Toxicity Testing in the 21st Century

Daniel Krewski, University of Ottawa
and
Mel Andersen, Hamner Institutes for Life Sciences

ICCVAM Ten-Year Anniversary Symposium
Bethesda, Maryland
February 5, 2008

BEST

Board on Environmental Studies and Toxicology

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

Committee on Toxicity Testing and Assessment of Environmental Agents

Board on Environmental Studies and Toxicology

Institute for Laboratory Animal Research

Division on Earth and Life Studies

National Research Council

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Committee Roster

Daniel Krewski (*Chair*), University of Ottawa, Ottawa, ON
Daniel Acosta, Jr., University of Cincinnati, Cincinnati, OH
Melvin Andersen, CIIT Centers for Health Research, Research Triangle Park, NC
Henry Anderson, Wisconsin Division of Public Health, Madison, WI
John Bailar III, University of Chicago, Chicago, IL
Kim Boekelheide, Brown University, Providence, RI
Robert Brent, Thomas Jefferson University, Wilmington, DE
Gail Charnley, HealthRisk Strategies, Washington, DC
Vivian Cheung, University of Pennsylvania, Philadelphia, PA
Sidney Green, Howard University, Washington, DC
Karl Kelsey, Harvard University, Boston, MA
Nancy Kerkvliet, Oregon State University, Corvallis, OR
Abby Li, Exponent, Inc., San Francisco, CA
Lawrence McCray, Massachusetts Institute of Technology, Cambridge MA
Otto Meyer, Danish Institute for Food and Veterinary Research, Søborg, Denmark
D. Reid Patterson, Reid Patterson Consulting, Inc., Grayslake, IL
William Pennie, Pfizer, Inc., Groton, CT
Robert Scala, Exxon Biomedical Sciences (Ret.), Tucson, AZ
Gina Solomon, Natural Resources Defense Council, San Francisco, CA
Martin Stephens, The Humane Society of the United States, Washington, DC
James Yager, Jr., Johns Hopkins University, Baltimore, MD
Lauren Zeise, California Environmental Protection Agency, Oakland, CA

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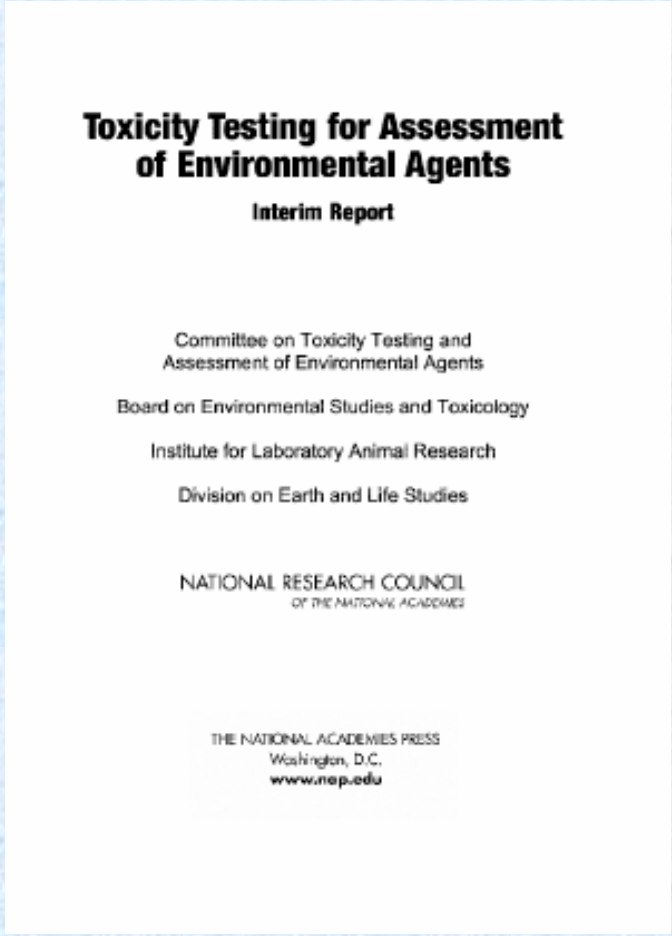
Interim Report



- Review current toxicity-testing protocols and strategies.
- Review current cancer and noncancer risk assessment guidelines to determine data needs for risk assessment.
- Review various documents (EPA, ILSI-HESI, NTP, and REACH) that propose improvements to toxicity-testing approaches.

www.nas.edu

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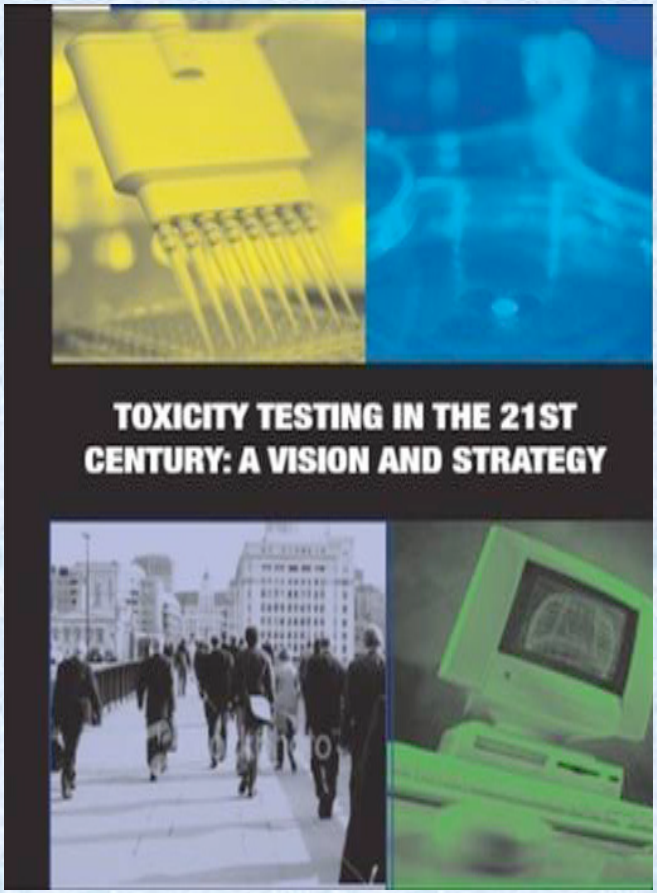
Statement of Task: Final Report



