Chemical Prioritization and Risk Assessment in the 21st Century – A Highly Personal Perspective

March 27, 2008

Chemical Prioritization Community of Practice
US EPA Research Triangle Park, NC

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The Hamner Institutes for Health Sciences

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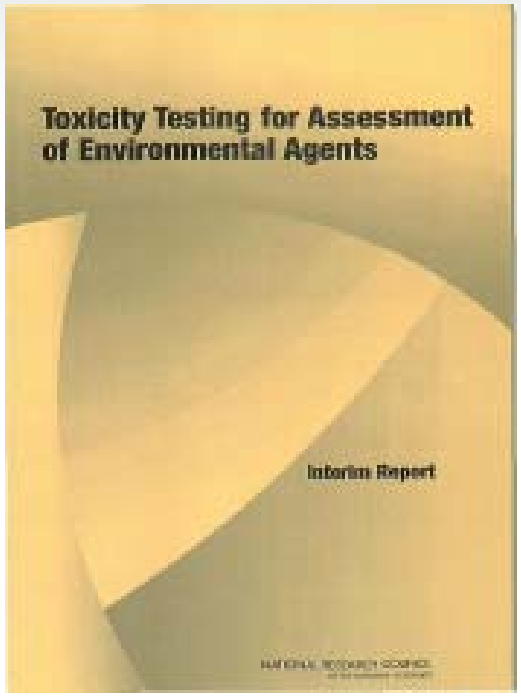
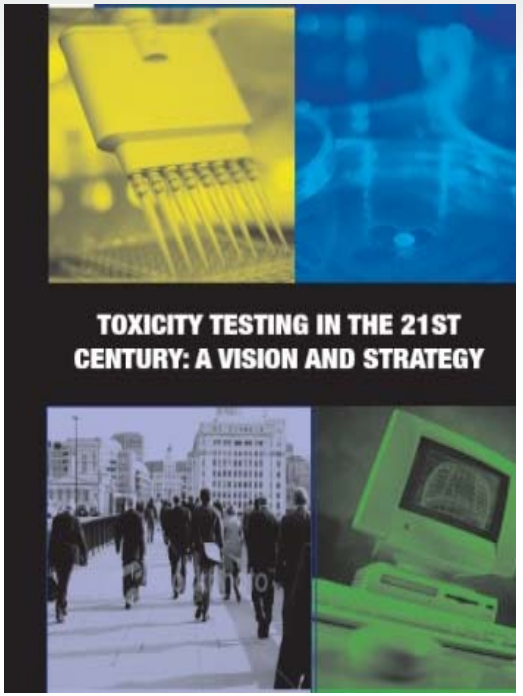
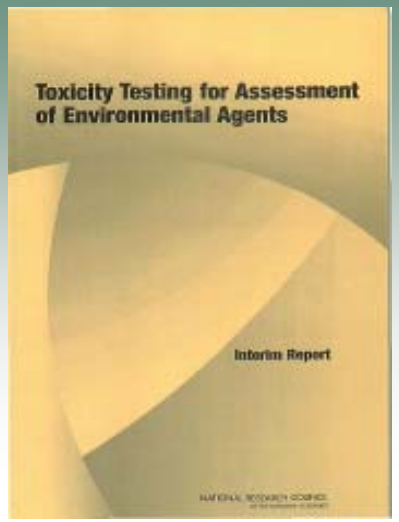
Martin Stephens, The Humane Society of the United States, Washington, DC
James Yager, Jr., Johns Hopkins University, Baltimore, MD
Lauren Zeise, California Environmental Protection Agency, Oakland, CA

Life Span: 2004 – 2007
With Two Products:







Design Criteria:
Toxicity Testing of Environmental Agents

Broadest coverage of chemicals, end points, life stages

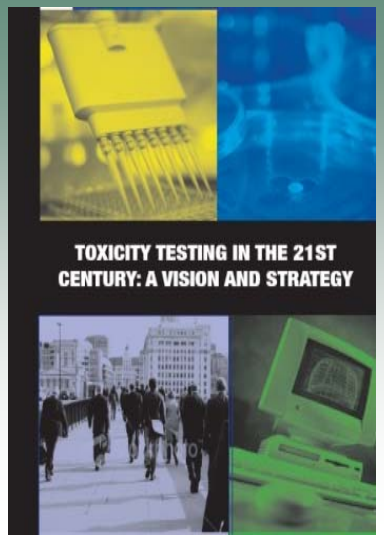
Fewest animals; least suffering for those used

Lowest cost; least time

Detailed **mode of action** and dose response information for **human** health risk assessment

Understatement

Chapter 2:



The goal of toxicity testing is to develop data that can ensure appropriate protection of public health from the adverse effects of exposures to environmental agents. Current approaches to toxicity testing rely primarily on observing adverse biologic responses in homogeneous groups of animals exposed to high doses of a test agent. However, the relevance of such animal studies for the assessment of risks to heterogeneous human populations exposed at much lower concentrations has been questioned.

Déjà view: A look back at a Dwane Powell cartoon that has resonance today.

