EPA
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Environmental Protection
Agency

Renoir, *On the Terrace*, 1881

Does Exposure Imitate Art: Exposure Science for 21st Century Toxicity Testing

Society of Toxicology Issues Session:

NAS Vision for Toxicity Testing in 21st Century

March 11, 2010

Elaine Cohen Hubal
National Center for Computational Toxicology

United States Environmental Protection Agency

The Context: Chemical Evaluation and Risk Assessment

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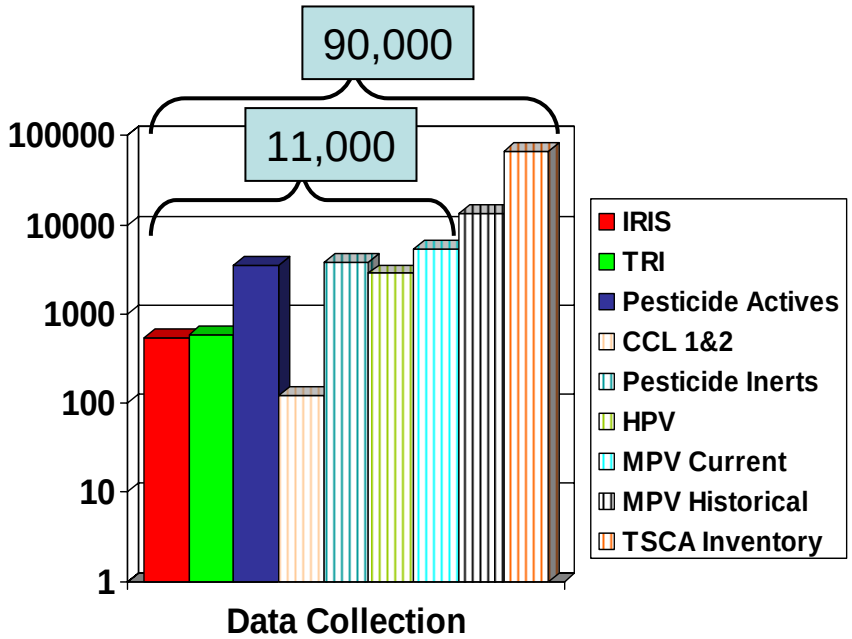
Mandate to Assess Thousands of Chemicals

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



Richard Judson, NCCT

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.

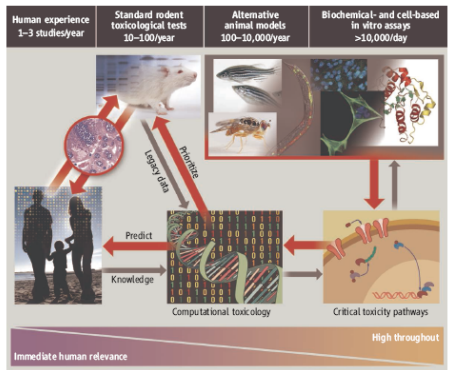
Francis S. Collins,*† George M. Gray,‡ John R. Bucher§

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.

