic QT interval. Consideration should be taken when selecting anesthetics for this model.

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**2196a**

**IG-001 Suppressed Hypersensitivity Reactions versus Taxol® in Beagle Dogs**

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Study Purpose: Hypersensitivity reactions (HRs) are known adverse effects of Taxol® chemotherapy. Taxol® is formulated in Cremophor EL (Cr-EL) which triggers HRs requiring premedication. Abraxane®, a Cr-EL-free albumin-bound formulation (nab-Pac), has low incidence of HRs. IG-001 is a novel Cr-EL-free, non-biologic nanoparticle formulation being developed as an alternative to nab-Pac. We report the suppressed HRs for IG-001 vs Taxol® in beagle dogs as the most sensitive species.

Methods: Sixty-five beagle dogs (33 M and 32 F) were evaluated in 3 GLP studies. In a dose-escalation study, 9 dogs received a 30 min intravenous (iv) infusion of IG-001 (1.1-4.5 mg/kg) or Taxol® (0.1-1.1 mg/kg). In the single (n = 24) or repeated 5-cycle (n = 32) dosing studies, dogs received IG-001 (1.5-4.5 mg/kg for 3 hr) or Taxol® (0.1-2.5 mg/kg for 30 min), the latter group with antihistamine premedication. Measurements included clinical observations, body weight, food consumption, hematology, blood chemistry, and histopathology. Results: Minimal clinical signs were noted at IG-001 doses of 1.1 to 2.2 mg/kg, but 4.5 mg/kg was associated with morbidity. Taxol® produced HRs at 0.1 mg/kg and above, necessitating premedication and shortening of infusion time from 3 hr to 30 min. The MTD was >2.2 mg/kg for IG-001 and 0.2 mg/kg for Taxol®. HRs to IG-001 was limited to only 2 of 32 (6.25%) dogs from the dose-escalation study. The HRs occurred after 3 or 4 doses within 3 or 5 weeks. No HR was noted with the IG-001 vehicle or those treated with IG-001 in the single- and repeated-dose studies. Excluding the severe HRs observed in the Taxol® group, body weight, food consumption, clinical signs, and hematology responses were comparable between IG-001 and Taxol®.

Conclusions: Taxol® infusion in the canine model resulted in severe HRs requiring >2.2 mg/kg premedication and a reduction in infusion time. IG-001 was better-tolerated without premedication at substantially higher doses and with a low potential to produce HRs. IG-001 could potentially be a safe and effective alternative to nab-Pac.

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**2196b**

**Repeat-Dose Rabbit Vaginal Tolerance/Toxicity Study of Diindolylmethane Cream for Topical Treatment of Cervical Intraepithelial Neoplasia**

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Cervical Intraepithelial Neoplasia (CIN) is a premalignant condition characterized by dysplastic changes in the cervix. Because it can be identified by screening and may progress to invasive malignancy, CIN is an attractive target for cancer prevention. Indole-3-carbinol and its condensation product, diindolylmethane (DIM), have chemopreventive activity in several carcinogenesis models. As a result of its superior stability, DIM is being evaluated in clinical trials for cancer prevention in several sites, including the cervix. A preclinical study was performed to characterize the local and systemic effects of intravaginal administration of DIM (DIM Vaginal Cream, BioResponse LLC, Boulder, CO [BR-DIM-VC]). Groups of 5 NZW rabbits received intravaginal instillation of the maximum feasible volume (4.0 ml) of BR-DIM-VC (0% [placebo], 2%, 4%, or 6%) on alternate days for two weeks. Exposure to BR-DIM-VC induced no mortality and had no effect on body weight. The results of clinical and physical observations, chemical analysis, hematology, and coagulation assays were comparable in all groups. Vaginal pH was unchanged by exposure to BR-DIM-VC. Vaginal irritation scoring demonstrated dose-related vaginal edema and erythema in rabbits treated with BR-DIM-VC. At 2%, 4% rabbits were normal; 1 rabbit demonstrated very slight edema (no erythema). At 4%, 2/5 rabbits were normal; 3 rabbits demonstrated very slight edema with barely perceptible erythema. At 6% 1/5 rabbits was normal, 2 rabbits demonstrated very slight edema with very slight erythema, and 2 rabbits demonstrated slight edema with well-defined erythema. No vaginal edema or erythema was seen in placebo-treated rabbits. Repeat-dose intravaginal administration of BR-DIM-VC on alternate days for two weeks induced no systemic toxicity in rabbits. BR-DIM-VC did induce very slight to slight vaginal irritation in a dose-related fashion. (HHSN261201200025F)

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**2196c**

**A Retrospective Analysis of the Effect of Housing Conditions on Body Weight, Food Consumption and Survival in Rat Carcinogenic Rat Studies**

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Rodent toxicity studies have historically been performed in wire-bottom cages. However, both the National Research Council (NRC) and Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, recommend housing rats in solid-bottom cages with bedding to prevent development of foot lesions and/or stress responses. A retrospective analysis of Sprague-Dawley rat carcinogenicity study data (n=25 control groups) compared the effects of individual vs. pair housing and wire-bottom vs. solid-bottom caging on body weight, food consumption and survival. Survival was analyzed by the Cox proportional hazards model; body weight was analyzed by the Gompertz non-linear model. For rats housed individually in solid-bottom cages, average body weights were higher compared to rats housed individually in wire-bottom cages. The body weight differences become significant around Week 25 in males and Week 45 in females, and continued until the end of study. At Week 104, the average difference in males was about 50 g or 5% and in females was about 55 g or 11%. The differences in average body weight did not correspond with a significant difference in average food consumption or in survival rates. For pair-housed rats in solid-bottom cages, average food consumption was lower (~7% in males; ~8% in females) than rats housed individually. This difference in average food consumption corresponded with a slightly lower average body weight (Week 104, ~3% in males; ~9% in females) and a marginal improvement in survival (in females only, Week 104, ~15%). In conclusion, this analysis demonstrates that housing conditions (individ-ual vs. paired and wire-bottom vs. solid-bottom) may affect body weight, food consumption, and survival on two-year rat carcinogenicity studies.
An international expert group which includes 32 organisations (pharmaceutical and biotechnology companies, contract research organisations and regulatory bodies) has shared data on the use of recovery animals in the assessment of pharmaceutical safety for early development. The group has used this data as an evidence-base to make recommendations on the inclusion of recovery animals in toxicology studies to best assess human safety, while reducing animal use. The initiative is led by the NC3Rs in collaboration with the Medicines and Healthcare products Regulatory Agency (MHRA).

Recovery animals are used in pharmaceutical development to provide information on the potential for a toxic effect observed in animals to translate into long-term human risk. They are included on toxicology studies to assess whether effects observed during dosing persist or reverse once treatment ends. It is a regulatory requirement to assess recovery at some point during drug development.

The group devised a questionnaire to collect information on the use of recovery animals in general regulatory toxicology studies to support first-in-human clinical studies. Questions focused on study design (e.g. species, recovery duration and the number of animals used), the rationale behind inclusion or exclusion (case-specific vs. default inclusion), and the impact this had on internal and regulatory decisions. Data on 137 compounds (53 biologicals and 78 small molecules) from 259 studies was provided by 22 companies and showed that there was wide variation in where, when and why recovery animals were included. We found that recovery animals were included on all studies for 85% biologicals and 65% small molecules.

Recovery animals were included on more than one dose group for 70% biologicals and 25% small molecules. The number of recovery animals used per compound ranged from 0 to over 100. An analysis of individual study and programme design shows that there are opportunities to reduce the use of recovery animals in certain circumstances which would not impact drug development.

Alkaline phosphatase (ALP, EC 3.1.3.1.) in serum is measured frequently in toxicity studies. ALP isoenzymes in serum are important items for the evaluation of organ toxicity, but they are also informative on underlying environmental contaminants-induced diabetes and identify AhR signal transduction as a potential therapeutic target.

Epidemiological studies have shown that exposure to persistent organic pollutants (POPs) is associated with increased risk of type 2 diabetes. Most of POPs function through activation of the aryl hydrocarbon receptor (AhR), and our previous studies have shown that AhR knockout (AhRKO) mice exhibit enhanced insulin sensitivity.

In this study, we explored the effects and mechanisms of AhR agonists on the insulin signaling pathway using wild-type (WT) and AhRKO mice, as well as hepatic tumours. Hepa-1c1c7 (C7). In C7 cells, AhR agonists 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and beta naphthoflavone (BNF) decreased insulin receptor substrate 1 (IRS-1) protein expression and inhibited Akt phosphorylation, but did not change IRS-1 mRNA, TNF-α or phospho-JNK levels. AhR knockdown with specific siRNA partially reversed the BNF or TCDD-mediated IRS-1 protein decrease. Furthermore, the proteasome inhibitor, MG132, partially restored AhR agonist-mediated IRS-1 protein degradation. In addition, AhR activation by its agonists compromised the insulin signaling pathway and caused hyperglycemia in WT mice, but not in AhRKO mice. Taken together, these data indicate that AhR activation induced IRS-1 ubiquitin-dependent degradation leading to insulin resistance and hyperglycemia. Our findings provide an insight into the mechanisms underlying environmental contaminants-induced diabetes and identify AhR signaling as a novel therapeutic target.
the mechanism causing these effects and their relevance to human disease is not well understood. In this study, we have used unique immortal human preadipocytes that can be differentiated into adipocytes to evaluate the effects of PCBs on adipocyte biology. Exposure of the preadipocytes to PCB126 at concentrations of 0.1 - 10 μM caused a dose-dependent decrease in subsequent differentiation to adipocytes. Reduced differentiation was marked by significant decreases in accumulation of lipids, mRNA expression of adipocyte differentiation markers (PPAR/2, CEBP/α, p2 and adiponectin; P < 0.05) and genes in the insulin signaling pathway (IRS-1 and GLUT4). Strikingly, the decrease in adipocyte differentiation was more profound in preadipocytes exposed to PCB126 before differentiation compared to exposure during differentiation, the latter being the treatment regimen used in previously reported studies. These results indicate that PCB126 may maintain pre-adipocytes in an undifferentiated state, possibly leading to expansion and metabolic disruption that can initiate metabolic syndrome. These interesting observations warrant further research in order to understand the mechanism by which PCB126 causes inhibition of adipocyte differentiation and the roles of adipose tissue in PCB126 induced toxicity.

2200 TCDD Treatment Enhances Hepatic Stellate Cell Activation during Experimental Liver Fibrosis

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related chemicals are widely spread environmental pollutants that elicit toxicity through the aryl hydrocarbon receptor. It was recently shown that exposure to TCDD enhances gross markers of liver damage during experimental liver fibrosis. Liver fibrosis is a wound healing response characterized by the deposition of excessive or abnormal extracellular matrix by myofibroblasts, namely hepatic stellate cells (HSCs). Previous studies in our laboratory revealed that TCDD treatment enhances activation of a human HSC line in vivo. The goal of the present study was to determine if TCDD increases HSC activation in vitro using two mouse models of liver fibrosis: bile duct ligation (BDL) and carbon tetrachloride (CCL4) administration. For the BDL model system, C57Bl/6 mice were treated with TCDD (20 μg/kg) or peanut oil (vehicle) one day prior to surgery and euthanized 3 and 7 days later. For the CCL4 model system, C57Bl/6 mice was administered twice a week for 6 weeks and TCDD (20 μg/kg) or vehicle was given during the last 2 weeks. In BDL mice, TCDD treatment elicited a 3- to 6-fold increase in plasma alanine aminotransferase levels and caused marked expansion of periportal bile ducts. Immunofluorescence staining revealed that TCDD treatment increased expression of the HSC activation marker, alpha-smooth muscle actin (αSMA), in areas surrounding the bile ducts, which coincided with a three-fold increase in αSMA transcript levels in the whole liver. In the CCL4 model, a 50% increase in liver-body weight ratio was observed in TCDD-treated mice compared to mice that received CCL4 alone. TCDD treatment also elicited a 5-fold increase in hepatic αSMA transcript levels and enhanced collagen deposition, as revealed by increased Sirius red staining. These results indicate that TCDD treatment enhances HSC activation in vivo, which may contribute to the exacerbation of liver damage observed in TCDD-treated mice during experimental liver fibrosis.

2201 Congener- and Species-Specific Differences in Relative Effect Potencies of Dioxin-Like Compounds between In Vitro-Exposed Human Peripheral Blood Lymphocytes and Mouse Splenic Cells


Human risk assessment for dioxin-like compounds is typically based on the concentration measured in blood serum multiplied by their assigned toxic equivalence factor (TEF). Consequently, the actual value of the TEF is very important for accurate human risk assessment. In this study we investigated the effect potencies of 3 polychlorinated dibenzo-p-dioxins (PCDDs), 6 polychlorinated dibenzofurans (PCDFs) and 10 polychlorinated biphenyls (PCBs) relative to the reference congener 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in vitro exposed primary human peripheral blood lymphocytes (PBLs) and mouse splenic cells. REPs were determined based on cytotoxicity in 96-well plates using CellTiter96® Aqueous One Solution assay (Promega). The aryl hydrocarbon receptor repressor (AhRR) gene expression as well as the TCF3 and REL gene expression. These factors interfere with understanding the toxic potential of this class of compounds, particularly in mixtures. Characterizing the non-carcinogenic toxicity of PAH compounds will contribute to toxicity assessments, understanding mixture interactions, and the management of human health risks. The objective of this study was to observe cellular responses from PAH concentrations. Clone-9 rat liver cells were grown in culture and tested to observe acute toxicity. In paired trials, cells were exposed to 15 parent and methylated PAHs for a period of 24 hours, with concentrations ranging from 0.25-20 parts per million (ppm). At 100% confluence, Janus green B staining was performed to observe cellular viability and proliferation. Many of the tested compounds displayed minimal toxicity, with no observed inhibition of proliferation or viability except at the highest concentration. Benzo[a]pyrene (BaP) displayed slight toxicity, with a decline in proliferation only at 20 ppm. There was no observed response from benzo[c]pyrene, benzo[b]fluoranthene, chrysene and phenanthrene. 3,6-dimethylphenanthrene, 7,12-dimethylbenz[a]anthracene and 9,10-di-menthylanthracene displayed severe toxicity, impairing both proliferation and viability in a dose-responsive manner. The results for the parent compounds concur with available literature, which acknowledge carcinogenesis as the primary health concern rather than non-carcinogenic toxicity at environmentally-relevant concentrations. However, our findings suggest that methylated PAHs can be much more toxic than the parent compounds. Further research will compare these results to trials using binary PAH mixtures for the purpose of characterizing mixture effects.

2202 Measurement of Protein Adducts Formed by Quinoid Metabolites of Polychlorinated Biphenyls (PCBs)

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Recently unexpected high levels of indoor PCB air concentrations were found in a large number of public elementary school classrooms. Most of the airborne PCBs are lower chlorinated congeners, from mono- to tri-chlorinated PCBs. Lower chlorinated biphenyls can be metabolized to dihydroxy metabolites and further oxidized to quinoid metabolites both in vivo and in vitro. Quinoid metabolites of PCBs may form adducts at nucleophilic sites of proteins. Using radioactively labeled PCB3, we previously had observed hemoglobin adducts in vivo and covalently bound adducts with nuclear protein in liver. Our current objective is to establish the methodologies to quantitatively measure the adducts formed by PCB3 quinones, and to determine the distribution of protein adducts and the target proteins in cells treated with PCB3 and metabolites. Monoclonal antibodies against PCB3 quinone protein adducts may be employed to screen tissues for such adducts and to isolate adducted proteins. Two types of immunogens of PCB3 quinones were synthesized. PCB3 quinones were linked with keyhole limpet hemocyanin (KLH) or N418 through N-succinimidyl 3-(2-pyridyldithio)-propionate (SDPD). These antigens were injected repeatedly into mice. Four hybridoma lines with strong antibody production were chosen for further analysis of antibody specificity. In addition a method is being developed to quantify protein adduction by detachment bound quinone molecules from the proteins, isolating and quantifying them. To establish this method, PCB3-para-quinone was incubated with bovine serum albumin (BSA). Protein adducts were hydrolyzed under mild acidic conditions and extracted with hexane. Quinoid PCB3 residues in the extracts were determined by HPLC and confirmed by mass spectrometry. In future experiments GC/MS or LC/MS will be used to increase detection limits. (Supported by NIEHS Superfund Program P42 ES013661)

2203 The Effects of Polycyclic Aromatic Hydrocarbons on Cellular Proliferation and Viability in Clone-9 Rat Liver Cells

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Polycyclic aromatic hydrocarbons (PAHs) have been labeled contaminated of concern due to their carcinogenic potential, insufficient toxicological data, ubiquity, and common presence in the environmental media. These factors interfere with understanding the toxic potential of this class of compounds, particularly in mixtures. Characterizing the non-carcinogenic toxicity of PAH compounds will contribute to toxicity assessments, understanding mixture interactions, and the management of human health risks. The objective of this study was to observe cellular responses from PAH concentrations. Clone-9 rat liver cells were grown in culture and tested to observe acute toxicity. In paired trials, cells were exposed to 15 parent and methylated PAHs for a period of 24 hours, with concentrations ranging from 0.25-20 parts per million (ppm). At 100% confluence, Janus green B staining was performed to observe cellular viability and proliferation. Many of the tested compounds displayed minimal toxicity, with no observed inhibition of proliferation or viability except at the highest concentration. Benzo[a]pyrene (BaP) displayed slight toxicity, with a decline in proliferation only at 20 ppm. There was no observed response from benzo[c]pyrene, benzo[b]fluoranthene, chrysene and phenanthrene. 3,6-dimethylphenanthrene, 7,12-dimethylbenz[a]anthracene and 9,10-di-menthylanthracene displayed severe toxicity, impairing both proliferation and viability in a dose-responsive manner. The results for the parent compounds concur with available literature, which acknowledge carcinogenesis as the primary health concern rather than non-carcinogenic toxicity at environmentally-relevant concentrations. However, our findings suggest that methylated PAHs can be much more toxic than the parent compounds. Further research will compare these results to trials using binary PAH mixtures for the purpose of characterizing mixture effects.
2204 Biotransformation of BDE-100 to Potentially Toxic Metabolites: Predominant Role of Human CYP2B6

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Polybrominated diphenyl ethers (PBDEs) are additive flame-retardants used in numerous consumer products, resulting in their presence as widespread environmental contaminants. Recent studies have suggested that hydroxylated metabolites of PBDEs accumulate in human serum at levels similar to or greater than their parent PBDEs. Furthermore, it has been suggested that bioactivation through oxidative metabolism may add to the neurotoxic potential of PBDEs, however, insufficient data exists on the metabolism of PBDEs in humans. The purpose of this study was to characterize the in vitro metabolism of 2,2',4,4',6,6'-hexabromodiphenyl ether (BDE-100), one of the most abundant PBDEs found in humans, by recombinant human cytochrome P450s (CYPs) and pooled human liver microsomes (HLMs). Recombinant CYPs (1A1, 1A2, 2A6, 3A4, 2B6, 2C8, 2C9, 2C19, 2D6, and 2E1) were individually incubated with 20 μM BDE-100 to monitor CYP-specific metabolism. For kinetic studies, recombinant CYP2B6 and HLMs were incubated with BDE-100 (0-60 μM). Analysis was completed by gas chromatography-mass spectrometry after derivatization of the hydroxylated-BDE metabolites (OH-BDEs) to their methylated derivatives using (trimethylsilyl) diazomethane. CYP2B6 was found to be the predominant CYP responsible for the formation of five mono-OH-BDE and two di-OH-BDE metabolites observed for BDE-100. One metabolite was identified as 6-OH-BDE-100 through use of an authenticated standard. Utilizing fragmentation characteristics of methoxylated-BDEs (MeO-BDEs), it is hypothesized that two meta-OH-BDEs and one para-OH-BDE are among the remaining OH-BDE metabolites. Kinetic studies of BDE-100 metabolism by CYP2B6 and HLMs were confirmed by Km values ranging from 3.7-5.9 μM and 4.7-5.6 μM, respectively, suggesting a high affinity towards the formation of OH-BDEs. These results will ultimately better inform future studies investigating the potential of PBDEs and their metabolites to produce neurotoxic and other health effects. (NI-EHS grant #ES021554)

2205 Enantioselective Disposition of 2, 2’, 3, 5’, 6-Hexachlorobiphenyl (PCB 95) and Its Metabolites in Mouse Dams Dosed during Pregnancy

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Racemic PCB 95 has been implicated as a developmental neurotoxicant. Although studies in adult female mice show a dose-dependent enantiomeric enrichment of PCB 95 and its hydroxylated metabolites (OH-PCBs), the effect of pregnancy on the enantioselective disposition of PCB 95 in laboratory animals has not been investigated. This is an important question since pregnancy-related changes in cytochrome P450 and enantioselective transfer of PCBs and OH-PCBs to the fetus and neonate could potentially modulate enantiomeric enrichment of PCB 95 and its metabolites. This study investigates dose-dependent differences in the enantioselective disposition of PCB 95 and OH-PCBs in female C57Bl/6 mice (8 weeks old) fed daily with racemic PCB95 (0.1, 1 or 6 mg/kg) or vehicle (peanut oil) in peanut butter, starting two weeks before mating and continuing throughout gestation and lactation. The levels and enantiomeric enrichment of PCB 95 and OH-PCBs were determined in adipose, blood, brain and liver. There was an increase in PCB 95 and OH-PCB levels with increasing dose in all tissues. Adipose had the highest level of the parent compound; blood had higher OH-PCBs levels than liver, and no metabolites were detected in brain or adipose. PCB 95 displayed clear enantiomeric enrichment in all tissues, with enantiomeric fractions (EF) ranging from 0.26 ± 0.01 (adipose) to 0.11 ± 0.02 (liver). These EF values are comparable to observed previously in PCB 95-treated adult mice. Our findings suggest that pregnancy-related changes in P450 enzyme levels and transfer to the offspring do not significantly change the enantiomeric enrichment of PCB 95 in adult female mice. Future studies are needed to better understand the toxicological implications of the enantiomeric enrichment of PCB 95 and its metabolites in mice and, ultimately, PCB exposed humans. [Supported by NIH grants, ES014901, ES017425 and ES007059]

2206 Decreased Hepatocyte Nuclear Factor 4alpha Activity As A Mechanism of Hepatomegaly Induced by PFOA and PFOS

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Perfluorooctanoate (PFOA) and Perfluoroctanesulfonate (PFOS) are synthetic perfluorinated carboxylic acids and are persistent organic pollutants with significant reproductive, hormonal and hepatic effects. PFOA and PFOS induce hepatomegaly in rodents, mechanisms of which are not completely clear. Recent in vitro studies indicate that PFOA and PFOS can bind and inhibit HNF4α activity in hepatic cell lines. Previous studies in our laboratory have shown that inhibition of HNF4α results in hepatomegaly. Based on these data, we investigated whether loss of HNF4α function is involved in hepatomegaly induced by PFOA and PFOS. Male CD1 mice treated with PFOA (3 mg/kg) and PFOS (10 mg/kg) once a day for seven days by oral gavage exhibited significant increase in liver to body weight ratio along with increased PCNA positive cells in the liver. PFOA and PFOS treatment decreased in HNF4α protein levels in the liver without affecting its mRNA levels. Western blot analysis showed increase in Cycillin D1 protein levels upon PFOA and PFOS treatment. Further, Real Time PCR analysis of several known and novel HNF4α target genes revealed that PFOA and PFOS exposure resulted in a significant increase in the expression of pro-mitogenic genes normally down regulated by HNF4α including cdkn3, egfr1, cyp2c37, ccdn1, akt1, bcl7a, myc, and uqcrbl1. Consistent with these observations, human hepatocyte treated for 48 hours with 100 μM PFOS showed an increase in mRNA of Cdon1, Ognin1, Cdkn3, Cds3, genes that are negative targets of HNF4α and a decrease in Apoa2, Fpol1, E2 genes which are positive targets of HNF4α. Taken together, these data indicate that PFOA and PFOS-induced decrease HNF4α protein and activity, may be involved in hepatomegaly induced by these chemicals.

2207 Low-Dose Perfluorooctanesulfonic Acid (PFOS) Induces Hepatic Lipid Accumulation and Dampens Caloric Restriction-Induced Lipid Loss in Mice

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A combination of caloric restriction (CR), dietary modification, and exercise is the recommended therapy to reverse nonalcoholic fatty liver disease. The ability to mount an effective response to caloric restriction required to effectively shift hepatic metabolism to fatty acid oxidation depends upon induction of Sirtuins, AMP-activated kinase, and Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α). PFOS, a fluorosurfactant previously used as a stain repellent and anti-stick material, is persistent in the environment and considered an “emerging contaminant” by the EPA. PFOS (1-10 mg PFOS/kg/day) induced hepatic lipid accumulation associated with altered lipid metabolism gene and protein expression in mice. We hypothesized that PFOS interferes with the beneficial effects of CR on hepatic lipid utilization and glucose homeostasis. Adult male C57BL/6 mice were fed ad libitum or 25% reduced calorie diet concomitant with 100 μg PFOS/kg/day for 6 weeks. PFOS significantly increased percent body weight after 4 weeks of administration, but did not significantly alter CR-induced percent weight loss over 6 weeks. Further studies indicated that PFOS (50 nm, 1, 5, 10, and 50 μM) increased adipogenesis in 3T3-L1 cells through induction CEBPs and PPARγ. PFOS also increased hepatic triglyceride accumulation and lipid loss after CR was lower in PFOS treated mice but not associated with significant changes in lipoprotein gene expression. We also evaluated PFOS effects on glucose tolerance and found that it interfered with CR-induced improvement of glucose tolerance. This was further associated with suppression of hepatic Glut-2 and IRS-1 mRNA expression and PFOS stimulation of glucose production in isolated hepatocytes. Overall, a relatively low sub-chronic administration of PFOS had disruptive effects for lipid and glucose homeostasis under ad libitum and CR conditions.

2208 Bile Salt Transporters Are Involved in the Disposition of Perfluoroalkyl Sulphonates in Rats and Humans

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Perfluoroalkyl sulphonates (PFSAs) are persistent environmental contaminants present in human serum. Pharmacokinetic studies in animal models suggest that the long half-lives of certain PFSAs such as perfluorohexanesulfonate (PFHxS) and
perfluorooctanesulfonate (PFOS) are in part, due to strong hepatic accumulation and slow renal elimination. Studies in rats have indicated that PFOS undergoes enterohepatic circulation and in humans, cholestyramine treatment appeared to enhance its fecal excretion. Given that the sodium dependent bile acid uptake transporters such as sodium-dependent bile acid transporter (ASBT) mainly expressed at the apical membrane of enterocytes and the bile salt efflux pump (BSEP) expressed at the apical membrane of hepatocytes are essential for the enterohepatic circulation of bile acids, we tested whether PFSAs are substrates of these transporters in rats and humans. We used CHO Flp-In cells stably expressing human NTCP and HEK293 cells transiently expressing rat NTCP, as well as rat and human ASBT to measure the uptake of perfluorobutanesulfonate (PFBS), PFHxS, and PFOS in the presence or absence of sodium. In addition, SP vesicles expressing human BSEP were used to test whether PFBS, PFHxS or PFOS would inhibit taurocholate transport by human BSEP. The results demonstrate that both rat and human NTCP can transport PFBS, PFHxS and PFOS. However, human ASBT only transports PFOS while rat ASBT transports none of the PFSAs. Transport of taurocholate by human BSEP was inhibited by both PFHxS and PFOS. We further characterized transport by performing time and concentration dependent uptake studies. In conclusion, these results suggest that the long half-life and the hepatic accumulation of PFOS in humans are, at least in part, due to transport by NTCP, ASBT and BSEP.

**2209 Evaluation of 6-Week Dietary Exposure to 100 ppm Perfluorooctane Sulfonate (PFOS) and Normal Choline or High Choline in Sprague-Dawley Rats**

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Perfluorooctane sulfonate (PFOS) is a bioaccumulative and environmentally persistent chemical that has been detected in the serum of occupationally exposed workers as well as the general population. In toxicology studies, PFOS administration (at doses several orders of magnitude greater than the general population exposure) has produced hepatocellular hypertrophy, hepatocellular vacuolation, increased liver triglycerides, and decreased serum cholesterol. Many of these observations are similar to those reported in choline deficient animal models used to investigate non-alcoholic hepatic steatosis. Choline is a nutrient used as the essential phospholipid building block and as a precursor of phosphorylcholine. PFOS creates an ion complex with choline in solution and in ex vivo liver which may constitute a choline sink leading to a choline deficient phenotype. The central hypothesis is that at high doses PFOS makes an ion complex with choline in the liver and produces a choline deficient phenotype in the treated animals which may be exacerbated by increased lipid β-oxidation. To investigate this hypothesis, Sprague Dawley rats (6/sex/group) received diets containing normal choline, high choline, 100 ppm PFOS with normal choline or 100 ppm PFOS with high choline for 6 weeks. The total consumed PFOS dose achieved approximately 200 mg/kg which approximates the acute oral LD50 in rats (i.e., 251 mg/kg). As such, moribund animals, i.e. those to light and touch were noted in PFOS-exposed animals. Hepatocellular vacuolation and microscopic evidence of peroxisome proliferation were noted in PFOS-exposed animals. The hepatic vacuoles were a result of lipid accumulation based on Oil Red O staining. Choline supplementation showed a trend towards decreased hepatic lipid concentration in PFOS-treated male rats. Additional studies are needed to further investigate this potential relationship between PFOS and choline.

**2210 Perfluoroalkyl Acids in River and Sea Water of Japan**

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Environmental waters such as river water (RW) and sea water (SW) are expected to be the major exposure sources of Perfluoroalkyl acids (PFAA) to humans via tap water and food fish. At the 2013 Annual Meeting of SOT, we reported that perfluorooctanoic acid (PFOA); a chemical that has an environmental presence with uncertain human health effects. We evaluated the mortality experience of employees of an APFO manufacturing plant. Methods: This cohort mortality study followed 9,027 employees including 4,668 from a site that produced PFOS from 1947-2000 and 4,359 from a non-APFO facility in the same geographic area. Yearly APFO exposure was estimated using a job and task-based APFO exposure matrix developed from industrial hygiene monitoring data, expert judgment, and production information. APFO production workers were categorized into quartiles of cumulative exposure with those from a non-APFO plant as a non-exposed reference population. Standardized mortality ratios (SMR) and 95 percent confidence intervals (95% CI) were estimated using state reference mortality data from 1960-2008. Standardized rate ratios (SRR) and 95% CI were estimated for the exposed population and each APFO exposure quartile in comparison to the non-exposed worker population. Study was approved by Univ of MN IRB. Results: We observed 2,954 deaths (SMR = 0.93 (95% CI=0.89-0.96)). Cancers of a priori interest (Obs total, Obs expected, SRR (95% CI)) included liver (15, 8, (1.5 (0.5-4.2)), pancreatic (48, 18, [0.8 (0.4-1.5)), kidney (24, 6, [0.4 (0.1-0.9)), bladder (16, 8, [1.3 (0.5-3.6)), and prostate (72, 23 [10.6 (4.1-10.0))]. There was little evidence of an exposure response association comparing exposure quartiles to the referent population. The SRR for prostate cancer in the highest exposure category was elevated, but imprecise (obs 6, SRR=2.2 (95% CI=0.6-7.7)). The SRR for heart disease was 0.81 (95% CI=0.70-0.94), with a SRR of 1.2 (95% CI=0.6-2.2) in the highest exposure category. Conclusion: In this study, occupational exposure to APFO was not associated with a greater risk of death from all causes or specific causes of death.

**2211 Mortality Experience of APFO Production Workers**


Background: The ammonium salt of perfluorooctanoic acid (APFO) is used in fluoropolymer production. APFO dissociates in biologic media to perfluorooctanoate (PFOA); a chemical that has an environmental presence with uncertain human health effects. We evaluated the mortality experience of employees of an APFO manufacturing plant. Methods: This cohort mortality study followed 9,027 employees including 4,668 from a site that produced PFOS from 1947-2000 and 4,359 from a non-APFO facility in the same geographic area. Yearly APFO exposure was estimated using a job and task-based APFO exposure matrix developed from industrial hygiene monitoring data, expert judgment, and production information. APFO production workers were categorized into quartiles of cumulative exposure with those from a non-APFO plant as a non-exposed reference population. Standardized mortality ratios (SMR) and 95 percent confidence intervals (95% CI) were estimated using state reference mortality data from 1960-2008. Standardized rate ratios (SRR) and 95% CI were estimated for the exposed population and each APFO exposure quartile in comparison to the non-exposed worker population. Study was approved by Univ of MN IRB. Results: We observed 2,954 deaths (SMR = 0.93 (95% CI=0.89-0.96)). Cancers of a priori interest (Obs total, Obs expected, SRR (95% CI)) included liver (15, 8, (1.5 (0.5-4.2)), pancreatic (48, 18, [0.8 (0.4-1.5)), kidney (24, 6, [0.4 (0.1-0.9)), bladder (16, 8, [1.3 (0.5-3.6)), and prostate (72, 23 [10.6 (4.1-10.0))]. There was little evidence of an exposure response association comparing exposure quartiles to the referent population. The SRR for prostate cancer in the highest exposure category was elevated, but imprecise (obs 6, SRR=2.2 (95% CI=0.6-7.7)). The SRR for heart disease was 0.81 (95% CI=0.70-0.94), with a SRR of 1.2 (95% CI=0.6-2.2) in the highest exposure category. Conclusion: In this study, occupational exposure to APFO was not associated with a greater risk of death from all causes or specific causes of death.

**2212 iTRAQ Protein Profile Analysis of Mouse Tissues after Perfluorooctanoate (PFOA) Exposure**

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Perfluorooctanoic acid (PFOA) is a stable man-made compound with many industrial and commercial uses. Concern has been raised that it may induce hepatotoxicity, immunotoxicity, developmental effects and some other toxicological effects. However, the testicular toxicity of PFOA and its underlying mechanism is not well known. In this study, male mice were exposed to 0.08, 0.31, 1.25, 5 and 20 mg/kg/day of PFOA for 28 days. Absolute testis weight was diminished at the highest dose, as well as testosterone and progesterone levels in serum and testis were all increased in a dose range of 1.25,5 and 20 mg/kg/day. In addition, the seminiferous tubules were damaged in a different degree after PFOA exposure. After iTRAQ labeling combined with two-dimensional liquid chromatography and tandem mass spectrometry (2DLC–MS/MS) analysis, 93 differentially expressed proteins between the control and the 5 mg/kg/d PFOA treated mice were successfully identified. These proteins were mainly involved in cell proliferation, lipid metabolism, steroid metabolism and spermatogenesis, nucleic acid metabolism, stress response and apoptosis, and others. In addition, serum and testis cholesterol were all reduced after PFOA exposure, and the mRNA and protein expressed levels of genes involving in cholesterol transport and testosterone synthesis were all inhibited by PFOA. Thus, we speculated that the decrease of serum cholesterol might play an important role in testicular toxicity of PFOA.
2213 Effects of Perfluorooctanoate (PFOA) and Perfluorooctanesulfonate (PFOS) on Cholesterol Efflux in Macrophage-Derived Foam Cells

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Cholesterol metabolism and efflux from peripheral cells, such as macrophages and foam cells, is a critical part of reverse cholesterol transport (RCT), a metabolic process that has implications for atherosclerosis and cardiovascular diseases. There have been several conflicting studies that suggest environmental and occupational exposure to Perfluorooctanoate (PFOA) and Perfluorooctanesulfonate (PFOS) affects RCT and cholesterol metabolism. However, the data from animal studies and from examination of potential mechanisms of action raise questions as to whether there is a direct causality of PFOA and PFOS exposure and hypercholesterolemia. In these studies, we have directly examined the effects of PFOA and PFOS on cholesterol efflux and in genes involved in this process. PFOA and PFOS are agonists for nuclear receptors that increase cholesterol efflux, such as the peroxisome proliferator-activated receptors (PPARs). Importantly, in THP-1 differentiated into macrophage-derived foam cells, PFOA and PFOS increased cholesterol efflux to either HDL or apoA. Comprehensive gene expression analysis by microarray is being pursued to determine the key genes being regulated by these perfluorinated compounds in foam cells that impact RCT. These studies will also assist in the identification of genes that are sensitive to PFOA and PFOS exposure that can be utilized in biomonitoring studies. Taken together, these studies suggest that PFOA and PFOS exposure affects cholesterol efflux, but not in a manner consistent with a negative health impact.