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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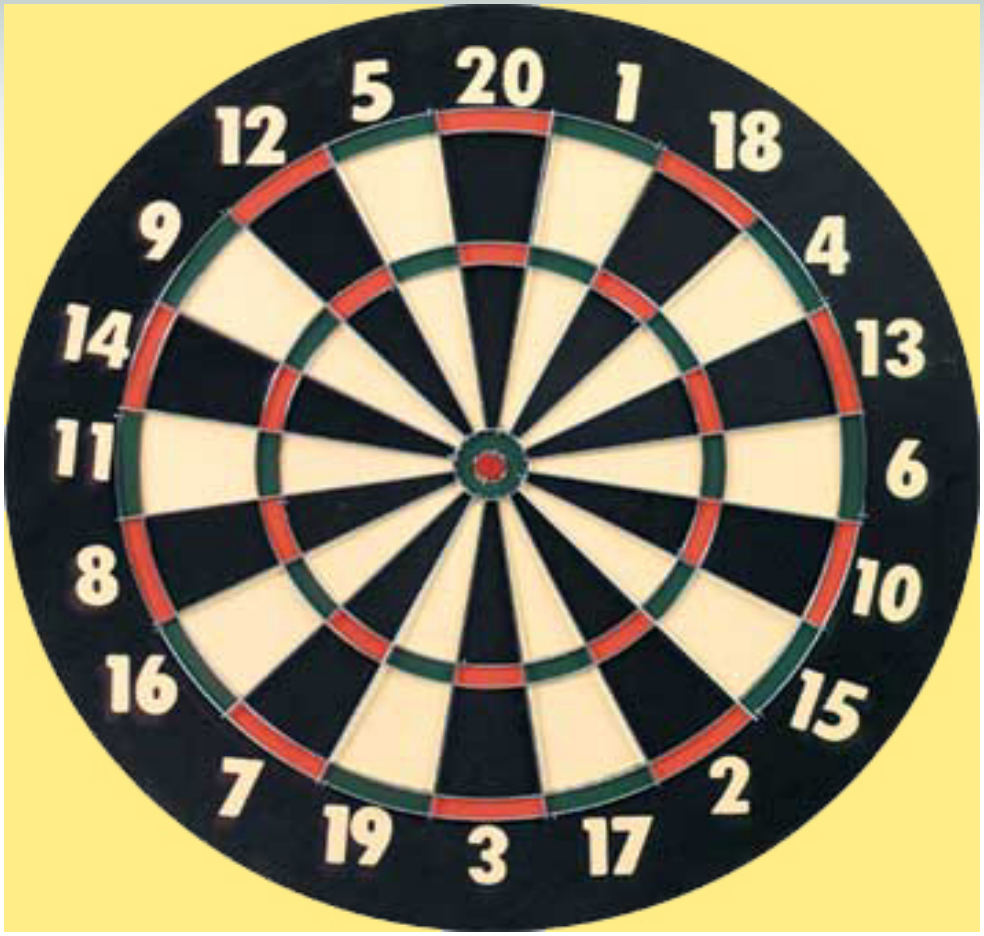
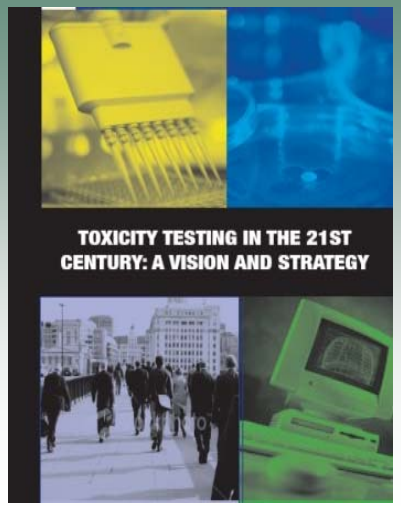
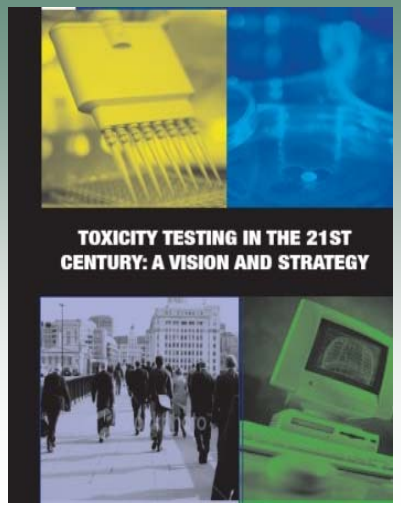
Creating a Target:





The Vision (toxicity testing)

This report envisions a not-so-distant future in which virtually all routine toxicity testing would be conducted in human cells or cell lines ***in vitro*** by evaluating perturbations of cellular responses in a suite of toxicity pathway assays using high throughput robotic-assisted methodologies.



The Vision (interpretation of tests)