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

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

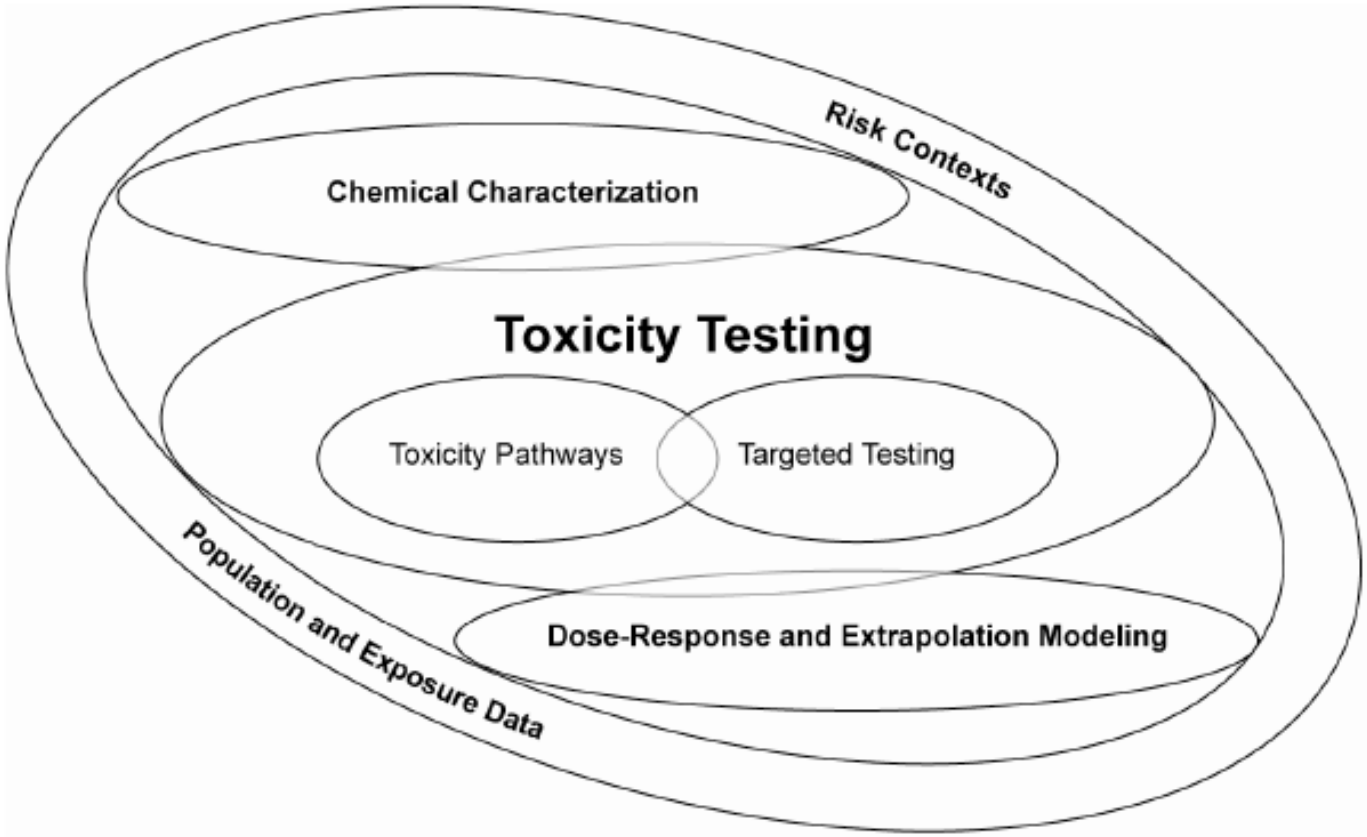

FIGURE 2-3 The committee's vision is a process that includes chemical characterization, toxicity testing, and dose-response and extrapolation modeling. At each step, population-based data and human exposure information are considered, as is the question of what data are needed for decision-making.

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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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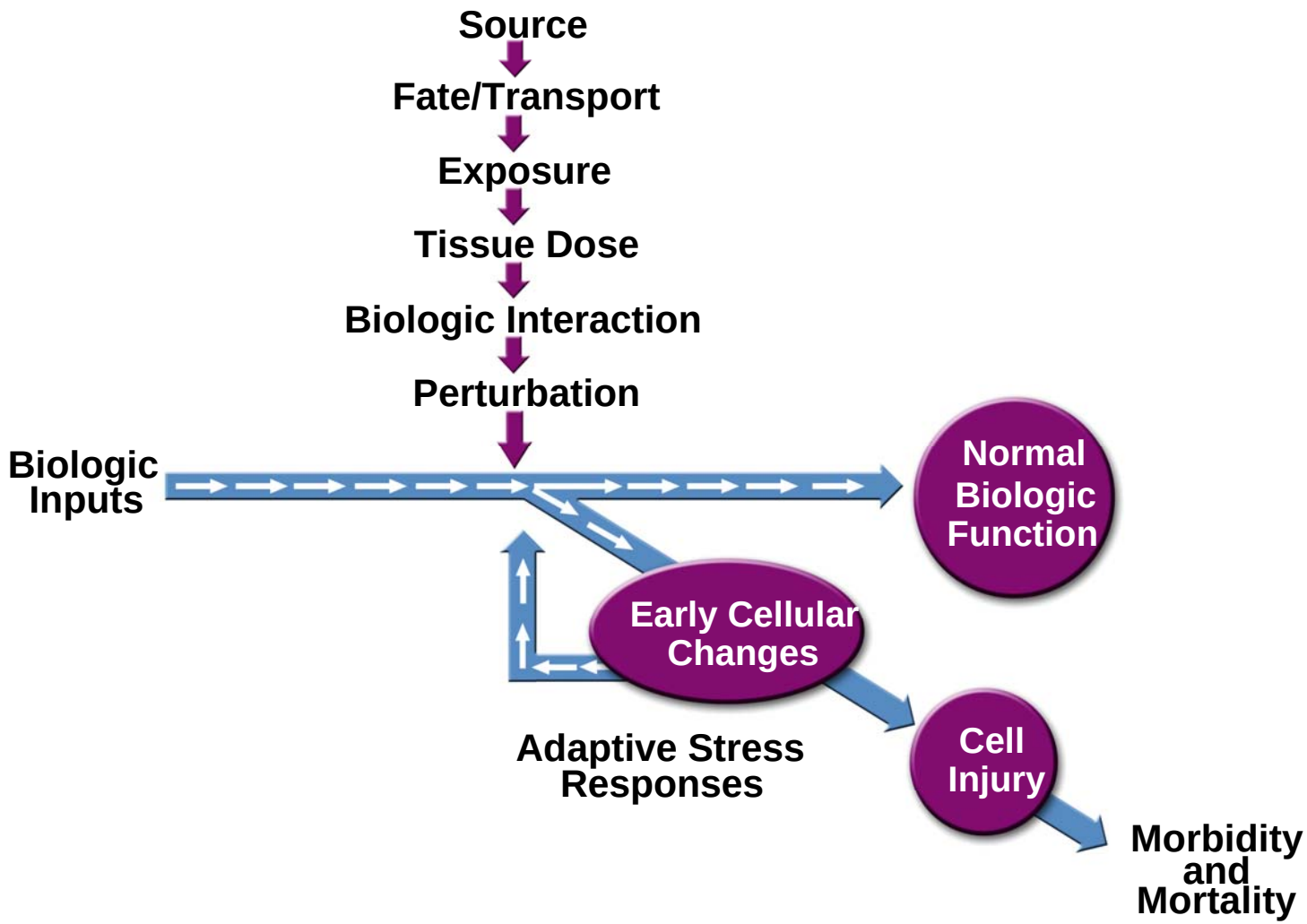
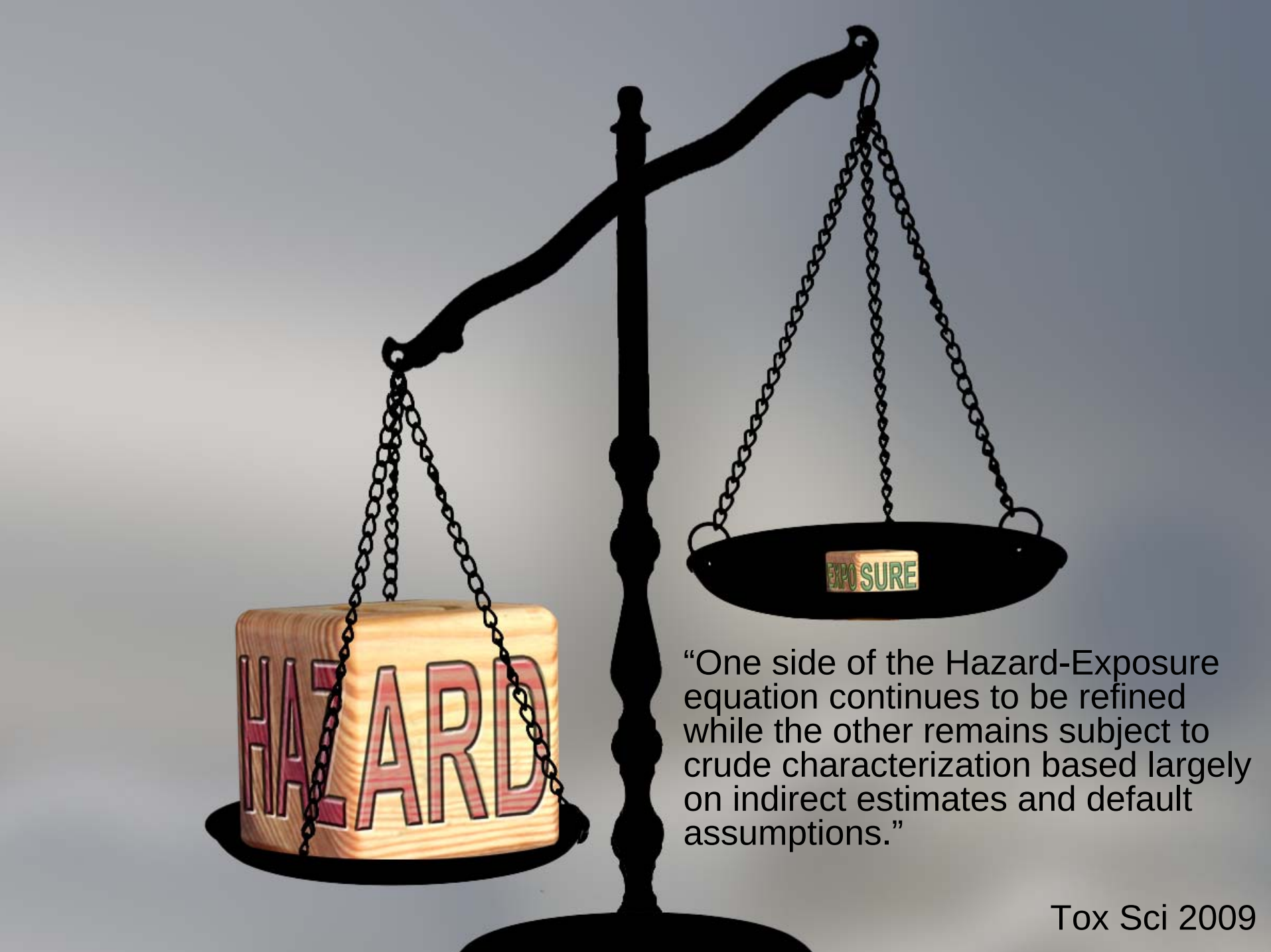
Exposure Science in NRC Vision



