Advancing Toxicity Testing Methods for the 21st Century

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Context:

- How have we been doing ‘toxicity testing’?
- What new approaches have been suggested – NRC 2007?
- What tools will be required to use these new toxicity testing results for risk and safety assessments?
- How might these new tools become part of ‘routine’ toxicity testing in the future?
Interspecies Adjustments (PK Modeling) in vivo human exposure ‘standard’ mg/kg/day

Approaches in setting RfC’s and RfD’s

Conduct a variety of animal toxicity studies
Select most sensitive result
Linear or threshold extrapolation
Point of Departure (BMD)
Estimate risks at lower doses

Interspecies Adjustments (PK Modeling)
General frustration with these approaches...

- Date to 1930’s
- Exorbitant in use of animals
- Low throughput; expensive
- Conservative extrapolation tools
- Questionable relevance to human risk
- Little use of modern biology
The National Research Council Committee for Toxicity Testing of Environmental Agents

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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
Committee Life Span: 2004 – 2007
With Two Products – Environmental Agents
The report argues that a transformative redefinition of toxicity testing is required to meet key design criteria.

http://www.nap.edu/catalog.php?record_id=11970
Design Criteria:

- Brodest coverage of chemicals, end points, life stages
- Fewest animals; least suffering for those used
- Detailed mode of action and dose response information for human health risk assessment
- Lowest cost; least time
... a not-so-distant future where all routine toxicity testing will be conducted in human cells or cell lines \textit{in vitro} by evaluating perturbations of cellular responses in a suite of toxicity pathway assays.......
Normal cellular signaling pathways that, when sufficiently perturbed, are expected to result in an adverse health effect.

Need assays eventually to cover the key pathways altered by chemical exposures
Nrf2 is primarily bound to the cytoplasmic protein Keap1

With exposures to oxidants, Nrf2 is released, moves to nucleus, activating various anti-oxidant genes
What are some others?

**Stress response signaling**

- HIF-1 - Hypoxia
- Heat-shock proteins
- p38 MAPK
- Hypo-osmolarity
- Metal stress
- p53-DNA damage
- Nrf2-Oxidative stress

**Receptor Mediated Signaling**

- PXR, CAR, PPAR and AhR receptors
- Steroid family receptors – ER, AR, T3 receptor
Commentaries in Toxicological Sciences

FORUM SERIES, PART I
Toxicity Testing in the 21st Century: Bringing the Vision to Life
Melvin E. Andersen* and Daniel Krewski†

Introduction plus 8 Commentaries

Holsapple, Afshari & Lehman-McKeeman, Editors (2009)

Meek & Doull (2009)  
Bus & Becker (2009)  
MacDonald & Robertson (2009)  
Hartung (2009)  
Hubal (2009)  
Chapin & Stedman (2009)  
Walker & Bucher (2009)  
Boekelheide & Campion (2010)

Authors responses:

FORUM
The Vision of Toxicity Testing in the 21st Century: Moving from Discussion to Action

TOXICOLOGICAL SCIENCES 107(2), 324–330 (2009)
doi:10.1093/toxsci/kfn255
Advance Access publication December 12, 2008

TOXICOLOGICAL SCIENCES 0(0), 1–8 (2010)
doi:10.1093/toxsci/kfq188
Advance Access publication June 23, 2010
Noted Some Overarching Issues in the Responses

1. **Defining adversity** from in vitro studies

2. **Predicting** in life **toxicity** testing results from in vitro tests

3. **Setting regulatory standards** *from in vitro* test results

4. **Making the transition** from current practices to new in vitro based approaches
Components of the NRC Vision

- Chemical Characterization
- Toxicity Testing
  - Toxicity Pathways
  - Targeted Testing
- Dose-response and Extrapolation Modeling

Risk Contexts

Population-based and Exposure Data

tools to assist interpretation

the assays
Perturbation of Toxicity Pathways

Exposure → Tissue Dose → Biologic Interaction → Perturbation → Normal Biologic Function

Biologic Inputs

Adaptive Stress Responses

Early Cellular Changes

Cell Injury

Cytotoxicity, Apoptosis, Necrosis, Proliferation, Mutation
The “Swiss cheese” model of adverse effects

- Chemical Exposure
- Chemical is electrophilic
- Irreversible changes
- Abrupt dose-response transition
- Mitochondrial dysfunction
- Active Failures
- Adverse Effect
- Apoptotic cell

Adapted from Boekelheide and Campion, Toxicol. Sci., 2010.
Defining *adversity* from panels of assays

- Chemical Exposure
  - Chemical reacts with DNA
  - Functional consequence at cellular/gene level with dose response
  - Coordinated cellular response through pathway cascades with dose-response and pathway mapping
  - Functional consequence: assessing degree of pathway perturbation necessary for adverse organ system outcome