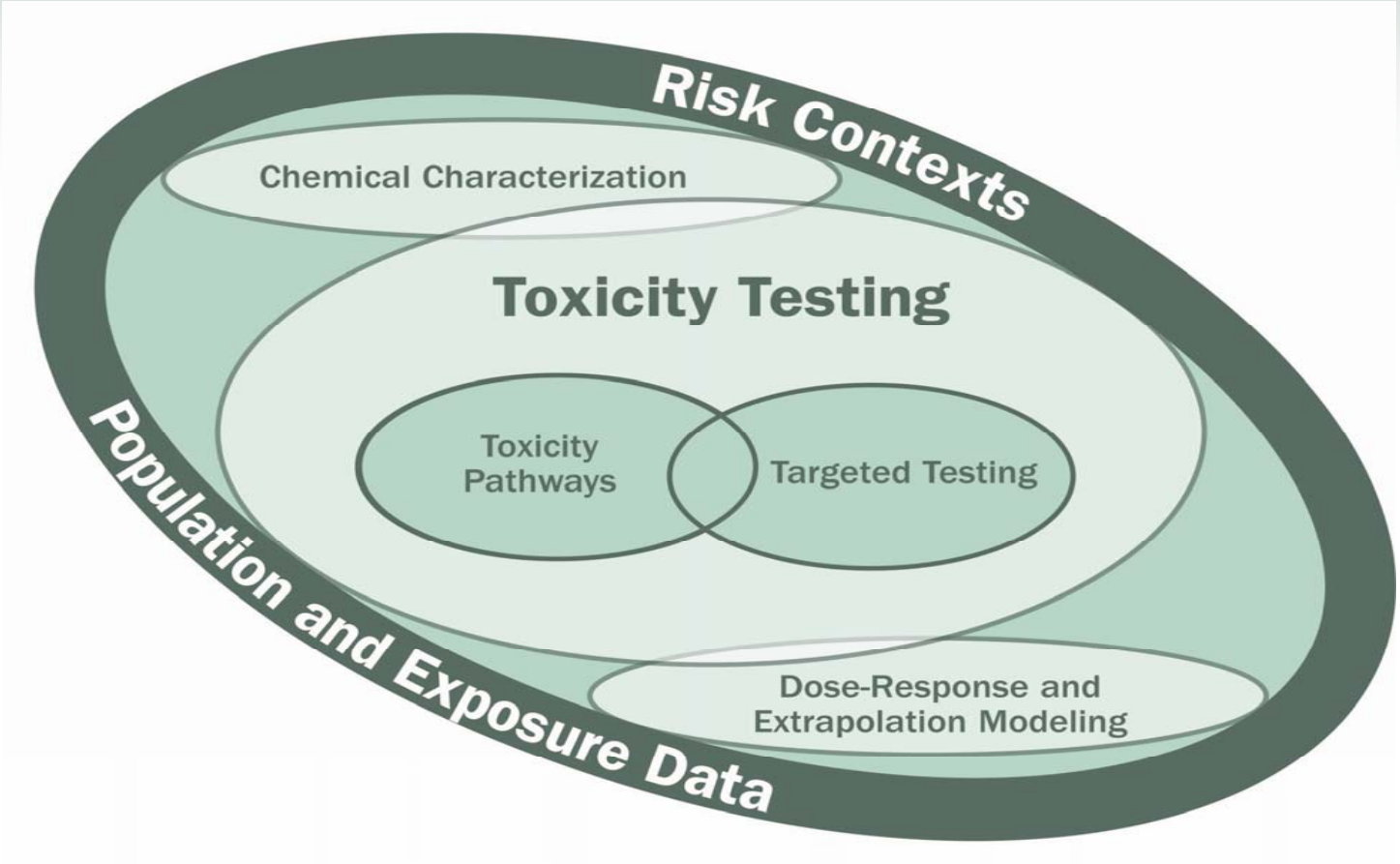
Dose response modeling of perturbations of pathway function would be organized around computational systems biology models of the circuitry underlying each toxicity pathway. *In vitro* to *in vivo* extrapolations would rely on pharmacokinetic models – ideally physiologically based pharmacokinetic models - that would predict human blood and tissue concentrations under specific exposure conditions.

What does the target look like?

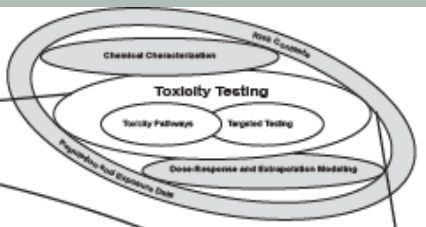
If the following steps are taken successfully, we can develop a new, more mode of action based approach for generating toxicity test results based on perturbations of human biology **AND** for using this information for a risk avoidance approach to regulation.

- toxicity pathway test protocols
- systems biology models for analysis of toxicity pathway response
- biokinetic dosimetry models for in vitro in vivo extrapolation
- legislation to focus on risk avoidance rather than risk assessment

Components of the Vision



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.

Toxicity Pathways

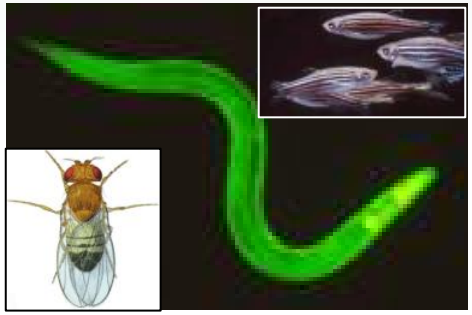
A cellular response pathway that, when sufficiently perturbed, is expected to result in an adverse health effect. How many will there be?

High Throughput Screening, or… What's wrong with this picture?

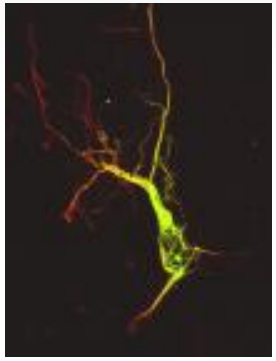
1-3/year

10's/year

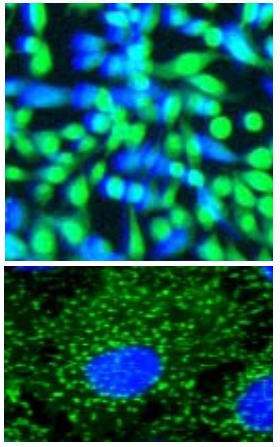
???