2213a Tetramethylbisphenol A versus Bisphenol A: Excitation-Contraction Coupling Impairment through Divergent Mechanisms

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Bisphenol A (BPA) and its brominated derivative tetramethylbisphenol A (TBBPA) are high production volume chemicals utilized in the manufacture of various consumer products. While these chemicals share the same chemical backbone, the halogenation on TBBPA may produce activity on a distinct set of molecular targets versus BPA. The targets we investigated are those involved with skeletal muscle excitation-contraction (EC) coupling and relaxation, namely 1) the dihydropyridine receptor (DHPR), 2) the type 1 ryanodine receptor (RyR1), and 3) the sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA). TBBPA indeed had concentration-dependent interactions with these targets: 1) it inhibited specific [3H]ryanodine binding to RyR1, achieving >1500% of vehicle control at 30 μM, and induced rapid efflux of Ca2+ from actively loaded microsomes; 3) it inhibited the biochemical activity of SERCA with an IC50 of ~13.3 μM. TBBPA did not modify baseline Ca2+ levels, and induced rapid efflux of Ca2+ from actively loaded microsomes; 2) it elicited an increase in specific [3H]ryanodine binding to RyR1, achieving >1500% of vehicle control at 30 μM, and induced rapid efflux of Ca2+ from actively loaded microsomes; and 3) it inhibited the biochemical activity of SERCA with an IC50 of ~13.3 μM. In contrast, BPA had no influence on specific [3H]PN200 binding, SERCA activity, and Ca2+ efflux. However, surprisingly, both TBBPA and BPA impaired EC coupling in primary skeletal myotubes. In field stimulated myotubes loaded with the Ca2+ indicator fluo-4, 10 μM TBBPA rapidly diminished evoked Ca2+ transient amplitudes, achieving complete transient abrogation within 15 min of exposure, and increased baseline intracellular Ca2+ levels. BPA did not modify baseline Ca2+ levels, and while BPA did diminish Ca2+ transient amplitudes, complete abrogation was not achieved. These results indicate that while TBBPA is able to potently modify Ca2+ dynamics in myotubes via interactions with DHPR, RyR1 and SERCA (which are indeed detrimental to muscular function), such interactions are not necessary for impairing skeletal EC coupling. The mechanism(s) by which BPA impairs skeletal EC coupling warrant further investigation. Sponsored by NIH P42 ES06099, P01 11269, U.S. EPA R833292, R829388, T32 HL 86350, and T32 ES 7099.

2213b An Evaluation of Gestational Exposure to Perfluorooctanoic Acid (PFOA): Effects on Body Composition and Physiological Factors


Exposure to environmental pollutants can be a factor for induction of metabolic disorders. This study examined if exposure to PFOA during development could alter body composition and other physiological outcomes. Study 1: Pregnant CD-1 mice were gavaged with BPA at 0, 0.001, 0.01, 0.1, or 0.3 μg/kg body weight (bw) from gestation day (GD) 1 – 17. At weaning, pups were fed a high fat (HFD) or control (CD) diet. Body composition, blood pressure (bp), and gene expression in tissues of offspring were examined. Male- BW increased in 0 mg PFOA+HFD vs 0 mg PFOA+CD and 0.01 mg PFOA+HFD vs 0.01 mg PFOA+CD. In HFD, bw decreased in 0.3 vs 0 mg PFOA. There were no effects on percent of body fat. At postnatal day (PND) 90, diastolic bp was decreased in 0.1 and 0.3 mg PFOA+HFD vs 0 mg PFOA+HFD and increased in 0.3 mg PFOA+HFD vs 0.3 mg PFOA+CD. The bp effects of 0.1 mg PFOA+HFD persisted to PND 180. Female- At 0 and 0.001 mg PFOA+HFD had increased weight gain vs CD. The %fat increased in 0.001 vs 0 mg PFOA+HFD. At PND 180, diastolic bp decreased in 0.01 and 0.3 mg PFOA+CD vs 0 mg PFOA+CD. Differential gene regulation was produced by HFD and PFOA in white fat and liver at 52 weeks of age. At 0.01 mg PFOA+HFD vs 0.01 mg PFOA+CD, 3 genes in white fat and liver were under-expressed while 14 genes in white fat and 19 in liver were over-expressed. At 0.01 mg PFOA+HFD vs 0.01 mg PFOA+CD, 3 genes in white fat and 4 genes in liver were under-expressed while 14 genes in white fat and 15 in liver were over-expressed. Study 2: Pregnant mice were fed NIH-31 (low phytoestrogen) diet and dosed with PFOA at 0.01, 0.1, or 1 mg/kg from GD 1 – 17. Pups remained on the NIH-31 diet. There were no age- or dose-related differences in bw or %body fat in either sex. In summary, exposure to PFOA and HFD produced sex-dependent changes in bw, body composition, and bp response in mouse offspring exposed to the chemical during perinatal development. This abstract does not necessarily reflect USEPA policy.

2214 Cardiovascular Effects of Lead, Inorganic Mercury, and Methylmercury: Potential Risk to Humans

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Humans are exposed to lead, inorganic Hg and methylmercury in the environment. The European Food Safety Authority (EFSA) estimated average human exposures of 0.68 μg Pb/kg-bw/d, 0.08 μg Hg(II)/kg-bw/d and 0.039 μg MeHg/kg-bw/d. Epidemiological studies show an association between exposure to Pb or Hg and hypertension. The study objective was to explore cardiovascular effects of sub-chronic Pb or Hg exposure in rats to determine NOAELs for human health risk assessments. Male rats (n=3-6) were exposed for 4 weeks to lead acetate (Pb: 4 – 45000 μg/kg-bw/d), mercuric chloride (Hg(II): 7 – 4000 μg/kg-bw/d) or mono-methylmercury chloride (MeHg; 4 – 1607 μg/kg-bw/d) in the drinking water. Echocardiography, carotid artery ultrasound and intra-arterial blood pressure were performed after 4 weeks.

Dose-response curves for most cardiovascular effects were U-shaped. Blood pressure was significantly increased for Pb ≥ 2000 μg/kg-bw/d (Δ20 mmHg systolic) as well as for MeHg ≥ 7 μg/kg-bw/d (Δ40 mmHg systolic and diastolic), while Hg(II) had no effect on blood pressure. MeHg ≥ 7 μg/kg-bw/d also increased pulse pressure and heart rate, while Pb and inHg had no effect. Pb ≤ 57 μg/kg-bw/d decreased blood pressure and increased carotid diameter (vasodilation) with compensatory increase in stroke volume. In contrast, Hg(II) ≤ 29 μg/kg-bw/d reduced carotid diameter (vasoconstriction) with compensatory decrease in stroke volume, while MeHg showed no effect.

Cardiovascular responses to Pb, Hg(II) and MeHg were U-shaped, but both Pb and MeHg increased blood pressure at higher doses. Based on the increased blood pressure response, NOAELs (safety factor of 100) for humans were estimated as 0.57 μg Pb/kg-bw/d, 0.29 μg Hg(II)/kg-bw/d and 0.04 μg MeHg/kg-bw/d. Thus, risk of cardiovascular disease might be increased by Pb in the general European population and by MeHg for high fish consumers.

2215 Stainless Steel Leaches Nickel and Chromium into Foods during Cooking

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Toxicological studies show that oral doses of nickel and chromium can cause cutaneous adverse reactions such as dermatitis. Additional dietary sources, such as leaching from stainless steel cookware during food preparation, are not well characterized. This study examined stainless steel grades, cooking time, repetitive cooking cycles, and multiple types of tomato sauces for their effects on nickel and chromium leaching. While a dose of only 67 μg of nickel was associated with cutaneous reactions in 40 percent of nickel sensitive participants, we found depending on cooking conditions 88 to 1,000 μg Ni per serving of tomato sauce cooking in stainless steel. Like nickel, we found chromium leached from stainless steel into tomato sauce, from 86 to 900 μg Cr in a single serving of tomato sauce. All tomato sauce samples that were cooked in the presence of stainless steel using typical cooking conditions.
procedures showed significantly elevated Ni and Cr concentrations. In addition to other natural dietary sources, stainless steel cookware can significantly contribute to overall nickel and chromium consumption.

2216  Oxidative Stress and Heavy Metal Toxicity in Work Place Exposure to Heavy Metals in Nigeria

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Heavy metals contamination of the environment is of major concern because of their toxicity and threat to human life and the environment. Studies have shown that the air in the work environment usually contains a number of chemicals, which when inhaled and absorbed by the body, pose a potential risk for workers’ health. Though, most metals are essential for proper functioning of the human biological system, humans are not adapted to dealing with them at concentrations higher than the reference values.

Presence of elevated concentrations of heavy metals can result in induction of oxidative stress via production of reactive oxygen species or reduction of antioxidant defenses, disruption of calcium homeostasis, induction of DNA damage, interaction with sulphydryl groups, disturbances in the heme biosynthetic pathway, and some are known human carcinogens.

Accumulating evidence also indicates that workplace exposure leading to generation of free radicals, accompanied by inadequate antioxidants in the body leading to oxidative stress on the increase. This study assesses workplace exposure to heavy metals in thirty-seven metal workers and thirty-six age and sex matched office workers serving as controls. Ten (10) ml of venous blood was collected from each subject into lithium-heparin bottle and this was used to determine Total Plasma Peroxides (TPP) by the FOX2 method while Cadmium (Cd), lead (Pb), zinc (Zn), Manganese (Mn), Ferritin were determined by atomic absorption spectrophotometer. There was significant increase p<0.05 in Lead (0.0159 ± 0.0029 µg/L and Mn (31.92 ± 7.79 µg/dl in the exposed group than the controls and significant positive correlation between TPP and Pb. Significant decrease p<0.05 in Ferritin (56.38 ± 8.22 µg/L in the exposed was also observed. Occupationally exposed workers are prone to heavy metal toxicity with increased free radical generation which may lead to increased chronic disease manifestations and even cancers. Increase intake of antioxidant supplements may be beneficial as well as using safety/protection apparels.

2217  Gene Expression Profiling in Saudi Individuals Exposed to Environmental Heavy Metals

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The aim of this study was to evaluate the effects of long-term exposure to environmental heavy metals on the gene expression profile of individuals live near to mining areas at Mahd Ad-Dahab region, Saudi Arabia. For this purpose, microarray analysis was conducted in an attempt to evaluate the alteration of gene expression profile of gene-regulated diseases and to identify potential biomarkers that may be involved in the response to heavy metals toxicity. Sixty healthy volunteers were divided to two groups; exposed group consisted of 40 male residents in the heavy metal-polluted area, whereas the control group consisted of 20 male residents in a non-polluted area (Riyadh). Our results showed that the plasma levels of Pb, Cd, and Hg were found to be significantly high in the heavy metal-exposed group. Total RNA was isolated from whole blood using PAXgene Blood RNA tubes and reversed transcribed and hybridized to the gene array using the Affymetrix U219 GeneChip. Microarray analysis identified 425 genes that are differentially expressed in heavy metal-exposed groups. The alteration of many genes of interest revealed by microarray analysis suggests that these genes play a role in heavy metal-induced toxicity. Some of these genes were confirmed by RT-PCR analysis. The commonly significant differentially expressed genes in the heavy metal-exposed groups were analyzed for network, functional, canonical pathways, and upstream regulators using the Ingenuity Pathway Analysis (IPA) software. Renal and urological diseases, developmental disorders, and hematological diseases were the most significant diseases that are associated with heavy metal-exposed groups. The following genes participate in renal disease induction (such as SNRPG, UQCRB and AIF1) could be used as early biomarkers for renal toxicity. IPA also indicated that MYC might be a sensitizer, corrosive or irritant.

2218 Are Metals in Rolling Tobacco Less Risky Than Cigarettes?

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Smoking is a major public health problem and an important source of exposure to toxic elements, including metals. The presence of metals in tobacco depends mainly on where the tobacco was grown and the production process.

Objectives: a) to quantify the concentrations of metals (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Si, V, Zn) in 23 brands of rolling tobacco and to compare them with the concentrations previously found in cigarettes, b) to compare metals levels across different brands and producers, and with/without additives.

Materials and Methods: 34 samples from 23 different brands of rolling tobacco sold on the island of Tenerife (Spain) were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

Results: Mean concentrations of metals in rolling tobacco were (mg/kg): Al (207.97); B (9.20); Ba (31.91); Ca (9189.74); Co (0.23); Cr (0.62); Cu (8.65); Fe (167.23); K (9767.13); Li (14.66); Mg (2257.74); Mn (56.29); Mo (0.09); Na (458.99); Ni (0.75); Si (644.28); V (0.29); Zn (17.45). Mean levels in rolling tobacco with/without additives were (mg/kg): Al (223.3/181.7); B (8.499/7.735); Ba (28.97/21.50); Ca (829/817873); Co (0.196/0.182); Cr (0.793/0.259); Cu (8.619/9.524); Fe (164.5/141.8); K (7567/10260); Li (19.05/13.69); Mg (1945/2449); Mn (42.54/53.71); Mo (0.075/0.171); Na (497.3/497.1); Ni (0.776/0.638); Si (649.7/629.5); V (0.358/0.434); Zn (12.92/20.12). Average Cd levels (mg/kg) were 0.396 (range 0.129–0.035); levels with/without additives were 0.2080/0.484. Average Pb levels (mg/kg) were 0.327 (range 0.131–0.759); levels with/without additives were 0.3390/0.326.

Conclusions: The levels of Al, Cr, Co, Mn, Ni, Pb and Cd in rolling tobacco are roughly half of those previously detected in tobacco cigarettes (mg/Kg; Al (428); Pb (0.56); Cr (1.442); Mn (112.026); Ni (2.238); Cd (0.810); and Pb (0.602).

The objective of this study is to identify the metals that are not well known in the production of rolling tobacco does not affect the level of metals significantly.

2219 Evaluation of Electric Arc Furnace-Processed Steel Slag for Dermal Corrosion, Irritation, and Sensitization

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Electric arc furnace (EAF) steel slag is alkaline (pH of ~11-12) and contains metals that are known dermal sensitizers, most notably chromium and nickel. As such, EAF slag has potential to cause dermal irritation and sensitization at sufficient dose, but no published study has characterized the potential for dermal effects associated with EAF slag. To assess dermal irritation, corrosion, and sensitizing potential of EAF slag, in vitro and in vivo dermal toxicity assays were conducted following Organisation for Economic Co-operation and Development (OECD) guidelines. In vitro dermal corrosion and irritation testing (OECD 431 and 439) of EAF slag was conducted using human epidermal model. In vivo dermal toxicity and delayed contact sensitization testing (OECD 404 and 406) were conducted in rabbits and guinea pigs. Chemical properties of EAF slag were also characterized. Calcium, iron, silicon and magnesium oxides were the major constituents of EAF slag, each contributing more than 10% of the slag composition. Other elements measured in the EAF slag at higher concentrations were manganese, aluminum and chromium, but nickel was measured at relatively low concentrations. Barium and chromium were the only constituents detected in the TCLP extraction leachate, and they were measured concentrations of 0.798 and 0.250 mg/l, respectively. EAF slag was not corrosive and not irritating in any in vitro and in vivo dermal toxicity tests. The results of the delayed contact dermal sensitization test indicate that EAF slag is not a dermal sensitizer. These findings are supported by the observation that metals in EAF slag occur as oxides of low solubility with leachates that are well below TCLP limits. Metals in slag are tightly fused to the physical matrix limiting the mobility of metal constituents that may be of concern for dermal effects. Based on these results and in accordance to the OECD guidelines, EAF slag is not considered a dermal sensitizer, corrosive or irritant.
Butte, Montana, a town of approximately 35,000 residents, is a part of the largest superfund site in the United States. One of the unique aspects of Butte’s history is that surface mining and smelting activities occurred, and continue today, in close proximity to Butte’s historically dense urban population. This has resulted in homes and neighborhoods in Butte with high concentrations of a variety of toxic metals (and metalloids) including arsenic, lead, copper, zinc, mercury and cadmium. Therefore it is likely that people living near areas of active mining, or the numerous areas of uncovered smelter wastes, may have been chronically exposed through air, water or soil to this suite of potential toxins. In this study, volunteers were recruited based upon mapping and demographic data to determine if potential chronic exposure to these metal mixtures could lead to changes in miRNA expression patterns. Human hair and nasal cell samples were collected from individuals of various ages, living within close proximity to current surface mining as well as near historic mine tailings (n=16). Control samples were collected from individuals living in Bozeman, MT (n=8) a town without historical or current mining practices. Elemental analysis was performed on the hair samples by ICP-MS and several metals were found to be elevated in the Butte population, including copper and molybdenum. Preliminary miRNA data show a number of up-regulated micro RNAs that are putative oncomirs (mir-93-5p, mir-106b-5p). There is also a down-regulation in the Butte population of miRNAs known to participate in regulation of inflammatory responses (mir-146b-5p and mir-146a-5p). These preliminary data illustrate the need for additional study and data collection of samples from individuals living downwind of an active open-pit copper mine.

**2221 Initial Characterization of Metal Exposures in Community Residents Living Adjacent to the Black Leaf Pesticide Manufacturing Complex**

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Black Leaf is an abandoned industrial site in Louisville, KY with exceptionally high-level soil contamination by organochlorine pesticides (OCP) – exceeding the Residential Screening Level (RSL) by up to 18,000-fold. However, the site is also contaminated to a lesser degree with arsenic, lead, and polycyclic aromatic hydrocarbons. Black leaf is surrounded by the Park Hill Neighborhood, and in 2013 EPA began remediating residential yards with soil contamination. The aim of this study was to determine if adult Park Hill residents living adjacent to Black Leaf had elevated levels of arsenic and lead; and to determine if levels correlated with soil mapping and pro-inflammatory cytokine elevation. Blood lead and creatinine (Crea) nine-adjusted random urine arsenic levels were determined by inductively coupled Plasma/Mass (ICP/MS). Serum pro-inflammatory cytokines were measured by Luminex analysis. The mean age of voluntary participants (n=53) was 52±14.6 years with 18.6±19.4 years of residence near Black Leaf. Participants were predominately female (66.0%) and African American (79.2%). Two participants living adjacent to an on-site arsenic “hotspot” had very high urine arsenic levels (175 and 60 mcg/g creat). These residents also had marked elevation in serum IL-8 levels.<ref>2222 Influence of Light/Dark Condition on Bioaccumulation and Toxic Severity of Heavy Metals in Mice**


We recently reported that cadmium (Cd)-induced mortality was markedly different by injection timing meaning light/dark cycle affect the severity of Cd-induced toxicity. In this study, we report further evidences for the diurnal variation of toxicities induced by heavy metals such as chromium and nickel in mice. Male C57BL/6J mice adapted for 14 days with assigned to 6 groups of 5 animals were administrated intraperitoneally (i.p.) with potassium dichromate (50 mg/kg) or nickel sulfate (60 mg/kg) at different hours in the day (10:00, 14:00, 18:00, 22:00, 2:00 and 6:00 h), describing as zeitgeber time (ZT); ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22. The mortality was observed until 14 days after the injection. As a result, mice were more sensitive to Ni during light phase (ZT6) while more resistant during dark phase as same as the result of Cd. In case of Cd, however, mice were more sensitive at late-night to mid-dark phase (ZT10 to ZT18). Therefore, 1) mice had clear diurnal variation to metal-induced toxicity, and 2) the daily fluctuation of metal sensitivity differed depending on metal compounds, probably depending on the valence of metal ion. We further investigated that Cd accumulation in the liver was significantly rose at light-phase exposure compared to dark-phase exposure. Our results clearly indicate that light/dark condition affects on severity of metal-induced toxicity and metal bioaccumulation.
Electronic cigarettes (EC) are novel nicotine delivery devices. Based on our earlier data, we hypothesized that new models of EC will vary in their performance characteristics and have heavy metals in their aerosol. We investigated this hypothesis using 10 brands of cartomizer EC and three approaches that included dissections, performance, and elemental analysis of EC aerosols. All 10 brands had the same basic design components, which consisted of wires, solder joints, air-tubes, a mouthpiece, fluid, and fibers. Four brands had been used prior to packaging, while six were in pristine condition. The flow rate required for aerosol production ranged from 4–21 mL/s. During “smoke out”, flow rate was constant for 5 brands, but had to be increased periodically to produce aerosol in 5 brands. Within the brands, pressure drop was relatively stable, but varied between brands (12-330 mm H2O). The elemental composition of EC aerosol and smoke from conventional cigarettes was compared using inductively coupled plasma optical emission spectroscopy. Of 62 elements screened, 21 were present in EC aerosols. Eight (Al, Ba, Na, Pb, Si, Sr, Zn) were, in general, less abundant in EC aerosol than in conventional cigarette smoke. In contrast 12 (Ag, Ca, Cr, Cu, Fe, Mg, Mn, Ni, Se, Sn, Ti, Zn) were more abundant as much silicon as the conventional brands and a different leading brand of EC had about 3.4 times as much tin in its aerosol than in smoke. One major selling brand had about 500 times as much tin in its aerosol than the conventional cigarette smoke.

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In Vietnam, pollution caused by small-scale smelting of automobile batteries into lead ingot is a growing concern. The smelting craft village of Ngïa Lo, Vietnam, has attracted the attention of health and environmental experts due to its extremely high lead levels in water, soil, dust, and foods. Despite the threat of toxic metal exposure, biomonitoring among susceptible populations, such as children, has not been previously conducted. In this pilot study, twenty children from Ngïa Lo ages 18 months to four years were selected for capillary whole blood and toenail biomonitoring. Blood lead levels and toenail levels of arsenic, cadmium, chromium, lead, manganese, and mercury were measured. All 20 children had detectable levels of blood lead and every child had levels that exceeded the CDC guideline level of 5 μg/dL. Eighty percent of tested subjects had blood lead levels higher than 10 μg/dL. Five children (25%) had lead levels greater than 45 μg/dL, the level of recommended medical intervention. Average toenail lead, manganese, and mercury levels were 157, 7.41, and 2.63 μg/g, respectively, well above levels previously reported in children. Significant correlations were found between toenail metal levels including chromium and manganese and lead and cadmium (r>0.65, p<0.001). Linear regression showed that closer residential distance to a smelter was associated with increased blood lead levels (p<0.07). Results support the urgent need to control toxic metal exposure sources related to domestic smelting and seek treatment for children residing in these villages with the highest blood lead levels.

The heavy metal residues of Fe, Zn, Cu, Ni and Mn were assessed in three food crops, barley Hordeum Vulgar (grain crop); broad beans Vicia faba (legume) and rape seed Brassica napus (oil crop) irrigated with municipal wastewater. Results showed that Mn and Ni were significantly higher at all crops irrigated by municipal wastewater. However, the effect of municipal wastewater irrigation was insignificant on Zn and Cu residues when compared to groundwater irrigation. The residues of Fe and Mn in straw of all wastewater irrigated crops were significantly higher than in grain and seeds. However, there were no significant differences between the residues of the heavy metals Cu, Zn and Ni in all crops irrigated by municipal wastewater or groundwater. The study approved the need of proper regulations for disposal, recycling and application of municipal wastewater due to agricultural lands.
2230 Novel Oral Detoxification of Mercury, Cadmium, and Lead with Thiol-Modified Mesoporous Silica

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Thiol modified mesoporous silica (SH-SAMMS) was developed as an oral drug for the prevention and treatment of heavy metal poisoning. SH-SAMMS was first evaluated for the removal of methyl mercury (MeHg(II)), inorganic mercury (Hg(III)), lead (Pb(II)), and cadmium (Cd(II)) in terms of binding capacity and rate in synthetic matrices mimicking conditions of gastric and intestinal fluids. SH-SAMMS was found to be superior to SAMMS with carboxylic and phosphonic acid based ligands as well as other commercially available chelating sorbents. Furthermore, SH-SAMMS could effectively remove soluble mercury from digested fish without significantly affecting the levels of most essential minerals found in fish. SH-SAMMS retained Hg(II) and MeHg(I) tightly inside the nano-size pores, preventing micron-size bacteria from converting the mercury species to more mobile forms. Rats fed with diet containing MeHg(I), Cd(II), and Pb(II) and SH-SAMMS for two weeks had blood Hg levels that were significantly lower than rats fed with the metal rich diet containing metals only. Upon cessation of the metal rich diet, continued administration of SH-SAMMS for two weeks facilitated the clearance of Hg faster and greater than those not fed with SH-SAMMS. Rats receiving SH-SAMMS were also protected from body weight loss associated with the metal poisoning. Retention of Hg and Cd in major organs was lowest in rats fed with SH SAMMS throughout the entire study of 4 weeks. For Pb, while its retention in most tissues of rats was negligible (with the exception of bone), the reduction of blood Pb by SH-SAMMS, but not more than that of rats receiving metal diets alone, was observed due to SH-SAMMS, but not more than that of rats receiving metal diets alone.

A child’s blood Pb assay is often the first realization that exposure to environmental Pb has occurred. A critical gap is an investigative protocol that preempts harmful childhood Pb exposure. Mapping soil Pb and blood Pb evokes a novel approach. New Orleans was split into high (≥100 mg/kg) and low (<100 mg/kg) soil Pb census tracts (CT). Four soil sample locations, busy streets, residential streets, house sides, and open spaces (away from streets and houses), represent each CT. Soil and blood Pb concentrations within the high and low Pb census tracts were analyzed by multi-response permutation procedures (MRPP) and p-Values were <0.05. Pre-Katrina children’s blood Pb ≥ 5 µg/dL in high and low Pb CTs decreased to 29.6% and 7.5%, respectively. In high Pb CTs, median soil Pb for busy streets, residential streets, house sides, and open space sample locations were 367, 313, 1228, and 103 mg/kg, respectively; in the low Pb CTs median soil Pb for busy streets, residential streets, house sides, and open spaces were 64, 46, 32, and 28 mg/kg, respectively. The best predictor of children’s blood Pb is residential street-side samples systematically collected throughout residential New Orleans. Soils in high Pb CTs lack a margin of safety. Low Pb CTs were safer by factors ranging from 3 to 38 depending on soil sample location. Patterns of lead from decades of Pb deposition were not worsened by post-Katrina renovations. Contrarily major improvements are materializing in selected communities. Low Pb soils are available at the city outskirts to remedy Pb contamination. All large cities probably present similar characteristics as New Orleans. Soil Pb maps are a diagnostic tool that assist with comprehending and promoting proactive childhood Pb exposure prevention at the community scale. Funding support by U.S. HUD (Grant # LALT0002-11) to Tulane University.

2231 Vulnerability of Frontal Cortex to Chronic Manganese Exposure in Welders

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Historically the basal ganglia were thought to be the main target of manganese (Mn) neurotoxicity. Recent evidences show significant effects of Mn exposure on the frontal cortex. The aim of this study is to test the vulnerability of the frontal cortex in Mn-exposed welders.

29 welders (14 from China and 15 from the U.S.) and 33 age- and gender-matched controls (23 from China, 10 from the U.S.) were recruited. Each subject underwent the same magnetic resonance imaging and spectroscopy exams. MR spectra were acquired from the frontal cortex, thalamus, motor cortex, and striatum in the U.S. cohort, while from the frontal cortex, thalamus, hippocampus and posterior cingulate cortex (PCC) in the Chinese cohort. Brain metabolites were quantified including myo-inositol (mI), glutamate (Glu), creatine (Cr). A series of high resolution inversion recovery images and multi-echo gradient echo images were acquired to calculate T1 and T2* values, which quantitatively measure brain Mn and Fe levels, respectively. Metabolic changes, T1 and T2* values of the same brain regions were compared between welders and controls within each cohort.

Both welder groups showed significantly reduced T1 values in many brain regions including the frontal cortex, and significantly reduced T2* values only in the frontal cortex. Chinese welders showed significant changes in Glu, Cr and macromolecules in the frontal cortex, mI in the thalamus and PCC, no changes in the hippocampus, while U.S. welders also showed more changes in the frontal cortex, without any changes in the other regions.

Overall, the frontal cortex of both welder groups showed more metabolic changes and Fe deposition assessed by reduced T2* values, indicating the frontal cortex may be particularly vulnerable to Mn exposure in welding fumes (Supported by NIH/NIEHS #R01 ES052059, National Science Foundation of China Grant #8107320).

2232 Measuring Community Lead (Pb) Toxicity: Filling the Prevention Gap to Advance from Reactive to Proactive Medicine


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The effect of Seasonal variation on the concentration of seven metals: cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) was evaluated in water, two species of most consumed fishes ( Tilapia nilotica & Clarias lazera), an aquatic plant ( Ceratophyllum demersum L), and soil sediments in Wadi Al Raiyan lakes, El-Fayoum, Egypt. Samples were collected in summer and winter 2010-2011. Metals concentrations were measured by Atomic Absorption Spectrophotometry.

2233 Correlation of Blood Cr (III) and Adverse Health Effects: Application of PBPK Modeling to Determine Nontoxic Blood Concentrations


Chromium (Cr) is a trace metal essential to human health and exposure typically occurs via the diet on a daily basis. Some groups of individuals, such as those consuming Cr(III) supplements or patients with Cr-containing implants, may have elevated blood Cr(III) concentrations. Although blood Cr(III) concentrations are thought to be an accurate metric of exposure, little is known about the relationship between these concentrations and possible adverse health risks. This study evaluated the various effects reported in animal and human epidemiological studies of Cr(III) exposure in an effort to correlate them with blood Cr(III) concentrations. The target endpoints identified in this analysis included the hematological, hepatic, and renal systems. Animal and human physiological based pharmacokinetic (PBPK) models were used to estimate steady state blood Cr(III) concentrations from a variety of dosing regimens. Our results suggest that blood Cr(III) concentrations as high as 480–580 µg/L are not associated with any responses. For each of the three health endpoints considered in this analysis (hematological, hepatic, and renal) no adverse effects were observed below 3,700 µg/L. Some hematological responses were observed at 3,700 µg/L, and adverse effects clearly occurred at 7,500 µg/L. These findings can be used to assess potential health risks to individuals with elevated blood Cr(III) concentrations.

2234 The Effect of Seasonal Variation on the Concentration of Metals in Water, Fish, Aquatic Plant, and Soil Sediment in El-Fayoum, Egypt

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The effect of Seasonal variation on the concentration of seven metals: cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) was evaluated in water, two species of most consumed fishes ( Tilapia nilotica & Clarias lazera), an aquatic plant ( Ceratophyllum demersum L), and soil sediments in Wadi Al Raiyan lakes, El-Fayoum, Egypt. Samples were collected in summer and winter 2010-2011. Metals concentrations were measured by Atomic Absorption Spectrophotometry.
Manganese (Mn) is a naturally occurring element, essential in trace amounts for living organisms, but is potentially toxic in high concentrations. Certain occupations including mining, welding and steel manufacturing can expose workers to chronically high levels of airborne Mn, leading to a clinical condition known as Manganism, which has Parkinson-like symptoms. Recent studies report excess dietary Mn can impair immune and reproductive functions in birds. Previously we showed Mn is present in some commercially available fertilizers. We hypothesized plants grown in soils high in Mn or supplemented with fertilizers containing Mn will accumulate Mn from the soil and that use of fertilizers with high concentrations of Mn will increase the accumulations, possibly creating a situation where animals and people ingesting the fruits and vegetables might be subjected to elevated Mn levels. This work was supported by grants 0516041071 of NYSDOE and 0622197 of the DUE Program of NSF.

Hundreds of pollutants are discharged into the environment every day. Of these, heavy metals are regarded as serious pollutants of the aquatic environment because of their environmental persistence and tendency to be concentrated in aquatic organisms. Abandoned mines are an important global concern and continue to pose potential threats to human health including environmental damage. There is not any specific regulation for mining wastes in Turkey and this situation puts the mining wastes into the dangerous category. Therefore, this study focuses on the potential causes of autoimmune disease (AD) as a follow-up to information generated from the total DINEH sample (N=1,304). Statistical modeling and geospatial data of abandoned U wastes and participants’ homes indicate an association between EU exposures and self-reported AD. To investigate immune system responses, we determined lymphocyte subpopulations and measured serum cytokines using flow cytometry. Whole blood samples were used to identify T cells (CD3+), T helper (CD4+), T cytotoxic (CD8+), B cells (CD19+), natural killer (NK) cells, and HLA-DR+ activated immune cells. Pro- and anti-inflammatory cytokines, as well as antimicrobial and specific autoantibodies, were measured in serum using enzyme-linked immunosorbent assays (ELISA). Regression modeling indicates that DINEH participants who reported increased EU exposure had increased proportion of activated T cells and decreased proportion of B cells and other antigen-presenting cells. Such cellular changes can lead to lower production of protective antibodies against invading pathogens. Alterations in cytokine production indicated an inflammatory response. These findings indicate altered immune response among the participants related to EU exposure.

Hundreds of workers with values more than 400 μg l−1 were invited on our clinic for mobilization treatment. We found important to monitor the exposed group regularly. We recommend to establish a regular medical control of exposure level.
Evaluation (BASE) database has been used extensively for comparative background values for indoor air in commercial buildings for risk assessments and soil vapor intrusion assessments. EPA’s BASE study sampled 100 buildings with a total of 298 sample sets. For this comparative study we collected indoor air samples from 37 sites and compared these data to the background values found in the BASE study. We collected a total of 794 indoor air sample sets that were analyzed for volatile organic compounds using EPA Method TO-14A and TO-15. The BASE study used a combination of two sample collection methods: 6 liter SUMMA canisters and multi-tube sorbents. This study utilized only SUMMA canisters and all samples were sent to a contract laboratory for analysis. The BASE method reporting limits ranged from 0.2 to 7.0 µg/m3. Our data set was then compared to the minimum, maximum, means, and the 25th, 50th, 75th, 90th, 95th and 99th percentile values in the BASE data set. Our results show that although similar to the background dataset there were enough differences to conclude that the "background" dataset is limited and should be expanded to include additional data. This additional data is readily available through State programs that have been collecting indoor air samples through a variety of programs including soil vapor intrusion assessments. This expanded dataset would be more reflective of actual background; allowing further evaluation of the data sets for more accurate risk assessment of the human population that it is being applied to.

When ocean water contaminants threaten the shoreline, risk to public health from contaminant exposure during recreational beach use must be evaluated and managed. This study was designed upon the assumption that users, including both skin surface area exposed and duration of contact. Currently, there are almost no data of this type available. The objective of this project is to determine the extent of water contact by the public during recreational beach use and how contact is influenced by weather and water conditions. Permanently mounted, high resolution video cameras are used to capture subjects' behavior on a public beach in Florida following an IRB approved protocol. Because the study involves passive observation of individuals in public, consent of subjects is not required. Video files are evaluated by an analyst post-hoc (i.e., not in real time). Potential subjects are restricted to those who spend their entire time on the beach within the field of view of the cameras so that the full extent of water contact during the day can be measured. Subjects are classified by gender and approximate age (adult, child, toddler) based upon appearance and are assigned a subject number. From the group of potential subjects identified for each study day, a subset is randomly selected for quantitative analysis. For each subject, the approximate extent and time of immersion are determined in time steps from the video files. The skin surface area exposed is assumed based upon age/gender classification and median body weight and surface area data from NHANES. An exposure time and average skin surface area in contact with water for the day is calculated for each subject. Ambient air and water temperatures are also recorded and will be used in a later phase of the study to develop a predictive model of water exposure on the beach as a function of weather conditions. Information derived from this study will be of value in risk assessment for estimating exposure to water borne contaminants on public beaches.

Exposure to the polynuclear diphenyl ethers flame retardants (PBDEs) causes concern for carcinogenicity, neurotoxicity, reproductive toxicity, and thyroid toxicity. Hydroxylated PBDE metabolites (HO-PBDEs) are of increasing toxicological interest because of their greater biological activity compared to parent compounds. Little is known about the extent and patterns of HO-PBDEs during pregnancy, especially in populations susceptible to heritable neurodevelopmental disorders. In this study, ExpoCast predicted emissions of 74 chemicals found in 32 flooring materials included a range of natural and synthetic floor coverings, upholstery, and articles of clothing. Depending on their physicalchemical properties, they may bioaccumulate in the indoor environment at higher magnitudes than in the outdoor environment, which is correlated with high indoor exposure rates. Halogenated flame retardants, such as polybrominated diphenyl ethers, are semi-volatile organic compounds (SVOCs) that are potentially harmful to humans. In this study, ExpoCast predicted emissions of 74 chemicals found in 32 flooring materials for which gas phase concentrations were measured by Wilke et al. (2004).1 Flooring materials include a range of natural and synthetic floor coverings, installations, and adhesives. Emissions calculations principally depend on the gas phase concentration of the SVOC in the material as well as the surface area of that source. A linear regression yielded R² and p values of approximately 0.3 and 2.0E-12, respectively, with logP and vapor pressure being the most significant predictors for gas-phase concentration followed by their presence in adhesives and resilient flooring. These results potentially allow for the forecasting of gas-phase concentrations of chemicals for which their analytical data in flooring materials are lacking. Data generated from high throughputs exposure methods are then combined with high-throughput chemical screening data from ToxCast projects to order to statistically assess risk in a time and cost effective manner. As a result, comprehensive risk assessment of indoor use chemicals may be achieved. This abstract does not necessarily reflect EPA policy.

Synthetic polynuclear diphenyl ethers (PBDEs) have been widely used as flame retardants in many consumer products including electronic devices. The most important routes of human exposure appear to be from contaminated food and contact with dust found in households and workplaces. Structurally related derivatives of PBDEs are the hydroxylated (OH-PBDEs) and methoxylated forms (MeO-PBDEs). Experimental evidence suggests the OH-PBDEs pose greater health risks than other forms of PBDEs. Certain OH-PBDEs and MeO-PBDEs are also marine natural products and it is unclear although likely, that marine fish and shellfish, which bioaccumulate these compounds serve as a vector for human exposures. In this study, we are measuring approximately 120 different PBDE, OH-PBDEs and MeO-PBDEs in household/workplace dust and blood plasma samples provided by human volunteers living in the Puget Sound region of Washington State and working in either the commercial fishing or recycling industries. The commercial fishing occupation is largely an outdoor activity that promotes above average seafood consumption while electronic recycling may expose workers to dust with higher than average levels of PBDEs. Thus, comparison of PBDE levels in samples associated with these occupations may provide insight on the relative importance of dust vs. food as a source of PBDEs. Initial results suggest the pattern of PBDE distribution in volunteers that consume low amounts of seafood are more comparable relative to those from volunteers consuming higher than average amounts of seafood. For example, 97% of OH-PBDEs were detected in volunteers consuming low amounts of seafood. Also, comparison of results from volunteers of white-European, African-American and Hispanic ethnicity suggested ethnicity was not an important variable in determining PBDE congener content of plasma relative to occupation and other lifestyle attributes. Supported by NIOSH Grant 1R21OH010259-01A1.

