Toxicity Testing for the 21st Century – Applications for the SRP

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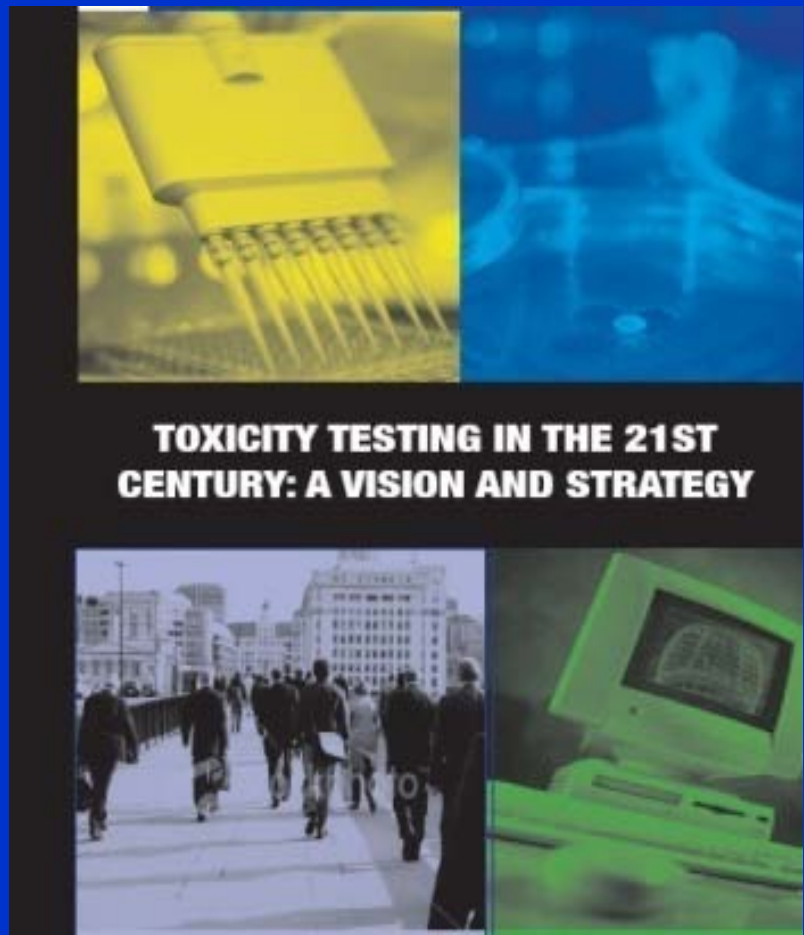
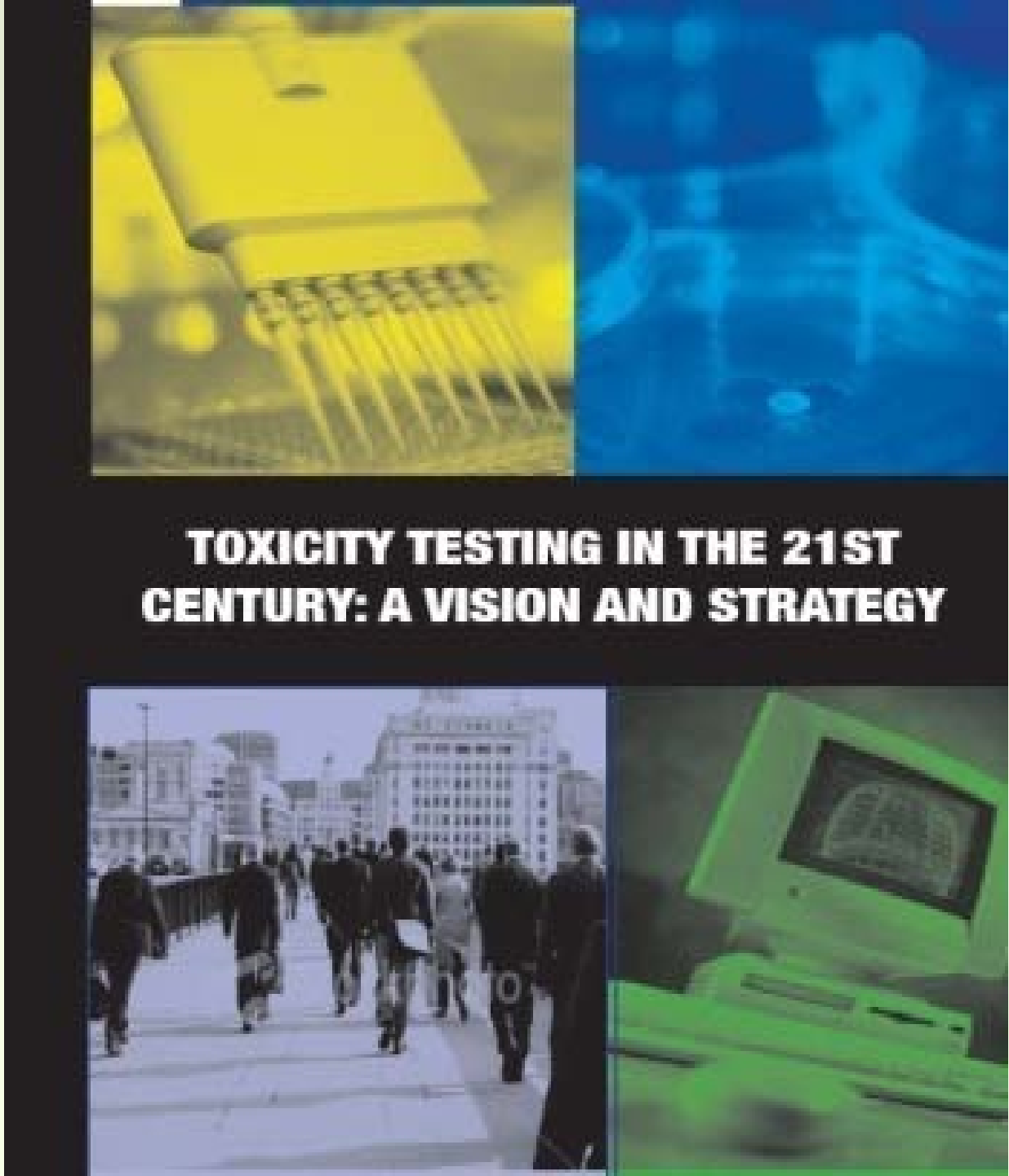


- **The Vision and Approach**
- **Implementation**
- **Applications for the SRP**

National Academy of Sciences 2007

"….. a not-so-distant future where all routine toxicity testing will be conducted in human cells or cell lines ***in vitro*** by evaluating perturbations of cellular responses in a suite of toxicity pathway assays….."