- Assessment of key exposures (life stages) and toxicity outcomes (neurotoxicity)
- State-of-the-science testing and assessment procedures (genomics, bioinformatics, pharmacokinetics)
- Efficient experimental design and reduced use of laboratory animals
- New and alternative test methods
- Computational and molecular techniques in risk assessment

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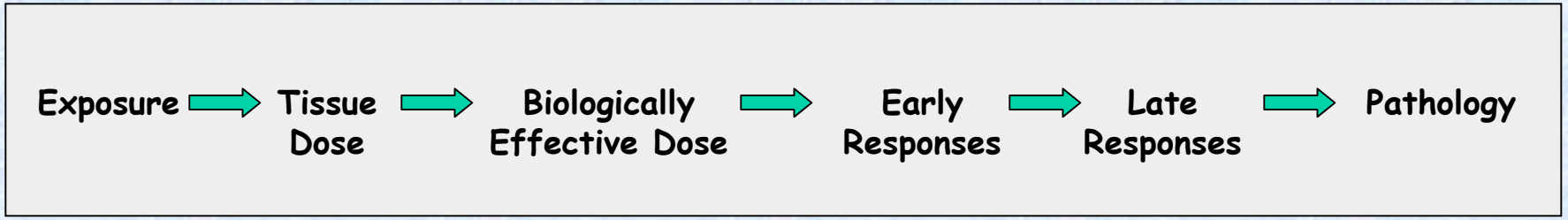
Current Paradigm: The Exposure-response Continuum



Physiologically Based Pharmacokinetic Models

Tissue Dose Metric

Mode of Action

Pharmacokinetics

Pharmacodynamics

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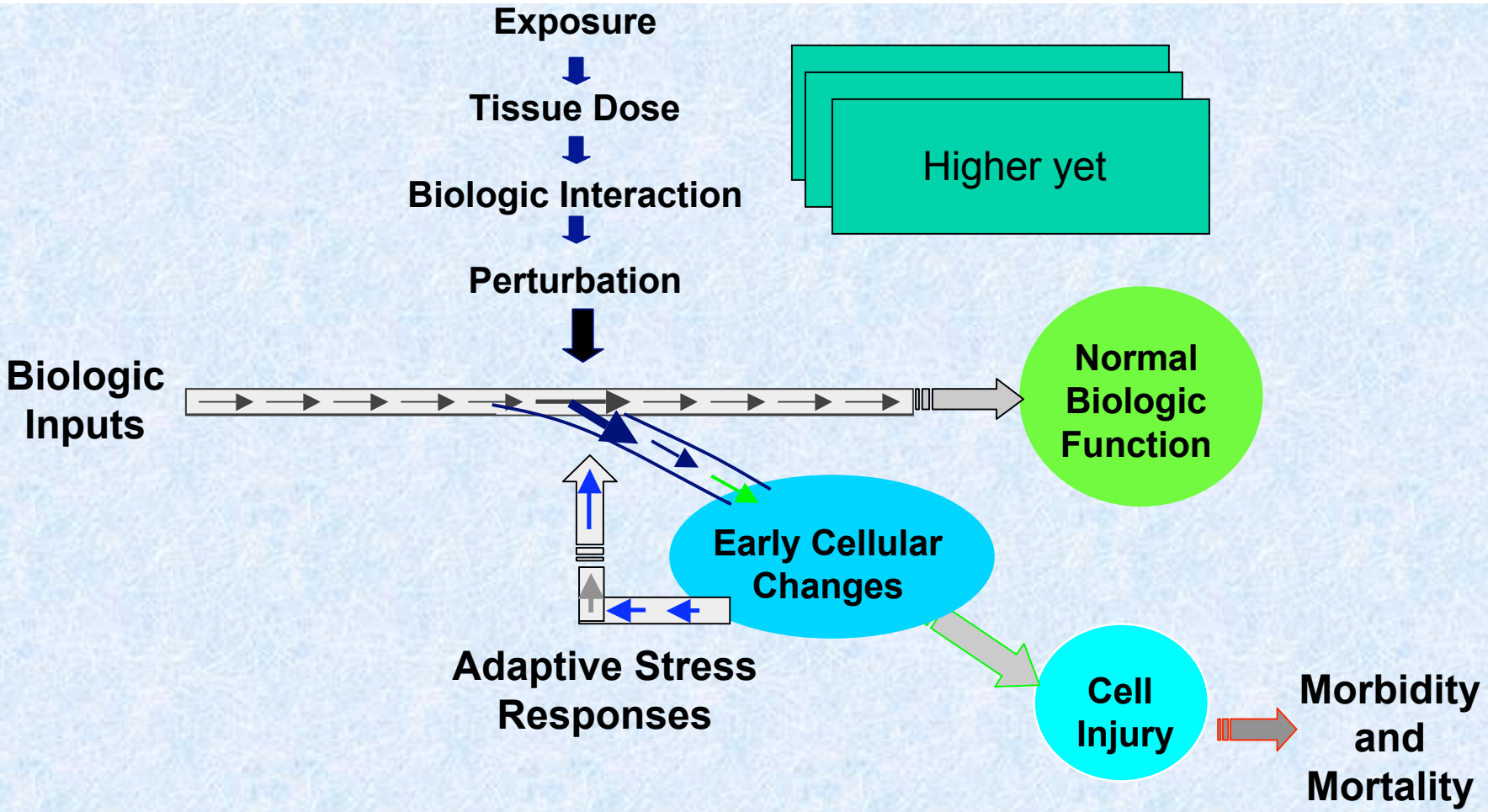
A New Paradigm: Activation of Toxicity Pathways



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Toxicity Pathways

Toxicity Pathway: A cellular response pathway that, when sufficiently perturbed, is expected to result in an adverse health effect.

