THE HAMNER INSTITUTES FOR HEALTH SCIENCES

WHERE GREAT MINDS & MEDICINE MEET

The Future of Toxicology as a Predictive Science

February , 2008

Workshop on Acute Chemical Toxicity Testing Bethesda, MD

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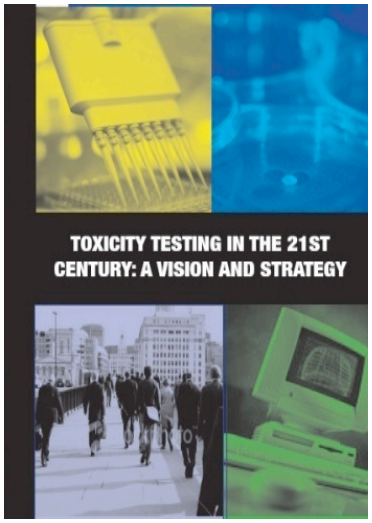
Director, Center for Dose Response Modeling
The Hamner Institutes for Health Sciences &

Professor, University of Ottawa

Toxicity Testing in the 21st Century: A Vision and 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



Committee Roster

Daniel Krewski (*Chair*), University of Ottawa, Ottawa, ON
Daniel Acosta, Jr., University of Cincinnati, Cincinnati, OH
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Henry Anderson, Wisconsin Division of Public Health, Madison, WI
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Gail Charnley, HealthRisk Strategies, Washington, DC
Vivian Cheung, University of Pennsylvania, Philadelphia, PA
Sidney Green, Howard University, Washington, DC
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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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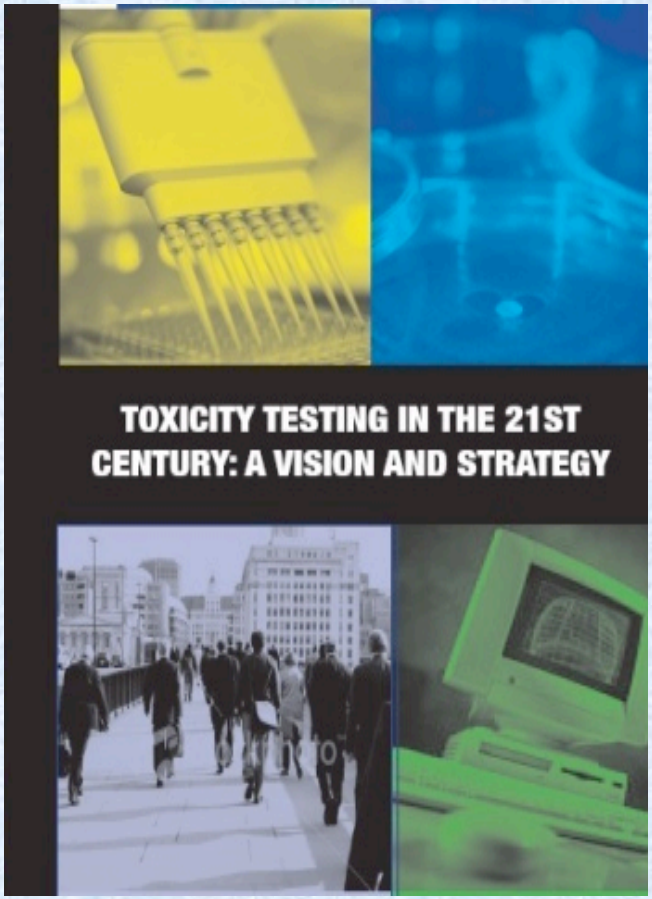
The Report
June 12, 2007



What's the best way to design a 'modern' toxicity testing program to assess potential human risks posed by exposures to environmental agents over a broad range of doses and compounds and to be in a position to use this information in quantitative human health risk assessment?

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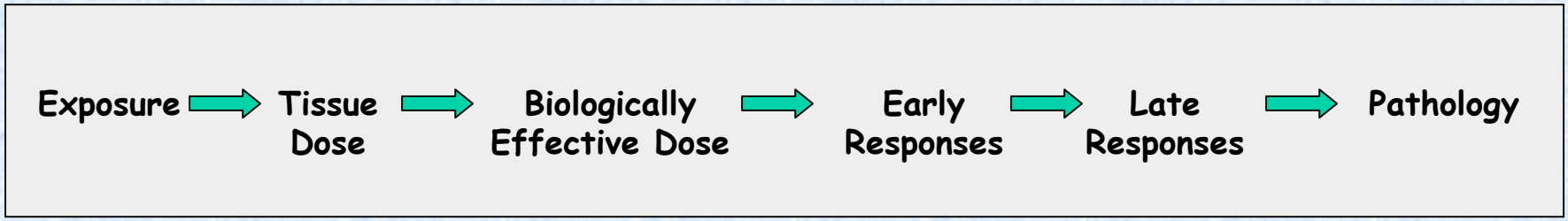
Design Criteria For New Approaches

A fundamental re-direction in toxicity testing is needed to achieve the following design criteria:

- To develop a more robust scientific basis for assessing health effects of environmental agents (mechanistic data)
- To provide broad coverage of chemicals, chemical mixtures, outcomes, and life stages
- To reduce the cost and time of testing
- To base decisions on human rather than rodent biology and focus on more relevant dose levels

Current Paradigm: The Exposure-response Continuum



Physiologically Based Pharmacokinetic Models

Tissue Dose Metric

Mode of Action

Pharmacokinetics

Pharmacodynamics

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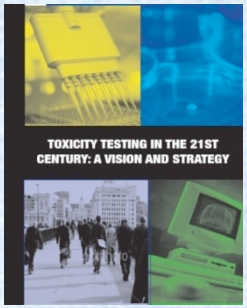
A New Paradigm: Activation of Toxicity Pathways