Risk due to chemical exposure is a function of chemical hazard and exposure. Proximate sources of exposure due to the presence of a chemical in consumer products are identified as key drivers of exposure and are not well quantified. The ExpoCast project is developing a model that forecasts indoor exposure to chemical additives in textiles. Flame retardant chemicals are found in flooring, upholstery, and articles of clothing. Depending on their physicochemical properties, they may bioaccumulate in the indoor environment at higher magnitudes than in the outdoor environment, which is correlated with high indoor exposure rates. Halogenated flame retardants, such as polybrominated diphenyl ethers, are semi-volatile organic compounds (SVOCs) that are potentially harmful to humans. In this study, ExpoCast predicted emissions of 74 chemicals found in 32 flooring materials for which gas phase concentrations were measured by Wilke et al. (2004).1 Flooring materials include a range of natural and synthetic floor coverings, installations, and adhesives. Emissions calculations principally depend on the gas phase concentration of the SVOC in the material as well as the surface area of that source. A linear regression yielded R² and p values of approximately 0.3 and 2.0E-12, respectively, with logP and vapor pressure being the most significant predictors for gas-phase concentration followed by their presence in adhesives and resilient flooring. These results potentially allow for the forecasting of gas-phase concentrations of chemicals for which their analytical data in flooring materials are lacking. Data generated from high throughputs exposure methods are then combined with high-throughput chemical screening data from ToxCast projects to order to statistically assess risk in a time and cost effective manner. As a result, comprehensive risk assessment of indoor use chemicals may be achieved. This abstract does not necessarily reflect EPA policy.
Bisphenol A (BPA) is an organic compound with a weak estrogenic property, the health effects of BPA remain inconclusive but it is agreed that it has low acute toxicity. Because of the ubiquitous presence of BPA in our daily lives, there is concern about its ability to leach from products into consumables creating adverse health effects. Such scenarios led “BPA free” products. We have characterized the presence of BPA in various plastic products: baby bottles, water and food containers; investigated the effects of temperature, and degree of use on BPA leachates. Various plastic baby bottles, water/sports bottles, and food containers were filled with water, boiled to 90, 95, or 100oc for 36, 48, 60 times/hours, sample collected along with 0.5gm from different plastics were extracted and analyzed along with standard using HPLC-Uv-Vis at 225-310 nm; with a mobile phase (acetonitrile: water (70:30 v/v)) and mass confirmed with MALDI-TOF for BPA and metabolites. HPLC: BPA elutes with a mean retention time (RT) of 7.35 +/- 0.05 min and quantification showed differential leaching of BPA from different plastic containers depending on the temperature and extent of use. Microswab water in plastic containers showed temperature and time-dependent concentration of BPA leachates. Analysis of different containers showed presence of BPA in all samples except from Ziploc Smart Snap. MALDI-TOF analysis of sample extracts confirmed presence of BPA and other derivatives. Most of the plastic containers tested leached BPA from baby bottle, water bottles and food containers even those labeled “BPA free” except Gumdrop baby bottle and Ziploc Smart Snap. Concern remains of how free and safe are plastic containers labeled “BPA free”? Though, BPA has low estrogenic activity and not considered a major threat to human health, but manufacturers of consumer products need to live up to their product standard and avoid the guessing game. However, more research is required to identify conditions that lead to BPA leach from products and the long term toxic effects of BPA.
Introduction: Inorganic arsenic (iAs) exposure is potential occupational hazard in copper mining and smelting. After absorption, As undergoes successive reductions and methylations metabolism. As excretion profile was related with environmental arsenic levels, individual arsenicism risk, sex, age, nutritional status and body weight. The current investigation was carried out to evaluate As exposure and the urine metabolite profiles of workers with different jobs in a copper mining and processing plant in China. Methods: A total of 170 male workers in 5 different departments (administrative employees (Group 1), copper ore miners (Group 2), copper ore grinding and milling (Group 3), electrolytic procession (Group 4) and copper smelters (Group 5)) of a copper mining and processing plant were randomly recruited. Subject age, job history, alcohol and smoking habit, dietary habits, et al. were collected. Blood pressure, height and weight were measured and body mass index (BMI) was calculated. Spot urine samples were collected and determined for iAs, MMA, DMA, and trimethylated arsenic (TMA) by atomic absorption spectrophotometer with an arsenic speciation pretreatment system. Results: The highest urinary levels of iAs, MMA, DMA and TMA were found in the Group 5. There was a statistically higher excretion of urinary As in the Group 4 and 5 compared to Group 1, 2 and 3 (p<0.05). In Group 4 and 5, 77.8% and 93.3% of workers had total urinary As values higher than 50μg/g Cr, and significantly higher than that of Group 1, 2 and 3. There was a statistically higher excretion of urinary DMA in the workers of BMI>25 and intake seafood in recent 3 days. The odds of subjects with urinary As exceeded 50μg/g Cr were positively and significantly associated with different production type (OR=1.907; CI=1.387-2.621; p=0.000) and BMI (OR=1.504; CI=1.009-2.242; p=0.045). Conclusion: The workers in copper production plant presented significantly elevated levels of iAs, MMA, DMA and TAs, especially for smelters and workers of electrolytic process.

In New Mexico (NM), arsenic in groundwater has been measured as high as 600 μg/L. Arsenic in drinking water is a potential public health concern in areas of NM where concentrations in groundwater exceed the Environmental Protection Agency maximum contaminant level of 10 μg/L. Approximately 90% of NM’s drinking water supply comes from groundwater. A biomarker of arsenic exposure could estimate the contribution of arsenic from drinking water. The analysis evaluated how well urinary arsenic concentration is associated with arsenic concentration in drinking water among NM biomonitoring project participants.

Data were utilized from three NM Department of Health projects conducted from 2004 through 2012, which included volunteer participants residing in 76 communities. For this analysis, only adults who provided samples of their drinking water, a spot urine sample and completed an exposure assessment survey were included (N=1009). Total arsenic concentrations from drinking water and urine samples were analyzed. Sample collections and analytical methods applied were similar among the biomonitoring projects, therefore, it was concluded that the results could be pooled for analysis. A multiple regression model was developed to evaluate the effect of drinking water arsenic concentration on urinary arsenic level, with adjustment for variables such as age, sex, dietary supplement use, tobacco use, fish/seafood consumption, and daily water consumption.

After adjusting for relevant covariates, for each 1 μg/L increase of total arsenic in drinking water, there was a 2.7% increase in creatinine-corrected total urinary arsenic concentration.

Urinary arsenic concentration can serve as a biomarker of exposure to arsenic in drinking water; however, other factors also contribute to urinary arsenic levels. Education about the importance of arsenic testing and methods of reducing arsenic in drinking water, especially for those on private wells, can positively affect health behavior of communities at risk for excessive exposure to arsenic.

Arsenic (As) can exist in the environment in several different forms, and each form has unique chemical characteristics that influence its toxicity. Within the last decade or so, the increased focus on speciated As (both the inorganic and organic forms) and its potential toxicity has resulted in a large body of literature on speciated As in different food types. We evaluated the state of knowledge of As speciation in food and calculated the average levels of several species of As measured in food. Because inorganic arsenic (iAs) is considered the most toxicologically important form of As, we focused our analysis on papers presenting information on total inAs and speciated inAs (inAs3+ or inAs5+). Other As species (e.g., monomethylarsonic acid [MMA], dimethylarsinic acid [DMA]) were also evaluated when presented with inAs information. Publications were drawn from the peer-reviewed literature and reports by authoritative health agencies. We compiled a large database of measurements, including over 6,000 unique inAs data points. Our analysis demonstrated that inAs in foods can vary widely by type and even by sample, with mean inAs concentrations ranging from undetectable (in milk) to 8,100 μg/kg (in seaweed/algae). After seaweed, the highest mean inAs concentrations were found in rice-based products such as flour and bran, followed by rice and rice cereals. We also found a high percentage of non-measurable As in many food types. We believe this is one of the first analyses to combine data from the available literature on speciated arsenic to calculate summary statistics for exposed and non-exposed individuals, and thus provide a more complete profile of the community exposure to arsenic.
We are exposed to thousands of chemicals over our lifetimes. A major challenge to risk assessors is to understand how and when chemical exposures occur, and which "exposure pathways" contribute the most. An informatics-driven approach to assigning "product-use" categories to product "ingredients" will help prioritize which chemicals will be given more scrutiny for a target population (life-stage, gender, ethnicity), identifying (a) human activities that result in increased chemical exposure while (b) reducing the dimensionality of hazard assessment to a tangible subset for risk characterization. How chemicals are used is directly related to their potential exposure routes, and in the sense of near-field source apportionment, to the identification of chemical-specific use-to-receptor exposure pathways. We have developed the Chemical/Products Categories Database (CPCat) to explore these pathways. CPCat is a relational database comprised of chemical "use category" information, including both consumer- and industrial-process-based uses for chemicals, using mined and curated data from 14 major sources from multiple countries, including data from regulatory bodies, manufacturers and retailers. CPCat contains information on 42k unique chemicals mapped to a set of >700 use categories. We provide an example of how CPCat can be used to link life-stage to use-pattern to products and their ingredients for children's exposure. CPCat can be used to identify chemicals that children would potentially be exposed to, and the major activity/source of such exposures; a database query of the "child" or "baby" keywords results in the identification of 1,300 chemicals, of which the majority are associated with the "child toy" CPCat category. This information can then be used to rank plausible exposures of chemicals which may be investigated for potential biological activity. The entire database and a set of tools will be made publicly available. The views expressed in this abstract are those of the authors and do not reflect the views or policies of the U.S. EPA.
black carbon content. Aging of radiolabelled benz(a)pyrene (BaP) on the soils was conducted for eight weekly cycles in which soils were wetted and dried. Eight- and 24-hr absorption experiments were then conducted in vitro using heat-separated human cadaver epithelids to assess the flux of BaP from each soil and to investigate the effect of soil characteristics and BaP concentrations on flux. Experiments were also conducted with BaP delivered in solvent to permit comparison to absorption from a soil matrix. Uptake (as assessed by radiolabel recovered from swabbed skin) of solvent-deposited BaP was more rapid than penetration to receptor fluid, reflecting lag time in skin. Uptake and penetration from aged soils at nominal initial concentrations of 5 and 10 ppm BaP were correspondingly lower, reflecting reduced thermodynamic activity in soil. This research is particularly relevant in light of EPA’s draft toxicity profile for BaP, which introduces a Dermal Cancer Slope Factor for use in risk assessment of BaP exposures, including exposures from soil.

2234x Hematotoxicity and Genotoxicity in Children Exposed to Benzene in a Petrochemical Area in México

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Background Children living in Tula, Hidalgo, México, are exposed to pollutants from petrochemical and chemical industries located in the area. The Secretaría de Medio Ambiente y Recursos Naturales (SEMARNA) reports emissions to environment of some toxic compounds. Benzene emission has been reported to the environment and it is well known that is a hematotoxic and genotoxic compound. Objective The aim of this study was to evaluate the genotoxicity and hematological effects in children environmentally exposed to benzene. Methods Samples of urine, blood and buccal epithelium were obtained from 26 children (6–8 years old). Exposure to benzene was assessed by determination of an urine metabolite: trans, trans-mucic acid (t,t-MA). Micronucleous frequency (MNf) was evaluated in buccal epithelial cells and hematological parameters were determined.

Results: Exposure assessment indicates that the 35% of children have levels above de Biological Exposure Index (500 ng/gCreatinine). The mean of the t,t-MA urinary levels was 460 ng/gCreatinine; this value is above of levels reported in previous studies in children from Italy, Thailand and México. In the hematological status assessment, 100% of children have some hematic parameter abnormal. The 78% have hemoglobin levels above normal values for the age group; 42% has altered lymphocytes (10% lymphopenia, 19% granulocytes alterations, 22% monocytes). The preliminary results (n = 8) the mean of MNf was 12.7/1000; this value is three times higher than those reported in other studies in children. No statistic significant correlation between benzene exposure and hematological parameters was found.

2234y Refined Exposure Assessment for Three Active Ingredients of Humidifier Disinfectants

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Exposure assessment for three major active ingredients used for humidifier disinfectants, polyhexamethylene guanidine (PHMG), oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride (PGH), and 5-chloro-2-methylisothiazol-3-(2H)-one/2-methylisothiazol-3-(2H)-one (CMIT/MIT) mixture, were conducted in a bedroom for good occupational hygiene, and for active health monitoring, the respiratory and skin exposure were assessed. The objective of this study was to evaluate disinfectant exposure from selected products with simulated laboratory use. An environmental chamber was used to simulate laboratory application of the products. Bulk samples of various products were tested before and after use. Industrial hygiene personal and area air samples were collected during all testing following NIOSH 7400/7402 methodologies. Phase contrast microscopy (PCM) and transmission electron microscopy (TEM) were used for sample analyses. Collection of personal and area air samples varied between 4 and 6 hours. Analysis of personal air samples using PCM, showed airborne fiber concentrations as high as 0.058 f/ml during use of tongs fitted with asbestos sleeves. Further analysis using TEM showed the highest asbestos level of 0.0036 f/ml. Manipulation of transit boxes resulted in personal TWA asbestos levels as high as 0.02 f/ml. Analysis of area air samples during heating of gauze pads using PCM showed airborne fibers concentrations ranging from less than 0.007 to 0.009 f/ml. When analyzed using TEM, asbestos levels ranged from 0.001 to 0.002 f/ml. Personal samples were in similar ranges. Some asbestos was lost from gauze pads during use. Asbestos-containing gauze pads produce de minimis levels of asbestos when heated. Testing of various asbestos containing materials used in research laboratories indicate low potential for asbestos fiber exposures.

2235 Asbestos Content of Heavy Equipment Brake-Wear Debris and Associated Airborne Exposures during Brake Work

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Asbestos-containing brake linings were commonly used in heavy construction equipment until the 1980s. However, asbestos exposures and potential health risks associated with brake repair on heavy equipment have not been comprehensively evaluated. The present study determined whether individuals working with heavy equipment brake components, or bystanders to such work, were exposed to chrysotile asbestos above exposure limits dependent upon chrysotile content of equipment brake wear debris (BWD) and type of machinery. Publicly available published and unpublished studies examining BWD asbestos content in various heavy equipment were identified. Variables potentially contributing to BWD and airborne asbestos concentrations included common brake job practices, infrequent
manipulative activities, cleaning methods, and brake component dimensionality. Tasks included combinations of changing brakes, inspection of brake pads, glazing brakes with sandpaper, and cleaning with solvent or compressed air. The degree to which airborne asbestos concentrations correlated to BWD composition and physical characteristics, the type of heavy machinery, and the brake work activities was determined. Most, but not all, BWD samples contained less than one percent chrysotile by weight. For air samples, phase contrast microscopy results for personal, bystander or area samples did not exceed 0.622 total fibers based on short-term, task-based sampling. 8-hr time weighted average concentrations did not exceed 0.045 asbestos fibers after adjusting for asbestos content using transmission electron microscopy. These results are consistent with those observed for smaller vehicles, including automobiles. This suggests that individuals working with these materials would not have been exposed to asbestos above contemporary or current exposure limits, regardless of BWD chrysotile content. It is unlikely that the dimension of brake components influences the degree to which mechanics working with such products were historically exposed to asbestos.

**2236 Determination of Air Conc. and Exposure to Spores from Microbe-Based Cleaning Products: Influence of Human Activity and Vacuuming**

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Microbe-based carpet cleaning products contain non-pathogenic, highly specialized microbes that produce appropriate enzymes in the presence of soils ceasing production when the stains or soils have been removed. These products are used in homes and institutions and potential for human exposure from residues in the air and on treated surfaces exists. The purpose of this study was to determine the air conc. of viable bacterial spores after use of two microbe-based carpet cleaning products based on a blend of Bacillus spores: a whole room consumer-use product and a spot treatment product using a trigger sprayer. The influence of choreographed human activities on airborne spore levels was investigated. Studies were conducted in a simulated residential exposure room carpeted with plush nylon carpet and equipped with impingers to collect airborne spores. Airborne spore levels were elevated after application of either product but quickly returned to values similar to the 95th percentile or less of field measurements of airborne spores in indoor settings. Vacuuming and passage of time continued to cause a decrease in airborne levels. The trigger sprayer for spot application produced levels of airborne spores slightly above the provisional threshold limit (TLV) of 1x10^4 CFU/m^3, established for airborne microbes in occupational settings. The spores are, however, mainly carried in non-inhalable water-based particles. So although airborne spore counts in the spot application exceeded the TLV, trigger sprayer products typically produce non-inspirable particles that, despite their presence, actually pose minimal potential for pulmonary exposure.

Based on the data from these exposure studies, and a critical evaluation of the published literature, it is concluded that carpet cleaning products containing a mix of Bacillus spores can be regarded as safe for the users.

**2237 A Continuing Legacy of Chlordane and Dieldrin—A Case Example of Pesticides in Well Water in Stamford, Connecticut**

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The Connecticut Department of Public Health (CT DPH), the Connecticut Department of Energy and Environmental Protection (CT DEEP), and the city of Stamford have been involved over the past four years with a number of contaminated private wells in Stamford. Banned pesticides, dieldrin and chlordane, were discovered in several private wells at levels exceeding Connecticut’s state drinking water guidelines. The source of the contamination was originally thought to be a nearby landfill, but it is now believed to be from either home pesticide treatment for termites or agricultural use several decades ago. The well contamination issue was originally thought to be limited to a small area of Stamford, but now it is known to be more widespread. To better understand the scope of the pesticide contamination in Stamford, the Stamford Health Department launched a small random sampling program and a subsidized city testing program. There are almost 2000 wells that have been tested to date where approximately 15% of wells tested have either dieldrin and/or chlordane present with 6% exceeding state drinking water guidelines. The maximum concentrations of chlordane and dieldrin in wells are 7.4 and 1.3 parts per billion (ppb), respectively, and 25 and 43 times the state drinking water guidelines. Using these maximum levels for chlordane and dieldrin, the lifetime cancer risk from drinking the contaminated water is up to 5 excess cancer cases out of a population of 10,000. The city of Stamford, with assistance from CT DEEP and CTDPH, took a proactive approach and placed many of these homes on whole house filters and later to municipal water. CT DEEP, CTDPH, and the city of Stamford have taken a number of actions to deal with the private well contamination and inform the community. The testing of contamination in Stamford raises broader questions about the past practice of treating home foundations for termite control using chlordane and dieldrin and the nature and scope of potential impacts to groundwater quality in Connecticut and possibly other states.

**2238 Surface Water Risk Assessment of Pesticides in Ethiopia**


Scenarios for future use in the pesticide registration procedure in Ethiopia have been devised for 3 separate Ethiopian locations, which are considered to be protective for 99% of Ethiopia. The scenarios estimate concentrations in surface water resulting from agricultural use of pesticides for a small stream and for 2 types of small ponds (Adriaanse et al., 2013). Preliminary results from scenario calculations will be presented.

On the basis of volume of use, application rate in Ethiopia and acute human toxicity the 7 pesticides considered to be of most risk to humans were selected, assuming exposure as a result of the consumption of surface water. Estimates of exposure concentrations in surface water were established using modelling software also applied in the EU registration procedure (PRZM and TOXSWA). Calculations generated the 99-percentile maximum concentration for use in human risk assessment and the 90-percentile maximum concentration for use in ecotoxicological risk assessment. Input variables included physicochemical properties, and data such as crop calendars, irrigation information, meteorological information and detailed application data which were specifically tailored to the Ethiopian situation. The results indicate that for all the pesticides investigated the acute human risk resulting from the consumption of surface water is low to negligible, whereas agricultural use of Chlorothalonil and Endosulfan may result in medium to high risk to aquatic species.

Key words: Risk assessment, pesticides, models simulation, aquatic ecosystem, Ethiopia

**2239 Human Health Risk Assessment of Metals in Fish in Ghana**

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Fish advisories have been established by many nations to protect the public from potentially harmful health effects linked to eating contaminated fish. Advisories are based on estimations of cancer and non-cancer health risks associated with concentrations of contaminating chemicals found in fish, and the average levels of consumption of fish in the population. Fish is an important source of nutrition in Ghana; however, little is known about potential health effects associated with eating fish in Ghana. The overall aim was to estimate the extent of contamination of the fishery resource so as to inform guidelines on fish consumption. We gathered information on levels of metals in fish in Ghana from scientific publications identified using the PubMed database. We estimated human health risk by calculating a hazard quotient based on the reported levels of mercury in fish and compared risk across different fish species and water bodies in Ghana. Mercury was the only metal in which there was sufficient data to estimate human health risk from eating fish caught in Ghana. The mean for all the recorded values for mercury concentrations in fish was 0.10 mg/kg wet weight. This concentration is associated with a hazard quotient of greater than 1, meaning that adverse health effects are possible. Generally, lower concentrations of mercury were found in fish from the lakes sampled while higher concentrations were detected in fish from rivers, particularly in gold mining areas of Ghana. Our results indicate mercury found in fish may be causing health effects, such as impaired neurological development. It is important for the government and relevant agencies in Ghana to consider a mechanism to effectively monitor pollution, issue fish consumption advisories when warranted, and determine and remediate the source of contamination, which likely includes mercury emissions from artisanal mining based on the heightened levels found in fish from rivers in the gold-mining region.
The lead (Pb) content consumer products has become an increasing concern over the past several years. In 2007, the Campaign for Safe Cosmetics published the test results of 33 lipstick samples and found that 61% contained lead, with a maximum Pb concentration of 0.65 ppm. In 2010 the US FDA tested 400 lipstick samples and found a median Pb concentration of 0.9 ppm and a maximum Pb concentration of 7.19 ppm. To assess the safety of these lipsticks in instances where children might intentionally or incidentally ingest lipstick products, we used the US EPA’s Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children to determine the blood Pb concentrations of children (aged 0-7) ingesting varying amounts of lipstick of different Pb concentrations. Modeled blood Pb concentrations were then compared to the estimated blood Pb concentration for the US Consumer Product Safety Commission’s (CPSC) guideline of 15 ug Pb/day (orally) and to the Centers for Disease Control (CDC) and US EPA actionable blood Pb levels (BLL) of 5 ug/dL and 10 ug/dL, respectively. The maximum estimated geometric mean blood Pb level resulting from the ingestion of the CPSC guideline was 3.8 ug/dL, assuming inclusion of background sources in the guidance value. The amount of the highest concentrated lipstick (7.19 ppm) required to yield a blood Pb concentration in the range generated by the CPSC guidance value and the US EPA BLL (3.8 – 10 ug/dL) was 0.25 – 4.94 g lipstick/day; this is equivalent to ingestion of around 1/50th to 2/5th of a 3.0 oz tube of lipstick/week, assuming concomitant background exposure. The amount of lipstick with the FDA median concentration of 0.9 ppm that would need to be ingested to reach this blood Pb concentration range was 2.0 – 39.4 g lipstick/day, or around 1/5th to three 3.0 oz tubes of lipstick/week. Our results suggest that the safety of these products and cosmetics should be assessed not only by the presence and amounts of hazardous contents, but also through an assessment of estimated exposures and comparison to health based standards.

Mercury is a naturally-occurring volatile contaminant of natural gas; concentrations range from <0.01 to 2000 μg/m³ worldwide depending on the geologic deposit. Hydraulic fracturing has revolutionized the scale of natural gas extraction, with certain shale deposits supporting thousands of active wells. At production platforms, large volumes of natural gas are flared (burned) to remove unwanted elements; in the process mercury may be released to ambient air. Mercury is a well-recognized toxin that can affect the nervous, immune, and other systems. We used a range of published natural gas mercury levels, well completion flared volumes, and a screening level dispersion model (SCREENS) to model mercury concentrations in ambient air near a model gas well. We speculated total mercury emissions into elemental and mercuric forms and considered soil deposition and mercury methylation. Potential health effects from inhalation, ingestion, and dermal exposure to different mercury species were evaluated by using USEPA exposure parameters and standard toxicity reference levels. Our results indicate that maximum annual average airborne concentrations of mercury occur approximately 1000 meters from the well. Based on modeled concentrations, mercury emitted from flaring during the completion phase of a single well does not pose chronic health concerns (health index [HI] <1). There are many uncertainties inherent in this screening-level assessment, including modeling emissions from single well, inclusion of only a single category of flare source, the mercury concentration of natural gas, the use of a screening air dispersion model, and mercury speciation and deposition. While preliminary, given the complexity of active high-density natural gas well fields with diverse mercury content, a refined risk assessment should be performed to ensure that health risk impacts are appropriately characterized.

New toxicological information has prompted USEPA to consider acute in utero toxicity at low levels of TCE in the air. Further, data reported from studies of vapor intrusion suggest both high variability in indoor levels, and little correlation with parameters such as temperature and pressure differentials, shallow soil vapor concentrations, precipitation, and soil characteristics. If acute effects from inhalation of TCE do occur, they may occur on few days per year, and these days cannot be reliably predicted. This dilemma presents challenges when assessing risks from acute exposure to TCE in air. Key questions include: how often will extreme concentrations occur; what extremes might be expected; how likely are these extremes to occur during the window for heart development; what data are needed to support risk assessment that addresses these issues; how should the results of the analysis be interpreted? These issues were considered where one unit of a building is used as a church. The unit is adjacent to a source of TCE contamination and overlies a “plume” of TCE vapors. Indoor air concentrations in this unit have varied somewhat over time, but have often been within an order of magnitude of the lowest air concentration of TCE being considered as a threshold for teratogenic effects (2 μg/m³). A one-dimensional stochastic analysis, using data from residential situations to estimate frequency and magnitude of TCE excursions, occupancy information appropriate for church use, a target of indoor air concentration of 2 μg/m³, and a window early in pregnancy for developmental effects on the heart, suggest low probability of pregnant women being present at times when TCE concentrations could be toxic. A “worst case” might suggest a few percent of days in a year; a “best case” might suggest a few percent of days over two or three years. Unless understanding of vapor intrusion improves substantially, risk managers may have to decide where along a continuum of days per unit time where risks become acceptable. The situation appears similar to that of lead, where an acceptable target is based on achieving target blood lead levels in percentage of children.
fied in raw biogas from these sources, including numerous straight and branched alkanes, alkenes, aldehydes, esters, alkyl benzenes, thiols, sulfides, organometallics, and many regulated toxic volatile organics. We collated risk-estimating criteria (i.e., toxicity values) for the biogas constituents from databases published by OEHHA, the U.S. EPA, and two other federal agencies. In some cases, we grouped chemicals whose toxicological properties were judged to be similar, and developed a single toxicity value for each group. Approximately 50 constituents lacked either individual or group risk criteria and could thus not be quantitatively assessed (e.g., several benzene derivatives and alkyl sulfides). We obtained air concentration estimates from CARB and evaluated cancer and non-cancer risks using standard assessment methods developed by OEHHA. Twelve biogas constituents were determined to be of health concern to consumers or workers based on screening thresholds for carcinogens (1 or 30-per-million additional risk, respectively) and non-carcinogens (a hazard quotient > 0.01 or 0.3, respectively). The contaminants were: Alkyl Thiols, Antimony, Arsenic, Copper, p-Dichlorobenzene, Ethylbenzene, Hydrogen Sulfide, Lead, Methanesulfonic Acid, Nitroso-di-n-Propylamine, Toluene, and Vinyl Chloride. As a result of the assessment, biomethane producers will be required to test for these chemicals to ensure that they are adequately removed from raw biogas during processing. OEHHA will update the risk assessment as new information becomes available.


Octamethylocyclotetrasiloxane (D4) and Decamethylocyclopentasiloxane (D5), two low molecular weight cyclic siloxanes, are used globally as intermediates in the production of several widely-used industrial and consumer products. Their global use requires consideration of consumer use information and risk assessment requirements from various sources and authoritative bodies. A global “harmonized” risk assessment was conducted to meet requirements for substance-specific risk assessments conducted by regulatory agencies such as the US EPA’s Integrated Risk Information System (IRIS) and Health Canada, various independent scientific committees of the European Commission, as well as provide guidance for chemical safety assessments under the REACH Regulation in Europe, and other relevant authoritative bodies. This risk assessment incorporates global exposure information combined with a Monte Carlo analyses to determine the most significant routes of exposure, utilization of a multi-species, multi-route physiologically-based pharmacokinetic (PBPK) model to estimate internal dose metrics, benchmark modeling to determine a point of departure (POD), and a Margin of Safety (MOS) evaluation to compare the estimates of intake with the POD. Because of the unusual pharmacokinetic behaviors of D4 and D5, including high lipophilicity, high volatility with low blood-to-air-partition coefficients, and extensive metabolic clearance that regulate tissue dose after exposure, the use of a PBPK model was essential to provide a comprehensive model that reflects these processes. The characterization of the potential for adverse effects after exposure to D4 and D5 using a MOS approach based on an internal dose metric removes the subjective application of uncertainty factors that may be applied across various regulatory agencies and allows examination of the differences between internal dose metrics associated with exposure and those associated with adverse effects.


Groundwater in agricultural regions often exceeds the nitrate Maximum Contaminant Level (MCL) of 10 mg/L as nitrogen (~45 mg/L as NO3) and reducing NO3 below the MCL can be costly. The MCL is based on a link to infant methemoglobinemia from studies circa 1950. While later research questions this relationship, the MCL is the legal limit. A risk assessment was completed to support the safety of groundwater NO3 levels above the MCL for use in distillation of plant oils for dietary supplement uses by children (age ≤3) and adults. The method considers the acceptable daily intake (ADI) for NO3 (mg/kg/d), body weight of consumer (BW, kg), fraction of total daily exposure from supplement use (allocation factor (AF), %), the daily supplement dose (g/d), volume of water (mL) needed to produce 1 g of oil (distillate ratio (DR), mL/g), and partitioning to the oil phase (partitioning factor (PF), %). Values used in this assessment: ADI=3.7 mg/kg/d (as NO3) from JECFA (since supplement is not for infants); BW=10 kg; AF=assumed 20%; Dose=4 g/d; DR=30 mL/g; PF=100% (worst case) or 0.0041% (reasonable case based on log Kow of ~4.39). The NO3 concentration in groundwater resulting in an exposure equal to 20% of the ADI from consumption of the supplement and assuming a worst case scenario of all NO3 from water ending up in the oil (PF=100%) was calculated to be 62 mg/L based on the following equation: $\text{Con}}_{\text{sup}} (\text{mg/L}) = \text{ADI (mg/kg/d)} \times \text{BW (kg) \times AF (\%)} \times 1000 \text{mL/L} / (\text{Dose (g/d) \times DR (mL/g) \times PF (\%)})$. However, as NO3 is a highly water soluble compound, the realistic PF value of 0.0041% (based on the log Kow of NO3) should be used in estimating the amount of NO3 that would partition to the oil. Using this PF value, the groundwater concentration of NO3 that would equal 20% of the ADI from use of the supplement is 1,594,065 mg NO3/L. This concentration could result in a severe health risk. Thus, as essentially zero NO3 in groundwater would likely partition to the oil, otherwise potable groundwater containing NO3 levels above the MCL may still be safe for oil distillation.


There has been and continues to be concern in the airline industry about pilot and flight attendant exposure to TOCP, a well-known neurotoxin. Oil used in jet engines may contain 1-3% tricresyl phosphate of which 0.1-1% is TOCP. The workplace 8 hour time weighted average OSHA Permissible Exposure Limit (PEL), NIOSH Recommended Exposure Limit (REL), and ACGIH Threshold Limit Value for TOCP are all 0.1 mg/cubic meter. Investigations of in-plane air contamination by aircraft turbine engine oil via engine bleed air supply systems have reported no levels of TOCP below limits of detection (LOD, 0.000009-0.0001059 mg/cubic meter) in the cockpit or cabins of sampled aircraft. Since there is no inhalation toxicity factor for TOCP, we performed a risk assessment comparing the acceptable daily intake based on an oral exposure with exposure at the LOD and PEL. Our assessment indicates that pilots and flight attendants are not exposed to sufficient TOCP to cause any adverse health effects. Our assessment is consistent with recent publications reporting that there are no detectable biomarkers of exposure to TOCP in flight crews.

2248 Exposure to Vitamin A (Retinol and Retinyl Esters) from Cosmetics Increases the Proportion of the Population That Exceeds the Upper Intake Level R. B. Hetland1, B. Granum1, J. E. Paulsen1, V. Thranie1, J. L. Lyche1, C. Luznow-Holm2, T. O. Fjordland1, I. L. Lillegaard1 and I. Steffensen1. 1Norwegian Scientific Committee for Food Safety, Oslo, Norway and 2External Expert, Oslo, Norway.

Vitamin A, a group of compounds including retinol (ROL) and retinyl esters, is required in numerous physiological functions. ROL and retinyl esters are widely used in cosmetics such as anti-wrinkle creams and moisturisers due to their positive effects in the skin. The Norwegian Scientific Committee for Food Safety (VKM, 2012) performed a risk assessment of ROL and retinyl esters in cosmetics based on the tolerable upper intake level (UL) of 3,000 μg ROL equivalents (RE)/day (SCF, 2002) and the lower guidance level (GL) of 1,500 μg RE/day for individuals at risk of osteoporosis and bone fracture (EFSF, 2008). Oral intake of vitamin A was estimated based on national food consumption surveys. Systemic exposure dose from cosmetics (with 0.05 and 0.3% RE) was estimated based on The Scientific Committee for Consumer Safety’s Notes of Guidance for the Testing of Cosmetic Ingredients (2010) and a study by Yourick et al. (2008). Intake of vitamin A is high in parts of the Norwegian population; the most important source is diet. Use of food supplements contributes significantly and increases the proportion of the population exceeding the UL. The contribution from cosmetics to the total intake of ROL and retinyl esters is most prominent for 13-year-olds (23% of UL) and adults (29% of UL). Excess exposure to vitamin A is of concern for individuals at risk of osteoporosis and bone fracture (e.g. post-menopausal women). About 10% of adult women in Norway exceed the GL of 1,500 μg RE/day by intake from food and food supplements alone. The additional contribution of retinol and retinyl esters from cosmetics increases this proportion to ca. 75% which means a significant increase in number of individuals at risk of osteoporosis and bone fracture. Acknowledgement. The assessment was performed by the VKM Panel on Food Additives, Flavoursings, Processing Aids, Materials in Contact with Food and Cosmetics.
2249 Assessment of Potential Health Risks on the Basis of the Character and Degree of Chemical Pollution of the Environment


Health risks to the population living in the protective action zone of the Maradykovsky Chemical Weapons Storage and Destruction Facility (CWSDF), Kirov Region, Russia, were assessed. The risk assessment process included four steps (hazard identification, dose–response evaluation, exposure assessment, and risk characterization) and involved ranking possible carcinogenic and non-carcinogenic hazards from exposure to pollutants emitted by the CWSDF, preliminary ranking potential carcinogens on the basis of the total annual emission and the weighing coefficient of carcinogenic activity, and emission dispersion modeling. Ranking the atmospheric pollutants of the CWSDF allowed identification of priority pollutants to be monitored, which included 1-13 non-carcinogens (sulfur dioxide, fuel-oil ash from power stations, nitrogen dioxide, PM 10, C12-C19 alkanes, nitrogen oxide, sodium sulfate, potassium sulfate, sodium carbonate, sodium dichromate, manganese and its compounds, and carbon monoxide); specific pollutants: RVX, 2-(diethylamino)ethyl isobutyl sulfide (DS), isobutyl methylphosphonic acid (IMP), and monoehanolamine; and carcinogens: soil, benz[a] pyrene, and gasoline.

The respiratory system, central nervous system, vision organs, and digestive tract were found to be the target organs for chronic inhalation exposure to the priority pollutants. The potential health risks were calculated using the average annual concentrations of the priority pollutants in the CWSDF emissions in the chosen reference points. The noncarcinogenic risks for specific priority pollutants were as follows: IMPA 0.000001–0.000086; DS 6.3E-008–2.2E-005; RVX 9.9E-007–2.4E-005. The carcinogenic risks varied in the ranges 1.5E-007–1.4E-007 for benz[a]pyrene and 5.9E-10–0.5E-09 for carbon (soot). The chronic non-carcinogenic and carcinogenic risk parameters were at acceptable levels for all reference points.

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2250 In Vivo Bioactivity in ToxCast Assays for Fruit and Vegetable Extracts

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The ToxCast and Tox21 programs have generated in vitro screening data for over 1000 chemicals to aid in hazard identification and setting chemical testing priorities. These data, together with high-throughput pharmacokinetic data, are used to infer possible toxic responses and external concentrations required to elicit these effects. There is only limited experience in evaluating dose-response for natural products in these assays. In this study, juices were extracted from 30 organically grown fruits and vegetables. These juices were screened in concentration-response format across multiple cell types using the BioMap platform to assess similarities of pathway responses of the extracts with those of database compounds. Bioactivities noted with unpeeled potato juice were immunomodulatory and tissue remodeling activities across endothelial, peripheral blood mononuclear and fibroblast cells. This pattern of response was similar to quercetin, a plant-derived flavonoid. Broccoli juice initiated anti-proliferative effects on endothelial, fibroblast and smooth muscle cells with significant similarity to mitomycin C (374 ng/mL; Pearson, r=0.806) and the fungicide mancozeb (40 μM; Pearson, r=0.776). To relate in vitro concentrations to administered dose, the filtered juice yield per g item was used with the plasma volume of a 70kg adult to approximate % juice present in systemic circulation after eating. This value was used as a surrogate for target tissue concentration. The activity described for broccoli was elicited at 0.5%, the amount of juice anticipated in the circulation following consumption of 2 cups of broccoli. Importantly, the bioactivities noted do not necessarily lead to adverse effects. These data provide context for assessing the in vivo relevance of in vitro concentration-response and bioactivity data generated in ToxCast and similar screening programs. This abstract does not necessarily reflect EPA policy.