Toxicity Response Pathways

Endogenous hormones

DNA damage

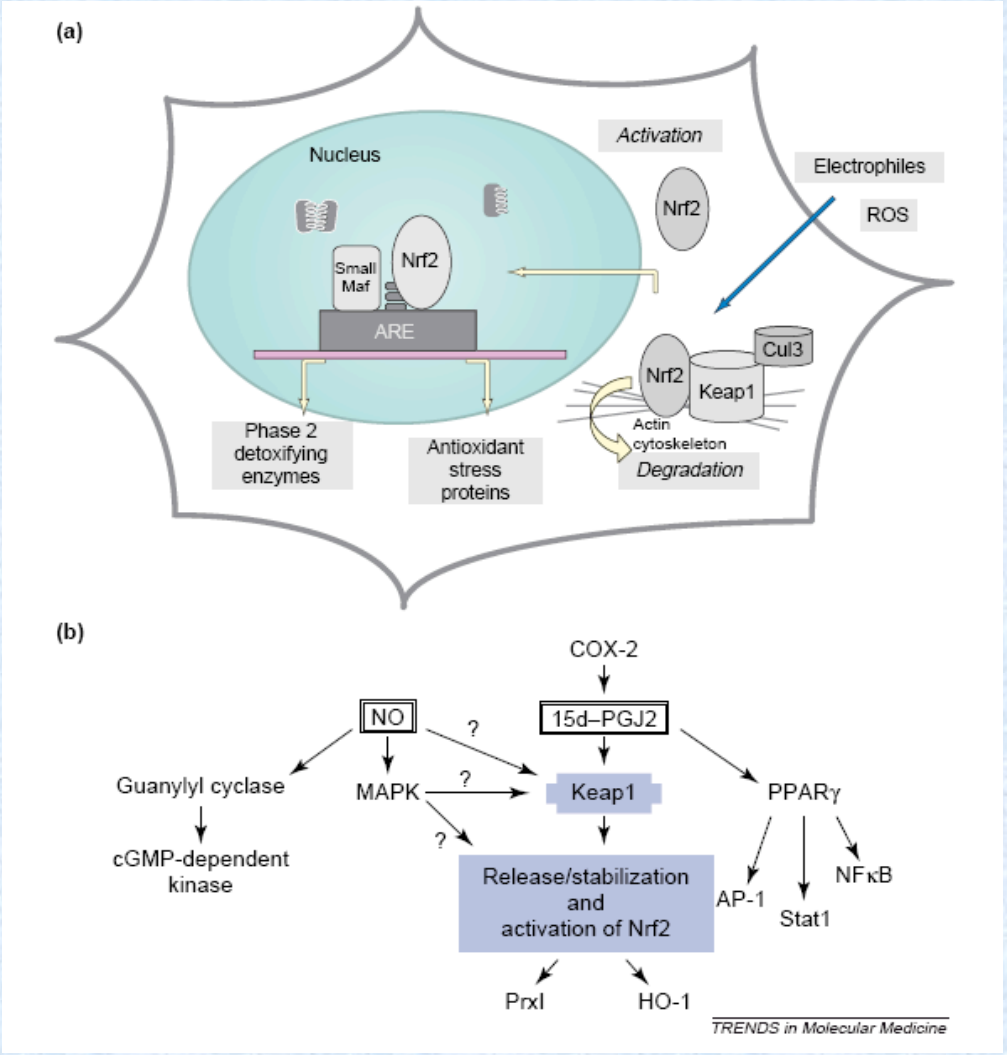
PXR, CAR, PPAR and AhR receptors

Hypo-osmolarity

Nrf2 oxidative stress

Heat-shock proteins

Nrf2 Antioxidant Response Pathway



In non-toxic environments, Nrf2 is bound to the cytoplasmic protein Keap1

In toxic environments, Nrf2 is released into the nucleus, leading to expression of antioxidant stress proteins

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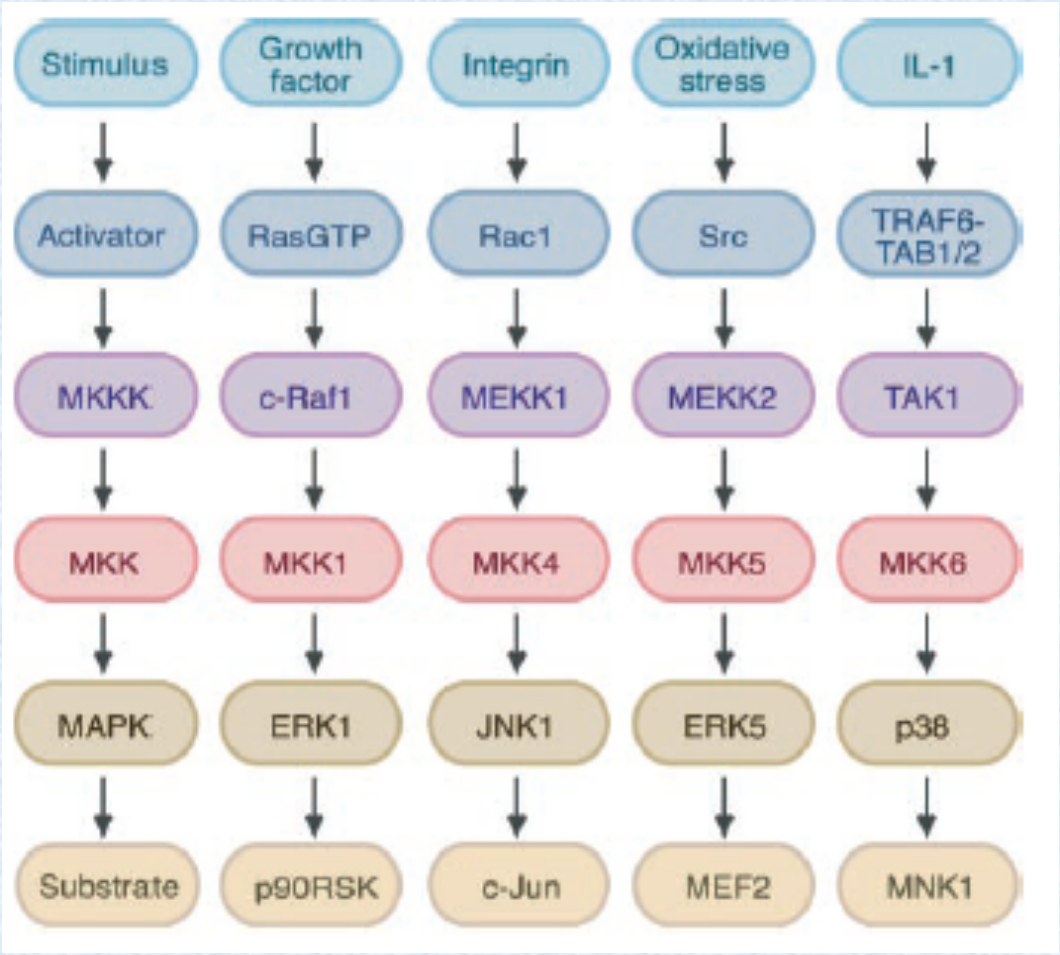
Integration of Cell Signaling Pathways



