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SUMMARY REPORT
State-of-the-Science Workshop on Chemically-induced Mouse Lung Tumors: Applications to Human Health Assessments

Held on January 7-8, 2014

US EPA Auditorium, Research Triangle Park, NC

Final – December 2014

National Center for Environmental Assessment

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Research Triangle Park, North Carolina

DISCLAIMER

This document reflects the proceedings of the workshop, including presentations made by invited speakers, the discussions consequent to those presentations, and summaries of the individual Sessions. Any statements included in this document which were made by the presenters or by participants in the discussions or in the session summary discussions are those of the individuals and should not be interpreted as statement of the US Environmental Protection Agency.

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Background

The two-day, state-of-the-science workshop covered a broad range of evidence from human, animal, and in vitro studies with a focus on specific chemicals (ethylbenzene, naphthalene, and styrene) causing lung tumors in mice and implicated in a proposed species-specific mode of action (MOA) based on metabolic and physiological susceptibility. The workshop was sponsored and organized by EPA with input from (1) a volunteer committee of outside experts (including representatives from academic institutions, State agencies, other Federal organizations, non-governmental organizations [NGOs], and industry), and (2) an internal working group of experts from EPA and other Federal partners. The workshop included four separate sessions examining individual topic areas in detail, beginning with and continually referring back to the human relevance of data from animal and in vitro studies.

This document is a brief summary of the proceedings of the workshop. A parallel effort to draft a more detailed version of the proceedings is also in development for publication in a peer-reviewed scientific journal.

The full title for the workshop (developed by a committee) is the "State-of-the-Science Workshop on Chemically-induced Mouse Lung Tumors: Applications to Human Health Assessments". This verbose title was shortened to the "Mouse Lung Tumor Workshop" and further reduced to the acronym "MLTW" which is used throughout this document in referring to the workshop.

Welcome and Introductory Remarks

Introductory remarks were presented by Dr. John Vandenberg, Director for the Research Triangle Park Division of the US EPA's National Center for Environmental Assessment (NCEA). Dr. Vandenberg also serves as National Program Director for the Human Health Risk Assessment Program within the Office of Research and Development; these are the research programs under which IRIS and other risk assessment activities within NCEA are conducted.

Dr. Vandenberg made note that the Mouse Lung Tumor Workshop was under development for more than a year and is part of the EPA IRIS program's aggressive improvements. The workshop is an example of enhancing engagement with stakeholders for the purpose of evaluating scientific evidence and interpreting that evidence in chemical risk assessments. The workshop was organized to set the stage for engagement on key scientific issues and to ensure that all of the relevant stakeholders and scientific disciplines were involved in the discussions. Dr. Vandenberg also acknowledged the sustained efforts of the NCEA scientists who organized and planned this workshop, with assistance from outside experts.

Dr. George Woodall provided an overview of the workshop, including its goals and scope, a review of the development of the program, and acknowledgment of all of the groups which had contributed to the organizational efforts for the workshop. Dr. Woodall served as the Project Lead for planning and organizing the workshop, and as the Workshop Chair. He was aided by Dr. Channa Keshava as the Co-lead, Dr. Paul Reinhart, and Dr. Nagu Keshava, who together formed the Core Team in organizing and planning of the workshop. Experts from within EPA and the National Institute for Environmental Health Science (NIEHS) formed an Internal Planning Committee which assisted in many planning activities and development of preliminary materials for participants to read prior to the convening of the workshop. An additional group of experts (the Peer Input Committee) was convened to ensure that perspectives from the major stakeholder groups (academia, industry, public interest groups, other federal agencies, and state

agencies) were also represented in the planning phase of the workshop. The Peer Input Committee also contributed through reviews of the preliminary reading materials described more fully below.

Context for the Workshop

Several chemical agents cause bronchiolar-alveolar adenomas and carcinomas (lung tumors) in mice. Three such agents are currently being assessed in the Integrated Risk Information (IRIS) Program within EPA, namely: ethylbenzene; naphthalene; and styrene. Other chemicals have been associated with similar types of tumors (cumene, coumarin, fluensulfone, benzene, and others), which may provide additional insights into potentially common mechanisms for tumor formation among the chemicals.

Goals of the Workshop

- Identify the evidence, from multiple scientific disciplines, regarding formation of chemically-induced lung tumors in mice
- Discuss analysis and interpretation of the evidence within the context of the EPA Cancer Guidelines
- Discuss how such evidence informs human health assessments
- Identify commonalities, linkages, or differences among the evidence from various disciplines [and across the chemicals]

Scope of the MLTW

- Inform the development of IRIS assessments for chemicals where mouse lung tumors are an issue: ethylbenzene, naphthalene, and styrene.
- EPA will not seek consensus, recommendations, or guidance during the workshop.
  - Application of a MOA framework to reach conclusions is not part of the scope of this meeting.
  - Identifying Key Events and whether they are Necessary Elements for application in a MOA are within the scope.
- Follow-on meetings may occur after the workshop to continue discussions related to the goals of the workshop

Dr. Woodall noted the need to ask the right questions to meet the goals of the workshop. In particular, the question of whether or not the information being discussed will affect a chemical-specific human health risk assessment for the three key chemicals was of prime importance to meet those goals.

Organizational Structure for the MLTW

The discussion sessions were organized to start with the human population and individual level, eventually working down to the level of cellular and subcellular effects.

- Population/Individual Level - Evidence in humans at the population and individual level (Session 1)
  - Epidemiological evidence for the key chemicals
  - Pathology of human tumor formation
- Tissue Level – A pathology-focused review of mouse models to predict human tumor formation (Session 2)
  - Includes issues of species/tissue concordance
- Mechanistic Level - Review of the biological mechanisms and metabolism of the key/related chemicals to form toxic by-products (Session 3)

  - Key enzymatic processes
  - Areas of commonality, and divergence
- Cellular/Subcellular Level - Genotoxicity, cytotoxicity, emerging molecular technologies (Session 4)

Key Discussion Topics

Dr. Woodall highlighted some of the discussion topics covered in the Session Abstracts document which the Internal Working Group identified as important considerations for the MLTW. These key topics are provided in the list below.

- Pharmacokinetic & Pharmacodynamic Considerations
  - PK: Do mice have a higher rate of creating the toxic moiety (or less capacity to detoxify) and are therefore "farther up" the dose-response curve?
  - PD: Is there is something specific that makes the mouse lung different from or more sensitive than humans?
    - The underlying disease processes for tumor formation are complex
    - Chemicals may disrupt processes in multiple pathways; multiple combinations of disruption may result in disease
  - Are the differences between species along a continuum or are they discrete?
- Tissue & Cellular Specificity
  - Localization
    - What is the evidence that Club (Clara) cells in particular are transformed in mice? What about type II pneumocytes (another likely and metabolically active potential target cells)?
    - Do cells adjacent to Club cells also become transformed (i.e., is there evidence that very local metabolism drives this effect)?
    - What is the evidence in humans and other species?
  - Concordance
    - There is not always one to one correspondence across species for tumor type.
    - Are mouse lung tumors a potential indicator for human tumors in the lung? In other tissues?
    - Are these particular types of mouse lung tumors predictive of human tumor biology, either in the lung or in some other tissue?
- Mode of action (MOA): Definitions based on 2005 EPA Cancer Guidelines (U.S. EPA, 2005)
  - Mode of action - "a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation."
  - Key event - "an empirically observable precursor step that is itself a **necessary element** of the mode of action or is a biologically based marker for such an element." *[emphasis added]*
  - In relation to the MLTW, MOA provides a useful framework for discussion of the data regarding formation of tumors via a set of **key events**.
  - Slight Differences in Application between organizations:
    - US EPA Cancer Guidelines Approach: quantitative differences can be used to adjust dose-response

    - WHO/IPCS Approach: quantitative differences can be used to dismiss relevance
- Regardless of which approach is taken, the basic information needs are the same.
- Are MOA considerations chemical-specific, or do they apply across all the key chemicals?
    - Cruzan et al. (2013); Cruzan et al. (2012); Cruzan et al. (2009) propose that a common MOA involving the CYP-2F2-mediated metabolic pathway as a key event applies to many chemicals causing mouse lung tumors.
    - Can data from multiple chemicals with the same purported MOA be used to bolster data gaps for other chemicals?
    - What weight of evidence (WOE) factors are important when considering whether a MOA is relevant (or not) in humans?
    - What factors should be considered when weighing whether a similar MOA is active for more than one chemical?

Preliminary Materials

As a part of the work done by the Internal Planning Group, a Sessions Abstract document (http://epa.gov/iris/irisworkshops/mltw/MLTW-SessionAbstracts-Final.pdf) was developed to provide background information related to each of the four planned sessions along with the major topics anticipated to be covered. Key references were identified for each session along with additional supplementary references to allow for a more thorough review of the literature related to the session topics. A list of discussion topics was also included in this preliminary document to help focus the discussion within the defined scope and with the objective of meeting the workshop goals. The Peer Input Committee also contributed to the development of the Session Abstracts document through reviews and insightful suggestions.

In addition to the Session Abstracts document, a project page for the MLTW was established in the Health and Environmental Research Online (HERO) database. The purpose of this project page was to facilitate the availability of references identified in development of the Session Abstracts document and in other planning activities - http://hero.epa.gov/index.cfm?action=landing.main&project_id=2190.

Logistical Considerations

The MLTW was convened with participation in-person or via webinar. Those on the webinar were given the opportunity to view the proceedings on their computer as well as listen in either via their computer speakers or through a teleconference line. At times, there were as many as 120 on-line participants and approximately 80 participants (including the Co-chairs, panelists, speakers, support staff, and public) in the EPA-RTP auditorium. The large number of on-line participants resulted in the inability of the current webinar and teleconference facilities to accommodate allowing on-line participants to speak; they were instead requested to use the webinar chat window to relay any questions for consideration. A full list of registered participants is included in Appendix C of this Summary Report.

Post Workshop Activities

At the end of the Opening Session, Dr. Woodall noted that a summary report of the workshop proceedings would be developed. In addition, review articles were anticipated to be developed for publication in a peer-reviewed journal for the MLTW overall and for the individual Sessions. Post-

workshop meetings may be considered to continue discussions related to the goals of the workshop; the goal of the final Summary Session was to identify potential follow-on activities and discussion topics.

Dr. Woodall reiterated that the primary goal of the MLTW was to help inform the development of IRIS assessment documents for the three key chemicals: ethylbenzene, naphthalene, and styrene. Discussion of the other related chemicals (coumarin, cumene, fluensulfone, and others) should be included only in so far as they help to inform aspects of the assessments on the key chemicals.

Session 1: Human Cancer – Epidemiology and Pathophysiology

Background and Introduction

Session Co-chairs: Jason Fritz (US EPA) and
Eric Garshick (VA Boston Healthcare System/Harvard Medical School)

Lung cancer is one of the leading causes of new cancer cases, accounting for 14% of all new cancer diagnoses (NCI, 2013c). Cancers of the lung and bronchus are by far the most common cause of cancer deaths in the United States, accounting for almost 30% of annual cancer mortality: as much as that resulting from breast, prostate, pancreas, colon & rectum cancer combined (American Cancer Society, 2014; Siegel et al., 2013). Although smoking is an important risk factor for lung cancer, contributing to roughly 80% of all lung cancer deaths in both women and men (Siegel et al., 2013; American Cancer Society, 2012), other risk factors include occupational and environmental exposures, particularly to second-hand smoke, asbestos, radiation (including radon), some organic chemicals, diesel exhaust, ambient air pollution and many others (American Cancer Society, 2014; IARC, 2013; NCI, 2013a, b). Genetic susceptibility also contributes to lung cancer development (American Cancer Society, 2014), although lung cancer-specific genetic factors have yet to be identified.

More than 80% of human lung cancer cases are classified as non-small cell lung cancer (NSCLC) (American Cancer Society, 2014; NCI, 2013a, b). The most common subtype of NSCLC is adenocarcinoma (AC), and occurs regardless of smoking status, whereas the second most common subtype, squamous cell carcinoma (SCC), is more frequently detected in current or former smokers (Lee and Forey, 2013; Travis et al., 2011a). In addition to classification and staging of lung cancer, the molecular characterization of the cancer cells is crucial for guiding therapy. Current lung cancer terminology affects how tumors are classified; how this terminology is applied across epidemiologic and individual human studies will be discussed. After a brief introduction to the lung cancer nomenclature and classification scheme, we will consider aspects central to the epidemiological evaluation of cancers in populations with occupational or environmental (i.e. inadvertent) exposure (Theme 1). Following this, evidence informing the association between occupational exposure to styrene, ethylbenzene, or naphthalene and lung cancer will be presented, and lung carcinogenesis will be described from level of individual pathology down to cellular and molecular biology (Theme 2). Many of the topics introduced in this session will establish a common foundation for more topical and detailed discussions in the sessions that follow.

Theme 1: Epidemiological study design and assessment of carcinogenicity

1.1 Approaches to Determining Carcinogenic Risks in Humans

Eric Garshick (VA Boston Healthcare System/Harvard Medical School)

The International Agency for Research on Cancer (IARC) provides independent scientific opinions after reviewing epidemiological studies, cancer bioassays, exposure, and mechanistic data and assigns a cancer classification based on the complete body of evidence:

- Group 1: Carcinogenic to humans
- Group 2A: Probably carcinogenic to humans
- Group 2B: Possibly carcinogenic to humans

- Group 3: Not classifiable
- Group 4: Probably not carcinogenic to humans

Additionally, IARC makes determinations regarding the strength of the human evidence for the association of exposure with cancers of specific tissues or systems (Table 1-1).

Table 1-1. IARC Human Cancer Weight of Evidence Descriptors

Descriptor	Rationale
Sufficient	Positive relationship between exposure and cancer; chance, bias and confounding is ruled out with reasonable confidence in studies
Limited	Chance, bias or confounding could not be ruled out with reasonable confidence
Inadequate	Insufficient quality, consistency or statistical power to permit a conclusion
Lack of risk	Several adequate studies; bias and confounding can be ruled out with reasonable confidence

Styrene, ethylbenzene, and naphthalene were classified by IARC as Group 2B. The related compound cumene was also recently classified as Group 2B in 2013, while coumarin was unclassifiable (Group 3). The National Toxicology Program (NTP) classified styrene, ethylbenzene, and naphthalene as "reasonably anticipated to be human carcinogens" based primarily on animal and/or mechanistic evidence. Both IARC and NTP categorized the available epidemiological evidence for lymphatic or hematopoietic tumors associated with styrene exposure as "limited". Evidence specifically regarding the association between human exposure to styrene, ethylbenzene, or naphthalene and lung cancer was either not evaluated or described as "inadequate".

Several challenges exist for the assessment of occupational exposure and associations with lung cancer: lung cancer has long latency (generally 20+ years) and prospective occupational health studies are difficult to perform. Reasons for this include reliance on occupational exposure records and limited historical exposure estimates, and the possibility of numerous confounders. Study population selection can also be problematic since relatively few persons may be exposed occupationally which diminishes statistical power. Furthermore, industry cohorts may also exhibit a "healthy worker survivor effect". This underestimates the effects of exposure as the healthier workers are retained and therefore exposed for longer periods. The common practice of comparing disease rates to general population rates also underestimates the true disease risk since persons who are employed are healthier (called the healthy worker effect).

In the evaluation of an epidemiologic study it is important to determine whether an exposure assessment has been conducted, or whether there is an assumption that employment in an industry is equivalent to exposure. The linkage between job title and duties with exposure should be assessed. The availability of study information will dictate some aspects of the outcome assessment. Death certificate-based mortality records used in retrospective cohort studies detect the majority of lung cancer cases since long term survival is uncommon. Cancer registry and hospital-based studies can provide detail regarding histology. Tissue can also be recovered from archived samples originally obtained for histology, but the use of such samples to assess novel biomarkers is limited.

Epidemiologic studies should be evaluated for potential confounding. The definition of a confounder is a factor which is both associated with lung cancer risk and the exposure of interest. Potential confounders

include smoking, or other environmental exposures contributing to lung cancer. Although cigarette smoking is often raised as a confounder in assessing occupational lung cancer risks, it is not likely to be differentially related to exposure within a single occupational cohort. Several methods exist to deal with potential confounding, such as conducting a nested case-control study within a cohort, and interviewing participants in registry-based case-control studies to obtain a smoking history or a history of other exposures.

Diesel engine exhaust exposure was used as an example where analyses included consideration of potential confounding:

- In the assessment of diesel exhaust as a lung carcinogen, 11 pooled lung cancer case-control studies in Europe/Canada were analyzed to illustrate adjustment for smoking (Olsson et al., 2011).
- Lung cancer mortality was assessed in a retrospective cohort study of trucking industry workers (Garshick et al., 2012; Garshick et al., 2008)and an exposure assessment based on elemental carbon was conducted (Davis et al., 2011; Davis et al., 2009; Davis et al., 2007; Davis et al., 2006) to illustrate an approach to exposure assessment.
- A healthy worker survivor effect was observed in the trucking industry cohort study since lung cancer risk decreased with total employment duration (Garshick et al., 2012).

Discussion: The resulting discussion included several questions regarding the healthy worker survivor effect, namely: frequency, likelihood and possible impact on an endpoint with high mortality such as lung cancer. It was noted that this effect could be observed, even for endpoints with short survival periods, since workers could be leaving the work force from health related factors unrelated to lung cancer. One way to evaluate it would entail determining if there was any specific health-related cause for workforce attrition (i.e. occupational asthma) which could force more susceptible individuals to leave the cohort and accumulate less exposure. While no common method may exist to quantify the healthy worker survivor effect, there are advanced statistical methods available. Others inquired about untangling possible synergistic interactions with smoking, which would require individual level smoking information. Participants noted that analyzing biomarkers for exposure to various agents including cigarette smoke could be possible in banked blood or tissue samples, and that this approach would not only permit further molecular characterization of polymorphisms present in the cohort, but could be employed prospectively as well. This question of choosing an appropriate marker or biomarker of exposure was raised in the context of exposure to styrene mixtures, specifically regarding the potential for alterations in enzyme activity, and participants re-emphasized using the relationship between exposure duration and disease pathogenesis to guide selection of appropriate exposure marker(s).

Human epidemiologic studies should assess the link between exposure and job title and duties, potential biases in a study population and reference group selection, and consideration of possible sources of confounding. Mechanistic information may contribute to the assessment of human carcinogenicity potential in the absence of adequate human epidemiologic data, and this approach has been used by IARC to upgrade some agents to Group 1 carcinogens (e.g. ethylene oxide).

1.2 Epidemiological Studies of Human Lung Cancer

Dan Krewski (University of Ottawa)

There is power in combining multiple studies and populations to see exposure-response relationships for very low exposures or cancers with low incidence. Past studies describing relationships between radon or $PM_{2.5}$ exposure and lung cancer risk serve as examples. An example examining radon studies demonstrated that combining high quality studies, defined by including those with the most accurate exposure assessment, provided more precise effect estimates (Turner et al., 2011; Krewski et al., 2005; Letourneau et al., 1994).

The relative contributions of various postulated environmental agents to human lung carcinogenesis were estimated, and presented as Table 1-2.

Table 1-2. The Environmental Contribution of Various Agents to Human Lung Cancer

Agent	Attributable Fraction	Reference(s)
Tobacco smoking	70-90%	ALS (2013); Parkin (2011); WHO (2013)
Residential radon	3–14%	Menzler et al. (2008); Brand et al. (2005); (WHO, 2013)
Particulate air pollution	5-12%	Evans et al. (2013); Vineis et al. (2007); (WHO, 2013)
Diesel emissions	6%	Vermeulen et al. (2014)
Other occupational exposures	3-15%	ALS (2013); Parkin (2011)
Environmental tobacco smoke	3%	ALS (2013)
Radiation	<1%	Parkin (2011)
Solvents	<<1%?	Vizcaya et al. (2013)

Since these are estimates, the individual contributions add up to greater than one. Above and beyond individual agent contributions to lung cancer risk, there is s a strong synergistic (greater than additive) effect between tobacco smoke and radon co-exposure on lung cancer risk.

Generally, in human occupational epidemiological studies, which are frequently retrospective, tissues for histology, molecular studies and biomarker evaluation are not available, limiting our understanding of lung cancer mechanisms. However, detailed histological categorization of lung cancer is available in some large studies, such as several from Canada obtained from a cancer registry and from hospital-based case series. In a large case-control study, some occupations were observed to have an increased risk for all histological subtypes of lung cancer (metal processing workers, bakers, ship deck crew), while other occupations experienced an increased risk for specific histological lung cancer subtypes (construction workers, chefs and cooks, medical workers). Such studies are hypothesis generating since the specific exposures are not known.

Discussion: The discussion initially focused on the evaluation of lung carcinogenesis in smoking or other high risk populations. The examination of numerous other factors (i.e. socioeconomic status) as covariates is highly desirable in the assessment of causality, since socioeconomic status is a surrogate for smoking habits. Regarding the contribution of tobacco smoking to human lung cancer, it was noted that there are thousands of chemicals in tobacco smoke, including some very potent known human or animal

carcinogens. Both genetic susceptibility and gene-environment interactions can further contribute to cancer, although no genetic factors/polymorphisms have been confidently identified as lung cancer-specific risk factors. One participant inquired about the apparent lack of dose-response in the radon data described (see slide presentation by Dan Krewski), and it was noted that the restricted data set relies only upon measured and not imputed data, and that any visual analysis of apparent relationships must take into account the significant uncertainty present within each set of measurements. Following up on a question raised in the previous discussion, it was noted that information on potentially susceptible subpopulations could be gleaned from individuals evaluated in drug safety and efficacy studies. A participant noted the considerable difficulty involved in interpreting negative epidemiological studies, and concluding that a given exposure does not cause an effect. The radon lung cancer studies were used to illustrate that although one large study provided negative results, combining results from multiple studies resulted in a positive association. It was acknowledged that it is difficult to conclusively demonstrate the lack of a health risk, and in fact, there is only one compound listed under Category 4 in IARC as probably not carcinogenic.

Theme 2: Available human and molecular data relevant to lung carcinogenesis following styrene, ethylbenzene, or naphthalene exposure

1.3 Lung Cancer Mortality: Workers Exposed to Styrene, Ethylbenzene, or Naphthalene

Jim Collins (Dow Chemical Company)

For the three chemicals of interest, there is human epidemiological data primarily for styrene from occupational exposure studies in three industries:

- Styrene-butadiene rubber
- Styrene-reinforced plastics
- Styrene monomer/polymer production

Of these cohorts, IARC judged the studies of glass fiber-reinforced plastics workers to be the most informative as these workers had higher styrene exposures and less potential for exposure to other substances than the other cohorts studied. IARC was primarily concerned with cancers of lymphatic and hematopoietic tissues given the available epidemiologic studies, and did not specifically evaluate lung cancer.

In the most reliable studies of styrene-exposed workers, the association between exposure and lung cancer was not strong or consistent: comparison of relative risk estimates show essentially no increased risk for overall styrene exposure.

- The Collins et al. (2013) study showed statistically significant increased risk but further analysis revealed a negative exposure-response relationship for lung cancer risk.
    - Exposure-response was flattened if adjusted for smoking (by considering bronchitis, asthma, and emphysema); no relationship was observed with increased latency.

- A similar response with exposure was observed in other smoking-associated cancer (bladder, kidney) & non-cancerous lesions (non-malignant respiratory disease, heart disease)

In a separate study, Ruder et al. (2004) showed workers ever exposed had higher levels of cancer compared to workers never exposed; but the lung cancer excess was observed in shorter term workers. In a third cohort, Kogevinas et al. (1994) showed no increase in lung cancer risk with increasing styrene exposure when estimate of exposure was based on longest job held job.

Studies assessing immunological effects potentially associated with styrene exposure did not control for smoking, and had a small sample size; IARC concluded that immune systems of workers were not affected by styrene exposure. The workers in the monomer and polymer studies of styrene-exposed workers described above also had ethyl benzene co-exposure: there are few studies describing ethyl benzene exposure, none with any quantitative estimates of exposure, and no evidence of increased lung cancer risk was reported. There are no studies useful in the causal assessment of lung cancer risk following naphthalene exposure.

Discussion: Regarding the average exposure duration for the styrene workers in the Collins et al. (2013) study, it was noted that although the average exposure was only 4 years, lung cancer was associated with short-term workers: 30-40% of the cohort was employed for <1 year. Other studies also had a few workers with long term exposure. One participant noted that observers should be cautioned not to discount the styrene-lung cancer association inverse dose-response relationship without a mechanistic explanation or investigation of cancer susceptibility. Ethylene oxide was suggested as an example where short term exposure induces *RAS* mutations (roughly 12% of all human lung cancers originate from *KRAS* mutations), whereas long term exposure appears to reduce mutation incidence. In response to questions regarding importance of exposure data quality, and in light that all data presented in the review of the styrene human epidemiological studies were mainly based on SMR analyses, it was noted that studies had internal comparisons with similar results as the SMR. Following questions on exposure assessment methodology and adjustment for smoking, it was noted that exposure assessment entailed visiting each of the plants, measuring and reconstructing styrene exposure. Painstaking attempts were made to reconstruct cumulative exposure-response for every worker over their entire career. Smoking adjustment in the Collins et al. (2013) study was done by adjusting the lung cancer risk by category (i.e., death from bronchitis, emphysema and asthma).

Further discussion revolved around the healthy worker survivor effect. It was proposed that the longer the workers stay in employment, the healthier they may be due to supplemental health programs, e.g. smoking cessation, fitness, etc. However, the panelist noted that while healthier people might work longer, they would also have higher cumulative exposure, inverting an apparent exposure-response relationship.