2250a Merging ExpoCast™ with ToxCast™: Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted In Vitro Bioactivity to Inform Toxicity Testing Strategies


Our previous work incorporating dosimetry and exposure with high-throughput screening (HTS) enhanced the utility of HTS data by translating in vitro bioactivity concentrations to external exposures required to achieve these levels internally. These oral equivalents were compared against exposure estimates to provide a margin of exposure (MOE). ToxCast Phase I chemicals are primarily food use pesticides for which regulatory estimates are available. As ToxCast efforts transition to chemicals lacking exposure information, exposure prediction tools become increasingly important. In this study, in vitro hepatic clearance and plasma protein binding were measured to estimate dosimetry-adjusted oral equivalents for Phase II chemicals. Chemical exposures were predicted from HT field mass balance models coupled with a predictor for near-field human exposure. Joint regression on all factors provided a calibrated consensus exposure prediction and a measure of variance conveying the uncertainty of the predictions. These values were compared against the calculated oral equivalents to assess the potential that actual exposures might elicit bioactivity. Exposure predictions across the lower and upper 95th confidence interval typically spanned eight orders of magnitude. Comparison of the minimum oral equivalent to the upper bound exposure prediction indicated that 49 and 95 of the 178 chemicals had MOEs < 1 and < 100, respectively. Application of these HT tools provides a first order approximation of MOEs to aid in prioritization of testing. Efforts are underway to reduce the uncertainty in the exposure predictions and increase the utility of this approach. This abstract does not necessarily reflect EPA policy.

2250b Development of Reverse Toxicokinetic Models to Correlate In Vitro and In Vivo Estrogen Receptor Activity

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Through the use of Tox21 high-throughput screening (HTS) program, efforts are underway to use quantitative high throughput in vitro assays to assess chemical effects across multiple cellular pathways, including the estrogen receptor (ER) pathway. HTS assays provide an efficient way of identifying potential biological targets for chemicals. However, the nominal in vitro assay concentrations may not accurately reflect the potential in vivo effects of these chemicals due to the differences in bioavailability and clearance. A set of pharmacokinetic models was developed to correlate in vitro concentrations with potential in vivo effects for Tox21 chemicals with potential to interact with the ER. These models estimate the daily oral doses in laboratory animals and humans for Tox21 ER active chemicals that would result in a steady-state in vivo blood concentration equivalent to the in vitro AC50 (concentration at 50% maximum activity) values identified using HTS assays that specifically target the ER pathway. These models were built using published experimental data and quantitative structure–activity relationship predictions for hepatic metabolic clearance and unbound plasma protein fraction for tested chemicals. The models were also adapted to incorporate infant physiology to include this most vulnerable human population. Using daily oral doses estimated from the model, Tox21 ER active chemicals were ranked, with chemicals having the lowest effective dose in these models being considered the most likely to interact with the ER, and further, as agonists or antagonists. The estimated oral dose for a subset of chemicals was also compared to the in vivo dose range reported to elicit ER-related effects. This project was funded in whole or in part with Federal funds from the NIEHS, NIH Under Contract No. N01-ES-35504.

2250c Estimation of Methylmercury Intake Doses in the South Korea Population Using a PBPK Model

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Recently, South Korea has measured total mercury (Hg) in blood as part of Korean National Environmental Health Survey (koNEHS) in 6311 subjects representing Korean general population. About 25 % of the biomarker measurements were above the German HBM1 (biomonitoring guidance value) of 5 μg Hg/L and...
about 1% was above the HBMD of 15 μg Hg/L. Among the various mercury-containing compounds, methylmercury (MeHg) is the most toxic because of its ability to be readily absorbed and to accumulate in the body. It has been linked to developmental deficits in children and increased risk of cardiovascular disease in adults. USEPA has a guidance value for MeHg exposure, but it is reported as a daily intake dose, requiring an exposure reconstruction step in order to compare the measured biomarker concentrations with average intake. Thus, the current study used these biomarker data to further investigate the potential of this population being exposed to MeHg at or above the U.S. Reference Dose (RfD) of 0.1 μg MeHg/day. First, total blood Hg concentrations were converted to MeHg using a randomly selected MeHg/Hg ratio from a previously reported distribution measured among South Korean. Next, these estimated blood MeHg concentrations were used to reconstruct MeHg intake amounts using a published physiologically based pharmacokinetic (PBPK) model for MeHg. Monte Carlo analysis was conducted to account for variability in physiology and pharmacokinetics in estimating the distribution of MeHg intake. The resulting mean amount was 2.88 μg/day, which is comparable to the estimate from the South Korean environmental monitoring report in 2011 based on MeHg residues in fish (2.8 μg/day). The estimated mean dose was 0.045 μg/kg/day, which was approximately half of the RfD, and above 10% of the estimated doses were higher than the RfD. Additional analysis will be conducted to examine what variables are associated with those biomarker measurements that are higher than the other guidance values.

**PS 2250d**  
Protecting Astronaut Health at First Entry into Vehicles  
Visiting the International Space Station: Insights from Whole-Module Offgas Testing  

NASA has accumulated considerable experience in offgas testing of whole modules prior to their docking with the International Space Station (ISS). Since 1998, the Space Toxicology Office has performed offgas testing of the Lab module, both MPL modules, US Airlock, Node 1, Node 2, Node 3, ATV1, HTV1, and three commercial vehicles. The goal of these tests is twofold: first, to protect the crew from adverse health effects of accumulated volatile pollutants when they first enter the module on orbit, and secondly, to determine the additional pollutant load that the ISS and its systems must handle. In order to predict the amount of accumulated pollutants, the module is sealed for at least 1/5th the worst-case time interval that could occur between the last clean air purge and final hatch closure on the ground and the crew’s first entry on orbit. This time range can come from a few days to a few months. Typically, triplicate samples are taken at pre-planned times throughout the test. Samples are then analyzed by gas chromatography and mass spectrometry, and the rate of accumulation of pollutants is then extrapolated over time. The analytical values are indexed against 7-day spacecraft maximum allowable concentrations (SMACs) to provide a prediction of the total toxicity value (T-value) at the time of first entry. This T-value and the toxicological effects of specific pollutants that contribute most to the overall toxicity are then used to guide first entry operations. Finally, results are compared to first entry samples collected on orbit to determine the predictive ability of the ground-based offgas test.

**PS 2250e**  
Public Health Concerns Surrounding Fine Particulate Matter Generated from Hydraulic Fracturing  
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To evaluate the potential impact of fine particulates on public health in communities relatively close to hydraulic fracturing (i.e., fracking) activities, a preliminary evaluation of available particulate matter (PM2.5) data from three air monitoring stations in the Dallas, TX area was conducted. Annual air quality monitoring data for 2013 was obtained from the Texas Commission of Environmental Quality (TCEQ) web site. Air quality monitoring stations in Texas Region 4 sampled and monitored ambient air and meteorological conditions to generate the hourly averages that were used for this data analysis. The monitoring stations selected for this evaluation were: CS6 at Denton Airport South; C52 near Midlothian, TX; and C1044 near Haltom City, TX. The stations were selected due to the fact that they recorded PM2.5 and wind direction data that were needed to evaluate any potential impacts from fracking activities. These locations were also identified due to their relative distance from the potential influence of the Dallas/Fort Worth metro area. Geometric means of annual PM2.5 concentrations were determined for each location, for both PM2.5 appearing to originate from heavy fracturing activity, and for PM2.5 appearing to originate from a direction relatively free of fracturing activity. Wind direction was used to estimate the origin of the PM2.5. At all three locations, the annual PM2.5 concentration from the direction of fracturing was significantly elevated in comparison to the PM2.5 concentration facing away from the heaviest fracturing activity in the area (i.e., CS6: Toward Fracking (TF) PM2.5=7.6 μg/m3 vs. Away from Fracking (AF) PM2.5=6.2 μg/m3, p<0.001; C52: TF PM2.5=9.0 μg/m3 vs. AF PM2.5=5.8 μg/m3, p<0.001; C1044: TF PM2.5=7.6 μg/m3 vs. AF PM2.5=6.3 μg/m3, p<0.001). No direction-specific concentrations were above the primary EPA annual PM2.5 air quality standard of 12 μg/m3. These preliminary data indicate that mean PM2.5 levels from the direction of fracking are significantly higher than those from the direction that is relatively free of nearby fracking activities; however, the levels are not above the annual EPA standard.

**2250f**  
Cancer Risk of PAHs in Particulate Matter from Biofuel Combustion  

The risk of cancer attributable to PAH exposure has been assessed, in the light of increased use of biomass for space heating associated to the financial crisis over the last couple of winters. Ambient air PMs was sampled in several urban sites, and analysed chemically for PAHs and levoglucosan, used here as the most specific tracer of biomass combustion. Internal exposure to PAHs was estimated taking into account the deposition of the respective PM fractions across the human respiratory tract (HRT). Deposition at different regions of the HRT was carried out using the Multiple-Path Particle Dosimetry (MPPD) model. MPPD calculates the deposition and clearance of mono- and poly-disperse aerosols in the respiratory tract of rats and human adults and children (deposition only) for particles ranging from ultratine (0.01 microns) to coarse (20 microns) sizes. Potential cancer risk due to exposure to the mixture of PAHs in urban ambient air was calculated according to the toxicity equivalence factor (TEF) approach using as basis the benzo[a]pyrene (Ba[a]P) cancer potency. The Ba[a]P-TEQ (Toxicity Equivalent Quotient) (carcinogenic equivalent, in ng/m3) was calculated by multiplying the concentrations of each compound in the PAH mixture with the respective TEF for cancer potency relative to Ba[a]P. To estimate cancer risk the TEQ of the respective fraction of particulate matter deposited across HRT daily, was multiplied to a dose-response function, derived from the [Ba[a]P] Inhalation Unit Risk (the latter is equal to 0.88E-6 (ng/m3)-1). Significant variation in risk was observed among different age groups, much larger than variation due to geospatial attributes. The risk for children is four times higher than for adults. Only limited difference was found between traffic and urban background sites in the winter (mean cancer risk for both is close to 10-6). The carcinogenic potency of PM emitted from biofuel combustion (main PM source of the urban background pollution) was slightly higher compared to the potency of traffic-originated PM when using conventional fuel types.

**2250g**  
Data Source Summary Documents (DSSDs) Derived for Updated Drinking Water Standards and Health Advisories (DWSHA) Tables  

The DWSHA tables, sponsored by the U.S. Environmental Protection Agency’s (EPA’s) Office of Water (OW), summarize the drinking water regulations and Health Advisory (HA) values as well as the reference dose (RfD) and cancer risk values for drinking water contaminants. The tables provide Maximum Contaminant Level Goals (MCLGs), Maximum Contaminant Levels (MCLs), and HAs. HAs in the tables are guidance values based on non-cancer health effects for different durations of exposure (one-day, ten-day, and lifetime) or a specific probability of cancer (10-4 – 10-6 cancer risks). HAs are recommendations for unregulated drinking water contaminants to protect public health as needed. Based on EPA policy, all DWSHA Tables published after 2012 will calculate lifetime HAs for all contaminants with RfDs, regardless of their carcinogenicity status. This will allow risk assessment managers to compare the noncancer and cancer values and determine which level provides a more meaningful scenario-specific risk reduction.

A systematic review of the DWSHA table was initiated in 2012 to ensure that the benchmark values are consistent with the most current EPA assessments and all contaminants with RfDs have lifetime HAs. A total of 75 RfD and oral slope factor updates were identified that needed to be confirmed. The associated DWEL, Lifetime HA, or Cancer Risk values are being recalculated accordingly. Additionally, 29 contaminants without lifetime HAs will be reviewed. These changes are summarized in Data Source Summary Documents (DSSDs), along with principal study information and uses and occurrence, and hypertexted to the DWSHA Tables. Quality assurance/control is an ongoing process to review.
Toluene and Dibutyl Phthalate in Nail Lacquers: A Proposition 65 Exposure Assessment

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Toluene and Dibutyl Phthalate (DBP) can be found in many consumer products, including nail lacquers. In 2012, the California Environmental Protection Agency (Cal EPA) evaluated 25 nail products and found that 64% of lacquer polishes contained toluene and 28% contained DBP. Concentrations of toluene and DBP ranged from 42 to 190,000 ppm and 14,000 to 88,000 ppm, respectively. Exposure to these chemicals is regulated by the California Safe Drinking Water and Toxic Enforcement Act of 1986 (commonly referred to as Prop 65). The purpose of this study was to estimate consumer exposure to toluene and DBP from nail lacquers via dermal and inhalation exposure routes. Using the results collected by the Cal EPA, a Prop 65 exposure assessment was performed based on minimum, median, and maximum toluene and DBP concentrations in nail lacquer. Inhalation exposure was estimated using standard conservative exposure assessment equations, and dermal exposure was calculated using SkinPerm Quantitative Structural Activity Relationship methodology. The Maximum Allowable Dose Level (MADL) of toluene is 7000 µg/day and assumes that 50% of the inhaled toluene is absorbed. To compare the total absorbed toluene concentration from nail polish application to the MADL, we combined the estimated dermal exposure with 50% of the calculated inhalation exposure. Our estimated absorbed toluene dose for a consumer ranged from 39.5 to 1.8 x 10^5 µg/day. Overall, most concentrations of toluene reported by the Cal EPA resulted in exposures that fell below the Proposition 65 MADL; however, higher toluene concentrations resulted in exposure values that exceeded the MADL. Given the negligible volatilization rate of DBP, only dermal exposure was evaluated. Consumer DBP exposure ranged from 3.4 x 10^3 to 4.2 x 10^3 µg/day. All estimated DBP exposures were above the MADL of 87.5 µg/day.

A Tiered Safety Assessment Approach for Evaluating Chemicals in Consumer Products and Applications for Asthma Risk Management

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Asthma is a complex syndrome with potentially severe consequences. The number of affected individuals is growing, although the reason for the increase is uncertain. Substantial evidence that exposure to some chemicals is linked to asthmatic responses increases the need for effective management of potential chemical exposures. A safety assessment approach tailored to the screening of asthma risks from consumer product ingredients was developed for use as a risk management tool. Several key features of the approach advance the use and integration of assessment resources often used for asthma investigations. First, a quantitative health benchmark for asthma or related endpoints (irritation and sensitization) is provided that extends the assessment beyond qualitative hazard classification methods. Second, a parallel structure includes methods for dose-response assessment of asthma endpoints and for scenario specific exposure estimation. The two parallel tracks are integrated in a risk characterization step. Third, a tiered assessment structure is provided to accommodate different amounts of data for both dose-response assessment (i.e., use of existing benchmarks, hazard banding, or the threshold of toxicological concern) and exposure estimation (i.e., use of empirical data, model estimates, or exposure categories). Tools building from traditional methods and resources have been adapted to address specific issues pertinent to asthma toxicity (e.g., mode-of-action and dose-response features) and the nature of consumer cleaning product use scenarios (e.g., product use patterns and exposure durations). A case study for acetic acid was used in various sentinel products and residential cleaning scenarios, was developed to refine and verify relationships among tiered approaches within the safety assessment methodology with the goal that each lower data tier in the approach provides a similar or greater margin of safety for a given scenario.

Use of Text-Mining and Machine Learning to Prioritize the Results of a Complex Literature Search

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As part of the effort to conduct various meta-analysis studies, literature reviews and technical reports, toxicologists routinely conduct complex searches of the scientific literature. These searches can yield thousands or tens of thousands of candidate documents. Human curation of this large literature set is resource intensive. To that end, we have developed and implemented, in collaboration with NIEHS, tools and procedures that can assist researchers in prioritizing, filtering and organizing these large literature search results. To demonstrate the effectiveness of this approach, we tested our software using a set of 48,638 articles identified by researchers as potentially relevant in a review of the transgenerational inheritance of health effects. Given only the title and abstract information available from PubMed, human curators applied complex inclusion and exclusion criteria to identify the relevant subset from these candidates. This time-consuming baseline approach identified 765 “included” documents. 47,873 “excluded” documents. Compared to the manual process, the system we have developed makes this baseline approach more efficient by (1) Prioritizing the results such that relevant documents are more likely to appear near the top of the ranked results list, and (2) Organizing/clustering search results according to major themes and topics in a manner that makes it more manageable to navigate the resulting literature. For example, using a randomly selected “seed” consisting of only 50 included and 50 excluded documents, our system ranked the remaining unlabeled documents such that 56% and 93% of the included documents occurred in the top 10th and 50th percentiles, respectively. Furthermore, the system conveniently organizes the document set for interactive browsing using MeSH terms and other user-defined categories. Together, these facilities will allow researchers to identify relevant literature more efficiently, and to spend more time on the analysis and interpretation of the resulting documents.

HAWC (Health Assessment Workspace Collaborative): A Modular Web-Based Interface to Facilitate Development of Human Health Assessments of Chemicals

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Regulatory agencies conduct assessments of the potential for chemicals to pose a threat to human health. Such assessments typically consist of a critical review of available studies to identify adverse health effects to characterize exposure-response relationships. In addition to the systematic review of pertinent studies and the
an analyses of available data, opportunities for public input and multiple levels of scientific review are also critical steps to engage stakeholders who may be affected. The overall framework for how assessments are performed and what information should be evaluated is well established, yet the format and the process for how assessments are conducted remain to be a notable and persistent challenge. In this project, we aimed at addressing these challenges by creating a modular, web-based content-management system to synthesize multiple data sources into overall human health assessments of chemicals. We created an online tool, HAWC (Health Assessment Workspace Collaborative, https://hawcproject.org/) which integrates and documents the overall workflow from literature search and review, to data extraction, evidence synthesis through visualization tools, dose-response analysis, uncertainty characterization, and customized reports. Crucial benefits of such a system include improved integrity of the data and analysis results, greater transparency, standardization of data presentation, and increased consistency. By utilizing a web-based workspace, assessment team members can collaborate on the same project rather than share files and track edits. Utilizing HAWC as a web-based portal for reviewers and stakeholders, all interested parties have dynamic access to completed and ongoing assessments. Overall, this project creates a clear summary of the results of the assessment, and a source for primary data and/or tabulated study summaries and visual aids that constitute the scientific justification for its conclusions.

2251 Toxic Effects of Secondhand Smoke-Revealing Mechanism by Systems Biology Approach
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The aim of this study was to investigate mechanism that enables tobacco smoke components to induce cardiovascular system toxicity. Secondhand smoke (or ETC) contains around 90 known toxic compounds emitted from tobacco combustion. We focused on 8 compounds labeled as harmful and potentially harmful constituents (HPHCs) by FDA and present mainly in the side-stream smoke with side-stream to mainstream smoke ratio of two. We created a computational model of biological pathways describing cellular processes activated by these compounds by manually annotating and processing molecular information from the literature from the public domain (PubMed articles and FDA reports) and made the data computable. We generated a comprehensive database of known side effects and protein targets of these 8 compounds. By applying bioinformatics analysis tools to this data we have identified several adverse effects regarding heart diseases, reproductive and developmental toxicity. For each of these adverse effects we generated the hypothesis of mechanism of action that is supported by current knowledge.

2252 Profiling Environmental Chemicals That Induce the Antioxidant Response Pathway Using Cell-Based Assays and Cheminformatics Tools
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Oxidative stress causes cell damage which can lead to a variety of neurodegenerative diseases. Exogenous compounds that induce oxidative stress cause cell damage which triggers the transcription of antioxidative genes located in the antioxidant response element (ARE) signaling pathway. This process is essential for alleviating cell injury, but the mechanisms that lead to cytotoxicity and further animal toxicity are still unclear. Previously, a cell-based ARE beta-lactamase reporter gene assay was used to screen 14,086 compounds in the National Toxicology Program (NTP) library in a Quantitative High Throughput Screening (qHTS) format. Then, compounds that induced the ARE pathway were identified. In this study, we first used various Quantitative Structure-Activity Relationship (QSAR) approaches to develop predictive ARE models. The resulting models were used to virtually screen other compounds of interest (e.g. Tox21 chemical library). Next, we used our in-house automatic profiling tools to gather and analyze available animal toxicity data for each of the compounds in both the NTP-14086 and the extended chemical library. The in vitro and in vivo relationship established by this step could be used to develop predictive models for complicated animal toxicity endpoints (e.g. those of neurotoxicity). The final alternative computational toxicity predictors could be used to prioritize potentially toxic compounds that cause oxidative stress and induce the ARE pathway.

2253 Improving the Development of Adverse Outcome Pathways: Lessons Learned from the AhR Rodent Liver Tumor and AhR Avian Teratogenicity/Embryolethality AOPs
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An adverse outcome pathway (AOP) represents the sequence & causal linkages between initial molecular events, key events and an adverse outcome. As part of the OECD AOP Programme, we separately developed an Aryl Hydrocarbon Receptor (AhR) AOP for rat liver tumor promotion (RLTP) & an AhR AOP for avian deformities/lethality & then collaborated to enter these in the AOP Wiki (beta). Differing levels of polymorphism-dependent AhR activation provides strong evidence identifying AhR activation during critical periods of avian development as the molecular initiating event (MIE) for avian teratogenicity/embryolethality. While AhR binding is the initial molecular event, there is strong evidence that sustained AhR activation for a significant portion of the lifespan is the MIE causally related to RLTP. In developing & adapting these for the AOP Wiki, we arrived at a recommendations applicable to other AOPs; these are 1) recognizing that while receptor binding & acute changes in gene transcription may be the IMEs, they do not always predict the MIE & the Wiki should be revised accordingly; 2) expanding the AOP Wiki structure beyond key events to include a) associative events (biomarkers that are not themselves necessary for the adverse outcome but are reliable indicators for key events) & b) modulating factors (e.g., aspects of homeostasis or other factors that may alter the occurrence &/or dose-response of the MIE or subsequent key events); 3) the need for a robust & transparent framework to document scientific confidence for use of the AOP & assays that measure responses related to each specific key event for predictive toxicology; & 4) the need to discuss explicitly application of the AOP for each different regulatory purpose: priority setting; chemical categorization; integrated approaches to testing & assessment (IATA); and screening level hazard/risk assessment.

2254 Data Mining Approach to Formulate Alerting Chemotypes for Liver Steatosis/Steatohepatitis/Fibrosis
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COSMOS oRepeatToxDB, oral repeat-dose toxicity database, is designed with an ontology describing toxicological effects at each dose level using controlled vocabulary, thus enabling mechanistic data mining. Observations are also coupled to organism-level sites and more specific effects at lower levels are formulated within hierarchical framework: organs/segments/tissues→cells/organelles. The majority of biological/chemical processes occur at the cell/organelle level, and so interactions between chemicals and proteins/gene are investigated in order to associate chemical structures with phenotypic effects resulting from related toxicity mechanisms. Furthermore, common structural fragments are extracted and refined into mechanistic chemotypes representing underlying molecular initiating events. We present a data mining view for liver steatosis, steatohepatitis and fibrosis. Over 20% of cosmetics-related chemicals in this database were associated with lipid deposition, fatty changes, cytoplasmic vacuolization, cellular infiltration and inflammation in various hepatocytes, ultimately leading to fibrosis. Combined phenotypic effects and morphological changes at various sites were mapped onto chemical compounds. Applying the ToxPrint chemotypes to these compounds, the set of alerting chemotypes for liver steatosis/steatohepatitis/fibrosis was identified. They include alcohols, diols, glycol ethers, aminophenols, tertiary amines, aromatic amines, polychlorinated short alkanes, halogenated amines, and Michael acceptors. Identification of these alerting chemotypes can be considered as the initial step in developing the categories used in safety/risk assessment. This approach also provides a way to investigate molecular pathways relevant to toxicological mechanisms. Supported by EU FP7 COSMOS Project.
Nephroticity and functional impairment in the kidney caused by drugs, have increased in recent years and become one of the major stumbling blocks in new drug candidates' safety. Currently, there are predominantly pre-clinical animal tests and in vitro models for assessing drug induced kidney injury (DIKI). The aim of this study is to develop, accelerate and ratify a unique computational model as a strategy in predicting drug induced organ injury, including DIKI. We manually annotated publicly available data (more than 10,000 articles) and proprietary company datasets (several thousand gene expression observations) related to nephrotoxicity, to build a complex molecular network of genes, proteins and chemicals within the knowledge base. We focused on known DIKI associated drugs, together with their metabolites, signaling molecules, nuclear hormone receptors and all known affected proteins for each relation within the knowledge base, with the ultimate goal in predicting possible kidney injury of a drug. We tested our system with the current standards of renal function, as blood urea nitrogen and serum creatinine, which were confirmed as proof of the concept for the DIKI. In addition, our model also confirmed recently discovered beta-2-microglobulin as molecule that could be used for detecting kidney injury.

Adverse outcome pathways (AOPs) describe the mechanistic link between a molecular initiating event (MIE) and an adverse toxicity outcome (AO). Anti-viral drugs, a class of compounds categorized as one of the most toxic in the FDA CIDER urinary tract toxicity dataset, were investigated to identify the mechanisms leading to renal toxicity. The FDA CIDER urinary tract toxicity dataset is based on the post-marketing adverse events of 1600 chemicals. Dataset clustering based on 70% sub-structure similarity provided five clusters covering purine and pyrimidine nucleosides, their corresponding phosphate nucleotide analogues, and other structurally related compounds. These classes were then investigated for the mechanistic pathways which lead to nephrotoxicity and the preparation of structural alerts for toxicity prediction. Four structural toxicity alerts were successfully implemented. These were based on publicly available in vivo reports of nephrotoxic events in man and other mammals, and data from relevant mechanistic in vitro assays. The collated data was also used to generate plausible mechanisms to describe the nephrotoxicity of anti-viral drugs in man and other mammals. We report the construction of AOPs to describe the mechanisms of the in vivo nephrotoxicity of anti-viral drugs. Structurally related nucleoside and nucleotide drugs lead to histologically and biochemically proven structural and functional alterations of both proximal tubular and glomerular cells. Proximal tubulopathies such as Fanconi syndrome and acute tubular necrosis, and glomerulopathies such as minimal change nephropathy and focal segmental glomerulosclerosis have been reported. Mitochondrial toxicity has been implicated in these highly metabolically active renal cells as one potential MIE, which is likely to be related to the clinical mode of action of these compounds in vivo. Furthermore, these compounds can act as potent inhibitors of mitochondrial polymerase gamma, which leads to mitochondrial toxicity.

We analysed rodent fetal dysmorphogenesis data (2141 chemicals) within the FDA CDER reproductive toxicity non-proprietary dataset. These data were investigated to identify pathways leading to teratogenic outcomes. Androgen receptor binding, estrogen receptor binding, 5alpha-reductase inhibition and aromatase inhibition were amongst the identified modes of action. Further literature work was undertaken to identify plausible supporting data, pathways and mechanisms. It was found that disruption of these pathways by xenobiotics and the subsequent mis-regulation of hormones can lead to teratogenic effects in developing sex organs and cause specific syndromes of defects in offspring. In rodent studies the most sensitive period of treatment is late organogenesis. Compounds may interact with various parts of these pathways rather than just at one point. Different receptor binding sites have a high degree of similarity and substrates may act as both agonists and antagonists depending on the conditions present. The sex which is predominantly affected can depend on the target and the type of binding. 5alpha-reductase inhibitors predominantly affect male offspring whereas the effects of estrogen receptor antagonism can predominantly be seen in female offspring. Investigations for compounds inhibiting the aromatase enzyme highlight data and knowledge gaps in the literature.

We report the construction of AOPs for in vivo teratogenicity, where the molecular initiating events are receptor based. The AOP framework helps to view how subtle differences in perturbation of the pathways can affect the final outcome.

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Breast cancer is the most common cause of death of middle-aged American women and few preventable risk factors have been identified. Identifying breast carcinogens could lead to risk reduction, but this has not been a focus of toxicological research. High throughput screening initiatives such as ToxCast and Tox21 hold great promise for filling in data gaps on chemical hazards. We investigated the coverage of current ToxCast and Tox21 assays with respect to breast cancer-relevant pathways such as endocrine and cancer hallmark endpoints identified previously by an expert panel, comparing the two to identify gaps. We systematically searched ToxCast and Tox21 assay descriptions for keywords reflecting endocrine, cancer hallmark, and related pathways. We also searched for assays conducted in breast cells or with proteins isolated from breast cells. We verified our results with members of the ToxCast team. ToxCast and Tox21 contain several assays intended to evaluate chemicals' effects on estrogen signaling pathways and cancer hallmark pathways, both categories of pathways believed to be important in breast cancer etiology. However, other im-
portant pathways and endpoints, including Her2, ER-β, and expression of breast cancer-relevant genes, are less well represented, whether because they were not prioritized, or because of difficulties in developing suitable high throughput assays. ToxCast and Tox21 currently include assays measuring ER, PR, AR, and aromatase, as well as cell growth kinetics, cell cycle perturbations, steroidogenesis, and apoptosis, in breast cells or proteins isolated from breast cells. Measurement of gene expression, growth signals, metabolism, and other endpoints in breast cells might provide additional important information about breast cancer risk. This disease-focused approach can be extended to other health effects, such as neurotoxicity or asthma, in order to suggest additional assays for high throughput screening projects.

2260 High-Content Screening of Rodent Mammary Gland Carcinogens in Two Breast Cell Lines
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Breast cancer is the most common cause of death of middle-aged American women and few preventable risk factors have been identified. Identifying breast carcinogens could lead to risk reduction, but this has not been a focus of toxicological research. High throughput testing methods, such as those being developed in US EPA ToxCast and NTP Tox21 programs, have the potential to identify carcinogens more quickly and cheaply than traditional rodent bioassays; the predictive power of these methods is still being evaluated. We used high content screening to measure the responses of two human mammary epithelial cell types, MCF7 (derived from a tumor) and MCF10A (derived from normal cells), to 24 and 72 hour exposures to a set of 111 chemicals relevant to breast cancer. Endpoints included measurements of DNA damage repair (p53, pH2AX), mitochondrial health, and cytoskeletal integrity (tubulin), among others, but no endocrine-related pathways. Because few human breast carcinogens have been identified, we tested 74 rodent mammary carcinogens (MCs), 19 ‘non-carcinogens’ that did not induce any tumors in NTP bioassays, and 18 chemicals that disrupt mammary gland development in rodents. Comparing the two time points, assays run with 72 hours of exposure were more sensitive than those measured after 24 hours of exposure in either cell line, with more chemicals showing activity and more of the active chemicals showing activity at lower doses. Comparing the two cell lines, most assays were more sensitive in MCF10A than in MCF7 after 24 hours, and more sensitive in MCF7 than in MCF10A after 72 hours. Chemicals previously demonstrated to be genotoxic without metabolic activation in the Ames assay did not show more activity than Ames-negative chemicals in the two assays reflecting DNA damage repair (p53 and pH2AX). Additional research is required to relate high content screening endpoints to standard toxicity tests such as genotoxicity batteries and Ames, and to understand the importance of cell line and exposure conditions.

2261 Re-Evaluation and Expansion of the US FDA/CDER Rodent Carcinogenicity Database for Structure-Based Analysis

A high-quality database of well-characterized toxicities linked to chemical structures is crucial to the development of structure-activity relationships. In order to have available the most accurate experimental data possible for structure-activity relationship development and read-across purposes, we re-analyzed the results of laboratory testing of the 1683 chemicals in the FDA/CDER rodent carcinogenicity database using contemporary standards, with a focus on extracting additional data fields and employing a more structured format for data archiving than in previous efforts. Test protocols were re-evaluated by OECD TE 451 and ICH S1B standards, including the selection of test animals, dosage, dosing duration and study duration, clinical examinations and histopathology evaluations. For pharmaceticals, statistical significance in pair-wise comparison to concurrent controls (Fisher’s Exact Test or an equivalent statistical analysis) was defined for rare tumors as \( p \leq 0.025 \) and common tumors as \( p \leq 0.005 \). For other chemicals, significance was defined for rare tumors as \( p \leq 0.05 \) and common tumors as \( p \leq 0.01 \). A numerical activity scale algorithm was designed to consistently quantify and stratify the results of the rodent carcinogenicity studies to enable more sophisticated computer analyses of structure-based trends. Among all reviewed chemicals, 190 are rodent carcinogens which cause tumors in male and female rats and mice, 364 are non-carcinogens. 98 out of the 190 carcinogens target the same organs across species and gender. The number of chemicals that are tumorigenic to male rats, female rats, male mice and female mice is 519, 482, 447, and 459, respectively. The most common tumor sites are the liver and lung. All organ-specific carcinogenicity data, supporting citations, and their respective chemical structures will be made available to the FDA/CDER community as a structurally searchable data repository, facilitating complex structure- and text-based queries, and supporting the development of quantitative structure-activity relationships.

2262 Establishing Best Practice for the Application of a Novel Statistical-Based Model to Evaluate Potential Mutagenic Impurities under ICH M7
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Under the emerging International Conference on Harmonisation (ICH) M7 guideline, a safety assessment of a pharmaceutical impurity may, in some instances, be performed using in silico-based methods without the need for in vitro testing. Such assessments should consist of a statistical-based method (QSAR) and an expert rule-based method (SAR), both of which should be consistent with OECD guidelines for the regulatory use of (Q)SARs. These models need to be appropriately applied to ensure accurate and trustworthy predictions are generated. Furthermore, model predictions should be transparent and scientifically explicable to support the expert assessment recommended under the guideline.

In this work, we present best practice for the application of a new statistical-based QSAR software, Sarah Nexus, for the assessment of the genotoxic potential of pharmaceutical impurities. Sarah Nexus utilizes a novel machine-learning approach based upon self-organizing hierarchical networks, and was used to construct a statistical model based upon Salmonella mutagenicity data. The methodology allows for the tuning of model performance to balance sensitivity against specificity, as well as overall accuracy against coverage. To establish best practice, optimal model application settings were assessed using two independently-derived external validation sets containing 513 (19% positive) and 809 (42% positive) compounds. The same optimal settings of 10% sensitivity (defines the relative impact of positive signals) and 10% equivocal (the threshold under which the signal is not strong enough for the model to make a call) were identified for both cases, resulting in balanced accuracy of 74 and 77%, sensitivity of 64 and 68%, and specificity of 86 and 85%, respectively, for the two validation sets. While coverage is reduced to 81 and 86% under these conditions, this is considered an acceptable trade-off in favor of overall improved accuracy and, in particular, improved sensitivity.

2263 Genotoxic Risk Assessment of Impurities in Drug Substances

CPMP/SWP/S199/02 (2006) guidelines for Europe and FDA draft guidance (2008) for US are the current main reference documents for dealing with genotoxic and carcinogenic impurities in drug substances. An international effort for addressing different approaches and sometimes inconsistencies or gaps in the multiple guidances or papers published from different international bodies (ICH, EMA, PhRMA, FDA) is ongoing by the International Harmonization Conference (ICH), which M7 guideline is currently in step 2.

In this poster focus is given to the genotoxic impurity issue, describing the internal (Aptakis) Genotoxic Risk Assessment (GRA) process and presenting an overview of the results obtained from GRAs performed in the past three years. A perspective and process change once M7 guideline is released is also presented/discussed. GRA is an integrated, multidisciplinary process, involving essentially chemists, analysts and toxicologists, with the aim to evaluate the potential presence of genotoxic (DNA reactive, mutagenic) impurities in drug substance. The chemical route is first evaluated for potentially mutagenic compounds as defined by the “Genotoxic Impurities of High Concern” list of functional groups. Intermediates, reagents, major by-products (including reaction of reagents with solvents) and degradants are considered. Chemical structure rising concern are submitted to Derek Nexus (Lhasa Limited) for identification of structural features alerting for mutagenicity and carcinogenicity; those with confirmed alerting structure are submitted to the Ames test. Genotoxins should be removed or controlled at TTC (Threshold of Toxicological Concern) level. The group has evaluated in the past three years 20 synthetic routes (133 chemical stages), for an overall amount of approx 800 chemical structures assessed, of which 97 structures were recommended to DEREK screen. For most of these structures a control strategy was developed and only 59 were actually submitted to the analysis in silico. From these, 16 were confirmed to have mutagenicity and/or carcinogenicity concern. Ames test resulted positive for 3 structures.
It has long been axiomatic that predictive toxicology models built on data available in the public domain can encounter problems when attempting to predict proprietary data, e.g. from a drug development program. Conversely, model performance against public data is often used to support the selection of models for specific applications and such analyses are often published. One outcome of some recent model development work has been to produce metrics that can be used to experimentally test the axiom and assess the validity of basing conclusions on public data. A large data set, based primarily on public data, was used to identify improvements to an expert knowledge base for (Ames test) mutagenicity. These improvements were implemented as new or modified structural alerts, and were incorporated into successive knowledge base iterations. The performance of each iterative build was evaluated against a large public data set (Benchmark) and a proprietary data set (Vitic Intermediates).

Our analysis showed that the addition (or modification) of structural alerts consistently improved the detection of mutagens within the Benchmark data set. However, this improvement was not mirrored against the Vitic Intermediates data set.

