United States Environmental Protection Agency

Exposure Considerations for Chemical Prioritization and Toxicity Testing

Elaine Cohen Hubal

ISEE-ISEA 2008 Joint Annual Conference
October 12-16, 2008

Office of Research and Development
National Center for Computational Toxicology

October 6, 2008

United States Environmental Protection Agency

Outline

- National Center for Computational Toxicology
- ToxCast ™ Prioritizing Toxicity Testing of Environmental Chemicals
- Toxicity Testing in the 21st Century
- Exposure Science for Toxicity Testing

United States Environmental Protection Agency

US EPA National Center for Computational Toxicology

- The emerging field of computational toxicology applies mathematical and computer models and molecular biological and chemical approaches to explore both qualitative and quantitative relationships between sources of environmental pollutant exposure and adverse health outcomes.

- The integration of modern computing with molecular biology and chemistry will allow scientists to better prioritize data, inform decision makers on chemical risk assessments, and understand a chemical's progression from the environment to the target tissue within an organism and ultimately to the key steps that trigger an adverse health effect.

http://www.epa.gov/comptox/

United States Environmental Protection Agency

Themes

- The research conducted in the NCCT is designed to address the need for:
  - (1) characterization of the target system across levels of biological organization;
  - (2) improved linkages across the source-to-outcome continuum; and
  - (3) a shift from linear source-to-dose paradigm to a systems-based approach.
- In addition, the complexity of the systems under study and the multidimensional nature of data produced using emerging technologies require extensive collaboration and advanced environmental informatic capabilities.

United States Environmental Protection Agency

NCCT Research Activities

- ToxCast ™: Prioritizing Toxicity Testing of Environmental Chemicals (David Dix and Keith Houck)
- Distributed Structure-Searchable Toxicity (DSSTox) Database Network and Aggregated Computational Toxicity Resource (ACToR): Informatics for Environmental Health Risk Assessment (Ann Richard and Richard Judson)
- Virtual Tissues: Characterizing Toxicity Pathways and Extrapolating Dose-Response (Imran Shah and Tom Knudsen)
- Mechanistic Indicators of Childhood Asthma (MICA) Study: Understanding Environmental Factors of Complex Disease (Jane Gallagher)

United States
Environmental Protection
Agency

ToxCast ™ Prioritizing Toxicity Testing of Environmental Chemicals

United States Environmental Protection Agency

Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

- The U.S. EPA has identified a clear need to develop methods to evaluate a large number of environmental chemicals for their potential toxicity.

- In 2007, EPA launched ToxCast™ in order to develop a cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals in a short period of time.

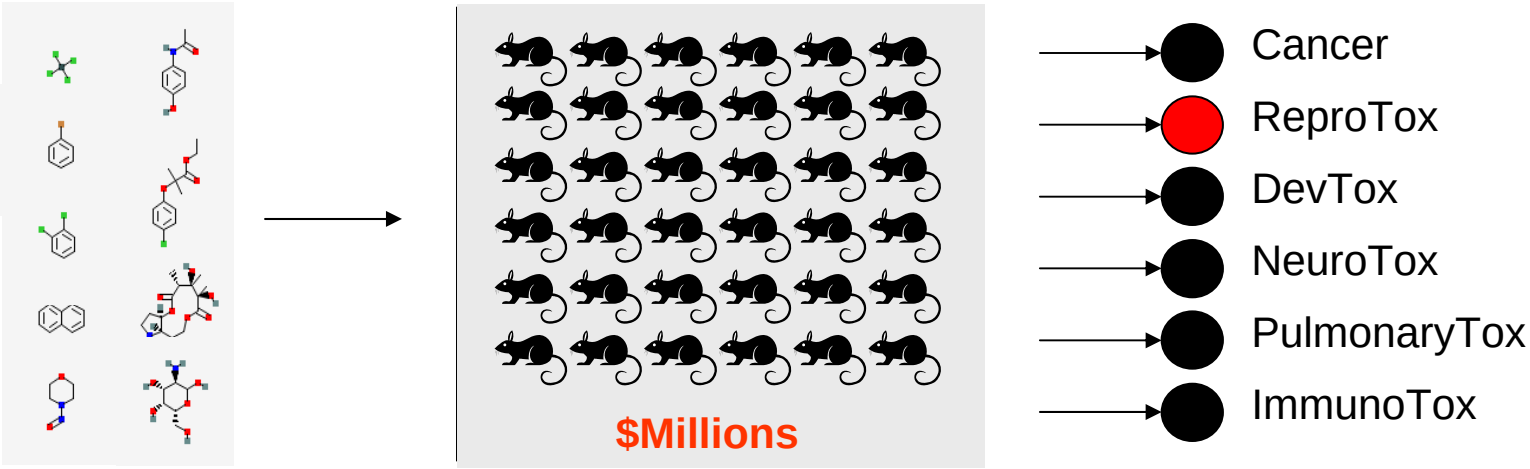
- Using data from state-of-the-art high throughput screening (HTS) bioassays developed in the pharmaceutical industry, ToxCast™ is building computational models to forecast the potential human toxicity of chemicals.