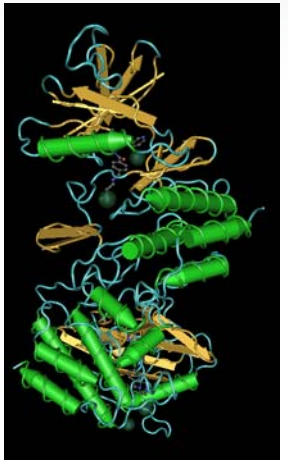


100's/year



10,000's/day

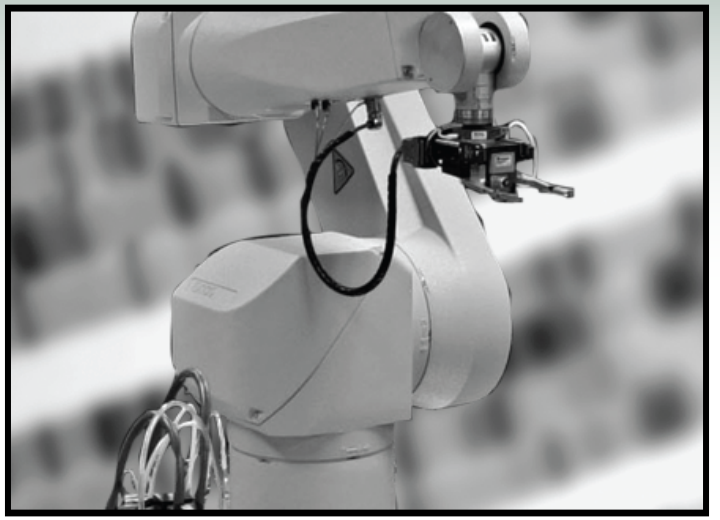




100,000's/day

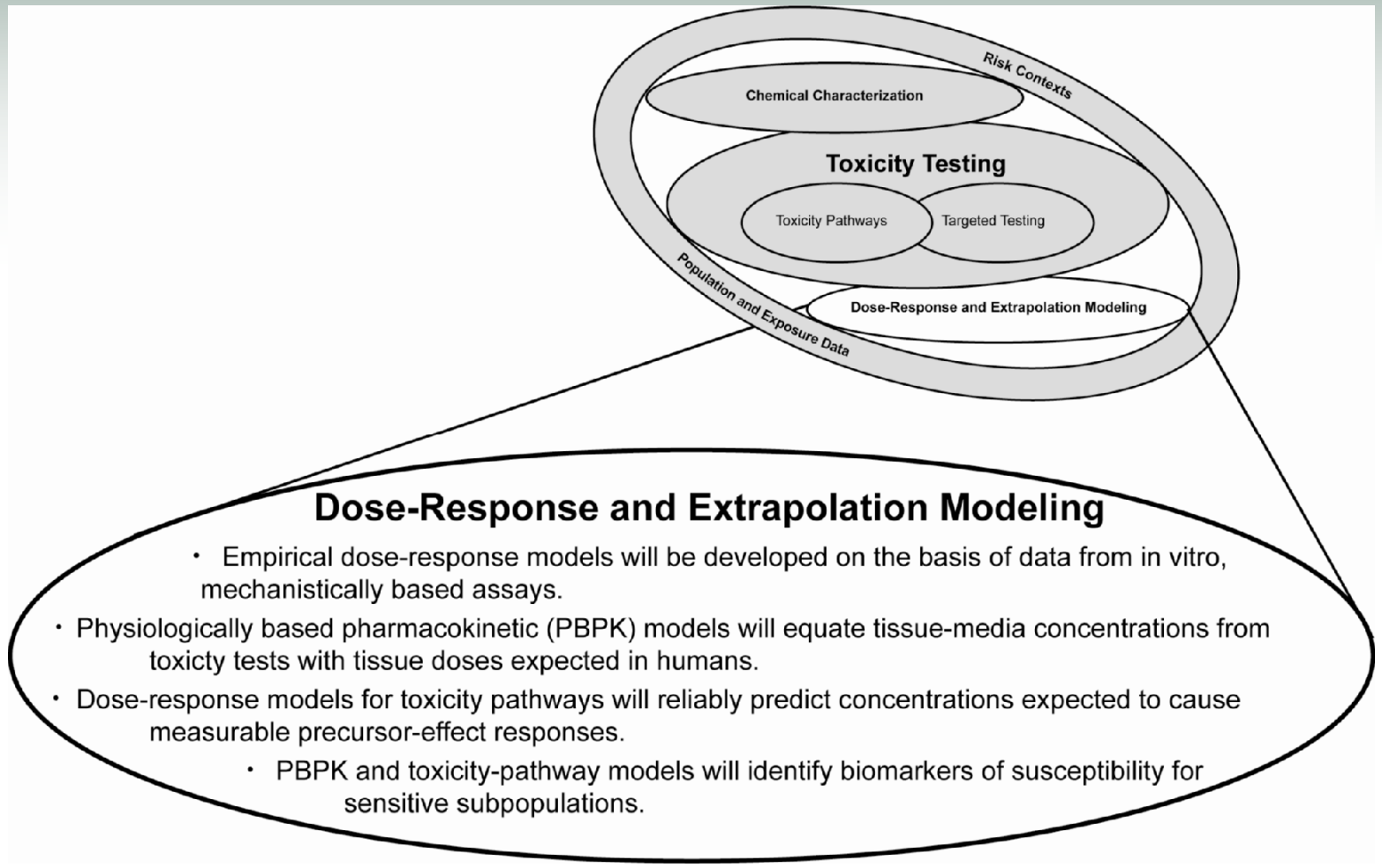
High Throughput
Molecular mechanism

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

Dose-Response and Extrapolation Modeling
Putting results into a risk perspective

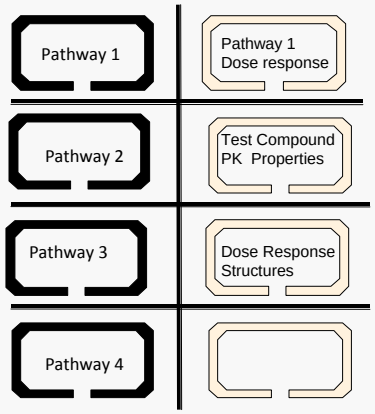


Dose-Response and Extrapolation Modeling

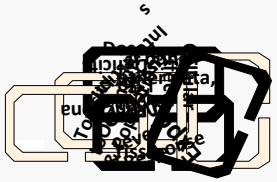
- Empirical dose-response models will be developed on the basis of data from in vitro, mechanistically based assays.
- Physiologically based pharmacokinetic (PBPK) models will equate tissue-media concentrations from toxicty tests with tissue doses expected in humans.
- Dose-response models for toxicity 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.