It can be concluded that performance against public data should be weighted accordingly when considering the use of models within a given chemical space. This experimentally supports the original axiom, at least in part, and suggests that building a model comprising only of public datasets (however large) may not be sufficient to allow accurate predictions within proprietary chemical space. However, further analyses would be required to verify the robustness of this conclusion (e.g. other pharmaceutical data sets) and also to read-across these results into other domains (e.g. agrochemicals or personal care products). Future work will focus on this, as data become available.

**Development of an In Silico Profiler for Mitochondrial Toxicity**

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Disruption of normal mitochondrial functioning can result in cell death and is implicated within a variety of diseases. There are a number of mechanisms by which normal functioning can be impaired these include, but are not limited to, uncoupling of oxidative phosphorylation, inhibition of the electron transport chain and membrane permeability induction. Mitochondrial toxicity has been identified as a source of chronic toxicity in a number of drugs leading to their withdrawal from the market. Thus, the ability to identify chemicals potentially capable of this type of toxicity would be of benefit in the risk assessment process. Despite this, few in silico methods have been developed capable of making such predictions. Therefore, the purpose of this study was to develop an in silico profiler consisting of a series of structural alerts related to the mechanisms involved in mitochondrial toxicity. Data for 288 compounds identified as being either toxic or non-toxic to mitochondria were extracted from the literature. Structural similarity was undertaken to group the data, producing fourteen categories, from which nine structural alerts were identified, covering nine chemical classes and five mechanisms of mitochondrial toxicity. The nine chemical classes covered include thiadiazolidinediones, anthracyclines and cholic acid derivatives. The identified mechanisms were uncoupling of oxidative phosphorylation, inhibition of the electron transport chain, induction of membrane permeability transition, alternative electron acceptance and initiation of the death receptor pathway. These structural alerts were supported by mechanistic information from the literature allowing the chemistry of the Molecular Initiating Event to be defined. In addition, a new structure knowledge representation method, chemotype, is also used to extract mechanistic alerts from the dataset. The alerting chemotypes can also be deployed in KNIME node or on ChemOtyper (free software from FDA). Supported by the EU FP7 COSMOS Project.
In silico prediction of off-target pharmacology and adverse effects for small molecule drugs has been hampered by small and/or poorly representative training sets, and subsequently low predictivity. Here we present the results of an evaluation of the Similarity Ensemble Approach (SEA) to large scale prediction of off-target pharmacology. SEA calculates whether a molecule will bind to a target based on the chemical features it shares with known ligands, using a statistical model to control for random similarity. An independent test of over 200 proprietary compounds was evaluated for off-target interactions in comparison to actual ligand binding or enzyme inhibition data across approximately 150 targets. Using a training set based on publically available data (CheMBL14), the prediction accuracy was poor and close to random (AUC of 0.53), however a second independent test set of over 850 well known drugs and chemicals had improved prediction accuracy (AUC of 0.79). As a result it was hypothesized that good prediction accuracy could be obtained if the training and test set compounds were structurally similar. To that end, we generated a training set based on a large comprehensive collection of internal drug-target interaction data and subsequently evaluated the algorithm using a 3-fold cross-validation approach. Using various affinity thresholds and compound fingerprinting methods, we consistently achieved high global prediction accuracy (AUC > 0.9). Sensitivity and specificity for individual off-targets could be optimized with altered thresholds to limit the number of false positives and still achieve a useful level of sensitivity. These results highlight the possibility of providing advanced knowledge of hundreds of off-targets based on structural information alone. It is anticipated such methods will be useful to guide selectivity screening strategies during lead optimization and provide novel insights to off-target toxicities not otherwise known.

**2270 Molecular Dynamic Simulation Studies of Bisphenol A and Its Analogs with Estrogen Related Receptor-Gamma and Human Androgen Receptor**

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Bisphenol A (BPA) and several of its analogs are high volume chemicals used mainly in the manufacturing of polycarbonate plastics. It has long been recognized that BPA and its analogs behave as xenoestrogens, and can cause reproductive disorders. We have recently determined the free energies of binding of BPA and its chlorinated and nitrated products to the estrogen-related receptor-gamma (ERRγ) and compared the binding energies observed with the natural ligand, estradiol. These studies hold promise for characterizing binding pockets and changes in free energy of binding for biologically-relevant bisphenols and their environmentally-generated derivatives. The present study focuses on extending the molecular docking calculations/simulations to various BPA analogs based on binding to not only ERRγ but also to human androgen receptor (hAR) by performing Free Energy Perturbation (FEP) using NAMD software. The absolute binding free energies of the ligand complexes are computed by running annihilations forward and backward with explicit solvent. Restraining potentials were removed during the simulations to observe changes in conformational and translational energies of the ligand and receptor upon binding. The whole system was minimized and equilibrated before running the said annihilations to remove steric hindrance(s) between atoms. The net binding free energies of BPA and its analogs thus calculated are analyzed and compared with those of experimental values which revealed strong binding affinity of compounds towards the receptors when compared to the natural ligand. The results from this study are promising as they can be used to characterize binding pockets and changes in free energy of binding of BPA and helps in identifying the most significant molecular targets for xenoestrogens in general and BPA in particular [Support from NSF (HRD1043316) and the US DoEd (PO31B040030) is acknowledged; corresponding author’s email: rao_uppu@sbur.edu].

**2271 Amoxillin- and Pefloxacin-Induced Ionoregulatory Disruptions in Rat Tissues**

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Antibiotics are among the commonly prescribed and abused drugs in medicine. Their use is often associated with a variety of side/toxic effects. In order to investigate whether amoxillin and pefloxacin perturb electrolyte homeostasis, rats were treated with therapeutic doses of each antibiotic for 5 and 10 days respectively. Twenty four hours and 5 days after antibiotic withdrawal, blood and other tissues (liver, kidney, brain, heart and spleen) were removed from the animals analysed for their electrolyte contents and activities of Na+-K+- and Ca2+-Mg2+-ATPases. Molecular docking of the antibiotics against rat’s alpha-3 subunit of Na+-K+-ATPase (NCBI accession number: gi6978547) and Ca2+-ATPase (NCBI accession number: gi6758008) was also performed. After 5 days of pefloxacin administration, the activity of Ca2+-Mg2+-ATPase in erythrocyte ghost, liver, kidney, brain and heart dropped to 75%, 71%, 91%, 92% and 42% of the control respectively. Na+-K+-ATPase activity was reduced to 48% and 59% of the control in the kidney and brain respectively. There was however a reversal in the activities of renal and cardiac Na+-K+-ATPase as well as splenic and cardiac Ca2+-Mg2+-ATPase 5 days after the withdrawal of amoxicillin treatment. These culminated in hyponatremia, hypokalemia, hypocalemia and hyponagemesia. By day 15 however, there was a resolution of the ionoregulatory disruption. In silico docking revealed that three hydrogen bonds are formed between amoxillin and Glu818, Tyr1012, Arg930 and Arg827 of Na+-K+-ATPase. Pefloxacin however forms only one hydrogen bond with Arg930 of the enzyme. Only Glu875 and Pro828 of Ca2+-ATPase form hydrogen bond with amoxillin and pefloxacin respectively. The findings of this study further refuel the pattern and mechanisms of ionoregulatory disruptions by these antibiotics.

**2272 Enhanced Alternatives Analysis: Automated Evaluation of Chemical Hazard Attributes to Identify Preferred Substitutes**

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Many companies, especially downstream users of chemicals, are moving toward improved chemicals management by establishing programs to better understand the chemicals in their products. In addition to collecting specific chemical ingredient information, companies are tasked with identifying chemicals of concern and prioritizing where to take action. One aspect of this process is to utilize Restricted Substance/Authoritative Lists (RSLs). A more robust approach focuses on understanding the underlying hazard data in the scientific literature to identify critical endpoints.

Benefits of an enhanced process for evaluating chemicals in products beyond basic RSL review includes predicting which chemicals are likely to be added to RSLs in the future and reducing cost of chemical substitution via proactive efforts. Our research made use of the SciVera Lens attributes-based chemical hazard assessment database and several accompanying automated analytical tools to identify the gap between chemicals on key RSLs and chemicals with reported high hazard (based on GHS criteria). The results are based on analysis of the set of over 135,000 chemicals in the SciVera Lens and European Chemical Agency (ECHA) databases and a group of over 40 common RSLs.

The analysis focused on the Developmental-Reproductive (D-R) endpoint due to the fact that several RSLs identify this endpoint as the rationale for listing. The results indicate that more than 30 percent of chemicals with reported scientific data for high D-R hazards were not on any RSL and greater than 50 percent when assessed against specific D-R lists.

This analysis confirms that a hazard attributes-based assessment, when combined with the efficiency and consistency of automated techniques, provides for a more robust and predictive approach for alternatives assessment, allowing users to identify chemicals of potential concern prior to inclusion on an RSL, thereby reducing the potential for regrettable substitutions.
We present an in silico workflow for evaluating chemical toxicity instigated upon dermal contact. The first step identifies compounds that will not be absorbed and removes these from further consideration. Compounds that are absorbed and cause skin irritation are identified next, while absorbed non-irritating compounds are passed on to the next module for evaluation of skin sensitization potential. Probable metabolites are added to the workflow along with the parent molecules. Skin sensitization potential is predicted by modeling mouse local lymph node assay (LLNA) data, with values ranging from non-sensitizing to weak, moderate, strong, or extreme. Covalent modification of proteins by sensitizers by well-known reaction mechanisms represents the molecular initiating event for the induction. ToxPrint chemotypes, structural fragments encoded with physicochemical properties and electronic system information, are used to categorize chemicals into MIE classes. Skin metabolic rules, coded in chemotypes, were also used to predict bioavailability and reactivity. Results from multimodal ordinal classification QSAR models for each MIE class are combined using a rigorous weight-of-evidence approach that explicitly quantifies the uncertainty associated with each prediction. Our approach has been externally validated using skin irritation and sensitization results from literature studies. The workflow effectively separates irritants from non-irritants. Approximately 70% of sensitizers are predicted in the correct category and better than 90% are predicted within one category or their reported experimental value. Further, all sensitizers are associated with chemotype alerts, illustrating the power of this approach in overcoming limitations of classical structural alerts. The workflow comprehensively addresses chemical toxicity via dermal contacts.

**2273a Computational Molecular Modeling for the Assessment of Nanoparticle Toxicity: Interactions with Biomolecules**

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Over the past two decades, nanotechnology has emerged as a key player in various disciplines of science and technology. A detailed understanding of the molecular details of interactions between nanoparticles (NP) and biomolecules is crucial for obtaining adequate information on mechanisms of action of nanomaterials and their possible toxicological outcomes. There has been a recent surge in the application of in silico based methods and approaches to address interactions of NPs with biomolecules providing insights into their mechanisms. To do this, we used structure-based computational modeling as a tool to predict the molecular interactions between carbon nanotubes and graphene with the carboxyl moieties on carbon nanotubes and graphene – positioning them in close proximity to the catalytic site of the enzyme – are essential for the effective catalysis and safe degradation of these materials in vivo. The current draft of the International Conference on Harmonisation (ICH) M7 guideline describes the use of (quantitative) structure-activity relationship (Q) SAR) models during drug safety evaluation. The guideline, however, does not specify the use of any particular model, but instead recommends that the models meet the general definition of statistical or rule-based methodologies, and allow the identification of structural alerts. In this study, we evaluated the performance of Toxtree, a freely-available, open source SAR model and two newly-released commercial (Q)SAR programs, Sarah Nexus and Leadscope Expert Alert System, as potential candidates for qualifying pharmaceutical impurities. To effectively assess the performance of Toxtree, an in-house Salmonella mutagenicity database of 3979 compounds (43% positive) and a highly-curved version of the Hansen dataset of 3734 compounds (58% positive) were used. Performance statistics for the two datasets ranged from 79% to 85% sensitivity and 85% to 73% negative predictivity, respectively. The performance of the statistical-based system, Sarah Nexus, was assessed using the in-house Salmonella dataset after removal of compounds that overlapped with the training set. The resulting dataset comprised 809 chemicals (42% positive), and yielded sensitivity and negative predictivity of 68% and 79%, respectively. The rule-based Leadscope Expert Alert System was evaluated using the curated Hansen dataset of 3734 chemicals (58% positive) and yielded performance of 85% sensitivity and 78% negative predictivity. These performance statistics compare favorably with those of the three most widely-used commercial model systems tested with the same data sets, indicating their suitability for the qualification of pharmaceutical impurities under ICH M7.

**2273d Metabolism Simulation and Toxicity Prediction in the Evaluation of Food Ingredients/Contaminant Safety**

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During the safety assessment of food additives and their impurities, metabolic knowledge becomes critical when in vivo data are unavailable for the specific compound. Inclusion of metabolism information into the in silico workflow is therefore a pre-requisite for the US FDA’s Chemical Evaluation and Risk Estimation System (CERES). The chemical space of food additives was profiled using public ToxPrint and metabolic (human liver and S9-fraction) chemotypes. Chemotypes are structural fragments encoded with physicochemical properties, and are considered as alerts when associated with a specific endpoint. The metabolic potential was predicted by applying a diverse set of over 100 phase I as well as more than 20 phase II conjugation reactions of human liver metabolic chemotypes. As an example, the effect of S9 metabolic chemotypes on Ames mutagenicity was analysed against the food additives. Although nearly 20% of the compounds were perceived as genotoxic carcinogens by chemotype alerts, only 4% of such compounds were actually predicted to be mutagenic under CERES mechanistic QSAR paradigm. Nearly 50% of the food additives predicted to be mutagenic were matched with chemotype alerts for genotoxic carcinogens. When repeating the analysis after removing the compounds that may be detoxified by S9, the reliability of the genotoxic alerts is increased almost 3 times. This study demonstrates the value of the use of metabolic rules in conjunction with known chemotype alerts to reduce the false positive rate of structural rules. Implementation of the metabolic rulebase in CERES is also presented. The research was funded by EU FP7 and Cosmetics Europe. (Grant no 266835).
Establishing relationships between gene expression and phenotypic endpoints is important to discover mechanisms of action of a toxic compound. In this study, pattern recognition methods for the identification of genes whose expression time-courses are associated with phenotypic endpoints are compared. Pattern recognition methods have been shown to be very effective in answering many biological questions, because biological entities belonging to the same cluster are assumed to be functionally related (1,2). To compare pattern recognition methods for identification of gene-phenotypic endpoint relationships from time-courses, four representative methods were chosen: k-means clustering (1), short time series expression miner (STEM)(3), linear mixed model (LMM) mixtures (4) and dynamic time warping (DTW4omics)(5). The four methods were applied on two published data sets on a human liver carcinoma cell line (HeP2): the response to benzo(a)pyrene (6) and menadione (7). Both data sets consist of time-series of both gene expression and phenotypic endpoints.

Lists of genes associated with an endpoint were identified and the overlap among the four methods determined. Gene Ontology (GO) and pathway analysis were applied on the gene lists. Gene, pathway and GO lists show low overlap among the methods and each method provides biologically relevant information (large overlap with the literature). Additionally, relationships not occurring in the literature represent new hypotheses. Testing these hypotheses experimentally can lead to the discovery of new mechanisms of action for a toxic compound.

Currently mandated testing for potential estrogenic activity will involve thousands of chemicals, cost millions of dollars, and take decades to complete using current validated tests. High-throughput screening and computational toxicology tools may streamline this process by the quick and cost-effective identification of endocrine-active chemicals (EACs). Access to a comprehensive database of high-quality in vivo EAC toxicity data is critical for the validation of in silico models and in vitro assays. The database can be used to prioritize chemicals for screening. Validation of HTS assays using the database will enable replacement of current validated in vitro screening assays with validated HTS assays. The results of these tests will better inform and target in vivo screening assays. To create such a database, we reviewed the current scientific literature, identified high-quality in vivo endocrine disruption testing data, and compiled the data into a single database. Initial review focused on the estrogenic effects of 52 reference chemicals selected by the EPA and NTP. Studies including data for these 52 chemicals on a number of different estrogenic endpoints (i.e., uterotrophic, pubertal, multigenerational, etc.) were identified. Data from the studies were extracted and compiled using a standardized ontology. An R script was developed to evaluate the quality of the data according to modified Klimisch criteria in an efficient and standardized manner. Data that were classified as reliable were added to the database, which is available on the NTP website (http://ntp2.niehs.nih.gov/go/40658). This database constitutes a critical resource for validating in vitro and in silico models of estrogenic activity. This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. N01-ES-35504.

Quantitative high-throughput screening (qHTS) assays from Phase II of the Tox21 collaboration provide concentration-response profiles for thousands of chemicals. It is necessary to be able to rank chemicals using data generated from such large-scale screening experiments in order to efficiently prioritize environmental agents for possible endocrine disruption effects. Rankings are typically based on parameter estimates derived from fits to a pre-specified model, such as the logistic Hill Equation model. In particular, chemicals are often ranked using AC50 parameter estimates from the Hill Equation model, where an AC50 value represents the concentration exhibiting 50% of maximal induction along a sigmoidal curve. However, rankings based on this metric can be unreliable due to large errors associated with the parameter estimates when response data does not adequately fit the assumed model structure. Here, we describe an alternative approach for ranking chemicals in qHTS experiments based on weighted entropy scores (WES). WES provides a measure of average activity level that can be calculated from the observed response values and the underlying assay detection limits. We use WES to rank chemicals based on 15-point concentration-response data obtained from Tox21 Phase II BGI ER-Luc estrogen receptor agonist and antagonist assays. A total of 276 substances in agonist mode, and 134 chemicals in antagonist mode, produced consistently high WES scores across all three experimental runs (WES ≥ 2 bits). Only 38 compounds in agonist mode, and 32 compounds in antagonist mode, were ranked in the top 1,000 compounds by WES and AC50. No significant correlation was observed when comparing ranks based on WES and AC50 for the 11,776 tested substances in agonist mode or antagonist mode.

FDA drug labels represent the consensus and combined experience of regulators, drug sponsors and manufacturers, and scientific experts with information about product indications, target populations, and adverse drug reactions (ADRs). The amount of information captured in drug labels has grown rapidly, and the total number has likewise increased with around 400 to 500 new or updated drug labels being added weekly from the SPL (Structured Product Labeling) group in the FDA. This rapid pace of change, along with the breadth and depth of information contained in drug labels, highlights the need for a powerful search tool. FDALabel (http://www.fda.gov/ScienceResearch/BioinformaticsTools/ucm289739.htm) is a freely available, web-based, comprehensive drug label database with powerful full-text query capabilities. The database contains the full set of approximately 50,000 FDA-approved drug labels downloaded weekly from the National Library of Medicine’s DailyMed archive (http://dailymed.nlm.nih.gov/). The simple search options in FDALabel include full-text search as well as searches within only the product or generic name. The advanced search enables query text based on any combination of specific sections, dosage forms, document types, market categories, market date and other information. We have selected study cases in this poster, including a pharmacogenomics biomarker study and also an ADR study which utilizes MedDRA standard terminologies. FDALabel provides researchers, regulators, drug developers, and physicians an effective and efficient means of accessing the rich amount of information contained in FDA drug labels. It also opens the possibility for new uses of drug labels in supporting the FDA’s goals of advancing translational and regulatory sciences, e.g., by identifying trends in ADRs or by identifying ADRs that are associated with increased risks to public health.

High-throughput screening (HTS) and high-content screening (HCS) assays have changed the pace of chemical data collection for gene-protein, cellular, whole-embryo, and pathway-based responses. However, without the necessary assay metadata, it becomes a challenge to communicate what kind of data an assay reports. Integrative semantic resources such as the BioAssay Ontology (BAO) were developed to facilitate the annotation of assay parameters. We have used BAO with expansions to its vocabulary to produce the ToxCast Assay Annotation, a metadata resource to aid in the communication and use of EPA’s ToxCast HTS and HCS assays. The ToxCast Assay Annotation describes 942 analyzed readouts (assay endpoints), which have complete Phase I and II disruption testing data (about 1000 chemicals). The annotation includes over 40 descriptor-classes (e.g., assay design type, detection technology type, and key assay reagent), which have been organized to aid in communication and analysis. In addition to BAO, various ontology sources such as the GPCR ontology and NCIt thesaurus were incorporated for an abstract view of the target annotations. Where applicable, the conceptual difference between the technological target (e.g., mRNA, chemical, or protein) and the intended target (e.g., transcription factor activity, enzyme activity, or signaling pathway activity, respectively) are separately annotated. This annotation allows for a clear and concise reference for interpreting the ToxCast data, allows for identification of comparable ToxCast assay endpoints, and offers the potential to link with other HTS data repositories. This abstract does not necessarily reflect Agency policy.
models on those test chemicals from publicly available toxicology databases and resources including OpenTox, ToxBank, Comparative Toxigenomics Database, TG-GATES, SEURAT, PubChem, and Tox21 databases for risk assessment and management decision-making supported by the remediation tool for contaminated sites and identified data gaps and limitations in applications. Disclaimer: “This poster abstract is based on a report to be finalized under contract to HC, Contaminated Sites Division; however, the abstract, the report and the poster does not necessarily reflect the opinion of HC nor is it HC guidance.”

**2273n Web Application Supporting Chemical Safety Decisions**

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**MVC architecture** that allows separation of backend support and frontend dis- sources of data into customized information displays. iCSS is built using both (iCSS) Application. iCSS is a web-based dashboard that synthesizes multiple data, the USEPA has developed the Interactive Chemical Safety for Sustainability datapoints for thousands of chemicals. To allow for easier public access to this abstract does not necessarily reflect U.S. EPA policy.

serve as a portal for ToxCast data access for risk managers and the public. This larger chemical subsets down to finely focused chemical-wise views. The iCSS will plays streamlines the toxicity assessment process and allows users to zoom from scores are carried over into a prioritization process where the chemicals are ranked attention. Users can adjust global score criteria or apply expert knowledge to mod- ify prioritization, and all decisions saved for sharing or later consideration. The scores are carried over into a prioritization process where the chemicals are ranked in a weight-of-evidence scheme to identify candidates for focused assessment. The aggregation of relevant data and use of web applications to generate custom displays streamlines the toxicity assessment process and allows users to zoom from larger chemical subsets down to finely focused chemical-wise views. The iCSS will serve as a portal for ToxCast data access for risk managers and the public. This abstract does not necessarily reflect U.S. EPA policy.

**2273q Screening Environmental Chemicals in Metabolically Competent Human-Derived Hepatocytes**

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ToxCast™ is the U.S. EPA’s screening program comprising hundreds of relatively inexpensive and high-throughput assays to compare thousands of chemicals toreference toxicants and evaluate potential bioactivity. A new suite of assays aimed at investigating gene specific perturbations elicited by environmental chemical exposure in metabolically-competent, human-derived HepaRG cell line was designed and optimized. Briefly, cells were treated with individual chemicals in an eight-point concentration-response format in duplicate for 48 hours in a 96-well plate format. Total RNA was collected for gene expression profiling via real-time quantitative polymerase chain reaction (qRT-PCR). We evaluated the expression of a diverse set of 93 genes representative of various biological processes and disease states including: cell proliferation, survival, and death; nuclear receptor (NR) mediated metabolism and transport; oxidative stress; steatosis and fibrosis. Many of the genes are known to be transcriptionally regulated by key NRs, including CAR, PXR, PPARα, and HNF4α. Additionally, we evaluated reference plates with dilutions from high concentrations (e.g. 1 mM) of known toxiancients phenobarbital (PB), alloxin B1, omeprazole (OMP), fenofibric acid, and chenodeoxycholic acid (CDCA). These results demonstrated that the HepaRG cells elicited classically reported expression responses for CAR/PXR and FXR activation upon exposure to PB, OMP and CDCA, respectively. Evaluating thousands of chemicals in this assay provides unique insight into endogenous gene signaling net-works intrinsic to key hepatocellular processes and disease states in humans. This abstract does not necessarily reflect U.S. federal government policy.

**2273r Prioritization of the Tox21 10K Library for Xenobiotic Metabolism and Toxicity Studies Using In Silico Metabolism Models**

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One of the challenges within the U.S. Tox21 high throughput screening program is the lack of in vitro approaches incorporating xenobiotic metabolism. To help address this issue, in silico methods (ADMET-Predictor) have been employed to

**2273o Estimation of Octanol/Water Partition Coefficient and Aqueous Solubility of Environmental Chemicals Using Molecular Fingerprints and Machine Learning Methods**

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Octanol/water partition coefficient (logP) and aqueous solubility (logS) are two im-portant parameters in pharmacology and toxicology studies, and experimental mea-surements are usually time-consuming and expensive. In the present research, novel methods are presented for the estimation of both logP and logS of environmentally interesting chemicals solely based upon simple binary molecular fingerprints on a single data set which consists of 993 training samples and 251 test samples. A group of quantitative structure-property relationship (QSPR) models were developed using four approaches with different complexity: multiple linear regression (MLR), random forest (RF) regression, partial least squares regression (PLSR), and support vector regression (SVR). Genetic algorithms (GA) and RF method were employed to select the most information-rich subset of descriptors for obtaining reliable and robust regression models with high prediction performance. It was found that MLR, PLSR and SVM exhibited satisfactory predictive results with low prediction errors and substantially outperformed RF. MLR coupled with GA for descriptor selection was clearly superior to all other approaches and achieved cor-relation coefficients of 0.936 and 0.927 between the calculated and experimental data on the validation set for logP and logS, respectively. The inclusion of logP and molecular weights (MW) as two descriptors into logS models significantly improved the prediction accuracy, especially for RF modeling. The present study demonstrates that molecular fingerprints are very useful descriptors, GA is a very efficient feature selection tool and the selected descriptors can effectively model the two properties, and simple methods such as MLR give better results than more complicated methods. These models can be used for rapidly and accurately predict-ing logP and logS of environmental chemicals. This abstract does not necessarily reflect U.S. EPA policy.

**2273s Pharmacokinetic Triage for Environmental Chemicals**

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Pharmacokinetic (PK) models aid in determining whether chemical exposures pro-duce potentially hazardous tissue concentrations. For bioactivity identified in vitro (e.g. ToxCast™) – hazardous or not – PK models can forecast exposure thresholds, below which no significant bioactivity is expected. Successful methods have been developed for pharmaceutical compounds to determine PK from limited in vitro measurements and chemical structure-derived property predictions. These high throughut (HT) PK methods: provide a more rapid and less resource-intensive alternative to traditional PK model development. Unfortunately, predictions from HTPK approaches have demonstrated mixed success for environmental chemicals when compared to predictions made by PK models developed with extensive in vitro data. Here we tested assumptions of previous HT PK approaches using a sim-ply physiologically-based PK (PBPK) model and in vitro data for 232 chemicals in human and 39 chemicals in rat. We then analyzed the discrepancy between the predictions of HTPK and in vitro literature PK data for 44, mostly pharmaceutical, chemicals to characterize those properties that correlate with poor predictive ability (e.g., in vitro HTPK data, physico-chemical descriptors, chemical structure, and predicted transporter affinities). We propose a framework for PK triage in stages: First, in vitro measurements and in silico predictions determine whether the simpl-est HTPK approaches are likely to be sufficient. Second, identify and collect any additional, targeted in vitro data that is needed. Third, identify those chemicals most likely to require traditional, in vitro PK methods. This methodology allows prioritization of PK resources and characterizes the confidence in HTPK model predictions for potentially thousands of environmental chemicals that currently have no PK data. This abstract does not necessarily reflect EPA policy.
identify and prioritize chemicals likely to undergo xenobiotic metabolism transforms (human) for studies in assays using metabolically-competent cells (e.g., primary hepatocytes, HepaRG). Three general criteria were used for prioritization: 1) chemicals predicted as substrates for human metabolism enzymes, 2) chemicals predicted to undergo extensive metabolism, and 3) chemicals predicted to generate metabolites with structural alerts for toxicity endpoints. Over the 18 xenobiotic metabolism enzymes (P450s and UGTs) predicted, true positive prediction rates of 85.7% (CYP1A2), 90.4% (CYP2C9), 76.6% (CYP2C19), 93.3% (CYP2D6), and 93.5% (CYP3A4) were observed for established substrates of 5 major xenobiotic metabolism pathways (quantitative models). Applying ADMET Predictor to 8,193 unique chemicals in the 10k library, 47,320 hits were predicted. Intrinsic clearance predictions were generated for each chemical and these data were combined using the ToxPi application to rank chemicals based on extent of metabolism weighted to relative expression levels in human liver. Using MedChem Studio, 168,805 unique metabolites were predicted. These structures were analyzed in various toxicity prediction models (e.g., estrogenic/androgenic activity, mutagenicity) to rank molecules based on anticipated toxicity. Rankings (Tox Risk) revealed various structures including 49 with nitroso groups (e.g. N-Nitrosopiperazine) out of the top 100 (96 predicted metabolites). 2,152 unique molecules made up the top 94% was reached with a non-error rate of 89% in fitting and 80% with 5-fold cross-validation. The U.S. EPA and Tox21 partners screened 1,877 chemicals, including pesticides; food, cosmetics and personal care ingredients; pharmaceuticals; and industrial chemicals. Testing used 782 in vitro assays across 7 technologies and biological formats (cell-free, cell lines and primary cells, multiple tissues). Assays were run in concentration-response (0.01 to 100 μM) with replicates and controls. We report several key findings. First, in 91% of the genes assayed for which we have reference chemicals (defined as a chemical with a known molecular target, >75 out of 313 genes total), a reference chemical is in the top quartile of potency. Second, 41% of chemicals show a “burst” of non-specific activity related to cell stress / cytotoxicity. On average, once ~5% of assays are activated by a chemical, there is a linear increase in the number of cytotoxic/cell-stress-related hits relative to hits in other assays (R2=0.84). This indicates a potential for non-specific assay activity. In a set of 40 chemicals with both ToxCast and in vitro pharmacokinetic data, the burst concentration corresponds roughly to concentrations predicted to be seen in vivo at the maximum tolerated dose (MTD) in rat 2 year chronic cancer studies (78% of MTDs were in the burst region). Cholinesterase inhibitors were a prominent exception, with MTDs all below the burst range, since the MTD is driven by cholinesterase activity rather than systemic effects. Finally, chemicals were ranked by relative potency and target specificity to provide a quantitative metric to prioritize chemicals for further study. About ~1% of chemical-gen combinations show potent and specific activity (~3 hits per chemical on average). This abstract does not necessarily reflect U.S. EPA policy.

**2273s In Vitro Screening of 1877 Industrial and Consumer Chemicals, Pesticides, and Pharmaceuticals in up to 782 Assays: ToxCast Phase I and II**

R. Judson1, K. Houck1, M. T. Martin1, A. M. Richard1, T. B. Knudsen1, N. S. Sipes1, I. Shah1, S. B. Little1, J. F. Wambaugh1, M. Linnenbrink1, M. C. Leung1, C. Strope1, L. Truong1, R. Thomas1, D. Smith1, D. Reif2, D. Rotroff2, N. Kleinstreuer4, M. Xia3 and R. Huang3.

The U.S. EPA and Tox21 partners screened 1,877 chemicals, including pesticides; food, cosmetics and personal care ingredients; pharmaceuticals; and industrial chemicals. Testing used 782 in vitro assays across 7 technologies and biological formats (cell-free, cell lines and primary cells, multiple tissues). Assays were run in concentration-response (0.01 to 100 μM) with replicates and controls. We report several key findings. First, in 91% of the genes assayed for which we have reference chemicals (defined as a chemical with a known molecular target, >75 out of 313 genes total), a reference chemical is in the top quartile of potency. Second, 41% of chemicals show a “burst” of non-specific activity related to cell stress / cytotoxicity. On average, once ~5% of assays are activated by a chemical, there is a linear increase in the number of cytotoxic/cell-stress-related hits relative to hits in other assays (R2=0.84). This indicates a potential for non-specific assay activity. In a set of 40 chemicals with both ToxCast and in vitro pharmacokinetic data, the burst concentration corresponds roughly to concentrations predicted to be seen in vivo at the maximum tolerated dose (MTD) in rat 2 year chronic cancer studies (78% of MTDs were in the burst region). Cholinesterase inhibitors were a prominent exception, with MTDs all below the burst range, since the MTD is driven by cholinesterase activity rather than systemic effects. Finally, chemicals were ranked by relative potency and target specificity to provide a quantitative metric to prioritize chemicals for further study. About ~1% of chemical-gen combinations show potent and specific activity (~3 hits per chemical on average). This abstract does not necessarily reflect U.S. EPA policy.

**2273t Using ToxCast Nuclear Receptor Activity to Predict Liver Toxicity**

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Nuclear receptors (NR) are molecular initiating events in pathways to rodent liver cancer. 260 out of 309 ToxCast Phase I chemicals induced liver lesions in rat according to guideline testing studies from ToxRefDB. We used supervised machine learning to systematically investigate the accuracy of 621 ToxCast Phase I high-throughput screening (HTS) assays for classifying 260 chemicals into 10 types of adverse chronic hepatic histopathological outcomes. Predictive models using a variety of classification algorithms (LDA, Naive Bayes, SVM) yielded a cross-validation balanced accuracy of 68.7% (±8.9%) for proliferative effects; 58% (±2.3%) for chronic hyperplasia and 63.2% (±3.8%) for hypertrophy. Univariate analysis of 621 HTS assays based on statistical correlation with corresponding in vivo endpoints revealed distinct associations between NR assays and liver endpoints categories. Next, all 91 NR assays out of 621 HTS assays were selected, including: retinoic X receptor-like (RXRβ/β); peroxisome proliferator-activated receptor-like (PPARα/γ/δ); constitutive androstane receptor (CAR; NR1H3); pregnane X receptor (PXR; NR1I2); liver X receptor (LXR; FXR; NR1H4); steroid receptor-like (SR; ERβ/α; ERα); RARα; RAR-related orphan receptor (RORα/β/γ); NR1F; thyroid hormone receptor (TRHα/β); vitamin D receptor (VDR; NR1I1). Using these 91 NR assays for supervised machine learning produced in classification accuracy to 73% (± 0.0%) for proliferative effects; 85% (±5.9%) for chronic hyperplasia and 65.2% (±4.2%) for hypertrophy. This work documents the accuracy of nuclear receptor activity for predicting non-genotoxic rodent hepatocarcinogens, and, more broadly, it highlights the relevance of using molecular initiating events for prioritizing environmental chemicals. This abstract does not represent EPA policy.

**2273v Predicting Toxic and Therapeutic Mechanisms of the ToxCast Chemical Library by Phenotypic Screening**

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Several thousand chemicals were tested in 700 toxicity-related in-vitro HTS biosays through the EPA’s ToxCast and Tox21 projects. This chemical set only covers a portion of the chemical space of interest for environmental exposure, leading to a need to fill data gaps with alternative methods. A cost effective and reliable approach to fulfill this task is to build Quantitative Structure-Activity Relationships (QSARs). In this work, a subset of 1800 ToxCast chemicals was used to build QSAR models for multiple ToxCast assays to predict activity of chemicals in a larger environmental database of ~30K structures. The initial molecular targets for this project were a set of 18 G-Protein Coupled Receptor (GPCR) assays. These assays are part of the aminergic category which was among the most active within the biochemical assays. The QSAR predictions were based on two levels; the first was a classification into active/non-active chemicals; then regression models were built to predict the AC50 potency values of the biosays for the active chemicals. Different software packages were used to calculate constitutional, topological and fingerprinted molecular descriptors based on two-dimensional structures. Then several classification and regression model-fitting methods including PLS-DA, SVM, MLR, PLS and KNN were tested. The overall approach also included variable selection techniques such as Genetic Algorithms that were applied in order to select the most predictive molecular descriptors for each assay. The models were evaluated using n-fold cross-validation and forward validation on a held-out subset of the initial data. Finally, the applicability domains of the models were defined. Using PLS-DA for the human histamine H1 GPCR assay, a classification accuracy of 94% was reached with an error rate of 8% in fitting and 10% with 5-fold cross-validation with only 2 latent variables. This work shows the promise of using in vitro data to develop structure-based models for use in predicting target activity across the diverse space of environmental chemicals. This abstract does not necessarily reflect U.S. EPA policy.

**2274 In Silico Study of ToxCast GPCR Assays by Quantitative Structure-Activity Relationships (QSARs) Modeling**

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SOT 2014 Annual Meeting 611
agos and mTOR inhibitors, all chemical classes with susceptibility to venous thrombosis. Further, structure-based analysis demonstrated associations between chemical categories and mechanism class predictions. Our results yielded an extensive list of potential toxicological targets and biological pathways that we are incorporating into a chemical prioritization strategy for chemicals of concern to the Agency. This work does not necessarily reflect official Agency policy.

**2273w Predictive Models for Mechanism-of-Action Classification of Chemicals Using Phenotypic Data**


Introduction. There is increasing interest in applying in vitro methods to characterize the potential toxicity of drugs and chemicals. Methods that would allow automatic classification of in vitro activity profiles into mechanism classes could be helpful in prioritizing novel agents. Methods. We have developed primary human cell-based assay panels in which chemical effects on the expression of protein biomarkers are measured. Eighty-eight selective, well-characterized tool compounds representing 28 distinct mechanism classes relevant to key biological and toxicity mechanisms were profiled in a standardized panel of 8 assay systems that had been previously used for testing >1000 chemicals and materials for the Environmental Protection Agency’s ToxCast program (Houck, 2009). The resulting dataset of 83 endpoint measurements was then used to build predictive models for each of the 28 mechanism classes using machine learning. A support vector machine (SVM) approach gave the best performance, and so was applied to build a series of two-class SVM models for each mechanism class. Results. These models were then applied to evaluate several libraries and collections of bioactive materials including diversity and kinase focused libraries, biologics and natural products. Compounds from all collections types were highly active in phenotypic assays (hit rates of 7-60%) at 1-5 μM. Analysis of a kinase-focused library and a set of environmental bio-actives revealed 48-57% of actives could be classified into one of the 28 tested mechanism classes. The most common mechanisms were mitochondrial inhibitors (14-27%) and proteasome inhibitors (4-10%). Proteasome inhibitors and AMP elevators were more frequent in the environmental bioactive collection. Conclusions. These results suggest that compounds with potentially undesirable mechanisms are surprisingly common in most compound collections including libraries used for high throughput screening. This method may be useful for library characterization, determining compound mechanisms of action, and chemical prioritization.