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

United States Environmental Protection Agency

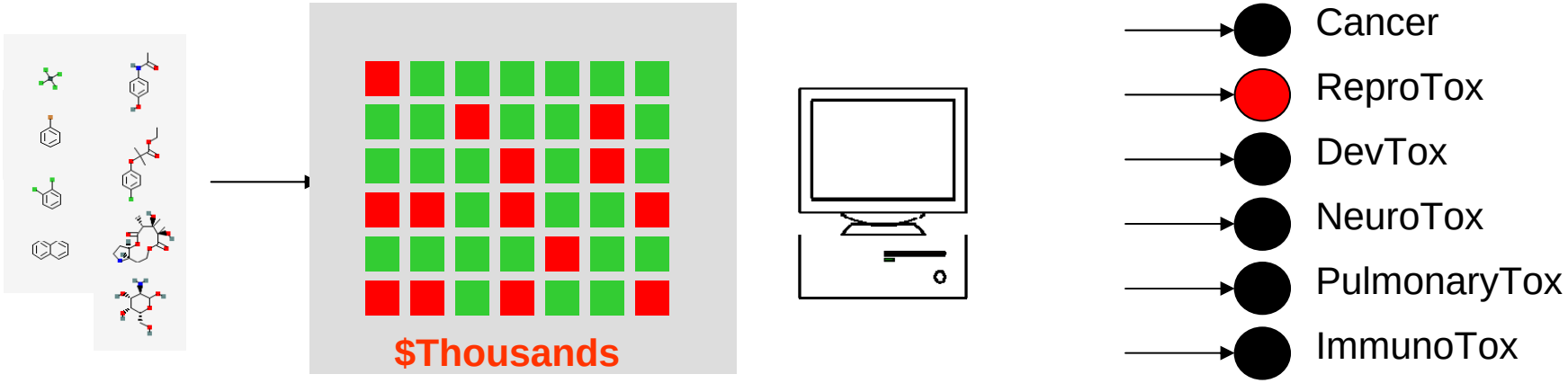
Current Approach for Toxicity Testing

in vivo testing



Future of Toxicity Testing

in vitro testing *in silico* analysis



HTS
-omics

Bioinformatics/
Machine Learning

United States Environmental Protection Agency

ToxCast™ - Phase I

- Profiling over 300 well-characterized chemicals (primarily pesticides) in over 400 HTS endpoints.
- Endpoints include biochemical assays of protein function, cell-based transcriptional reporter assays, multi-cell interaction assays, transcriptomics on primary cell cultures, and developmental assays in zebrafish embryos.
- Most Phase I compounds have been tested in traditional toxicology tests
- In Phase II, ToxCast™ will screen additional compounds representing broader chemical structure and use classes, in order to evaluate the predictive bioactivity signatures developed in Phase I.

United States
Environmental Protection
Agency

Toxicity Testing in the 21st Century

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

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

National Academy of Sciences • National Academy of Engineering • Institute of Medicine • National Research Council

POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

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.