Using Toxicity Pathway Test Results for Risk Assessment

Hazard ID data sets

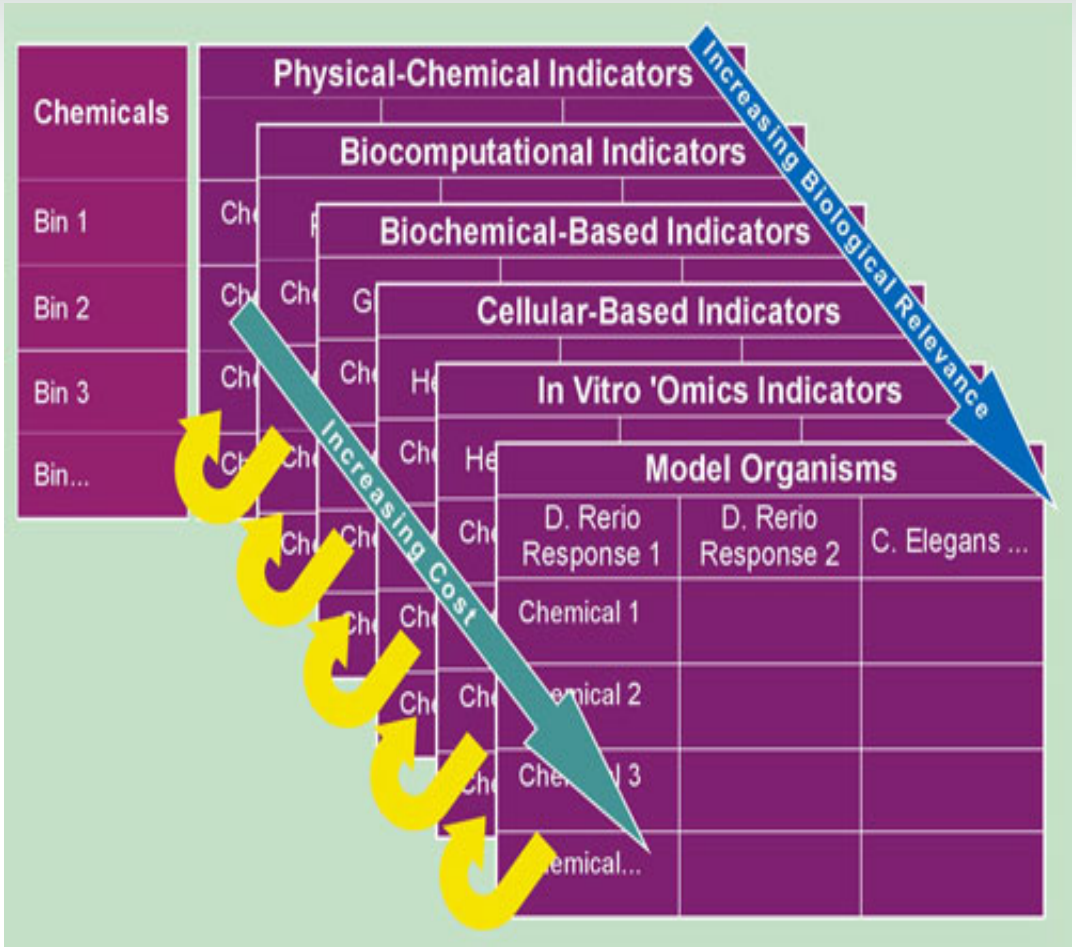


DECISION MAKER CHALLENGE



Studies on multiple pathways in vitro and dose-response behaviors need to be interpreted in relation to expected risks in humans at relevant exposures. How can this be accomplished?

