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ExpoCast™: Exposure Science for Prioritization and Toxicity Testing

International Society of Exposure Science 2009 Annual Meeting
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November 4, 2009

Elaine Cohen Hubal
National Center for Computational Toxicology

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The Context: Chemical Evaluation for Risk Management

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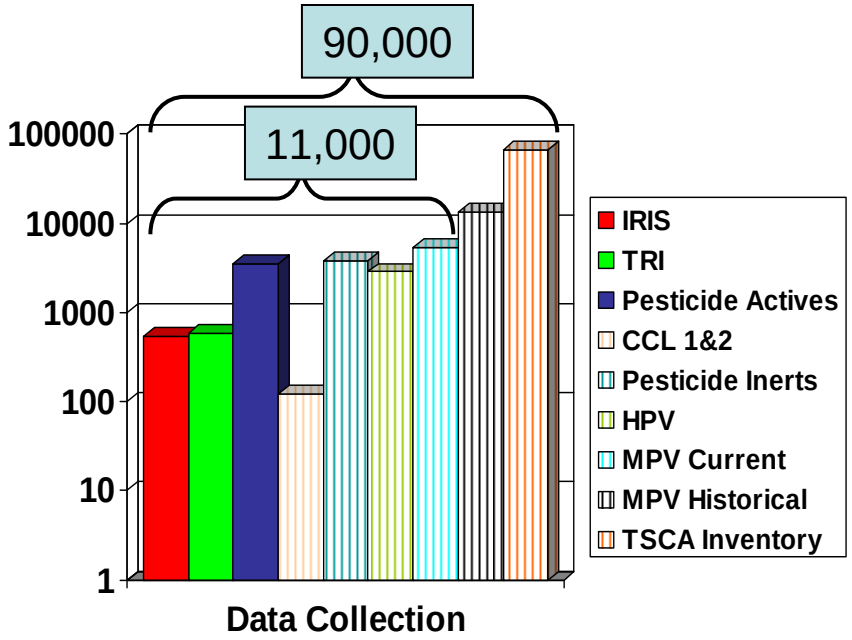
Mandate to Assess Thousands of Chemicals

Need to develop methods to evaluate a large number of environmental chemicals for potential human-health risks



Richard Judson

Transforming Toxicology

THE NATIONAL ACADEMIES

REPORT IN BRIEF

July 2007

Toxicity Testing in the 21st Century: A Vision and a Strategy

Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research Council report creates a far-reaching vision for the future of toxicity testing.

Toxicity tests on laboratory animals are conducted to evaluate chemicals—including medicines, food additives, and industrial, consumer, and agricultural chemicals—for their potential to cause cancer, birth defects, and other adverse health effects. Information from toxicity testing serves as an important part of the basis for public health and regulatory decisions concerning toxic chemicals. Current test methods were developed incrementally over the past 50 to 60 years and are conducted using laboratory animals, such as rats and mice. Using the results of animal tests to predict human health effects involves a number of assumptions and extrapolations that remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about effects at lower doses or exposures. Test animals are typically observed for overt signs of adverse health effects, which provide little information about biological changes leading to such health effects. Often controversial uncertainty factors must be applied to account for differences between test animals and humans. Finally, use of animals in testing is expensive and time consuming, and it sometimes raises ethical issues.

Today, toxicological evaluation of chemicals is poised to take advantage of the on-going revolution in biology and biotechnology. This revolution is making it increasingly possible to study the effects of chemicals using cells, cellular components, and tissues—preferably of human origin—rather than whole animals. These powerful new approaches should help to address a number of challenges facing the

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We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

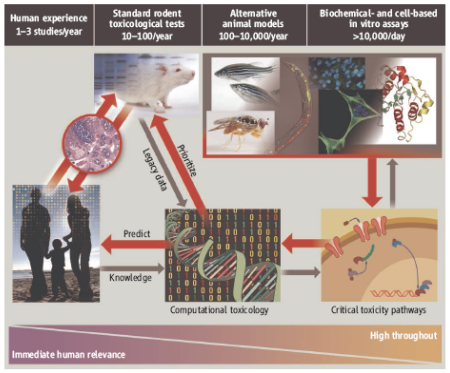
Francis S. Collins,*† George M. Gray,‡* John R. Bucher§*

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,



Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

[1]Director, National Human Genome Research Institute (NHGRI), National Institutes of Health, Bethesda, MD 20892; [2]Assistant Administrator for the Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460; [3]Associate Director, U.S. National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709, USA.

*The views expressed here are those of the individual authors and do not necessarily reflect the views and policies of their respective agencies.