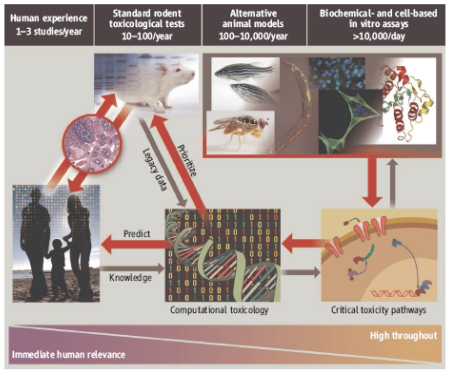
Francis S. Collins,*† George M. Gray,‡* John R. Bucher§*

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.

*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

United States Environmental Protection Agency

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

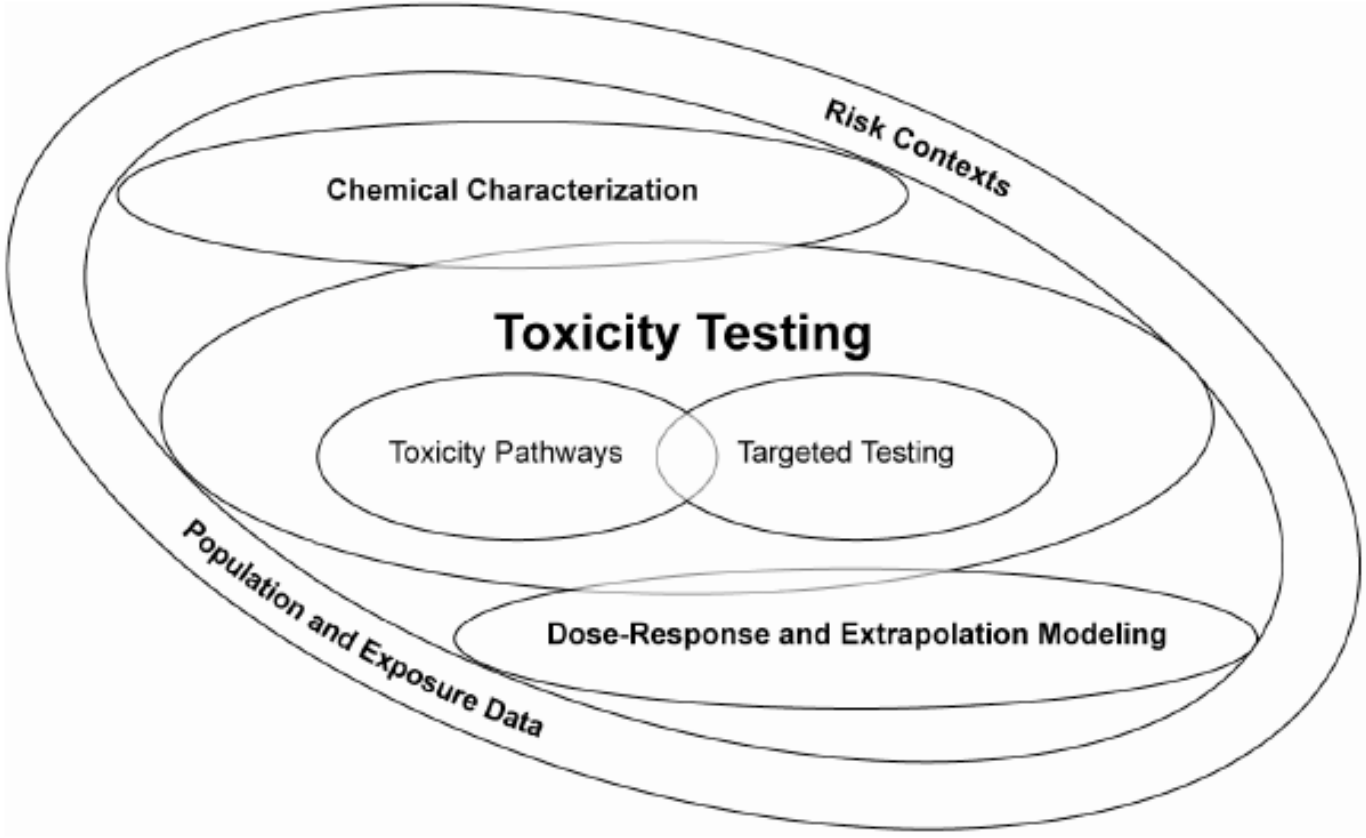


FIGURE 2-3 The committee's vision is a process that includes chemical characterization, toxicity testing, and dose-response and extrapolation modeling. At each step, population-based data and human exposure information are considered, as is the question of what data are needed for decision-making.