ToxCast Cascade



Andersen's Dose-Response and Extrapolation Modeling Cascade

I. *in vitro* high throughput toxicity pathway tests ($\sum_{n=1}^{132}$)

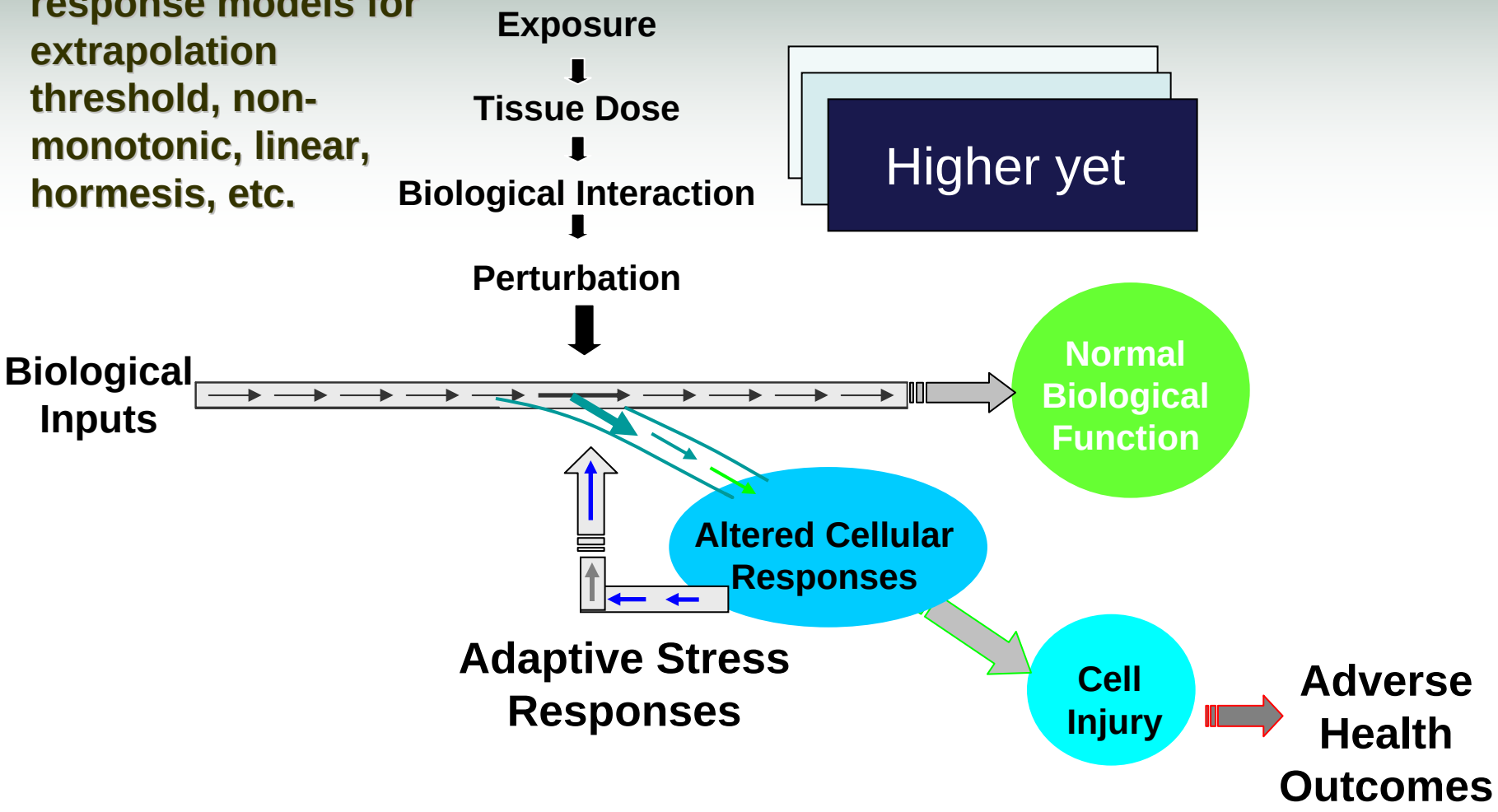
II. Computational systems biology description of pathway circuitry for dose response modeling and dose response models – thresholds, non-mo

III. Dose dependent transition models for sequential pathway activation to understand links from perturbations to toxic responses

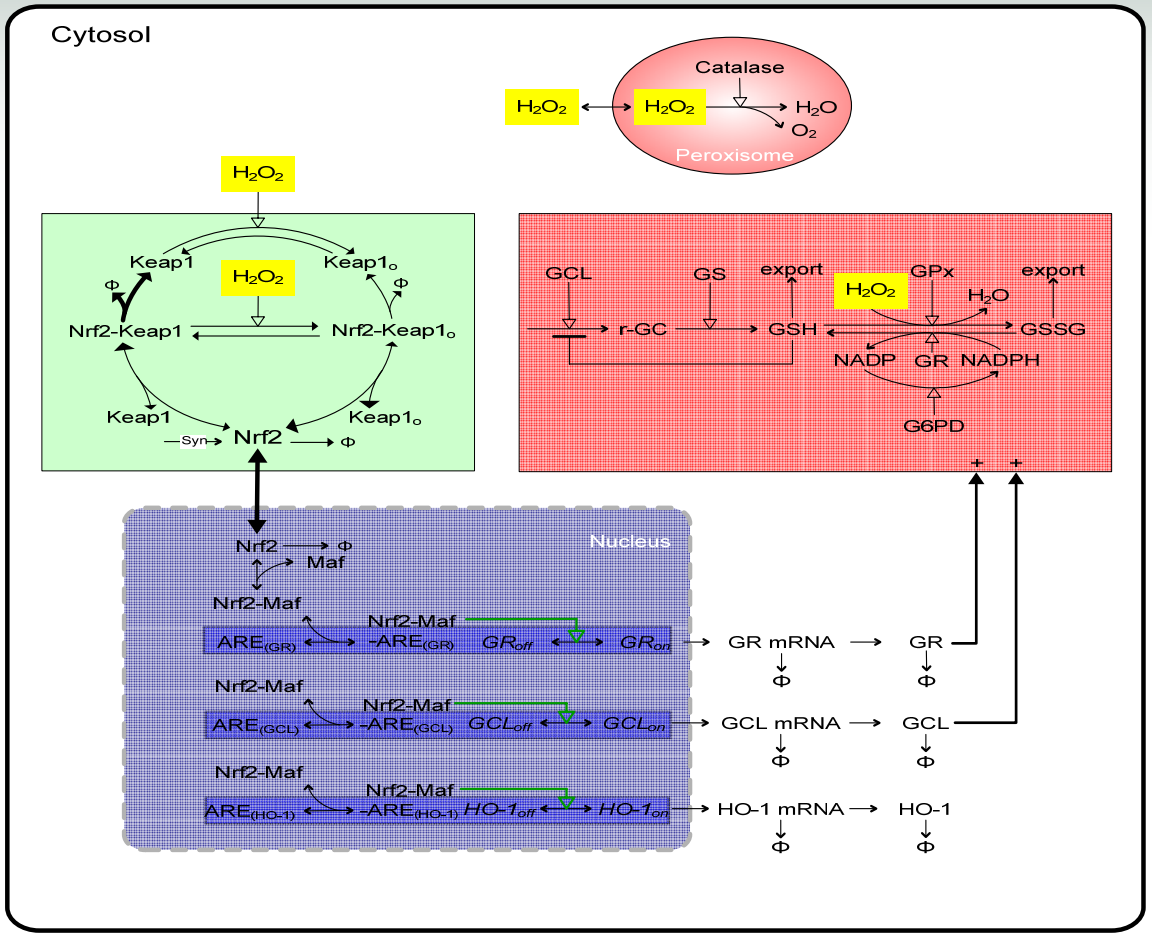
IV. PBPK Modules – Compound specific or class specific for *in vitro- in vivo* extrapolation, interpreting biomonitoring studies and inferring relationship of expected use patterns and doses to human populations