Mitogen-activated protein kinase (MAPK) cascades integrate cell signaling pathways that govern cell kinetics

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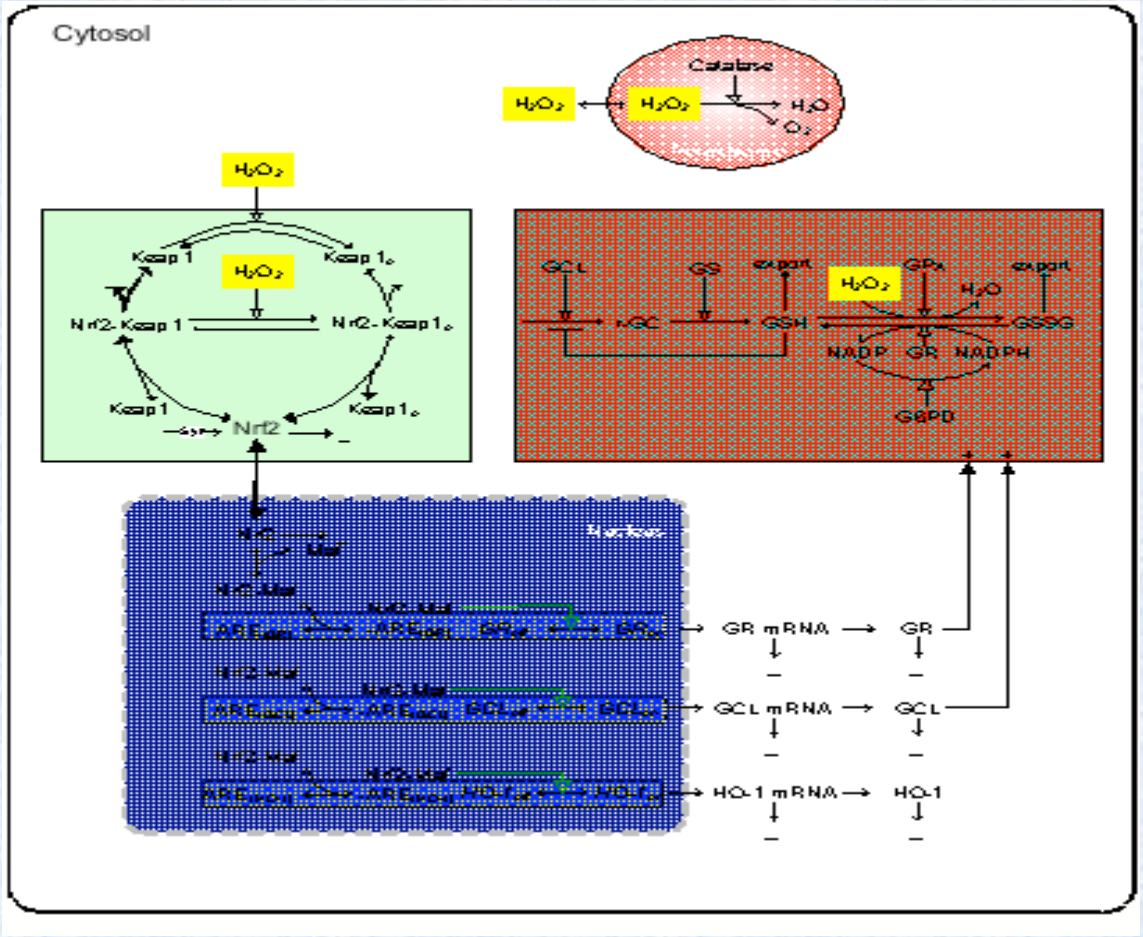
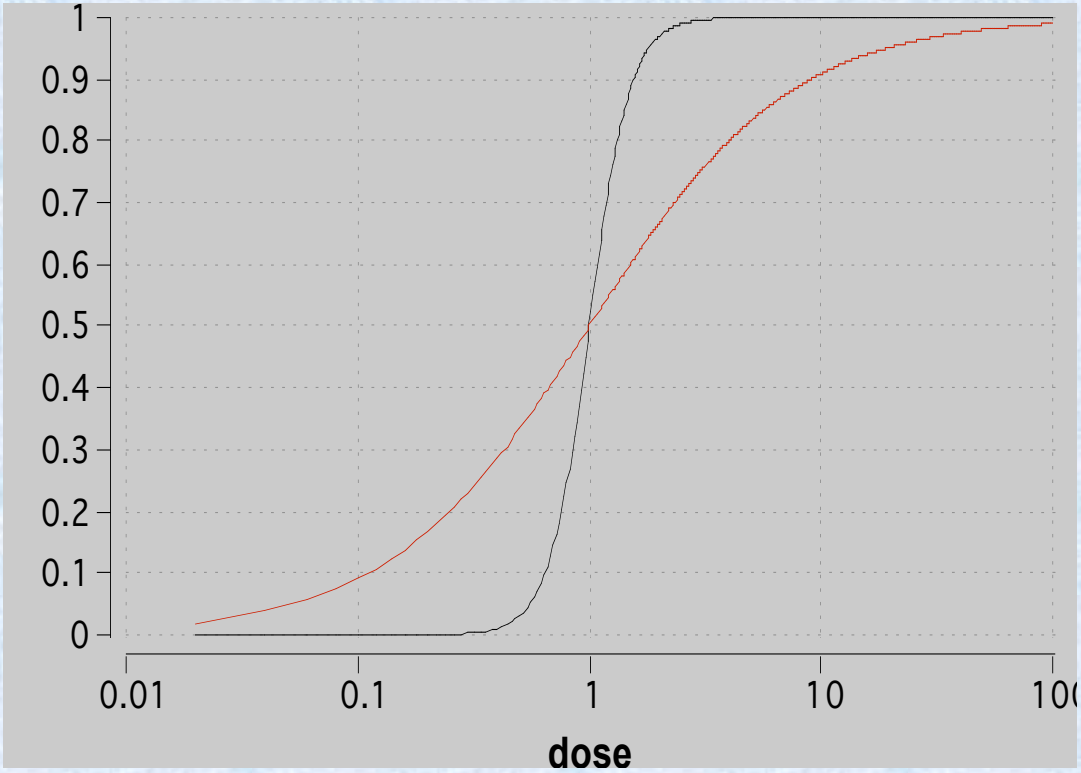
Computational Systems Biology