Modified from NRC, 2007



"One side of the Hazard-Exposure equation continues to be refined while the other remains subject to crude characterization based largely on indirect estimates and default assumptions."

Tox Sci 2009

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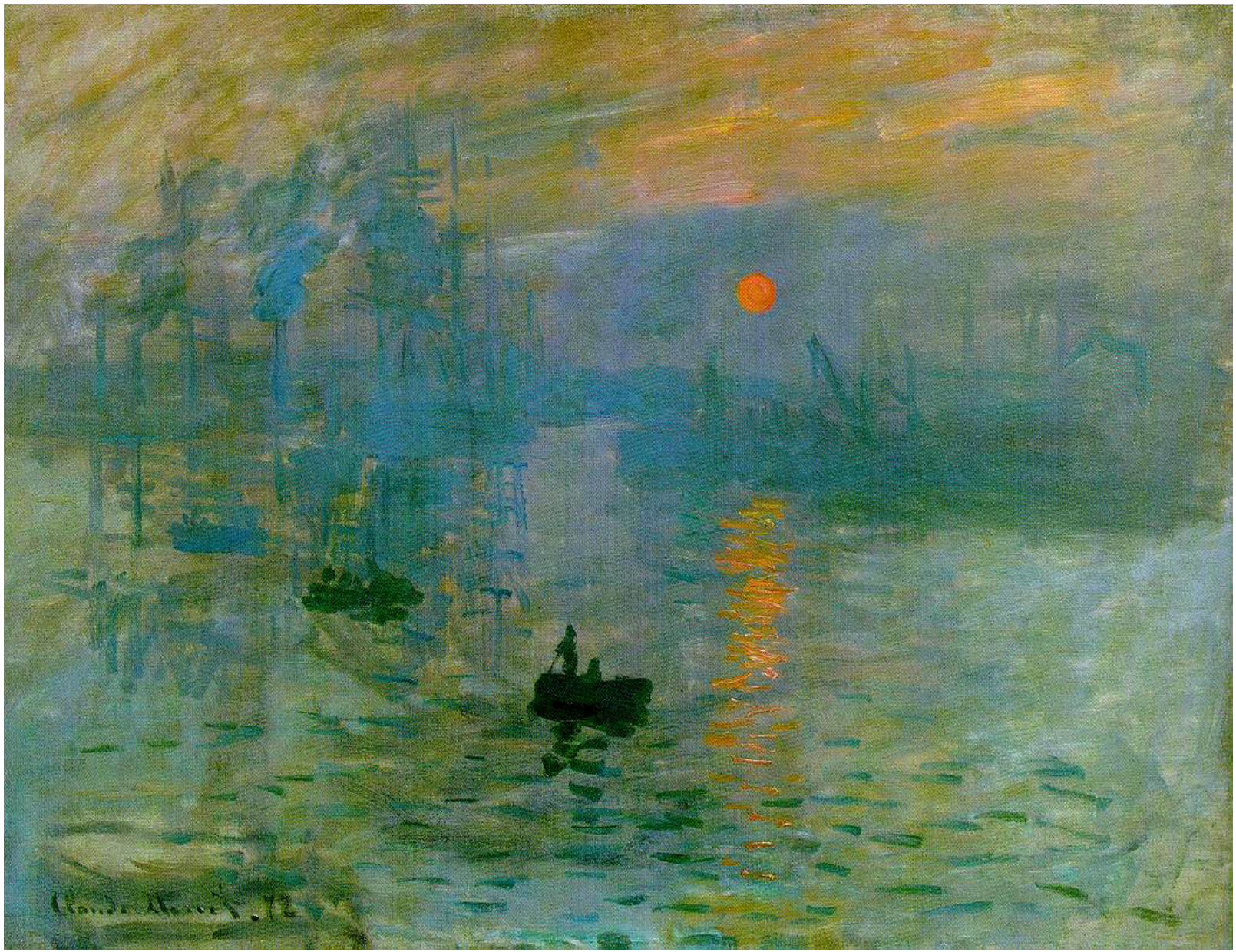
Will fundamental knowledge of toxicity pathways improve understanding of real-world human-health risk?