United States Environmental Protection Agency

Toxicity Testing in the Twenty-first Century

- The key aspect of the NRC vision and the proposed paradigm shift in Toxicity Testing is that new tools are available to examine toxicity pathways in a depth and breadth that has not been possible before.

- Efforts underway to apply high-throughput-screening (HTS) approaches for chemical prioritization and toxicity testing have been accelerated in response to NRC reports.

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

United States Environmental Protection Agency

Exposure Science for Toxicity Testing

- Now a paradigm shift in exposure science commensurate with that seen for toxicity testing is required to realize the potential of these tools for improved risk assessment and improved public health.

- Shift from resource and time intensive measurement and modeling to rapid, inexpensive approaches for characterizing and predicting biologically relevant exposure.

- Significant investments in exposure science will be required to provide the critical real-world information for interpreting these toxicity data, predicting risks and informing risk management decisions.

United States
Environmental Protection
Agency

Exposure Science for Toxicity Testing

United States Environmental Protection Agency

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

"The vision emphasizes the generation and use of population-based and human exposure data where possible for interpreting test results and encourages the collection of such data on important chemicals with biomonitoring, surveillance, and epidemiologic studies. Population-based and human exposure data, along with the risk context, will play a role in both guiding and using the toxicity information that is produced."

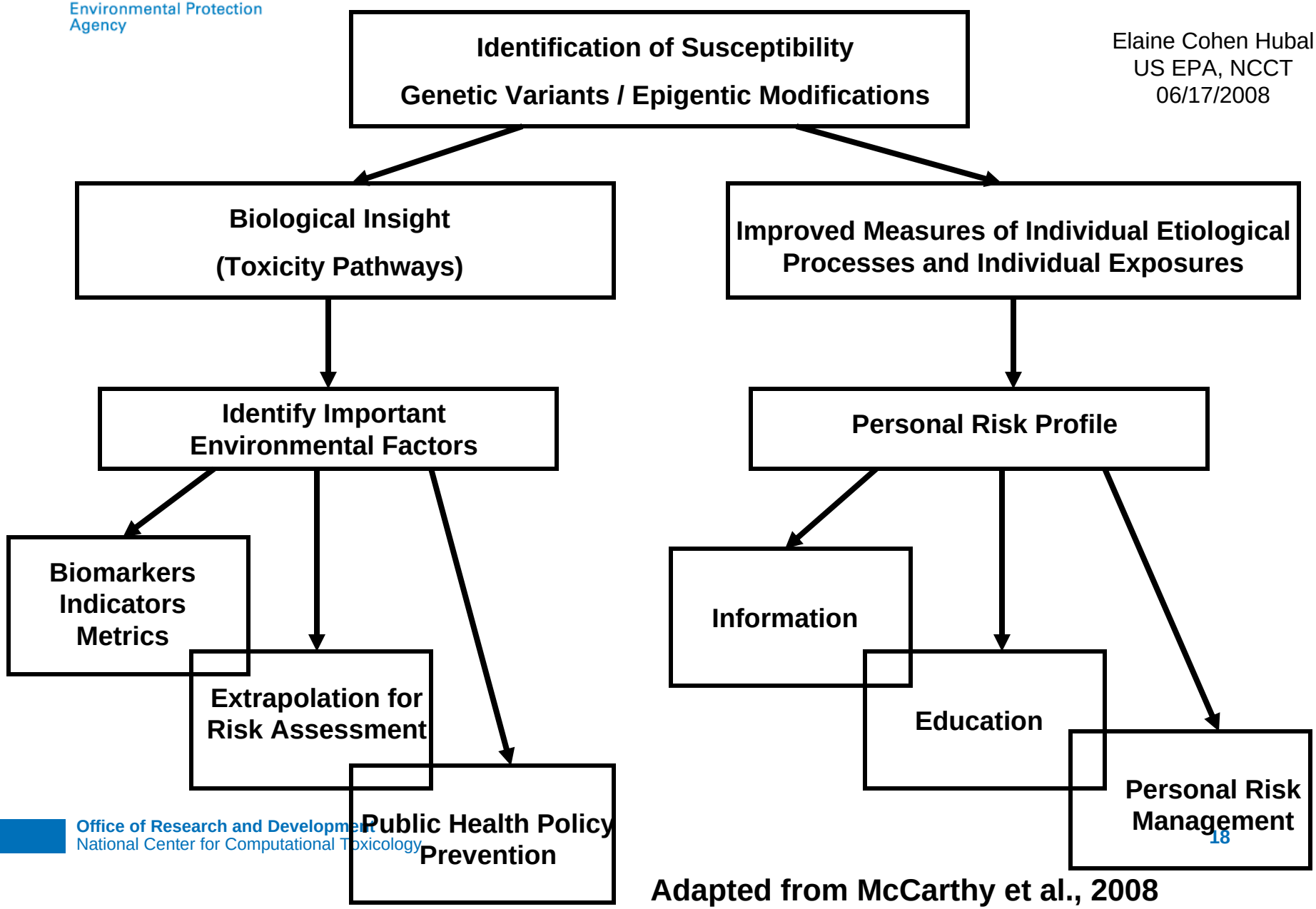
United States Environmental Protection Agency