**2273x Read-Across Driven by Molecular Initiating Events: Comparison of In Silico Profiling with Experimental Results for Reactivity**


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Grouping or category formation allows the filling of data gaps by read-across to predict toxicity. Ideally it should be based on mechanistic similarities. Adverse Outcome Pathways (AOPs) link the chemistry of the Molecular Initiating Event (MIE) to effects and thus provide a mechanistic framework for the category formation. Covalent binding to biological proteins is an important MIE for toxicological endpoints including skin and respiratory sensitization, liver toxicity and elevated acute toxicity. A profiler (a collection of 108 structural alerts based on organic reaction mechanisms) has been developed to group compounds according to their protein binding capability. However, the alerting potential has not been evaluated in terms of the applicability domain and ability to create robust categories. Therefore, the ability of the alerts to profile 27 sulfur-containing industrial chemicals from a range of chemical classes (mercaptans, sulfides, disulfides, sulfoxides, sulfones, sulfoxides, sulfoxides; saturated and unsaturated) was investigated. To achieve this aim, in silico predictions were compared with experimental results. Compounds were considered to be protein binders when reactive towards glutathione in chemico and demonstrating excess toxicity in the Tetrahymena pyriformis assay. The prediction of protein binding for thiols, disulfides, saturated sulfoxides and sulfates (via S2 reaction), unsaturated sulfoxides, sulfones and sulfonates (via Michael addition) was verified experimentally with distinct excess toxicity for Michael addition. The in silico profiler was able to predict reactivity and differentiate reaction mechanisms related to reaction potency; this was associated with the degree of saturation of the compounds. The knowledge from this analysis was coded into a new set of chemotypes to better group compounds to perform read-across. Supported by the EU FP7 COSMOS Project and ECHA.

**2273y ToxBank Integrated Data Analysis of SEURAT-1 Reference Compounds**

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The SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing-1) research cluster is comprised of seven EU FP7 Health projects and is co-financed by Cosmetics Europe. The SEURAT-1 strategy is to adopt a mode-of-action framework to describe repeated dose toxicity to derive predictions of in vivo toxicity responses. ToxBank is the cross-cluster infrastructure project which provides a web-accessible shared repository of research data and protocols. Experiments generate dose response data over multiple timepoints using different omics platforms including transcriptomics, proteomics, metabolomics, and epigenetics over different cell lines and a common set of reference compounds (details available at wiki.toxbank.net). Data is also generated from functional assays and bioreactors and supplemented with in silico approaches. This complex and heterogeneous data is consolidated and harmonized through the ToxBank data warehouse in order to perform an integrated data analysis. We describe for 14 reference compounds the meta-analysis of multiple types of time-dependent dose response omics and functional data combined with in vitro and in vivo background knowledge including consideration of modeling variations in biokinetic responses. Open TG-GATEs human in vitro liver data of the reference compounds includes reactive compounds (e.g., acetaminophen, CCH4), mitochondrial disruptors (e.g., Rotenone), promiscuous binders (e.g., valproic acid, amiodarone), nuclear hormone receptor ligands (e.g., tamoxifen, WY14643), selective binders (e.g. fluoxetine and cardioxotins (e.g., Doxorubicin, Nifedipine). Adverse events of interest that are represented include cytotoxicity, fibrosis, steatosis, cholesterol and phospholipidosis. Overall we obtained 31,717 differential expression results with 14 compounds from the 45 comparisons, with Doxorubicin providing over 5000 results. Pathway enrichment analysis of Doxorubicin identified a number of key pathways including mismatch repair after 24 hours treatment and TNF-signaling at high doses after 24 hours.
Profiling (especially weak, which tend to be less reproducible), which then can be phosphate flame retardants. Many of the known chemical-target interactions demonstrate the in vitro activity profiling results using 49 brominated or organo-
the actual number of active compounds noticeably drops (by as much as 7%). We applying the signal processing protocol, signal reproducibility greatly improves (by
weak signals, which may serve as early indicators for toxicity, is also challenging. To
data for in vitro activity profiling is challenging due to presence of non-reproduc-
and NCATS, is currently screening a 10K (~8,300 unique) compound library
The finalized results have been uploaded into the ToxCast Dashboard for data
integration and analysis, as well as to serve as the primary portal for publication and
data release. This work does not necessarily reflect Agency policy.

Tox21, a federal inter-agency collaboration involving NIEHS/NTP, EPA, FDA,
capabilities, and outlier detection/masking, concentration activity estimates using dose-response
modeling, and identifying & filtering confounded activity calls based on other
assays. To increase curve-fitting and hit-calling accuracy, the ToxCast workflow incorporates a data-scan feature in the early stage of processing to identify noise and response variation. Moreover, the ToxCast workflow standardizes the analysis of highly-heterogeneous chemical assay data sets by accepting heterogeneous data formats, allowing for rapid processing with a convenient interface enabling easier access and interpretation of data at all levels for repeatable and transparent analyses. The finalized results have been uploaded into the ToxCast Dashboard for data integration and analysis, as well as to serve as the primary portal for publication and data release. This work does not necessarily reflect Agency policy.

Drug-induced liver injury (DILI) remains the primary cause of drug failures during clinical development and post-marketing. It is estimated that up to 40% of poten-
tially hepatotoxic compounds in humans go undetected in preclinical studies that utilize conventional biomarkers. Serum alanine aminotransferase (ALT) activity is a widely used clinical biomarker to assess the risk of liver injury during drug development and approvals by regulatory agencies. Since ALT increases may be transient, thus less clinically relevant, the development of alternative biomarker strategies capable of differentiating transient ALT increases from those that progress to severe DILI is essential. Several biomarkers identified by evidence from peer-reviewed literature and datasets at various institutions are being evaluated as potential DILI biomarkers by individual scientists and international research consortia such as Innovative Medicines Initiative and Critical Path Institute’s Predictive Safety Testing Consortium. In this symposium we will discuss gaps and opportunities for clinical evaluation of DILI biomarkers and their application in DILI biomarkers strategies. Special attention will be given to the evaluation of protein- and miRNA-based biomarkers for detection of DILI in the clinic including their potential to facilitate the understanding of underlying toxic mechanisms. Furthermore, we will introduce the application of NextGen Sequencing in DILI biomarker research in human subjects.

Drug-induced liver injury is a serious complication in drug therapy that is a pri-
mary cause of drug failure during clinical development. Commonly used biomark-
ers, particularly the serum transaminases (ALT, AST) and bilirubin (TBil) are used as useful indicators of hepatocellular of cholestatic liver injury. Because ALT is a very sensitive biomarkers of DILI, the differentiation of ALT increases that sponta-
neously resolve from the ones that signal serious liver injury is very difficult. On the
other hand, increases of TBil levels that are considered a hallmark of severe DILI
occur only after significant damage has already occurred. Therefore the develop-
ment of new biomarker strategies capable of assessing the risk of clinically relevant liver injury in early stages is essential. Because prospective clinical studies evaluating performance of biomarkers of DILI are ethically not feasible, biomarker research of DILI in human subjects requires new approaches. Here we will focus on current status, gaps and opportunities in the development and evaluation of new clinical DILI biomarker strategies including the utilization of samples from subjects with variety of liver impairments via retrospective study design as well as validation and qualification process including the impact of international collaborative efforts.
Hepatotoxicity remains a major challenge in drug development. Although alanine aminotransferase (ALT) remains the gold standard biomarker of liver injury, alternative biomarker strategies to better predict the potential for severe drug-induced liver injury (DILI) are essential. Here, we evaluated the utility of glutamate dehydrogenase (GLDH), purine nucleoside phosphorylase (PNP), malate dehydrogenase (MDH), and paraxonase 1 (PON1) as indicators of liver injury in cohorts of human subjects, including healthy subjects across age and gender, subjects with a variety of liver impairments, and several cases of acetaminophen poisoning. GLDH and MDH had a strong correlation with elevated ALT levels and possessed a high predictive power for liver injury, as determined by ROC analysis. In contrast, PON1 and PNP did not detect liver injury in our study. Finally, evaluation of patients with acetaminophen-induced liver injury provided evidence that both GLDH and MDH might have utility as biomarkers of DILI in humans. This study is the first to evaluate GLDH, MDH, PON1, and PNP in a large number of human subjects and, and it provides an impetus for prospective clinical studies to fully evaluate the diagnostic value of GLDH and MDH for detection of liver injury.

**Mechanistic Biomarkers of Mitochondrial Dysfunction during Drug-Induced Liver Injury in Humans**

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Extensive progress has been made during the last decade in the understanding of mechanisms of drug-induced liver injury (DILI), especially acetaminophen (APAP) hepatotoxicity, in experimental animals. Binding of reactive metabolites to cellular, in particular mitochondrial proteins, mitochondrial dysfunction and oxidant stress, MAP kinase activation, nuclear DNA fragmentation and mitophagy are critical events. Mitochondria clearly emerged as the converging point of various signaling pathways leading to cell death. Despite the progress in animals, the understanding of DILI mechanisms in humans is limited. The presentation will focus on a mechanistic serum biomarker approach to study mechanisms of cell injury, especially mitochondrial dysfunction, in human APAP overdose patients and in mice. Biomarkers that are being used in patients after validation in experimental animals (APAP, furasamide toxicity), include the mitochondrial matrix enzyme GLDH, mitochondrial DNA, nuclear DNA fragments (generated by endonuclease G from mitochondria), and various acylcarnitines. These studies have demonstrated the successful use and some caveats of mechanistic biomarkers in humans suggesting that mitochondrial damage and nuclear DNA fragmentation are critical components of liver injury mechanisms in patients. In addition, the use of these biomarkers as predictors for acute liver failure will be discussed.

**Novel Liver Biomarkers Provide Insight into Benign Drug-Induced ALT Elevations in the Clinic**

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Aminotransferase elevations can be an important indicator of overt liver injury in the clinic; however, there are several drugs in clinical use that cause benign alanine aminotransferase (ALT) elevations yet have no injury risk. Novel biomarkers are currently in development to more accurately discern acute drug-induced liver injury potential and we hypothesized that these newer biomarkers would not be elevated in the context of benign drug-induced ALT elevations. To test this hypothesis, ALT, glutamate dehydrogenase (GLDH), sorbitol dehydrogenase (SDH), high mobility group box 1 protein (HMGB1), cytokerin 18, and miRNA(miR)-122 were assessed in the serum of healthy volunteers enrolled in two separate clinical studies of hepatitis or cholestatics administration. Elevations in all of the novel biomarkers were found to be coincident with ALT in both clinical studies. We conclude that the novel biomarkers may have less utility to be predictive of overt hepatotoxicity that previously assumed. Yet, it is clear that the pattern of biomarker elevations provide important insights into the mechanisms underlying the low-level liver injury that occurs following exposure to hepatic (i.e. necrosis with secondary activation of an innate immune response) and cholestatics (i.e. necroapoptosis).

**Evaluation of Emerging Biomarkers of DILI in Human Populations**

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MicroRNAs are stable and reliably detectable in blood. Recently, levels of circulating microRNAs such as miR122 and miR192 have been shown to respond to treatment with hepatotoxicants. Since microRNAs are either organ specific and/or associated with molecular pathways, microRNA-based biomarkers provide exciting opportunities. Deep sequencing technologies for microRNAs (smallRNA-seq) provide the most unbiased assessment of the many microRNA variants that can exist. In this study, we have compared profiles of circulating microRNA in human subjects with accidental acetaminophen (APAP) overdose with matched controlled subjects. We have identified over 30 microRNA species with time dependent response to APAP overdose. The set included known microRNAs such as mir122 and mir192 as well as novel, so far unknown, microRNAs whose levels drastically increased upon the hepatotoxic exposure. We have confirmed the identified microRNAs by independent RT-PCR methods, and evaluated their levels in other subjects with liver impairments.

**Does Evolution Matter in Toxicity Testing? Rethinking Cross-Species Extrapolation in Hazard Characterization**


Training in evolutionary thinking can help biomedical researchers, clinicians, and toxicologists ask useful questions that they might not otherwise pose. Emerging high-throughput technologies, predictive toxicology, and bioinformatics, as well as alternative toxicity models such as bacteria, yeast, C. elegans, zebrafish, and stem cells, provide new information across the phylogenetic tree at a molecular scale to support human health risk assessments. These novel approaches also create an opportunity to consider what role evolution plays in toxicity testing, particularly in cross-species toxicity extrapolation of pollutants and manufactured chemicals. This symposium aims to generate discussion on evolution and three specific aspects of toxicity testing: (1) how can the phylogeny of species-xenobiotics interaction influence the design and interpretation of high-throughput screens with microbes and alternative animal models; (2) how can the molecular evolution of naturally-occurring toxins and species interaction in the natural world inform the hazard characterization of pollutants and manufactured chemicals; and (3) how can comparative physiology in both vertebrates and invertebrates influence the development of adverse outcome pathways for human health risk assessments.

**Application of NextGen Sequencing for Discovery of Novel miR-Based Candidate Biomarkers of DILI in Human Subjects**


MicroRNAs are stable and reliably detectable in blood. Recently, levels of circulating microRNAs such as miR122 and miR192 have been shown to respond to treatment with hepatotoxicants. Since microRNAs are either organ specific and/or associated with molecular pathways, microRNA-based biomarkers provide exciting opportunities. Deep sequencing technologies for microRNAs (smallRNA-seq) provide the most unbiased assessment of the many microRNA variants that can exist. In this study, we have compared profiles of circulating microRNA in human subjects with accidental acetaminophen (APAP) overdose with matched controlled subjects. We have identified over 30 microRNA species with time dependent response to APAP overdose. The set included known microRNAs such as mir122 and mir192 as well as novel, so far unknown, microRNAs whose levels drastically increased upon the hepatotoxic exposure. We have confirmed the identified microRNAs by independent RT-PCR methods, and evaluated their levels in other subjects with liver impairments.

**Evolutionary Approaches in Cross-Species Toxicity Extrapolation: A Naturalist’s Perspective**


A basic tenet of evolutionary biology is that species adapt to changes in its environment, including chemical exposures, over extended time and multiple generations. The mechanism of natural selection allows their progeny to eventually survive and reproduce in the changed environment (i.e. evolutionary fitness). While the choice of test animal species in laboratory toxicity testing is often based on the conservatism of biology with humans, a better understanding in molecular evolution and comparative toxicology of naturally-occurring toxins can also inform the hazard characterization of pollutants and manufactured chemicals. This presentation will introduce the principal concepts of evolutionary biology that are applicable in toxicological sciences, including site directed mutagenesis, phylogenetic inference, purifying selection, and plant-herbivore coevolution. Specific examples of living poisonous and venomous species will be given, including toxicological cocktails (e.g. venoms or suites of alkaloids acting in concert) as well as proteinaceous toxins (e.g. enzymes or physiological pathway inhibitors). Their relevance to hazard characterization of environmental toxicants will be discussed.
Both the evolution of species and the clonal selection of cells into tumors require a balance between genomic stability and diversification. DNA repair is essential for preserving DNA sequence information, and many DNA repair processes have been conserved across species. During evolution from microbes to mammals, key repair activities were not only conserved, but they were also leveraged to address selective pressures specific to mammals, such as antibody diversification. The role of base excision repair (BER) in these contexts will be presented. To learn more about BER in humans, we recently developed a novel high-throughput DNA repair platforms for assessing DNA repair capacity in primary human cells. The "CometChip" leverages photolithography to enable hundreds of samples to be assessed for their DNA damage levels in parallel, enabling studies of inter-individual variation in DNA repair. Here we show that BER is highly variable in cells from ethnically diverse individuals. In addition to BER, we are also very interested in repair that is facilitated by homologous recombination (HR). Ironically, the BER pathway, while critical for removing damaged bases, also creates single strand breaks as repair intermediates. HR is required to tolerate the presence of these DNA repair intermediates. Here we describe the development of novel fluorescence based animal models in which rare cells that have undergone HR can be detected within intact tissues. A fundamental discovery resulting from this model is that BER in mammals puts pressure on HR under normal physiological conditions, raising the possibility that one DNA repair pathway creates selective pressure for another.

Small fish models such as the zebrafish are commonly used in toxicity and pharmacological testing, particularly to screen for early life stage effects, as well as in mechanistic studies. It is important to consider the evolutionary relationship between these fish models and humans, as fish have undergone at least one genome duplication event, and for many mammalian transcription factors fish often have duplicate copies (co-orthologs). Here, we will present the comparative evolutionary relationship of two families of genes encoding transcriptionally relevant transcription factors—the aryl hydrocarbon receptor (Ahr) and nfe2-related (Nrf) gene families—and their associated signaling pathways. We will compare the Ahr and Nrf genes in two small fish models, the killifish (Fundulus heteroclitus) and zebrafish (Danio rerio), with those in humans and rodents, focusing on multiple fish Ahr (Ahr1a, Ahr1b, Ahr2a, Ahr2b) and Nrf2 (Nrf2a, Nrf2b) genes. We will discuss the implications of transcription factor multiplicity for biomedical and environmental toxicology, including both the challenges and the opportunities provided by differences in gene number and function among fish and mammals. [NIH grants F32ES017585, R01ES016366, and R01ES006272]

This symposium will highlight advances in our understanding of the complex causal relationships between early-life environmental manganese (Mn) exposure and lasting neurobehavioral impairment, gained through the coordinated integration of epidemiological and animal model studies. Behavior and cognitive function are among the most important public health outcomes, since the potential loss of neurological functioning early in life due to toxic exposures may result in diminished academic and economic productivity that can persist over the life span. Motor abnormalities are likewise relevant in relation to neurodegenerative disorders, leading to Parkinsonian disturbances in the aged. Multidisciplinary investigations that synthesize advances in developmental testing, animal toxicology, exposure assessment, statistical modeling, and epidemiology are necessary to gain insights into the impacts of early-life Mn exposure—impacts that have direct implications for public health. This symposium will address this research need through presentation of coordinated human and animal research that focuses on comparable neurophenotypes across species and age ranges, and that incorporates novel exposure-assessment tools, such as Mn levels in shed deciduous teeth, to assess the impacts of early-life and lifelong Mn exposure over distinct developmental windows/life stages.

This presentation will highlight a critical issue in the neurotoxicology of manganese (Mn) - the translation of animal results to humans. Because human randomized trials of Mn exposure cannot be conducted for Mn or any potentially neurotoxic agent, translation of animal study neurotoxicology research to humans must involve epidemiologic observational studies. Animal/human studies planned in tandem could also better elucidate mechanisms of action in the CNS. Human and animal studies seldom measure the same functional domains or address exposures at the same life stages, at least not in a coordinated fashion. Although there are several caveats when extrapolating results from animals to humans, one caveat, the use of different neurophenotypes, can be overcome given the development of computerized tests of animal/human behavior, such as the virtual radial arm maze (VRAM), the delayed spatial alternation and temporal response differentiation test models for specific research questions. This presentation will discuss the comparative data content in CTD, provide examples of cross-species analysis capabilities for mechanistic studies and describe future curation and integration initiatives that will further expand the comparative analysis capacity of CTD.
(a fixed interval response), and the continuous performance test (CPT), among others. In preliminary analysis of Italian children performing the Virtual Radial Arm Maze Increased hair Mn was not associated with latency (2.8 sec 95%CI 1.3-6.7) but was associated with increased total distance traveled (0.6 95%CI 0.0-1.3) and increased working memory count ratio (1.68, 95% CI 1.09-2.01), suggesting worse performance overall on this task in Mn-exposed children. In the delayed spatial alternation task performed in a Mexico cohort of 2 year olds, blood Mn was inversely associated with total number of errors performed correctly (-0.84, 95% CI -1.5, -0.15). Potential links to animal studies with similar designs will be discussed.

2288 Neurodevelopmental Effects of Ambient Exposure to Manganese in Appalachian Children Residing near a Ferromanganese Refinery
E. Haynes. Department of Environmental Health, University of Cincinnati, Cincinnati, OH. Sponsor: D. Smith.

Airborne manganese (Mn) exposure can result in neurotoxicity in workers and adults exposed to high levels of Mn, yet there is limited data on its effects on children. Marietta, Ohio has the longest operating ferromanganese refinery in the United States. The goals of the research were to characterize environmental exposures to Mn and determine the effect of chronic exposure on neurobehavior in children. A total of 408 children ages 7-9 years were enrolled in the Marietta Community Actively Researching Exposure Study (CARES). Internal dose measures were determined for lead, Mn, and secondhand tobacco smoke exposure. Children with hair Mn concentrations greater 610 ng/g had a 3 point mean decrease in full scale IQ compared to children with hair Mn in the low and middle tertiles. In backward elimination regression modeling, in hair Mn was also statistically and inversely associated with full scale IQ (β = -1.38; p=0.008) after adjusting for parent IQ, parent education, and gender. Additionally, in blood Mn was also significantly associated with internalizing problems (p<0.03), but not ln hair Mn. Increases in hair and blood Mn and a decrease in time-weighted distance from the refinery were also significantly associated with poor postural instability. Presentation of these results will include discussion of SFS, an alternative source of Mn, and will be integrated similar epidemiological studies in Mexico and Italy, as well as the animal model studies presented in this symposium.

2289 Manganese Exposure across Different Life Stages Produces Comparable Deficits in Neuromotor Function and Olfactory Discrimination
R. Lucchini1,2. 1Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, NY and 2Occupational Medicine, University of Brescia, Brescia, Italy.

Results from our cross-sectional epidemiological study on environmental metal exposure in children and elderly living in the vicinity of previously and currently active ferroalloy plant emissions in northern Italy will be presented, and integrated with other epidemiological studies in Mexico and Ohio, as well as the coordinated animal model studies presented in this symposium. Our studies in Italy have comprehensively assessed Mn exposure over the lifespan, as well as olfactory function (using the Sniffin’ Sticks test for odor identification) and motor function (using the Virtual Radial Arm Maze and the Ladder Rung Walking tests) among children and the elderly. To address this, we undertook detailed studies on rats exposed to controlled doses of Mn for different durations including early life exposure (low and high doses) and lifelong exposure (low and high doses). Results show that tooth-Mn levels accurately reflect both short- and long-term exposure and are also predictive of levels in other tissues including bone and brain. To validate this in humans, we undertook a prospective study comparing Mn levels in environmental and biological matrices with tooth-Mn levels measured using a laser ablation method that allowed us to develop a temporal map of Mn exposure over the prenatal and early postnatal periods. Results confirm that the tooth-Mn biomarker can be used to reliably reconstruct perinatal Mn exposure.

2290 Validation of Novel Exposure Biomarkers in Manganese-Exposed Rats and in a Cohort of Children at Risk of Environmental Manganese Exposure
M. Arora. Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York City, NY. Sponsor: D. Smith.

This presentation will integrate assessment and validation of novel Mn exposure biomarkers across the animal model and epidemiological studies presented in this symposium. Transfer of manganese (Mn) across the placenta is tightly regulated and maternal blood levels do not accurately reflect fetal exposure and furthermore, because of complex kinetics, blood Mn in children may not precisely measure postnatal exposure. Consequently, measuring Mn exposure during the prenatal and early childhood periods remains a major challenge in studies exploring health outcomes of early Mn exposure. To address this, we undertook detailed studies on rats exposed to controlled doses of Mn for different durations including early life exposure (low and high doses) and lifelong exposure (low and high doses). Results show that tooth-Mn levels accurately reflect both short- and long-term exposure and are also predictive of levels in other tissues including bone and brain. To validate this in humans, we undertook a prospective study comparing Mn levels in environmental and biological matrices with tooth-Mn levels measured using a laser ablation method that allowed us to develop a temporal map of Mn exposure over the prenatal and early postnatal periods. Results confirm that the tooth-Mn biomarker can be used to reliably reconstruct perinatal Mn exposure.

2291 Fine Neuromotor Deficits following Early and Lifelong Manganese Exposure, and the Efficacy of Oral Methylphenidate Treatment to Alleviate Motor Function Deficits
D. Smith. Microbiology and Environmental Toxicology, University of California Santa Cruz, Santa Cruz, CA.

Results from the epidemiological studies presented above indicate that environmental manganese (Mn) exposure produces lasting deficits in fine motor function in children and the elderly. To address this and establish exposure causality, we used a rodent model and the Montoya staircase and Ladder Rung Walking tests to determine whether developmental Mn exposure produces lasting deficits in sensorimotor performance in adulthood. Long-Evans male neonate rats were exposed daily to oral Mn from postnatal day 1-21 (early life only), or from PND 1 - throughout life. Results revealed that early life Mn exposure alone caused long-lasting impairments in fine motor control of reaching skills at the higher, but not lower Mn dose, lifelong Mn exposure led to widespread impairment in reaching and grasping/retrieval performance in adult rats, with the lower Mn dose group showing the greatest impairment, and lifelong Mn exposure produced similar (higher Mn group) or more severe (lower Mn group) impairments compared to their early life-only Mn exposed counterparts. Results of the Ladder Walking test, and the efficacy of oral methylphenidate treatment to alleviate lasting deficits will also be presented. These results substantiate the emerging clinical evidence in children reported above showing associations between environmental Mn exposure and deficits in fine sensorimotor function.

2292 Attentional Deficits in Early-Life and Lifelong Manganese-Exposed Rats and Their Reversal with Oral Methylphenidate Treatment
S. A. Beaudin. Microbiology and Environmental Toxicology, University of California Santa Cruz, Santa Cruz, CA.

As suggested by results from the epidemiological studies presented above and elsewhere, overexposure to manganese (Mn) in early life is associated with attention deficit with hyperactivity disorder in children, but the specific nature and causal relationship of these attentional deficits have yet to be demonstrated. To address this, we determined whether early life Mn exposed rats exhibited persistent deficits in visual attentional processes and impulsive behavior in adulthood, and if lifelong Mn exposure caused similar or more severe attention deficits than early life exposure alone. The Mn-exposed groups showed normal visual attention performance in vigilance tasks. However, the Mn-exposed groups displayed deficits in attentional accuracy when required to attend selectively to visual cues in the presence of olfactory distractors. Notably, impulse control was not measurably impaired in the Mn groups. The results substantiate the emerging epidemiological evidence and show that developmental Mn exposure can cause selective attentional deficits across a range of doses and exposure durations, including lasting susceptibility to greater distractibility in adulthood. The efficacy of oral methylphenidate to ameliorate the attention deficits will also be presented – these latter results will inform the mechanistic basis for these deficits as well as the potential efficacy of MPH for treating Mn-related attentional deficits in children.
Are Biofuels More or Less Toxic than Conventional Fuels and What Are the Implications for Human Exposure and Risk?

A. M. van Erp and M. C. Madden. Health Effects Institute, Boston, MA and US EPA, Research Triangle Park, NC.

During the past decade, the use of biofuels such as biodiesel and bioethanol has been steadily increasing as a viable alternative to the use of petroleum-based fuels. Although there are clear advantages in terms of energy security and climate change, there are several unknowns about the long-term effects of the use of biofuels on the environment and on human health. Because there are many different biofuels and biofuel blends that originate from different feedstocks, evaluating their effects on the environment and human health over their entire lifecycle becomes rather complex. Although there may be reductions in emissions of certain compounds, there may also be unintended consequences. For example, adding bioethanol to gasoline reduces emissions of benzene and other hydrocarbons, but increases the levels of toxic aldehydes in the engine exhaust, and also leads to increased evaporative emissions. While first-generation biofuels such as ethanol derived from corn are now widespread, next-generation fuels such as those produced by microorganisms are under development. The workshop will start with an overview of the types of biofuels that are currently available and recent results of comparative emissions-testing programs in the US. We will then present the latest results from several research programs to evaluate the emissions and comparative toxicity of biofuels, including in vitro testing for genotoxicity, in vivo evaluation of pulmonary and cardiovascular effects, and the toxicity of fatty acid methyl esters (FAME) that are found in biodiesel. In addition to new results regarding emissions testing, the workshop will discuss different approaches and harmonization of toxicity testing in vitro and in vivo. Finally, we will conclude with approaches to assess human exposure and health impacts. (This may not represent official US EPA policy.)

Biofuel Usage, Composition, and Engine Emissions


This presentation will provide an overview of biofuels in the United States, with a focus on biodiesel and other biodistillate fuels. In recent years, the volume of biofuels has increased steadily due to a variety of legal and regulatory drivers for the promotion of biofuels. These drivers and their influence on current and predicted future volumes of different kinds of biofuels will be presented. Currently, there are many different biodistillate fuels in use or under development but it is difficult to predict what their effect will be in terms of future market shares. This presentation will provide an overview of the main kinds of biofuels, including the major categories of biodistillates, explaining their terminology, feedstocks, manufacturing processes, compositions, and properties. Fuel composition is inevitably linked to vehicle emissions and their potential air quality and public health impacts. Emissions standards for diesel engines and vehicles have become much more stringent over time. Compliance with these standards has been achieved by a combination of engine and fuel modification and utilization of emissions control systems. The incremental emissions impacts of biodiesel compared to conventional diesel fuel have been investigated extensively. There is widespread agreement that in most situations, biodiesel blends provide modest reductions in emissions of carbon monoxide (CO), total hydrocarbons (HC), and fine particulate matter (PM), but slight increases (or no change) in emissions of nitrogen oxides (NOx). The effects of other biodistillate fuels upon these so-called "criteria emissions" have not been studied as thoroughly, but are probably similar to the effects of biodiesel.

Emissions of non-criteria pollutants including mobile source air toxic (MSAT) emissions are also of concern, especially in terms of the use of bioethanol and the formation of aldehydes. However, much less information is available regarding the impacts of biodiesel, and other biodistillates, on MSAT emissions. This area will be discussed, other open issues will be identified, and recommendations for additional work will be presented.

In Vitro Toxicological Evaluations of Emissions from Heavy-Duty Vehicles Using Biofuel Blends

N. Kado and C. F. Vogel. Environmental Toxicology and Center for Health and the Environment, University of California Davis, Davis, CA.

The evaluation of the potential health effects from exposure to emissions from motor vehicles presents numerous challenges because these emissions are complex mixtures of gases, vary with fuel and engine type, and the emissions may be altered due to evolving control strategies and technologies. One approach toward evaluating these complex particle and vapor-phase mixtures is to use screening tools, such as directed chemical analyses and in vitro biological analyses. An example of this approach is the detailed emissions study of biodiesel, renewable diesel, and ultra-low sulfur diesel fuels, which was conducted as part of a multi-investigator and multi-institutional study on regulated emissions as well as unregulated toxic emissions. Samples from heavy-duty trucks following standard test cycles on a chassis dynamometer were evaluated chemically and in a screening effort using in vitro assays. These assays included mutagenicity tests and expressions of inflammatory and oxidative stress markers in human macrophage cells and lung epithelial cells. The trend for many of the tests/species compared to ultra-low sulfur diesel fuel was lower with increasing biofuel blends, but with some tests/species, results were highly dependent on biofuel blend. This presentation will provide a brief overview of some of the in vitro evaluations with focus on heavy-duty vehicle tests. (The abstract and opinions are of the authors and do not represent policy of the California Air Resources Board or the University of California)

Comparative Toxicity and Mutagenicity of Biodiesel Exhaust


Biodiesel (BD) is commercially made from the transesterification of plant and animal derived oils, and the composition of biodiesel exhaust (BE) depends on the type of fuel, the blend ratio and the engine and operating conditions. While numerous studies have characterized the health effects associated with petroleum diesel (PD) emissions following controlled animal and human exposures, information on pure and blended BD emissions are far more limited. We developed a test program to compare the mutagenicity and toxicity of 100% (B100) or a 20% mix (B20) of soy-based BD exhaust to emissions from conventional PD (B0). Exhaust was generated by a 3.2 L Yanmar engine driving a 3.8 kW Pramac generator with a constant load of 3 kW and diluted to target concentrations of 0, 50, 150, or 500 ppm/m3 as determined by TEOM. Average NO (ppm) at the 500 ppm/m3 level were 15.2 (B100), 12.7 (B20) and 14.8 (B0). Rats and mice were exposed independently for 4 hours per day for up to 6 weeks depending on the experimental protocol and model system. In general, B0 had the greatest pro-inflammatory effects in normal mice while both B0 and B100 potentiated plaque formation in Apo E mice. The B0 and B100 also caused changes in heart rate variability and systemic inflammatory responses in hypertensive rats. Organic extracts of B0 were the most mutagenic with respect to both the Ames Salmonella assay and the U.S. Environmental Protection Agency’s Ames test. This presentation will provide an overview of biofuels in the United States, with a focus on biodiesel and other biodistillate fuels. In recent years, the volume of biofuels has increased steadily due to a variety of legal and regulatory drivers for the promotion of biofuels. These drivers and their influence on current and predicted future volumes of different kinds of biofuels will be presented. Currently, there are many different biodistillate fuels in use or under development but it is difficult to predict what their effect will be in terms of future market shares. This presentation will provide an overview of the main kinds of biofuels, including the major categories of biodistillates, explaining their terminology, feedstocks, manufacturing processes, compositions, and properties. Fuel composition is inevitably linked to vehicle emissions and their potential air quality and public health impacts. Emissions standards for diesel engines and vehicles have become much more stringent over time. Compliance with these standards has been achieved by a combination of engine and fuel modification and utilization of emissions control systems. The incremental emissions impacts of biodiesel compared to conventional diesel fuel have been investigated extensively. There is widespread agreement that in most situations, biodiesel blends provide modest reductions in emissions of carbon monoxide (CO), total hydrocarbons (HC), and fine particulate matter (PM), but slight increases (or no change) in emissions of nitrogen oxides (NOx). The effects of other biodistillate fuels upon these so-called "criteria emissions" have not been studied as thoroughly, but are probably similar to the effects of biodiesel.

Emissions of non-criteria pollutants including mobile source air toxic (MSAT) emissions are also of concern, especially in terms of the use of bioethanol and the formation of aldehydes. However, much less information is available regarding the impacts of biodiesel, and other biodistillates, on MSAT emissions. This area will be discussed, other open issues will be identified, and recommendations for additional work will be presented.

Approaches to Comparative Biofuels Toxicity Testing and Recent Results from Europe


On a regular basis new combustion or after treatment technology is brought on the market to meet the new stricter standards or to reduce the costs to meet the current regulations. The use of new (bio)fuels and fuel additives is also increasing to reduce the emission of NOx and to be less dependent on natural petroleum. The current type approvals are mostly not sufficient to examine the effects for man and the environment. This implies that when harmfulness is examined for a specific biofuel, opposite outcomes could be reached by different institutes due to diverse measurement set up and risk assessment. This presentation will discuss the difficulties in testing toxicity of emissions associated with combustion of biofuels, given the large number of permutations of possible fuel and engine combinations. With regard to measuring/limiting air pollution exposure and its health impact caused by automotive emissions, and more specifically biofuel combustion, there are no common testing guidelines and standards on which to base policy. Clearly, it would be preferable to come to agreement on a harmonized approach for in vitro and in vivo testing, so that new fuels that come to market can be assessed relatively quickly and in standardized way. One of the main questions is what biological pathways and health outcomes should be covered in such "screening" evaluations. So far, mutagenicity and genotoxicity have been a major focus but other end points should be considered as well, given the increasing evidence of cardiovascular and other health effects associated with inhalation of combustion products. In addition, a standardization approach is needed for the generation of combustion aerosols and exposure conditions to really support comparative hazard screening. Examples of recent results from Europe including human exposure studies will be shown as illustrations.
2298 Human Health Impact Assessment of Biofuels


Transportation fuel use represents a key source of air pollutant exposure, particularly in urban areas where mobile source emissions and large populations overlap. Changes in fuel composition can substantially alter the complex mixture of combustion emissions. The increased use of biofuel blends over the past decade and development of policies to promote their uptake necessitate detailed evaluation of the associated health implications. Health Canada (HC) has developed a risk assessment framework to evaluate health impacts of non-conventional fuels and applied it to biofuels. The approach used is: 1) comparative (basecase vs. new fuel) in order to estimate the incremental health risks/benefits of biofuels; and 2) comprehensive, with a focus on principal pathways of risk. The recent HC biodiesel (BD) assessment includes a review of the relative toxicity of BD emissions compared to diesel emissions, and modeling of the impact of the use of BD blends (5% or 20% by volume in ultra low sulphur diesel) in on-road heavy duty vehicles on Canadian air emissions, air quality and resultant health impacts. Incremental health impacts are estimated based on concentration-response functions linking ambient air pollutants with increased risk of premature mortality, hospital admissions, emergency room visits and respiratory outcomes. It is estimated that BD blend use in Canada would reduce emissions of particulate matter, carbon monoxide, volatile organic compounds and polycyclic aromatic hydrocarbons, and increase emissions of nitrogen oxides. These effects are expected to attenuate by 2020 due to the incorporation of new technology vehicles in the fleet. Changes in air pollutant levels due to BD use are expected to be small (<1%), and the associated health impacts minimal. The health effects literature on the comparative toxicity of BD emissions was limited for most endpoints. It was concluded that compared to diesel exhaust, BD exhaust is unlikely to elicit greater respiratory toxicity and may be less mutagenic. Biofuel research to address risk assessment needs and a comparison with the US approach will be reviewed.