Exposure

↓

Tissue Dose

↓

Biologic Interaction

↓

Perturbation

↓

Higher yet

Biologic Inputs

Normal Biologic Function

Early Cellular Changes

Adaptive Stress Responses

Cell Injury

Morbidity and Mortality

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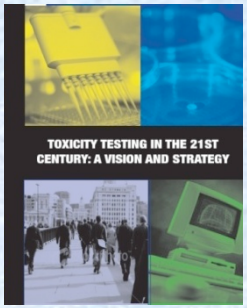
Toxicity Pathways



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

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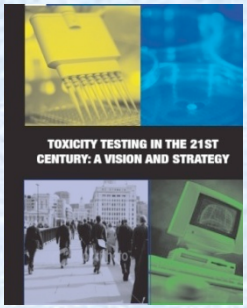
Toxicity Pathways



Endogenous hormones

DNA damage

PXR, CAR, PPAR and AhR receptors

Hypo-osmolarity

Nrf2 oxidative stress

Heat-shock proteins

P38 MAPK

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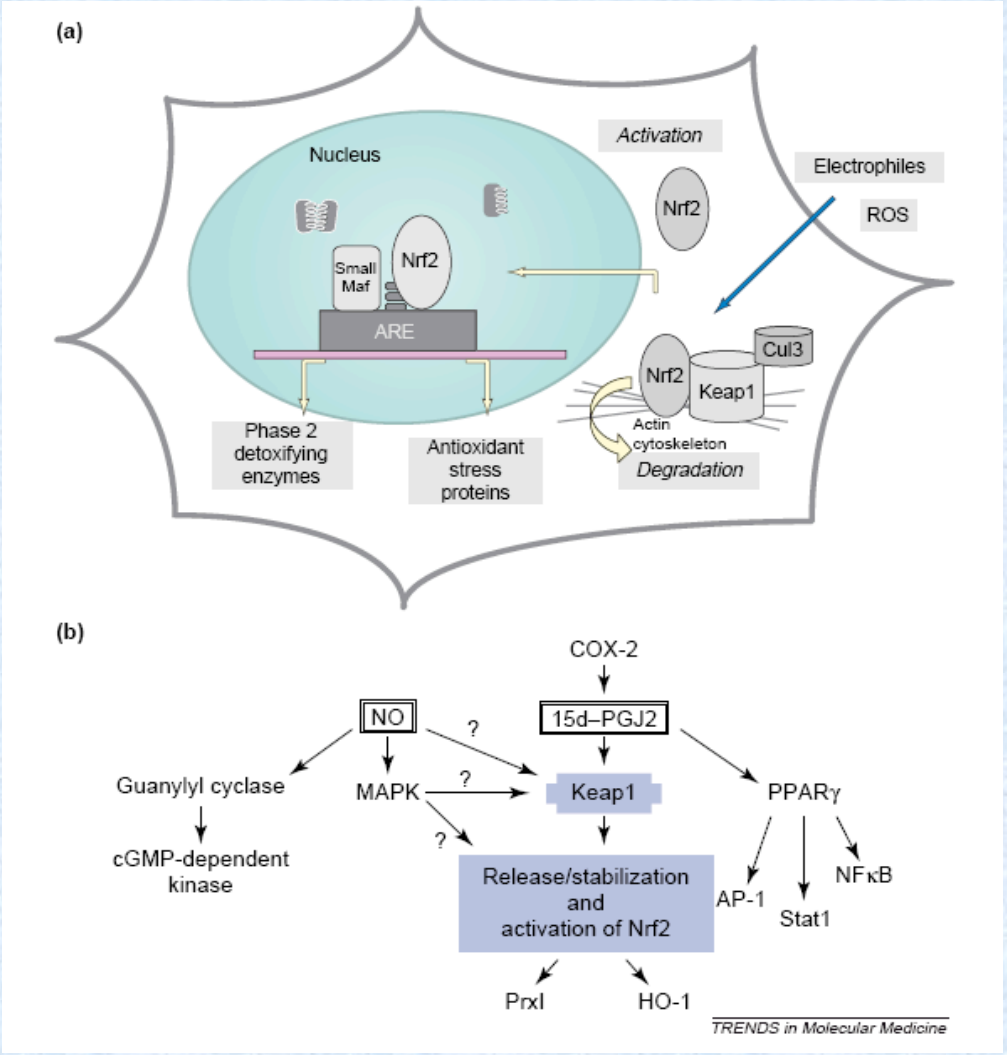
Antioxidant Response Pathway



Normally, Nrf2 is bound to the cytoplasmic protein Keap1

When challenged with oxidant stressors, Nrf2 is released, going to the nucleus and guides expression of antioxidant stress genes