Thinking from Biological Perturbations to Responses

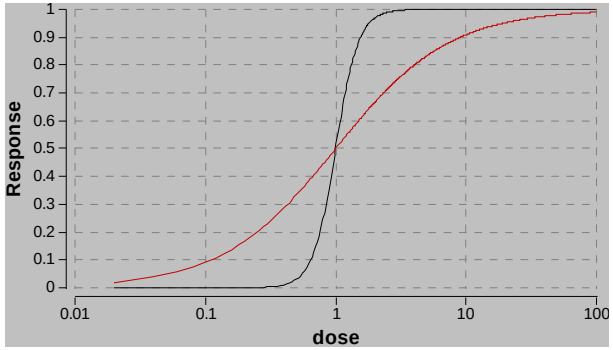
Various dose-response models for extrapolation threshold, non-monotonic, linear, hormesis, etc.



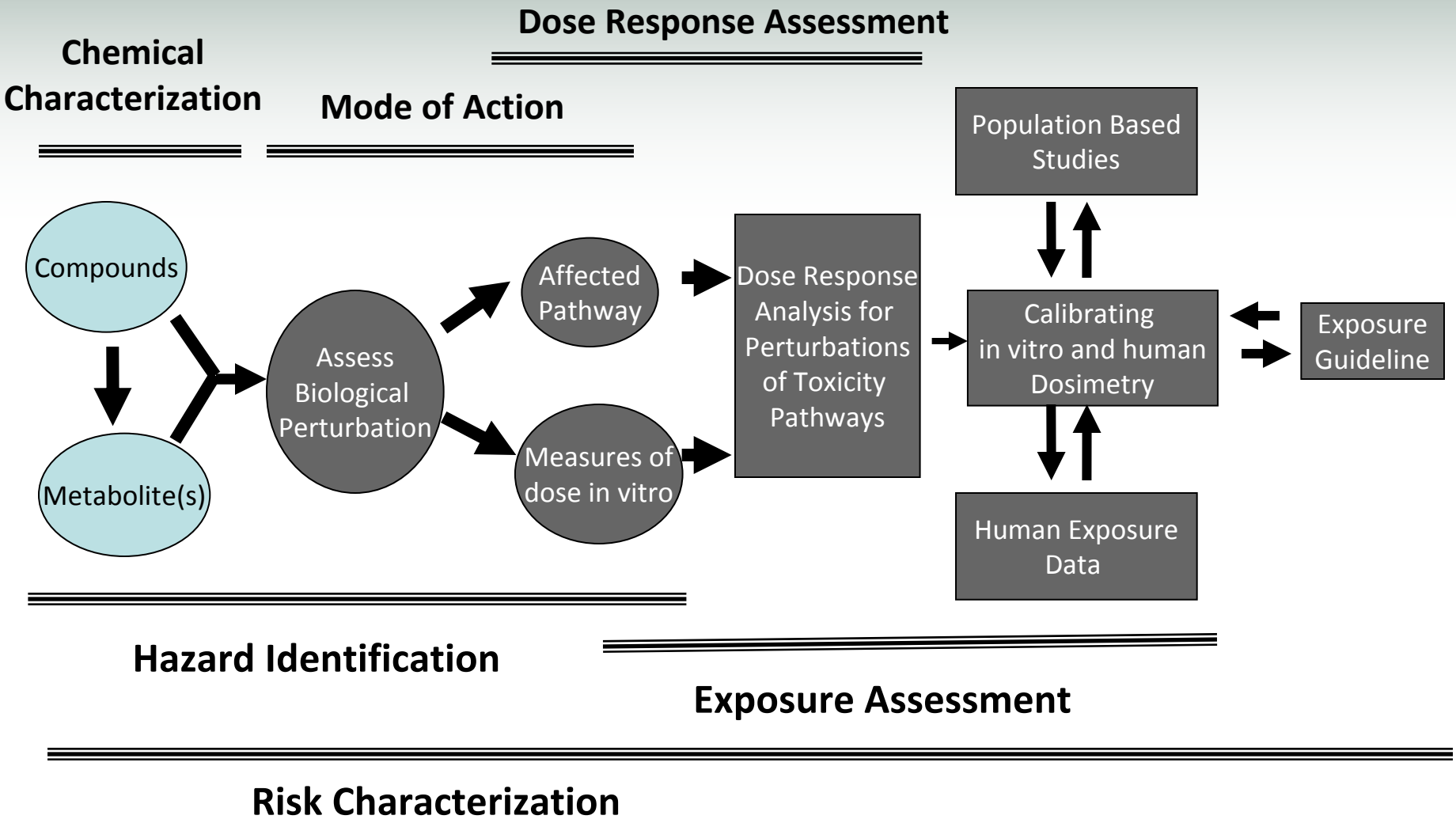
Computational Systems Biology Model for the Circuitry and the Output