21st Century Exposure Research Questions

- How do we use information on host susceptibility and background exposures to interpret and extrapolate in vitro test results?
- How do we use human exposure data to select doses for toxicity testing so we develop information on biological effects at environmentally relevant exposures?
- How can we relate human exposure data from biomonitoring surveys to concentrations that perturb toxicity pathways to identify potentially important exposures?

Translation of Findings from Toxicity Testing

Elaine Cohen Hubal
US EPA, NCCT
06/17/2008

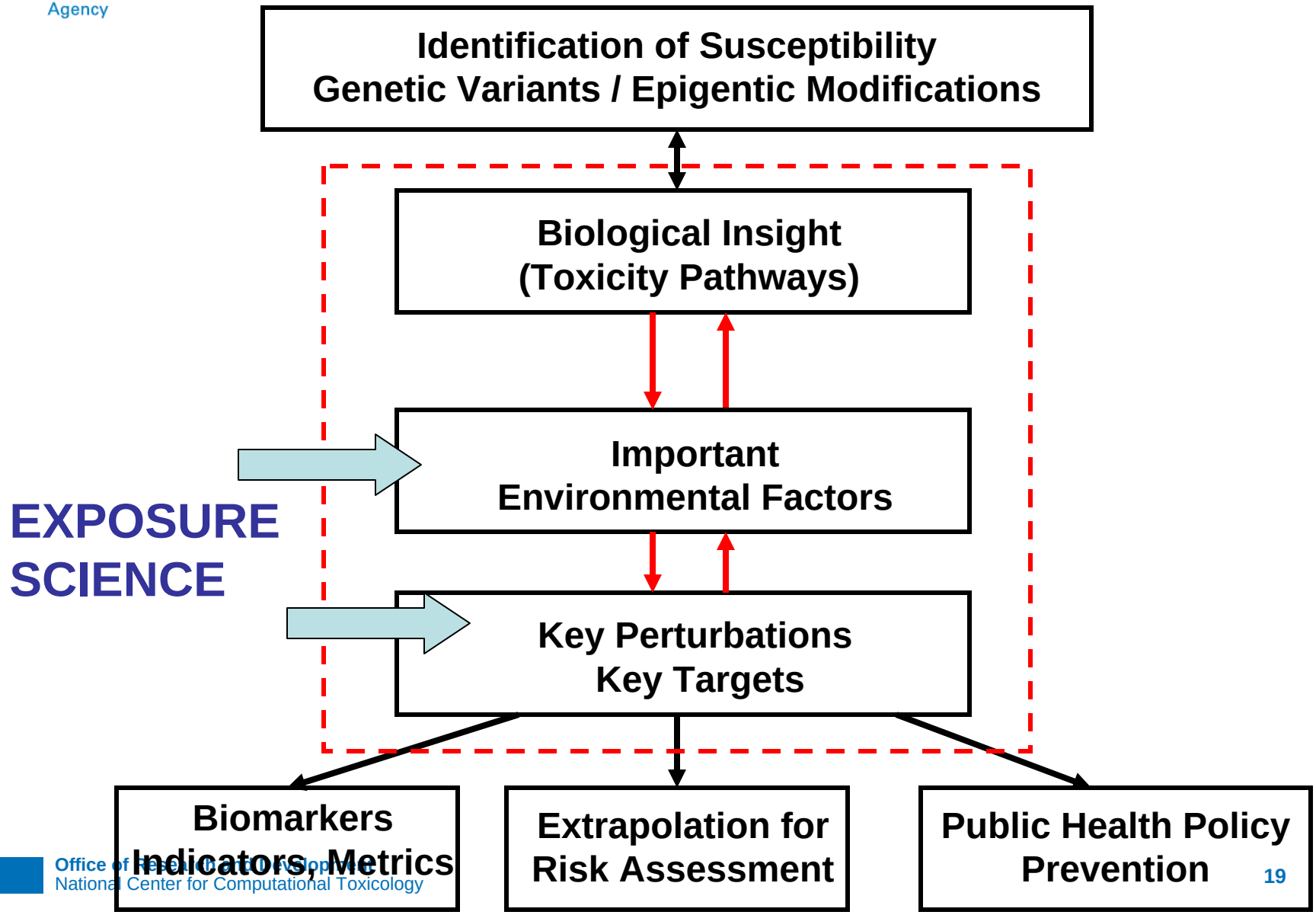


Adapted from McCarthy et al., 2008

Translation: Population Level (Government)