Feedback controlled adaptive stress responses govern activation and perturbation of signaling pathways

Dose-response Modeling of Nrf2 Pathway Activation



Nfr2 activation represents an important biological perturbation of a general toxicity pathway

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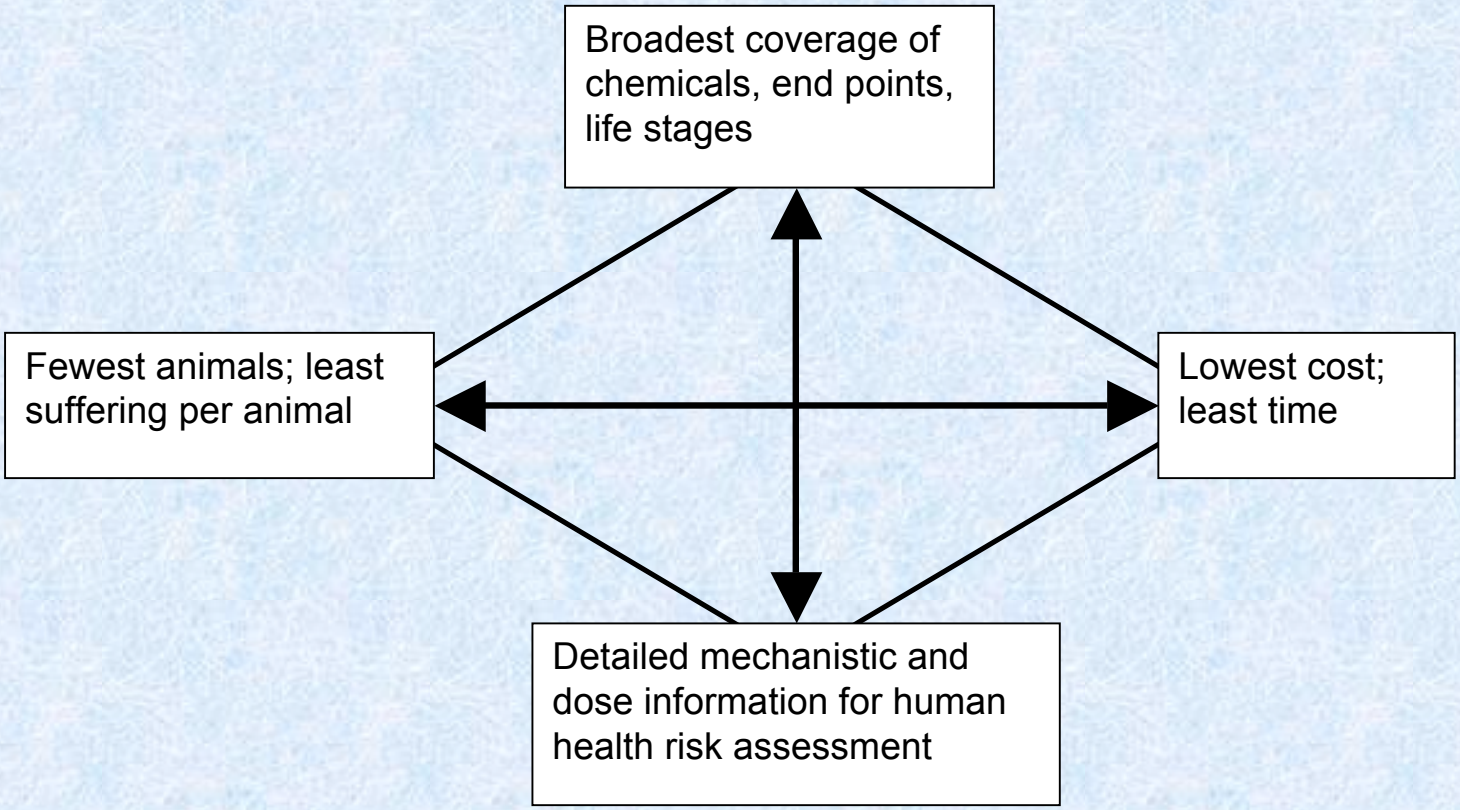
Design Criteria: Objectives of Toxicity Testing