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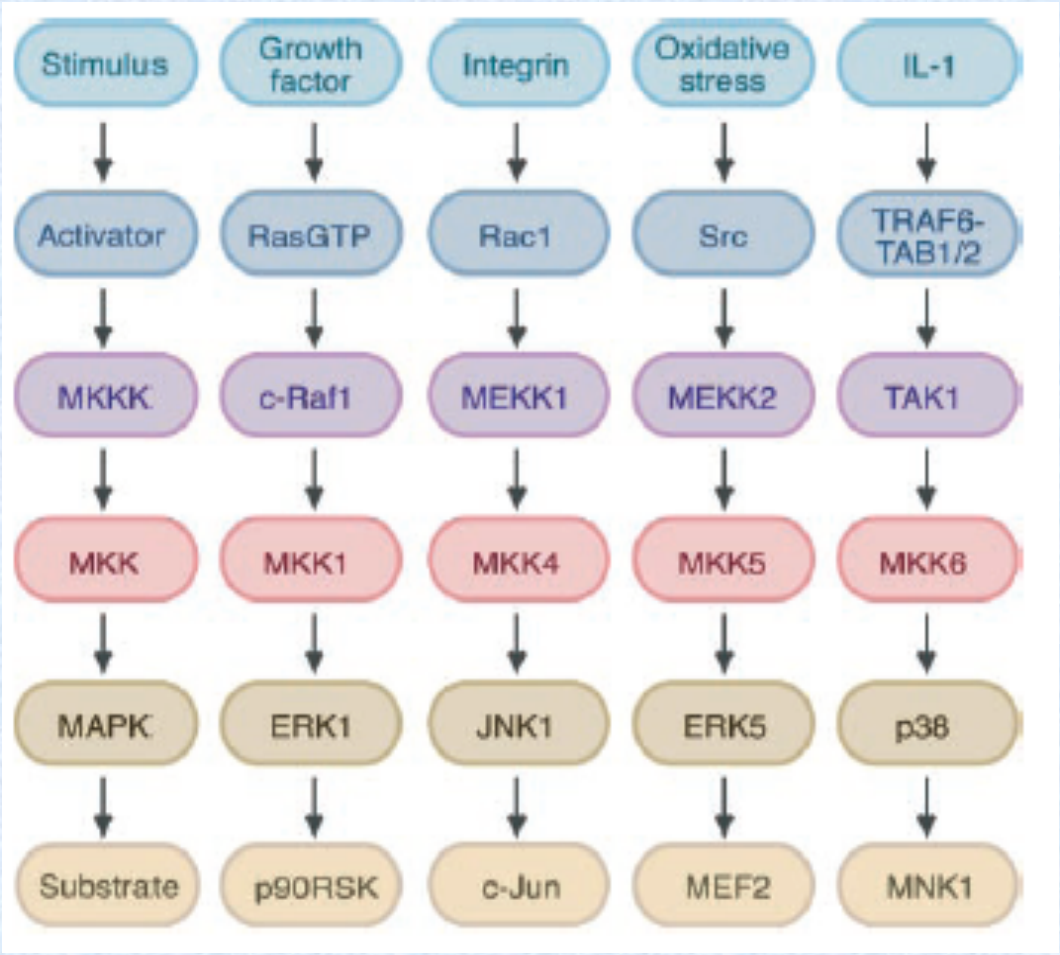
Integration of Cell Signaling Pathways



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

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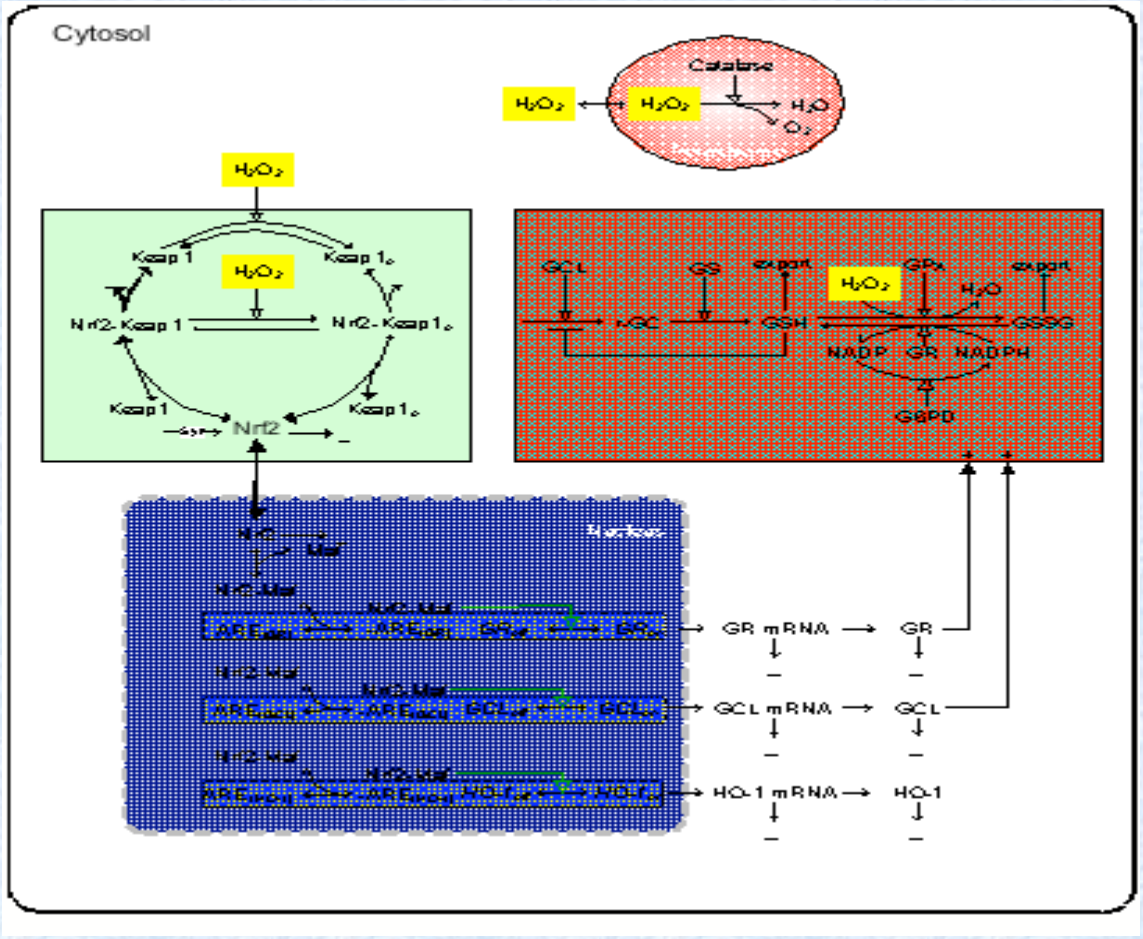
Computational Systems Biology