†Author for correspondence. E-mail: francisc@mail.nih.gov

Science, Feb 15, 2008

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Toxicity Testing in the Twenty-first Century

- Key aspect of the NRC vision is that new tools are available to examine toxicity pathways in a depth and breadth that has not been possible

- Efforts to apply high-throughput-screening (HTS) approaches for **chemical prioritization** and **toxicity testing** have been accelerated

- An explosion of HTS data for *in vitro* toxicity assays will become available over the next few years ---- **Data are available now!**

- **How will this new toxicity information be *translated* to assess potential for real-world human health risk?**

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ToxCast ™ Program for Prioritizing Toxicity Testing of Environmental Chemicals

- In 2007, EPA launched ToxCast™ to develop an efficient, cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals

- Using data from state-of-the-art high throughput screening (HTS) bioassays, ToxCast™ is building computational models to forecast the potential human **toxicity** of chemicals

- New technologies must be applied to ***BOTH*** toxicology and exposure science if the ultimate goal of screening chemicals for **risk** is to be achieved

http://epa.gov/ncct/toxcast/

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Toxicity Testing in the Twenty-first Century: A Vision and a Strategy

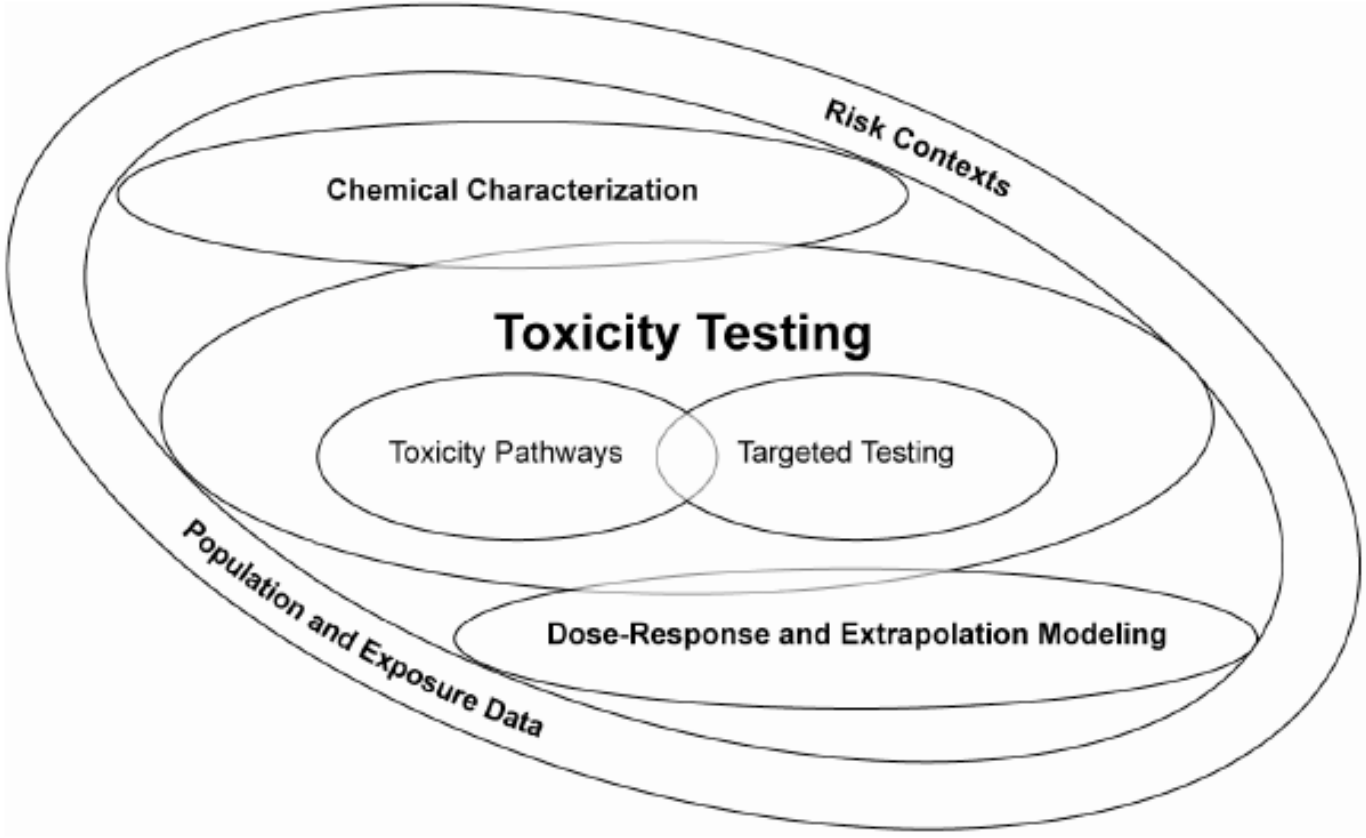

FIGURE 2-3 The committee's vision is a process that includes chemical characterization, toxicity testing, and dose-response and extrapolation modeling. At each step, population-based data and human exposure information are considered, as is the question of what data are needed for decision-making.

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Exposure Science in NRC Vision - TRANSLATION

- Population-based data and human exposure information critical for guiding development and use of toxicity information

- Components include:
  - Use of information on host susceptibility and background exposures to interpret and extrapolate *in vitro* test results.
  - Use of human exposure data to select doses for toxicity testing so we develop hazard information on **environmentally-relevant effects**.
  - Use of biomonitoring data to relate real-world human exposures with concentrations that perturb toxicity pathways to identify potentially important (**biologically-relevant**) exposures.