- Assessing complex human-health risks requires that **hazard**, **susceptibility**, and **exposure** are all reliably characterized.
- Currently, balance of efforts to improve measuring hazard and exposure less than ideal.
- Accurate assessment of many environmental exposures remains an outstanding and largely unmet challenge in toxicology and risk assessment.
- **To realize the NRC vision, we face a critical need for advanced exposure science.**

Adapted from Wild, 2005



Claude Monet, ***Impression,*** *soleil levant****,*** 1872

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Impressionism a Transformational Movement

- Radicals–broke the rules of painting

- Developed new techniques - different way of seeing
  - Plein-air, open composition (system definition)
  - Immediacy and movement (interplay of subject and environment, dynamics)
  - Light expressed in a bright and varied use of color (key determinants)

- Captured a fresh and original vision
  - Re-created the sensation in the eye that views the subject, rather than recreating the subject.

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Does Exposure Imitate Art?

- System
  - Moved from studio out into modern world
  - Open compositions, realistic scenes
- Resolution
  - Exquisite detail (smoothly blended) of surrogate representation
  - Abstraction (distillation) of key determinants to address mechanism
  - Free brush strokes of pure color to emphasize vivid overall effects rather than details
- Determinants
  - Light (changing qualities)
  - Color (bright and varied)
  - Form (loose brush strokes)

Open System, Relevant Resolution

Jacques-Louis David, The Comtesse Vilain XIIII and Her Daughter (1816)

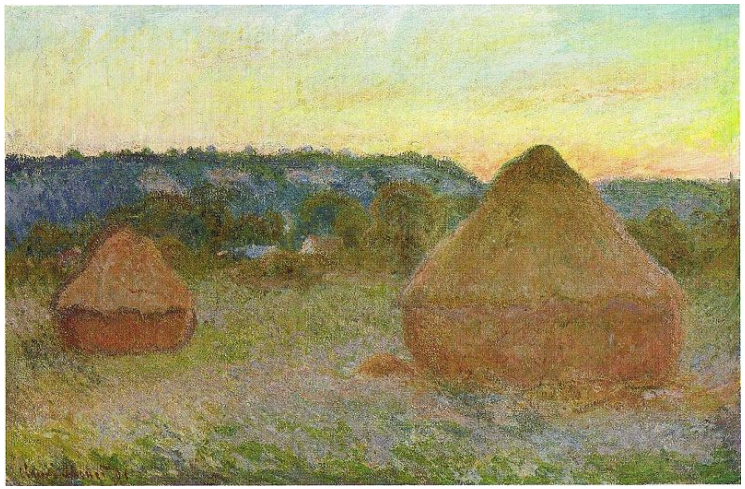
Pierre-Auguste Renoir, Le Moulin de la Galette, 1876