1.4 Human Lung Cancer Pathology and Cellular Biology

Brigitte Gomperts (University of California, Los Angeles)

Lung structure and function: Mammalian lungs are a very complex structure as they are composed of more than 42 different cell types. The main structural compartments of the lung include the cartilaginous tracheobronchial airways and associated submucosal glands, respiratory and terminal bronchioles, and

alveoli. It is critically important to consider all compartments, each composed of different epithelial, endothelial and mesenchymal cell constituents, and it is inadvisable to think about one cell type as acting independently of others. The large cartilaginous airways are lined by a pseudo-stratified columnar epithelium, and the basal cells of this epithelium are stem cells, capable of self-renewal and differentiation to the cell types of the airway epithelium, namely ciliated cells, Club (Clara) cells and mucus cells. Submucosal glands are also present throughout the cartilaginous airways in humans. The bronchioles (small airways) are lined with Club and ciliated epithelial cells, and the alveoli are composed of type I and II pneumocytes (alveolar epithelial cells). At the bronchoalveolar duct junctions are the bronchoalveolar stem cells (BASC) that express both Club [Clara] Cell Secretory Protein (CCSP; Club cell marker) and Surfactant Protein C (SPC; type II pneumocyte marker). A few differences in human vs. mouse lung anatomy were highlighted in Table 1-3.

Table 1-3. Key Differences between Mouse and Human Lung Anatomy, Pathology

Physiological aspect	Human	Mouse
Submucosal glands, location	Throughout cartilaginous airways	Apical 3rd portion of trachea
Goblet cells, location	Throughout large airways	Rarely found
Stem cell turnover	More rapid in response to environmental injury	Slow
Likely precursor to adenocarcinoma (AC)	Atypical adenomatous hyperplasia (AAH)	Adenoma (AD) and AAH

Lung cancer histopathology: In human lung cancer, approximately 20% is of the Small Cell variety (SCLC), while the remaining 80% are Non-Small Cell Lung Cancers (NSCLC) which can be further categorized as adenocarcinoma (40%) and squamous cell carcinoma (25%), with other more rare histological subtypes (adenosquamous or "mixed" carcinoma, carcinoid) each occurring in <5% of cases. The updated 2011 IASLC/ATS/ERS classification of adenocarcinomas now includes histology of pre-neoplastic (AAH) lesions; a refined classification for small biopsies and cytology specimens; and stresses the importance of differentiating between adenocarcinomas and squamous cell carcinomas due to differences in therapeutic strategies (Austin et al., 2013; Travis et al., 2013a; Travis et al., 2013b; Travis et al., 2013c).

Stepwise progression of lung cancer: In the cartilaginous airways, premalignant airway lesions, such as squamous metaplasia and dysplasia, develop after injury of the airway but we think that most actually spontaneously resolve. However, some lesions persist and progress stepwise to squamous cell carcinomas. It is not known why some lesions persist and how they can progress to invasive squamous lung cancer. Stepwise progression can also be observed in adenocarcinoma from atypical adenomatous hyperplasia, and some data is available to predict if tumors will form.

"Field cancerization" in epithelial tumors is the term used to describe the epithelium around the tumor that appears histologically normal but has genetic and epigenetic changes, some of which are also found in the tumor. The hypothesis is that the "field cancerization" develops in the airway epithelium after injury, and during the repair process, the epithelium develops genetic and/or epigenetic changes that expand and displace the normal epithelium (Gomperts et al., 2013; Kadara et al., 2013; Gomperts et al., 2011). Continued injury and repair, along with continued proliferation, leads to pre-malignant lesions and potentially neoplasia. Gene expression and miRNA signatures from bronchial brushings in the "cancer

field" can predict whether an indeterminate nodule is cancerous or not (Bogen et al., 2008). Thus future monitoring of temporal-spatial changes in the airway epithelium might predict cancer, and may hold predictive utility as biomarkers of these cancer field effects.

Cell of origin of lung adenocarcinoma: There is some controversy as to the cell of origin for lung cancer. Carla Kim originally published in 2005 that the BASC cell is the cell of origin for lung adenocarcinomas in the mouse oncogenic KRAS model (employing intratracheal adenoviral cre-recombinase delivery) (Kim et al., 2005b). However, Mark Onaiti's group showed, with cell-specific inducible expression of cre-recombinase, that type II alveolar cells are the cell of origin of lung adenocarcinomas in the oncogenic KRAS mouse model, and that BASC cells undergo some hyperplasia but do not form cancers (Xu et al., 2012a). Interestingly, the mouse adenocarcinomas from the Onaitis group showed a good correlation in gene expression with human lung adenocarcinomas expressing mutant *K-Ras*. CD133-expressing cells may represent a cell of origin for human lung adenocarcinomas, but this area still remains controversial (Eramo et al., 2008).

Inflammation in lung cancer: Inflammation can be both pro- and anti- tumorigenic in the lung. Programmed cell death-1/ programmed cell death ligand-1 (PD1/PDL1) are emerging as new therapeutic targets used to direct the immune system against tumor cells (Creelan, 2014). While radical oxygen/nitrogen species (ROS/RNS) can be employed by inflammatory leukocytes to trigger the destruction of neoplastic cells, ROS/RNS at low levels serve as a cell signaling mediators, and at moderate levels could trigger transient proliferation in a variety of epithelial and progenitor cells. Excessive ROS can drive hyperproliferation of mouse epithelium, especially in cells where the homeostatic regulation is dysfunctional (e.g. tumor cells). Inflammatory cytokines released by both myeloid and lymphoid effector cells can trigger oncogenes and inactivate tumor suppression genes within nascent tumor cells, both of which are important in driving pre-malignant lesion progression.

Oncogenes and tumor suppressors: Current molecular mechanisms of human lung carcinogenesis appear to largely involve oncogenes associated with the Ras pathway, either by mutations in KRAS itself, or by upstream mutations in EGFR/HER2 and EML4-ALK fusions, or downstream in MEK. In addition to oncogene activation, the tumor suppressors P53, PTEN and LKB-1 are typically inactivated during progression to malignancy (Cooper et al., 2013).

Discussion: During the discussion, a similarity in response was proposed between humans occupationally exposed to styrene, and mice experimentally exposed to tobacco smoke; namely, the inverse relationship between exposure duration and association with lung cancer reported in both situations. Another participant noted that oxidative stress, one effect of pulmonary exposure to tobacco smoke, is also associated with styrene/ethylbenzene metabolism. It was proposed that paraquat (PQ), a widely studied herbicide, might serve as a useful model for understanding the role of oxidative stress and cytotoxicity in lung cancer, since PQ exposure causes oxidative stress and lung toxicity, but not lung cancer. This observation suggests that continuous exposure to ROS-generating chemicals, such as those in tobacco smoke, may kill off nascent tumor cells. The field cancer model suggests that cycles of continuous injury and repair may lead to tumorigenesis; however, these feedback mechanisms may stop if exposure is reduced, or when the source of injury is removed. In cases where ROS and lung toxicity appear insufficient to cause lung tumorigenesis, as is the case for PQ, another genetic "hit" past ROS generation may need to be considered.

It was noted that different chemicals likely affect different cell types in the airway, and that different signaling pathways may also be subsequently affected in a chemical-specific manner. One caveat to these species comparisons may be the inherent differences in stem cell turnover rate, and perhaps the stimuli required to initiate stem cell division/differentiation, as human pulmonary cells proliferate much more frequently than mouse cells. Specifically regarding BASC cells in a lung injury setting, BASC cells are known to proliferate for repair and differentiate into Club cells, but it is controversial as to whether BASC cells in injured airways can also give rise to type II pneumocytes. Some injury models in mice show both cell types arising from BASCs and some not. Carla Kim used Sca1 as a marker in mouse studies (Kim et al., 2005b), but this is a murine protein without any described human homolog, so this method cannot be employed to sort BASC cells from human tissues. Alveolar type II cells seem to be the more likely cell of origin for the tumors in the oncogenic *KRAS* mouse models as shown in the elegant work from Mark Onaitis' group (Xu et al., 2012a).

On an individual patient level, clinical assessment of human lung cancer has changed from assessing histology alone to molecular profiling. This is particularly important in lung adenocarcinomas where molecular alterations are dictating targeted therapies. Human adenomas are not thought to progress to neoplastic lesions, unlike the clear progression from adenoma to adenocarcinoma in mice. Instead AAH lesions are the important premalignant lesions in humans. However, identifying similar molecular profiles (field gene expression, spectrum of mutations, epigenetic changes) in animal bioassays may inform mouse-human tumor type concordance. In addition, identifying specific cells that are targeted by chemicals and examining the effects that the chemicals have on these cells will be helpful in identifying the cell of origin of lung cancer in animals and humans, and may provide mechanistic insights into lung carcinogenesis.

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Session 2: Comparative Pathological Evidence

Background

Session Co-chairs: Charles E. Wood (US EPA) and
Mark Steven Miller (Wake Forest School of Medicine)

Lung tumors in mice share numerous morphological and molecular characteristics with human lung cancer. However, species differences also exist which may influence the human relevance assessment of mouse lung tumors. While lung tumors can arise spontaneously in mice, as in humans, mouse lung tumorigenesis can also be experimentally induced by chemical exposure, radiation, or direct genetic manipulation through molecular biology and selective breeding. For chemical exposures, lung is the second most frequent tumor site reported in pathology databases of the EPA and National Toxicology Program. In particular, mouse bronchiolar-alveolar tumors are proposed for some chemicals to originate in type II pneumocytes or club (Clara) cells via pathways that might be species-specific. While rodent lung tumors are reported primarily in the mouse, they have also been observed in treatment-related response in the rat and other species. Genetically engineered mouse models (GEMMs) of lung cancer have also been developed which demonstrate a dramatic incidence and rapid progression of lung tumors in mice bred to contain specifically-mutated genes. These mice typically developed aggressive lung tumors within weeks to months, versus the months to years generally reported following exposure for chemically-induced mouse lung tumors. Molecular pathology analyses have revealed shared biological targets and pathways between mouse and human lung tumors; however, the human health relevance of lung tumors in mouse studies remains unclear. In this session we will review the comparative biology of mouse lung tumors, associated pathologic effects of known mouse lung tumorigens, and issues related to tissue and species concordance.

2.1 Introduction

Charles E. Wood (US EPA)

Mouse lung tumors are an important issue for risk assessment at EPA and other health and regulatory agencies. These tumors are commonly reported in guideline mouse carcinogenicity bioassays and often cited in risk assessments and cancer classifications, yet their relevance to human health remains unclear. In the US EPA Toxicity Reference Database, lung is the second most common site for treatment-related tumor outcomes in the mouse, representing ~7-8% of compounds tested. In a review of over 400 compounds evaluated by the EPA Cancer Assessment Review Committee, 27 had lung tumor outcomes cited in risk assessment classification but only one had an accepted lung tumor mode of action (MOA) (related to mitogenicity). So currently there is little precedent for regulatory acceptance of non-genotoxic MOAs for mouse lung tumors, which is an important reason for this workshop.

The comparative pathology of mouse lung tumors is a central consideration for both MOA and human relevance evaluation. Mouse lung tumors, at least in traditional bioassay strains, often have a distinctive location in the terminal bronchiolar region of the lung and a distinctive cuboidal cellular morphology characteristic of either type II pneumocytes or club cells. These tumors are generally classified as bronchiolar-alveolar or A/B tumors, which share features with certain types of human lung tumors but not

others. An important goal of this session is to explore some of these pathologic differences and similarities between mouse and human lung cancers.

A hallmark of mouse lung tumors is the marked variation in incidence for both spontaneous and chemically-induced tumors across different strains of mice, indicating the importance of model selection. As an example, older data from bioassays investigating urethane effects illustrate the marked differences in response that are possible across different mouse strains for similar exposures. In this session we will talk further about this variation and present general considerations for GEMMs of lung tumorigenesis and the role of genetic background as a determinant of tumor responses and interpretation.

The mode of action framework is a cornerstone of human relevance assessments at EPA. This construct was described in the 2005 EPA Cancer Assessment Guidelines (U.S. EPA, 2005) and subsequently by the WHO International Programme on Chemical Safety (IPCS), which defined MOA as a series of key events and processes leading to cancer or other adverse health outcomes (Boobis et al., 2006). An important distinction for our discussions is to separate MOA (i.e. key events driving mouse lung tumors) and human relevance questions (i.e. comparative tumor features and whether key events in the mouse are observed in human, rat, and other species).

The primary proposed MOA to be discussed in this session starts with metabolism of parent compounds to a cytotoxic intermediate by CYP2F2 in club cells in the mouse lung, followed by local regenerative proliferation in the terminal bronchiolar epithelial cells, and, over time, hyperplasia, adenomas, and carcinoma. To better understand this MOA, our goals here are to discuss strain and model considerations for evaluating mouse lung tumors and whether lung cytotoxicity, proliferation, and hyperplasia are consistent findings across the different compounds of interest. On the human relevance side, we will discuss the morphologic features of mouse lung tumors compared to other species, cell of origin considerations for mouse lung tumors, and tissue and species concordance issues for mouse lung tumors.

2.2 Comparative pathology of mouse lung tumors

Gary A. Boorman (Covance Inc.)

Lung tumors in mice share numerous morphological and molecular characteristics with human lung cancer. However, species differences also exist which may influence the relevance of mouse lung tumors in risk assessment. While lung tumors arise spontaneously in mice, as in humans, mouse lung tumorigenesis can also be experimentally induced by chemical exposure, radiation, or direct genetic manipulation through molecular biology and selective breeding. For chemical exposures, lung is the second most frequent tumor site reported in studies conducted by the EPA and National Toxicology Program.

The mouse lung has a single left lobe and four right lobes as compared to the human lung with three right and two left lobes. Microscopically the human lung has multiple generations of well-defined respiratory bronchioles, while in the mouse lung, the terminal bronchiole generally transitions into an alveolar duct without an intervening respiratory bronchiole. In the terminal bronchiole in the mouse 60 – 80% of the lining epithelium is comprised of club (Clara) cells. Club cells are not found in the distal airways in humans but are located in the proximal airways.

Proliferative lesions in the mouse lung often appear to originate in the distal airways at the junction of the terminal bronchiole and alveolar duct. The proliferation of airway lining epithelium extends in the

centriacinar region filling adjacent alveoli. A small focal lesion where the underlying alveolar structure is intact is diagnosed as bronchiolar-alveolar hyperplasia. When these proliferative changes (hyperplasias) become solid aggregates of cells, underlying alveolar architecture is lost and there is compression of the adjacent parenchyma, the lesions are diagnosed as bronchiolar-alveolar adenomas. Adenomas may appear as a solid pattern of uniform round cells with abundant cytoplasm similar to the Type II cells lining the airway and grow in a more tubular or linear pattern or appear as combination of the two cellular patterns. Special histochemical stains and ultrastructural examination have revealed characteristics of Type II and/or club cells in the tumors. As neoplastic cells become more pleomorphic, extend into vessels and/or metastasize to the lung and/or distal organs, these lesions are diagnosed as bronchiolar-alveolar carcinomas. In nearly all mouse strains, bronchiolar-alveolar adenomas are more common than bronchiolar-alveolar carcinomas and both tumors tend to be more common in males that females. The incidence of these tumors are quite variable; as an example, bronchiolar-alveolar adenomas in controls can vary from 8 – 36% in male B6C3F1 mice and 8 – 38% in male CD1 mice.

Both spontaneous and induced mouse bronchiolar-alveolar tumors appear to originate in Type II pneumocytes or club (Clara) cells via pathways that might be species-specific. While rodent lung tumors are reported primarily in the mouse, they have also been observed as a treatment-related response in the rat and other species. The cell of origin/location for mice may differ from human lung tumors, which are often more centrally located near the pulmonary hilus and are often squamous cell carcinomas. However, with changes in smoking habits and composition of cigarettes more peripheral adenocarcinomas are being reported.

The differences with human pulmonary neoplasia and the variable rates for both adenomas and carcinomas in mice make the use of pulmonary tumors as a screening tool for safety assessment problematic. However, genetically engineered mouse models (GEMMs) of lung cancer have also been developed which may be useful to test specific hypotheses. For example, mice bred to contain specifically-mutated genes have been shown to develop aggressive lung tumors within weeks to months, versus the months to years generally reported following exposure for chemically-induced mouse lung tumors. Molecular pathology analyses have revealed shared biological targets and pathways between mouse and human lung tumors; however, the human health relevance of lung tumors in standard mouse screening studies remains unclear.

Other discussion points included the high variability in lung tumor incidence across mouse strains and often across studies. It was noted that genetic factors have been identified but that this variation is often difficult to explain. There is high association of mouse lung adenoma with carcinoma (with likely progression) but this is not typically seen with human lung carcinomas. It was not clear, however, whether data were available to support this idea specifically for human lung adenocarcinomas. Participants also noted the higher numbers of club cells in the terminal bronchioles in the mouse lung compared to the human lung but that club cells are still present in the human lung. Lastly, participants noted that there is a higher rate of metastasis for human compared to mouse lung tumors.

2.3 Mouse Lung Tumor Model Considerations

Mark Steven Miller (Wake Forest School of Medicine)

The majority of mouse lung tumor models produce adenocarcinomas, a histological subtype of non-small cell lung cancer (Shaw et al., 2005; Malkinson, 1998; Dragani et al., 1995). While this subtype is the most

prevalent in the human population (constitutes approximately 40% of all lung cancer patients), the applicability of the findings in mouse models may only apply to this subgroup of lung cancer patients. It is also important to keep in mind that not all mice are created equally – mice exhibit strain-specific differences in their susceptibility to specific cancers (Dragani, 2003; Bauer et al., 2001; Malkinson, 1989). Lung tumor susceptibility varies from the highly resistant C57BL/6 mouse, which exhibits a tumor multiplicity of <1 tumor/mouse, to the highly susceptible A/J mice, which exhibits a tumor multiplicity of >25 tumors/mouse. There is a wide range of susceptibilities reported for the intermediate strains. Many of the strains used for the construction of transgenic and knockout mice - such as the FVB/N, 129, and O20 strains – and the B6C3F1/N hybrid used by the NTP, exhibit intermediate susceptibility.

Murine susceptibility to lung cancer is due to differences at a number of genetic loci. The *Pulmonary adenoma susceptibility 1* (*PAS1*) locus on chromosome 6 appears to account for 75% of inherited susceptibility (Manenti and Dragani, 2005; Ryan et al., 1987; Malkinson et al., 1985). While this gene locus has been associated with a polymorphism in the Ki-*ras* locus, work by Dragani's group has identified 6 genes in the *PAS1* locus, suggesting susceptibility may be mediated by multiple genes (Manenti et al., 2004). The *Pulmonary adenoma resistance 2 (Par2)* locus on chromosome 18 may code for *Poh*, an error prone DNA polymerase. The *Pas1* and *Par2* loci play dominant roles in determining tumor incidence and multiplicity. In addition, the *Susceptibility to Lung Cancer* (*Sluc*) (Tripodis et al., 2001; Fijneman et al., 1998), *Pulmonary Adenoma Progression* (*Papg*) (Zhang et al., 2002; Herzog et al., 1999; Herzog and You, 1997; Herzog et al., 1995), and *Pulmonary Adenoma Histiogenesis Type* (*Paht*) (Malkinson, 1999) loci play roles in modifying susceptibility, progression, and the histology of lung tumors. Several studies have suggested that susceptibility loci in mice can be mapped to the equivalent susceptibility loci for lung cancer in humans (Li et al., 2003; Yanagitani et al., 2002; Dragani et al., 2000; Abujiang et al., 1998; Manenti et al., 1997).

There are a large number of Genetically Engineered Mouse Models (GEMMs) that contain activated oncogenes or knockouts of tumor suppressor genes. In using these models, one needs to consider a number of factors that can alter ones interpretation of the experiments - transgenic lines that have similar constructs can produce different results. One should consider:

- If the transgene is derived from mice, humans, or another species?
- What is the copy number and is the transgene expressed at physiological or supra-physiological levels?
- If the transgene is a mutant form of the gene, what is the mutation and how does that influence gene function?
- If a knockout model, is the gene truly knocked out or is it expressed as a truncated protein that may have unexpected effects?
- Is the transgene constitutively or conditionally expressed?
- Is the gene expressed from its natural promoter or an exogenous promoter?
- What is the gene's location in the genome?
- Is the gene expressed ubiquitously or in an organ-specific manner?
- Is the gene expressed from an inducible promoter that will allow for temporal and/or concentration dependent expression?

Transgenic mice often develop tumors with decreased latency and increased multiplicity, providing greater statistical power with fewer mice. Many chemicals may be weak carcinogens or can work through

non-genotoxic or epigenetic mechanisms that influence later events in the carcinogenic process, such as tumor progression. These chemicals may not yield positive responses in standard rodent bioassays, which are specifically designed to identify genotoxic chemicals. However, these chemicals may synergize with genetic alterations to enhance cancer formation. The use of GEMMs and tumor promotion protocols can thus be powerful tools in assessing the potential carcinogenicity of chemicals and determining their mode of action.

The key take home message is that *the genetic background of the strain you are using can influence the outcome/interpretation of your results*. Thus, in experimental design, it is important to keep in mind the target organ and question that one is asking.

Key questions raised in the discussion included the following:

- One must take into account potential strain-specific differences in sensitivity to lung tumor formation.
  - What are the key factors?
    - Differences in CYP induction/metabolism, such as Cyp2F2?
    - Differences in DNA repair?
    - Other genetic mechanisms of action?
- Chemicals can cause lung cancer via epigenetic MOAs.
  - Consider promotion as well as initiation in assessing lung cancer induction.
- Should we be using multiple strains to make final assessments of the potential for lung carcinogenicity of unknown test chemicals?
- How can we incorporate GEMMS into a regulatory framework?

2.4 Rodent Lung Tumors in National Toxicology Program Studies

Arun Pandiri (Experimental Pathology Laboratories, Inc.)

Disclaimer: The data interpretation and opinions expressed in this summary are those of the author Dr. Arun Pandiri and do not necessarily reflect the position of the National Toxicology Program, NIEHS.

Lung tumors are the second most common target site of neoplasia in the National Toxicology Program (NTP) bioassays (Dixon et al., 2008). The common non-neoplastic pulmonary lesions are hyperplasia and inflammation, and the most common neoplastic lesions are alveolar/bronchiolar adenomas/carcinomas. The incidences of spontaneous lung tumors (in vehicle controls) are higher in the B6C3F1 mouse (n=950/sex; 28% male, 9.5% female) than in the F344 rat (n=700/sex; 3.6% male, 1.4% female) (NTP, 2013b, c).

For this workshop, NTP studies with a significant lung tumor response in B6C3F1 mice and/or F344/N rats were evaluated. There were 67/580 NTP bioassays where there was a chemically-induced lung tumor response in either species when the same chemical was tested in both species. The incidence of chemically-induced lung tumor responses (n=67) with clear or some evidence of carcinogenicity was higher in the B6C3F1 mouse (male 51%, female 60%) compared to the F344 rat (male 21%, female, 21%). However, when the evidence of carcinogenicity in any organ from these 67/580 studies were considered, both species had a comparable evidence of carcinogenicity (mouse: male 63%, female 76% and rat: male 69%, female 70%). A positive lung tumor response was seen in both mice and rats in only 21% (14/67) of the NTP chronic bioassays.

In the NTP chronic bioassay, chemicals that were structurally related to styrene and naphthalene and which showed evidence of pulmonary carcinogenicity were reviewed (Table 2-1). The selected NTP bioassays (styrene, naphthalene, coumarin, ethylbenzene, cumene, divinylbenzene, and benzofuran) resulted in a significant lung tumor response only in mice (in parenthesis - Table 2-1) but not in rats. With the exception of styrene, naphthalene and ethylbenzene, other tumors at multiple sites were noted in the same species for the other chemicals that caused lung tumors.

Preliminary immunohistochemistry data was generated to evaluate the pulmonary target cell (type II cells (SPC) or Club (Clara) cells (CC10) in B6C3F1 mice exposed to styrene, ethylbenzene and cumene for 13 weeks (n=20/study, control and high dose). In addition, lung tumors (n=20/study) resulting from 2-year exposures to ethylbenzene and cumene were evaluated. In the 13-week studies, Club cell loss was noted in bronchioles with styrene but not with ethylbenzene or cumene exposures. In addition, no pulmonary histological lesions were observed in the 13-week ethylbenzene and cumene studies (NTP, 2009; Chan, 1992). The lack of pulmonary histological lesions in the 13-week ethylbenzene study was surprising since Stott et al., Stott et al. (2003) demonstrated elevated S-phase synthesis rates (BrdU stain) in terminal bronchiolar epithelium in both male and female B6C3F1 mice exposed to 750 ppm for 4 weeks. Further investigations are needed to clarify this possible discrepancy. In the 2-year studies, neoplastic cells predominantly expressed SPC (type II cell phenotype) while the expression of CC10 (Club cell phenotype) was minimal to absent in neoplastic cells.

There were higher incidences of mutations in *KRAS* (87% *vs* 14%) and *Tp53* (52% *vs* 0%) in lung tumors resulting from chronic cumene exposure when compared to lung tumors arising spontaneously in vehicle controls. The predominant *KRAS* mutation was detected in codon 12 (G>T transversion) in lung tumors resulting from chronic cumene exposure (21%) compared to spontaneous lung tumors (0.008%). The predominant *Tp53* mutations were noted in exon 5 and were detected in only lung tumors resulting from cumene exposure (46%) but not in spontaneous lung tumors (0%) (Hong et al., 2008).

Discussion: In summary, chemically-induced and spontaneous lung tumor incidences in NTP studies were higher in B6C3F1 mice than in F344 rats. With a few exceptions, chemically-induced mouse lung tumors were usually associated with primary tumors originating in multiple organs. Molecular analysis of chemically-induced and spontaneous lung tumors may provide some insight into chemical specific effects associated with tumorigenesis. Panelists indicated that that the Type II and, less commonly, club cell markers (e.g. SPC, CC10) are expressed in lung tumors of standard mouse strains but that the immunophenotype of lungs tumors induced by compounds of interest was not known. Further, it was not clear how mouse lung tumor immunophenotypes compared to those in human lung adenocarcinomas. Finally, although structurally related chemicals may cause lung tumors in the B6C3F1 mouse, the mechanisms of tumorigenesis may not be similar.