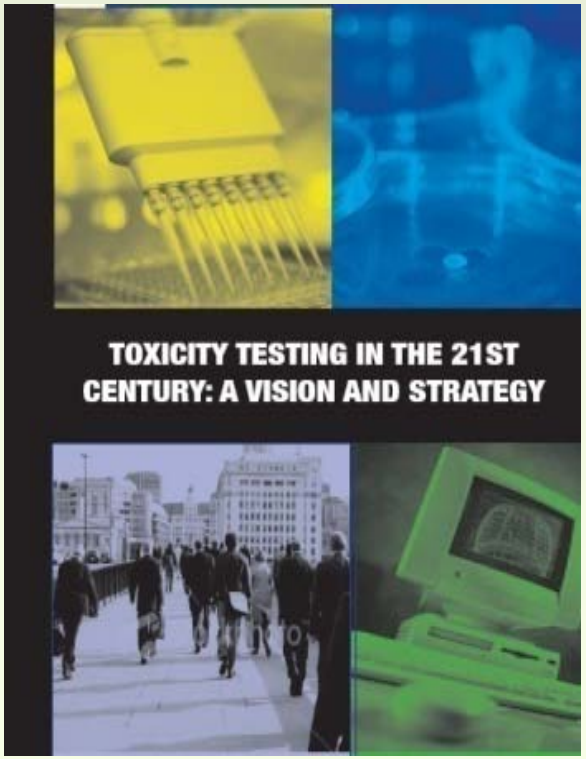
Andersen and Krewski (2009). Toxicity Testing in the 21st Century: Bringing the Vision to Life. Tox. Sci., 107, 324-330.



The Vision and Approach

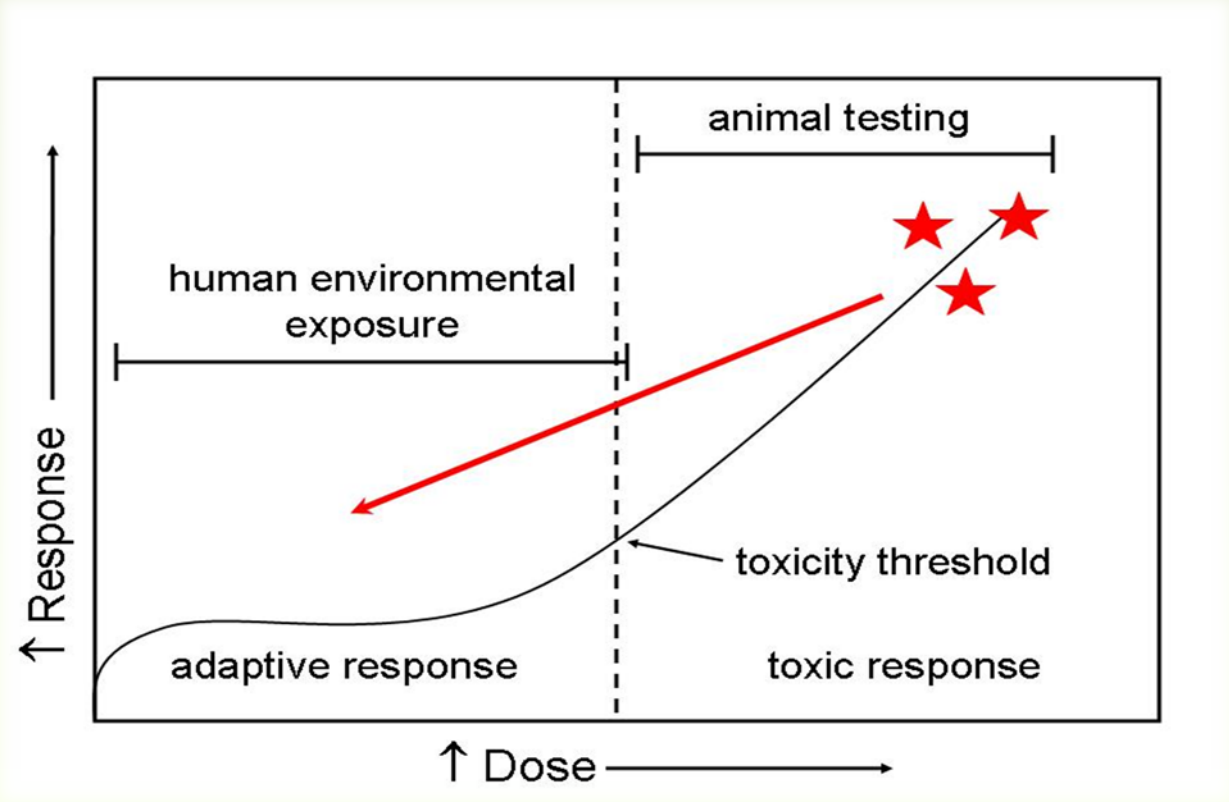
Contents

1. Introduction
2. Vision
3. Components of Vision
4. Tools and Technologies
5. Developing the Science Base and Assays to Implement the Vision
6. Prerequisites for Implementing the Vision in Regulatory Contexts