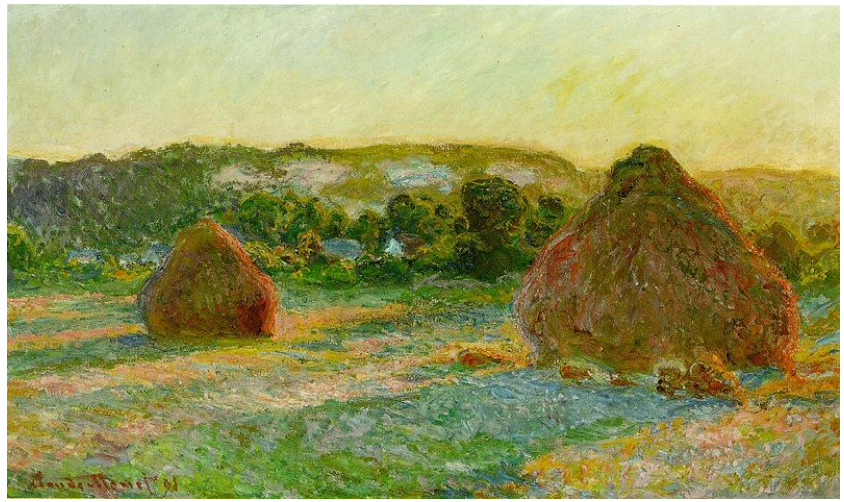
Key Determinants

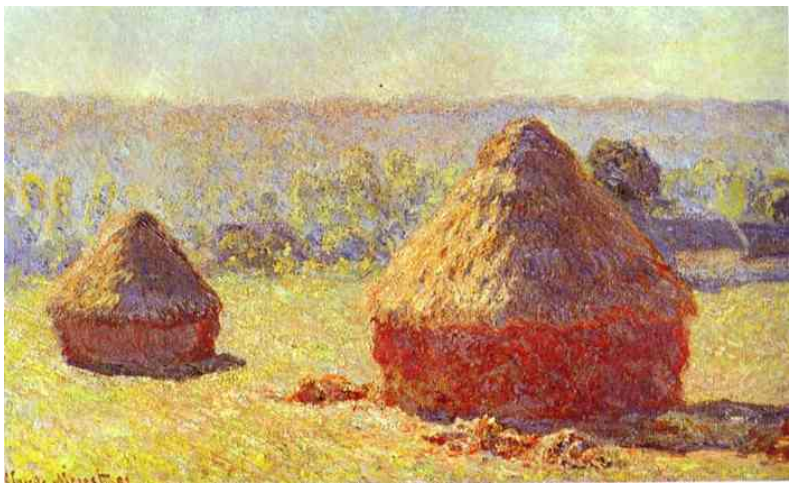
Fragonard, The Swing, 1767

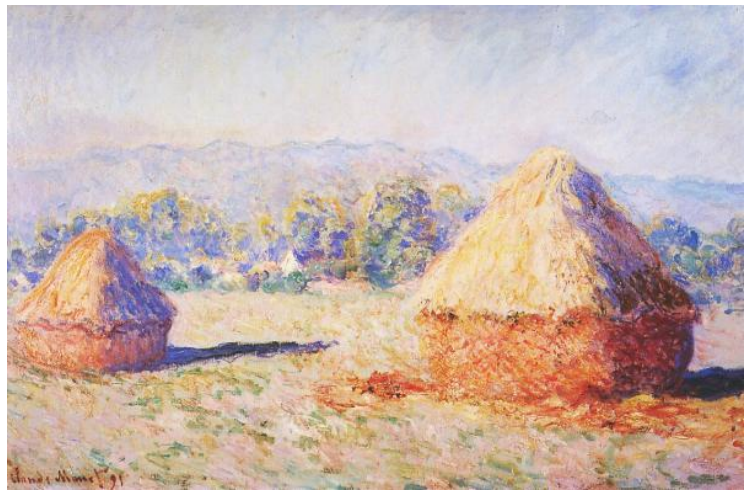
Renoir, The Swing (La Balançoire), 1876

Variability, Vulnerability and Life-stage Aspects Integral









Monet, Grainstacks 1890-1890

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Exposure Science Research Questions

- What does the real world look like?
  - What are the critical elements of exposure in a given context?
  - What are the key metrics for characterizing these exposure elements in that context?
  - What is the required resolution for measuring key metrics and modeling exposures so that these are relevant for developing and interpreting hazard information to assess health risks?

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

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Transforming Exposure Science for Toxicity Testing

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New technologies must be applied to *BOTH* toxicology and exposure science if the ultimate goal of evaluating chemical safety is to be achieved.

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

Cohen Hubal, Tox Sci, 2009

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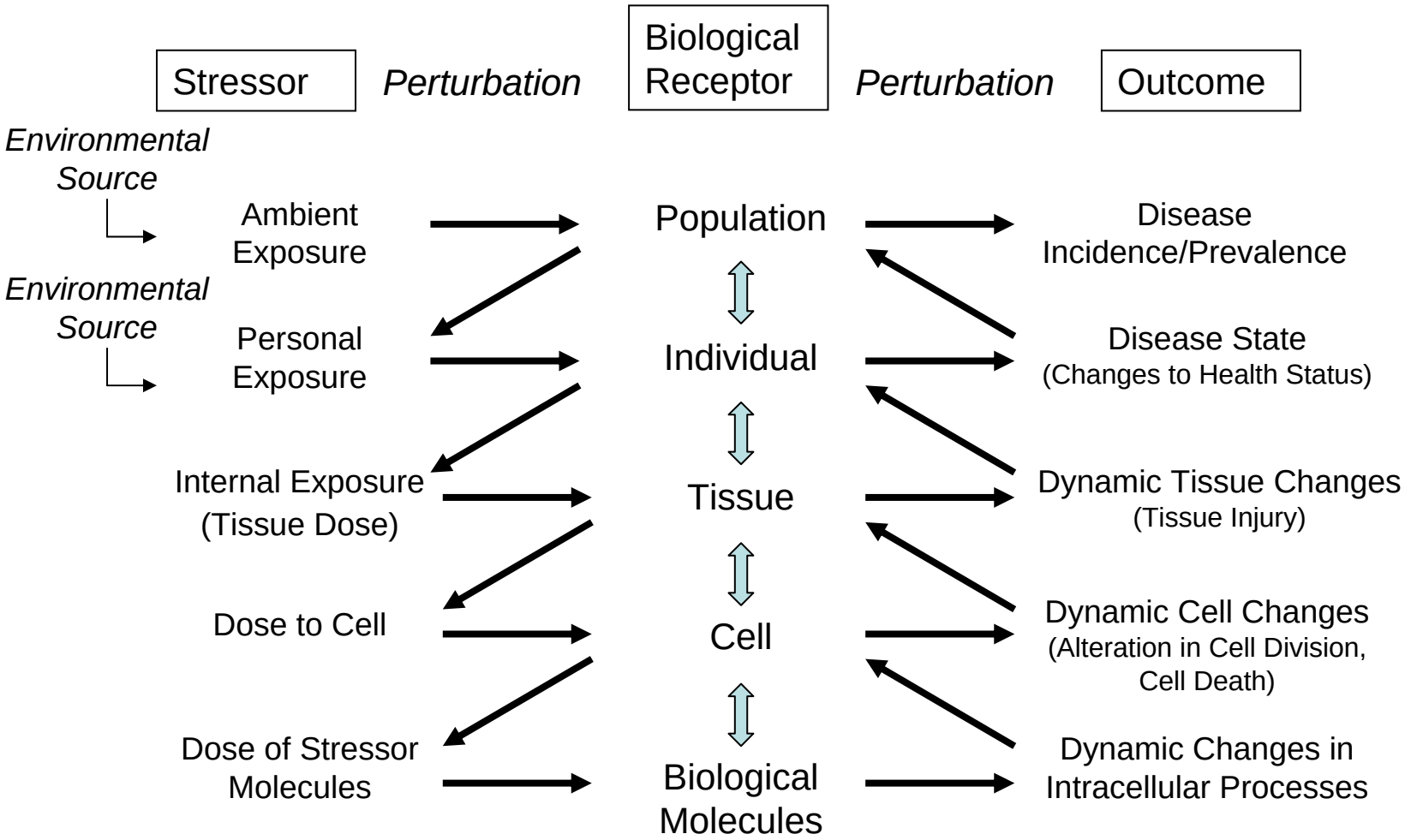
Systems Biology: Exposure at All Levels of Biological Organization