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Exposure Science Research Questions

- What does the real world look like?
  - What are the critical elements of exposure space and how do these compare with the elements of toxicologically-based chemical space?
  - What are key determinants required to characterize critical elements of exposure?

- How can we leverage new scientific understanding and tools in molecular, computational, and information sciences to develop **rapid**, **inexpensive** approaches for characterizing biologically-relevant exposure?

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Exposure Science for Computational Toxicology

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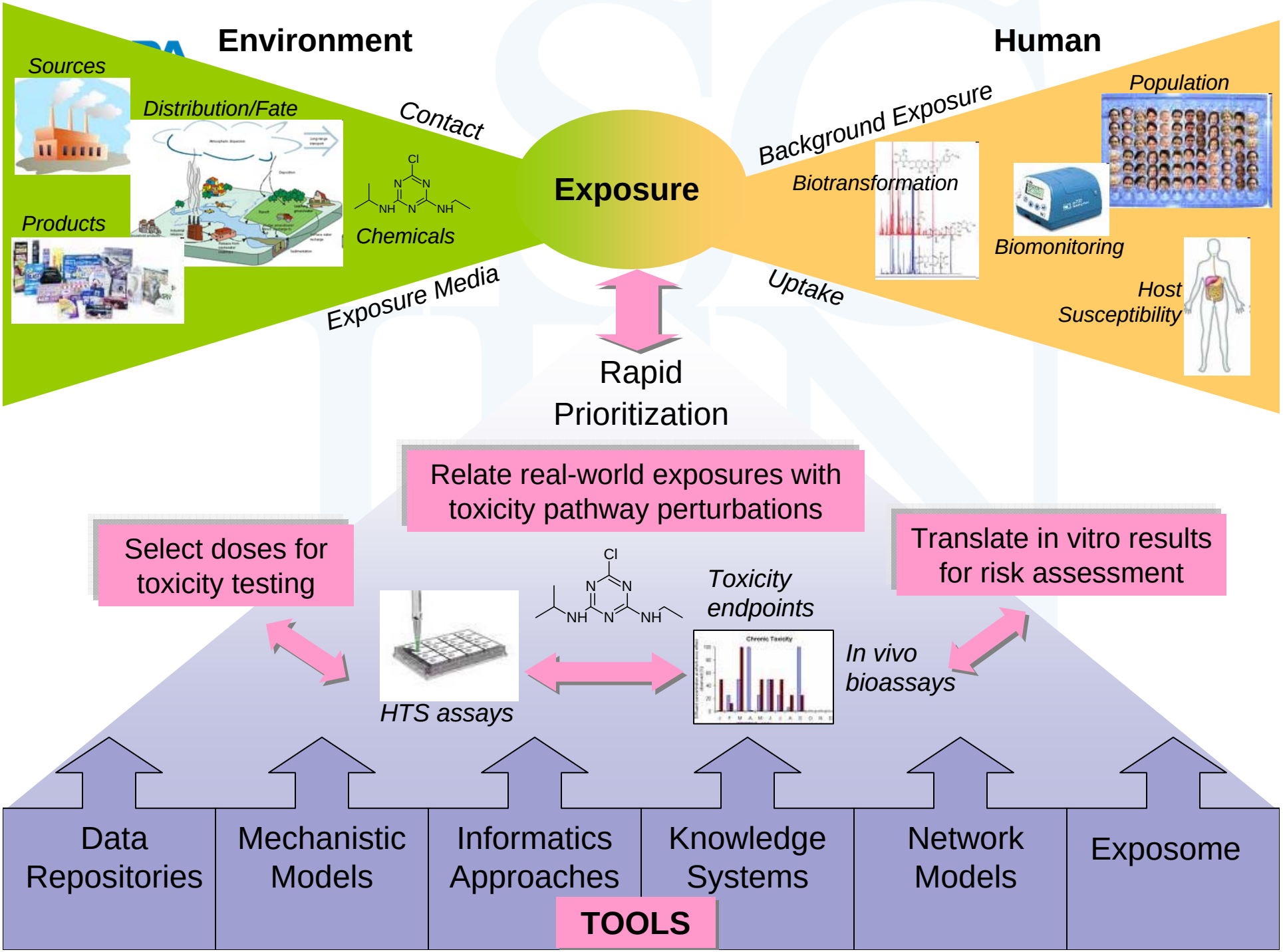
ExpoCast™: Exposure Science for Prioritization and Toxicity Testing

- Purpose
  - Advance characterization of exposure required to ***translate*** advances and findings in computational toxicology to information that can be directly used to support risk assessment for decision making and improved public health.

- Objective
  - Develop novel approaches and tools for evaluating and classifying chemicals, based on potential for ***biologically-relevant*** human exposure, to inform prioritization and toxicity testing.