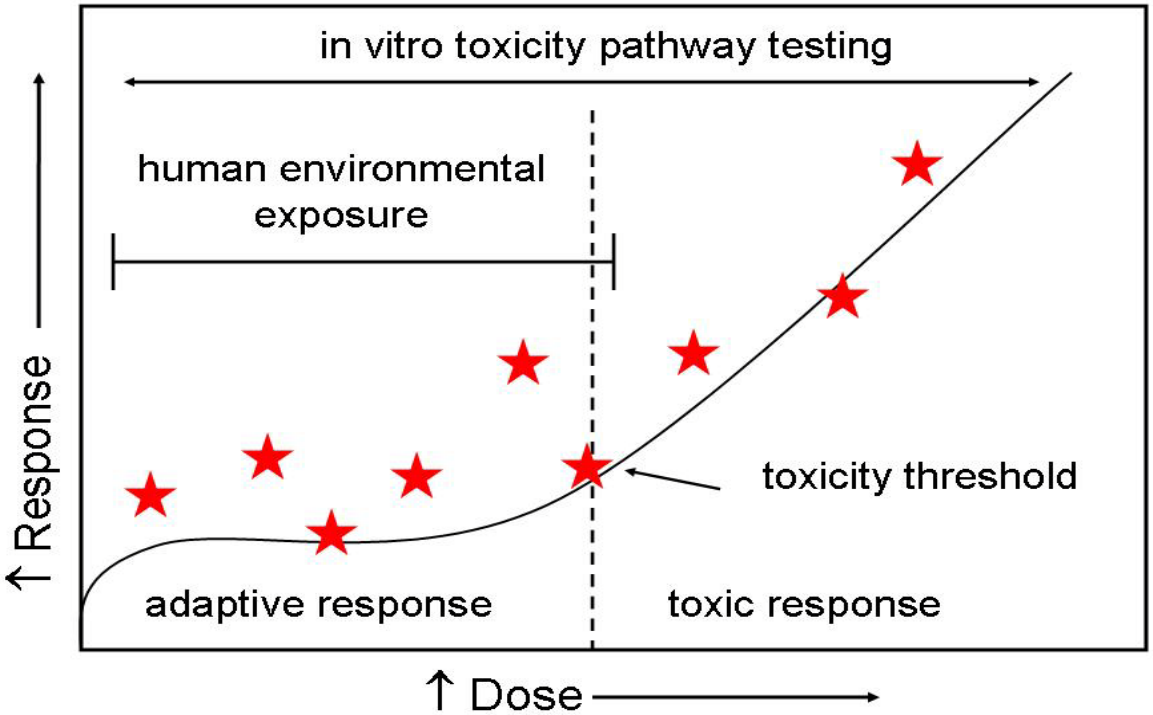
Increasing frustration with current approaches to toxicity testing …

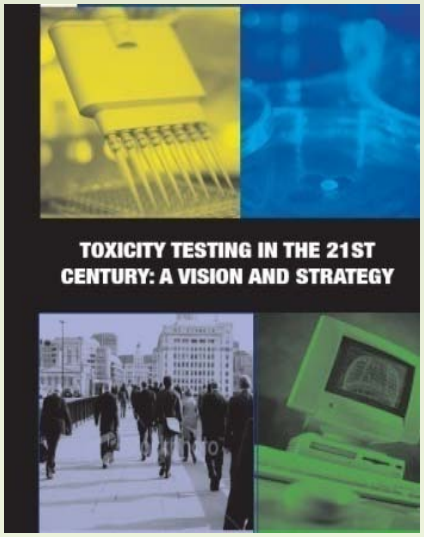
- Low throughput; expensive
- Questionable relevance to actual human risks
- Conservative extrapolation defaults, including safety factors
- Traditional approaches dating to 1930's
- Reliance on animals
- Little use of modern biology, mode of action
- Requires:
  1. Extrapolation across species
  2. Extrapolation from high-to-low dose



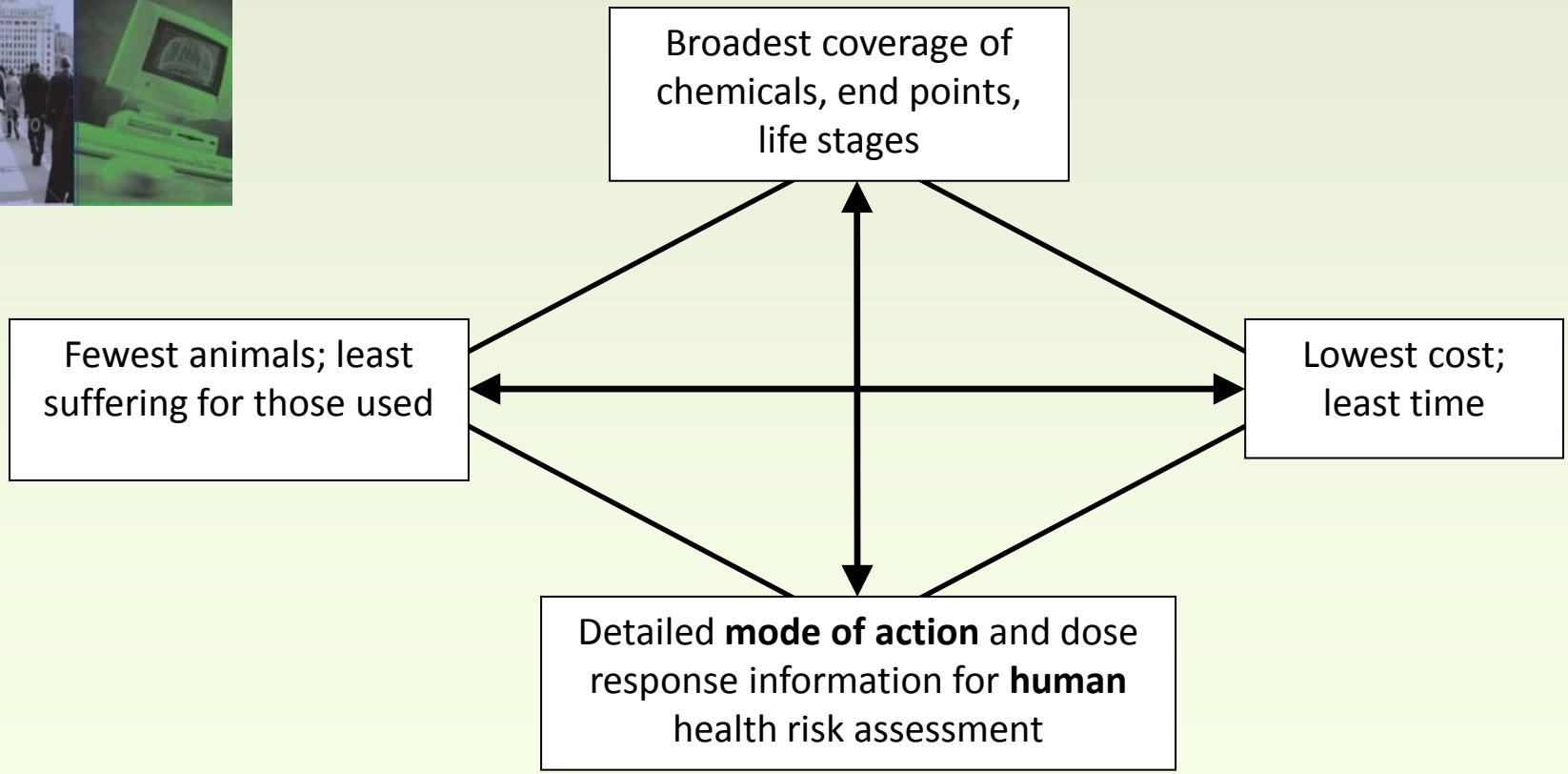
Vision of a future of toxicity testing based on a very different paradigm……