Cohen Hubal, JESEE, 2008

Figure 1

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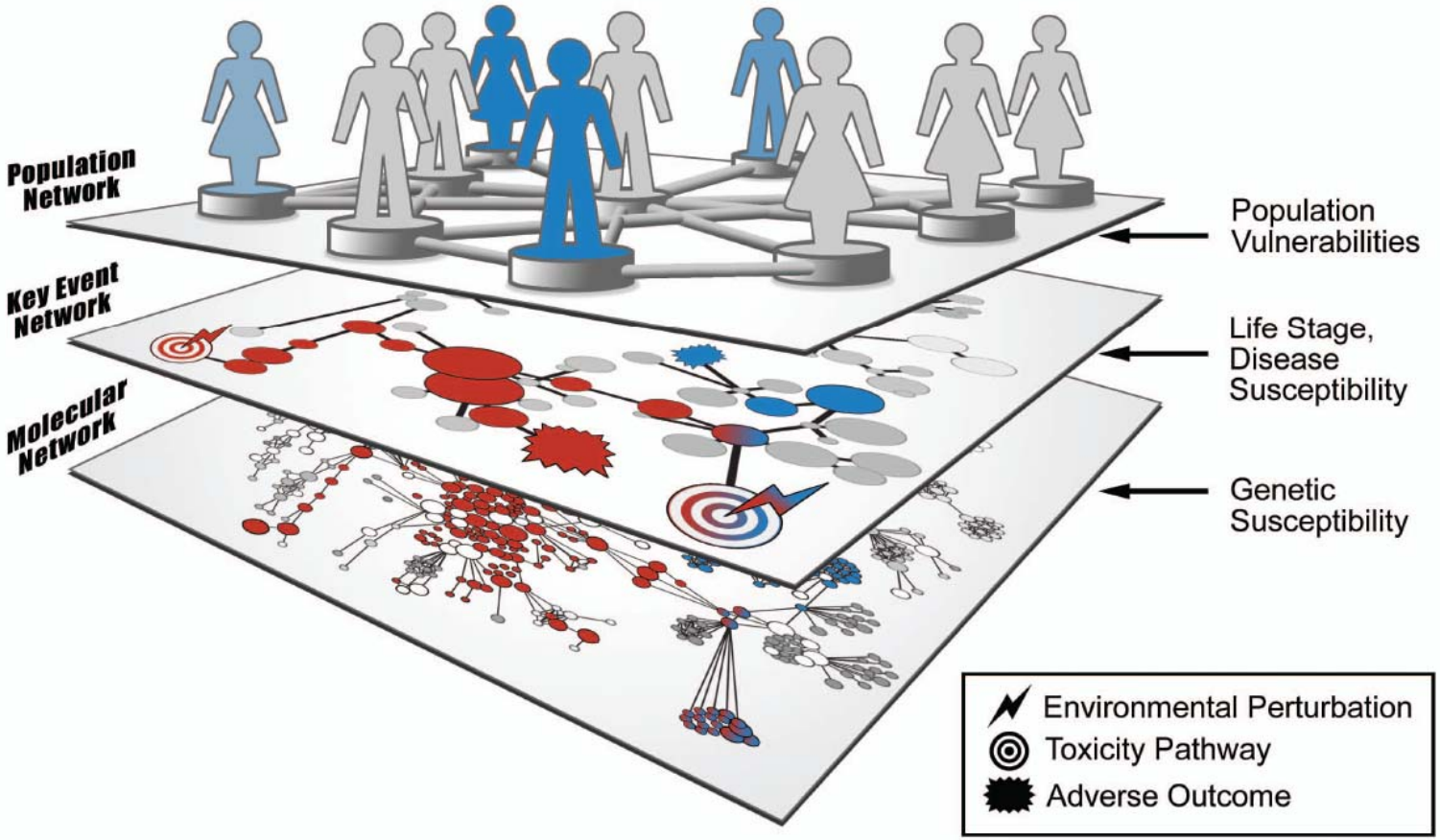
Systems Biology: Extending Network Analysis to Develop the Exposome

Consider coupled networks spanning multiple levels of biological organization



Adapted from Edwards & Preston (2008), Tox Sci, 106(2):312-318

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Systems Biology: Extending Network Analysis to Inform Risk Assessment

- 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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Biologically-Relevant Exposure Metrics

Markers required that can be directly associated with key events in disease processes and with individual exposure profiles

- '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))

Investment in this area of research required to provide important approaches for assessing real-world exposures

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Computational Techniques – Two Branches

- A combination of discovery and engineering (mechanistic)-based modeling approaches for hypothesis development and testing are required.