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Options for Future Toxicity Testing Strategies

Option I **In Vivo**	**Option II** **Tiered In Vivo**	**Option III** **In Vitro/In Vivo**	**Option IV** **In vitro**
Animal biology	Animal biology	Primarily human biology	Primarily human biology
High doses	High doses	Broad range of doses	Broad range of doses
Low throughput	Improved throughput	High and medium throughput	High throughput
Expensive	Less expensive	Less expensive	Less expensive
Time consuming	Less time consuming	Less time consuming	Less time consuming
Relative large number of animals	Fewer animals	Substantially fewer animals	Virtually no animals
Apical endpoints	Apical endpoints	Perturbations of toxicity pathways	Perturbations of toxicity pathways
	Some *in silico* and *in vitro* screens	*In silico* screens possible	*In silico* screens

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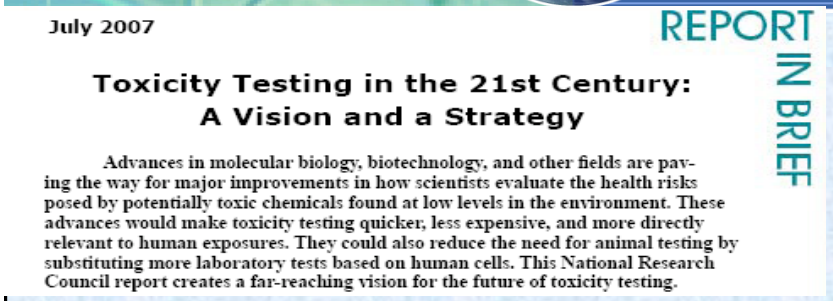
The Committee's Vision



July 2007

REPORT IN BRIEF

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

Final Report Released June 12, 2007

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Components of the Vision

Chemical Characterization

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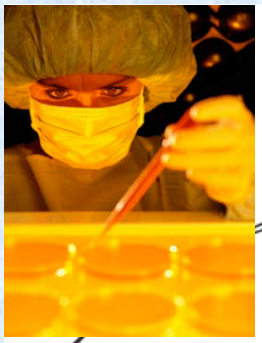
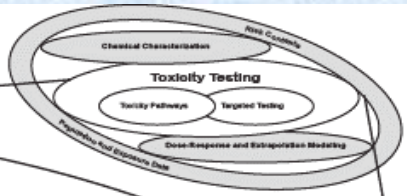
Toxicity Testing





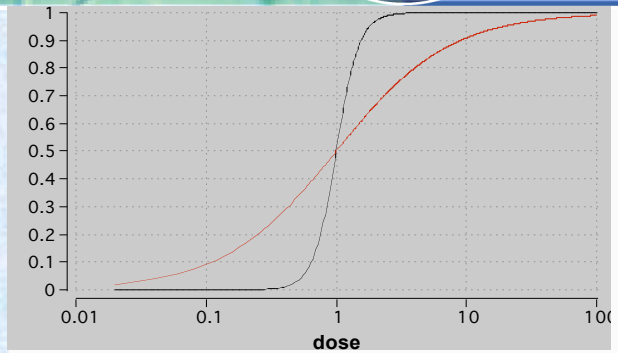
Toxicity Pathways

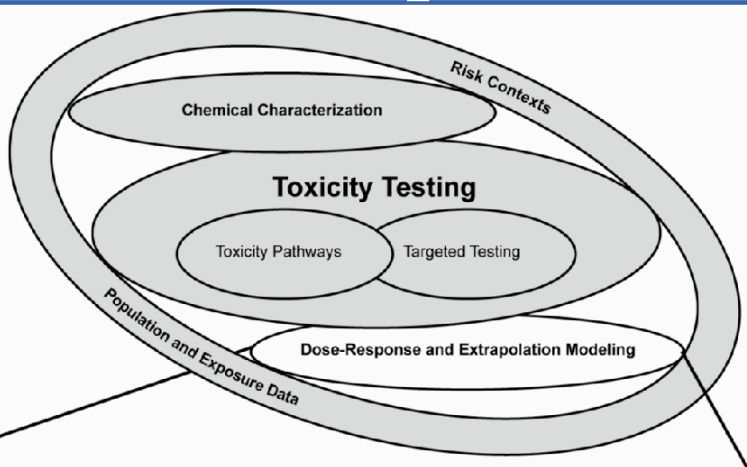
- Evaluation of perturbations in toxicity pathways rather than apical end points.
- Emphasis on high-throughput approaches using cells or cell lines, preferably of human origin.
- Use of medium-throughput assays of more integrated cellular responses.

Targeted Testing

- Testing conducted to evaluate metabolites, assess target tissues, and develop understanding of affected cellular processes at genomics level.
- Limited types and duration of in vivo studies, focusing on up to 14-day exposures.
- More extensive testing for representative compounds in novel chemical classes.