2299 Role of Circulating Factors in Mediating Systemic Toxicity of Inhaled Substances

L. Chen1 and M. Campen2. 1Environmental Medicine, New York University School of Medicine, Tuxedo Park, NY and 2Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.

Air pollution has been known to cause chronic and acute systemic health effects, including cardiovascular and possibly neurodegenerative diseases, but the pathway by which inhaled substances drive effects beyond the lung is unclear. The lung provides an effective barrier against most gaseous and particulate components of air pollution, and those that are taken up systemically are often in remarkably low concentrations. Moreover, several studies fail to see the same effects at relevant concentrations when particulates are delivered by gavage, intravenously, or in cell culture studies, implying that there is a lung-specific reaction that occurs to drive systemic effects. Recent studies have revealed that inhalation exposures to a variety of pollutants can lead to the generation of circulating bioactive factors that drive endothelial cell activation and may also be responsible for neuroinflammation. Circulatory changes include generation of adducted proteins, altered metabolites and lipids, and altered function of lipoproteins. The identity of the causal components(s) in the circulation remains unclear, but ongoing lipidomic and metabonomic research is providing important insights. This workshop will highlight a number of advances in this area related to exposures to particulate matter, metals, ozone, combustion mixtures, and nanomaterials.

2300 Influence of PM2.5 Exposure on the Receptor for Advanced Glycation End Products: Insight into an Emerging Risk for Diabetes

J. M. Vaughan1, B. Narayanan1, A. Schmidt2 and L. Chen1. 1Environmental Medicine, New York University, New York City, NY and 2Medicine, New York University, New York City, NY.

Diabetics are a particularly vulnerable population to the adverse cardiopulmonary effects of particulate air pollution (PM)- this is often attributed to enhanced inflammation and endothelial dysfunction. Recent reports have implicated activated receptors for advanced glycation end-products (RAGE) as an integral factor in the inflammatory processes in cardiovascular dysfunction and diabetes; nonetheless, it is unclear whether ambient air pollutants (PM) alone/or in combination with other endogenous factors may contribute to the activation of RAGE. In this study, we have determined that RAGE was significantly (P=0.0358) higher in serum samples of residents in Jinchang than that in Zhangye (452.6±29.1ng/ml). This change was due to higher levels of Ni, Cu, As, and Se in PM2.5 in Jinchang than that in Zhangye. We also found a dose dependent increase in cell proliferation and small increases in sRAGE activity when human vascular endothelial cells exposed to ambient PM. Immunofluorescence detection showed an elevated number of cells positive for membranous RAGE expression; accompanied with a two-fold increase in mRNA for RAGE and NF-κB. Furthermore, results also showed a >2 fold increase of ATP4 and NF-kB in cells treated with PM+BSA. We also measured the expression of other non-glycated ligands (S100, HMGBl). Using siRNA to silence RAGE in human endothelial cells and RAGE knock out mice, we have confirmed the plausible interaction between PM and RAGE and verified that RAGE is a potential inflammatory mediator of inhaled ambient PM.

2301 Carbon-Based Engineered Nanomaterial Exposure Alters Circulating Factors that Induce Endothelial Activation and Impairment of Nitric Oxide Synthesis

A. Eredi1 and M. Aragon2. 1CDR-NIOSH, Morgantown, WV and 2University of New Mexico, Albuquerque, NM.

Carbon-based engineered nanomaterials (ENM), including carbon nanotubes (CNT), graphene, and fullerenes, have profound utility in medicine, electronics, and composites. While the surface is just being scratched on the diverse applications, a growing workforce is subject to exposure by inhalation. In vitro studies identify pulmonary cytotoxicity, inflammation, and fibrosis as a result of carbon-based ENM exposure. In addition to the pulmonary response, effects related to cardiovascular dysfunction included vascular inflammation and oxidative stress, endothelial dysfunction, and increased atherosclerotic plaque formation. The mechanisms linking the initial pulmonary exposure to resultant cardiovascular dysfunction are under investigation. Given the apparent toxicity of certain ENM and the vast number of ENM including various permutations of each, controlled toxicology studies in humans are not practical. Therefore, a translational in vitro screening assay was developed for rodents to assess the potential cardiovascular toxicity of ENM. Specifically, this particular study explored the ability of circulating factors after pulmonary exposure to affect mechanisms related to vascular dysfunction. Serum collected from mice exposed to carbon black, multi-walled CNT, or varying types of graphene were incubated with murine primary endothelial cells. The serum from exposed mice increased endothelial cell surface expression of VCAM-1 and ICAM-1 as early as 4 hr post-exposure when compared to respective shams. In addition, the serum from exposed mice reduced ATP-stimulated endothelial cell nitric oxide production by 25%. These results complement end point cardiovascular effects and showed the presence of circulating factors contributing to vascular dysfunction following carbon-based ENM exposure. Furthermore, the methodological design allows for interpretation of systemic effects in an unbiased approach while providing a platform for comparative studies of differing ENM in a translational context.

2302 Endothelial Cell Pattern Recognition Receptors, CD36 and LOX-1, Contribute to Responses to Pollution-Induced Circulating Factors

M. Campen. Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.

Our laboratory initially discovered that the serum from human volunteers contains a bioactive component following controlled exposures to diesel or nitrogen dioxide that can activate inflammatory endothelial cell responses. While we have eliminated the possibility that common cytokines are the driving factor(s) in this response, the identity of the biochemical ligands that drive such responses remains unknown. However, we have considered the possibility that multiligand pattern recognition receptors (PRRs), as mediators of response to extracellular damage, could play a specific role in endothelial cell responses to pollution-induced circulating factors. Two specific receptors have been investigated: cluster of differentiation 36 (CD36) and the lecinth-like oxidized low density lipoprotein receptor-1 (LOX-1). These observations help explain systemic vascular inflammatory effects of diverse inhaled pollutants such as ozone and PM, along with complex mixtures from combustion sources. Ongoing translational work enables cumulative response patterns in controlled settings to improve assessment of relative toxicity of individual components and mixtures of pollutants.
We and others have shown that exposure to concentrated ambient particles and motor vehicle emissions induce lipid peroxidation in the lungs, circulating blood and systemic tissues such as the liver and the aorta. It is unclear however, whether effects induced in the lungs are required for the induction of systemic vascular effects. It is possible that circulating factors such as plasma lipoproteins could be altered and serve as mediators of the systemic toxicity of inhaled substances. This presentation will focus on our studies designed to evaluate the functional changes induced by air pollutants on plasma HDL lipoproteins, their relationship to lipid peroxidation in the lungs, circulating blood and systemic tissues and the activation of endogenous peroxidation pathways.

### Systemic Effects of Inhalation Exposure Mediated by ‘Omic Perturbation in Serum

**A. K. Ottens. Neuroscience, Virginia Commonwealth University, Richmond, VA. Sponsor: M. Campen.**

Recent studies suggest that inhalation exposure to ozone generates circulating factors in blood that can result in systemic health consequences, including activation of microglia and inflammatory responses in the brain; however, the nature and complexity of these products have remained a mystery. As ozone rapidly reacts in the lung surfactant, the generation of pulmonary-derived modified lipids, proteins, or small molecules may be a plausible outcome that leads to systemic effects. Through recent development of a label-free quantitative data-independent Omic platform, analysis of serum factors reveals a diverse and dose-dependent molecular perturbation with broad biochemical implications. Specific effects observed in coronary vascular and nervous systems will be discussed related to the compositional alterations in the serum.

### The Use of Dogs and Minipigs As an Alternative to the Nonhuman Primate in Nonclinical Safety Assessment of Biopharmaceuticals

**J. A. Wieler1 and J. Bluemel1. 1MedImmune, Gaithersburg, MD and 2Amgen, Thousand Oaks, CA.**

Appropriate species selection is paramount for the nonclinical safety evaluation of biopharmaceuticals. Regulatory guidance requires the use of a pharmacological responder species, in particular for biopharmaceuticals. Most often the nonhuman primate (NHP) is considered as the only pharmacologically relevant species, and especially the cynomolgus monkey is frequently used for nonclinical safety studies. In recent years, ethical concerns, increased public scrutiny, and issues of availability and transportation of NHPs have put considerable pressure on researchers worldwide to improve study designs and to intensify the search for alternative species. Besides recent activities to improve study designs to reduce the number of animals used, there is a sense of urgency to intensify the search for alternative species and consider nontraditional approaches beyond the use of “standard” toxicology species. Species like the dog and minipig are meanwhile widely used in toxicity studies for chemical-derived pharmaceuticals but rarely considered for nonclinical safety evaluation of biopharmaceuticals. The limited availability of scientific background data, e.g., pharmacogenomic or physiology comparisons, and limited regulatory experiences are considered as major obstacles for a wider use of dogs and minipigs for biopharmaceutical safety testing. A better understanding of factors like sequence homology, target/ligand expression, downstream signaling, effector functions, and antibody kinetics, as well as the availability of tools like species-specific background databases, in vitro assays, or reagents, would greatly facilitate the use of dogs and minipigs. Increased use of dogs and minipigs for nonclinical safety assessment of biopharmaceuticals would in turn increase the regulatory confidence and experience. The objective of this workshop is to review and discuss recent progression and challenges in the scientific characterization of dogs and minipigs and discuss their utility and limitations for regulatory safety testing of biopharmaceuticals.

### The Immune System and Immune Function Testing in the Dog and Minipig


Reactivity with canine and minipig targets and to some extent the level of immunogenicity of human/humanized proteins in these species may be limitations to their use with regards to safety assessment of biopharmaceuticals. However, tools are available or can be developed to address immunology-related questions. The availability of appropriate knowledge and analytical tools regarding the immune system may be critical for proper nonclinical safety assessment of biopharmaceuticals in the dog and in the minipig from the following standpoints: demonstration of species relevance (on target specific binding, pharmacodynamics); conduct of immunotoxicology assessments (including immunophenotyping of subpopulations of lymphocytes and functional assessments [e.g., T cell-dependent antibody response [TDAR], cytotoxic T cell activity, NK function]; assessment of immunogenicity and documentation of immunogenicity-related toxicity. This presentation will provide a comparison of the human, dog, and minipig immune system (overall structural and functional organization, ontogeny, innate immunity, humoral and cell-mediated immunity). It will then provide an overview of the tools available to document and characterize immunotoxicology in the dog and minipig (with a focus on TDAR and immunophenotyping), as well as existing gaps. It will then discuss available methods for documentation of anti-drug antibodies, circulating or tissue immune complexes.

### The Dog and Minipig As Pharmacology Models to Evaluate Biopharmaceuticals

**N. Dybdal. Safety Assessment Pathology, Genentech, South San Francisco, CA.**

Whether being considered for use in general toxicology or pharmacology studies, the many features of the dog and minipig which make them attractive for consideration as a non-rodent toxicology species must never overshadow the need to first determine if their use is appropriate and justified biologically. First is the species specificity of the biopharmaceutical which must be characterized and relevance in dog and/or minipig must be established (e.g. is the protein appropriately cross-reactive with the target – receptor/ligand). The biological pathway of concern needs to be characterized and comparative activity and control of the pathway understood (e.g. downregulation, upregulation, effector function). Although the potential immunogenicity of the biopharmaceutical may present a challenge, duration of dosing, dose or schedule can often be managed to help avoid this issue. Immunogenicity of foreign proteins reflects a challenge to the feasibility of conducting studies in dogs and minipigs and should not be confused with an opportunity for use of these species as predictive models of immunogenicity in humans. This presentation will provide case examples of the use of dogs and minipigs as pharmacology models. In these examples the obstacles remaining will be balanced against the opportunities and growing feasibility of fully realizing the benefits of conducting pharmacology studies of biopharmaceuticals in these species.

### Animal Model Genomic Data Aids Species Selection in Pharmaceutical Discovery and Development

**J. J. Vamathévan. Computational Biology, GlaxoSmithKline, Stevenage, United Kingdom. Sponsor: J. Wieler.**

Improving drug attrition remains a challenge in pharmacological discovery and development. A major cause of early attrition is the demonstration of safety signals which can negate any therapeutic index previously established. Safety attrition needs to be put in context of clinical translation (i.e. human relevance) and is negatively impacted by differences between animal models and human. In order to minimize such an impact, an earlier assessment of pharmacological target homology across animal model species will enhance understanding of the context of animal safety signals and aid species selection during later regulatory toxicology studies. This talk will cover the genomes of the scrofa Göttingen minipig and the Canis familiaris beagle. Comparative analysis of these genomes with other key model organisms, namely mouse, rat, cynomolgus macaque, rhesus macaque, two related breeds (S. scrofa Duroc and C. familiaris boxer) and human reveal considerable variation in gene content. Key genes in toxicology and metabolism studies, such as the UGT2 family, CYP2D6, and SLC01A2, displayed unique duplication patterns. The use of simple phylogenetic tools allow for the comparisons of known human drug targets prior to starting safety studies. Example studies of such drug targets have revealed surprising variation such as species-specific positive selection, duplication and pseudogenized targets. These data will facilitate the more effective use of animals in biomedical research as well as enhance the understanding of the context of animal safety signals and aid species selection during later regulatory toxicology studies.
Animal welfare is enshrined in the Treaty of the EU. The revision of the Directive on animal protection in the EU was discussed intensely under the framework of the European Commission, because the use of animals, particularly the nonhuman primate (NHP), for testing purposes is a sensitive issue in Europe. The European Community funded a Specific Support Action (SSA) to evaluate the potential impact of toxicity testing in minipig as an alternative approach in regulatory toxicity testing, with the aim of contributing to replacement, refinement and reduction of animal testing. This SSA was named the RETHINK project. The use of NHP and minipig as animal models have been discussed in different forums and this presentation focuses on the minipig as an alternative and draws extensively on RETHINK. The EU launched the Directive 2010/63 indicating that the use of NHP in biomedical research is of the greatest concern to the general public. It is recognized that the use of live animals continues to be necessary to protect human health, but the use of NHP should be restricted to potentially life-threatening conditions in humans or in relation to cases of debilitating conditions. An important application of NHP is in the area of biotechnology-derived proteins. ICH S6(R1) guidance recommends the use of a relevant species, i.e., a species that does respond to the pharmacological action of the product. Because of the high specificity of biopharmaceuticals there is an important restriction in the choice of species. In most cases the cynomolgus monkey is the default species being chosen. Based on the EU-legal restrictions mentioned above the minipig might be considered as an important ‘alternative’ species. There are several opportunities and challenges in using minipig as the nonrodent species of choice.

- Application of minipigs in the testing strategy of dermally-applied products
- Limitations in reproductive and developmental toxicity testing of biopharmaceuticals
- Cardiovascular functioning in minipigs as a model for humans.

These topics will be discussed using case reports.
**RI 2314** Effects of *In Utero* and Early Postnatal Exposures to Metal-Containing Dusts

R. Lantz. Dept of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ.

In the arid Southwest of the United States, dusts, especially downwind of legacy mine tailings and smelters, can contain high levels of metals, including arsenic. The levels of arsenic in these dusts can be an order of magnitude above ambient levels. In addition, because of high levels of arsenic in drinking water, simultaneous exposure to arsenic from water and from airborne particulate dust occurs. Exposure to airborne toxicants, that are downwind of toxic sources, can cause adverse health effects. Exposure of pregnant female mice to air pollutants has been shown to alter lung function and to enhance sensitization and airway reactivity in offspring. *In utero* and/or postnatal exposure to cigarette smoke, ambient urban air particles or metals leads to increased airway reactivity, decreased surface to volume ratios in the lung and altered lung function in the offspring. In this study, we examined the effects of inhalation of arsenic compounds during sensitive developmental time periods. Mice were exposed by inhalation to dusts containing various forms of arsenic that can be found in mine tailings or downwind of smelters, either during *in utero* development, after birth or both. Pulmonary function, structure and inflammation were analyzed to determine the effects of these exposures on the developing lung. The effect of combined inhalation and ingestion was also analyzed. Alterations in lung resistance, elastance and airway reactivity were seen. These changes correlated with alterations that occurred in the connective tissue around airways. The responses depended on the form of arsenic that was inhaled as well as the developmental time when the exposures occurred. Children living in an arid region of Mexico who would be expected to be exposed both through ingestion and inhalation of arsenic showed decrements in lung function. These data indicate that inhalation of dusts containing arsenic during sensitive developmental times may result in altered pulmonary function and structure later in life.

**PI 2316** Metabolic Pathways Related to Inflammation Are Disrupted by Arsenic in the Livers of Mice Fed a Western Diet

W. H. Watson1, X. Shi, X. Wei2, I. Koo2, R. H. Schmidt1, X. Yin2, S. Kim2, A. Vaughn3, C. McClain1, G. E. Arter2 and X. Zhang2. 1Department of Medicine,Geisel School of Medicine at Dartmouth, Hanover, NH. 2Chemistry, University of Louisville, Louisville, KY. 3Pharmacology & Toxicology, University of Louisville, Louisville, KY and 4Robby Rex VAMC, Louisville, KY.

Arsenic is a widely-distributed environmental component that is associated with a variety of cancer and non-cancer adverse health effects. Additional lifestyle factors, such as diet, contribute to the manifestation of disease. Recently, arsenic was found to increase inflammation and liver injury in a dietary model of fatty liver disease. The purpose of the present study was to investigate potential mechanisms of this diet-environment interaction via a high throughput metabolomics approach. GC×GC-TOF MS was used to identify metabolites that were significantly increased or decreased in the livers of mice fed a Western diet (a diet high in fat and cholesterol) and co-exposed to arsenic-contaminated drinking water. The results showed that there are distinct hepatic metabolomic profiles associated with eating a high fat diet, drinking arsenic-contaminated water, and the combination of the two. The high fat diet significantly altered nine metabolites, and the addition of arsenic resulted in further changes to three of these metabolites as well as introducing changes in six additional metabolites. The profiles of altered metabolites were consistent with perturbations in the metabolism of glutathione, short-chain fatty acids and medium-chain fatty acids. Of particular interest was the apparent block in the synthesis of glycine when arsenic was combined with a high fat diet. Glycine can inhibit inflammatory signaling by hepatic Kupffer cells in other model systems, and the decrease in glycine levels seen in our model may account for the observed increase in inflammatory cytokines and markers of Kupffer cell activation. These results point to potentially novel mechanisms by which arsenic can promote the progression of fatty liver disease.

**RI 2315** Metals, the West, and Translational Science

J. L. Lewis. Community Environmental Health Program, University of New Mexico, Albuquerque, NM.

The western US has been heavily mined for a range of heavy metals, leaving the majority of the 500,000 abandoned mines in the US behind. Among these abandoned mines are also more than 10,400 abandoned uranium mine waste sites scattered throughout the west, with more than 1100 located on the Navajo Nation. Arsenic, both naturally occurring and as a co-contaminant in mine waste, also complicates exposures through its presence in unregulated water sources relied on by up to 30% of the populations in some of these regions. Evaluating the impact of these mixed-metal exposures in Western communities is further complicated by the fact that the sites often impact rural and tribal communities that have not been adequately represented in the research base from which toxicity has been evaluated. Evaluating the health impacts of the unique mixtures in the waste within the context of existing lifestyle and risk factors presents unique challenges, and an opportunity for developing novel approaches that integrate disciplines including population science, toxicology, and clinical care with physical sciences and novel statistical approaches to understand the complex interactions. We have been able to build on a diversity of strengths to develop an integrated transdisciplinary response to these issues and to begin not only characterizing the problems, but identifying interventions, informing clinical care, and informing policy to reduce the impact on our communities. In Navajo communities, we have found that while exposures in communities during the active phase of mining increased the risk for kidney disease, chronic lower dose exposures to legacy waste increase the likelihood of hypertension, autoimmune disease and immune dysfunction in adults. We are currently investigating the relationship between these exposures to mixed metals in legacy waste, reproduction, and development in Navajo children. These community-based approaches also represent efforts to integrate Western science with Native learning models and underscore the importance and impact of translational science in meeting the needs of improving community health.

**PI 2317** Organic Species of Arsenic Have Adverse Effects on *Pseudomonas aeruginosa*-Induced Immune Response

E. Notch, R. Barnaby, B. Coutermarch, V. Taylor, B. Jackson and B. Stanton. Geisel School of Medicine at Dartmouth, Hanover, NH.

Arsenic is the number one environmental contaminant of concern with regard to human health. Epidemiological studies have linked arsenic exposure with lung disease, including bacterial infections, and chronic obstructive pulmonary disease, which are associated with *Pseudomonas aeruginosa* (Pa) infection. Animal models have also shown that arsenic alters the immune response. Little is known about effects of low doses relevant to US population exposure or the contributions of organic arsenic to altered immune response. This study examined impacts of inorganic sodium arsenite (iAsIII) and two major metabolites, monomethylarsonous acid (MMAIII) and dimethylarsenic acid (DMAIII), on Pa induced cytokine secretion by primary human bronchial epithelial cells (HBEC). Exposure of HBEC to 1μg (10ppb) did not alter Pa induced cytokine secretion. In contrast, 5ppb DMA significantly decreased IL-8, IL-6, CXCL1 and CXCL2 secretion after Pa stimulation compared to cells exposed to Pa alone. Exposure to 5ppb MMA increased Pa induced IL-8, and CXCL2 secretion. These alterations in HBEC proinflammatory cytokine secretion can impact macrophage recruitment or amplification of chemotactic stimulus through reduced macrophage cytokine secretion. Differentiated THP-1 cells (macrophages), were exposed to purified CXCL1 and CXCL2 at concentrations based on HBEC secretion. Lower concentrations of CXCL1 and CXCL2, equivalent to those released by HBEC exposed to DMA and Pa, reduced THP-1 IL-8 and IL-1β secretion. Higher concentrations of CXCL1 and CXCL2, equivalent to those released by HBEC exposed to MMA and Pa, significantly increased THP-1 IL-1β secretion. This suggests that MMA will enhance inflammatory response potentially leading to lung damage and DMA will suppress inflammatory response resulting in increased pathogen burden. These results demonstrate that exposure to low levels of organic arsenic, that may occur from ingestion of rice and rice based products, have untoward effects on the respiratory immune response to Pa infection.
Arsenic Exposure and Cell-Mediated Immunity in Preschool Children in Rural Bangladesh

S. Ahmed1,2, Y. Wagatsuma3, M. Kippler4, R. Raqi1 and M. E. Vaheri1, 1Institute of Environmental Medicine (IEM), Karolinska Institutet, Stockholm, Sweden, 2International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh and 3University of Tsukuba, Tsukuba, Japan.

Prenatal arsenic exposure is associated with reduced infant’s thymic size and function and increased morbidity, indicating arsenic-related impaired immune maturation. In this follow-up study, we evaluated the persistence of arsenic-related impaired immunity by measuring the delayed type hypersensitivity response (DTH) in pre-school aged children, in relation to prenatal and concurrent arsenic exposure. Children born in a longitudinal mother-child cohort in rural Bangladesh were studied at 4 years of age (n=577). Exposure to arsenic was assessed by the concentrations of arsenic metabolites in maternal urine (U-As) collected in gestational week 8 (GW8), as well as in children’s concurrent urine. U-As was measured by HPLC online with ICP-MS. Children were given an intradermal injection of purified protein derivative (PPD), and the diameter (mm) of induration was measured after 48 hours. Plasma levels of 27 cytokines were analyzed by multiplex cytokine assay. Concurrent, but not prenatal, arsenic exposure was associated with increased risk of not responding to PPD (induration <5mm) injection. The odds ratio (OR) of not responding to PPD was 1.91, (95% confidence interval (CI): 1.18, 3.07) in children in the highest quartile of U-As (125-1228 μg/L) compared with lowest quartile (12-34 μg/L). The associations were stronger in underweight children and those in families with low socioeconomic status. Concurrent arsenic exposure was inversely associated with Th1 cytokines (IL-2 and TNF-α) (regression coefficient (95% CI): -0.38 (-0.69, -0.06); and -1.17 (-2.38, 0.05), respectively) and, thus, Th1/Th2 ratios (IL-2/IL-4 and IL-2/IL-10). In subsequent stratified analyses, these associations were more evident in underweight children and those in families with low socioeconomic status. In conclusion, arsenic exposure appeared to impair cell-mediated immunity in children by reducing the DTH, presumably linked to reduced Th1 cytokines.

Inhibition of Arsenic-Induced Unfolded Protein Response Prevents Endothelial Activation and Inhibits Atherosclerosis in APO E-Knockout Mice


Epidemiological and animal studies suggest that exposure to arsenic exacerbates atherosclerosis. However, the mechanisms by which arsenic exerts itsatherogenic effects are not known. We observed that exposure of endothelial cells to sodium arsenite for 4 days increased the expression of pro-inflammatory cytokine IL-8 by 5-fold and molecular chaperones of protein folding - GRP78 and HERP by 2-fold. Moreover, acute exposure of sodium arsenite (2-6 hours) to endothelial cells increased the surface expression of adhesion molecules ICAM-1, VCAM-1 and E-Selectin by 1.2-1.5-fold; leukocyte adhesion by 1.3-2.5-fold; leukocyte trans-endothelial migration by 2.5-4.0-fold; and expression of pro-inflammatory cytokines and chemokines by 27-150-fold. Acute exposure of endothelial cells to sodium arsenite also activated the IRE-1 and ATF-6 arms of unfolded protein response (UPR) and increased the expression of molecular chaperones. Knockdown of IRE-1 by siRNA and adenosine transfection with ATF-6 prevented sodium arsenite-induced endothelial activation. Similarly, phenyl butyric acid (PBA), a chemical chaperone of protein folding, prevented sodium arsenite-induced endothelial activation. Moreover, feeding PBA to apoE-null mice for 16 weeks inhibited sodium arsenite-induced UPR in atherosclerotic lesions; expression of adhesion molecules on endothelial cells lining the atherosclerotic lesions; lesional and systemic inflammation and prevented the arsenic-induced exacerbation of lesion formation in the aortic valve. Together, these data suggest that arsenic causes endothelial activation and exacerbation of atherosclerosis by triggering UPR and chemical chaperones of protein folding could prevent arsenic-induced exacerbation of atherogenesis and inflammation.

Characteristics of Activated Lymphocytes in Silicosis Differing in Th17 Phenotype from Systemic Scleroderma

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Inhalation of silica causes lung pneumonia, silicosis, the patients with which occasionally suffer from autoimmune diseases including systemic scleroderma (SSc). We hypothesized an immunological effect of silica exposure, and examined immunologically patients with silicosis and SSc using flow cytometry, realtime PCR and cell culture techniques. Both the patients with silicosis and SSc showed increases in CTLA-4, CXCR3 and IL-1R1 on CD4+ T cells and CD25, FasL, CXCR3 and HLA-DR on CD8+ T cells. Additionally, silicosis showed increases in CD45RO on CD8+ T cells and FasL on NK cells, but SSc did not. Foxp3 mRNA level was high in CD4+ T cells of silicosis as well as SSc, but IL-1R1 mRNA was high only in SSc. Silicosis showed high TNF-α mRNA in stimulated CD4+ T cells,
whereas SSC showed high IL-17A mRNA. Both stimulated CD8+ T cells of silicosis and SSC showed high mRNA levels of granzyme B, IFN-γ and FasL, IP-10, MCP-1 and TNF-α levels in plasma were high in silicosis as well as SSC. These results indicate that silicosis and SSC patients have several similar characteristics of increases in regulated on activation, normal T cell expressed and secreted (RAGE) and inflammatory cytokine, and that Th17 function increases in CD4+ T cells of SSC patients. The similarities between silicosis and SSC suggest a potentially immune-activating effect of exposure to silica, leading to autoimmune diseases. The difference in characteristics observed in both the diseases might contribute to understanding pathogenesis of autoimmune diseases following exposure to silica.

### 2323 Transient Receptor Potential Cation Channel A1 (TRPA1) Mediates Decrements in Cardiac Mechanical Function and Dysrhythmia Caused by a Single Air Pollution Exposure in Mice

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Time-series studies indicate that the risk of cardiac events and arrhythmia increase significantly in the hours to days directly following exposure to air pollution. Previous studies have demonstrated that pharmacological inhibition of transient receptor potential cation A1 (TRPA1) attenuates diesel exhaust-induced cardiac arrhythmia. In this study, we sought to further confirm the role of TRPA1 in acute cardiovascular dysfunction due to air pollution exposure by utilizing a TRPA1 knockout mouse model. We hypothesized that acrolein and ozone (O3), both potent TRPA1 agonists, would cause decrements in cardiac function and electrical disturbances via TRPA1 mediated mechanisms. Conscious, unrestrained C57BL/6 (wt) and TRPA1 knockout (ko) mice were exposed to 3ppm acrolein for 3 hours or 0.3ppm O3 for 4 hours; separate groups were exposed to filtered air (FA). Electrocardiogram (ECG) was recorded continuously before, during and after exposure. 24 hours post-exposure, cardiac function was assessed using a Langendorff cardiac perfusion preparation. Acrolein exposed wt mice demonstrated a significant decrease in HR during exposure as well as an increased incidence of arrhythmia compared with FA controls. Acrolein also produced a significant increase in ventricular relaxation, whereas exposure to O3 produced a significant decrease in contractility and relaxation in wt mice. No effects were seen in acrolein or O3 exposed ko mice. These data indicate that TRPA1 mediates, at least in part, the development of acute cardiac function decrements and dysrhythmia following exposure to either of these two relatively ubiquitous pollutants. While both acrolein and O3 are TRPA1 agonists, they appear to cause divergent cardiac responses, which suggest the underlying mechanisms are more complex than mere sensory activation. (This abstract does not reflect EPA policy)

### 2324 Inhalation Exposures to Ozone Induce Insulin Resistance and Pulmonary Pathology in Type II Diabetes-Prene Mice

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Introduction: Epidemiological studies suggest that diabetics may be more susceptible to the adverse health effects of air pollution. Mice chronically exposed to particulate air pollutants induce insulin resistance (IR), an early indicator of type II diabetes (T2D). In the present study we tested the hypothesis that episodic inhalation exposures to a common gaseous air pollutant, ozone (O3), will induce early onset of IR in mice genetically prone to develop T2D.

Methods: Male C57BL/6, KK, and KKAY mice were exposed to 0 ppm (filtered air; FA) or 0.5 ppm O3, 4h/day, for 13 weeks. Two hours after the last exposure, mice were subjected to insulin tolerance tests (ITT) and then sacrificed 4 hours postexposure. Mice received a single intraperitoneal injection of insulin and blood samples were collected at 0, 10, 20, 30, 60, 90, 120 and 130 minutes after injection. Bronchoalveolar lavage fluid (BALF) was analyzed for inflammatory cells, and lungs were processed for light microscopy.

Results: Normoglycemic, C57BL/6 mice exposed to FA or O3 had normal ITT. In these mice, O3 caused modest increases in the number of BALF inflammatory cells and minimal to mild pulmonary pathology. In contrast, O3 induced greater increases in BALF inflammatory cells in the hyperglycemic KK mice, along with more severe pulmonary pathology. Marked IR was present in O3-, but not FA-, exposed KK mice. Both FA- and O3-exposed, hyperglycemic KKAY mice developed IR. KKAY mice exposed to O3 had the greatest number of BALF inflammatory cells and the most severe pulmonary pathology of all the mice on study.

Conclusions: Episodic O3 exposures caused an early onset of IR in mice genetically prone to T2D. These hyperglycemic animals had greater O3-induced lung injury and inflammation compared to normoglycemic, insulin responsive mice. These results suggest that people at risk for T2D may be more susceptible to respiratory and metabolic health effects caused by elevated concentrations of ambient O3. Funded by USEPA RD83479701.

### 2325 Ozone-Induced Impairment of Systemic Metabolic Processes: Influence of Prior Ozone Exposure and Metformin Pretreatment on Aged Wistar Kyoto (WKY) Rats


Ozone (O3) exposure is associated with adverse cardiopulmonary health effects in humans and thought to produce metabolic impairment, such as insulin resistance. Recently, we showed that acute O3 exposure caused glucose intolerance and increased serum leptin and epinephrine in aged Brown Norway rats. We hypothesized that 1) subchronic pre-exposure to O3 at a young age would predispose rats to exacerbated metabolic effects of subsequent O3 exposure later in life and 2) metabolic effects of O3 would be different in aged rats pretreated with metformin to improve glucose uptake in tissue. WKY rats were exposed to O3 at 0 or 0.5 ppm 6h/day×2days/week x 13 weeks starting at 3 months of age. To study the effects of pre-exposure, the same rats were re-exposed to 0 or 1 ppm ozone acutely for 6h at 14 months of age. To examine if treatment to improve glucose uptake would reduce the effects of O3, another group of 18 month old WKY rats were treated with the anti-diabetic drug metformin in drinking water (3g/L) for 2 weeks prior to a 6h/day x 2 day acute exposure to 0 or 1ppm ozone. Glucose tolerance tests, serum, and Bronchoalveolar lavage fluid (BALF) analyses were done immediately after exposure in both studies. Marked age-related impairment of glucose tolerance was observed. Additionally, O3 exposure caused further impairment of glucose tolerance. O3 exposure resulted in acute lung injury/inflammation and increases in serum levels of leptin, insulin, cholesterol and triglycerides in aged WKY rats. Pre-exposure to O3 had no significant impact on any of these parameters. Treatment with metformin did not reduce O3-induced lung injury or glucose intolerance, but decreased serum leptin and insulin in all rats. Our results suggest that pre-exposure to O3 does not impact response to re-exposure at an older age. We believe that the metabolic effects of O3 are not mediated by impaired cellular glucose regulation. (Does not reflect US EPA policy).

### 2326 Serum Metabolomic Profiling and Liver Transcriptomic Analysis Provides Mechanistic Evidence of Ozone (O3)- Induced Systemic Metabolic Impairment


Recently, air pollution has been linked to insulin resistance and obesity but the mechanisms remain to be elucidated. We have recently shown that acute O3 exposure induces glucose intolerance, hyperglycemia and increases in leptin and epinephrine in rats. Here, we hypothesized that the metabolic effects of O3 are associated with global changes in liver fatty acid and amino acid metabolism and that global metabolomic and transcriptomic approaches will provide insight into the mechanisms by which O3 might contribute to increased insulin resistance and/or obesity. Male Wistar Kyoto (WKY) rats were exposed to air or 1 ppm O3, 6 hrs/day for 1 or 2 days and liver gene expression and serum metabolomic analysis were performed immediately after. Metabolomic analysis identified O3 effects on numerous small metabolite biomarkers. O3 increased blood glucose, long chain free fatty acids, cholesterol and decreased 1,5-anhydroglucitol (AG); biomarkers of insulin resistance. Metabolites of glycosylation were increased, however; intermediates of the TCA cycle and bile acids were decreased in the serum by O3. Branched chain amino acids were increased, suggesting muscle amino acid catabolism. Of particular interest, metabolites that are associated with gut microbiome (e.g. phe- nylacetylglycine) were also markedly affected by O3. Liver gene expression profile after O3 exposure correlated with the serum metabolite changes. For example, elevated serum fatty acids coincided with decreased expression of lipid biosynthetic genes, suggesting that the liver might have initiated homeostatic control for metabolic changes. In conclusion, our metabolomic and genomic analyses show that O3 induces acute systemic alterations reflective of changes in glucose, lipid, and amino acid metabolism which upon chronic exposure might contribute to insulin resistance and obesity. (Does not reflect EPA Policy).
Ccr2 is a chemokine receptor that mediates the migration of macrophages (MP) to sites of injury. In these studies we analyzed the role of Ccr2 in ozone-induced inflammatory cell trafficking to the lung. Treatment of C57Bl/6 mice with ozone (0.8 ppm, 3 h) resulted in increased bronchoalveolar lavage (BAL) protein after 24-48 h, indicative of alveolar epithelial injury. This was associated with a time-related increase in CD11b+ inflammatory cells in the lung. The majority of these cells expressed CD11c and F4/80 indicating that they are mature lung MP. Ly6C is expressed at high levels on proinflammatory MP. CD11b+F4/80+Ly6Chi proinflammatory MP increased in the lung after ozone, peaking at 24-48 h. CD11b+F4/80+Ly6C midinflammatory MP also increased in the lung, peaking 48-72 h after ozone. Loss of Ccr2 resulted in a decrease in ozone-induced accumulation of CD11b+ cells in the lung. This was correlated with decreases in CD11b+F4/80+Ly6Chi and CD11b+F4/80+Ly6C midipopulations. Whereas decreases in proinflammatory Ly6C midMP persisted for at least 72 h post ozone, decreases in antiinflammatory Ly6C midMP were transient, and by 72 h were near control levels. CD11b+F4/80+Ly6g+Ly6c+ myeloid-derived suppressor cells (MDSC), which display antiinflammatory activity and are known to migrate to sites of injury, were increased in the lung 48 h after ozone. Loss of Ccr2 resulted in a persistent increase in MDSC. Changes in lung MP and MDSC were linked to reduced toxicity of ozone, measured by BAL protein levels. The effects of loss of Ccr2 on splenic monocytes, which function as an extramedullary source of inflammatory cells were also analyzed. Ozone caused a decrease in CD11b+F4/80+Ly6Chi proinflammatory monocytes in the spleen; this response was attenuated in Ccr2−/− mice 24-72 h after ozone. These results demonstrate a key role of Ccr2 in ozone-induced inflammatory cell migration into the lung and toxicity. Moreover, it appears that the effects of Ccr2 are predominantly on proinflammatory MP.