- Knowledge-discovery
  - Data-collection, mining, and analysis
  - Required to extract information from extant data on critical exposure determinants, link exposure information with toxicity data, and identify limitations and gaps in exposure data.

- Mechanistic (dynamic) simulation
  - Mathematical modeling at various levels of detail
  - Required to model the human-environment system and to test our understanding of this system.

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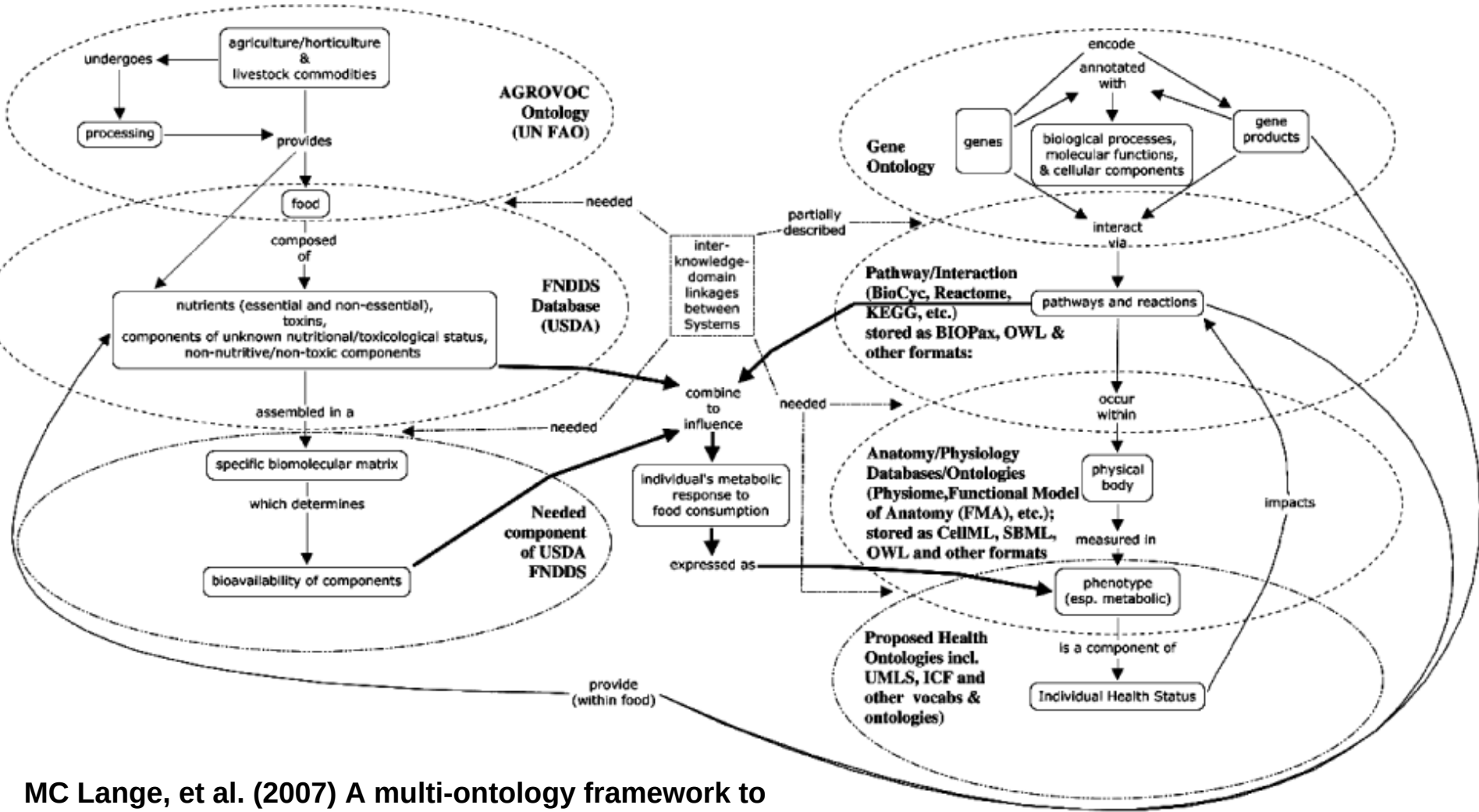
Exposure-Hazard Knowledge System

- 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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Mapping the Exposome

Exposome: (Chris Wild, 2005)

Measurement of the life-course of environmental exposures

- Need for a "step change" in exposure assessment to provide the evidence base for public health decisions to address environmental health
- Articulates a vision for exposure measurement commensurate with that of NRC vision for toxicity testing

- The Exposome: A powerful approach for evaluating environmental exposures and their influences on human disease (NAS, Feb 25-26)

- Sophisticated new tools are available to measure response
  - **Broad-based investment is required to develop commensurate exposure measurement capacity**

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Exposure Science for Computational Toxicology

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ExpoCast™: Exposure Science for Prioritization and Toxicity Testing