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

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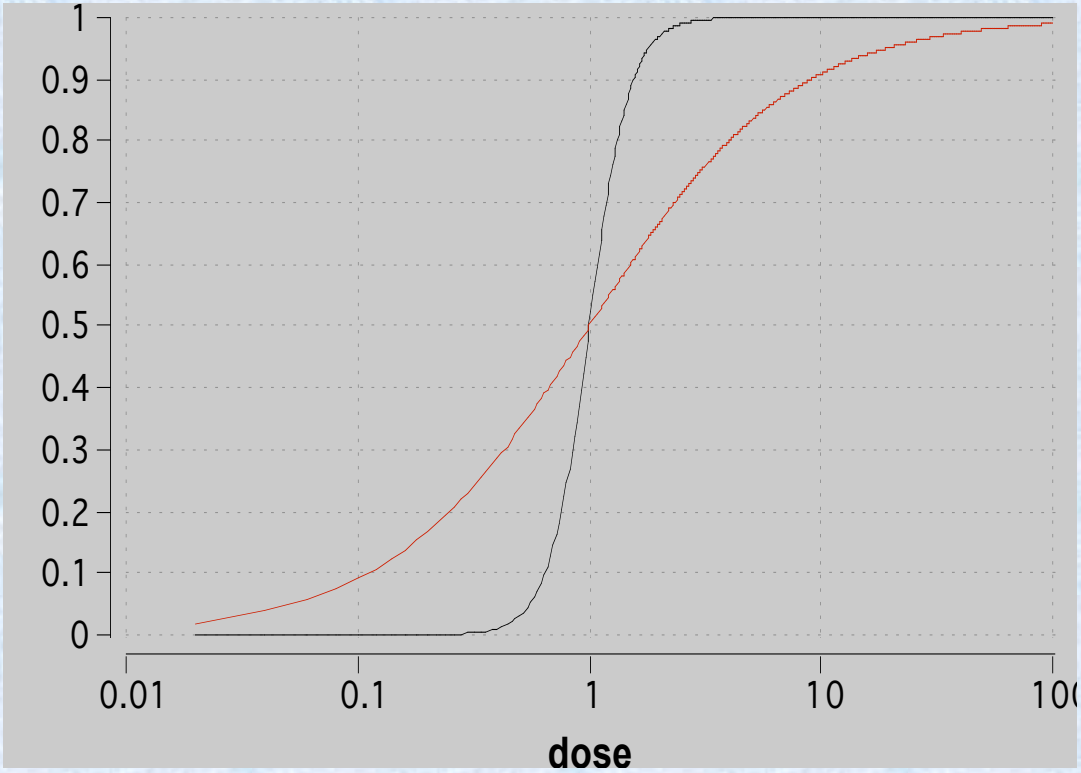
Dose-response Modeling of Nrf2 Pathway Activation



Nfr2 activation represents an important biological perturbation of a general “toxicity pathway”. Need tools to assess dose response.

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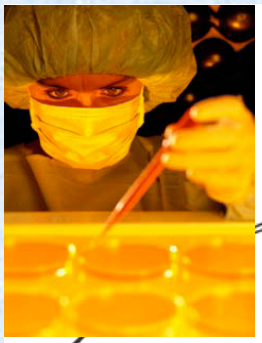
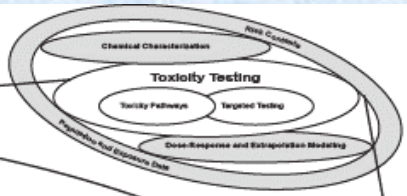
Options for Future Toxicity Testing Strategies

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

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.

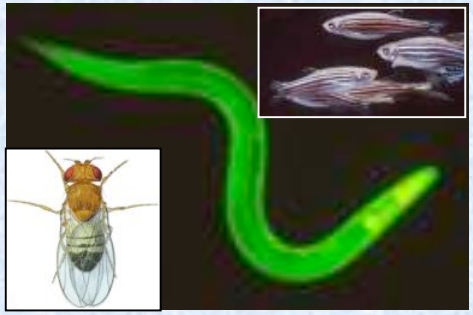
High Throughput Screening

BEST

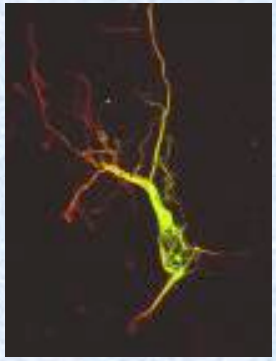
Board on Environmental Studies and Toxicology