- Multiple doses in vitro
- Defined number of toxicity pathways
- High throughput
- Expensive to develop, cheap to do
- Fast
- Mechanistic endpoints
- Based on human biology
- Requires:
  1. Extrapolation across levels of biological organization
  2. Extrapolation of in vitro-to-in vivo dose response



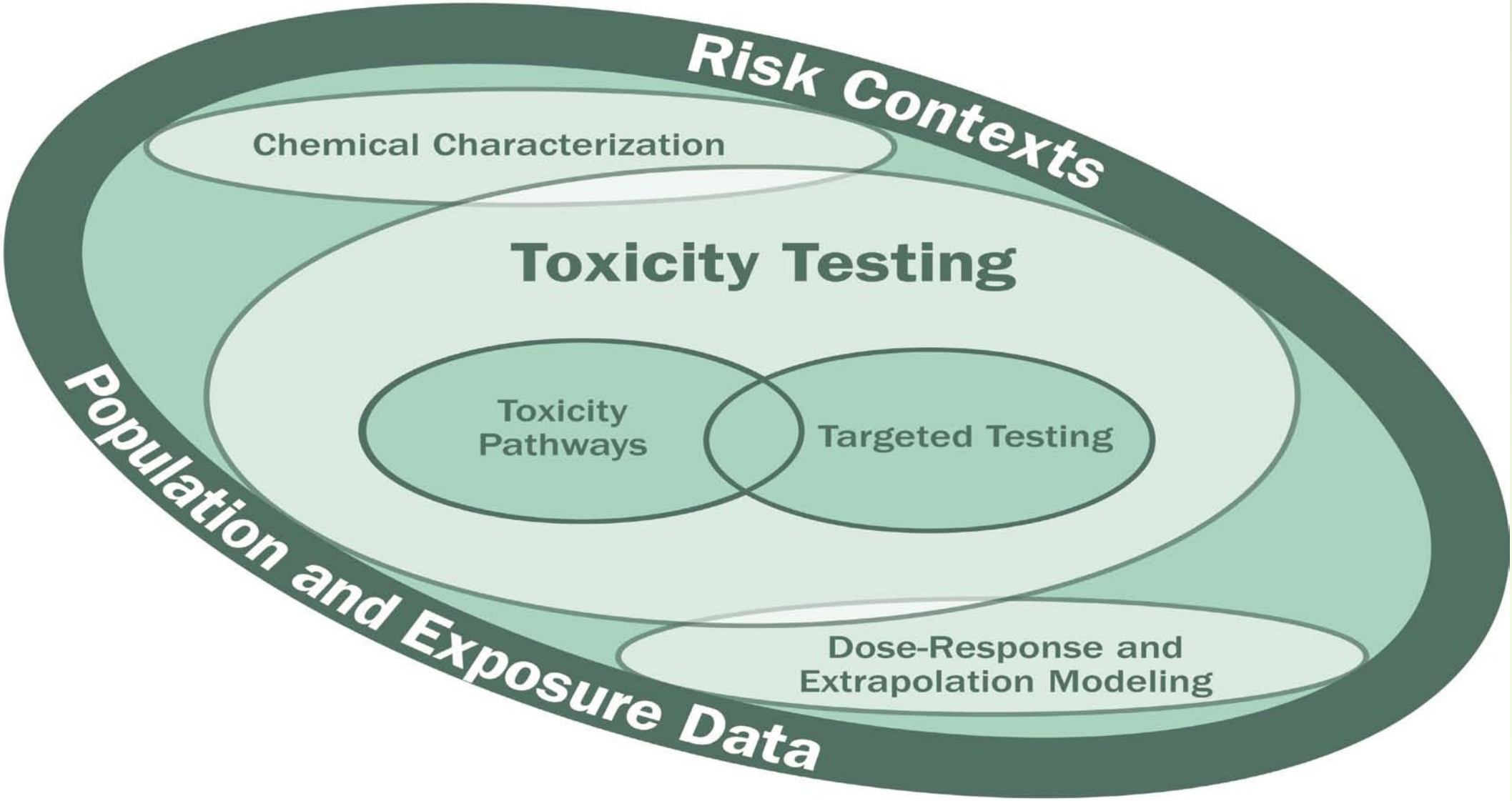


Design Criteria:
Toxicity Testing of Environmental Agents



Options for Future Toxicity Testing Strategies Table 2-1

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


Risk Contexts
Chemical Characterization
Toxicity Testing
Toxicity Pathways
Targeted Testing
Dose-Response and Extrapolation Modeling
Population and Exposure Data

Toxicity Pathways

What are the toxicity pathways?

- A cellular response pathway that, when sufficiently perturbed, is expected to result in an adverse health effect.
- Just a normal biological signaling pathway and its components

How many are there?

Endogenous hormones

PXR, CAR, PPAR and AhR receptors

DNA damage

Heat-shock proteins

Nrf2 oxidative stress

p38 MAPK

Hypo-osmolarity

Designing Toxicity Pathway Assays

In vitro, rapidly performed toxicity pathway tests in primary human cells, cell lines, or tissue aggregates

Rapid progress since completing the report:

- Human stem cell biology
- Better access to human cells
- Bioengineered tissues

Possible approach

Isolate cells from patients with and without genetic diseases

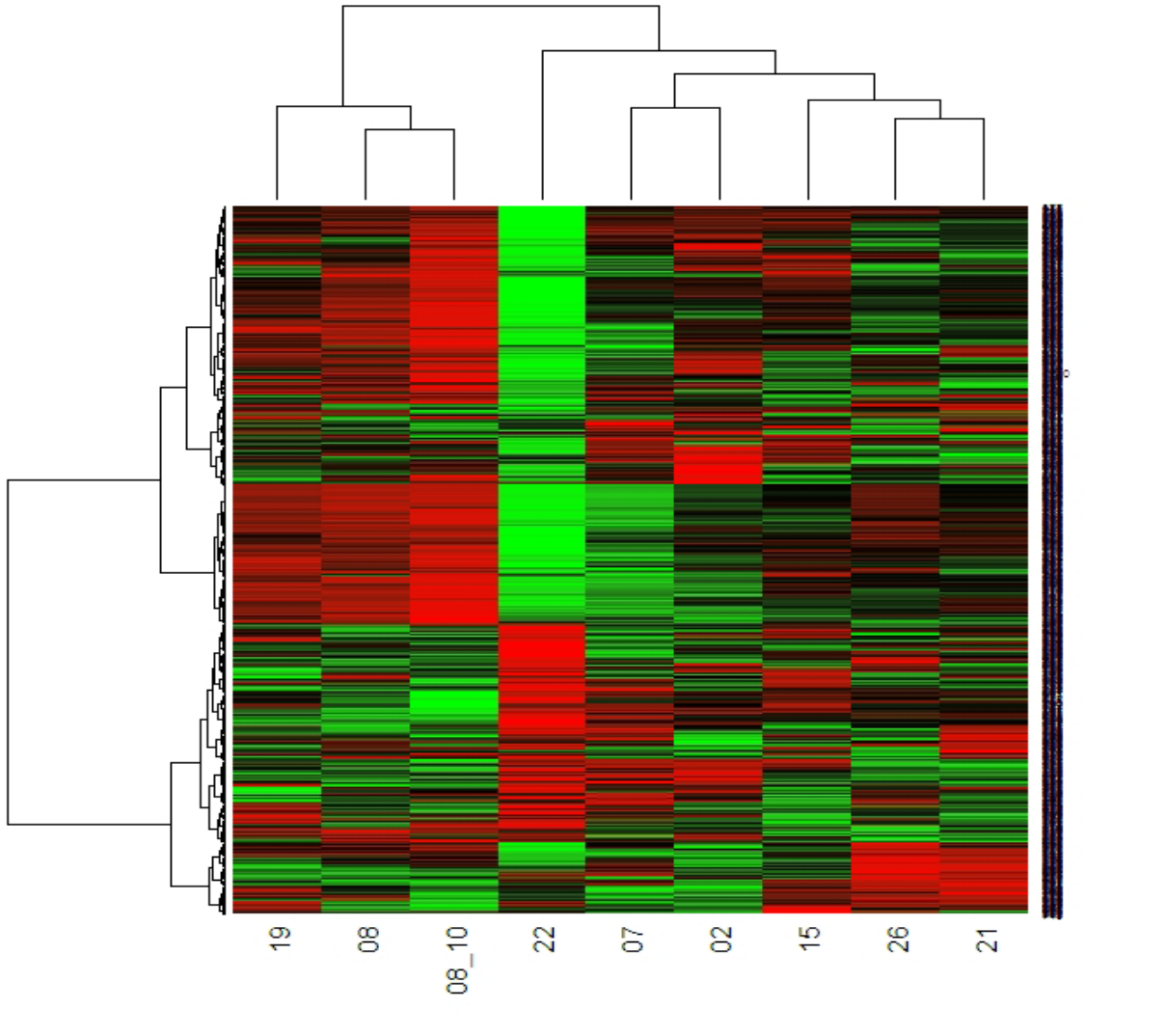
↓

Transfect and generate pluripotent stem cells

↓

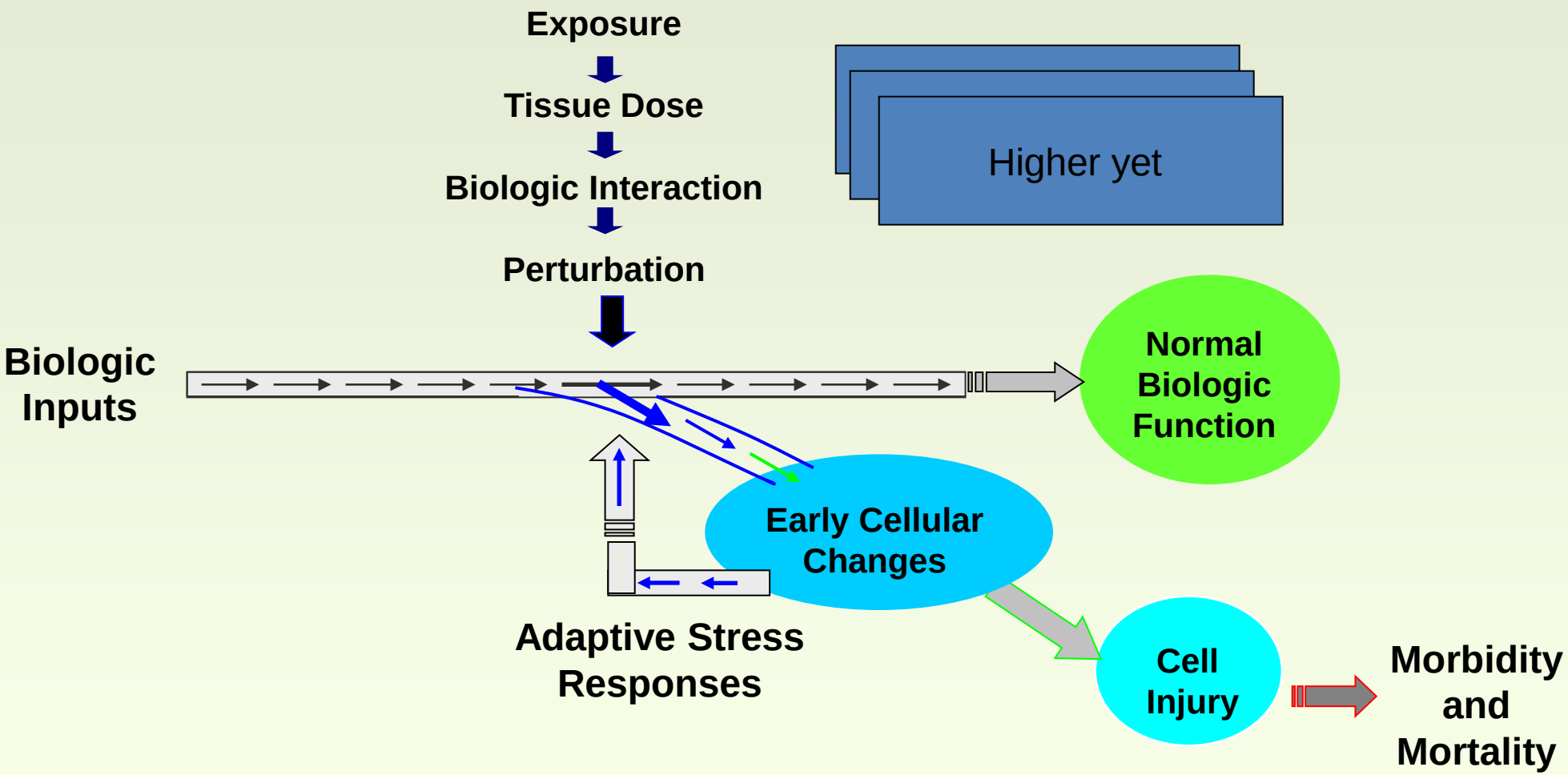
Generate differentiated tissues with and without known genetic defects

Pathway Testing – toxicogenomics, etc.