Environment
Human
Sources
Distribution/Fate
Products
Chemicals
Contact
Exposure
Exposure Media
Uptake
Background Exposure
Biotransformation
Population
Biomonitoring
Host Susceptibility
Rapid Prioritization
Relate real-world exposures with toxicity pathway perturbations
Select doses for toxicity testing
Translate in vitro results for risk assessment
Toxicity endpoints
HTS assays
In vivo bioassays
Data Repositories
Mechanistic Models
Informatics Approaches
Knowledge Systems
Network Models
Exposome
TOOLS

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Priority Exposure Research for Computational Toxicology

- Accessible and linkable exposure databases
- Exposure screening tools for accelerated chemical prioritization
- Advanced computational approaches for interpreting *in vitro* toxicity data in the context of individual and population health
- Biologically-relevant exposure metrics (biomarkers reliably associated with exposure)

Human vulnerability and life-stage aspects are integral to each of these.

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New technologies must be applied to *BOTH* toxicology and exposure science if the ultimate goal of screening chemicals for risk is to be achieved.

- Systems exposure science
- Biologically-relevant exposure metrics
- Environmental informatics and advanced computational models

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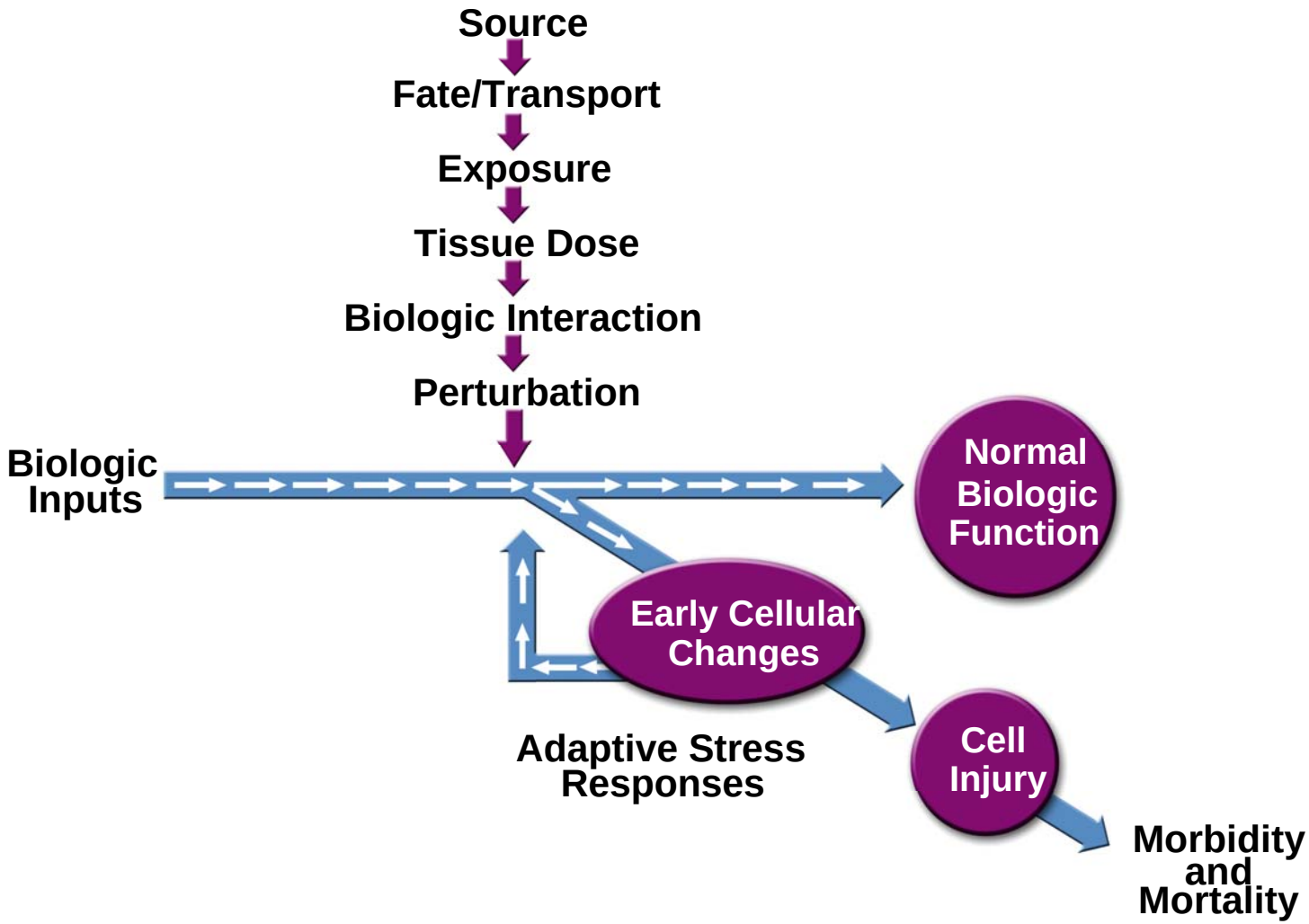
Systems Biology: Exposure at All Levels of Biological Organization