Table 2-1. Summary of structurally related chemicals tested in the NTP bioassay that resulted in alveolar bronchiolar tumors

TR #	Chemical	Ames	Route	Male Rat	Female Rat	Male Mouse	Female Mouse	Multiple sites
TR-185	Styrene	-	Gavage	NE	NE	**(EE)**	NE	No
TR-410	Naphthalene	-	Inhaled	CE	CE	NE	**(SE)**	No
TR-422	Coumarin	+	Gavage	SE	EE	**(SE)**	**(CE)**	Yes
TR-466	Ethylbenzene	-	Inhaled	CE	SE	**(SE)**	SE	No
TR-542	Cumene	-	Inhaled	CE	SE	**(CE)**	**(CE)**	Yes
TR-534	Divinylbenzene	-	Inhaled	EE	NE	NE	**(EE)**	Yes
TR-370	Benzofuran	-	Gavage	NE	SE	**(CE)**	**(CE)**	Yes

**NTP evidence of carcinogenicity; CE=clear evidence, SE=some evidence, EE=equivocal evidence and NE=no evidence; parenthesis indicates a lung tumor response*

2.5 Species differences in compound responses and cell of origin considerations

Laura Van Winkle (University of California – Davis)

Several chemicals with on-going or completed EPA assessments have bioassay data indicating the development of treatment-related bronchiolar-alveolar lung tumors in mice. This type of tumor is prevalent in several mouse strains and purported to originate in club cells via a MOA which is species-specific. The occurrence of similar effects in other species has been investigated, particularly in rats, monkeys, and humans. Among the chemical agents involved are compounds with a vinyl group (styrene), alkyl aromatics (ethyl benzene, cumene) and others (coumarin and naphthalene). Analysis of the available data and interpretation of results of chemically-induced mouse lung tumors and the relevance of such mouse lung tumors to human cancer risk has been a topic of debate. Here we discussed current evidence related to the cytotoxic effects of these chemicals, metabolic influences on the MOA of chemically-induced mouse lung tumors, and cell of origin issues for these tumors.

The anatomy of the lung varies by species, with variations in airway cell types and by their location in the lung. A particularly important aspect is the lung epithelial cell-type composition (basal cells, goblet cells, club [Clara] cells, ciliated cells) which was compared in three regions (proximal bronchus, midlevel and terminal bronchioles) in the lungs of mice and monkeys – see slides 2 and 3 of Dr. Van Winkle's presentation. These differences lead to variation in local chemical deposition patterns, susceptibility to injury, and the capability to repair cellular damage.

Naphthalene (slides 4-14): Naphthalene is toxic to club cells, regardless of the route of exposure –e.g., club cells are affected in the mouse by intraperitoneal (IP) exposure. A summary of the differences in toxicity in various regions of the lung in mice and rats (24 hours post exposure) showed sensitivity of mouse trachea and distal bronchioles with no effects in those tissues in rats, and greater sensitivity of the rat in the olfactory epithelium (a tissue with no Club cells) when compared to mice.

There is evidence of increased cellular proliferation from acute IP exposure to naphthalene and distal bronchiole is exquisitely sensitive by both inhalation and IP routes; which cells are proliferating is yet to

be determined. She also noted that female mice are more susceptible than males, and neonatal mice are 5-10 fold more susceptible than adults.

Dr. Van Winkle also highlighted the cycle of injury and repair from acute naphthalene exposure. Club cell injury, as evidenced by formation of vacuoles, follows exposure to naphthalene after which ciliated cells flatten (squamation), and there is a reduced presence of secretory granules. This is then followed by a wave of proliferation which peaks at 2-3 days post exposure, followed by migration of cells to infill thinned tissue and differentiation leading to a return to a morphologically normal steady state. If there is a repeated pattern of exposure, the cycle is disrupted, the epithelium doesn't re-differentiate as noted by a lack of Club cell markers.

Repeated inhalation or IP exposure to naphthalene can lead to tolerance, which is defined as resistance to a high challenge dose following a week or more of exposure to repeated doses well below the LD50. There is no evidence this was due to something going on in the liver.

There were a number of other observations related to MOA for naphthalene:

- Glutathione depletion occurs early in the process, before toxicity becomes apparent
- P-450 is required
- Reactive metabolites bind to protein
- Naphthalene epoxide and downstream metabolites are toxic to Club cells, as noted by Chichester et al. (1994)
- CYP 2F2 contributes to mouse lung Club cell toxicity
- Female mice are more susceptible to acute toxicity than male mice

Ethylbenzene: (Slides 15-17) Information regarding the carcinogenicity of ethylbenzene comes from an NTP-sponsored study which concluded that ethylbenzene showed "some evidence of carcinogenic activity in male mice based on increased incidence of alveolar/bronchiolar neoplasms." A study by Stott et al. (2003) investigated S-phase DNA synthesis in the lung (small airways and alveoli) in 1 week and 4 week studies with B6C3F1 mice. Statistically significant differences were noted in the labeling index with increases in the small airways in both sexes in the 1-week study and a reduction in labeling in male alveoli in the longer study.

Styrene: (Slides 18-24) Evidence from a 24 month study (Cruzan et al., 2002) showed a dose-related increase in bronchiolar epithelial hyperplasia in both male and female mice, and a dose-related increase in hyperplasia in the bronchiolar-alveolar region of male mice. A key question is whether the Club cell is a target. Comparative images of the terminal bronchioles show crowding in mice exposed for 104 weeks to 160 ppm styrene versus controls (Cruzan et al., 2001), and additional images showed increased expression of CC10 in areas with hyperplasia, but the evidence that Club cells are the target is incomplete.

There is some question whether or not Cyp2F is the key metabolic enzyme for styrene – Yuan et al. (2010) provided data showing that cells with increased Cyp2E led to increases in protein covalent binding when compared with wild type animals expressing Cyp2F. Shen et al. (2014) showed a greater decrease in LDH activity in BALF (a marker of lung injury) in Cyp2F2-null mice versus Cyp2E1-null mice.

One last consideration was the role of liver in metabolism for styrene. Carlson (2012) found that hepatic P450 reductase knockout mice were protected from styrene toxicity when compared to wild type mice.

Summary and Discussion Points on Species Differences

[Note: the points and conclusory statements below are those of the speaker.]

- Is there clear morphologic evidence of club cell cytotoxicity?
  - Naphthalene-yes
  - Styrene – not in vivo, some evidence from in vitro biochemical studies with isolated cells
  - Ethylbenzene – no
- Is there a clear temporal distinction between cytotoxicity (from electron micrography [EM] or histopathology) and proliferation in terminal bronchiolar epithelial cells?
  - Naphthalene – yes, acutely. Not clear that these are separate under conditions of repeated exposure and likely overlaps.
  - Styrene – no, cytotoxicity not well defined on a cellular basis in intact tissue
  - Ethylbenzene – no, cytotoxicity not well defined on a cellular basis in intact tissue
- Are there species differences in response in the lung?
  - Naphthalene-yes for both cytotoxicity and tumors in lungs of mice (female) and not rats
  - Styrene – tumors in mice but not rat lungs. Cytotoxicity unclear
  - Ethylbenzene-tumors in mice (male) but not rat lungs. Cytotoxicity unclear
- Cyp2F2 (mouse) has the highest catalytic activities with naphthalene; the catalytic activities of Cyp2F4 (rat) are identical.
- CYP2F1 (human), 2F5 (Rhesus) are difficult to express as catalytically active proteins.
- Rat vs mouse differences in metabolism and susceptibility can be accounted for, in part, by substantial differences in quantities of CYP2F protein present (Baldwin et al., 2004).
  - High susceptibility of the mouse lung to naphthalene appears driven by CYP2F2.
- Excellent correlation between the catalytic efficiency of naphthalene metabolism in microsomes from different species and in toxicity of selective portions of the respiratory tract of rodents.
  - In non-human primates catalytic efficiencies are low.
- Formation of covalent protein adducts correlates with toxicity but whether this is a key step is not clear.
  - Monkeys have a higher than expected level of covalent binding in the lung in comparison to the measured rate of metabolism.
- GSH depletion is a necessary but not sufficient step to cause lung toxicity (e.g. just depleting GSH does not cause club cell necrosis).
- The role of the liver in toxicity, and possibly carcinogenesis, is not well defined.
- The relative abundance of metabolites in intact systems (not microsomes), and following repeated exposures, is not well understood.
- *Cytotoxicity*: Participants discussed key intermediate events for compounds of interest, namely terminal bronchiolar cell cytotoxicity, proliferation, and hyperplasia, and noted limited data gaps related to evidence for specific club cell toxicity (for styrene and ethylbenzene).

2.6 Animal and Human Tumour Site Concordance

Dan Krewski, (University of Ottawa)

Dr. Krewski is the lead of an expert group within IARC performing an analysis of animal and human tumor site concordance. The analysis is limited to IARC Group 1 agents – those known to cause human

cancer. The preliminary evaluation reported here included 95 agents identified through Volume 106, but excluded biologicals and all radiation; 12 agents were placed in Group 1 without sufficient evidence of carcinogenicity in humans based on "mechanistic upgrades" of sufficient animal evidence. Another 25 agents were without sufficient data in animals, according to the IARC weight of evidence criteria.

How do we compare tumors in animals and tumors in humans? A method had to be developed to translate animal and human tumors. Tumor sites were grouped and a tumor site concordance database was built. The tumor sites were coded for both humans and animals and resulted in 39 tumor sites being identified in 9 organ and tissue systems from an abstraction of animal and human tumors for Group 1 agents in the IARC monographs.

The distribution of tumor sites in humans and in animals was illustrated in stacked bar charts (slides 11-14 of Dr. Krewski's slides) which show the distribution for the Group 1 agents. Color coding for the type of agent involved was used for the bars in the figures, and color coding of the site (horizontal axis) discriminated between agents causing tumors in both animals and humans, in humans alone, or in animals alone. Separate figures showed the number of Group 1 agents inducing tumors in humans, animals, mice, and rats, respectively. Color coding for the type of agent involved was used for the bars in the figures, and color coding of the site (horizontal axis) discriminated between agents causing tumors in both animals and humans, in humans alone, or in animals alone. The lung was the most frequently affected tumor site for humans, all animals, and rats; skin was the most frequently affected site for mice.

Slide 16 shows a difficult to read heat map of tumor concordance between animals and humans. On the vertical axis, 95 group one agents were tallied and on the horizontal axis were the 39 tumor sites. Red is the most pervasive, blue less. Strong association become visually apparent using this approach. Heat maps linking the strength of the association between Group 1 agents and different tumor sites identified particularly strong associations between asbestos and lung tumors, between Pu-239 and skin tumors, and between 2-napthylamine and urinary tract/uroendothelial tumors, where in each case the same tumors are induced in humans and in four animal species.

A set of analyses similar to those performed on the 39 tumor sites (as shown in slides 11-16) was also performed on the 9 organ and tissue systems. The results of the histograms indicated similar findings: tumors of the upper aerodigestive tract and respiratory system were most frequently seen in humans, animals, and rats; tumors of the skin and connective tissue were most frequently seen in mice. The visual patterns apparent in the organ and tissue systems (slides 23-24) identified the upper aerodigestive tract and respiratory system as the system in which tumors were induced by Group 1 agents most often in both humans and animals, x-rays and gamma radiation affected 7 of the 9 tissue and organ systems in both animals and humans, and tobacco smoking affected multiple organ and tissue systems in humans.

An analysis of the selected quantitative measures of concordance was covered last. It was noted that these quantitative measures may be specific, but may also underestimate the concordance; IARC has a high bar for acceptance and one study is not enough to reach a conclusion. The analysis compared the concordance of humans with five animal species. The results showed the strongest concordance between tumor sites in humans and rats in the lung, mesothelium, nose and thyroid; and between mice and humans for hard connective tissue, skin and lower reproductive tract. When the analysis was performed on concordance by organ system: both rat and mice were moderately to substantially related for nervous and endocrine systems; the rat for upper aerodigestive and respiratory system, and the urinary system; and the mouse for the lymphoid and hematopoetic system, and female breast and reproductive organs and tract.

In a review of concordance between mice and rats, it was noted that the overall concordance between mice and rats in 266 NTP bioassays was 74% (Haseman et al., 1998); Gold et al. (1989) reported a similar overall concordance between mice and rats of 76% in 392 experiments in their Carcinogenic Potency Databases; and Piegorsch et al. (1992) determined that, considering experimental error, the maximum observable concordance is limited to about 80% under the NCI/NTP bioassay protocol.

A parallel project seeks to develop a tumor mechanisms database, which will be based on additional data outside the IARC monographs to try to identify 10 major mechanisms by which humans get cancer.

Discussion:

- All routes of exposure were considered in this analysis because the monographs did not have enough info to do it systematically so this was done by the experts.
- A question arose on whether or not the IARC mechanisms database will track with the Office of Economic Cooperation and Development (OECD) effort to develop an Adverse Outcome Pathway (AOP) database. The OECD effort will begin with a focus on cancer because that's where most of the effort has been put in with MOA/AOP efforts to date and is being done in collaboration with WHO IPCS as part of its harmonization program.
    - 24 mechanistic endpoints were identified in the OECD collaboration with WHO, IPCS, and IARC
    - In this effort, IARC did not pay attention to WHO IPCS AOP work and created de novo 10 new mechanistic pathways; it will be interesting to compare with what IPCS is doing, which is much more in-depth on 113 agents – a lot of data.
- One participant noted that a new manuscript is in development comparing when lung tumors are shown in either rats or mice alone, or in both species and how that informations should relate to safety assessments.
- It was also noted that non-concordance may be explainable with sufficient mechanistic information, and that the development of the mechanistic database may help in analysis along these lines.
- A question on whether any chemicals in this analysis represent the key chemicals for this workshop (i.e., any solvents like ethylbenzene, naphthalene, styrene, benzene, or related chemicals like Coumarin, or fluensulfone). None of the Group 1 agents match up with the 6 mentioned by additional analysis could investigate if any of the Group 1 solvents provide insights. The focus was on group 1 agents, but it may be that expansion to group 2 agents may be helpful.
- A number of additional analyses are possible with the developing concordance database, including analysis of substances for which there is convincing evidence in humans and in animals but not at the same site
- It was also noted that the database will be available for public access after IARC approval.

Session 2 Summary Discussion

- There was much discussion on K-Ras, which is already mutated so one wouldn't expect a change. Supposition is there is a proliferative cytotoxic action. What other transgenic models could we look at to answer those questions?

  - Picking the right model and asking the right question and trying to use an MOA we think describes how it works might be the best way to go. We may need to try one or two different models to test what best fits the data.
- Cell of origin – is it important whether it is Club versus Type 2 cells and does it really matter for a given compound and given MOA?
  - We currently can't say if the cell of origin affects aggressiveness of a tumor.
  - Regarding the cell type, it is important to be asking the right questions. If initial toxicity is in club cell do we care if that is the target cell transformed or if it is a field effect?
  - If the question is how to protect an individual, determining the potential target cell may affect potential interventions. Once it has become a tumor, it might not matter any longer. Early mechanism for chemoprevention or therapy, might not matter where the cell of origin is. It is unlikely to be a simple black/white, yes/no question.
  - In humans, P16 will be more likely to be mutated and hyper-methylated. In mouse, methylation is important and one is more likely to see changes in P14. Data on the early stages in the cancer progression process may lead to better interventions and may be important in defining critical markers. Early changes might be quite different than later.
  - The question is relevant at two different levels: human health risk assessment and mechanistic toxicology. In terms of humans, we don't really care. In respect to mechanistic toxicology, it matters somewhat because it helps us handle dose response. It could be that we find none of the targets in the animal are in the human. On the other hand, we might find that there are a number of biochemical peculiarities and there might be differences in repair mechanisms. In the end, if it comes down to one particular piece of information in the MOA, then we can elucidate whether or not that is expressed.
- Genomic expression data may be useful to identify significant changes. It may not be the same set of genes in each species or population, but rather specific networks of genes that control the process.
- Animal models don't take into account metastasis, which is what kills humans. Rarely is metastasis observed in mice. The need is to develop a mouse or rat model where metastasis is a frequent event, then test an agent from hyperplasia to metastasis and determine how we can intervene.
- One panelist noted that the cell of origin is really important for basic biology of carcinogenicity – the problem is we don't have good ways to sort the cells.
- Another panelist offered that not only does the mouse have Club cells, but that it has a lot of them. Mouse has Cyp2F2; it has a lot of Cyp2F2. The rat has good detoxification enzymes which the mouse lacks. Which enzyme, where it occurs and how much all matter.
- In discussing data gaps, a panelist offered that electron microscopy on mouse lung tissue shows cytotoxicity due to styrene exposure, but that those data have not been published.
- A panelist offered that there is a lack of the morphologic component. We need multiple modalities in the tissue.
- Another important issue to highlight are temporal aspects.
- One area we did not get to hear about is stem cell biology and the role it might have in tumorigenesis.
  - It was noted that many articles were posted on the MLTW HERO project page.

- On the question of tolerance (both for benzene and naphthalene), the cells do eventually become susceptible again. Once it is tolerant, it does not stay that way forever. The timeframe depends on the dose. It is over a 4-7 day period that tolerance is lost.
- On the IARC Concordance Database: It may be possible to mine the database for other chemicals that might elucidate what we are looking at for these three chemicals. However, the human data do not have histology in this database, so that would be a limitation.

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Session 3: Biological Mechanisms

Background and Introduction

Session Co-chairs: Paul Schlosser (US EPA) and Ron Melnick (Ron Melnick Consulting)

The approach taken in this session is summarized in the three themes:

1) Mode of Action Analysis;
2) Pharmacokinetics and Pharmacodynamics of Ethylbenzene, Naphthalene, and Styrene; and
3) Evidence from Related Chemicals and Integration of Cross-Cutting Issues

Each theme builds on the foundation of the discussions which proceed it, leading in the end to a discussion of cross-cutting issues (including issues identified in prior sessions). This format lead to a lively discussion to conclude this session. The focus in Theme 2 on the three key chemicals was in keeping with their critical importance to the EPA in supporting the assessment of those chemicals.

Theme 1: Mode of action analysis

3.1 A Framework for Considering the CYP2F2 MOA Hypothesis & Relevance of Mouse Lung

Ron Melnick (Ron Melnick Consulting)

Dr. Melnick presented a framework for considering the CYP2F2 mode-of-action (MOA) hypothesis and the relevance of mouse lung tumors to humans. In the absence of convincing data to the contrary, the International Agency for Research on Cancer (IARC), the US National Toxicology Program (NTP), and the US EPA consider animal tumor findings relevant to evaluations of human risk. Countering this basic public health perspective requires sufficient and valid evidence for a species-specific cancer response. To establish the CYP2F2 MOA for each particular chemical, at least three fundamental issues need to be thoroughly addressed: 1) demonstration that CYP2F2-mediated metabolites are the determinants of the mouse lung tumor response, 2) demonstration that these reactive metabolites are produced by CYP2F2 only in the mouse lung and not systemically distributed (or are not distributed from other tissues in sufficient quantity to cause cytotoxicity), and 3) demonstration that the relationship between hypothesized essential precursor events (cytotoxicity and sustained regenerative hyperplasia) in the mouse lung and the tumor response in that organ is consistent (i.e., that tumors do not occur at exposure levels for which cytotoxicity does not occur), since genotoxicity produced by non-CYP2F2-mediated metabolites could be carcinogenic for some CYP2F2 substrates and not for others. To establish the proposed MOA as a general one which can be extrapolated to other chemicals, well-defined conditions for when it can be extrapolated need to be provided, and consistency among several individual chemicals would need to be demonstrated.

To address these basic issues and evaluate human relevance, pharmacokinetic (PK) and pharmacokinetic (PD) data are needed on the chemicals that are expected to act via the hypothesized MOA. Critical information needs from PK studies include: 1) characterization of lung dosimetry of toxic and carcinogenic metabolites produced by CYP2F2 and other CYP450 enzymes (e.g., CYP2E1), 2) characterization of the lung dosimetry and systemic distribution of key metabolites after repeated exposures (the latter point is important because an essential feature of the MOA is cytotoxicity to Club (Clara) cells where CYP2F2 is predominantly expressed), 3) characterization of human variability (e.g.,

genetic differences in expression of enzymes that affect lung dosimetry of key metabolites, age/life stage, and effects of other exposures that might affect expression of metabolizing enzymes), and 4) characterization of the kinetics and tissue distribution of corresponding ring oxidation enzymes in humans.

A scientifically justifiable MOA requires identification of the key events and processes that result in cancer formation (U.S. EPA, 2005). Mechanistic data are vital to the identification and characterization of key events leading to the induction of lung cytotoxicity and carcinogenicity. According to the proposed MOA, metabolites produced by CYP2F2-mediated ring oxidation (leading to ring-open metabolites) in the mouse lung cause local cytotoxicity, which is followed by sustained cell proliferation and subsequent lung tumor development; however, a shortcoming of the hypothesis is that the specific reactive intermediate(s) that cause cytotoxicity and mouse lung tumors have not been identified. Furthermore, potential involvements of alternative processes (GSH depletion, reactive oxygen species, protein binding, topoisomerase inhibition, genotoxicity) have not been fully evaluated. Studies showing the absence of carcinogenicity when a key event is blocked strengthens the evidence for a causal association (U.S. EPA, 2005); demonstration that the lack of a lung tumor response by styrene, naphthalene and ethylbenzene in CYP2F2-null mice of a sensitive strain would add significant support for the hypothesized MOA. Studies of protein or DNA adducts and characterizations of the genetic profile of tumors induced by these agents (e.g., frequencies and types of oncogene or tumor suppressor gene mutations) would aid in identifying specific reactive intermediates and evaluating the involvement of genotoxic and nongenotoxic processes. Demonstrating consistency in the relationship between key precursor events (e.g., sustained cell proliferation rate) and lung tumor outcome is necessary for each chemical purported to act by this MOA in order for it to be accepted on a chemical-by-chemical basis. Demonstrating this consistency for multiple chemicals is essential for establishing general biological plausibility and coherence for the proposed MOA.

Finally, the MOA must account for findings of related compounds. For example, benzene, a human carcinogen that is metabolized predominantly by CYP2F2 and CYP2E1 induces lung tumors in mice but not in rats – are the mouse lung tumor findings for benzene relevant to humans? This issue is important because of reported increases in lung tumor risk among workers exposed to benzene.

3.2 Hypothesis-driven MOA Analysis

George Cruzan (ToxWorks)

Dr. George Cruzan presented a summary of the hypothesis and evidence for the CYP2F2 MOA. The basic hypothesis is that mouse lung tumors induced by styrene, ethylbenzene, and naphthalene result from CYP2F2-generated ring-oxidized metabolites that cause club cell toxicity leading to regenerative hyperplasia and subsequent tumor development. Observations that support a mouse-specific lung tumor response for chemicals that are metabolized by CYP2F2 include:

1) Evidence is strong for lung tumors induced in mice after inhalation exposure to styrene and suggestive for lung tumors in mice after gavage treatment, while the evidence for styrene-induced tumors in rats was generally negative,
2) Cytotoxicity in terminal bronchioles of mice occurs after a single oral exposures to styrene (increased LDH, protein, and cells in bronchoalveolar lavage fluid), after 2 weeks exposure (increased BrdU labeling and decreased Club cell secretory protein), and after 3 months of

exposure (decreased staining of Club cells); hyperplasia in terminal bronchioles was observed in mice after 12 months of exposure and extended into alveolar ducts by 18 months. In contrast, lung toxicity was not observed in rats even at exposures up to 160 ppm styrene for 2 years,

3) Analyses of urinary metabolites from rodents exposed to styrene indicated that mice produced 8 to 20-fold more metabolites through the ring-oxidized pathway than rats.

Though styrene oxide is a genotoxic chemical, observations indicating that this metabolite of styrene is not involved in the mouse lung tumor response include:

1) Lack of lung tumor initiation by styrene in A/J mice,
2) Lack of increased chromosomal aberrations in lungs of B6C3F1 mice exposed to styrene,
3) Lack of an increase in BrdU labeling index in terminal bronchioles of CYP2F2-knockout mice treated with styrene oxide by ip for 5 days,
4) Ethylbenzene, which does not form a side-chain epoxide and is not genotoxic, also induces lung tumors in mice.

Additional mechanistic findings supporting the hypothesis that mouse lung tumors induced by styrene are due to lung toxicity resulting from CYP2F2-mediated ring oxidized metabolites include:

1) Metabolism of styrene by CYP2F2 in the mouse lung produces ring-oxidized metabolites that are toxic to Club cells,
2) Exposure to styrene or 4-hydroxystyrene causes lung toxicity in mice, but not in rats
3) Neither styrene nor styrene oxide caused lung toxicity or increased BrdU labeling in the absence of CYP2F2 metabolism (using CYP2F2 knockout mice),
4) 4-hydroxystyrene is toxic to mouse lung Club cells at 50-fold lower dose than styrene, while 2-hydroxystyrene and 3- hydroxystyrene are not toxic to the mouse lung and the styrene analogs, 3-methylstyrene and 4-methylstyrene (ring oxidation at the 4 position is impossible), do not cause increases in mouse lung tumors,
5) 4-hydroxyethylbenzene (the ring oxidized metabolite of ethylbenzene), but not 1-phenylethanol, also causes increased BrdU labeling in the mouse lung.

The proposed lack of human relevance for lung tumors induced in mice by chemicals that are metabolized by CYP2F2 is based on the much lower metabolic activity of the human isoform CYP2F1 for naphthalene compared to mouse CYP2F2 or rat CYP2F4, the low metabolism of styrene in CYP2F2 knock-out mice that have an inserted transgene containing DNA for human CYP2F1, and the lack of lung toxicity in this transgenic mouse model after exposure to styrene or styrene oxide.