Assess pathways, integrate tissue responses, and evaluate metabolites

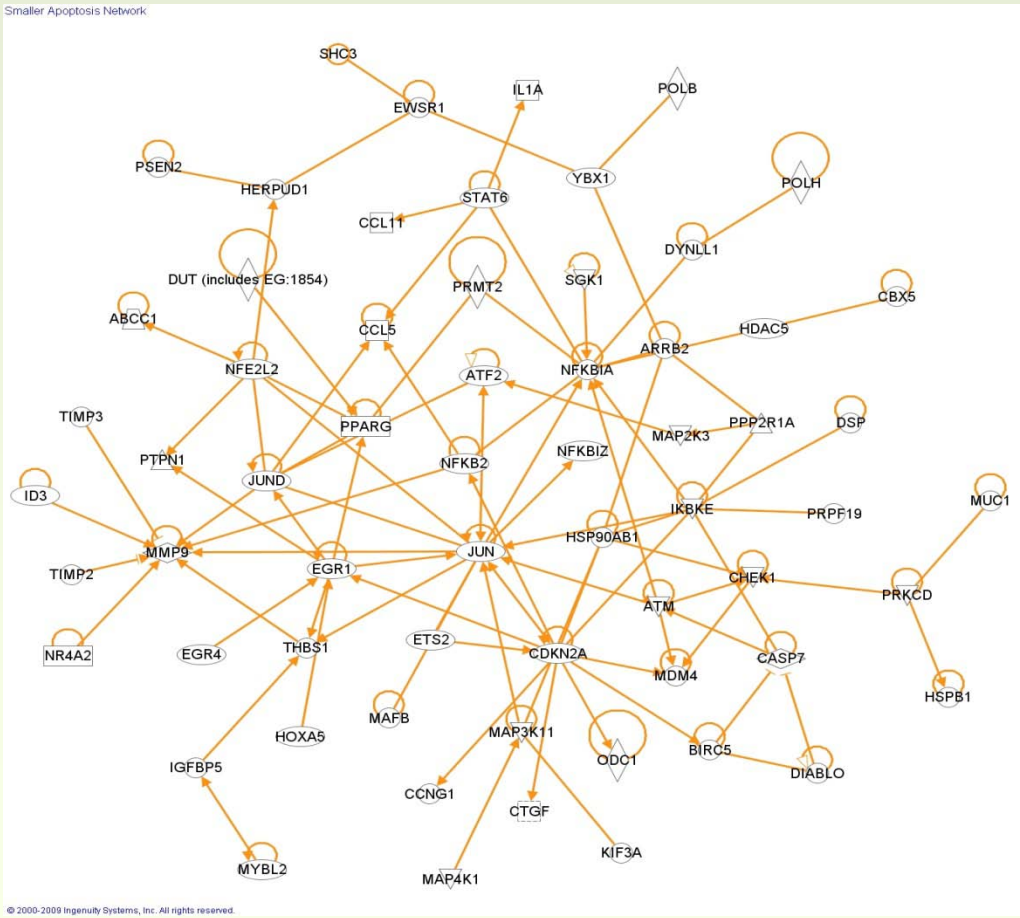
Perturbation of Toxicity Pathways



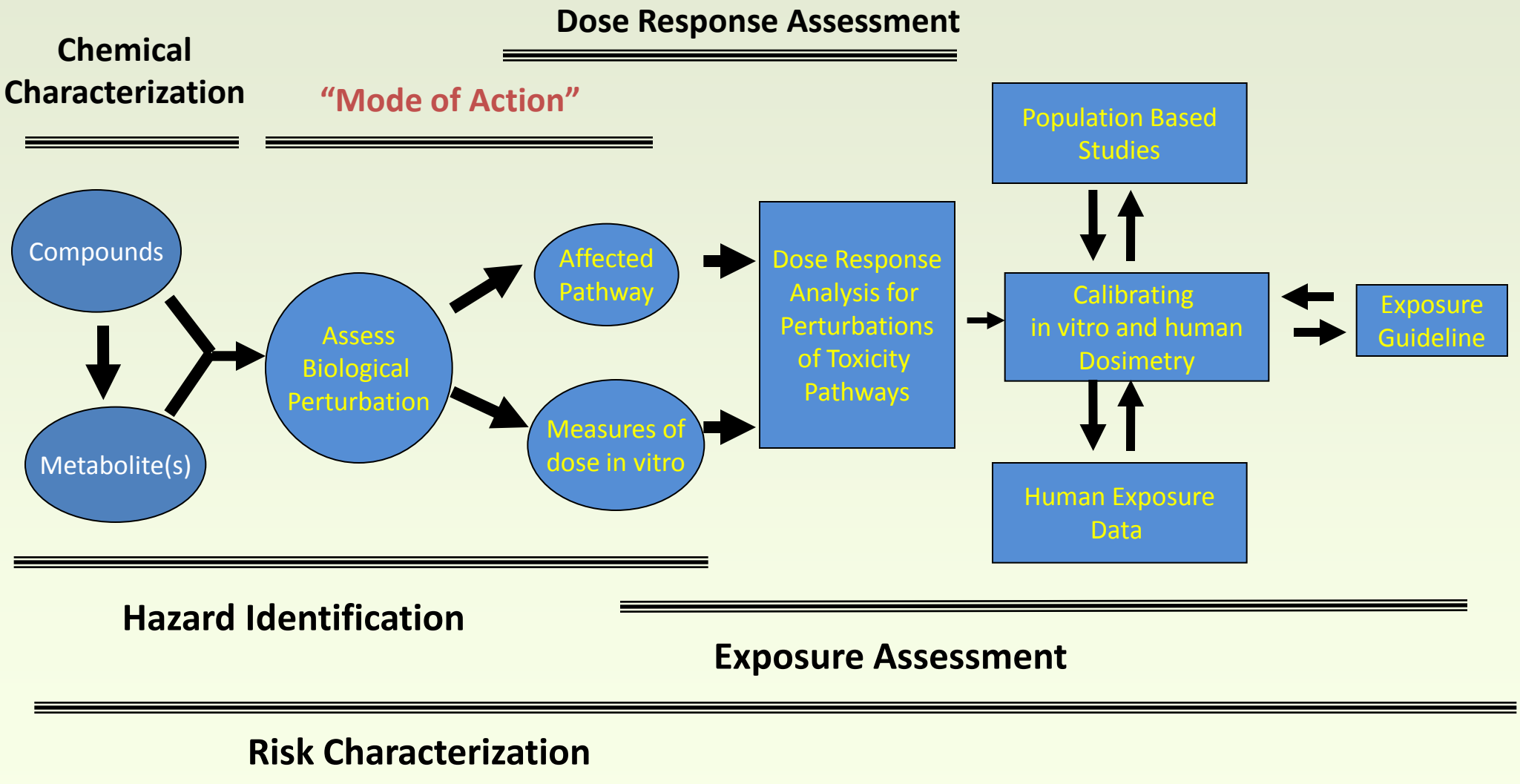
Dose Response Models Linking Perturbations to more Integrated Responses