Modified from NRC, 2007

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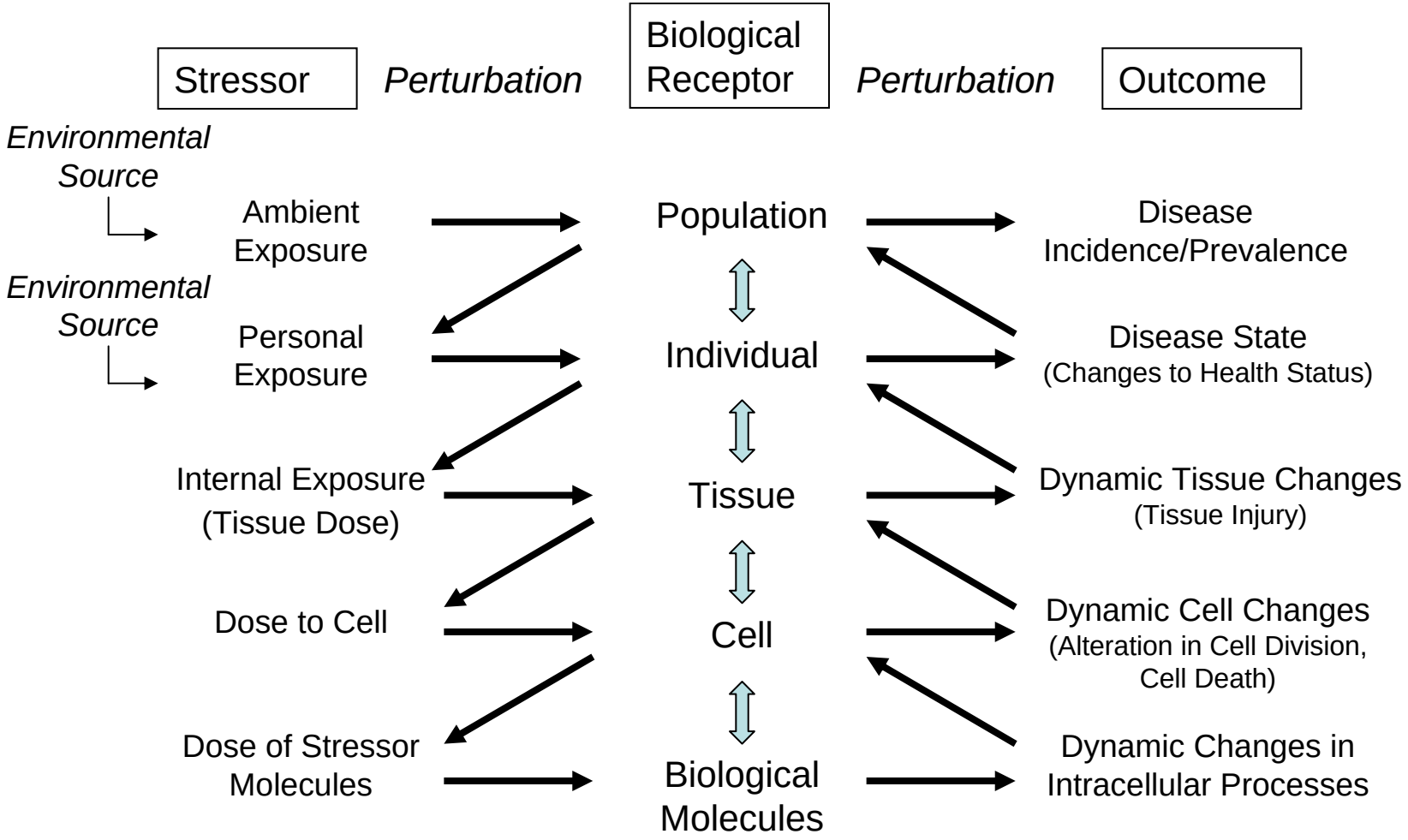
Systems Biology: Exposure at All Levels of Biological Organization



JESEE, 2008

Figure 1

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Systems Biology: Extending Network Analysis to Inform Risk Assessment

- Consider coupled networks spanning multiple levels of biological organization
- Mechanistic understanding derived by characterizing networks and impacts of perturbations
- Networks at different levels used to merge molecular-level changes with measured events at the individual or population level
  - Molecular networks based on data from 'omic measurements
  - Key event networks, where each node ideally represents a toxicity pathway, abstracted from molecular network based on biological interpretation and targeted experimentation
  - Adverse outcome driven by impact of an individual's genetics, epigenetics and exposure profile
  - Connectivity at the population level driven by common genetics, lifestyle, environment