Discussion of Theme 1: Mode of Action

The cell of origin of lung tumors in mice is not known, though cells within the tumors stained for surfactant (a marker of normal alveolar cells) and only weakly for CCSP (marker for normal Club cells). Club cell involvement is proposed because all early toxicity responses, including exfoliation, cell replication, and hyperplasia occurs in Club cells.

Regarding mouse strain differences, the inhalation carcinogenicity study was conducted in CD-1 mice, which are very susceptible to spontaneous and chemical induced lung tumors, while the knock-out and transgenic models were developed in C57BL/6 mice, which have a lower spontaneous rate of lung tumor

formation. In spite of this difference, 4-hydroxystyrene did not induce a greater BrdU labeling in CD-1 mice than in C57BL/6 mice.

Regarding the genotoxicity of styrene oxide and styrene, styrene oxide is positive in a number of in vitro genotoxicity assays but gave mixed results in in vivo studies; assays of mutagenicity and clastogenicity in the mouse lung were negative for styrene.

Theme 2: Pharmacokinetics and pharmacodynamics of ethylbenzene, naphthalene, and styrene

3.3 Pharmacokinetics and Pharmacodynamics of Ethylbenzene

Ernest Hodgson (North Carolina State University)

Dr. Ernest Hodgson discussed the pharmacokinetics and pharmacodynamics of ethylbenzene and stated that the database on this chemical is small and inadequate to reach a definitive conclusion on the human relevance of lung tumors caused by ethylbenzene in B6C3F1 mice. Inhalation exposure to ethylbenzene induced kidney tumors in male and female rats, liver tumors in female mice, and lung tumors in male mice (NTP, 1999b). The lung tumor findings in male mice included a positive dose-related trend and a significant increase in the high exposure group. Alkyl oxidation is the major metabolic pathway of ethylbenzene elimination in rats, mice, and humans. Reactive metabolites of ethylbenzene are produced by CYP2F2- and by CYP2E1-mediated oxidation. Ethylbenzene undergoes ring oxidation to reactive intermediates in liver microsomes from rats, mice, and humans, and in lung microsomes from rats and mice; these metabolites may cause P450 suicidal inhibition in rat and mouse lung microsomes. CYP2F1 is inducible in human lung cell lines. In Session 2 (Comparative Pathology) Pandiri reported that after 13 weeks of inhalation exposure to ethylbenzene, Club cells in mice were not affected nor were any chemically related histopathological lesions observed in the mouse lung. The paucity of pharmacokinetic and mechanistic data on ethylbenzene, including the lack of evidence for sustained cytotoxicity or a sustained increase in cell proliferation in the mouse lung, the putative key precursor events for the mouse lung tumor response, weakens any judgment on the linkage between the hypothesized MOA and induction of mouse lung tumors. The human relevance of the mouse lung tumor findings cannot be justifiably dismissed because no key precursor events have been identified, because of the greater variability of PK and PD parameters in humans compared to inbred mouse strains, and because human exposures to ethylbenzene are usually part of a complex mixture.

Discussion of Ethylbenzene

It was noted that the mouse strain in question was B6C3F1 and that there appears to be no evidence as to whether EB is toxic or not to Club cells. It was also pointed out that some of the studies listed in the presentation [e.g., those of Saghir et al. (2009)] evaluated metabolism and showed that EB is a ring oxidant in mice but not in rats. The ring oxidants are highly reactive. Also, there is suicide inhibition of P450 probably caused by 2F2 ring metabolites. Tumors seen with EB were adenomas, nonmalignant. Styrene is more potent than EB and EB only appears to induce tumors at high dose.

3.4 Pharmacokinetics and Pharmacodynamics of Naphthalene

Laura Van Winkle (University of California, Davis)

Dr. Laura van Winkle discussed the mode of action for naphthalene-induced toxicity and cancer. In contrast to ethylbenzene, there are a lot of data for naphthalene (NA). An initial oxidation step is obligate for NA-induced effects. Warren et al. (1982) provided evidence for reactive, P450-generated GSH-depleting metabolism. Piperonyl butoxide, a known inhibitor of CYP, reduced NA-induced airway epithelial injury, while diethyl maleate, which depletes glutathione, enhanced NA-induced injury. Li et al. (2011) demonstrated that lung microsomes from CYP2F2-null mice have roughly 160-fold decreased catalytic efficiency for NA compared to wild-type. Warren et al. (1982) also showed correlative changes in whole lung covalent binding (protein adducts) with the metabolic changes induced by piperonyl butoxide (reduced binding) and diethyl maleate (increased binding). Hence the overall reactive metabolite binding correlates with toxicity. This binding precedes the earliest signs of toxicity and is distributed with airflow patterns in the lung: much higher in the airway epithelium than in residual lung. Binding to critical proteins is thought to be a common mechanism for toxicities associated with acetaminophen, 4-ipomeanol, and other compounds. On the other hand, DNA adducts following in vivo or ex vivo NA treatment have not been reported in the lung.

Nonlinearity & Species Differences: In the mouse lung, while the formation of protein adducts has a low, approximately linear shape at lower exposure levels (< 200 mg/kg ip), there is a continuous decrease in GSH with NA and a transition to a much higher rate of adduct formation per unit dose of NA above that level (Warren et al., 1982). A comparison of protein binding rates with nasal tissue explants from rats and monkeys showed similar covalent binding ex vivo (Destefano-Shields et al., 2010). However when mouse lung tissues were incubated with NA (Cho et al., 1994) the protein binding was about 3-fold higher than seen in similar experiments with the monkey (Boland et al., 2004). Unfortunately, these data do not include measurements with rat lung tissues ex vivo, which would allow for a more direct comparison among all three species. However a comparison with lung airway microsomes showed mouse metaolic activity to be about 4-times higher than the rat and 100-times higher than the monkey (Buckpitt et al., 2013).

Proposed MOA: Thus a possible sequence of events for NA-induced cytotoxicity is:

1. NA oxidized to reactive metabolites (via CYP, including CYP2F2)
2. Reactive metabolites deplete GSH, the cell's normal protective mechanism
3. With GSH depletion, protein thiol oxidation accelerates, leading to protein unfolding
4. Because critical proteins involved in protein folding are damaged, cell cannot recover
5. Cytotoxicity occurs

In particular, the lower levels of protein binding observed at exposure levels that only cause slight GSH depletion can be repaired by the cell, but it is only when there is significant GSH depletion that accelerated damage and cytotoxicity are observed.

It has also been shown that repeated NA inhalation exposure in mice leads to a level of tolerance, apparently due to induction of GSH synthesis (West et al., 2003).

Metabolite Specificity: With regard to metabolite specificity, all of the potentially reactive metabolites, including the epoxides, have some activity. Pham et al. (2012a; 2012b) showed that naphthalene oxide, naphthalene diolepoxide, and both 1,4- and 1,2-naphthoquinone form adducts with model peptides, though the oxide adducted fewer sites. Previously, Zheng et al. Zheng et al. (1997) had shown that binding of the epoxide to sulfur nucleophiles was minor relative to 1.2-naphthoquinone in isolated Club cell incubations with NA.

Dosimetry: While a full PK/ADME study for *chronic* NA inhalation in mice has not been done, blood levels have been measured after a single exposure and shown to decline quickly in mice (~ 30 min half-life) and rats (~ 40 min half-life) (NTP, 2000a, 1992a, b). After a rapid uptake of NA into the blood (P blood:air = 571), male and female rats appear to have an equal capacity for metabolism in the lungs, as do male and female mice. However, saturation of the metabolism occurs at lower NA blood concentrations in female mice than in male mice. Similarly, the liver metabolic pathway represented by the Michaelis-Menten equation shows the same metabolic capacity and saturation level in male and female rats, but the metabolic capacity and saturation levels are lower in female mice than in male mice. In the isolated perfused mouse lung NA generated dihydrodiol and GSH conjugates as 70% of total metabolites in the perfusate, indicating that circulating NA is metabolized in the mouse lung and that a significant amount of inhaled NA may be metabolized in the lung before reaching the blood (Kanekal et al., 1991). On a per mg microsomal protein basis, mouse liver metabolizes naphthalene at a total rate similar to mouse lung (Buckpitt et al., 1987).

The data also show that the steady-state concentration of naphthalene in the lungs of rats is not very different from that of mice exposed to equivalent concentrations. However, rates of metabolism and the cumulative metabolism of naphthalene in the lung were markedly greater in mice than in rats. Rates of naphthalene metabolism did not increase proportionally with increasing exposure concentration, indicating metabolic saturation in this organ. Metabolic saturation was more evident in the rat lung than in the mouse lung.

NA metabolism was also greater in the mouse liver than in the rat liver; however, the species difference in liver metabolism was not as marked as that in the lung. Metabolic saturation was only apparent in the liver of rats exposed to 60 ppm. For both species, 65-75% of the metabolic clearance occurred during the 6-h exposure periods; only in the 60 ppm rats was metabolic clearance reduced to 50% of the total inhaled dose, probably due to metabolic saturation. Elimination of liver CYP2F2 in the HRN mouse increased circulating NA, but did not decrease circulating NA-GSH metabolites, indicating that the liver also has a key role in detoxification (Li et al., 2011).

Human liver microsomes metabolize naphthalene to a cytotoxic, nongenotoxic, protein reactive metabolite – reduced by addition of GSH (Tingle et al., 1993). In particular, human liver microsomes convert NA to the dihydrodiol, 1-naphthol, and 2-naphthol (Cho et al., 2006). Liver metabolism can be a significant factor because the metabolites of naphthalene are stable enough to travel through the circulation and impact the lung. NA oxide can escape hepatocytes and in the presence of protein has a half-life of 11 min. (Kanekal et al., 1991; Buonarati et al., 1989; Richieri and Buckpitt, 1987). As stated previously, all metabolites cause changes in isolated perfused mouse lung, but differ in potency (Kanekal et al., 1991, 1990):

- NA: decreased GSH, Club cell toxicity, increased reactive metabolites
- NA oxide: decreased GSH, Club cell toxicity
- napthoquinone and dihydrodiol also caused Club cell toxicity and an increase in vacuolated cells but this was much less than the NA oxide or NA
- 1-naphthol did not cause Club cell toxicity.

Hence the liver could contribute to but is not *required* for lung toxicity (in the mouse), since Club cells in the lung are still a target in ex vivo systems.

Since it has been shown that NA is toxicologically inert without metabolic conversion to the epoxide, the kinetics of specific CYPs should also be considered. Since human exposure levels are likely to be low, enzymes with high µM or low mM Km values are unlikely to be important. Overall P450 levels in the primate lung are low, so the enzymes present would need to have a high catalytic efficiency (V_{max}/K_m) to be important. The amounts of catalytically active protein in specific cells (i.e., Club cells) will be important to the response of those cells. Human CYPs are expressed in different areas of the respiratory tract, as shown in Table 3-1.

Table 3-1. Human CYP Expression in the Respiratory Tract

Tissue	CYPs detected*
Nasal mucosa	2A6, 2A13, 2B6, 2C, 2J2, 3A
Trachea	2A6, 2A13, 2B6, 2S1
Lung	1A1, 1A2, 1B1, 2A6, 2A13, 2B6, 2C8, 2C18, 2D6, 2E1, 2F1, 2J2,2S1, 3A4, 3A5, 4B1
Esophagus	1A1, 1A2, 2A, 2E1, 2J2, 3A5

* From Ding and Kaminsky (2003)

The kinetics of NA metabolism with recombinant human CYPs and mouse CYP 2F2 have also been evaluated. As shown in Table 3-2, not only is the intrinsic Vmax of mouse CYP2F2 much higher than any of the human isoforms, but the Km is much lower.

Table 3-2. Intrinsic Vmax and Km of mouse CYP2F2

P450 isoform	Vmax (pmol/pmol/min)*	Km (mM)*	Vmax/Km
1A1	9.1	111	0.08
1A2	35.8	73	0.49
2B6	20.2	58.6	0.34
2E1	8.4	10.1	0.83
3A4	8.1	60.7	0.13
2F2 (mouse)	107	3	36

* From Cho et al. (2006)

Summary: Mouse CYP2F2 has the highest catalytic activity for naphthalene *and* high expression in the lung. The high susceptibility of the mouse lung to naphthalene appears driven by CYP2F2. The catalytic activity of rat CYP2F4 is identical to mouse CYP2F2, but the total catalytic activity in the mouse lung is

much less than the rat. Thus rat vs. mouse differences in metabolism and susceptibility can be accounted for, in part, by substantial differences in quantities of CYP2F protein present (Baldwin et al., 2004). There is excellent correlation between the catalytic efficiency of naphthalene metabolism in microsomes from different species and in toxicity of selective portions of the respiratory tract of rodents. Human CYP2F1 and Rhesus monkey CYP2F5 are difficult to express as catalytically active proteins. In nonhuman primates catalytic efficiencies are low, but monkeys have a higher than expected level of covalent binding in the lung in comparison to the measured rate of metabolism.

Formation of covalent protein adducts correlates with toxicity but whether this is a key step is not clear. GSH depletion appears to be a necessary but not sufficient step for lung toxicity (i.e., just depleting GSH does not cause Club cell necrosis). The role of the liver in toxicity, and possibly carcinogenesis, is not well defined. The relative abundance of metabolites in intact systems (not microsomes), and following repeated exposures, is not well understood.

Discussion of Naphthalene

A participant asked if the variability in GSH among species had been measured and noted that the source of the GSH could matter. For example the kinetics of GSH production in Club cells, as a function of repeated exposure, would be interesting. Dr. van Winkle responded that a depletion assay has been conducted in mice, but not in other species, and noted the study from West et al. (2003) indicating that induction of GSH synthesis was a mechanism for NA tolerance in the mouse lung after repeated exposure.

A participant noted that among humans, the number of variations in glutathione transferases is dramatic, but these are only expressed in liver. There can be 5-7 additional copy numbers in some populations, based on genetics. He asked if this factor has been considered. In response, the high capacity of the liver for detoxifying NA is already known and there is also a similar wide variation among rhesus monkeys. But the extent to which this affects lung dosimetry and toxicity is not yet known. Research is currently under way, but not yet ready for publication. Variation among humans will be an important issue.

Another participant noted that naphthoquinone is mutagenic and that humans can form the epoxide and (to a smaller extent) the quinone, then asked how this might affect susceptibility. Dr. Van Winkle responded that studies were under way with epoxide hydrolase knockout mice, but were not ready for publication. Information is also available from isolated Club cells (Chichester et al., 1994) incubated with different metabolites. Naphthalene epoxide was found to be the most rapid binder, more rapid than the quinones, and also more potent in terms of toxicity. If the quinone was extremely toxic, that would have been seen. However a better understanding of the total mass balance (competition) between GSH and epoxide hydrolase is needed. Studying metabolism in the human lung is difficult.

3.5 Pharmacokinetics and Pharmacodynamics of Styrene

Tim Fennell (Research Triangle Institute)

The general issue first discussed was in establishing how a chemical is carcinogenic, and in particular for chemicals that are carcinogenic in the mouse lung, determining the applicability of the mechanism to humans. The presentation focused on whether the key reactive metabolite produced in the target organ is specific to that organ and animal species.

General questions are:

- Is the key metabolite mutagenic or non-mutagenic?
- Is the chemical carcinogenic in just one organ? Just one species?
- Or is it carcinogenic in multiple organs or species?
- Does the mechanism involve (highly) reactive metabolites?
- Are these cytotoxic?

Styrene is clearly carcinogenic in the mouse lung. Metabolism of styrene via CYP2F2 in the mouse lung has been shown to be significant and there are more Club cells (where this metabolism is concentrated) in the mouse lung than rat or human. The mouse lung differs from other species in metabolizing styrene via ring oxidation. Styrene exposures to mice lead to increased cell replication and decreased Club cell secretory protein (CCSP).

In support of the proposed MOA being specific to the mouse lung are the data on species differences in metabolism and that acute toxicity and induced cell proliferation are significantly reduced or eliminated in CYP2F2 knockout mice. This leads to a key question of whether styrene would be carcinogenic in CYP2F2 knockout mice.

With regard to human relevance, besides the questions above regarding species, organ and reactive metabolite specificity, one should determine if a specific enzyme is involved, whether that enzyme or one with comparable specificity exists at all in humans, and whether there is activity for the enzyme (in the organ of concern). One can also address pharmacodynamic applicability with respect to specific mechanisms: mutagenicity, cell proliferation, and cytotoxicity.

With regard to the proposed (mouse-specific) MOA, Dr. Fennel reiterated the primary knowledge that it is taken up from the air and metabolized in the lung and liver. However, he stated that the metabolite of concern was unclear (uncertain). Possibilities include vinylphenol and metabolites of the vinylphenol, a catechol, a ring-opened metabolite, and an epoxide. Data key to supporting the MOA would be demonstration of the metabolite in vitro or in vivo. To not be relevant for humans, one would need to show that the metabolic pathway is not active.

Metabolic data in humans include results from 13C labelled styrene exposure followed by NMR spectroscopy of urine samples (Johanson et al., 2000). This technique allows for the observation of all metabolites in a sample. Substantial differences between rats, mice, and humans are seen (Manini et al., 2002b). For humans, in the region where one expects to see ring opened metabolites or their GSH conjugates, very little quantity was found. LC-MS analysis of styrene metabolites following workplace exposure showed 4-vinylphenol glucuronide and sulfate (Manini et al., 2002b), similar to what is seen in rats.

The role of specific CYPs in the metabolism of styrene can be examined by a range of techniques: chemical ligand inhibition of metabolism, antibody inhibition, expressed recombinant CYPs, and studies in knockout mice. Styrene is metabolized by both CYP2E1 and CYP 2F2. However, it is also a substrate for other CYPs and most of the analyses of activity have focused on the oxidation of styrene to styrene oxide or to styrene glycol. The review article by Cruzan et al. (2009) refers to the earlier review of Carlson (2008), as supporting the statement that human CYP2F1 is expressed at levels lower than in the rat and that in vitro studies in BEAS-2B human lung cells overexpressing CYP2F1 showed little activity of that enzyme towards styrene. However, Carlson (2008) actually states that “unpublished

studies have not been able to demonstrate the metabolism of styrene by this CYP2F1 containing system." Thus the data to support these statements have not been published. In a much earlier study, Nakajima et al. (1994) did in fact demonstrate a high activity for CYP2F1 in the conversion of styrene to styrene glycol.

A recent paper by Shen et al. (2014) showed that the capacity to convert styrene to styrene glycol and 4-vinylphenol in CYP2E1 knockout mouse lung microsomes was the same as in wild type mice, showing little role for CYP2E1 in that tissue, while these rates were significantly reduced with liver microsomes of the CYP2E1 knockouts. On the other hand metabolism to styrene glycol was reduced by about 65% in CYP2F2 knockout mouse lung microsomes vs. wild type and metabolism to 4-vinylphenol reduced to below the detection limit. In the CYP2F2 knockout liver, metabolism to styrene glycol was only reduced about 25%, but again metabolism to 4-vinylphenol was reduced to below the detection limit. The Cyp2F2-null mice were resistant to styrene-induced pulmonary toxicity.

A final set of studies was described involving CYP2F1 humanized mice, on a CYP2F2 knockout transgenic strain, in comparison to wild type mice (Cruzan et al., 2013). No cytotoxicity or no increase in BrDU labeling with styrene or styrene oxide was observed in the transgenic mice compared with the wild type after exposure to 200 mg/kg/day ip for 5 days. Further, decreased BrDU labeling occurred in the knockout and humanized mice vs. wild type when administered 4-vinylphenol. These data could be interpreted as showing a lack of metabolism via human CYP2F1 in mice. However, the results are ambiguous, and changes could also result from alterations in metabolism resulting from the CYP2A13 or 2B6 isoforms, both of which can oxidize styrene (Fukami et al., 2008; Nakajima et al., 1994).

Having examined the existing data, a set of more refined questions can now be posed and, to a degree, answered:

- Is there a species difference in lung metabolism? Most likely.
- Are the toxic metabolites so reactive that they have to be produced in situ? That might be the case.
- Are vinylphenols mutagenic with activation by lung microsomes?
- Are the toxic metabolites so reactive that they cannot be detected directly? If not, can they be detected indirectly?
- Can a marker be developed that indicates they were formed? Protein or DNA adduct?

In summary, Dr. Fennel stated that he still had questions such as these and, that he was not entirely convinced of the proposed MOA. He concluded by describing a number of approaches that could be used to exactly determine lung metabolism:

- Gene expression
- Protein expression
- Protein modification: blood protein adducts and tissue adducts
- Metabolomics
- Dose and time response. Is there a correlation with covalent binding and GSH depletion?

Discussion of Styrene

A session panelist noted that in previous mechanistic studies, Shen et al. (2014) had only seen significant toxicity from 4-vinylphenol, not the other vinylphenols. At the doses administered by Shen et al. (2014),

100 mg/kg, styrene itself did not have a significant effect. He further elaborated that the studies were done by ip administration, and that the 2-, 3-, and 4-vinylphenol will all travel in the circulation. In contrast, 4-dihydoxystyrene can't get to lungs by this route because it is too reactive.

In response to another question, it was noted that CYP2F1 is polymorphic.

Another participant asked about oxidation to polyphenols, if there were peroxidases in Club cells. The answer is unknown. Another participant noted that polyphenols suicide-inhibit the enzyme producing them. Catechol metabolites auto-oxidize and will react with the enzymes (and other proteins).

Theme 3: Evidence from Related Chemicals and Integration of Cross-Cutting Issues

3.6 Related Chemicals: CYP2F2 Substrates & Other Mouse Lung Tumorigens

Paul Schlosser (US EPA)

Dr. Paul Schlosser described data for other chemicals that are either mouse tumorigens or known to be CYP2F2 substrates, which can inform the generality of the proposed hypothesis and the set of mechanistic data needed to determine the likely specificity of a mouse lung tumor endpoint to that species vs. humans. Four chemicals that were briefly discussed are methylene chloride (MC), benzene, fluensulfone, and trichloroethylene.

Methylene chloride (MC): MC causes liver and lung tumors in mice, but at levels that don't cause overt cytotoxicity (NTP, 1986; Serota et al., 1986). There is transient vacuolation of Club cells and lung cell proliferation (not secondary to cytotoxicity) that appears to be CYP-mediated, but existing data do not indicate a specific role for CYP2F2. Current PBPK models of MC dosimetry assume that oxidation occurs exclusively by CYP2E1, but these are semi-empirical and the saturation constant for oxidation fitted by PBPK modeling does not match that determined in vitro. The vacuolation and proliferation responses appear to be CYP-related and the fact that they are not sustained can be explained by the protective depression of CYP activity that occurs with continued exposure (U.S. EPA, 2011).

While MC does not cause lung tumors in rats, it does cause mammary tumors in that species, indicating that the overall cancer risk is not species-specific. Likewise, while human occupational exposures may not be associated with lung cancer, they have been associated with several cancers, including brain, liver, biliary tract, non-Hodgkin lymphoma, and multiple myeloma (Cooper et al., 2011). The cancer risk is thought to be glutathione-S-transferase (GST)-mediated, leading to formation of reactive metabolites, including genotoxic products. The relative rate of GST-mediated metabolism in the rat lung is about 14% of that in the mouse, at least partly explaining the relative sensitivity for that tissue. Given these observations, the sensitivity of the mouse lung vs. the rat lung cannot be attributed to expression of CYP2F2 in the mouse and in fact higher rates of MC oxidation would be expected to reduce, not increase cancer risk, for comparable levels of GST activity. Humans who carry the null allele for the key enzyme, GST-T1, would be assumed to have zero cancer risk. But most of the population is predicted to have a non-zero risk, although this may be quantitatively low.

Benzene: Benzene is a multi-site carcinogen in rats and mice when animals are exposed orally, but caused lung tumors in mice only. Inhalation exposure caused lung cancer in CD-1 and CBA/Ca mice, but this was only seen in stop- or intermittent-exposure study designs. Benzene is well known as a human

leukemogen and some epidemiological studies show lung cancer associations in exposed workers. Thus there is a clear cancer risk in humans with evidence for a site-concordant effect in the lung.

Acute benzene toxicity appears to require CYP-mediated metabolism, with CYP2E1 long assumed to be primarily responsible. However CYP2F2 has been shown to contribute almost equally to CYP2E1 in mouse lung oxidative activity. But CYP2F2 is not known to create distinct metabolites from CYP2E1, and in the human lung CYP2F1 has comparable activity to CYP2E1, though these activities are much lower than in the mouse lung. A specific role for Club cells has not been suggested for benzene, but the high expression of CYPs in those cells would clearly be a risk factor.

Oxidative (CYP2E1) activity in the rat lung is extremely low, so the species sensitivity difference for that tissue can at least be partly attributed to this quantitative difference. On the other hand benzene's oxidative metabolites can circulate in the blood, so hepatic metabolism should also contribute to lung dosimetry. Hence the differential sensitivity of the mouse lung after oral exposures could be due to higher hepatic activity in that species. But benzene does cause cancer in other sites in the rat, indicating that part of the explanation may also be mouse-specific sensitivity of the lung which is not related to metabolism.

Fluensulfone: Fluensulfone causes alveolar and bronchiolar hyperplasia and adenomas in CD-1 mouse, with Club cells considered the likely cell of origin. It does not cause cancer in Wistar rats, but the extent of a proliferative response has not been evaluated in this species. There are no observations or associations for humans. A range of mutagenicity tests were negative for fluensulfone, suggesting that the proliferative response is a key event for the MOA

Mouse lung microsomes showed significant metabolic activity. About 20% of this activity is attributable to CYP2F2 and 5% to CYP2E1, but the enzyme(s) responsible for the remaining 75% have not been identified. There was no elimination with human microsomes. However, the active metabolite is unknown and there has been no comparison of effects in CYP2F2 knockout mice vs. wild type mice. Hence it is possible that, like MC, another (conjugation) pathway could be the activation step. Metabolic conversion has not been tested by rat microsomes or any lung cytosolic preparation.