Computational systems biology description of pathway circuitry for creating biologically realistic dose response models

Dose dependent transition studies for sequential pathway activation to understand linkage to cell and tissue level responses (perturbations to adaptation to adversity)



How does the new 'risk' paradigm compare to the 1983 Red Book for environmental agents?



Advantages…….

- Toxicity testing more focused on human biology; not an uncertain reflection of high dose animal studies for what is expected at low doses in humans
- Creates detailed understanding of pathway targets, functional design of pathway circuitry , more diverse dose response models for target and integrated cellular responses for ties to possible outcomes

Challenges…….

- Assay Design/Development for Toxicity Pathways
- Improved methods to identify (predict) and test metabolites – targeted testing
- Co-ordinate development of ‘functional genomic tools’ to map and model pathways and use results to establish safe levels of exposures
- Train toxicologists and regulators about need for new approach and then in the tools and methods that will be involved in the transformation

Implementation

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2. Vision
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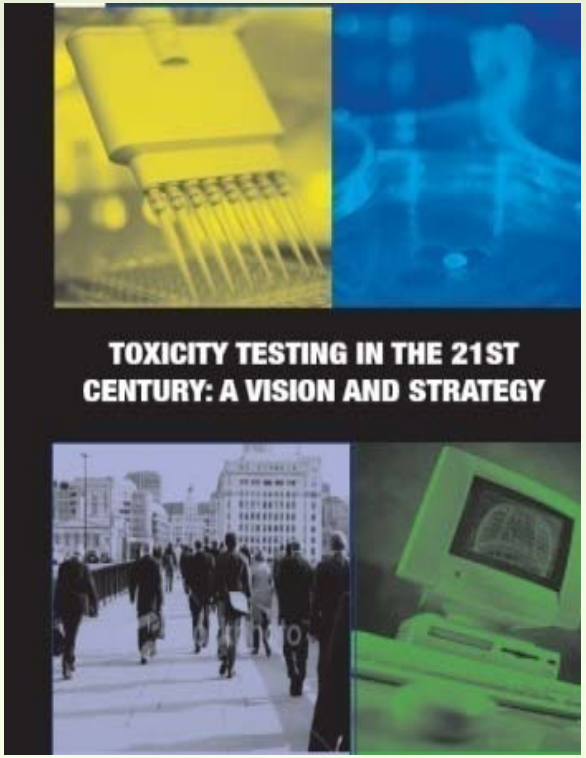


Developing the Science and Assays

Toxicity-pathway elucidation	Phase I	➢ Elucidate toxicity pathways. ➢ Establish data-storing and -management systems. ➢ Establish practices for assay conduct and reporting. ➢ Plan human-surveillance and -biomonitoring strategy.
Assay development & validation	Phase II	➢ Develop suite of representative human cell lines and cultures. ➢ Develop and validate high- and medium-throughput assays. ➢ Develop biomarkers for exposure, susceptibility, and effect for human surveillance and biomonitoring.
Assay relevance & validity trial	Phase III	➢ Gain experience though testing mechanistic assays • In parallel with traditional apical tests. • On chemicals with large datasets of apical tests. • By screening chemicals that would not otherwise be tested. ➢ Begin biomonitoring and surveillance of human populations.
Assembly & validation of test batteries	Phase IV	➢ Propose then validate suites of assays for use in place of identified apical tests.

Program Time Line

Building a transformative research program

- **Instituting focused research**
  - "A long-term, large-scale concerted effort is needed to bring the new toxicity testing paradigm to fruition…many years and hundreds of millions of dollars"
- **Interdisciplinarity, adaptability, and timeline**
  - "…an institutional structure that encourages and coordinates the necessarily multidsciplinary research"
  - "…the testing paradigm would be progressively elaborated over many years or decades…noticeable changes in toxicity-testing practices within 10 years…within 20 years, testing approaches will more closely reflect the proposed vision than current approaches"
- **Cross-institution and sector linkages**
- **Funding**

Institutional framework

- "…a research institute that fosters multidisciplinary research intramurally and extramurally…funded primarily by the federal government"
- "Should it be an existing entity…..or a new entity devoted exclusively to the vision?"
- "…housing the proposed research institute in a regulatory agency….could be problematic"
  - Regulatory budgets are under stress
  - Scale is too large
  - Need to insulate institute from the short-term orientation of regulatory-agency programs that depend upon the results of toxicologic testing

Implementation challenges….my observations

- A human- and mechanism-based in vitro testing system is inevitable

- We are still very early in the process

- The EPA has started (ToxCast), and the NIEHS is exploring the intellectual framework for the new paradigm

- The EU have jumped in and started working, although their efforts have been distributed among many locations, people, countries and centers with little organization (a “bottom-up” model)

- A different model is that proposed by the NAS committee – something like the Human Genome Project (a “top-down” model) with the expectation that it will take 20 years and $2B

Implementation challenges….my observations

PROBLEMS

- **Academics as leaders of big efforts**
  - **Typically interested in basic, not applied, research**
  - **Have many duties – teaching, training, other grants, consulting**
  - **The answer is always "more research is needed"**

- **Too many, loosely organized participants means repeating mistakes**

- **There are many common issues that need to be addressed by focused efforts and these issues should not be in every project**
  - **Metabolism**
  - **Genetic variability**
  - **hESC differentiation protocols**
  - **In silico approaches**

Implementation challenges….my observations

BIG QUESTIONS

- **Balance of assay convenience versus re-capitulating the differentiated whole human organism in vitro**
  - **For INVITROHEART, an EU effort, a model of combined atrial+ventricular tissue to better know about conduction is proposed....Is this necessary?**
  - **Simplicity is an important goal**