Edwards and Preston (2008) Tox Sci, 106;312-318

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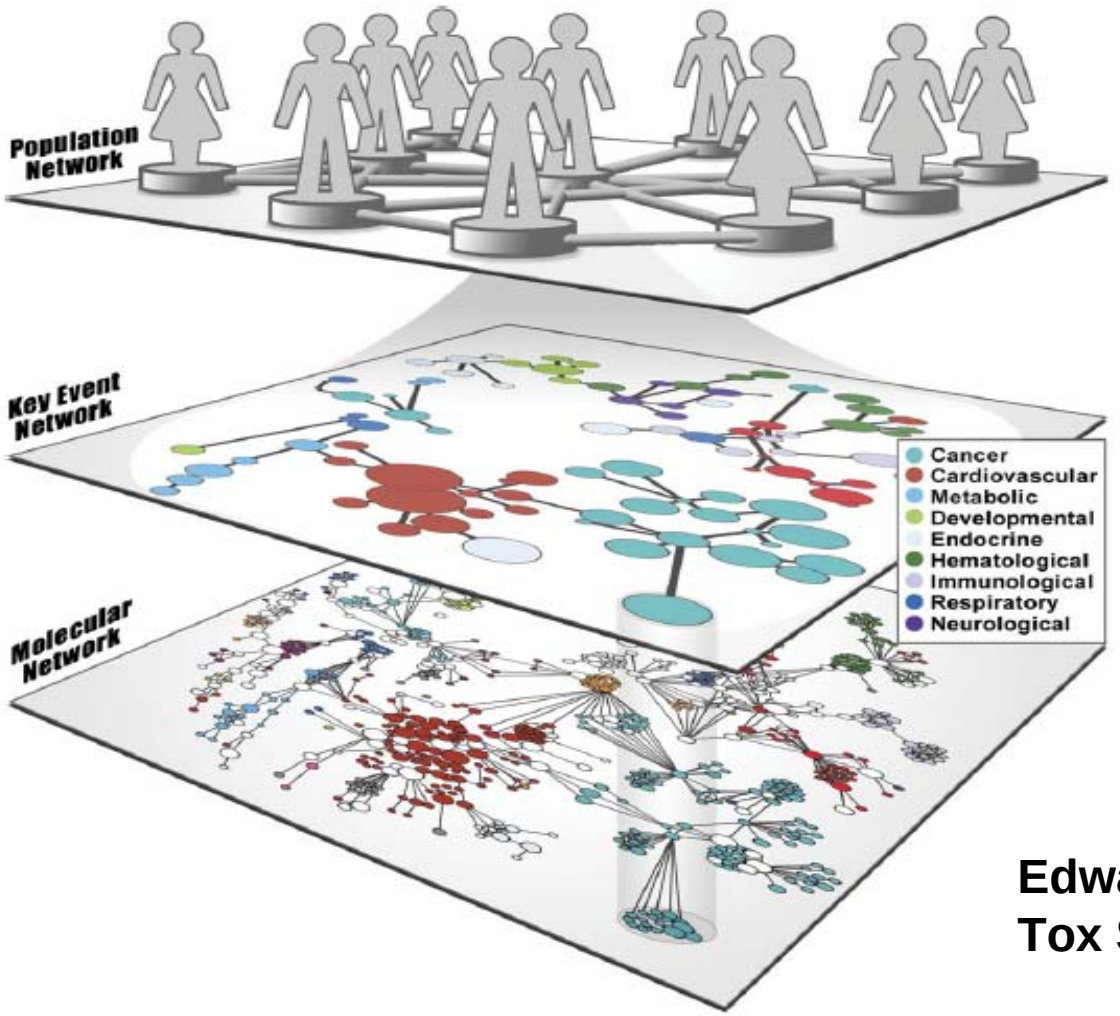