1-3/year

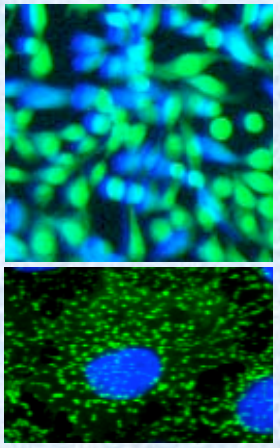
10's/year



100's/year



10,000's/day



100,000's/day

High Throughput
Molecular mechanism

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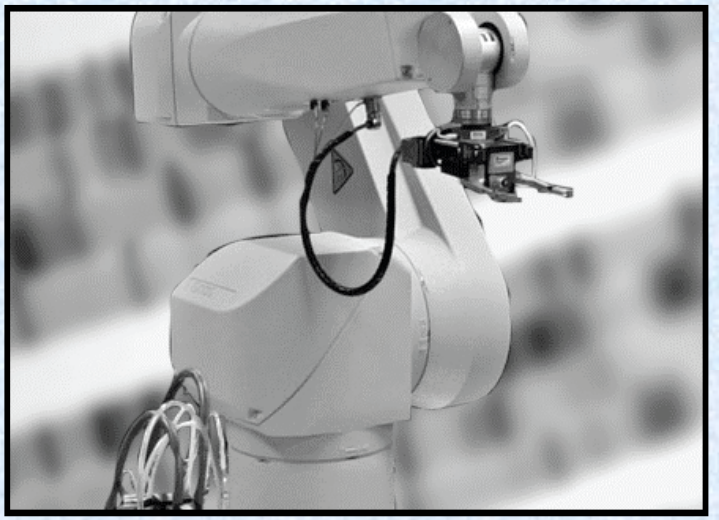
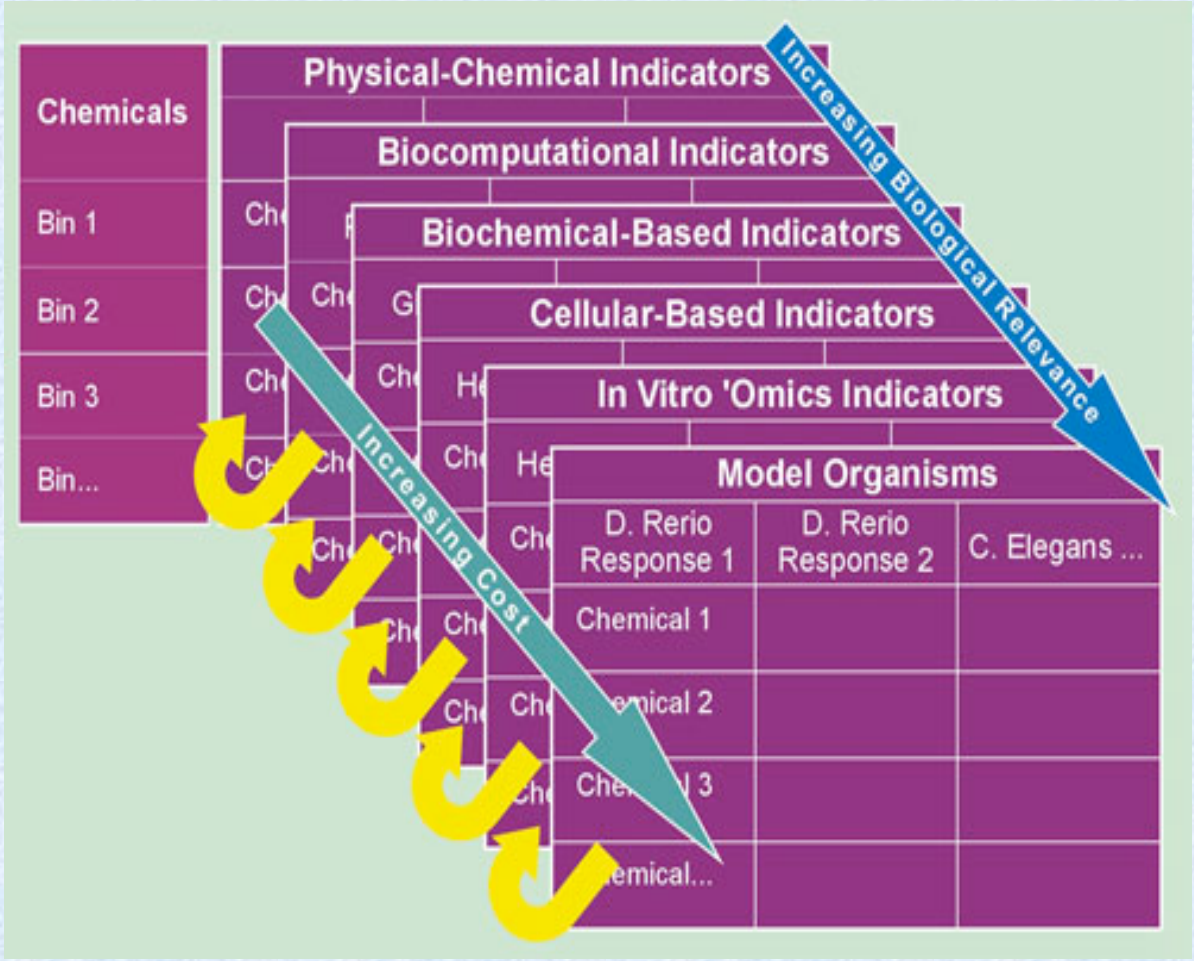
Implementing the Vision: NIH National Chemical Genomics Center



- 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)
- Various reporter based assays

EPA's ToxCast™ Program



Forecast toxicity based on bioactivity profiling. Could forecast human targets

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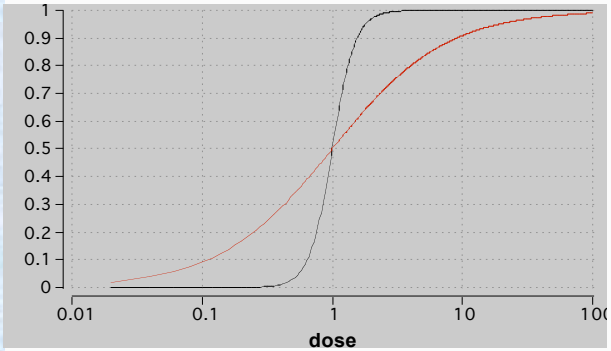
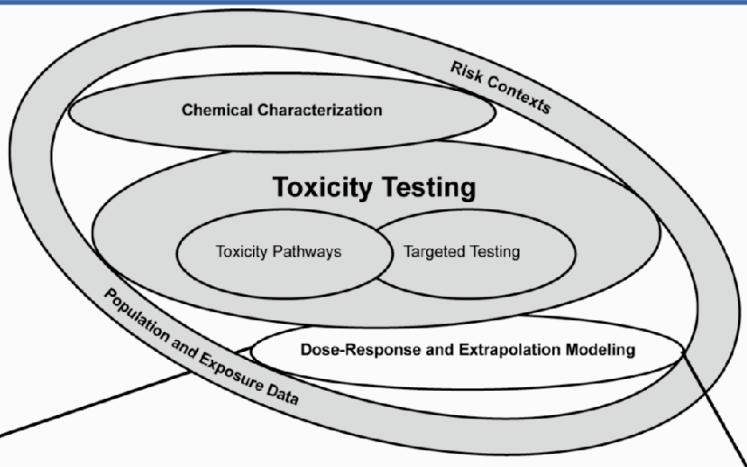
Dose-Response and Extrapolation Modeling