Trichloroethylene (TCE): TCE causes lung tumors in mice, but not rats or hamsters. It also causes liver tumors in mice and kidney tumors in rats by both inhalation and oral exposures. The kidney response rate in rats is low, but this is otherwise a rare tumor and the response is consistent with human observations. There is also limited evidence for lympho-hematopoietic cancers in rats and mice, and testicular tumors in rats. In humans the strongest epidemiological evidence is for kidney cancer, with more limited evidence for non-Hodgkin lymphoma and liver cancer. Thus, as for other chemicals discussed here, the lack of site concordance does not mean that there is no human cancer risk.

The key toxic metabolite of TCE is chloral hydrate (CH) and CYP2E1 is a significant but not exclusive mediator of the TCE's metabolism to CH. Hence production of CH is not CYP2F2-specific and the specific activities of different CYP isozymes, as defined by the ratio of Vmax to Km for that enzyme, are ranked as follows:

rat 2E1 > rat 2F4 > mouse 2F2 > human 2E1

Thus differences between species in the rate of CH production are expected to be quantitative, rather than being all-or-none. However the in vivo metabolic difference also depends on total expression of the corresponding CYPs. There was limited CH production with human lung microsomes, consistent with the low CYP2E1 activity in the human lung.

The sensitivity of the mouse lung to TCE-induced tumors therefore appears to be due to the quantitative difference in bioactivation. The much lower activity in human lungs would indicate a much lower risk, but not a zero risk.

Discussion on Related Chemicals

A further difference between fluensulfone and other chemicals proposed to fit the CYP2F2 hypothesis is that the fluensulfone-induced increase in cell proliferation reported by Strupp et al. (2012) was temporary, with an increase after 3 days of exposure but a return to control levels by 7 days. For other chemicals such as styrene the increase in proliferation is sustained over time. The temporary increase in proliferation from fluensulfone could be a mitogenic effect, rather than regenerative proliferation that is secondary to cytotoxicity, with an adaptive mechanism (tolerance) arising after 7 days of exposure. In that regard the response is not consistent with the CYP2F2 hypothesis for styrene.

It was noted that increased cell proliferation increases the numbers of cells at risk for carcinogenic transformation and so lead to an increase in the cancer rate even without an increased mutation rate. However, if there is an increase in mutation rate simultaneous with increased proliferation, it will amplify the risk relative to either occurring. Also, in the rodent phenobarbital has a transient mitogenic effect and promotes liver tumors. Hence a transient increase in proliferation can be significant, but in the case of phenobarbital-induced effects in the liver that significance is demonstrated in conjunction with an initiating agent.

3.7 Integration of Cross-Cutting Issues

John Lipscomb, PhD (US EPA)

Dr. John Lipscomb then described a set of cross cutting issues that relate to the consideration of the mouse lung hypothesis and how it might impact human health risk assessment. He reminded the participants that human health risk assessment (HHRA) is intended to inform policy decisions and intended to be health protective. The policies and procedures for risk management are specific to different programs in which it is conducted but conform to common risk assessment guidelines; cost benefit analysis is not consistently included. Although common policies apply across the U.S. EPA, different programs may interpret the policies differently. Further, each program can have its own interpretation of a given data set.

Having an MOA is both qualitatively and quantitatively valuable. Knowledge of the MOA serves as basis for cancer risk quantitation method. There are multiple frameworks available for evaluating the MOA, in particular for human relevance. What a framework cannot necessarily resolve is the fact that well-qualified scientists differ in their interpretations of data.

As is the case for the mouse lung tumor hypothesis, bioactivation is frequently a key step in MOAs. It may or may not depend on a single enzyme. Many individual enzymes can metabolize multiple substrates and individual substrates can often be metabolized by multiple enzymes. The degree of substrate/enzyme overlap is often concentration-dependent (e.g., one enzyme may predominate at low concentrations while several have significant activity at higher levels). Further, toxicity may or may not depend on a single bioactive metabolite. Thus the role of bioactivation is difficult to assign to a single enzyme.

Regarding the mouse lung tumor MOA, a first consideration is that chemically-induced tumors may have multiple MOAs, not all of which may be known. Some may be operative in humans while others are not. Obtaining sufficient data to prove that a MOA does *not* exist may be difficult.

Mechanisms that could lead to mouse-specific sensitivity would either be toxicokinetic (TK, related to dosimetry), or toxicodynamic (TD, related to response). The proposed MOA is that a difference in bioactivation, a TK factor, determines the specificity. Possible mouse-specific TD factors would be a qualitative difference in endogenous biochemistry, something unique to the mouse related to the response development. Expression of CYP2F2 has been demonstrated in the mouse lung and the comparable human enzyme (CYP2F1) is only expressed at very low levels in the lung. So a key question is whether CYP2F2 is solely responsible for bioactivation of the substrate. For several compounds it has been shown to produce unique metabolites with high cytotoxic activity, so the proposed MOA is plausible. Two quantities that could assist in determining the role of CYP2F2 are the Vmax and Km for its bioactivation.

For those metabolites that are unique to CYP2F2 (vs. other mouse enzymes) measurements in exposed humans or with human preparations for their presence should be made. If the hypothesis that the sensitivity of the mouse stems from its ability to produce these metabolites, this could be tested in part by determining the toxic effect of the metabolites in other animal species. If the sensitivity is not due to TD differences, then another species should respond similarly to a similar dose of the key metabolite.

Other experiments that would provide useful data would be to determine the toxic response, both of precursor events and lung tumors, in 2F2-knockout mice. In vitro metabolic studies with recombinantly expressed CYP2F2 (in a system lacking other CYPs) would allow for unambiguous characterization of its specificity and kinetic properties. Comparing metabolism with mouse and human lung microsomes would provide a direct quantitative comparison of metabolite production. Information from such experiments could be integrated into species-specific PBPK models. Finally, genotoxicity data at concentrations relevant to the tumorigenic response are necessary to rule that out as a secondary MOA.

Key questions to be addressed regarding TK are then as follows:

1) Are some key events seen for tumors not associated with CYP2F2 bioactivation?
2) Regardless of CYP2F2 expression, can human lung microsomal protein metabolize these substrates? If so, do they form the same metabolites as mouse lung microsomal protein?
3) Can we determine what level (rate of formation or concentration) of metabolites corresponds to the induction of tumors in mice?
4) Can we compare human rates of metabolism to rates of metabolism in mice at lung tumor inducing exposures?

To address the relevance of the mouse lung tumor MOA to humans:

1) Can other metabolites also contribute to the toxicity (regardless of whether through the same MOA)?
2) Is the bioactive metabolite only formed by CYP2F2?
3) Is the metabolite seen in humans, regardless of CYP2F2 expression? If yes, then the MOA is qualitatively applicable.
4) Can we determine a “threshold” level of metabolite formation responsible for tumors?

The quantitative risk or exposure limit for humans under different mechanistic scenarios can be estimated and compared in advance:

1) **Standard "nonlinear" approach:** If one assumes the proposed MOA is relevant to humans, this would involve determining the point of departure (POD) for the non-cancer precursor effect (e.g., cytotoxicity or induced proliferation) and using the default procedures to estimate the human equivalent concentration (HEC). Typically the POD would be identified as the lower confidence limit on dose for a specified level of effect from benchmark dose (BMD) analysis; i.e., the $BMDL_{10}$. For a category I gas with a portal of entry effect, the adjustment for ventilation per surface area (VE/SA) would be made and appropriate uncertainty factors (UFs) applied to arrive at an RfC.
2) **Standard "linear" approach:** On the other hand, if one assumes an unknown, low-dose linear (e.g., genotoxic) MOA, then one would estimate a cancer POD from mouse data using the multistage statistical model, use the same (category 1 gas) adjustment to extrapolate to humans, then obtain an inhalation unit risk from that HEC (i.e., 0.1/HEC if the POD was the lower confidence limit on 10% cancer risk in mice).
3) **Bioactivation/PBPK approach:** A more advanced and complex analysis could be conducted based on the assumed bioactivation (CYP2F2)-based MOA. For this approach rates of metabolism in mice and humans would need to be quantified and incorporated into respective PBPK models. The mouse model would be used to estimate rates of metabolism in the mouse lung at the exposure levels used in the cancer bioassay(s), which in turn would be used as dose inputs for BMD modeling of the cancer response. The mouse metabolism-metric $BMDL_{10}$ would then be assumed to apply to (or be scaled to) a human metabolism equivalent dose (HED). The human PBPK model would then be used to identify the corresponding human equivalent inhalation concentration (metabolism-metric HEC), which can be compared directly to the HEC obtained by the standard approach, or further converted to a bioactivation-based RfC and compared to that from the standard approach. (Since a PBPK model was used, the UF for animal-human TK conversion would be set to 1.)
4) **Harmonized approach**: Finally, a harmonized approach to cancer and noncancer risk assessment could be considered. As in the nonlinear approach described above, this would assume a threshold-based MOA, with a non-carcinogenic precursor step that is necessary but not sufficient (fully causative) for the cancer outcome. Exposures that only activate this precursor step to a limited extent would have to not produce tumors, while higher (or longer) exposures do. One could then conduct a nonlinear cancer risk assessment similar to the RfC method, including application of UFs. The result could be compared to the standard RfC. If higher than the standard RfC, one might choose to document the difference and use the standard RfC to be health protective.

In summary, Dr. Lipscomb suggested that a chemical-by-chemical approach would be less problematic than assuming a generalized CYP2F2-based MOA at present. To accept the proposed MOA for a specific chemical one would need to demonstrate specificity of the key metabolite to CYP2F2, specificity of the tumorigenic response to the CYP2F2 metabolite, and to conclusively demonstrate lack of formation of specific metabolite in humans, regardless of CYP2F2 expression. Alternative approaches that can be considered are a PBPK-based approach based on quantified rates of metabolism and a harmonized approach based on an established nonlinear MOA and identified precursor events. One also should consider the possibility that there are TD factors unique to mouse lung that affect response (e.g., similar to alpha 2 μ-globulin and male rat kidney tumors).

Session 3 Summary Discussion

Focus on CYP2F2 and 2F1? A participant questioned the need to specifically evaluate the expression and activity of CYP2F2 and 2F1. More specifically, using microsomal preparations a net rate of metabolism could simply be measured and used to evaluate relative risk. In response it was suggested that this would require knowledge that microsomal metabolism (and specific products of it) are the causative agents. It is possible that multiple metabolites are causative, some having proliferative effects and others being genotoxic. But if you have enough human data to account for possible polymorphisms and variation, such an approach could work. As a specific example, human lung microsomes are definitely known to metabolize naphthalene; in rhesus monkeys the rate is much lower than mice.

For styrene it has been shown that CYP2F2-mediated metabolism causes toxicity in the mouse lung, but there is also a circulating mutagen coming predominantly from the liver. How does EPA determine whether the tumor response is strictly due to cytotoxicity without the contribution of styrene oxide? If humans have much less 2F2 than mice, how would that factor in? Is that a quantitative adjustment? How does EPA address this? In response it was noted that there are quantitative methods for comparing dose-response data from animals and species. One would have to identify a precursor event or a measure of toxicity that can be evaluated in relation to the adverse outcome. Comparative data could be obtained in vivo and in some cases in vitro, depending on the relevance to humans. The dose-response data would be evaluated relative to the concentration of the active metabolite. Precedence for such applications exists (e.g., U.S. EPA health risk assessment for EGBE on the IRIS website).

Regarding styrene, the alternate opinion on the genotoxicity of circulating styrene oxide (SO) was voiced, noting that there are two genotoxicity studies in mouse lung that are both negative. SO did not cause an induction of lung tumors in AJ mice or chromosomal aberrations. However another participant stated that there are studies which demonstrate styrene adducts in the mouse lung. In particular, there is evidence in mouse lung tissue among different routes of administration in vivo that DNA is damaged, and there are consequences to that.

A participant asked if the focus should be on the quantity (protein expression level) of the CYPs or their species-specific activity. The focus on overall activity, which results from the combination of species-specific enzyme activity and expression, is likely most predictive of risk. In response to a follow-up question, it was noted that species-specific differences in either Km and/or Vmax of the CYP could be relevant (when only one enzyme produces significant activity); both are a quantitative differences that effect how much of the metabolite is produced. If you have competing metabolic pathways, the analysis becomes more complex; a simple measure of relative activity would not be sufficient to determine relative risk.

Types of genotoxic damage: A participant asked if oxidative adducts (e.g., O^6-methylguanine) had been evaluated, in the case that it is not a direct metabolite but an oxidative effect. Also, some weak carcinogens might be good tumor promoters, and those compounds may not have been tested in these types of systems. In the case of a weak carcinogen, it might not be one metabolite acting, but rather a mix of several compounds, such as occurs in cigarette smoking.

Human variability: A participant urged caution since we are comparing a genetically diverse human population to results from a few inbred strains of rodents. When you look at variation across the mouse genome, you have as much variation as you do in humans, but for the chemicals under consideration there

is a very small subset. The full species difference cannot be determined without fully evaluating variability among both mice and humans. For example, other mouse strains may express CYP2F2 but be resistant to lung-tumor induction from these chemicals. Only specific mouse phenotypes have been evaluated, when they are polygenic. In terms of toxicity, it is not just a question of production of the reactive metabolite, but how the animal handles it (i.e., pharmacodynamics).

Combination of effects*:* Reflecting earlier statements and discussion, it was noted that cytotoxic and genotoxic mechanisms are not mutually exclusive; a mechanism can incorporate both. A reactive metabolite may cause a mutation, cell proliferation and additional mutations. There are mathematical models of carcinogenesis that allow for proliferation and mutation separately, which have not been integrated into an assessment for these chemicals yet. Use of such models would be an approach to examine the impact of the two mechanisms together. A particular paper that examined the dual mode of action for naphthalene was mentioned (Bogen, 2008).

Alternate dosimetric tools*:* A participant asked if there are there any alternate tools like functional MRI to measure metabolite levels in tissues in situ, rather than relying on microsomal fractions or other artificial assays. An *in vivo* method of that type for lung dosimetry may not exist, but one alternate is to use tissue explants. For naphthalene, there are ongoing studies of this type. One would prefer to come as close as possible to the intact tissue. Micro-dialysis is another technique that could be considered.

Neonatal mice*:* A statement had been made about neonatal nice being more susceptible. A participant cautioned that evaluating quantitative susceptibility requires consideration of the dose level used. At higher doses neonatal mice were more susceptible because they could not excrete the compound, but at lower doses they were less susceptible. Another participant responded that a dose with high activity in the neonatal was a very small dose for an adult animal, but that elimination is an important factor. Dose transitions have been seen in other settings.

Focus on mouse lung*:* A participant noted that when health assessments are performed, mouse lung tumors would not be considered separately from other tissues and health effects. For each chemical the response in all tumor sites would be evaluated, and how they relate to each other. Where appropriate the analysis would extend across chemicals. The evaluation would consider both noncancer and cancer effects, and the MOA is intertwined in all of this.

Concern for animal welfare: A participant expressed a strong concern regarding the number of animal experiments being discussed in order to evaluate a single MOA. If one evaluates each substance separately, the amount of work and animals involved would be quite large. We do not have a general understanding of the key events, for which agreement is desired. Use of cell lines or culture systems that are close to the in vivo situation was suggested as a way past this dilemma.

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Session 4: Evidence for Cellular, Genetic, and Molecular Toxicity

Background and Introduction

Session Co-chairs: Nagu Keshava (US EPA) and Gary Stoner, PhD (Medical College of Wisconsin)

Carcinogenesis involves a complex series of events that alter the cell signals from its extracellular environment, thereby promoting uncontrolled growth. These alterations could induce cell proliferation leading to tumor development. Knowledge of the biochemical and biological changes that precede tumor development may provide important insights for determining whether a cancer hazard exists. Thus, understanding the range of key steps in the carcinogenic process (whether it be mutagenesis, increased cell proliferation, cytotoxicity, or receptor activation) becomes essential for evaluating the MOA of a particular agent. EPA has developed a framework for evaluating hypothesized carcinogenic MOA (U.S. EPA, 2005).

Most simply, genetic toxicity or genotoxicity can be defined as adverse effects occurring on genetic material and their associated mechanisms within the cell. Genetic materials include the DNA and supporting structures (histones) which assist in packaging DNA into higher level organizational structures known as chromosomes. Various cellular machinery, used to translate, replicate, and repair the genetic code stored in DNA, can also be affected and can lead to genotoxic outcomes. In general, genotoxic chemicals may be mutagenic or clastogenic. In either case, cell transformation from a normally functioning cell may lead to formation of a cancerous cell if the altered cell does not go through a normal programmed death (apoptosis) to remove the threat. It is well known that genotoxicity play a significant role in the development of tumor formation. Mutations in somatic cells can play a key role early in cancer initiation and might affect other stages of the carcinogenic process. All cancer cells acquire multiple mutations during carcinogenesis, therefore mutation induction or acquisition can be key events at some stage in all cancers.

In addition to genetic alterations, the study of epigenetics has been providing additional insight into mechanisms of carcinogenesis. Epigenetics includes methylation/demethylation processes of DNA, histone modifications, and micro RNA activation or inactivation. Evidence is recently emerging on the potential role of epigenetics in the MOA of mouse lung tumors. Although limited data base is available on epigenetic mechanism, any such data can be evaluated in the realm of MOA and weight of evidence for evaluating carcinogenesis or lung tumors. Furthermore, molecular and high throughput data are being generated that may be useful to better understand the adverse outcome pathways leading to formation of mouse lung tumors, and perhaps make comparisons to similar outcomes in humans.

4.1 An Overview of the Genotoxicity of Aromatic Hydrocarbons and their Reactive Intermediates

Stephen Nesnow (Independent Consultant)

Genotoxicity studies of the four aromatic compounds of interest (cumene, ethylbenzene, naphthalene and styrene) and some of their known reactive intermediates were discussed. The results presented were

obtained from review documents such as the NTP Report on Carcinogens, IARC Monographs, Cal/EPA reports, literature reviews and original peer-reviewed articles.

The genotoxicity database for ethylbenzene is limited both in terms of number of studies and the types of assays/endpoints evaluated. Most studies with ethylbenzene, gave negative results, but there were some weak positive responses. For example, human peripheral blood lymphocytes showed increased sister chromatid exchanges in the presence of S9 as did mouse lymphoma L5178Y cells. Similarly, Syrian hamster embryo cells were positive for micronucleus and cell transformation endpoints. Limited data was available (only DNA adducts and oxidized DNA studies) using the metabolites of ethyl benzene: 2-ethylhydroquinone and 4-ethylcatechol.

Cumene, the second compound discussed gave negative responses in bacteria and rodent cells in vitro, humans and human cells in vitro. All positive results came from rodent data. DNA damage was reported in the lungs of B6C3F1 mice and in the livers of F344 rats after gavage treatment, while negative responses were observed in several other organs. Micronuclei were also observed in F344 rat bone marrow cells when exposed by intraperitoneal injections. Cumene induced mouse lung tumors both in males and females, liver tumors in female mice and rat kidney tumors in males. Mutations in lung tumors were observed in both the K-*ras* and *p53* genes. α-Methylstyrene, a metabolite of cumene, induced liver tumors in mice and kidney tumors in rats. α-Methylstyrene did not induce mutations in bacterial cells or chromosomal aberrations in CHO cells, possibly due of lack of the appropriate tissue type used for metabolic activation. Mixed effects in the micronucleus assay in lymphocytes from B6C3F1 mice exposed by inhalation (females had positive results and male had negative results). α-Methylstyrene oxide, a metabolite of α-methylstyrene, was positive in bacterial mutation assays. It was concluded that for cumene that the α-methylstyrene metabolic pathway was consistent with the induction of mouse liver tumors but not mouse lung tumors.

Naphthalene induced lung tumors in mice (females) and nasal tumors in rats (both males and females). Naphthalene is not a mouse skin tumorigen, but it did form 1,2-naphthoquinone-DNA adducts in the skin of treated mice. Genotoxicity studies of naphthalene have provided both positive and negative results. Naphthalene did not induce mutation in bacterial or mammalian cells but was positive in the fruit fly. Although naphthalene did not induce DNA damage in bacteria or rat hepatocytes, positive effects were observed in vivo in both rats and mice, in liver and brain tissues. Chromosomal aberrations and sister chromatid exchange assays for naphthalene were positive in Chinese hamster embryo cells, but negative in human peripheral blood lymphocytes. Micronucleus studies were positive in the newt and in human MCL-5B cells, but negative in rodents. Naphthalene was negative in cell transformation assays. The genotoxicity of two major metabolites of naphthalene: 1,2-naphthoquinone and 1,4-naphthoquinone have been studied to a limited extent. 1,2-Naphthoquinone formed 1,2-naphthoquinone-DNA adducts in the skin of mice treated with this metabolite. The same DNA adducts were found in the skin of mice treated with naphthalene. 1,2-Naphthoquinone was positive in bacterial mutation assays with and without metabolic activation and positive in human cells for DNA damage and sister-chromatid exchanges. 1,4-Naphthoquinone was negative for mutation in mammalian cells, gave mixed responses for sister-chromatid exchanges in mammalian cells and was positive in the micronucleus assay in mammalian cells. It is possible that 1,2-naphthoquinone is a candidate for a reactive metabolite of naphthalene as a genotoxic compound. For the metabolite 1,4 naphthoquinone, there is little evidence for genotoxicity because of lack of studies required to make that conclusion.

Styrene induced mouse lung tumors both in males and females, and lymphohematopoietic cancers in humans. There were a number of studies in lung and other tissues where DNA adducts were found after administration of styrene to mice and rats using different routes of administration including inhalation and intraperitoneal administration. Most studies showed the formation of styrene oxide-DNA adducts. 8-Oxodeoxyguanosine adducts were also detected. It should be noted that the type of DNA adduct analytical method used, the route of exposure and the species may influence the formation and detection of DNA adducts. The results of assays for mutation were mixed; both positive and negative studies were available. Styrene was negative in bacterial assays and in *S. pombe*, and positive in the fruit fly and in *S. cerevisiae.* Styrene induced mutations in V79 cells in the presence of S9, but in L5178Y cells it produced negative response. Similarly, in humans, mixed results were obtained depending on the target cells and indicator gene. DNA damage was observed in vitro and in vivo assays. The available studies were mostly positive for DNA damage when tested among different cell types and routes of exposure. The results of DNA damage in were mixed in the human studies. Chromosomal aberrations were observed in plants, mammalian cells, in human peripheral blood lymphocytes and in Wistar rat bone marrow after inhalation exposure. However, negative results were reported in mice, Chinese hamsters and other rat strains. Also, negative results for chromosomal aberrations were reported in the B6C3F1 mouse lung. Micronucleus and sister chromatid exchange assays were mostly positive in all systems tested except in humans that reported mixed results, i.e. both positive and negative results were obtained. Assays for unscheduled DNA synthesis and cell transformation were negative.

Styrene oxide formed DNA adducts in several human cell types and in mice tissues after inhalation exposure. It was mutagenic in bacteria and in rodent and human cells. Styrene oxide induced DNA damage in rodent and human cells and in multiple tissues of mice in vivo. It induced chromosomal aberrations in plants, mammalian cells in culture, in human peripheral blood lymphocytes and in mice, but not in Chinese hamsters. It induced sister-chromatid exchanges in mammalian cells in culture, in human peripheral blood lymphocytes, and gave mixed responses in mice. Micronucleus test results for styrene oxide were positive in plants, mammalian cells in culture and in human peripheral blood lymphocytes, but negative in mice. One assay for unscheduled DNA synthesis was positive and one assay for cell transformation was negative.

It was concluded that unlike some strong genotoxins, aromatic hydrocarbons give a mixed pattern of responses seemingly dependent on many factors (e.g. metabolic capability, cell type, species, strain, gender, tissue, route of administration). In some cases they were only partially active across the breadth of bioassays for DNA adducts, DNA damage, mutation, chromosomal effects and related endpoints. For genotoxic activity, they may require specific groups of enzymes that are only induced by the parent chemical for their genotoxic responses (e.g. α-methylstyrene). The lack of substantial data on some of these agents hinders a full evaluation of their genotoxic potential. There is some evidence that ROS can contribute to the genotoxicity of several of these agents (e.g. ethylbenzene, naphthalene and styrene). In mouse lung, styrene induced styrene oxide-DNA adducts, 8-oxo-deoxyguanosine-DNA adducts, DNA damage and sister-chromatid exchanges. In mouse lung styrene oxide bound to DNA, induced 8-oxo-deoxyguanosine-DNA adducts, and DNA damage. Thus, there is evidence that styrene possesses genotoxic activity in mouse lung that could contribute to its MOA of lung tumor formation.

4.2 Mouse Lung Carcinogens, Reactive Metabolites, and Toxicity

David Eastmond (University of California, Riverside)

The presentation began with a discussion of the carcinogenicity of four compounds – benzene, ethylbenzene, naphthalene and styrene. The results from National Toxicology Program were provided which indicated that there was clear evidence for lung tumors including all tumor sites for benzene (gavage), some evidence for ethylbenzene and naphthalene (inhalation) and suggestive, but not convincing evidence for styrene (gavage). Significant increases in alveolar/bronchiolar adenoma or carcinoma were observed both in male and female mice for benzene, a significant increase in alveolar/bronchiolar adenoma or carcinoma was seen at the high dose in male mice for ethylbenzene, a significant increase in alveolar/bronchiolar adenoma was seen in female mice for naphthalene and a significant increase in lung adenomas and carcinomas combined was seen in male mice. Other non-NTP studies demonstrated a slight increase in lung toxicity for benzene, increases in DNA synthesis and decreased in metabolic enzymes in lungs in short-term studies for ethylbenzene, damage in mouse lung in multiple studies and selective damage in Club cells – particularly in the distal bronchioles for naphthalene; and lung tumors and hyperplasia in mice and other studies for styrene.