Edwards and Preston (2008)
Tox Sci, 106;312-318

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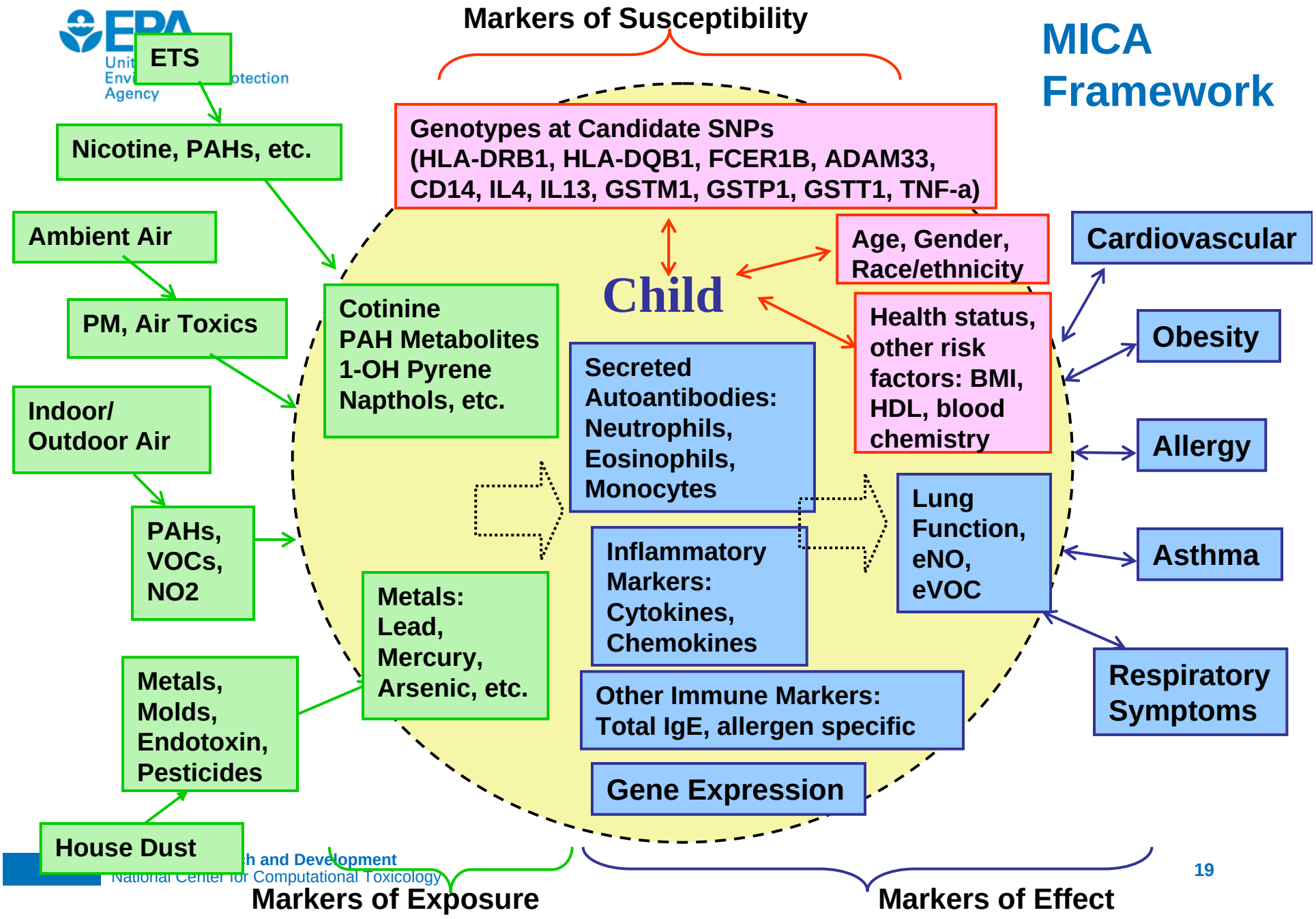
Biologically-Relevant Exposure Metrics

- Markers required that can be directly associated with key events in a disease process and with an individuals exposure profile
  - 'Omic technologies showing potential to yield a new generation of exposure metrics (Wild, 2009)
  - Altered global gene expression associated with exposures to arsenic, cigarette smoke, benzene, metal fumes and air pollution

- Better environmental biosensors required to study gene-environment interactions associated with complex disease (Collins 2007)
  - Nano-scale sensor arrays can be developed to detect specific sets of environmental agents (Andreescu et al, 2009)


Markers of Susceptibility
MICA Framework
ETS
Nicotine, PAHs, etc.
Ambient Air
PM, Air Toxics
Indoor/ Outdoor Air
PAHs, VOCs, NO2
Metals, Molds, Endotoxin, Pesticides
House Dust
Genotypes at Candidate SNPs (HLA-DRB1, HLA-DQB1, FCER1B, ADAM33, CD14, IL4, IL13, GSTM1, GSTP1, GSTT1, TNF-a)
Child
Age, Gender, Race/ethnicity
Health status, other risk factors: BMI, HDL, blood chemistry
Cotinine
PAH Metabolites
1-OH Pyrene
Napthols, etc.
Secreted Autoantibodies: Neutrophils, Eosinophils, Monocytes
Metals: Lead, Mercury, Arsenic, etc.
Inflammatory Markers: Cytokines, Chemokines
Other Immune Markers: Total IgE, allergen specific
Gene Expression
Lung Function, eNO, eVOC
Cardiovascular
Obesity
Allergy
Asthma
Respiratory Symptoms
Markers of Exposure
Markers of Effect

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Computational Techniques – Two Branches

- Knowledge-discovery
  - Data-collection, mining, and analysis
- Mechanistic (dynamic) simulation
  - Mathematical modeling at various levels of detail
- Both required