- **Does a test for carcinogenicity need to look at different tissues?**

- **Does a test for neurotoxicity need to include neurons?**

- **Is prioritization for further testing (using animals) the right goal, or should we be working on a stand-alone system from the beginning?**

Implementation challenges….my observations

RECOMMENDATIONS

- **It is important not to over-promise and fail to deliver, because this is a long-term effort**
- **Experience is important and cumulative... this will take time, patience, and accepting wrong turns**
- **A centralized-enough effort is very important**
- **More focus on pathways and commonality of responses**
- **Taking a 'systems' approach to human biology that relies on mechanisms and pathways has great value for society, contributing both to improved toxicity testing and to the fundamental molecular elucidation of human disease**

The goal is to do the best science....with a purpose; and the purpose is improved human health and safety

EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

***Use of Emerging Science and Technologies to Explore Epigenetic Mechanisms Underlying the Developmental Basis for Disease*, July 30-31, 2009**

***Computational Toxicology: From Data to Analyses to Applications*, September 21-22, 2009**

***The Exposome: A Powerful Approach for Evaluating Environmental Exposures and Their Influences on Human Disease*, February 25-26, 2010**

***Stem Cell Models for Environmental Health*, June 3-4, 2010**

***The Use of In Utero and Post-natal Indicators to Predict Health Outcomes Later in Life*, October 14-15, 2010**

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

Applications for the SRP

Contents

1. The SRP ethos
2. Our collective skills
3. Our team approach
4. Our track record of success
5. My recommendations

Superfund Research Program

The SRP ethos

Points from 2003 HGP Lessons Paper

- Build the best teams
- Process must be science-driven
- Meet managerial challenges
- International participation important
- Explicit milestones and quality assessment are valuable
- Technology matters
- Rapid prepublication data release demonstrates value to community
- Social consequences should be included as part of project (ELSI program)

Borrowed from Chris Austin and Collins et al., Science 300:286, 2003

Our collective skills

- toxicology • epidemiology • health and disease
- biomarkers • molecular and cellular pathways
- systems approaches • in vitro, model organisms, mammals • bioinformatics and computational biology
- technology development • engineering expertise
- research translation • community engagement

State-of-the-art science

Interdisciplinarity

Capacity to tackle big problems

Scientific and organizational nimbleness

Training the next generation of interdisciplinary scientists

Strong commitment to research translation, community outreach, USEPA, and local environmental management and health agencies

Our team approach

Our track record of success

The 2009 External Advisory Panel says our:

"...quality research has increased knowledge, reduced uncertainty in risk assessment, and has helped incorporate scientific evidence into environmental policy and decision-making…"

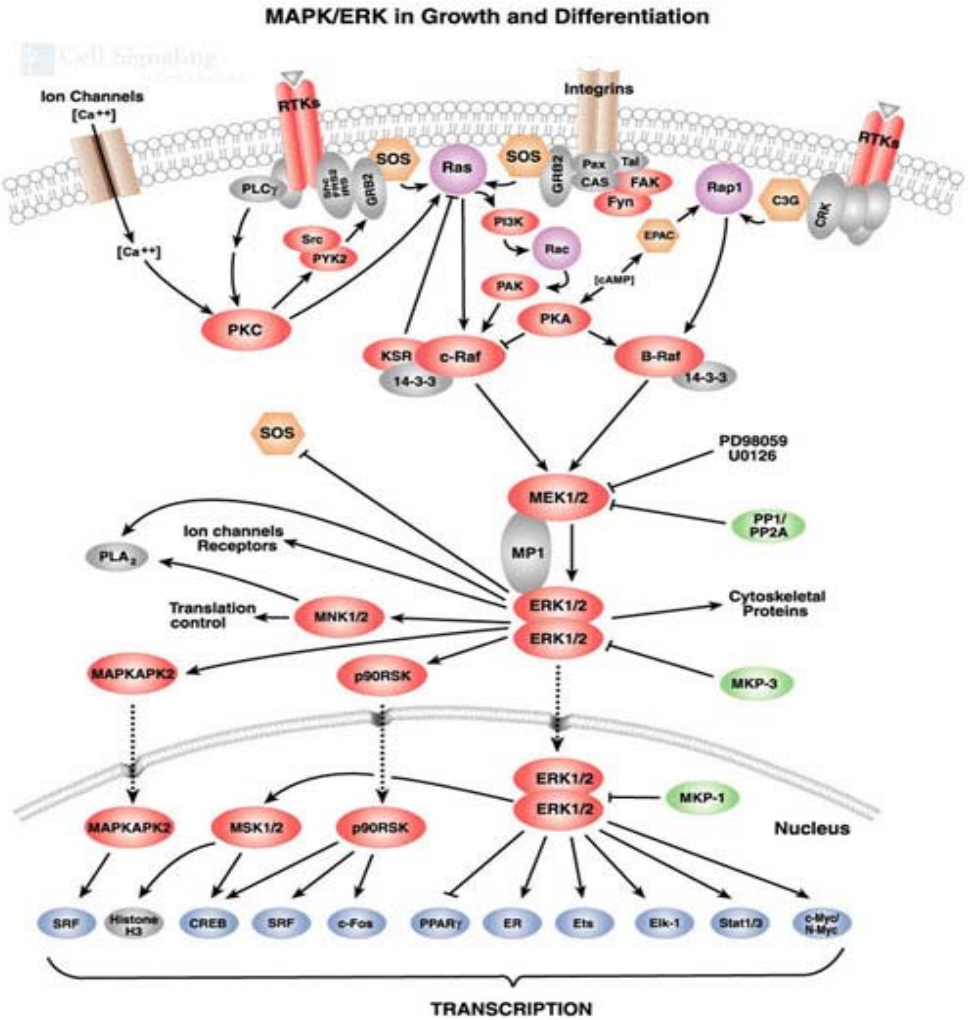
"…activities fill an important niche in the science needs for site assessment and remediation, have had a positive impact on public health…"

ALMOST A QUARTER OF A CENTURY OF SERVING THE PUBLIC BY DOING THE BEST PURPOSEFUL SCIENCE

My recommendations

- Always keep the application of our research to risk assessment in mind
- Where appropriate and possible, integrate 21st century approaches into our projects
- Include 21st century approaches as required elements in future RFAs
- Encourage SRP applications with 21st century approaches as the central theme

The 21st Century Is Here!

THEN

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