Specific discussion on benzene included that benzene is known to be a human leukemia agent, and is also lung carcinogen in mice. Some reports of lung cancer in humans are available; however, this is not widely accepted. Multiple metabolic pathways, and most likely, multiple mechanisms of action are involved in benzene's carcinogenic effects including the development of lung tumors. However, the critical mode of action is yet to be determined. MOA for styrene and its metabolites was also discussed. Styrene is metabolized to a number of epoxides, as well as aldehyde metabolites. The metabolic pathway for both humans and animals was discussed. The two major types of reactions of quinones and epoxides were also discussed. For quinones, arylation reactions, common to smaller quinones, result in thiol and amino adducts. However, for larger quinones, redox cycling is more common and can result in reactive oxygen species. Epoxides are electrophiles which can bind to DNA and proteins leading to multiple types of adducts. For some epoxides, it has been reported that a large percentage of the recovered adducts (~95%) are N7 guanine adducts. On the other hand, protein binding can result in amino and thiol adducts. Common reactions for aldehydes were also discussed. Aldehydes involved in protein binding can form Schiff bases so that the binding is reversible. Aldehydes can also bind to DNA leading to multiple adducts including protein-DNA crosslinks. The real challenge in all of these reactions is to identify which metabolites are involved and the importance of their involvement in toxicity. It is possible that different mechanisms are involved with different compounds. Examples of epoxide or epoxide-forming mouse lung carcinogens are ethylene oxide, glycidol, acrylamide, butadiene, chloroprene, urethane and vinyl chloride. Examples of mouse lung carcinogens due to bioactivation involving quinones, epoxides, or aldehydes include benzene, benzofuran, cumene, ethyl benzene, naphthalene and styrene.

The discussion continued on the importance of cytotoxicity and genotoxicity. The interrelationship between cytotoxicity and genotoxicity was discussed at some length. It was concluded that the relevance of genotoxicity results, as influenced by cytotoxicity, existed along a continuum, and that using a single cut-off point, as is commonly done, is overly simplistic. Further discussion of other mechanisms, other than genotoxicity and cytotoxicity, were presented which included apoptosis, necrosis and DNA breakage.

4.3 Overview of New and Developing Omic Technologies: Assessing Molecular Toxicity and Disease Susceptibility

Brian Chorley (US EPA, RTP)

Use of new and developing 'omic technologies in risk assessment were briefly discussed. The risk assessment challenges that the 'omic technology may help address include: (a) relevance to human condition and disease etiology, (b) susceptibility to disease, (c) defining early key events and biomarkers of MOA, (d) understanding adverse versus adaptive responses.

Discussion then focused on recent technological advances that have greatly improved the ability to measure genomic, epigenomic, proteomic, and metabolomic ('omic) alterations in both quantitative and cost effect manners. The significance and relevance of specific technologies were discussed. Further, 'traditional', current, and future technologies for genome-wide assessment were compared and contrasted. Specifically, a case study was presented that compared the results of RNA-sequencing and microarray-based data. Of particular interest, the example data was generated from a toxicological rat study. The differences seen with the two technologies were possibly due to the dynamic range limitations of each method and differences in normalization methods applied, which altered the perceived expression levels of gene at the extreme high and low ends.

Discussion led to describing advanced 'omic technologies, i.e., single molecule sequencing (third generation sequencing). Advantages of these technologies included increase throughput and lower costs, longer reads, detection of DNA modifications in real-time.

Brief discussion described developing tools that are used to delineate susceptibility to disease and exposures. An example of functional single nucleotide polymorphism discovery was given. The speaker also described the practice of genetic screens using inbred mouse strains to assess genetic susceptibility. While popular, the point made was that the resolution of such methods is limited. A comparison of traditional inbred mouse screens and next generation mouse genetic screens, such as the Collaborative Cross initiative, were discussed.

Discussion transitioned to focus on new technologies that can assess epigenetic alterations including chromatin changes, DNA methylation, non-coding RNA, biomarkers, and others. Examples of these genome-wide assessments of epigenetic alterations featured array and 2nd generation sequencing-based technologies. Of specific relevance to the workshop, data on importance of microRNA expression in lung cancer was presented. Importantly, several studies have indicated the role of microRNA (or 'oncomiRNA') in lung cancer, particularly in non-small-cell lung cancer.

The presentation concluded that there is real potential of utilizing 'omics-based data for chemical risk assessment, although some hurdles remain in terms of standardization, reproducibility, and acceptance.

4.4 Metabolomics

Timothy Fennel (RTI International)

The presentation included further use and relevance of new technologies in risk assessment. Metabolomics involving the broad spectrum analysis of the low molecular weight complement of cells, tissues, or biological fluids was discussed. Metabon(l)omics is used to determine the pattern of changes (and related metabolites) arising from a disease, dysfunction, disorder, or from the therapeutic or adverse effects of xenobiotics; including applications in plant and mammalian studies. The discussion included the difference between metabolomics and metabonomics which could be used interchangeably. Metabolomics can identify specific genes that define individuals at risk for a disease, dysfunction, or disorder, or response to treatments. Importance of metabolites and their role was also briefly discussed. Furthermore, examples of how metabolomics technology is being used in rodents exposed to chemicals such as benzene was discussed.

Current institutions/centers that are conducting research in the area of metabolomics was provided and highlighted. The six regional comprehensive metabolomics resource core were funded by National Institute of Health with a goal to increase national capacity to provide metabolomics profiling and data analysis services to basic, translational, and clinical investigations; to foster collaborative efforts that will advance translational research using metabolomics approaches; to facilitate institutional development of pioneering research, metabolomics training, and outreach; and to establish national standards.

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Workshop Summary Session

Dr. Woodall convened the final, summary session for the workshop with a reiteration of the goals of the workshop. All of the Session Co-chairs were brought to the front of the room to provide the key points from the discussions in their respective Sessions. Those final points are provided in Table 5-1, below.

Following the presentation of key points from the individual sessions, there was an open discussion to help define the potential for follow-on activities from the MLTW to pursue in the near future. A list of the identified activities was captured in real-time and projected both in the room and on the webinar. It was noted that there would need to be a prioritization of the listed candidate activities, and that the time constraints for the final session would require that prioritization take place after the workshop had adjourned. The list provided below has been somewhat revised from the list captured during the workshop to reflect some clarifications made post-meeting, and re-ordered into related topic areas.

Parking Lot of Other Issues

A number of issues were placed into a "parking lot" for later consideration, but were not necessarily ready to pursue as follow-on activities. The list below includes those parking lot issues.

- A review across strains and doses may be informative
- Are there additional stains that might help determine cell of origin?
- Does severity of final tumor matter for risk assessment?
- Is adenoma a pre-cursor to adenocarcinoma in mice?
- Robert Sills (NIEHS) is currently performing a review on cobalt dust, which may be useful for consideration of lung tumor formation processes
- Differences between chemically-induced tumors and spontaneous tumors may be useful
  - Mutational spectra were mentioned as a potential tool to elucidate those differences, if they exist.

Workshop Outcomes

Dr. Woodall noted that there were two indirect outcomes which were reported on during the MLTW and brought about through the planning for the workshop. The histopathological analyses of historical NTP tissue samples, reported by Dr. Pandiri, were instigated in anticipation of presentation at this event. A second outcome was the first public presentation of the IARC tissue concordance research by Dr. Krewski. Drs. Krewski and Woodall discussed the potential for such a presentation while at another meeting, which led Dr. Krewski to request permission from IARC to present these findings.

More directly, it was noted that one of the primary goals of the workshop was to have an open discussion to identify the key elements which would go into a future application in a MOA framework. One participant mentioned the following points: to not apply the discussed information in an MOA framework would be a missed opportunity; much of the discussion was related to mechanisms as opposed to mode of action; and pursuing an integrated discussion around an MOA framework would help identify "data gaps" for risk assessment purposes versus "data needs" from a research perspective. Dr. Woodall agreed that application of the information discussed during the MLTW into a MOA framework on a chemical by chemical basis would be a logical follow-on activity; however, Dr. Woodall also noted that the more open discussion from multiple perspectives, as accomplished in the MLTW, was a necessary first step before

taking on such a task. It was also noted during the discussions in multiple sessions of a lack of a strong basis for a common MOA for the three key chemicals, or with any of the chemicals with potentially similar mechanisms for tumor formation.

Closing

The MLTW was adjourned noting that additional discussions of the proposed follow-on activities may be considered. Thanks and gratitude were also relayed for contributions from the Co-chairs, Panelists, Speakers, and Participants to making the MLTW a successful event.

Table 5-1. Compiled List of Candidate Follow-on Activities

PBPK model development (identified as a follow-on activity in the planning stages)
Web-based discussion of needs and priorities [1]
Epidemiological studies
Update review of epi studies since last IARC review (assume styrene)
Meta-analysis with styrene epi studies? Collins et al. study was of a single cohort.
Consider observations in SBR workers. Issue of co-exposure to butadiene.
Better characterization of potential confounders in epi studies (e.g., SES of workers in Collins et al. study)
Genetic Toxicology
More complete analysis of Genetic Toxicology data, using work by Nesnow and Kligerman and as the basis.
Perform meta-analysis of sister chromatid and micronuclei data in humans.
Analysis of temporality of genotoxic event; may be more important than mutation leading to tumor
P-53-knockout to evaluate genotoxicity (existing data?)
Omics Technologies
Genomics analysis of existing data to determine relevancy of 2f2-mediated carcinogenesis to humans.
Probes/other analyses of historical NTP samples

1 PBPK Considerations include the following:

- Focus on models for risk assessment first; models developed for hypothesis testing are secondary
- 2F2 not yet incorporated into existing PBPK models. Is it needed?
- What empirical data are needed for model validation?

Histopathology
Complete analysis for samples for "other" chemicals
Do chemicals that induce lung tumors in mice increase 8-oxo-deoxyguanosine and levels of other indicators of ROS in cells?
MOA
Implement MOA framework on a Chemical-specific basis [2]
Apply the Onco-gene model to evaluate MOA for the key chemicals
Cross-cutting Issues
Evaluate differences between spontaneous and chemically-induced tumors
Systematic review of similarities and differences between human and mouse lung tumor
Review Studies of CYP2F1 polymorphisms in ethnic populations [3]
General Topics
Evaluate historic NTP samples – do we see these kinds of tumors more often now than previously? Is it strain specific?
Workshop on advancing cancer risk assessment, including consideration of chemicals which have cancer risks below RfC or RfD
Is there a threshold below which cancer is not induced?
6 chemicals produced tumors in rats only; 7 in mice only: evaluate, then discuss.
Analyze RAS pathway using transgenic model for accelerated cancer studies

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2 MOA Considerations:

- Integrated discussion of relationships between available data sets with focus on “mode” rather than “mechanism.”
- Will help to identify data “gaps” versus “needs” to move forward and use available data
- Need to schedule initiation with IRIS process to ensure all relevant studies/data are considered. If this is completed too far in advance, it may become outdated.

3 Polymorphism for CYP 2F1

- Studies suggest production of truncated (inactive?) proteins.
- Examine this literature to infer whether any evidence exists for Cyp 2F2-like quantitative metabolic characteristics with polymorphisms in humans.
- Use information on human variability to create data-derived uncertainty factors

APPENDICES

APPENDIX A: Panelists, Speakers and Project Core Team

Provided below are short biographical sketches for the Co-chairs, Panelists, and Invited Speakers for each Workshop Session. Bio-sketches are also provided for the Workshop Project Core Team.

Session 1. Human Cancer Epidemiology and Pathophysiology

Co-Chairs: Jason Fritz (US EPA) and Eric Garshick (Harvard Medical School/VA Boston Healthcare System)

Panelist	Title	Affiliation
Jason Fritz, PhD	Toxicologist	US Environmental Protection Agency, National Center for Environmental Assessment

After service as a U.S. Marine, Jason Fritz received his baccalaureate in Biochemistry from the University of Denver, and then completed his graduate training at the University of Colorado Anschutz Medical Campus, where he received a Ph.D. in Toxicology studying the effects of chronic inflammation on lung carcinogenesis. Dr. Fritz also received post-doctoral training at UC-AMC, prior to a fellowship in the National Center for Environmental Assessment, part of the Office of Research and Development, within the U.S. Environmental Protection Agency. He is an actively contributing member of the Society of Toxicology, the American Association for Cancer Research, and the Society for Risk Analysis. He also serves as an ad-hoc reviewer for the journal Carcinogenesis, and as reviewer and member of the editorial board for Toxicology Mechanisms and Methods. Currently, Dr. Fritz is a staff Toxicologist, assessment manager and co-chair of the Toxicity Pathways workgroup within the Integrated Risk Information System (IRIS) Division, where he has been engaged in evaluating the health hazards associated with chronic exposure to agents such as acrylonitrile, formaldehyde, and benzo(a)pyrene. He has also advised on recent promulgations of National Emission Standards for Hazardous Air Pollutants regarding the production of acrylic/modacrylic fibers, polymers and resins, in support of EPA's ongoing mission to protect human health and the environment.

Eric Garshick, MD, MOH	Associate Professor of Medicine/Physician	Harvard Medical School/VA Boston Healthcare System

Dr. Garshick received his Bachelor's degree in Chemical Engineering and Biology in 1975 and his MD degree in 1979, all from Tufts University. He received training in epidemiology at the Channing laboratory, Brigham and Women's Hospital, and received a Masters of Occupational Health degree from the Harvard School of Public Health in 1984. He trained in Internal Medicine at Beth Israel Hospital in Boston and in Pulmonary Medicine at the Brigham and Women's, Beth Israel, and West Roxbury VA Hospitals, and is Board Certified in Internal Medicine, Pulmonary Diseases, and Critical Care Medicine.

In addition to practicing Pulmonary and Critical Care Medicine at VA Boston, he has been the principal investigator of two NIH studies examining lung cancer mortality in relation to diesel exhaust exposure in railroad workers and trucking company workers and participated in the IARC assessment regarding diesel exhaust and cancer. He served as a consultant to the EPA Clean Air Scientific Advisory Committee regarding diesel exhaust, and served on the Institute of Medicine's Committee on Gulf War and Health assessment of environmental particulates and pollutants. He has also served a grant reviewer for NIH from 2005-2011 as a member of the Infectious Diseases, Reproductive Health, Asthma, and other Pulmonary Diseases Study Section, and has served as a reviewer on the VA Merit Review Panel for the Rehabilitation Research and Development since 2008. He has published 98 peer-reviewed papers.

James J. Collins, PhD	Director of Epidemiology	Dow Chemical Company

Dr. James Collins received his PhD in 1981 from the University of Illinois at Urbana-Champaign and is a Fellow in the American College of Epidemiology. He is currently the Director of Epidemiology at the Dow Chemical Company in Midland, Michigan. He is also an Adjunct Research Professor at the University of Pittsburgh, School of Public Health and at Saginaw Valley State University. Prior to joining Dow, he directed epidemiology programs at Solutia, Monsanto, Ford, and American Cyanamid and worked at Argonne National Laboratory. His major research interest is the impact of occupational and environmental exposures on health including exposures from dioxins, benzene, acrylonitrile, acrylamide, formaldehyde, styrene, and glutaraldehyde. He has published more than 100 papers in these areas. He is currently on the Editorial Boards for Environmental Health Perspectives, Journal of Environmental and Occupational Medicine, and the Open Epidemiology Journal. He has also has served on several science advisory committees.

Brigitte Gomperts, MD	Associate Professor	University of California-Los Angeles, Department of Pediatrics

Dr. Brigitte Gomperts received her medical degree from the University of the Witwatersrand, Johannesburg, South Africa, and her training as a Pediatric Hematologist-Oncologist at Washington University in St. Louis. She is currently an Associate Professor at the University of California, Los Angeles and a member of the Jonsson Comprehensive Cancer Center and the Broad Stem Cell Research Center. She is also a member of the American Thoracic Society. Her lab studies lung repair and regeneration from stem cells in health and disease. She has published more than 30 peer-reviewed papers in this area. Her lab is interested in understanding the mechanisms of airway basal stem cell repair and how this process goes awry during the development of premalignant lesions. She is also interested in identifying driver mutations that are associated with the stepwise progression of premalignant lesions to squamous non-small cell lung cancer. Her lab has developed novel in vivo and in vitro human and mouse models to study the process of stepwise progression to lung cancer in order to study these processes.

Daniel Krewski, MHA, MSc, PhD	Director	McLaughlin Centre for Population Health Risk Assessment, University of Ottawa

Dr. Daniel Krewski is Professor and Director of the R. Samuel McLaughlin Centre for Population Health Risk Assessment at the University of Ottawa, where he is involved in a number of activities in population health risk assessment within the new Institute of Population Health. Dr. Krewski has also served as Adjunct Research Professor of Statistics in the Department of Mathematics and Statistics at Carleton University since 1984. Prior to joining the Faculty of Medicine at the University of Ottawa in 1998, Dr. Krewski was Director, Risk Management in the Health Protection Branch of Health Canada. While with Health Canada, he also served as Acting Director of the Bureau of Chemical Hazards and as Chief of the Biostatistics Division in the Environmental Health Directorate. Dr. Krewski obtained his Ph.D. in statistics from Carleton University and subsequently completed an M.H.A. at the University of Ottawa. His professional interests include epidemiology, biostatistics, risk assessment, and risk management.

Session 2. Comparative Pathological Evidence for Lung Tumors

Co-Chairs: Charles Wood (US EPA) and Mark S. Miller (Wake Forest University)

Panelist	Title	Affiliation
Charles E. Wood, DVM, PhD, DACVP	Research Biologist	US Environmental Protection Agency, National Health Effects and Environmental Research Laboratory
Dr. Wood is a research scientist and pathologist within the Carcinogenesis Branch of the National Health and Environmental Effects Research Laboratory at the US Environmental Protection Agency (EPA) in Research Triangle Park, NC. He received his DVM from the University of Georgia, College of Veterinary Medicine and completed a fellowship in Comparative Pathology and PhD in Molecular and Cellular Pathobiology from the Wake Forest University School of Medicine, where he then served as a faculty member in the Department of Pathology with a joint appointment in Translational Science prior to joining EPA. Dr. Wood's background is in comparative/translational pathology and cancer biology. His research interests relate broadly to cancer risk modeling and mechanisms of carcinogenesis, with recent emphasis on prospective applications of the mode of action framework to improve chemical prioritization efforts. This work supports EPA programs related to chemical safety and water and air quality. In recent years he has served on various scientific advisory boards, expert review panels, and grant review sections related to this work. He currently serves as a member of the Cancer Assessment Review Committee for the US EPA Office of Pesticide Programs and as an ad hoc pathology advisor for several EPA science councils. Other professional activities include participation in various pathology work groups and scientific societies.		
Mark Steven Miller, PhD	Professor of Cancer Biology	Wake Forest School of Medicine
Dr. Miller received his Bachelor's degree in the Biological Sciences from Fordham University in New York and then completed his graduate training at Columbia University, where he received a PhD in Pharmacology in 1983. Dr. Miller received additional postdoctoral training in the Laboratory of Toxicology at the Massachusetts Institute of Technology from 1983 to 1986 and in the Laboratory of Comparative Carcinogenesis at the National Cancer Institute from 1986-1990. He previously held a faculty position at the University of Tennessee and joined the faculty at the Wake Forest University School of Medicine in 1996, where he currently holds the position of Professor of Cancer Biology and Physiology/Pharmacology. Dr. Miller has served on NIH, DOD, and EPA grant review panels, as well as serving as Chair of the IRIS Assessment of Nitrobenzene for the EPA; as a member of the Alcohol and Toxicology study section for the NIH, is an ad hoc reviewer for several journals and NIH and EPA study sections, and has served as an officer in the Society of Toxicology and the Genetic and Environmental Mutagenesis Society. He has published more than 75 articles in peer-reviewed journals. His research interests have focused on the interaction between environmental and genetic factors in determining the molecular pathogenesis of lung cancer utilizing a variety of in vivo animal models. Recent studies from have focused on (1) determining environmental/genetic interactions that affect an organism's susceptibility to lung cancer formation, particularly as it relates to the effects of environmental chemicals on the developing fetus; (2) the development of novel chemopreventive agents to prevent lung cancer formation in high risk individuals; and (3) use of imaging techniques to assess early lesion development. He has expertise with murine models of lung cancer and the analysis of tumors by biochemical and molecular techniques.		

Gary A. Boorman, DVM, PhD, DABT, DACLAM, DACVP	Toxicologic Pathologist	Covance, Inc.
Gary Boorman received his Doctorate of Veterinary Medicine from the University of Minnesota, spent a year in mixed practice in Wisconsin followed by a Laboratory Animal Residency at the University of Michigan where he received a Masters in Pathology. This was followed by four years at the Institute for Experimental Gerontology, TNO in Rijswijk, The Netherlands. Gary did a pathology residency at the University of California, Davis where he received his PhD in Pathology. He spent 30 years at the National Toxicology Program (NTP) at the National Institute of Environmental Health Sciences (NIEHS) with a focus mainly on Rodent Carcinogenicity Studies. Gary currently works with non-clinical studies at Covance Inc. located in Chantilly, Virginia. Gary is a Diplomate of American Board of Toxicology (ABT), American College of Veterinary Pathologists (ACVP), and the American College of Laboratory Animal Medicine (ACLAM), and a Fellow, International Academy of Toxicologic Pathology (IATP). In 2006, Gary was recognized as a Distinguished Research Alumnus of the College of Veterinary Medicine, University of Minnesota. He was recognized as a Distinguished Member, American College of Veterinary Pathologists in 2010. In 2012 Gary was given the Lifetime Achievement Award by the Society of Toxicologic Pathology. Gary's currents interests are rodent pathology and the use of genomic technologies to enhance our understanding of the morphological changes we find in non-clinical studies.		
Laura Van Winkle, PhD	Adjunct Professor	University of California at Davis
Dr. Laura Van Winkle is currently an Adjunct Professor in the Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California (UC) at Davis. She is also a Research Cell Biologist in the Center for Health and the Environment, John Muir Institute of the Environment also at UC Davis. She received her PhD in 1995 and has been a Diplomate of the American Board of Toxicology since 2002. She is a respiratory toxicologist with specialized training in airway cell biology, respiratory disease, and pathology of conducting airway epithelial injury and repair. Her research includes the study of air pollutants, ingested chemicals, allergens, and engineered nanomaterials and their effects on the adult and developing lung. She served as a peer review panel member of the EPA review of naphthalene carcinogenicity in 2004. She has reviewed grants for NIH and the Florida Department of Health and served as a councilor for the Inhalation and Respiratory Specialty Section of the Society of Toxicology. Professional affiliations include the American Society for Cell Biology, Society of Toxicology, American Physiologic Society and the American Thoracic Society, where she currently serves on the ATS Environmental Health Policy Committee. She has published over 60 papers in peer reviewed journals. She has 20 years of experience studying naphthalene pathology and mechanism of action.		
Arun Pandiri, BVSc&AH, MS, PhD, Diplomate ACVP, ABT	Pathologist	Experimental Pathology Laboratories, Inc. (Contractor to NTP, NIEHS)
Dr. Arun Pandiri is a pathologist at the Experimental Pathology Laboratories, Inc. in Research Triangle Park, North Carolina and provides on-site contract support for the Cellular and Molecular Pathology Branch of the National Toxicology Program within the National Institute of Environmental Health Sciences. He received his Veterinary Medical degree from ANGR Agricultural University, Hyderabad, India, and his PhD from Michigan State University. He completed his pathology residency training at North Carolina State University and is a diplomate of the American College of Veterinary Pathologists. He has interests in chemical-induced tumorigenesis and lung and gastrointestinal pathology. He is an active member in the Societies of Toxicologic pathology (STP) and Toxicology (SOT).		

Session 3: Biological Mechanisms

Co-chairs: Paul Schlosser (US EPA) and Ronald Melnick (Ron Melnick Consulting)

Panelist	Title	Affiliation
Paul Schlosser, PhD	Environmental Health Scientist	US Environmental Protection Agency, National Center for Environmental Assessment
Paul Schlosser received his Bachelors of Science (1982) and PhD (1988) from the University of Rochester, with a Masters of Applied Science (1984) from the University of Toronto, all in Chemical Engineering. He then conducted three years of postdoctoral research in Biochemical Engineering at the California Institute of Technology, developing methods to identify limiting factors in biochemical pathways used in industrial fermentation and cell cultures. In 1991 Paul joined the Chemical Industry Institute of Toxicology (later the CIIT Centers for Health Research, now The Hamner Institutes), and conducted research on the modeling of xenobiotic metabolism and dosimetry, with applications in risk assessment. Because of his background training in chemical engineering which includes transport phenomena, one focus of this work was inhalation dosimetry, particularly that of formaldehyde. Dr. Schlosser came to the U.S. EPA, National Center for Environmental Assessment (NCEA) in 2004 as an Environmental Health Scientist. Dr. Schlosser now co-chairs the NCEA's Pharmacokinetic Workgroup (PKWG), which is tasked with evaluating and guiding or conducting the application of PBPK and PK models in risk assessment. He has been a primary contributor to the completed Toxicological Reviews for dichloromethane and methanol (non-cancer). Paul also works on developing methods to quantify variability and uncertainty in PBPK model predictions. In professional society service, he has served as councilor of Biological Modeling Specialty Section (BMSS) of the Society of Toxicology (SOT); secretary/treasurer of North Carolina Chapter, SOT; vice-president, president-cycle and trustee-at-large of the DRSG; and president-cycle and board member of the Research Triangle Chapter, SRA.		
Ronald Melnick, PhD	Director	Ron Melnick Consulting, LLC
Ron Melnick received his Ph.D. from the University of Massachusetts in Amherst and was a postdoctoral fellow at the University of California in Berkeley. He was an assistant professor of Life Sciences at the Polytechnic Institute of New York, and then spent 28+ years as a toxicologist at the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program. At NIEHS, he was involved in the design, monitoring and interpretation of toxicity and carcinogenesis studies, and conducted mechanistic research to characterize potential health effects of environmental and occupational agents. He spent a year as an agency representative to the White House Office of Science and Technology Policy to work on interagency assessments of health risks of environmental agents and on risk assessment research needs in the federal government. Dr. Melnick has organized several national and international symposiums and workshops on health risks associated with exposure to toxins. After retiring from NIEHS, he established Ron Melnick Consulting, LLC. He has served on numerous scientific review boards and advisory panels, including those of the International Agency for Research on Cancer and EPA. He was the recipient of the American Public Health Association's 2007 David P. Rall Award for science-based advocacy in public health.		