Dose-Response and Extrapolation Modeling

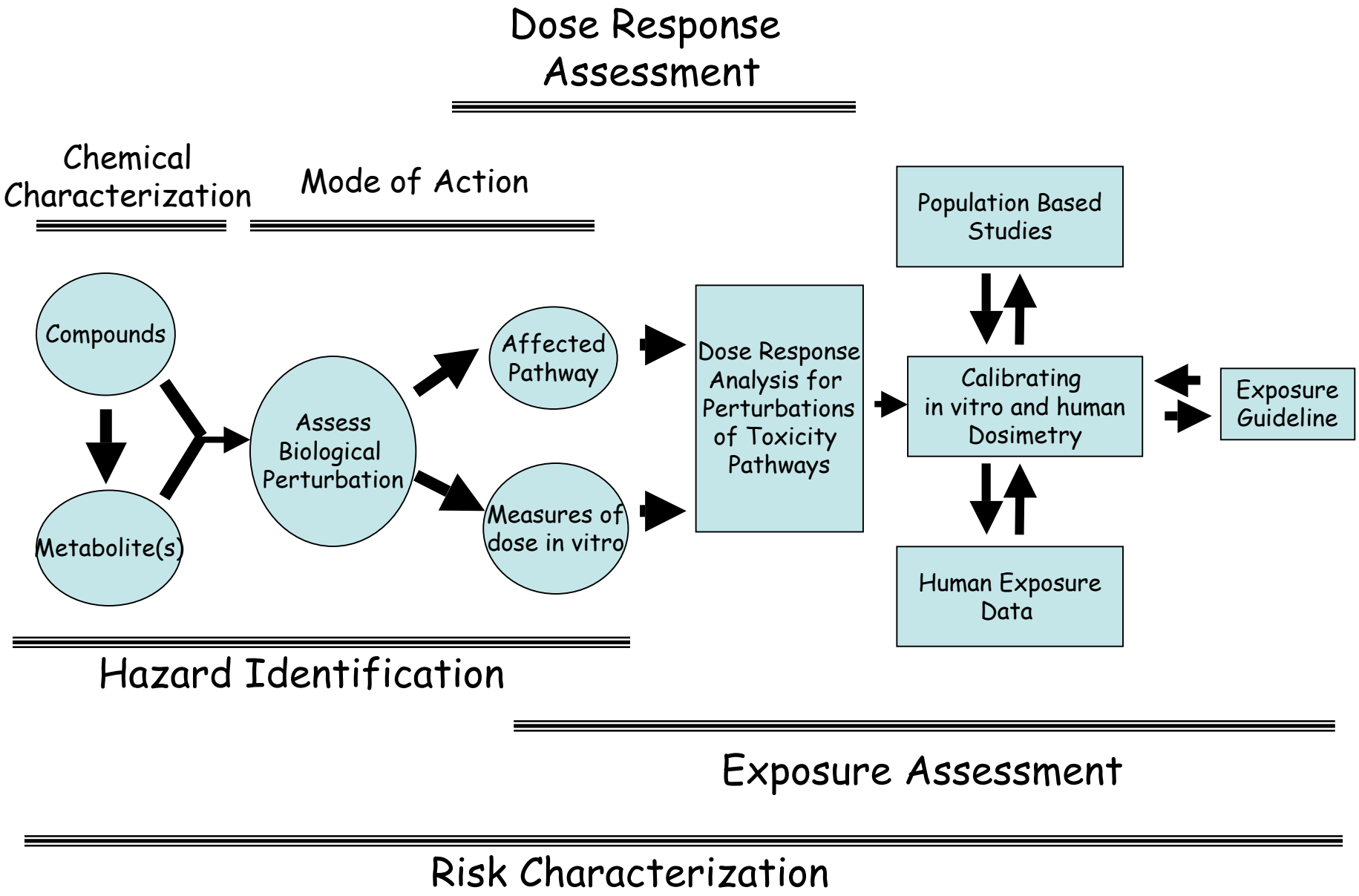




Dose-Response and Extrapolation Modeling

- Empirical dose-response models will be developed on the basis of data from in vitro, mechanistically based assays.
- Physiologically based pharmacokinetic (PBPK) models will equate tissue-media concentrations from toxicty tests with tissue doses expected in humans.
- Dose-response models for toxicity pathways will reliably predict concentrations expected to cause measurable precursor-effect responses.
- PBPK and toxicity-pathway models will identify biomarkers of susceptibility for sensitive subpopulations.

Toxicity Testing and Risk Assessment



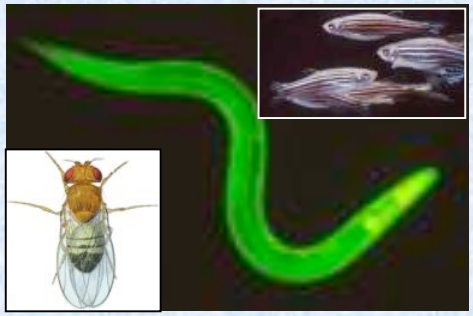
Implementation Strategy Requirements

- Understanding and mapping of toxicity pathways
- Comprehensive suite of in vitro tests, preferably based on human cells, cell lines, or components to identify pathway perturbations
- Evidence justifying that toxicity-pathway approach is adequately predictive of adverse health outcomes to use in decision-making
- Computational models of toxicity pathways to support application of in vitro test results in risk assessments.
- Targeted animal tests to complement in vitro tests
- Infrastructure changes to support basic and applied research needed to develop the tests and pathway models.

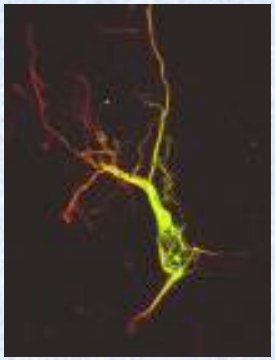
Implementing the Vision: NIEHS High Throughput Screens