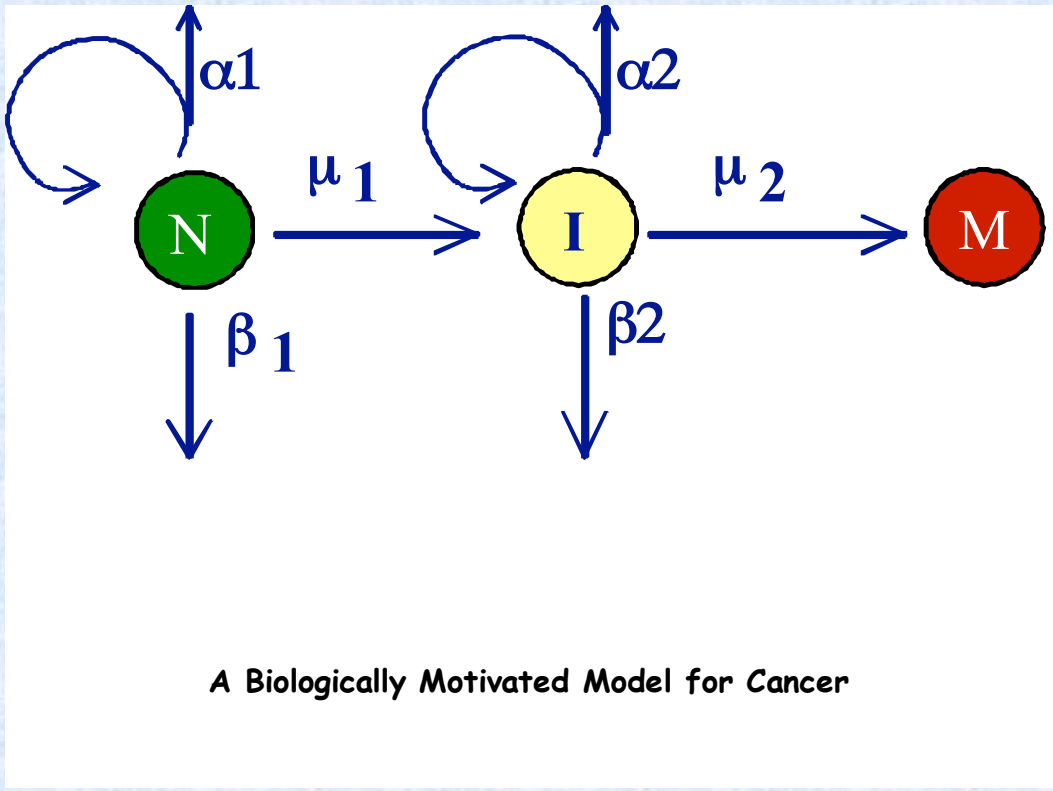




Dose-Response and Extrapolation Modeling

- 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.