2327 Ccr2 Regulates Inflammatory Cell Accumulation in the Lung and Tissue Injury following Ozone Exposure
A. M. Groves, M. Francis, M. Mandal, H. Choi, J. D. Laskin and D. L. Laskin, Rutgers University, Piscataway, NJ.


Introduction: Ozone (O3) is an oxidant air pollutant in photochemical smog. While nasal epithelial injury and remodelling have been reported in laboratory animals repeatedly exposed to O3, associated granulocytic rhinitis and pro-inflammatory cytokine expression have not been fully characterized. We investigated the temporal changes in granulocyte influx, cytokine gene expression, and epithelial remodelling in the nasal mucosa of mice episodically exposed to O3. Methods: Male mice were exposed to 0 or 0.5 ppm O3 for 1, 2, 4, 9, or 24 weeks (4h/day). Airway mucosa from nasal turbinates and lateral wall were analyzed for cytokine and epithelial gene expression. Nasal tissues were prepared for light microscopy and morphometry. Immunohistochemistry was used to identify neutrophils, eosinophils, and chitinase-like proteins (Ym1/Ym2). Epithelial mucous and goblet cell surfaces were histochemically detected. Results: 1-day-O3 exposure induced a neutrophilic influx with few eosinophils and concurrent epithelial necrosis. These responses were associated with overexpression of KC, MIP-2, IL-1β, IFNγ, IL-5, IL-6 and eotaxin genes. After repeated O3 exposures, neutrophils waned and eosinophils increased, along with epithelial regeneration and remodelling. 9-day-O3 mice had marked eosinophilic rhinitis with few neutrophils, mucous cell metaplasia and increased epithelial Ym1/Ym2 proteins. Concurrently there was overexpression of Gob5, Muc5AC, IL4, IL5, eotaxin and Ym2 genes. 24-day-O3 mice developed marked eosinophilic rhinitis, epithelial hyperplasia, mucous cell metaplasia, hyalinosis, and increased Ym1/Ym2 expression.

Conclusion: Repeated, episodic O3 exposures in mice induce Th2 cytokine overexpression, eosinophilic rhinitis, epithelial remodelling, and increased epithelial chitinase-like proteins. These results suggest an etiologic role of ambient ozone in the development of nonallergic eosinophilic rhinitis. Funded by USEPA R83479701.
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</tr>
<tr>
<td>Cech, L</td>
<td>245, 685*, 1316</td>
</tr>
<tr>
<td>Cech, L</td>
<td>1429*</td>
</tr>
<tr>
<td>Cech, D</td>
<td>954*, 1087</td>
</tr>
<tr>
<td>Cech, G</td>
<td>23, 1922, 2222</td>
</tr>
<tr>
<td>Cech, H</td>
<td>1530, 1589, 1811, 1812, 2521, 2001</td>
</tr>
<tr>
<td>Cech, Z</td>
<td>263</td>
</tr>
<tr>
<td>Cheng, G</td>
<td>1005</td>
</tr>
<tr>
<td>Cheng, H</td>
<td>1888</td>
</tr>
<tr>
<td>Cheng, P</td>
<td>22504</td>
</tr>
<tr>
<td>Cheng, S</td>
<td>786*, 1853</td>
</tr>
<tr>
<td>Cheng, T</td>
<td>1324*</td>
</tr>
<tr>
<td>Cheng, W</td>
<td>440*, 4440</td>
</tr>
<tr>
<td>Cheng, X</td>
<td>910</td>
</tr>
<tr>
<td>Cheng, Y</td>
<td>223*, 1106*</td>
</tr>
<tr>
<td>Chepeler, N</td>
<td>1478</td>
</tr>
<tr>
<td>Chepeler, N</td>
<td>954a</td>
</tr>
<tr>
<td>Chepeler, N</td>
<td>547, 735, 1183, 1327c, 1701, 2061</td>
</tr>
<tr>
<td>Cherry, D</td>
<td>1522, 2334</td>
</tr>
<tr>
<td>Cherry, W</td>
<td>338</td>
</tr>
<tr>
<td>Chevovna, Y</td>
<td>1301</td>
</tr>
<tr>
<td>Chevovna, Y</td>
<td>1150, 1617</td>
</tr>
<tr>
<td>Chetham-Spooner, J</td>
<td>1998</td>
</tr>
<tr>
<td>Chen, M</td>
<td>269</td>
</tr>
<tr>
<td>Chen, M</td>
<td>269</td>
</tr>
<tr>
<td>Cheung, F</td>
<td>408</td>
</tr>
<tr>
<td>Chevallier, A</td>
<td>1158</td>
</tr>
<tr>
<td>Chimmswal, Y</td>
<td>798, 399</td>
</tr>
<tr>
<td>Chiang, C</td>
<td>2054</td>
</tr>
<tr>
<td>Chiang, H</td>
<td>511*</td>
</tr>
<tr>
<td>Chiang, Y</td>
<td>939</td>
</tr>
<tr>
<td>Chihbou, S</td>
<td>873, 1618, 2056</td>
</tr>
<tr>
<td>Chigrupati, S Y</td>
<td>934*</td>
</tr>
<tr>
<td>Chihara, K</td>
<td>201</td>
</tr>
<tr>
<td>Child, J</td>
<td>641</td>
</tr>
<tr>
<td>Child, M</td>
<td>1053</td>
</tr>
<tr>
<td>Challilid, R</td>
<td>825</td>
</tr>
<tr>
<td>Chilton, J</td>
<td>1058</td>
</tr>
<tr>
<td>Chimote, G</td>
<td>1639a</td>
</tr>
<tr>
<td>Chin, M</td>
<td>1639b</td>
</tr>
<tr>
<td>Chin, M</td>
<td>1539</td>
</tr>
<tr>
<td>Chin, N</td>
<td>1539</td>
</tr>
<tr>
<td>Chinatagi, N</td>
<td>1539</td>
</tr>
<tr>
<td>Chipinda, P</td>
<td>2036</td>
</tr>
<tr>
<td>Chinone, Y</td>
<td>1106</td>
</tr>
<tr>
<td>Chimrule, N</td>
<td>1539</td>
</tr>
<tr>
<td>Chisholm, C</td>
<td>1981</td>
</tr>
<tr>
<td>Chitrakar, R</td>
<td>1229e*</td>
</tr>
<tr>
<td>Chithiboyvin, S</td>
<td>548</td>
</tr>
<tr>
<td>Chiu, C</td>
<td>1325</td>
</tr>
<tr>
<td>Chiu, W</td>
<td>630, 1831</td>
</tr>
<tr>
<td>Chivers, S</td>
<td>278</td>
</tr>
<tr>
<td>Cho, A K</td>
<td>1716</td>
</tr>
<tr>
<td>Cho, H</td>
<td>1804*</td>
</tr>
<tr>
<td>Cho, K</td>
<td>194</td>
</tr>
<tr>
<td>Cho, M</td>
<td>290, 1951</td>
</tr>
<tr>
<td>Cho, M</td>
<td>641</td>
</tr>
<tr>
<td>Choi, B</td>
<td>942*, 1327e</td>
</tr>
<tr>
<td>Choi, C</td>
<td>1166, 1167</td>
</tr>
<tr>
<td>Choi, H</td>
<td>395*, 1005, 1327</td>
</tr>
<tr>
<td>Choi, J</td>
<td>506, 787, 795, 799*, 799a</td>
</tr>
<tr>
<td>Choi, K</td>
<td>1087e, 1087g, 1223, 1233, 2234</td>
</tr>
<tr>
<td>Choi, S</td>
<td>1033, 1243, 1996*</td>
</tr>
<tr>
<td>Choi, T A</td>
<td>894*</td>
</tr>
<tr>
<td>Choi, Y</td>
<td>353, 790, 1125*, 1190</td>
</tr>
</tbody>
</table>
The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author’s names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author’s names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
AUTHOR INDEX (Continued)

McKeon, M .................. 1943a, 1943b
McKim, J M ................. 166, 170, 343*, 845, 1037
McKim, K ..................... 265a
McKinney, G ................ 1943b
McKinney, W ................ 603d, 1229a, 1368a, 1731u
McKinney, W ................ 5
McKone, T ..................... 1405
McLanahan, E D ......... 96b, 1766, 1830
McLaurin, K W .......... 440a*, 2273s
McLellan, C J ........... 606, 607, 608
McLaughlin, C.E.; 488, 496, 842e*, 1242a
McMahan, R S ........... 416
McMahan, R S .......... 416
McMullen, P D .......... 94, 160, 162, 1140*
McNally, K ................. 440f
McNamee, P ............... 1027
McNerney, M ................ 110e
McNerney, M E .......... 2191
McNeill, N ................. 581a, 1922c*, 1922d
McPherson, S ............. 287*, 473, 2142, 2156
McPherson, S .......... 287*, 473, 2142, 2156
McPherson, S ............. 327
McQueen, C A .......... 735*
McQuistan, T ............. 882
McShann, W .............. 979
McVay, G ................. 1042
Meade, B .................. 141, 142, 2025
Meade, M L ............... 573*
Means, J C ................. 309, 922a, 922b
Medeiros, R M ........... 152a
Medici, S ................. 1297e
Medina-Reyes, E I ....... 1996
Medvedev, A .............. 1635
Medvedev, M ............. 1253a, 1909
Mechee, R ................. 818
Meek, B ...................... 6
Meek, E C .................. 556, 568, 1515*, 2198
Meggars, V .............. 528, 568
Meehan, C ................ 818
Mecagni, G ............... 1042
Mehr, M ................. 1297e
Mehrzad, N ............... 344
Megill, J .................. 162
Mehendale, H M .......... 1164, 1165*
Mehrpourpaya, P ....... 1713s
Mehta, A .................. 1943e
Mehta, P .................... 347, 403*, 1801, 1810
Mehta, R D ............... 445
Mehta, V .................... 905, 1087s
Mei, C ..................... 508
Mei, N ....................... 319*, 1893, 1943b, 1973c
Meighan, T ................ 1229a
Meijer, M .................. 1834*
Meier, M .................. 1834*
Meirelles, G ................ 664
Meisenheimer, P ........ 527, 1784
Meister, A .................. 159
Mekenyan, O .......... 435
Melchers, D ............... 1150, 1154
Melchling-Kollmuss, S .... 1150
Mellert, W ................ 120, 634, 1591, 1871
Mello, F ................... 1638*
Melnik, S B ............... 1343
Mendard, A I ............. 581*
Mendard, S ................ 133
Mencalha, A ............... 466
Mendell, J T ............... 734*
Mendero, A M ........... 840a*
Mender, M .................. 1321, 1314
Mender, M A ............... 603i
Mendez-Rosa, R ........ 1218
Mendrick, D L .......... 11, 19, 813d
Meng, Q ................... 1275
Mengel, M ................. 863
Menke, A ................... 154
Menon, S .................. 1502
Mensing, T ................ 205
Mercado, F ............... 603i
Merck, K E ................. 254
Merrcer, R .................. 2046b
Merrcer, W C ............. 1561
Mercola, M ................ 767a
Meredith, C ............... 71, 237, 443, 1530
Mereness, I .............. 452, 1929
Mergart, A ................ 1920
Merrill, M D .............. 520*
Merrick, B .................. 1297t

The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's name refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's names refer to the abstract numbers. The asterisk after the abstract number indicates the author is the first presenter.
<table>
<thead>
<tr>
<th>Author</th>
<th>Abstract Number(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas, M A</td>
<td>1760</td>
</tr>
<tr>
<td>Thomas, N</td>
<td>768*</td>
</tr>
<tr>
<td>Thomas, R</td>
<td>2256a, 2273s</td>
</tr>
<tr>
<td>Thomas, R S</td>
<td>146, 1132, 1905, 2020, 2250, 2273o</td>
</tr>
<tr>
<td>Thomas, S</td>
<td>1898</td>
</tr>
<tr>
<td>Thompson, B</td>
<td>1116</td>
</tr>
<tr>
<td>Thompson, B</td>
<td>2037</td>
</tr>
<tr>
<td>Thompson, C M</td>
<td>622, 623, 628, 179, 1290</td>
</tr>
<tr>
<td>Thompson, D</td>
<td>552, 1095</td>
</tr>
<tr>
<td>Thompson, D C</td>
<td>2067</td>
</tr>
<tr>
<td>Thompson, E</td>
<td>582, 583, 584*</td>
</tr>
<tr>
<td>Thompson, J T</td>
<td>248, 2213</td>
</tr>
<tr>
<td>Thompson, R</td>
<td>632</td>
</tr>
<tr>
<td>Thompson, T A</td>
<td>1144</td>
</tr>
<tr>
<td>Thompson, W D</td>
<td>1303</td>
</tr>
<tr>
<td>Thompson, J</td>
<td>715*</td>
</tr>
<tr>
<td>Thornton, D</td>
<td>443</td>
</tr>
<tr>
<td>Thorne, G</td>
<td>1802*</td>
</tr>
<tr>
<td>Thorne, P S</td>
<td>516, 895, 1767</td>
</tr>
<tr>
<td>Thornton, C</td>
<td>208, 1683, 1987, 1988</td>
</tr>
<tr>
<td>Thorpe, R</td>
<td>989</td>
</tr>
<tr>
<td>Thoras, H R</td>
<td>893</td>
</tr>
<tr>
<td>Thorsrud, A B</td>
<td>331, 341, 342, 343, 845*</td>
</tr>
<tr>
<td>Thota, B</td>
<td>88, 1059, 1899</td>
</tr>
<tr>
<td>Thrall, B D</td>
<td>602, 814, 1963, 1980</td>
</tr>
<tr>
<td>Thrall, K D</td>
<td>1525</td>
</tr>
<tr>
<td>Thran, V</td>
<td>2238</td>
</tr>
<tr>
<td>Threadgill, D W</td>
<td>261</td>
</tr>
<tr>
<td>Tidball, A</td>
<td>1610*</td>
</tr>
<tr>
<td>Thuman, D</td>
<td>27</td>
</tr>
<tr>
<td>Thurmond, S</td>
<td>1394*</td>
</tr>
<tr>
<td>Thurston, G D</td>
<td>1210</td>
</tr>
<tr>
<td>Tiede, D</td>
<td>5</td>
</tr>
<tr>
<td>Tian, W</td>
<td>1129*</td>
</tr>
<tr>
<td>Tian, C</td>
<td>989</td>
</tr>
<tr>
<td>Tian, Y</td>
<td>1971</td>
</tr>
<tr>
<td>Tipton, B</td>
<td>91</td>
</tr>
<tr>
<td>Tibbits, B</td>
<td>1659</td>
</tr>
<tr>
<td>Tice, R R</td>
<td>122, 173b, 173h, 444, 467g, 958, 1063, 1616g, 1736a, 2273h, 2273e</td>
</tr>
<tr>
<td>Tichener, J N</td>
<td>1699</td>
</tr>
<tr>
<td>Tidball, A</td>
<td>1353, 1354</td>
</tr>
<tr>
<td>Tien, E</td>
<td>2065*</td>
</tr>
<tr>
<td>Tien, L</td>
<td>369*, 2060</td>
</tr>
<tr>
<td>Tierney, K</td>
<td>654</td>
</tr>
<tr>
<td>Tietjen, J P</td>
<td>146</td>
</tr>
<tr>
<td>Tierholck, A H</td>
<td>1752*</td>
</tr>
<tr>
<td>Tijani, A Y</td>
<td>807</td>
</tr>
<tr>
<td>Tijara, P B</td>
<td>824*</td>
</tr>
<tr>
<td>Tollmann, T</td>
<td>600</td>
</tr>
<tr>
<td>Tilly, T B</td>
<td>1664</td>
</tr>
<tr>
<td>Tilley, S C</td>
<td>27, 602</td>
</tr>
<tr>
<td>Timchalk, C</td>
<td>1573*</td>
</tr>
<tr>
<td>Timme-Laragy, R A</td>
<td>209, 2283*</td>
</tr>
<tr>
<td>Tirkkonen, J</td>
<td>983*</td>
</tr>
<tr>
<td>Tirmenstein, M</td>
<td>109, 1089*</td>
</tr>
<tr>
<td>Tither, S</td>
<td>102</td>
</tr>
<tr>
<td>Tuchschman, S</td>
<td>1153, 2197</td>
</tr>
<tr>
<td>Titorenko, V</td>
<td>306</td>
</tr>
<tr>
<td>Tluczkiewicz, I</td>
<td>631</td>
</tr>
<tr>
<td>Tobita, K</td>
<td>2076</td>
</tr>
<tr>
<td>Todd, S W</td>
<td>1559</td>
</tr>
<tr>
<td>Todorov, T</td>
<td>1559*</td>
</tr>
<tr>
<td>Todt, C E</td>
<td>1848*, 1849</td>
</tr>
<tr>
<td>Tohanyi, A F</td>
<td>1853</td>
</tr>
<tr>
<td>Tohanyi, C</td>
<td>200, 978B, 1346, 1747b, 2163</td>
</tr>
<tr>
<td>Tomita, T</td>
<td>1062a, 1087d</td>
</tr>
<tr>
<td>Tokar, J</td>
<td>12*, 958, 1297n, 1297t, 1297k</td>
</tr>
<tr>
<td>Toki, T</td>
<td>263</td>
</tr>
<tr>
<td>Tolbo, M F</td>
<td>976d, 1110a, 2258</td>
</tr>
<tr>
<td>Tolbert, P</td>
<td>868</td>
</tr>
<tr>
<td>Tollefson, L M</td>
<td>605c</td>
</tr>
<tr>
<td>Tomar, S</td>
<td>922</td>
</tr>
<tr>
<td>Tomblin, J F</td>
<td>999</td>
</tr>
<tr>
<td>Tomkinson, A</td>
<td>1205</td>
</tr>
<tr>
<td>Tomlinson, C</td>
<td>1163a</td>
</tr>
<tr>
<td>Toms, A</td>
<td>955</td>
</tr>
<tr>
<td>Toneva, M</td>
<td>286</td>
</tr>
<tr>
<td>Tong, F</td>
<td>671</td>
</tr>
<tr>
<td>Tong, S</td>
<td>177</td>
</tr>
<tr>
<td>Tong, W</td>
<td>603, 173b, 418e, 552e, 387a, 1509, 1887, 2273f</td>
</tr>
<tr>
<td>Tong, Z</td>
<td>1200</td>
</tr>
<tr>
<td>Tonkin, E G</td>
<td>567</td>
</tr>
<tr>
<td>Tonomura, Y</td>
<td>1866*</td>
</tr>
<tr>
<td>Tongyong, K</td>
<td>579</td>
</tr>
<tr>
<td>Took, J</td>
<td>1648, 2126a*</td>
</tr>
<tr>
<td>Topinka, J</td>
<td>706, 844, 1943a</td>
</tr>
<tr>
<td>Topiwala, K S</td>
<td>3212</td>
</tr>
<tr>
<td>Topper, M J</td>
<td>794</td>
</tr>
<tr>
<td>Topping, V</td>
<td>173, 1297a, 1973b, 1973c, 1973j</td>
</tr>
<tr>
<td>Torri, M</td>
<td>1174, 1866</td>
</tr>
<tr>
<td>Torrie, R</td>
<td>1419*</td>
</tr>
<tr>
<td>Torrie, M</td>
<td>278</td>
</tr>
<tr>
<td>Tornier, C</td>
<td>2177, 2179a</td>
</tr>
<tr>
<td>Torok, E</td>
<td>1949</td>
</tr>
<tr>
<td>Torok, D K</td>
<td>452</td>
</tr>
<tr>
<td>Torres, G B</td>
<td>343a</td>
</tr>
<tr>
<td>Torres, O</td>
<td>343b</td>
</tr>
<tr>
<td>Torres-Saravieda, P</td>
<td>479</td>
</tr>
<tr>
<td>Toscano, I</td>
<td>2151d</td>
</tr>
<tr>
<td>Toselli, P</td>
<td>1250</td>
</tr>
<tr>
<td>Totah, R</td>
<td>1129*</td>
</tr>
<tr>
<td>Tovar-Jensen, B</td>
<td>659</td>
</tr>
<tr>
<td>Towery, K L</td>
<td>1191</td>
</tr>
<tr>
<td>Toyama, T</td>
<td>1258</td>
</tr>
<tr>
<td>Tran, D</td>
<td>1066</td>
</tr>
<tr>
<td>Tran, H</td>
<td>505</td>
</tr>
<tr>
<td>Tran, K</td>
<td>837*</td>
</tr>
<tr>
<td>Tran, T</td>
<td>353</td>
</tr>
<tr>
<td>Tran, V</td>
<td>422*, 868</td>
</tr>
<tr>
<td>Tranchementage, Z</td>
<td>382</td>
</tr>
<tr>
<td>Trask, O</td>
<td>1873p, 1934</td>
</tr>
<tr>
<td>Trasich, U</td>
<td>952*</td>
</tr>
<tr>
<td>Traver, S</td>
<td>1363</td>
</tr>
<tr>
<td>Travles, G</td>
<td>1087n, 1110b, 1616c</td>
</tr>
<tr>
<td>Tremblay, C</td>
<td>93</td>
</tr>
<tr>
<td>Tremblay, M</td>
<td>238</td>
</tr>
<tr>
<td>Tret, J O</td>
<td>304</td>
</tr>
<tr>
<td>Tret'yakova, N</td>
<td>978</td>
</tr>
<tr>
<td>Treumann, S K</td>
<td>1992</td>
</tr>
<tr>
<td>Trevaskis, A</td>
<td>2124</td>
</tr>
<tr>
<td>Trickler, W</td>
<td>353</td>
</tr>
<tr>
<td>Trincor, M</td>
<td>1502</td>
</tr>
<tr>
<td>Trine, V</td>
<td>2196</td>
</tr>
<tr>
<td>Trinh, M</td>
<td>1345</td>
</tr>
<tr>
<td>Trindad, J</td>
<td>932, 942a, 1378</td>
</tr>
<tr>
<td>Tripodi, G</td>
<td>1600</td>
</tr>
<tr>
<td>Tripodi, N</td>
<td>209*, 1973</td>
</tr>
<tr>
<td>Troese, M</td>
<td>572, 580q, 657</td>
</tr>
<tr>
<td>Trompetta, L D</td>
<td>486, 1261, 1852</td>
</tr>
<tr>
<td>Trotey, A</td>
<td>274, 275, 471</td>
</tr>
<tr>
<td>Tropea, A</td>
<td>2126c, 2273o</td>
</tr>
<tr>
<td>Troshok, J</td>
<td>1900</td>
</tr>
<tr>
<td>Trost, C</td>
<td>273</td>
</tr>
<tr>
<td>Trost, L</td>
<td>2121</td>
</tr>
<tr>
<td>Troth, K</td>
<td>2196o</td>
</tr>
<tr>
<td>Troub, K J</td>
<td>1852</td>
</tr>
<tr>
<td>Troubridge, J F</td>
<td>2250d</td>
</tr>
<tr>
<td>Truchon, G</td>
<td>829</td>
</tr>
<tr>
<td>Trubis, B</td>
<td>1179</td>
</tr>
<tr>
<td>Trueblood, E</td>
<td>1157t, 1641</td>
</tr>
<tr>
<td>Truesdell, G</td>
<td>1644</td>
</tr>
<tr>
<td>Truong, K M</td>
<td>682*, 2205</td>
</tr>
<tr>
<td>Truong, L</td>
<td>31, 173e, 181, 2067, 440a, 1747b, 2273l, 2273s</td>
</tr>
<tr>
<td>Trush, M A</td>
<td>1792</td>
</tr>
<tr>
<td>Trush, P</td>
<td>1112</td>
</tr>
<tr>
<td>Tsengyan, Y K</td>
<td>956*, 957l, 9513</td>
</tr>
<tr>
<td>Tse, V</td>
<td>2277b, 306, 312a, 2149</td>
</tr>
<tr>
<td>Tseng, F</td>
<td>1502</td>
</tr>
<tr>
<td>Tseng, H</td>
<td>1636c*</td>
</tr>
<tr>
<td>Tseng, T</td>
<td>2008h</td>
</tr>
<tr>
<td>Tron, T</td>
<td>511</td>
</tr>
<tr>
<td>Trub, I</td>
<td>1900</td>
</tr>
</tbody>
</table>

The numerals following the author’s names refer to the abstract numbers.

The asterisk after the abstract number indicates the author is the first presenter.
Author Index (Continued)

Zhao, F  .................................................... 1300*
Zhang, M  .......................... 1189, 1194*, 1804a
Zhao, Q  ..................................................... 2034
Zhao, J  ..................................... 444, 649, 1146,
Zhao, G  ......................................... 1519, 1549*
Zhao, Q J  ........................................................ 6*
Zhao, X ........................................................  590
Zhang, J  ........................  418e, 639*, 962, 963,
Zhang, H ..........  874, 997, 1101, 1229c*, 1794,
Zhang, Y  ............................ 598*, 603b, 1163*,
Zhang, X L  ................................................ 1289
Zhang, L W  .................................... 820, 1616*
Zhang, L ..........  659*, 920, 1087*, 1141*, 1170
Zhao, C  ............................... 1188*, 1189, 1348
Zhao, R  ....................... 122h, 265e, 482*, 942a, 964,
Zhao, Z .................................................. 1297f
Zhao, B  .................................................. 92c
Zhao, C  .............................. 1188*, 1189, 1348
Zhao, D  ................................................. 2196*
Zhao, F  ................................................. 1300*
Zhao, G  ................................................. 1519, 1549*
Zhao, J  ............................. 444, 649, 1146,
1149, 1821, 2273cc
Zhao, L  .................................................. 1556, 1737
Zhao, Q  .................................................. 2034
Zhao, Q J  .................................................. 6*
Zhao, R  ................................................. 1804a*
Zhao, W  ................................................. 855, 2208*
Zhao, X  .................................................. 590
Zhao, Y  ......................... 75*, 266, 1250*, 1908, 2125*, 2191
Zhao, Z  .................................................. 157
Zhan, L  .......................................... 321b
Zheng, M .............................................. 813, 1250
Zheng, T  .............................................. 1276, 1303
Zheng, W  ......................... 122g*, 1346, 1291, 1349, 1361,
1362*, 1367, 1390, 1549, 2103*, 2107*
Zheng, X  .............................................. 594*, 1467
Zheng, Y  .............................................. 482, 1780
Zhuk, A  .............................................. 1268*, 1269, 1926
Zhong, F  ................................................ 273
Zhong, M  .............................................. 1282
Zhong, W  .............................................. 2269
Zhong, X  .............................................. 48, 49*
Zhou, C ................................................. 2184
Zhou, H .......... 813, 874, 1185, 1268
Zhou, M ................................................. 1628
Zhou, P  ................................................. 581, 131d, 1935*
Zhou, S  ................................................. 480*, 482, 483, 1580
Zhou, T ................................................. 1794, 2004c
Zhou, W ................................................. 1632, 1784
Zhou, X ................................................. 244, 533, 842, 1336*,
1327t, 1449, 1730, 2127*
Zhou, Y  ................................................. 418c
Zhou, Z ................................................. 1874, 2002
Zhu, B ................................................. 245, 247
Zhu, C ..................................................... 1303
Zhu, Q ................................................. 1229e, 1713t, 1790, 1792,
2252
Zhu, J ................................................. 224, 1903, 1922a
Zhu, L ..................................................... 1730
Zhu, S ..................................................... 1108
Zhu, W ..................................................... 1504
Zhu, X  ................................................. 986*, 2273h
Ziegler, T ................................................. 536, 855, 1781
Zielinger, T ............................................. 1713a
Ziemer, C ................................................. 601
Zierau, O .................................................. 173
Zikopoulos, D ........................................... 2250f
Zimmerman, J B  ..................................... 1831f
Zimmermann, I ......................................... 900
Zimprich, C A ............................................ 167, 291
Zink, D ..................................................... 718*
Zitzow, J .................................................. 2208
Ziv-Gal, A .................................................. 2184*
Zmierzchowski, D ....................................... 1009
Zollinger, T ................................................. 971
Zeng, M .................................................... 1087
Zordo, A ..................................................... 1279
Zu, K ......................................................... 1537*
Zucker, R .................................................. 1973d
Zur, M ....................................................... 2026
Zurinden, T ................................................. 64*, 137
Zwicky, C M ................................................. 434
Zywinski, K ................................................. 316*
Zykowski, J ................................................. 1297f

The asterisk after the abstract number indicates the author is the first presenter.
The numerals following the author's names refer to the abstract numbers.

SOT 2014 Annual Meeting 653
Abstracts

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
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Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).

Abstracts

SOT 2014 Annual Meeting

655

The,numerals,following,each,keyword,refer,to
the,relevant,abstract,number(s).
### Abstract Keyword Index (Continued)

| Pharmacodynamics                           | 901c, 1729, 1735 |
| Pharmacogenetics                           | 546 |
| Pharmacogenomic                            | 1480 |
| Pharmacogenomic                            | 552c |
| Pharmacokinetics                           | 880 |
| Pharmacokinetic                            | 70, 818, 881, 2163a |
| Pharmacokinetic Modeling                   | 2234m |
| Pharmacokinetic Models                     | 1876 |
| Pharmacokinetic Variability                | 92 |
| Pharmacokinetics                           | 652, 71, 78, 122, 276, 878, 884, 887, 889, 891, 894, 901d, 901, 1296, 1440 |
| Pharmacology                               | 2307 |
| Pharmacophore                              | 1090 |
| Phase II Conjugation Reaction              | 521 |
| Phenycyclidine                             | 1648 |
| Phenobarbital                              | 265c, 1200f, 1856, 1869 |
| Phencyclidine                              | 791 |
| Physiologic                                | 879 |
| Physiologic Eye                            | 879 |
| Phenotype Prediction                       | 545 |
| Phenyl Ethyl Alcohol                       | 1597 |
| Phenylethylamines                          | 1597 |
| Phenylpropanoid                            | 523 |
| PhIP                                      | 6, 93, 1110e |
| Phorbol Ester                              | 669a |
| Phorbol Esters                             | 669b |
| Phosgene                                   | 570, 581k, 1212, 2163b |
| Phosphatase                                | 1736 |
| Phospholipid                               | 1266, 1757 |
| Photo-Activated Cow Urine                  | 1943 |
| Photocaging                                | 2180 |
| Photodamage                               | 709 |
| Photodamage                                | 709 |
| Phototherapy                               | 2151a |
| Phototoxicity                              | 796, 761, 763, 1600 |
| Phototoxicity                              | 64, 761 |
| Photostability                             | 2281 |
| Physiologically Based Pharmacokinetic Model | 89, 96 |
| Physiologically Based Pharmokinetic Models  | 891 |
| Phytocannabinoids                          | 436, 2267 |
| Phytochemicals                             | 1075, 1087e |
| Phytoestrogens                             | 211, 1069, 1616e |
| Pig                                       | 6, 93, 1110e |
| Pig-a                                      | 452, 1943j |
| Pig-a Mutation                            | 445 |
| Piglet Model                              | 331 |
| Pigmentation                              | 219 |
| Pigmentary                                | 1173 |
| Piglotez                                  | 1178 |
| Pipeline                                   | 1767 |
| Piperoxyl Butyrate                         | 1173 |
| PIRC Rat                                   | 1109 |
| Pituitary                                  | 1071 |
| PK                                        | 961 |
| PKD1                                      | 936 |
| PKP Modeling                               | 581 |
| Plac1                                      | 1087n |
| Placenta                                   | 97, 974d, 994, 1497 |
| Placental Barrier                         | 1101e |
| Placental Exposure                        | 1101e |
| Placental Insufficiency                    | 1706 |
| Placental Transfer                         | 278 |
| Plagiozance                                | 725, 726, 727, 728, 729 |
| Plant Food Supplements                      | 1831e |
| Plant Toxins                               | 805 |
| Plasma                                     | 2151a |
| Plasma Microparticle                       | 901f |
| Plasma Microparticle                       | 1542 |
| Plasma Micronihoclastic Vestigation        | 1297c |
| Plastics                                   | 2234j, 2268 |
| Platelet Activation                        | 1127 |
| Platelets                                  | 1191, 1461, 1645 |
| Platinum                                  | 1266, 145a |
| Platycodon grandiflorum                   | 799, 800, 801, 802 |
| Pleural Fibrosis                           | 130 |
| Pleural Pleauchales                        | 1811 |

The numerals following each keyword refer to the relevant abstract number(s).
Abstract Keyword Index (Continued)

The numerals following each keyword refer to the relevant abstract number(s).

Reconstructed Human Corneal Epithelium Model .................................................. 1026
Reconstructed Human Epidermis Model .............................................................. 2168
Reconstructed Vaginal Tissue Model .................................................................. 173k
Recovery .................................................. 1566
Recovery Animals .......................................................... 2196aa
Recovery Guidelines ................................................................................. 244
Red Blood Cells ....................................................................................... 1768
Red Ginseng ......................................................................................... 1200e
Red Seabeam ......................................................................................... 653
Red Bloodology .................................................. 1102, 1195, 2019
Red Cycling ............................................................................................... 1083
Red Environment .................................................................................... 194
Red Potential .............................................................................................. 591
Redox Signaling .......................................................... 1452, 1778
Redox Status ............................................................................................. 129k
Reduction .......................................................... 1296, 1311, 1313
Reference Clustering .......................................................... 182k
Reference Concentration .................................................. 632j
Reference Dose .................................................. 777
Refusal Function ...................................................................................... 2012
Refinement .......................................................... 1226
Regional Divergence .......................................................... 1885
Regepahenf .......................................................... 1200a
Regulation ................................................................................................. 210
Regulation Groups .......................................................... 208k
Regulatory Acceptance .......................................................... 635
Regulatory and Safety Evaluation ................................................................. 1441, 1442, 1443, 1444, 1445, 1446
Regulatory Considerations ................................................................. 465
Regulatory Guidelines .......................................................... 456, 2250g
Regulatory Risk Assessment .............................................................. 631
Regulatory Safety Evaluation ................................................................. 2109, 2114
Regulatory Science .......................................................... 2110
Regulatory Testing ................................................................................. 2112
Regulatory Toxicity Testing ................................................................. 2306
Regulatory Toxicology .......................................................... 185, 639, 1415
Reintegrated Behavioral Sensitivity .......................................................... 369
Release ................................................................................................. 641
Remediation .......................................................... 1467d
Renal ...................................................................................................... 1543
Renal Biomarkers .......................................................... 1544
Renal Cell Carcinogenesis ................................................................. 253, 251
Renal Dysfunction .................................................................................. 2012
Renal Function ........................................................................................... 2049
Renal Proximal Tubule Cells ................................................................. 320
Renal Toxicity .......................................................... 649, 718, 1546
Renal Transport ....................................................................................... 2066
Renal Transporters .................................................................................. 2067
Renal Tumor .......................................................... 2196e, 2196g, 2196i
Renovirus ................................................................................................. 2037
Repair ................................................................................................. 1936
Repeal Dose .............................................................................................. 280
Repealed-Dose Toxicity ........................................................................ 164, 1625, 2192
Repealed-Dose Toxicity Study ................................................................. 164, 1601
Repealed-Dose Toxicology Studies ........................................................... 1992
Replication Stress ..................................................................................... 1293
Replication ............................................................................................... 26
Refill Fluid ................................................................................................. 1612
Repeat Dose ............................................................................................... 26
Referring Dose ............................................................................................ 777
Regulation of Safety Evaluation ................................................................. 1441
Renal Dysfunction ..................................................................................... 2045
Redox Biology .......................................................... 1102, 1795, 2039
Redox Status ............................................................................................. 1456
Redox Potential ........................................................................................... 591
Redox Cycling ............................................................................................. 1083
Red Blood Cells .......................................................................................... 1768
Reconstructed Vaginal Tissue ........................................................................... 467
Regulatory and Safety Evaluation ................................................................. 1441, 1442, 1443, 1444, 1445, 1446
Regulatory Risk Assessment ................................................................. 631
Regulatory Safety Evaluation ................................................................. 2109, 2114
Regulatory Science ................................................................................... 2110
Regulatory Testing .................................................................................... 2112
Regulatory Toxicity Testing ................................................................. 2306
Regulatory Toxicology .......................................................... 185, 639, 1415
Reintegrated Behavioral Sensitivity .......................................................... 369
Release ................................................................................................. 641
Remediation .......................................................... 1467d
Renal ...................................................................................................... 1543
Renal Biomarkers .......................................................... 1544
Renal Cell Carcinogenesis ................................................................. 253, 251
Renal Dysfunction .................................................................................. 2012
Renal Function ........................................................................................... 2049
Renal Proximal Tubule Cells ................................................................. 320
Renal Toxicity .......................................................... 649, 718, 1546
Renal Transport ....................................................................................... 2066
Renal Transporters .................................................................................. 2067
Renal Tumor .......................................................... 2196e, 2196g, 2196i
Renovirus ................................................................................................. 2037
Repair ................................................................................................. 1936
Repeal Dose .............................................................................................. 280
Repealed-Dose Toxicity ........................................................................ 164, 1605, 2192
Repealed-Dose Toxicity Study ................................................................. 164, 1601
Repealed-Dose Toxicology Studies ........................................................... 1992
Replication Stress ..................................................................................... 1293
Replication ............................................................................................... 26
Refill Fluid ................................................................................................. 1612
Repeat Dose ............................................................................................... 26
Referring Dose ............................................................................................ 777
Regulation of Safety Evaluation ................................................................. 1441
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