Feedback Controlled Adaptive Stress Response control activation and perturbations in the signaling pathway



Toxicity Testing in the 21st Century in relation to the 1983 Red Book



Toxicity Pathway Perturbations A frequently voiced Concern

- Might this approach lead to excessively conservative guidelines. Maybe…
- The discipline needs to be developing the interpretive tools from the beginning, not after the hazard id data start accumulating

Regulatory Context

- Shift in focus away from apical outcomes in experimental animals towards avoiding excessive perturbations of toxicity pathways

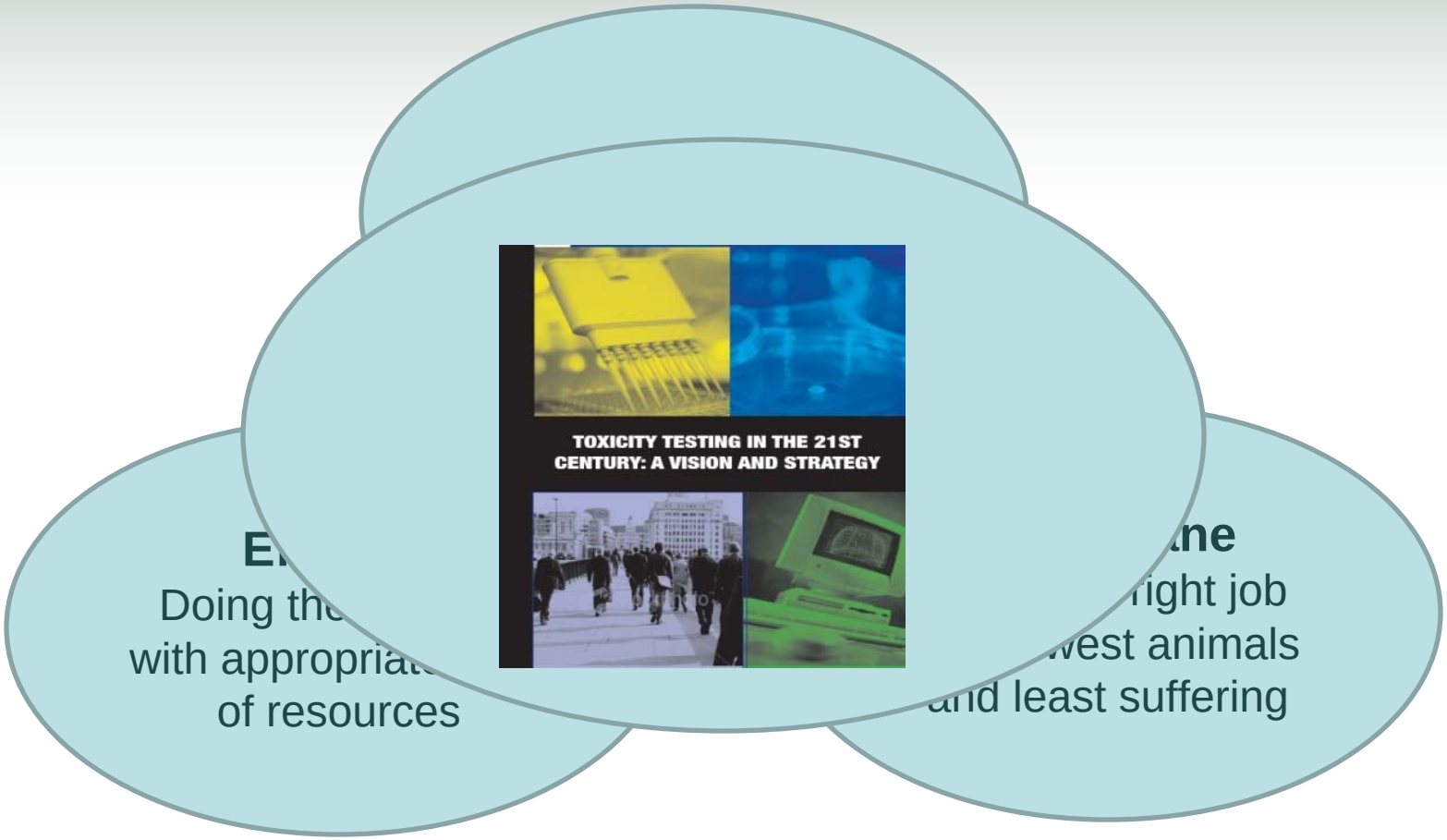
- Development of risk assessment practices based on pathway perturbations, out of the box

- Re-interpretation or possible re-writing of regulatory statues under which risk assessments are conducted – probably not

Conclusions

- Paradigm shift away from apical endpoints to perturbation of toxicity pathways
- Will provide much broader coverage of the universe of targets for 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
- Careful considerations of communicating risk assessment objectives and criteria

Considerations in Designing a Contemporary Strategy for Toxicity Testing with Environmental Agents



Final Comments

- Design a new toxicity test approach from a blank sheet – a daunting task even with 4 years and 23 colleagues – try it!
- The vision came as a surprise (**to me**): the current animal intensive paradigm for testing with **environmental agents** is really not addressing the right problem, *i.e.,* it's traditional, but not effective
- A contemporary program should focus on human biology, broad ranges of dose and perturbations of biological pathways
- A key issue for me has involved my feelings about the ethics of usage of animals when the data generated are not optimal for the decision making process in assessing likely human risks at relevant exposure concentrations