Timothy Fennel, PhD	Director of the RTI Center for Nanotechnology Health Implications Research	Research Triangle Institute
Timothy Fennell trained as a biochemist and has extensive experience in understanding the metabolism of chemicals and the role of metabolism in toxicity. He has conducted investigations of the metabolism of a wide variety of chemicals, including styrene. He has more than 30 years of experience in mechanistic-based research and is recognized as an expert in biomarkers, particularly in the area of reactive chemicals/metabolites and exposure assessment via protein- and DNA-adduct measurement. Dr. Fennell serves as the director of the RTI Center for Nanotechnology Health Implications Research funded by the National Institute of Environmental Health Sciences. He holds a PhD in Biochemistry and a BSc in Biochemistry (Honors) from the University of Surrey, Great Britain.		
Kathleen Burns, PhD	Director	ScienceCorps LLC
Dr. Burns is a toxicologist who specializes in chemical risk assessment focused on mode of action, threshold, elevated susceptibility and related concepts. She worked for state and federal agencies for 20 years before founding Sciencecorps in 2004. She assisted EPA in the development of air and water regulations, the TRI program, TSCA regulations, cost benefit methods, RSEI and other agency programs. She manages investigative teams and conducts environmental justice and epidemiological studies and risk assessments of chemical and radiological contamination of air, water, soil, consumer products, food and workplaces. She provides public health support to communities, litigation support on water contamination cases and assisted in drafting state and federal legislation. Dr. Burns has policy, science and public health degrees and training from the University of Chicago, the University of Illinois Medical Center in Chicago, Harvard University, and Northwestern University Medical School. She is a member of the American College of Occupational and Environmental Medicine and the International Society for Environmental Epidemiology.		
Ernest Hodgson, PhD	Distinguished Professor Emeritus	Department of Environmental & Molecular Toxicology, NC State University
Dr. Ernest Hodgson is a Distinguished Professor Emeritus at North Carolina State University and Executive Director of the Foundation for Toxicology and Agromedicine. Dr. Hodgson has conducted research on xenobiotic biochemistry for several decades, has authored c. 400 peer-reviewed papers in this area, and is editor and part author of several monographs. Most recently his research has focused on human studies utilizing human hepatocytes and sub-cellular preparations. He is currently involved in RNAseq studies of genome-wide effects of environmental chemicals. Dr. Hodgson is also editor and contributing author of toxicology textbooks (***Textbook of Modern Toxicology and Molecular and Biochemical Toxicology***, both currently in their 4th editions). He has been recognized by awards from the Society of Toxicology, the American Chemical Society, the International Society for the Study of Xenobiotics, the Consolidated University of North Carolina, and North Carolina State University.		

Invited Speaker	Title	Affiliation
George Cruzan, PhD	President	ToxWorks
Dr. George Cruzan provides toxicology consulting to a variety of companies and trade associations. Projects have included regulatory interactions and comments on proposed actions; toxicologic evaluation, including assessment of database, design of research programs, monitoring of studies, and integration into mode of action presentations, and presentations to regulatory agencies. Styrene health effects and mode of action (MOA) has been a major focus of his activities since 1989. He served as chairman of the Science and Technology Task Group of the Styrene Information and Research Center (SIRC) from 1991 to 1995, and has provided science consulting to SIRC since 1995. He was a member of the IARC Panel that reviewed styrene and naphthalene in 2002. He is the lead author on five publications on the MOA of mouse lung tumors from styrene. He has been certified in Toxicology by the American Board of Toxicology since 1980 and a member of the Society of Toxicology since 1986.		
John Lipscomb, PhD	Toxicologist	US Environmental Protection Agency, National Center for Environmental Assessment
Dr. Lipscomb is a toxicologist and risk assessor. His activities and interests center on replacing default options with science-based decisions. He has over 20 years experience in toxicology and risk assessment, including the US EPA, US FDA/NCTR and uniformed service in the US Air Force. He has published 62 articles, 10 book chapters, 31 government reports, edited a text on Toxicokinetics and Risk Assessment, written risk assessment guidance for the US EPA and the WHO's International Programme on Chemical Safety, and served as a technical advisor to the American Water Works Association for its research on drinking water disinfection byproducts.		

Session 4. Evidence for Cellular, Genetic, and Molecular Toxicity

Co-chairs: Nagu Keshava (US EPA) and Gary Stoner, PhD (Medical College of Wisconsin, Division of Hematology and Oncology)

Panelist	Title	Affiliation
Nagu Keshava, PhD	Toxicologist	US Environmental Protection Agency, National Center for Environmental Assessment

Dr. Keshava is currently a Senior Toxicologist at the National Center for Environmental Assessment, Office of Research and Development (ORD), Environmental Protection Agency (EPA), Washington DC, USA. Prior to moving to EPA, she was at Centers for Disease Control - National Institute for Occupational Safety and Health (CDC/NIOSH). She graduated with a Ph.D. from West Virginia University majoring in Genetics and Developmental Biology. Her areas of scientific expertise and interests include genetic toxicology, mode of action, risk assessment and cancer biology. At EPA, she has led or contributed to risk assessments of various chemicals including 1,2-dichloroethane, trichloroethylene, ethylene oxide, tetrachloroethylene, and formaldehyde. Dr. Keshava has provided scientific support to program offices within EPA and other federal agencies. She has received several awards including the Gold and Bronze medals from U.S. Environmental Protection Agency. She is a member of professional societies including Environmental Mutagenesis and Genomics Society, Society of Toxicology, Genetics and Environmental Mutagenesis Society (GEMS). She has authored or co-authored over 40 peer-reviewed articles and book chapters in journals including Cancer Research and Proceedings of National Academy of Sciences. She has also contributed to numerous governmental and intergovernmental reports. Dr. Keshava has served on several committees, organized and chaired workshops and symposium at the Environmental Mutagenesis and Genomics Society, Genetics and Environmental Mutagenesis society. She is a past president of GEMS.

Gary Stoner, PhD	Professor of Medicine	Medical College of Wisconsin, Division of Hematology and Oncology

Dr. Gary Stoner is Professor of Medicine at the Medical College of Wisconsin Division of Hematology and Oncology, specializing in the fields of chemical carcinogenesis and cancer chemoprevention. He currently serves as Director of the Molecular Carcinogenesis and Chemoprevention Program in the newly developing Cancer Center. Dr. Stoner received his PhD in microbiology from the University of Michigan in 1970 and subsequently became a post-doctoral fellow and research scientist at the University of California-San Diego. While at UCSD, his research focused on the development of the A/J mouse model of lung cancer for identification of environmental carcinogens and mechanistic studies of lung carcinogenesis. He then joined the Laboratory of Human Carcinogenesis at the National Cancer Institute where he conducted research on the metabolism of tobacco carcinogens in human lung tissues and developed human lung cell culture systems for investigations of carcinogen/oncogene-induced cell transformation. He later became involved in chemoprevention research at the Medical College of Ohio. His research is documented in more than 350 peer-reviewed publications and book chapters, and he has edited several books. Dr. Stoner has served on several grant and contract review committees including the NIH Chemical Pathology Study Section, the NCI Cancer Biology and Immunology Contract Review Committee, and as Chair of the NIH Chemo/Dietary Prevention Study Section and the American Cancer Society Advisory Committee on Carcinogenesis, Environment and Nutrition. He has also served as President of the Carcinogenesis and Molecular Biology Specialty Sections of the American Society of Toxicology and of the Ohio Valley Society of Toxicology. He has received numerous awards including the NIH MERIT award, and the Distinguished Alumni Award and Honorary Doctorate from Montana State University. He is also a Fellow in the American Association for the Advancement of Science.

David Eastmond, PhD	Chair, Cell Biology & Neuroscience; Professor of Cell Biology & Toxicologist	University of California Riverside

Dr. David A. Eastmond is a professor and chair of the Department of Cell Biology and Neuroscience at the UC Riverside. He received his B.S. and M.S. degrees from Brigham Young University in Provo, Utah and his Ph.D. from the University of California, Berkeley. From 1987 to 1989, he was appointed as an Alexander Hollaender Distinguished Postdoctoral Fellow at Lawrence Livermore National Laboratory. Shortly thereafter, Dr. Eastmond joined the faculty at UC Riverside where he is actively involved in research and teaching in the areas of toxicology and risk assessment. The research in Dr. Eastmond's laboratory focuses on the mechanisms involved in the toxicity and carcinogenesis of environmental chemicals. His research has centered on the metabolism and chromosome-damaging effects of various environmental chemicals including benzene, a widely used industrial chemical and environmental pollutant, and ortho-phenylphenol, a commonly used fungicide and disinfectant. Dr. Eastmond served as the president of the Environmental Mutagen Society and as a Jefferson Science Fellow in the State Department. He has also participated on a variety of review or advisory panels related to chemical mutagenesis, carcinogenesis and risk assessment including panels for EPA, the US Food and Drug Administration, the National Toxicology Program, the International Programme for Chemical Safety, the International Agency for Research on Cancer, the Organisation for Economic Cooperation and Development, Health Canada, and the International Working Group for Genotoxicity Testing. He currently serves as a member of the Carcinogen Identification Committee for the California Environmental Protection Agency.

Andrew Salmon, PhD	Scientific Advisor	California EPA, Office of Environmental Health Hazard Assessment

Dr. Salmon is currently a Scientific Advisor in Cal/EPA's Office of Environmental Health Hazard Assessment (OEHHA), Scientific Affairs Division, working on special assignments for research collaboration, recruitment and training. Previously, he was Chief of the Air Toxicology and Risk Assessment Unit, in the Air Toxicology and Epidemiology Section of OEHHA. He has worked in OEHHA for the past 25 years doing public health risk assessment, initially for Proposition 65 and more recently in support of the California Air Resources Board's Toxic Air Contaminants program. Current interests include mechanism of action of inhaled toxicants, methodology for cancer and non-cancer risk assessment, identification and estimation of special risks to children's health from air pollutants and potentially toxic contaminants in biogas. He was previously a Lecturer in Industrial Toxicology in the TUC Centenary Institute of Occupational Health at the London School of Hygiene and Tropical Medicine. He has also worked on the metabolism and toxicity of carcinogenic chemicals at University College Hospital Medical School, London, at the University of California, Berkeley and for an industrial toxicology research laboratory in England. Dr. Salmon holds an undergraduate degree in Biochemistry, and a doctorate, from the University of Oxford, England.

Andrew Kligerman, PhD	Research Biologist/Genetic Toxicologist/Cytogeneticist	EPA National Health and Environmental Effects Research Laboratory

Dr. Andrew Kligerman is a research biologist in the Integrated Systems Toxicology Division at EPA in Research Triangle Park, NC. He has been a cytogeneticist and genetic toxicologist at the EPA for more than 24 years. He is currently doing an informal rotation with the National Center for Computational Toxicology at EPA, where he is investigating the sensitivity and specificity of high-throughput tests for determining the genetic toxicology of chemicals. For the vast majority of his research career at EPA, he has studied the genotoxicity of important environmental and commodity chemicals. For the previous 10 years, his research has concentrated on investigating the mode of action of arsenicals in inducing genetic damage and cancer. Prior to joining EPA, Dr. Kligerman was a program leader at EHRT, Inc. and staff cytogeneticist at CIIT. Dr. Kligerman received his BS from Duke University in Zoology (1971). He attended Cornell University where he obtained an MS (1974) and PhD (1977) in Animal Cytogenetics in the Laboratory of Dr. Stephen Bloom studying SCEs and chromosome breakage in the mudminnow. Dr. Kligerman completed a Post-doctoral fellowship at Duke University in the Department of Pathology under Dr. George Michalopolous developing co-culture methods using primary rat hepatocytes and human fibroblasts to study genetic damage. Dr. Kligerman has received EPA's Bronze Medal and Levels I and II Scientific Achievement Awards and the EMS Special Service Award.

Invited Speaker	Title	Affiliation
Stephen Nesnow, PhD	Director	Stephen Nesnow, Consulting
Stephen Nesnow, Ph.D., is a retired Senior Scientist from the U.S. Environmental Protection Agency's (EPA) National Health and Environmental Effects Research Laboratory. Dr. Nesnow received his Ph.D. from New York University. After post-doctoral fellowships at the Sloan-Kettering Institute for Cancer Research and the McArdle Laboratory for Cancer Research, he joined the faculties of the University of Wisconsin and then the University of North Carolina. Dr. Nesnow served as the Branch Chief of the Biochemistry and Pathobiology Branch, EPA for over 20 years and then as a Senior Scientist until retirement. Dr. Nesnow has published more than 240 scientific publications in the area of chemical carcinogenesis, with specialties in metabolism, tumorigenesis, DNA adducts, toxicogenomics, pesticides, and complex mixtures. Dr. Nesnow has been an invited speaker to many national and international symposia and has served as organizer and session chairman at many of these meetings. He has served on national and international panels and committees including many International Agency for Research on Cancer (IARC) Working Groups as a member and as a Workgroup Sub-Chair. He has received awards from the EPA including a Distinguished Career Service Award, two Bronze Medals, and fourteen Scientific and Technological Achievement Awards. He currently serves as Director of Stephen Nesnow, Consulting.		
Brian Chorley, PhD	Molecular Biologist	US Environmental Protection Agency, National Health Effects and Environmental Research Laboratory
Dr. Brian Chorley is molecular biologist with fourteen years of laboratory research training in cellular biology and genomics. Dr. Chorley completed his PhD in 2005 from North Carolina State University under the mentorship of Dr. Kenneth Adler where he studied the signaling mechanisms of inflammation and mucin production in airway epithelial cells. He continued his research as a postdoctoral fellow at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, NC where he studied NRF2 antioxidant signaling pathway activation and single nucleotide polymorphisms which can alter regulation of NRF2 target genes. During his time at NIEHS, Dr. Chorley became interested in the environmental effects on human health and individual genetic susceptibility to disease and other adverse outcomes. In 2010, this experience led him to his current position as Principal Investigator in the National Health and Environmental Effects Research Laboratory (NHEERL) at the US Environmental Protection Agency where he currently studies genetic and epigenetic biomarkers of adverse outcomes after chemical exposure. He is a member of the American Association for the Advancement of Science (AAAS), American Association for Cancer Research (AACR) and a lifetime GEMS member and current councilor. Dr. Chorley currently lives in Raleigh, NC with his wife and two sons.		

Workshop Project Core Team

Team Member	Title	Affiliation
George M. Woodall, Jr., PhD	Workshop Chair and Project Lead	US Environmental Protection Agency, National Center for Environmental Assessment

Dr. Woodall has been working in environmental and public health for over twenty-five years. He received his doctorate in Toxicology from North Carolina State University in 1996, and previously attained a Masters of Science in Environmental Health from East Tennessee State University (1985) and a Bachelor of Science in Microbiology and Cell Science (1983) from the University of Florida. Dr. Woodall currently serves as a Toxicologist at the National Center for Environmental Assessment (NCEA) of the US EPA, where he works under the Human Health Risk Assessment Program in performing chemical risk assessments, and in developing and improving risk assessment methods. He is the current Chemical Manager for the IRIS assessment for styrene and has been active in review and analysis of the potential neurotoxic and cancer effects from styrene exposure. He also provides scientific support to the Office of Air Quality Planning and Standards of the US EPA for the Risk and Technology Review program for regulation of hazardous air pollutants. He also actively co-leads an interagency Information Management Working Group which strives to provide a basis for collaborative approaches and sharing of the key information relevant to developing human health risk assessments. Dr. Woodall has served on the National Advisory Committee for Acute Exposure Guideline Levels (AEGLs) for the EPA, and has served on or chaired several expert panels for the OECD. He received the 2008 Science and Technology Achievement Award for the paper: A review of the mutagenicity and rodent carcinogenicity of ambient air, co-authored with Larry Claxton. He previously held the position of Senior Toxicologist with the American Petroleum Institute (API); while in that position, he led the organization for and chaired a symposium (Co-sponsored by the API, the US EPA, the Chemical Industry Institute of Toxicology, and the American Forest & Paper Association) on health research and risk assessment for hydrogen sulfide. He is author or co-author of over 20 peer-reviewed articles and book chapters, and numerous governmental and intergovernmental reports. Dr. Woodall is also active as an officer in the Risk Assessment Specialty Section of the Society of Toxicology (current Secretary-Treasurer; SOT Member since 1988), is a current Councilor for the Genetic and Environmental Mutagenesis Society (GEMS), and a past-chair of the Dose Response Specialty Group of the Society for Risk Analysis (SRA; member since 2002).

Channa Keshava, PhD	Project Co-Lead	US Environmental Protection Agency, National Center for Environmental Assessment
Dr. Keshava is currently a Senior Health Scientist at the U.S Environmental Protection Agency (EPA), Office of Research and development (ORD), National Center for Environmental Assessment (NCEA), Integrated Risk Information System (IRIS) with a background in genetic toxicology and toxicogenomics. He obtained his Ph.D. in 1995 from West Virginia University, Department of Genetics and Developmental Biology Program. Following his postdoctoral training at the Emory University School of Medicine, Atlanta, GA, he joined as a staff scientist at the National Institute for Occupational Safety and Health (NIOSH), Center for Disease Control and Prevention, Morgantown WV. At NIOSH, he conducted research on understanding the carcinogenic effects of environmental pollutants including benzo(a)pyrene, diesel particulate matter, asphalt fumes etc. Dr. Keshava then moved to EPA in 2004 and continued to work in the fields of genetic toxicology, toxicogenomics and risk assessment. He is currently, works under the IRIS Program, which is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. Through the IRIS Program, EPA provides the highest quality science-based human health assessments to support the Agency's regulatory activities. Dr. Keshava is currently chemical manager for naphthalene IRIS assessment. He also provides genetic toxicology support to other IRIS assessments. Dr. Keshava serves as an ad-hoc reviewer for several journal articles including Mutation Research, Environmental Molecular Mutagenesis, Polycyclic Aromatic Compounds, and Carcinogenesis. He is a member of professional societies including Environmental Mutagenesis and Genomics Society, Society of Toxicology, and Genetics and Environmental Mutagenesis Society. He has received many awards including the Bronze Medal from EPA and the Distinguished Alumni Award from West Virginia University. He has made several invited presentations at the national and international meetings and organized and chaired sessions in the area of genetic toxicology and toxicogenomics. Dr. Keshava has led and participated in technical panels, scientific committees and risk assessment work groups. He is current President-elect for the Genetic and Environmental Mutagenesis Society. He has published over 30 peer reviewed journal articles in the field of genetic toxicology and toxicogenomics.		
Paul Reinhart, PhD	Workshop Support	US Environmental Protection Agency, National Center for Environmental Assessment
Dr. Reinhart received his PhD in Toxicology from the University of Kentucky in 1993 followed by several years of post-doctoral study at Wayne State University, Detroit, Michigan. His research has focused on the cellular and molecular components of pulmonary toxicity from a variety of agents. Dr. Reinhart is a long-standing member of the Society of Toxicology and is a Diplomate of the American Board of Toxicology. He has been a Toxicologist with the USEPA since 2005 and serves as the Chemical Manager for ethylbenzene.		

Nagu Keshava, PhD	Planning Liaison; Co-chair for Session 4	US Environmental Protection Agency, National Center for Environmental Assessment

Dr. Keshava is currently a Senior Toxicologist at the National Center for Environmental Assessment, Office of Research and Development (ORD), Environmental Protection Agency (EPA), Washington DC, USA. Prior to moving to EPA, she was at Centers for Disease Control - National Institute for Occupational Safety and Health (CDC/NIOSH). She graduated with a Ph.D. from West Virginia University majoring in Genetics and Developmental Biology. Her areas of scientific expertise and interests include genetic toxicology, mode of action, risk assessment and cancer biology. At EPA, she has led or contributed to risk assessments of various chemicals including 1,2-dichloroethane, trichloroethylene, ethylene oxide, tetrachloroethylene, and formaldehyde. Dr. Keshava has provided scientific support to program offices within EPA and other federal agencies. She has received several awards including the Gold and Bronze medals from U.S. Environmental Protection Agency. She is a member of professional societies including Environmental Mutagenesis and Genomics Society, Society of Toxicology, Genetics and Environmental Mutagenesis Society (GEMS). She has authored or co-authored over 40 peer-reviewed articles and book chapters in journals including Cancer Research and Proceedings of National Academy of Sciences. She has also contributed to numerous governmental and intergovernmental reports. Dr. Keshava has served on several committees, organized and chaired workshops and symposium at the Environmental Mutagenesis and Genomics Society, Genetics and Environmental Mutagenesis society. She is a past president of GEMS.

APPENDIX B: Workshop On-site Participants and On-line Registrants

Below is the list of on-site participants (including panelists, speakers, and staff), and those who indicated participation remotely. Actual remote participation was variable and in acknowledgement of the difficulty in monitoring participation via the webinar, all who registered for on-line participation are listed. Sponsorship is noted for those who indicated their participation was being supported by an organization other than their affiliation.

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Samir Abdel-Ghafar		Ministry of the Environment, Ontario, Canada			remotely
Richard Adamson	American Beverage Association	TPN Associates, LLC			remotely
Shanna Alexander		Georgia EPD			remotely
Dan Arrieta		Chevron Phillips Chemical Company LP			remotely
Stan Atwood		ILS, Inc.	x	x	in person
Lisa Bailey		Gradient			remotely
Jim Ball		EPA/ORD/NCEA			remotely
Deborah Banas		Experimental Pathology Laboratories, Inc.	x	x	in person
Marcy Banton		LyondellBasell	x	x	in person
Rodger Vincent Battersby		EBRC	x		in person
Alison Bauer		UC Denver AMC			remotely
Patrick Beatty		American Petroleum Institute	x	x	in person
Nancy Beck		American Chemistry Council			remotely
Rick Becker		American Chemistry Council			remotely
John Bell		Halogenated Solvents Industry Alliance, Inc.			remotely
Ted Berner		EPA/ORD/NCEA			remotely
Norman Birchfield		EPA/ORD/NCEA			remotely
Kenneth Bogen		Exponent, Health Sciences			remotely
Meta Bonner		EPA/ORD/NCER	x	x	in person
Gary Boorman		Covance, Inc.	x	x	in person
Susan Borghoff		ToxStrategies, Inc.	x		in person
Janice Britt		Toxstrategies			remotely

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Cecil Brownie		North Carolina State University	x	x	in person
Alyssia Bryant		Keller and Heckman LLP			remotely
Annie Buard		Solvay USA Inc.			remotely
Lyle Burgoon		EPA/ORD/NCEA	x		in person
James Bus	SIRC (Styene Information & Research Center)	Exponent, Inc.	x	x	in person
John Butala		Toxicology Consultants, Inc.	x	x	in person
Jane Caldwell		EPA/ORD/NCEA			remotely
Andrea Candara		New York State Department of Health			remotely
Anne Chappelle		Global Isocyanates			remotely
Guosheng Chen		Health Canada			remotely
Itai Chipinda		Phillips 66			remotely
Arthur Chiu		EPA/ORD/NCEA			remotely
Nancy Chiu		EPA/OW/OST			remotely
Kyoungju Choi		The Hamner Institutes	x		in person
Brian Chorley		EPA/NHEERL	x	x	in person
Evan Coffman		ORISE	x	x	in person
James Collins		Dow Chemical Company			remotely
Johanna Congleton		EWG			remotely
Torrie Crabbs		Experimental Pathology Laboratories, Inc.			both
George Cruzan	SIRC	ToxWorks	x	x	in person
Helen Cunny				x	in person
David Dankovic		NIOSH			remotely
Ghazi Dannan		EPA/ORD/NCEA-W			remotely
Laura Datko-Williams		ORISE	x	x	in person
Peter de la Cruz		Keller and Heckman			remotely
Yoshihito Deguchi		Sumitomo Chemical America Inc.			remotely
Steven DeSantis		NYSDEC			remotely
Xinxin Ding		NYSDOH			remotely

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Darol Dodd		Hamner Institutes for Health Sciences		x	both
David Eastmond		University of California, Riverside	x	x	in person
Chuck Elkins		Styrene Information & Research Center	x	x	in person
Caroline English		NSF International			remotely
Andrew Ewens		ILS, Inc.	x	x	in person
Bryan Eya		California EPA			remotely
Anna Fan		CalEPA/OEHHA			remotely
William Farland	Styrene Information & Research Center	Colorado State University			remotely
Susan Felter		Procter & Gamble	x	x	in person
Tim Fennell		Research Triangle Institute	x	x	in person
Penelope Fenner-Crisp		Independent Consultant			remotely
Gordon Flake		NIEHS/NTP	x		in person
Lynn Flowers		EPA/ORD/NCEA	x	x	in person
Paul Foster		NIEHS/NTP		x	in person
John French		TOXGEN/Toxicogenetics	x	x	in person
Jason Fritz		EPA/ORD/NCEA	x	x	in person
Sarah Gallagher		EPA/OSWER/PARMS			remotely
Sanford Garner		ILS, Inc.	x	x	in person
Eric Garshick		Harvard Medical School / VA Boston	x	x	in person
Andrew Ghio		EPA			remotely
Catherine Gibbons		EPA/ORD/NCEA/IRIS Program			remotely
Jeff Gift		EPA./ORD/NCEA	x		in person
Jonathan Gledhill		Policy Navigation Group		x	in person
Bala Gollapudi		Exponent			remotely
Brigitte Gomperts		University of California, Los Angeles	x	x	in person
Mike Guo		Cal/EPA/DPR			remotely
Linda Hall		California Department of Pesticide Regulation			remotely
Maria Hegstad		Risk Policy Report			remotely
Paul Hinderliter		Syngenta	x	x	in person