- Recognizes critical need for exposure information to inform
  - Chemical design and evaluation
  - Health risk management
- Goal
  - Advance characterization of exposure required to ***translate*** findings in computational toxicology to support exposure and risk assessment
  - Together with ToxCast™ help EPA determine priority chemicals
- Approach
  - Mine and apply scientific advances and tools in a broad range of fields
  - Develop novel approaches for evaluating chemicals based on potential for ***biologically-relevant*** human exposure

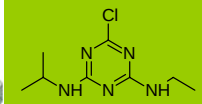
Environment

Sources

Distribution/Fate

Products

Contact



Chemicals

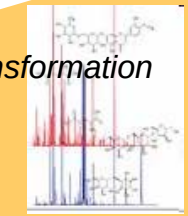
Exposure Media

Exposure

Uptake

Background Exposure

Biotransformation



Biomonitoring

Population

Host Susceptibility

Human

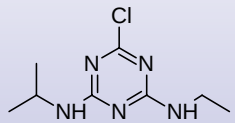
Rapid Prioritization

Relate real-world exposures with toxicity pathway perturbations

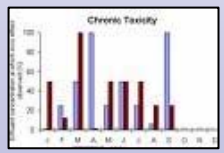
Select doses for toxicity testing

Translate in vitro results for risk assessment

HTS assays



Toxicity endpoints

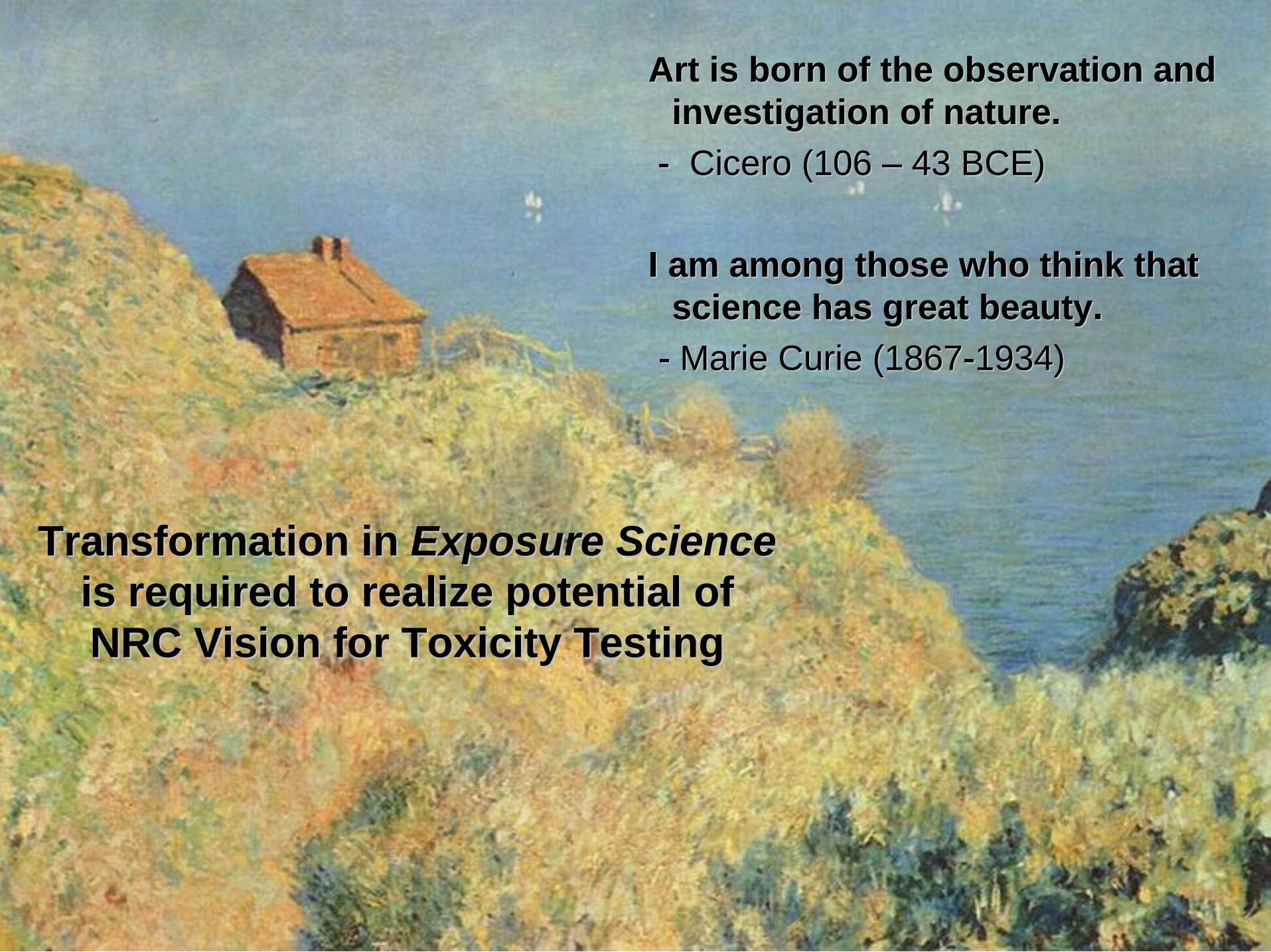


In vivo bioassays

Data Repositories | Mechanistic Models | Informatics Approaches | Knowledge Systems | Network Models | Exposome

TOOLS

Ann Richard, NCCT



Art is born of the observation and investigation of nature.

- Cicero (106 – 43 BCE)

I am among those who think that science has great beauty.

- Marie Curie (1867-1934)

Transformation in *Exposure Science* is required to realize potential of NRC Vision for Toxicity Testing

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Disclaimer

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