Elaine Cohen Hubal
US EPA, NCCT
06/17/2008

United States Environmental Protection Agency

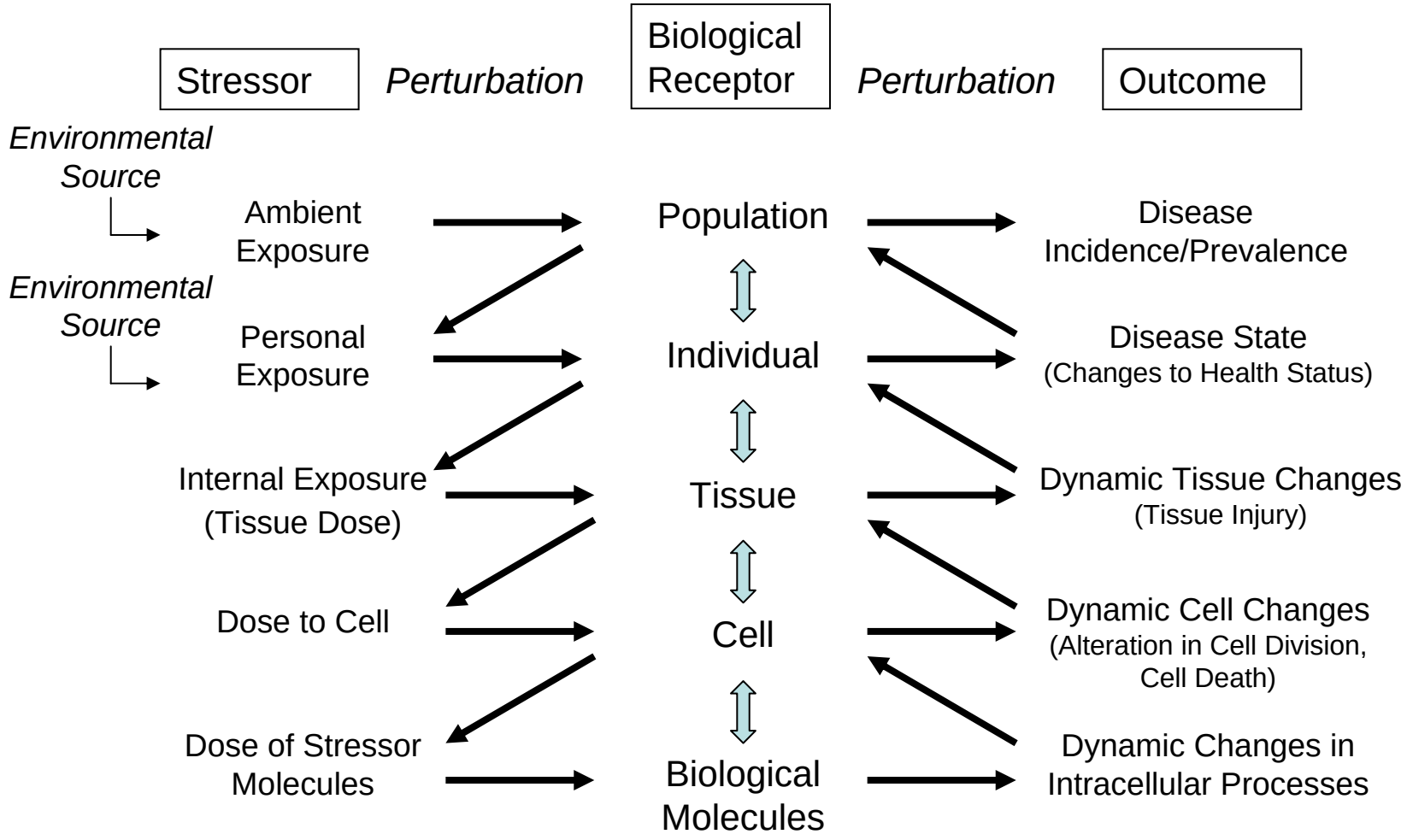


United States Environmental Protection Agency

Anchoring stressors to real-world human exposure

- **Important opportunity for exposure scientists**
- Consider analogies in hazard assessment to inform our path forward.
- The NRC Vision of a shift to characterizing toxicity pathways requires a commensurate shift to characterizing exposure across all levels of biological organization.
- Interpretation of toxicogenomic hazard data requires contextual relevance. Pathways identified using HTS approaches are being anchored to apical endpoints using conventional toxicity data.
- Similarly, understanding relevant perturbations leading to these toxicogenomic endpoints require anchoring stressors to real-world human exposure (e.g., biomonitoring data and other conventional exposure metrics).
- New approaches to risk assessment require exposure science to predict exposures down to the molecular level. Requires systems-based consideration of interactions between exposure and effect.

Cascade of exposure-response processes for integrating exposure science and toxicogenomic mode-of-action information.



United States Environmental Protection Agency

Exposure Science Research Needs

- **New and innovative tools required to characterize human exposure.**
  - Easily **accessible**, chemically indexed exposure databases that can be **linked** with toxicity databases are required to facilitate application of environmental informatics tools for risk assessment.
  - Screening-level indices for **efficient** screening of chemicals based on potential for exposure are required for toxicity-testing prioritization.
  - **Coordinated** development efforts are required to provide exposure, dose-response, and biological pathway models that use common programming languages to facilitate links across the source-to-outcome continuum.
  - Application of advance computational approaches for exposure and dose reconstruction is required to relate biomarkers of exposure in populations with administered concentrations of *in vitro* HTS assays.

United States Environmental Protection Agency

Exposure Science Research Needs

- **Significant advances required in mechanistic understanding of exposure processes and determinants.**
  - Translation of advanced toxicogenomic and biosensor technologies provides opportunity to develop efficient and affordable tools for measuring **biologically relevant exposures** and identifying susceptible individuals.
  - Data collected using these advanced monitoring protocols will provide mechanistic underpinning and support interpretation of toxicity test results.

United States Environmental Protection Agency

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 over 40 individuals from over 15 public and private sector organizations
- http://epa.gov/ncct/practice_community/exposure_science.html

United States Environmental Protection Agency

Acknowledgements

- NCCT – Bob Kavlock, Jerry Blancato
- ToxCast ™ - David Dix and Keith Houck
- NHEERL - Stephen Edwards

Disclaimer

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