Biologically Based Dose Response Models

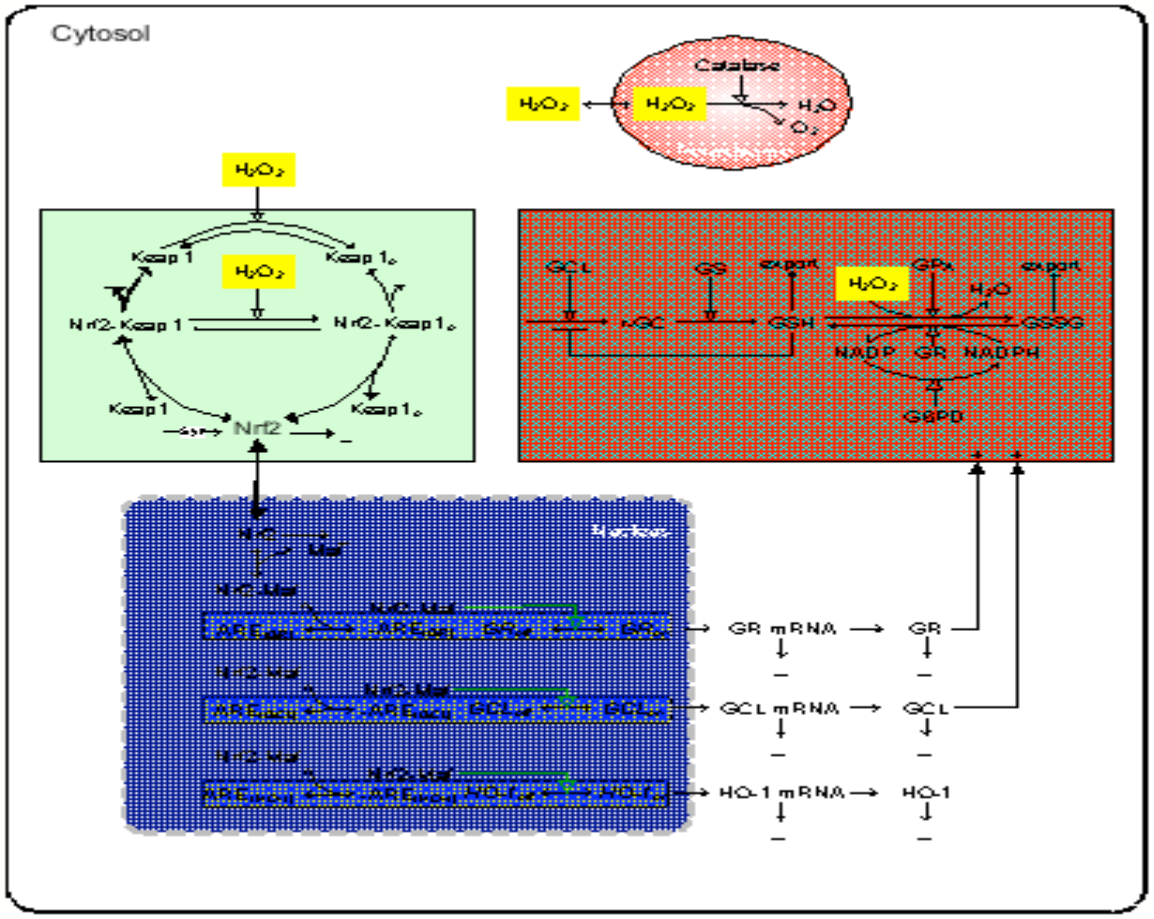


A Biologically Motivated Model for Cancer

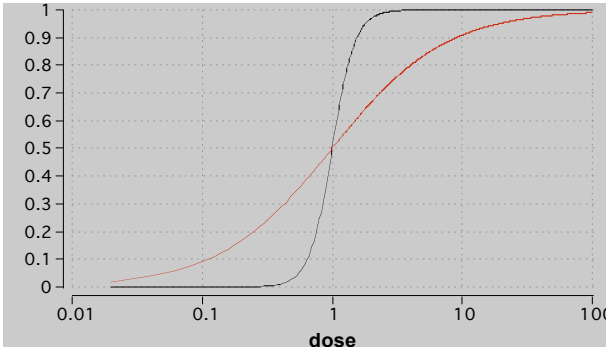
Capture dose-dependencies of main processes although lacking in specific biological detail.

Has been difficult to understand toxicity from top-down

Computational Systems Biology Model for the Circuitry and the Output



Circuitry model developed for all key assays to support dose response assessment, from bottom up



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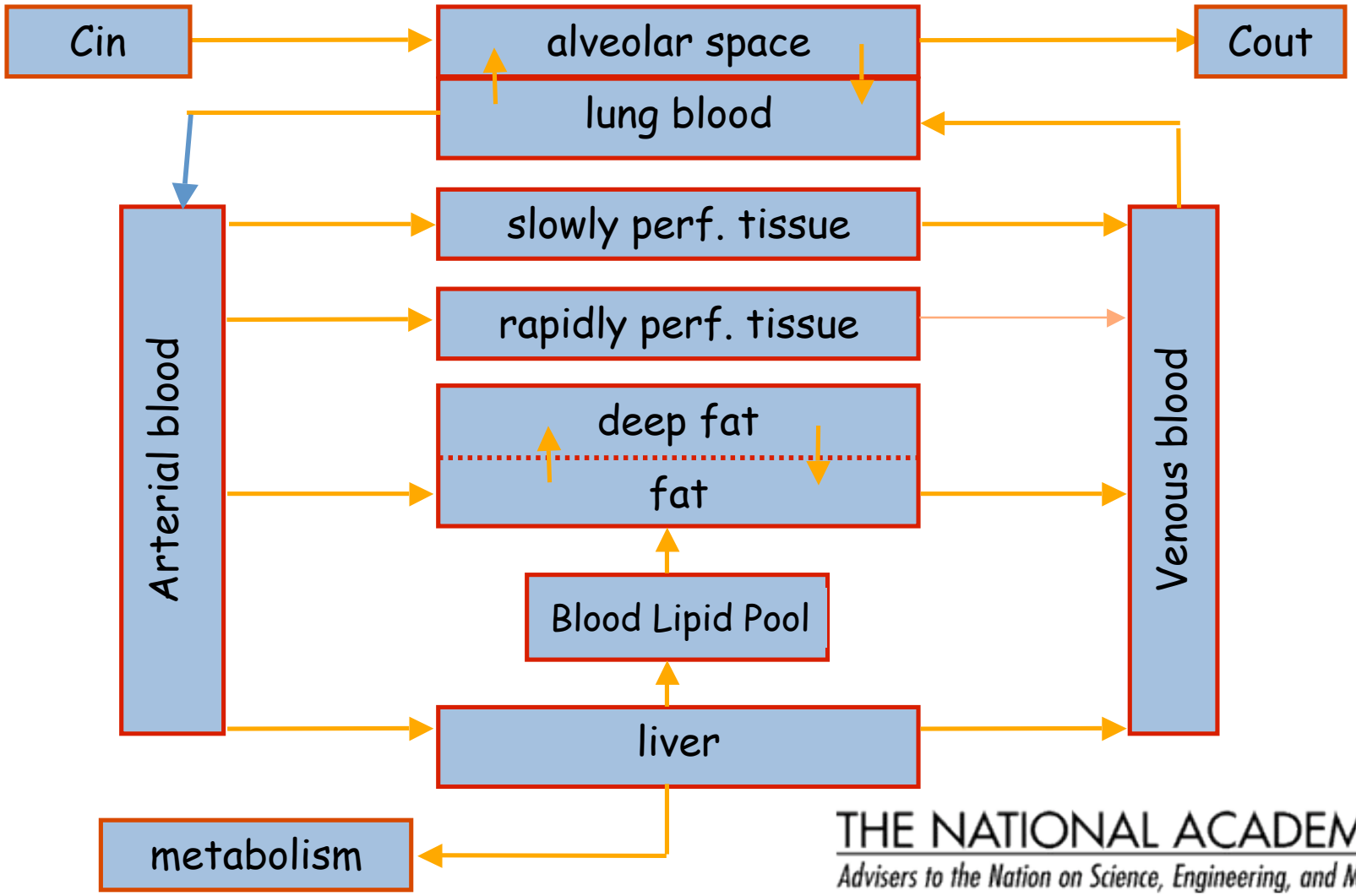
In vitro to in vivo extrapolations with PK and PBPK models