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Martin Hoagland		FDA			remotely
Ernest Hodgson		North Carolina State University	x	x	in person
julia Hoeng		Philip Morris internation			remotely
Karen Hogan		EPA/ORD/NCEA/IRIS			remotely
Jennifer Hsieh		Cal/EPA			remotely
Janis Hulla		Army Corps of Engineers			remotely
Ruth Hummel		EPA/OCSPP/RAD			remotely
Annette Iannucci		OSHA			remotely
Cheryl Itkin		EPA/ORD/NCEA			remotely
Gloria Jahnke		NIEHS/NTP	x	x	in person
Bill Jameson		CWJ Consulting			remotely
Kyathanahalli Janardhan		ILS, Inc.	x	x	in person
Annie Jarabek		EPA/ORD/NCEA	x	x	both
Sophie Jia		Chevron Phillips Chemical Company LP			remotely
Jennifer Jinot		EPA/ORD/NCEA			remotely
Lauren Joca		ORISE	x	x	in person
Tom Johnson		New York State Department of Heath			remotely
Samantha Jones		EPA/ORD/NCEA			remotely
Rhonda Kaetzel		Public Health Seattle King County			remotely
Robert Kavlock		EPA/ORD/IOAA			remotely
Dan Kelly		Marathon Petroleum LP			remotely
Channa Keshava		EPA/ORD/NCEA	x	x	in person
Nagu Keshava		EPA/ORD/NCEA	x	x	in person
Elaine Khan		CalEPA/OEHHA			remotely
Abu Khan		FDA/CFSAN/OFAS/DPR	x	x	in person
Andrew Kligerman		EPA/NHEERL	x	x	in person
Svetlana Koshlukova		CalEPA, DPR, Sacramento, CA			remotely
Renata Kowara		Health Canada			remotely
Daniel Krewski		University of Ottawa	x	x	in person
Joel Kronenberg		Monsanto Company			remotely

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Eric Kwok		California Department of Pesticide Regulation			remotely
David Lai		EPA/OPPTS/RAD			remotely
Juleen Lam		Johns Hopkins			remotely
Janice Lee		EPA./ORD/NCEA	x	x	in person
Carolyn Lewis		Dept. Pesticide Regulation, CalEPA			remotely
Jenny Li		EPA			remotely
Lori Lim		California EPA/OEHHA			remotely
John Lipscomb		EPA/ORD/NCEA	x	x	in person
Craig Llewellyn		The Coca-Cola Company			remotely
Pete Lohstroh		Dept. Pesticide Regulation, CalEPA			remotely
Ming Lu		HC			remotely
Karsta Luettich		Philip Morris Products SA			remotely
April Luke		EPA/ORD/NCEA/IRIS			remotely
Ruth Lunn		NIEHS/NIH	x	x	both
Brian MacGillivray		Cardiff University			remotely
Judith MacGregor		Toxicology Consulting Services	x	x	in person
Kathleen MacMahon		CDC/NIOSH			remotely
Toshihiko Makino	Daiichisankyo Co., Ltd.	NIEHS/NTP	x	x	in person
David Malarkey		NIEHS/NTP/CMPB	x		in person
Ellen Mantus		NAS			remotely
Brian Marable		Bayer	x		in person
Binney McCague		CDC/NIOSH			remotely
Peter McClure		SRC Inc			remotely
Ernest (Gene) McConnell		ToxPath, Inc.		x	in person
Barry McIntyre		NIEHS/NTP			both
Jenna McKenzie		CalEPA/CDPR			remotely
Connie Meacham		EPA/ORD/NCEA	x		in person
Ron Melnick		Ron Melnick Consulting	x	x	in person
Rodney Miller		Experimental Pathology Laboratories, Inc.	x	x	in person

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Mark Miller		Wake Forest University	x	x	in person
Martha Moore		ENVIRON International Corporation			remotely
Sandy Mort		NC Div of Public Health			remotely
Anuradha Mudipalli		EPA/ORD/NCEA	x	x	in person
Kelly Neal		EEC DEP DWM HWB			remotely
Stephen Nesnow		Independent Consultant	x	x	in person
Kathleen Newhouse		EPA/ORD/NCEA			remotely
Lori Nield		University of Colorado			remotely
Jorge Nina	Environmental Quality Board				remotely
Bob Nocco		Chevron			remotely
Adriana Oller		NiPERA	x		in person
Kim Osborn		ICF International	x	x	in person
Ines Pagan		EPA/OAR/HEID	x		in person
Christine Palermo		ExxonMobil	x	x	in person
Arun Pandiri		Experimental Pathology Laboratories, Inc.	x	x	in person
Sang Ki Park		FDA/CFSAN			remotely
Ann Parker		TERA			remotely
Barbara Parsons		National Center for Toxicological Research	x	x	in person
Geoff Patton		FDA			remotely
Amanda Persad		EPA./ORD/NCEA	x		in person
Vincent Piccirillo	Naphthalene Science Team	VJP Consulting	x	x	in person
Charles Plopper		Univ. of Calif., Davis			remotely
Solomon Pollard		EPA/R4			remotely
Lynn Pottenger		The Dow Chemical Company			remotely
Christy Powers		EPA/ORD/NCEA	x		in person
Resha Putzrath		NMCPHC, US Navy			remotely
Santhini Ramasamy		EPA/OW			both
Flora Ratpan		industry			remotely
Leslie Recio		ILS, Inc.	x	x	in person
Jon Reid		EPA/ORD/NCEA			remotely
Paul Reinhart		EPA/ORD/NCEA	x	x	in person

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Fred Reitman		Shell	x	x	in person
Lorenz Rhomberg		Gradient	x	x	in person
Divinia Ries		MI-DEQ			remotely
Susan Rieth		EPA/ORD/NCEA			remotely
Pat Rizzuto		BNA			remotely
Stephen Roberts		Univ. of Florida			remotely
Jim Rollins		Policy Navigation Group	x		in person
Avima Ruder		CDC/NIOSH			remotely
Shawn Sager		ARCADIS	x		both
Andrew Salmon		CalEPA/OEHHA	x	x	in person
Satinder Sarang		Shell	x		in person
Linda Sargent		CDC/NIOSH			remotely
Riz Sarmiento		Gilbane Co.			remotely
Brian Sayers		NTP			remotely
Val Schaeffer		OSHA			remotely
Tamar Schlekat		ARCADIS	x	x	in person
Paul Schlosser		EPA/ORD/NCEA	x	x	in person
Rita Schoeny		EPA/ORD/OSP			remotely
Cheryl Scott		EPA/ORD/NCEA			remotely
Jun Sekizawa		Communication Center for Food and Health Sciences			remotely
Dahnish Shams		EPA/ORD/NCEA			remotely
Robert Sills		NIEHS/NTP			both
Marilyn Silva		California Dept of Pesticide Regulation			remotely
Courtney Skuce		ICF International	x	x	in person
Wesley Smith		CalEPA/OEHHA			remotely
Jack Snyder		Styrene Information & Research Center	x	x	in person
Maria Spassova		EPA			remotely
Lauren Staska		ILS, Inc.	x		in person
Todd Stedeford		EPA/OCSPP/OPPT/RAD			remotely
Tom Steinbach		Experimental Pathology Laboratories, Inc.	x		in person
Mark Stelljes		SLR International Corporation			remotely

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Teri Sterner		US Air Force, 711HPW/RHDJ			remotely
Gary Stoner		Medical College of Wisconsin	x	x	in person
Matthew Stout		NIEHS/DNTP			remotely
Harlee Strauss		H Strauss Associates, Inc			remotely
Christian Strupp		Feinchemie Schwebda GmbH	x	x	in person
Scott Sudweeks	EPA R8	CDC\ATSDR			remotely
Meng Sun		Cal/EPA			remotely
True-Jenn Sun		Chevron			remotely
Katherine Sutherland-Ashley		OEHHA			remotely
Marja Talikka		Philip Morris			remotely
Matthew Taylor		Ashland			remotely
Michael Taylor		NiPERA			both
Sheau-Fung Thai		EPA/ORD/NHEERL	x	x	in person
Feng Tsai		Cal EPA			remotely
Alethea Tsui-Bowen		EPA Region 6			remotely
Molly Vallant		NIEHS/NIH			remotely
Laura Van Winkle		University of California, Davis	x	x	in person
John Vandenberg		EPA/ORD/NCEA	x	x	in person
Marylou Verder-Carlos		Cal-EPA, CDPR			remotely
Jane Vergnes		Acta Group			remotely
Sury Vulimiri		EPA./ORD/NCEA	x	x	in person
Tina Walker		FDA/CFSAN/OFAS			remotely
Katherine Walker		Health Effects Institute			remotely
Debra Walsh		EPA./ORD/NCEA	x	x	in person
Mark Walton		SCC			remotely
Bill Ward		EPA/NHEERL	x	x	in person
Teresa Washington		EPA/OPPT/RAD			remotely
James Weaver		EPA/ORD/NCEA	x	x	in person
Catherine Whiteside		FDA/CFSAN/OFAS/DPR	x	x	both

Full Name	Sponsor	Affiliations (Clean)	Attended in-person Jan 7	Attended in-person Jan 8	FINAL Attendance
Miglena Wilbur		CalEPA			remotely
Adrien Wilkie		ORISE	x	x	in person
Patty Wong		CalEPA/OEHHA			remotely
Yintak Woo		EPA/OCSPP/RAD			remotely
Charles Wood		EPA	x	x	in person
George Woodall		EPA/ORD/NCEA	x	x	in person
Mike Wright		EPA			remotely
Haruhiro Yamashita		NIEHS/NIH			remotely
Hui-Min Yang		EPA/ORD/NCEA			remotely
Chengfeng Yang		Michigan State University			remotely
Brianna Young		EPA/ORD/NCEA	x	x	in person
Melanie Young		EPA/OW/OST			remotely
Cynthia Yund		EPA/ORD/NCEA			remotely
Janet Zang		FDA			remotely

On-line Participants

The table below lists the names provided by individuals who logged into the webinar portion of the meeting. Where known, additional details were provided and duplicate entries were omitted; however, for many entries it was not possible to determine the full identification of the participant or if it was a duplicate entry.

Participant Name	7-Jan	8-Jan
Adriana Oller (NiPERA)		X
Alethea Tsui-Bowen	X	X
Alison Bauer	X	
Alyssia Bryant - Keller and Heckman LLP	X	
Andy	X	
Ann Parker - TERA	X	X
Anna Fan	X	X
Anne Chappelle (III)	X	X
Annie Buard	X	X
AnnieJ	X	
Avima Ruder (NIOSH)	X	X
B. Bhaskar Gollapudi	X	X
Bauer		X
Binney McCague (NIOSH)	X	

Participant Name	7-Jan	8-Jan
Bob Nocco (Chevron)	X	X
Brian Marable (Bayer CropScience)		X
Brian Sayers, NTP	X	X
Bryan Eya	X	X
Caroline English (NSF Int)	X	
Carolyn Lewis, CDPR	X	X
Catherine Gibbons (EPA)	X	X
Cheryl Itkin	X	
Craig Llewellyn	X	X
D. Arrieta	X	X
Dahnish Shams	X	X
Dan Kelly, Marathon Petroleum Company LP		X
Darol Dodd (The Hamner Institutes)	X	
Dave	X	
David Dankovic (CDC/NIOSH)	X	X
David Mattie 711 HPW/RHDJ	X	X
desantis (nysdec)		X
Dr. Ken Bogen, Exponent	X	X
Dr. Solomon Pollard		X
Elaine Khan	X	X
Eric Kwok	X	X
Feng Tsai	X	X
Flora Ratpan Nova Chemicals	X	X
Geoff Patton FDA/CFSAN	X	X
Ghazi Dannan	X	X
Guosheng Chen (Health Canada)	X	X
Harlee Strauss	X	X
Haruhiro Yamashita (NIEHS)	X	
Helen Cunny		X
Hui-Min Yang	X	X
Itai Chipinda	X	X
J. Bell HSIA	X	X
Jan Hulla, US Army Corps of Engineers		X
Jane Caldwell	X	X
Jane Vergnes (Acta)	X	X
Janet Zang	X	X
Janice Lee	X	
Janis Hulla US Army Corps of Engineers- Sacramento	X	X
Jenna McKenzie CDPR	X	
Jennifer Hsieh (Cal/EPA)	X	X

Participant Name	7-Jan	8-Jan
Jennifer Jinot, EPA	X	X
Jim Ball	X	X
Jim Collins	X	X
Joel Kronenberg	X	X
Johanna Congleton	X	X
John Schweitzer ACMA		X
Jon Reid	X	X
Karen Hogan (EPA)	X	
Karsta Luettich	X	X
Katherine Sutherland-Ashley (OEHHA)	X	X
Katherine Walker	X	
Kathleen MacMahon (CDC/NIOSH)	X	X
Kathleen Newhouse (US EPA)	X	X
Laurie Staska - ILS		X
Linda Hall (DPR)	X	X
Lisa Bailey (Gradient)	X	X
Lori Lim (OEHHA CalEPA)	X	X
Lori Nield (UC Denver)	X	X
Lou D'Amico EPA/ORD/NCEA/IRIS		X
Lynn Pottenger	X	X
M. Madden		X
Margarita	X	
Maria Hegstad (Inside EPA)		X
Maria Spassova	X	X
Mark Stelljes, SLR International	X	X
Mark Walton (SCC)	X	X
Martha Moore ENVIRON		X
Martin Hoagland, FDA CFSAN	X	X
Matt Howe SIRC		X
Matt Stout (NIEHS/NTP)	X	X
Matt Taylor - Ashland	X	X
Maureen Johnson	X	X
Meagan Madden	X	
Melanie Young	X	X
Meng Sun CAL/EPA	X	X
Michael Taylor (NiPERA)	X	X
Miglena Wilbur		
Miglena Wilbur (DPR, CalEPA)	X	X
mike	X	
Mike Guo	X	X

Participant Name	7-Jan	8-Jan
ming lu	X	X
Nancy B	X	X
Nancy Beck		X
Norman Birchfield EPA	X	X
nysdec		X
Pat Rizzuto	X	X
Patty Wong (CalEPA)	X	X
Penelope Fenner-Crisp	X	X
Pete Lohstroh (CDPR)	X	X
Peter de la Cruz	X	X
Peter McClure (SRC)	X	X
R. Becker ACC	X	
Ravi Subramaniam, NCEA-EPA	X	X
Richard H. Adamson TPN Associates LLC	X	X
Robert Kavlock (USEPA)		X
Ron Hampton - Gradient		X
Ruth Hummel (EPA/OCSPP)	X	
Ruth Lunn (NIEHS)	X	
Samantha Jones	X	X
Sandy Mort, NC Public Health	X	
Sang Ki Park, FDA/CFSAN	X	X
Santhini Ramasamy	X	
Schweitzer, John, Amer Composites Mfgrs Assn	X	
Shanna Alexander	X	X
Shawn Sager		X
sonya	X	
Sophie Jia (Chevron Phillips chemical company)	X	X
SRC	X	
Steve Roberts	X	X
Steven DeSantis (NYSDEC)	X	X
svetlana koshlukova	X	X
Tamar Schlekat		X
Todd Stedeford	X	X
Tom Osimitz	X	
Torrie Crabbs (EPL)	X	X
True-Jenn Sun	X	
W Smith/ OEHHA	X	X
William Farland (Colorado State University)	X	X
Xinxin Ding (Wadsworth)	X	X
Yinatao Woo	X	

Participant Name	7-Jan	8-Jan
Yintak Woo	X	X
Yoshi Deguchi (Sumitomo Chemical)	X	X
Total Number of Participants by day:	**114**	**107**

APPENDIX C: Workshop Final Agenda with Hyperlinks to Presentation Slides

State-of-the-Science Workshop on Chemically-induced Mouse Lung Tumors: Applications to Human Health Assessments

January 7-8, 2014
8:30am-5:00pm

U.S. EPA Auditorium C111
109 T.W. Alexander Drive
Research Triangle Park, NC 27711

Links to individual slide sets or a more detailed abstract for a presentation are provided; click on the title of a presentation to open the link.

Tuesday, January 7, 2013

Opening and Overview

8:30 am	Registration
9:00 am	Welcome and Introductory Remarks - John Vandenberg, PhD; NCEA RTP Division Director
9:20 am	Goals and Scope of the Workshop (PDF) (24 pp, 294K) - George Woodall, PhD; Workshop Chair and Project Leader
9:50 am	Workshop Logistics (On-site and On-line Interactions) - Channa Keshava, PhD; Project Co-Leader

Session 1: Human Cancer Epidemiology and Pathophysiology

Co-Chairs: Jason Fritz | *US EPA* Eric Garshick | *Harvard Medical School/VA Boston Healthcare System*	

10:00 am	**Session Overview (PDF)** (4 pp, 304K) Jason Fritz and Eric Garshick • Brief statement of session goals, presentation/discussion format • Introduction of co-chairs, panel members • Listing of discussion topics
10:05 am	**Approaches to Determining Carcinogenic Risks in Humans (PDF)** (19 pp, 477K) Eric Garshick | *Harvard Medical School/VA Boston Healthcare System* • IARC criteria for assessment of human carcinogenicity • Approach to epidemiologic study design for lung cancer • Exposure assessment • Outcome assessment - level of pathological/histological detail available in epidemiologic studies • Confounding in the assessment of lung cancer risk
10:20 am	Guided discussion
10:30 am	**Epidemiological Studies of Human Lung Cancer (PDF)** (22 pp, 1.13M) Dan Krewski | *University of Ottawa* • Known IARC Group 1 carcinogens/lung carcinogens • Causes of human lung cancer with attributable fractions • Concordance between human lung cancer with rodent and mouse models; note of mechanisms - to be further discussed during session 2 • Specific examples of human lung cancer studies highlighting approaches to exposure and outcome assessment • Highlight examples where specific histological data impacted on epidemiologic study interpretation
10:45 am	Guided discussion

10:55 am	**Lung Cancer Mortality: Worker Exposed to Styrene, Ethylbenzene, or Naphthalene (PDF)** (32 pp, 1.23M) Jim Collins | *Dow Chemical Company* • Discuss human epidemiologic cancer data for the chemicals of interest - including evidence for immunological effects in styrene-exposed workers • Limitations of current studies, including approach to exposure assessment, biomonitoring, effects at the molecular level • Limitations of human epidemiologic database - are data sufficient to draw conclusions?
11:10 am	Guided discussion
11:20 am	**Human Lung Cancer Pathology and Cellular Biology (PDF)** (18 pg, 1.12M) Brigitte Gomperts | *University of California, Los Angeles* • Lung cancer pathology, histopathology • Biology of the origins of lung cancer, including state of knowledge regarding cell types of origin for lung cancer • Molecular biology of lung cancer, introduction to inflammation, genetics & epigenetics – to be further discussed during session 4 "Molecular Toxicity, Epigenetics, genetic polymorphisms" • Discussion of immune-related effects relevant to/found in workers exposed to Styrene – for further discussion during session 4 • Relevant mutations/polymorphisms regarding chemicals of interest - for further discussion during sessions 2-4.
11:35 am	Guided discussion
11:45 am	**Session Summary Discussion**
12:00 pm	**Lunch**

Session 2: Comparative Pathology

Co-Chairs: Charles Wood | *US EPA* Mark Miller | *Wake Forest University*	**Panelists:** Gary A. Boorman | *Covance, Inc.* Laura Van Winkle | *University of California, Davis* Arun Pandiri | *Experimental Pathology Laboratories, Inc.*
1:00 pm	**Session Overview (PDF)** (19 pp, 1.89M) Charles Wood and Mark Miller

1:15 pm	**Comparative lung pathology (PDF)** (16 pp, 1.01M) Gary Boorman | *Covance, Inc.*
1:30 pm	Guided discussion
1:45 pm	**Mouse lung tumor model considerations (PDF)** (22 pp, 4.87M) Mark Miller | *Wake Forest University* • Discuss strain differences for wild-type mice as well as • Genetically Engineered Mouse Models (GEMMs) in lung cancer research • Use of mouse models to study mode of action (MOA): initiation and promotion - to be further discussed during session 3
2:00 pm	Guided discussion
2:15 pm	**Rodent lung tumors in NTP studies (PDF)** (25 pp, 2.33M) Arun Pandiri | *Experimental Pathology Laboratories, Inc.*
2:30 pm	Guided discussion
2:45 pm	**Break**
3:00 pm	**Species Difference in Response and Cell of Origin (PDF)** (25 pp, 9.41M) Laura Van Winkle | *University of California, Davis*
3:15 pm	Guided discussion
3:30 pm	**Animal and Human Tumour Site Concordance (PDF)** (30 pp, 2.34M) Dan Krewski | *University of Ottawa*
3:45 pm	Guided discussion
4:00 pm	**Session Summary Discussion**
5:00 pm	**Adjourn for the Day**

Wednesday, January 8, 2013

Session 3: Biological Mechanisms

Co-Chairs:	**Panelists:**
Paul Schlosser | *US EPA* Ron Melnick | *Ron Melnick Consulting*	Tim Fennell | *Research Triangle Institute* Kathy Burns | *ScienceCorps LLC* Ernest Hodgson | *North Carolina State University*

8:30 am	**Session Overview (PDF)** (5 pp, 1.06M) Paul Schlosser | *EPA* • Introduction of Co-Chairs, Panelists, and Presenters • Session Goals • Agenda • Discussion Topics
8:35 am	**A Framework for Considering the CYP2F2 MOA Hypothesis & Relevance of Mouse Lung Tumors to Humans (PDF)** (7 pp, 677K) Ron Melnick | *Ron Melnick Consulting*
8:45 am	**Hypothesis-Driven MOA Analysis: CYP2F2 (PDF)** (24 pp, 833K) George Cruzan | *ToxWorks*
9:05 am	Clarifying Q&A
9:15 am	**Pharmacokinetics and Pharmacodynamics of Ethylbenzene (PDF)** (16 pp, 1.01M) Ernest Hodgson | *North Carolina State University*
9:25 am	Clarifying Q&A
9:30 am	**Pharmacokinetics and Pharmacodynamics of Naphthalene (PDF)** (20 pp, 1.06M) Laura Van Winkle | *University of California, Davis*
9:40 am	Clarifying Q&A
9:45 am	**Pharmacokinetics and Pharmacodynamics of Styrene (PDF)** (21 pp, 978K) Tim Fennell | *Research Triangle Institute*
10:00 am	Clarifying Q&A
10:10 am	**Break**
10:30 am	**Related Chemicals: CYP2F2 Substrates & Other Mouse Lung Tumorigens (PDF)** (9 pp, 1.79M) Paul Schlosser | *US EPA* • Methylene chloride • Benzene • Fluensulfone • Trichloroethylene
10:40 am	Clarifying Q&A

10:45 am	**Integration of Cross Cutting Issues (PDF)** (4 pp, 75K) John Lipscomb, PhD | *US EPA*
10:55 am	**Session-wide Open Discussion**
11:30 am	**Lunch**

Session 4: Evidence for Cellular, Genetic, and Molecular Toxicity

Co-Chairs: Nagu Keshava | *US EPA* Gary Stoner, PhD | *Medical College of Wisconsin*	**Panelists:** David Eastmond, PhD | *University of California, Riverside* Andrew Kligerman, PhD | *US EPA, NHEERL* Andrew Salmon, PhD | *CalEPA, OEHHA*
12:30 pm	**Session Overview (PDF)** (5 pp, 73K) Nagu Keshava and Gary Stoner • Introduction of Co-Chairs, panelists, and Presenters • Session Goals • Agenda • Discussion Topics
12:40 pm	**An Overview of the Genotoxicity of Aromatic Hydrocarbons and their Reactive Intermediates (PDF)** (12 pp, 367K) Stephen Nesnow | *Independent Consultant* • Parent compounds, intermediates (stability, formation) and their effects on genotoxicity • Specific genotoxicity induced by chemicals of interest and their intermediates • Discuss individual chemicals and commonalities and relationship to MLT genesis
1:00 pm	Guided discussion

1:10 pm	**Mouse Lung Carcinogens, Reactive Metabolites and Toxicity (PDF)** (26 pp, 708K) David Eastmond | *University of California, Riverside* • Mouse carcinogen toxicity and metabolism • In vitro and in vivo cytotoxicity of chemicals and their intermediates • Postulated metabolism and mode of action of interested chemicals • Common reactions of quinones, epoxides etc. • Interrelationship between cytotoxicity and genotoxicity
1:30 pm	Guided discussion
1:40 pm	**Overview of New and Developing Omic Technologies: Assessing Molecular Toxicity and Disease Susceptibility (PDF)** (28 pp, 2.39M) Brian Chorley| *US Environmental Protection Agency, RTP* • Contribution of data from new technologies in understanding the mode of action • Strengths and limitations of these technologies in terms of pathway analysis • Discuss commonalities and relationship to MLT genesis • Discuss available data and identify data gaps with respect to these new technologies
2:00 pm	**Metabolomics (PDF)** (9 pp, 1.09M) Timothy Fennel | *RTI International*
2:10 pm	**Break**
2:25 pm	**Integration of Sessions 3 and 4** Gary Stoner | *Medical College of Wisconsin*
2:45 pm	Guided discussion
3:00 pm	**Session Summary Discussion**

Summary Session

Workshop Chair:
George Woodall \| *US EPA*

3:30 pm	**Summaries of Key Points from each Workshop Session** Session Co-chairs and Workshop Chair • Recap of Key Points from each Session • Identify Topics Needing Additional Consideration/Discussion
4:30 pm	**Next Steps** Workshop Chair to lead the discussion • Planning for Follow-on Virtual Workshops • Developing a Workshop Summary Report and Peer-review publications
5:00 pm	**Adjourn Workshop**

APPENDIX D: Comprehensive Reference List

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