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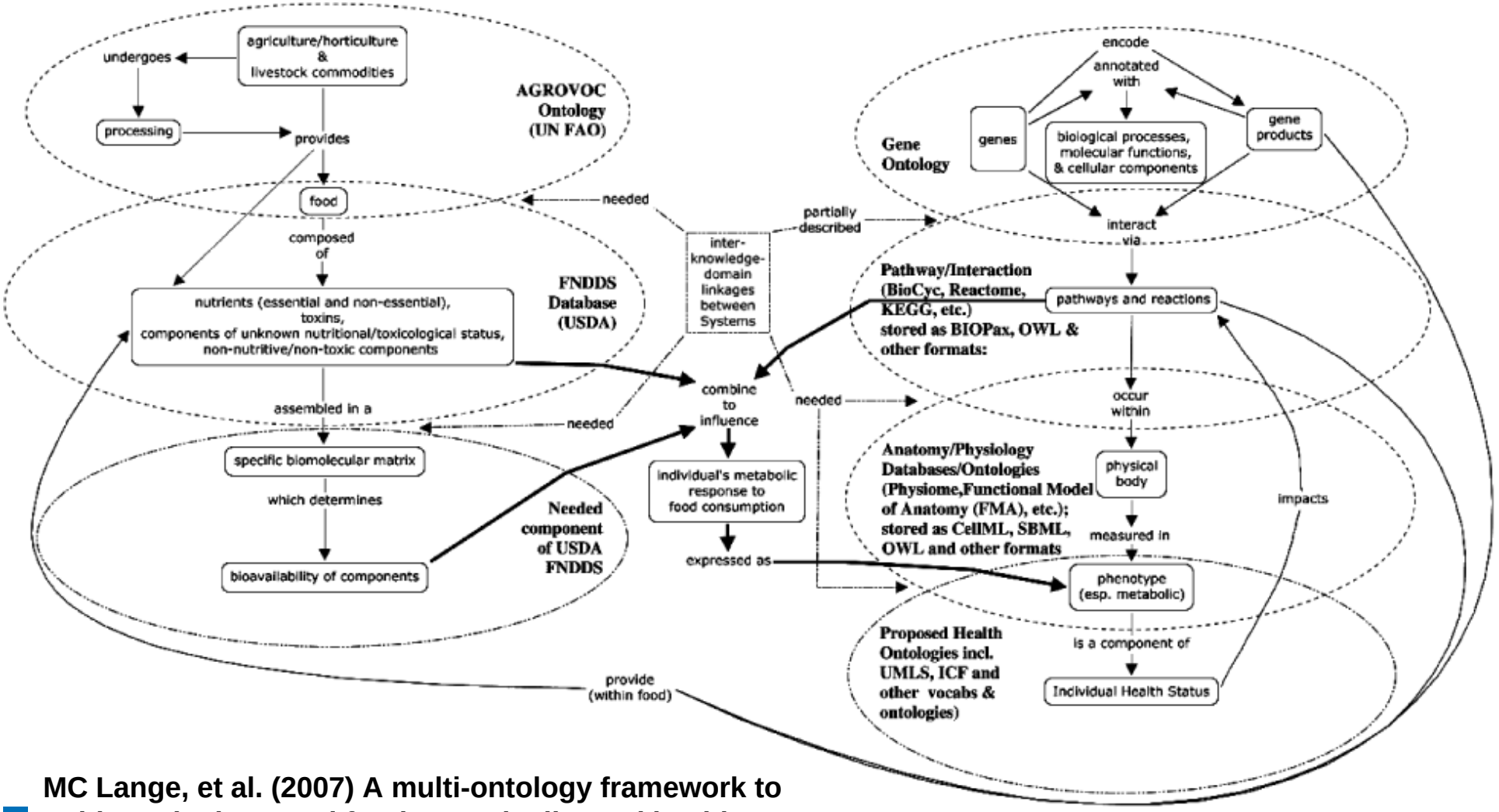
Exposure-Hazard Knowledge System

- Translation of HTP hazard information requires holistic risk assessment knowledge system
  - Include ontologies, databases, linkages
  - Facilitate computerized collection, organization, and retrieval of exposure, hazard, and susceptibility information
- Standardized exposure ontologies required to
  - Define relationships, allow automated reasoning, facilitate meta analyses
  - Develop biologically-relevant exposure metrics
  - Design *in vitro* toxicity tests to measure environmentally-relevant hazard
  - Incorporate information on susceptibility and background exposures to individual and population-level risks

Schematic of ontologies, databases and ontology/database linkages needed for the efficient development of a Foods-for-Health Knowledge System



MC Lange, et al. (2007) A multi-ontology framework to guide agriculture and food towards diet and health. J Sci Food and Ag 87(8)1427-34.

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Exposure Science for Prioritization

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Accessible and Linkable Exposure Databases

- Develop accessible, chemically indexed exposure databases linked with toxicity databases to facilitate application of environmental informatics tools for risk assessment
  - Link to ACToR to evaluate current state of knowledge
  - Link with DSSTox to relate chemical structure with both hazard and exposure
  - Link to publicly available use data
- Develop human exposure knowledgebase
  - Formal representation of key concepts and relationships
  - Ontology to define exposure domain and data structure

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Exposure Screening Tools for Accelerated Chemical Prioritization

- Workshop to review existing tools for exposure-based screening, identify critical gaps (NERL cosponsor)
- Develop standardized protocols and rapid/automated approaches for
  - collecting publicly available product use information from the web
  - selecting sentinel products
  - selecting and implementing scenarios
  - selection of screening models/modules
- Development streamlined generalized modules

- Application of informatic approaches to analyze extant exposure data
  - identify the critical metrics
  - develop simple indices for representing personal exposure over time, place, lifestage, and lifestyle or behavior

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EPA Community of Practice: Exposure Science for Toxicity Testing, Screening, and Prioritization

- The primary purpose of the EPA Exposure Science Community of Practice (ExpoCoP) is to provide a forum for promoting the advancement and utilization of exposure science to address Agency needs for chemical screening, prioritization and toxicity testing.

- Membership of well over 70 individuals from over 30 public and private sector organizations

- http://epa.gov/ncct/practice_community/exposure_science.html

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Disclaimer

Although this work was reviewed by EPA and approved for presentation, it may not necessarily reflect official Agency policy.