1-3/year

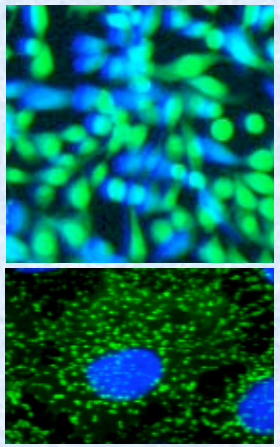
10's/year



100's/year



10,000's/day



100,000's/day

High Throughput
Molecular mechanism

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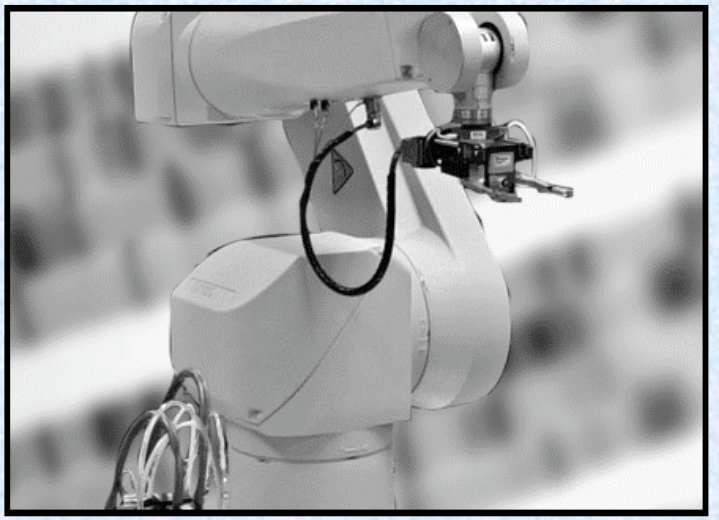
Implementing the Vision: NIH National Chemical Genomics Center



- Enzymatic assays
- Receptor binding assays
- GTPγS binding Assays
- Tissue culture assays
- Cell-based Elisa and Western Blots (for quantitative antigen detection)
- FLIPR™ Assays (GPCR and ion channel targets)
- Immunoassays

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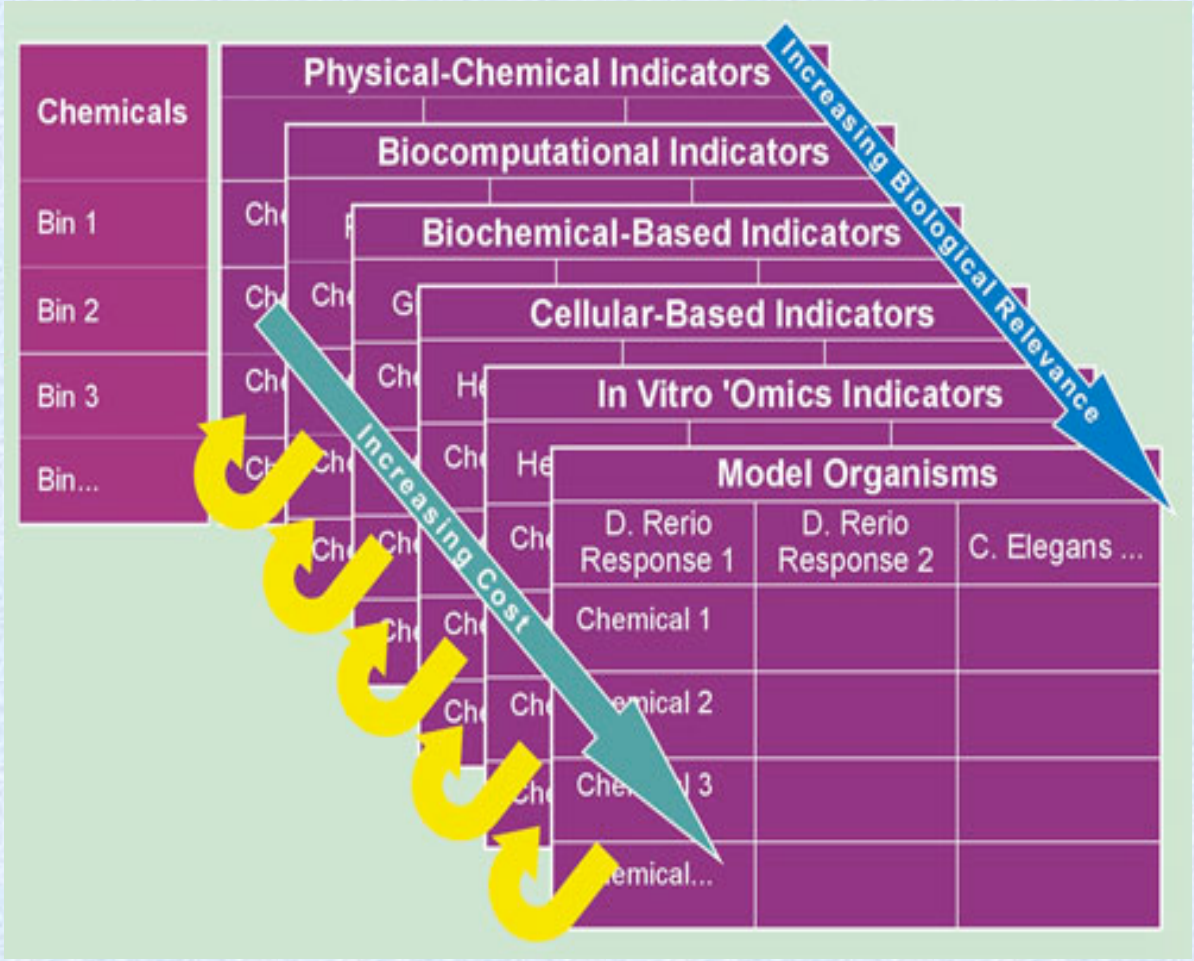
Implementing the Vision: EPA's ToxCast™ Program



Forecast toxicity based on bioactivity profiling

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Regulatory Context

- Shift in focus away from apical outcomes in experimental animals towards important perturbations of toxicity pathways
- Development of risk assessment practices based on pathway perturbations
- Re-interpretation or possible re-writing of regulatory statues under which risk assessments are conducted

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Conclusions

- Paradigm shift away from apical endpoints to perturbation of toxicity pathways
- More extensive use of computational toxicology and high throughput in vitro screening tests
- Will provide much broader coverage of the universe of environmental agents
- Substantial commitment of resources will be required to implement the vision
- Will require support of the scientific community, regulators, law-makers, industry, and the public
- *Effective communication of the vision is key to its success*