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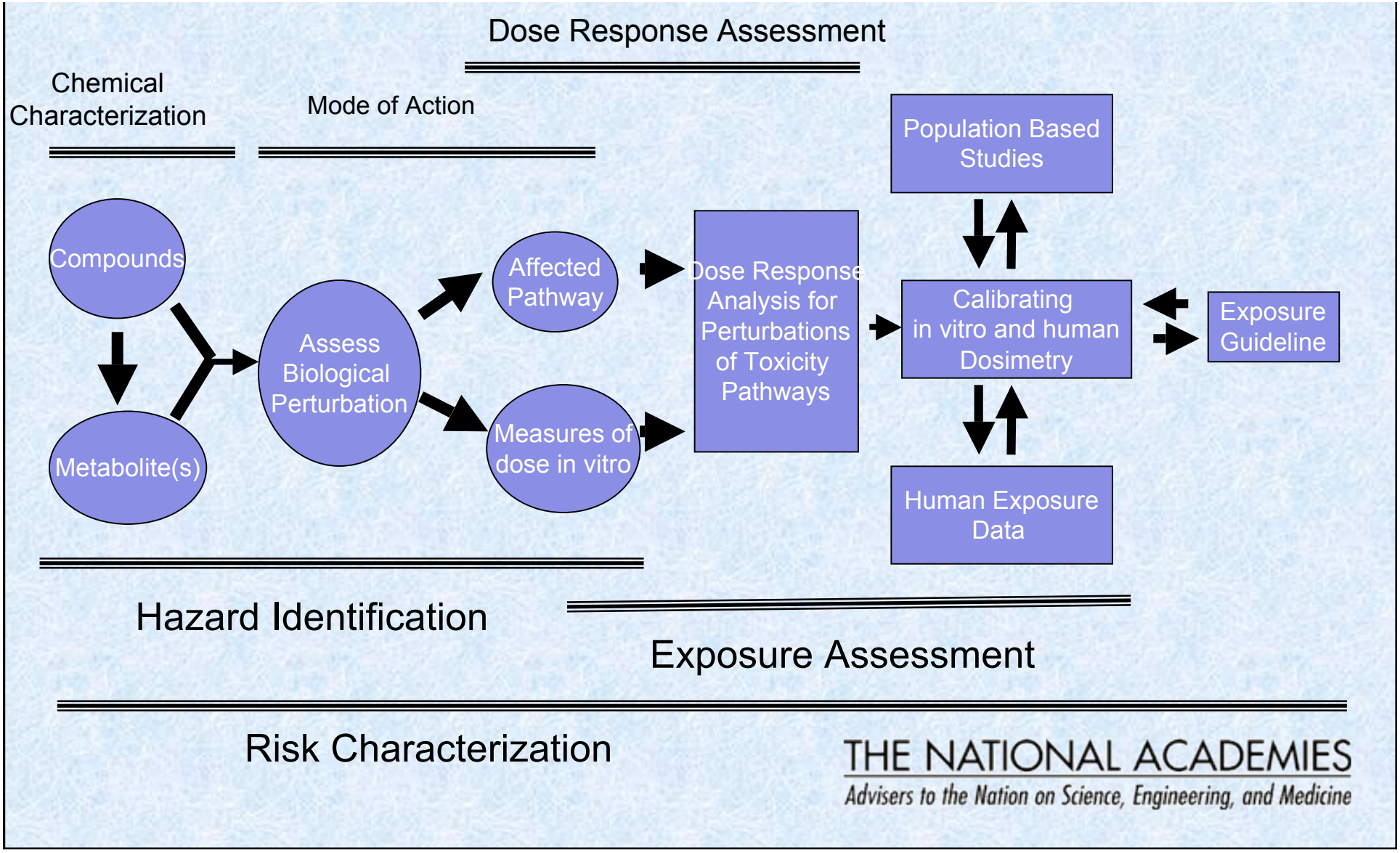
Implementation of Strategy

- Comprehensive suite of in vitro tests, preferably based on human cells, cell lines, or components.
- Computational models of signal transduction in toxicity pathways to support application of in vitro test results in risk assessments.
- Physiologically based pharmacokinetic (PBPK) models to assist in vitro to in vivo extrapolations
- Validation of toxicity pathway tests and test strategies

Method Development Focus

- Methods to predict metabolism
- Chemical-characterization & in silico tools
- High throughput assays
- Appropriate number of assays
- Approaches to uncover cell circuitry
- Mechanistic models for pharmacokinetics and for perturbations of cell signaling pathways

Toxicity Testing versus Risk Assessment Red Book



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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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What it is and what it isn't.

- Approach based on in vitro, high throughput tests to assess perturbations of 'toxicity pathways' of relevance for human biology and to interpret them in a dose-response context

- Assessed over wide range of doses and interpreted in relation to exposures that are not expected to cause significant perturbations of these key pathways

- **IT IS NOT** an approach to use suites of in vitro tests to predict high dose animal toxicity – i.e., it is not in principle like ECVAM, ICCVAM, US EPA ToxCast, or NIEHS high throughput approaches.

Conclusions

- Paradigm shift away from apical endpoints in test animals to perturbation of toxicity pathways in human cells
- Providing much broader coverage of the universe of environmental agents that warrant our attention
- Has consequences for toxicity testing and in the search for alternatives to animal testing
- Already, at this point in time, this vision is an applied sciences problem rather than a research-driven process
- Also topsy-turvy – testing in vitro based – research in vivo based