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

Melvin Andersen, PhD
The Hamner Institutes for Health Sciences
NAS Toxicity Testing 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
Life Span: 2004 – 2007
With Two Products:
Design Criteria: Toxicity Testing of Environmental Agents

- Brodest coverage of chemicals, end points, life stages
- Lowest cost; least time
- Detailed mode of action and dose response information for human health risk assessment
- Fewest animals; least suffering for those used
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.

Institute of what could get you today

It's conclusive to me... prepare a news release stating that these things can harm rats.

Stuff to watch out for:
- Sugar
- Eggs
- Meat
- Pesticides
- Your dog
- Your house
- Your wife
- Falling bricks
- Air
- Water
### Options for Future Toxicity Testing Strategies

<table>
<thead>
<tr>
<th>Option I</th>
<th>Option II Tiered In Vivo</th>
<th>Option III In Vitro/In Vivo</th>
<th>Option IV In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal biology</td>
<td>Animal biology</td>
<td>Primarily human biology</td>
<td>Primarily human biology</td>
</tr>
<tr>
<td>High doses</td>
<td>High doses</td>
<td>Broad range of doses</td>
<td>Broad range of doses</td>
</tr>
<tr>
<td>Low throughput</td>
<td>Improved throughput</td>
<td>High and medium throughput</td>
<td>High throughput</td>
</tr>
<tr>
<td>Expensive</td>
<td>Less expensive</td>
<td>Less expensive</td>
<td>Less expensive</td>
</tr>
<tr>
<td>Time consuming</td>
<td>Less time consuming</td>
<td>Less time consuming</td>
<td>Less time consuming</td>
</tr>
<tr>
<td>Relative large number of animals</td>
<td>Fewer animals</td>
<td>Substantially fewer animals</td>
<td>Virtually no animals</td>
</tr>
<tr>
<td>Apical endpoints</td>
<td>Apical endpoints</td>
<td>Perturbations of toxicity pathways</td>
<td>Perturbations of toxicity pathways</td>
</tr>
<tr>
<td>Some <em>in silico</em> and <em>in vitro</em> screens</td>
<td><em>In silico</em> screens possible</td>
<td></td>
<td><em>In silico</em> screens</td>
</tr>
<tr>
<td>Options for Future Toxicity Testing Strategies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Option I</strong> In Vivo</td>
<td><strong>Option II Tiered In Vivo</strong></td>
<td><strong>Option III In Vitro/In Vivo</strong></td>
<td><strong>Option IV In vitro</strong></td>
</tr>
<tr>
<td>Animal biology</td>
<td>Animal biology</td>
<td>Primarily human biology</td>
<td>Primarily human biology</td>
</tr>
<tr>
<td>High doses</td>
<td>High doses</td>
<td>Broad range of doses</td>
<td>Broad range of doses</td>
</tr>
<tr>
<td>Low throughput</td>
<td>Improved throughput</td>
<td>High and medium throughput</td>
<td>High throughput</td>
</tr>
<tr>
<td>Expensive</td>
<td>Less expensive</td>
<td>Less expensive</td>
<td>Less expensive</td>
</tr>
<tr>
<td>Time consuming</td>
<td>Less time consuming</td>
<td>Less time consuming</td>
<td>Less time consuming</td>
</tr>
<tr>
<td>Relative large number of animals</td>
<td>Fewer animals</td>
<td>Substantially fewer animals</td>
<td>Virtually no animals</td>
</tr>
<tr>
<td>Apical endpoints</td>
<td>Apical endpoints</td>
<td>Perturbations of toxicity pathways</td>
<td>Perturbations of toxicity pathways</td>
</tr>
<tr>
<td></td>
<td><em>Some in silico and in vitro screens</em></td>
<td><em>In silico screens possible</em></td>
<td><em>In silico screens</em></td>
</tr>
</tbody>
</table>
### Options for Future Toxicity Testing Strategies

<table>
<thead>
<tr>
<th>Option I In Vivo</th>
<th>Option II Tiered In Vivo</th>
<th>Option III In Vitro/In Vivo</th>
<th>Option IV In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal biology</td>
<td>Animal biology</td>
<td>Primarily human biology</td>
<td>Primarily human biology</td>
</tr>
<tr>
<td>High doses</td>
<td>High doses</td>
<td>Broad range of doses</td>
<td>Broad range of doses</td>
</tr>
<tr>
<td>Low throughput</td>
<td>Improved throughput</td>
<td>High and medium throughput</td>
<td>High throughput</td>
</tr>
<tr>
<td>Expensive</td>
<td>Less expensive</td>
<td>Less expensive</td>
<td>Less expensive</td>
</tr>
<tr>
<td>Time consuming</td>
<td>Less time consuming</td>
<td>Less time consuming</td>
<td>Less time consuming</td>
</tr>
<tr>
<td>Relative large number of animals</td>
<td>Fewer animals</td>
<td>Substantially fewer animals</td>
<td>Virtually no animals</td>
</tr>
<tr>
<td>Apical endpoints</td>
<td>Apical endpoints</td>
<td>Perturbations of toxicity pathways</td>
<td>Perturbations of toxicity pathways</td>
</tr>
<tr>
<td></td>
<td>Some <em>in silico</em> and <em>in vitro</em> screens</td>
<td><em>In silico</em> screens possible</td>
<td><em>In silico</em> screens</td>
</tr>
</tbody>
</table>
## Options for Future Toxicity Testing Strategies

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

Risk Contexts
- Chemical Characterization
- Toxicty Pathways
- Targeted Testing
- Dose-Response and Extrapolation Modeling

Population and Exposure Data
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

- 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 toxicity 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.
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 \( \left( \sum_{n=1}^{132} \right) 

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

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

- Exposure
- Tissue Dose
- Biological Interaction

Perturbation

Biological Inputs → Adaptive Stress Responses → Altered Cellular Responses

Thinking from Biological Perturbations to Responses

Higher yet

Normal Biological Function

Adverse Health Outcomes

Cell Injury
Computational Systems Biology Model for the Circuitry and the Output

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

**Chemical Characterization**
- Compounds
- Metabolite(s)

**Assess Biological Perturbation**
- Affected Pathway
- Measures of dose in vitro

**Dose Response Assessment**
- Dose Response Analysis for Perturbations of Toxicity Pathways

**Mode of Action**

**Hazard Identification**

**Exposure Assessment**
- Population Based Studies
- Calibrating in vitro and human Dosimetry
- Human Exposure Data

**Risk Characterization**

The Hamner Institutes for Health Sciences
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

Effective
Doing the right job with appropriate level of resources

Efficient
Doing the right job with fewest animals and least suffering

Humane
Doing the right job with least suffering
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.