- DNA-reactivity
- Genomic responses to exposure
- DNA-damage pathway upregulation
- Mutation

- Chemical level
- Genomic level
- Cellular Response level
- In vitro adversity

Assess Likelihood of high dose carcinogenicity

Mutated cell

Boekelheide and Andersen, ALTEX, in press, December 2010.
## Qualitative Approaches

<table>
<thead>
<tr>
<th>in silico methods</th>
<th>in vitro assays</th>
<th>DNA damage mutagenicity Assays (a-d)</th>
<th>Qualitative Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimating</td>
<td>1</td>
<td>a</td>
<td>low EC50</td>
</tr>
<tr>
<td>likelihood of toxicity</td>
<td>b</td>
<td>-</td>
<td>Compound likely to have mutagenic mode of action and likely to be a carcinogen at high doses – i.e., likely with “high degree of perturbation”</td>
</tr>
<tr>
<td>metabolism, cancer, etc.</td>
<td>c</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>d</td>
<td>-</td>
<td></td>
</tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>n</td>
<td>highest EC50</td>
<td></td>
</tr>
</tbody>
</table>
One proposal for using the in vitro test results in risk assessment – biostatisticians viewpoint

- Collect in vitro results
- Apply new uncertainty factors for severity & mode of Action

The Future Use of *In vitro Data in Risk Assessment to Set Human Exposure Standards – Challenging Problems and Familiar Solutions*

Kenny S. Crump, Chao Chen, Thomas A. Louis

doi: 10.1289/ehp.1001931 (available at http://dx.doi.org/)
Online 18 June 2010
Visualizing this option

Assessing adversity *in vitro*

Uncertainty Factors – both old and new

Panel of pathway assays → Point of Departure (concentration) → Acceptable concentration *in vitro* (ug/l)

In vivo human exposure ‘standard’ mg/kg/day → In vitro-in vivo adjustments
There is, of course, still hope that this will occur, as the basic premise under which BBDR modeling was pursued for risk estimation is conceptually valid. However, it will take a technical breakthrough to meaningfully overcome the problems discussed in this paper.”
There have been the technical breakthroughs to allow change from our 1970’s approaches to a new way of doing business,
The 21\textsuperscript{st} Century; started with the sequencing the human genome
Key New Technologies for the Transition have blossomed over the past decade

- Stem cell biology – cells, aggregates, 3-dimensional-models (initiatives in regenerative medicine)

- Pathway mapping to understand circuitry and and validate assays

- Computational Systems Biology & Dose Response Modeling
Simplicity in biology

Networks of interactions between thousands of molecules within cells seem to defy comprehension, but shared principles of design may simplify the picture.

Filtering out transients
Coherent feed forward loop
SUMMARY POINTS

1. The living cell is an information-processing system.

2. Information is processed by complex networks of interacting genes, proteins, and metabolites.

3. These networks can be decomposed into small interaction motifs that carry out specific information-processing functions.

4. The basic functions are signal transduction, homeostasis, noise suppression, logic gate, adaptation, cock-and-fire, toggle switch, and oscillation.

5. Examples of all these basic motifs, functioning as expected, are found in the macromolecular regulatory networks of living cells.

6. Understanding the functional motifs employed by cells will be crucial to future efforts to predict and intervene in their decision-making capabilities.
Common structure of cellular stress pathways containing sensors, transcriptional factors and transducers

Basic Components of Major Stress Response Pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>TF</th>
<th>Sensor</th>
<th>Major transducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress</td>
<td>Nrf2</td>
<td>Keap1</td>
<td>MAPK, ERK, p38, PKC</td>
</tr>
<tr>
<td>Heat shock response</td>
<td>HSF-1</td>
<td>Hsp90</td>
<td>CaMK2, CK2</td>
</tr>
<tr>
<td>DNA damage</td>
<td>p53</td>
<td>MDM2</td>
<td>ATM, JNK, Chk1, Chk2</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>HIF-1</td>
<td>VHL</td>
<td>p38, PI3K</td>
</tr>
<tr>
<td>ER stress</td>
<td>XBP-1, ATF6, ATF4</td>
<td>BiP</td>
<td>IRE1α, S2P</td>
</tr>
<tr>
<td>Metal stress</td>
<td>MTF-1</td>
<td>None</td>
<td>PKC, CKII, TKs</td>
</tr>
<tr>
<td>Inflammation</td>
<td>NF-κB</td>
<td>IκB</td>
<td>IKK</td>
</tr>
<tr>
<td>Osmotic stress</td>
<td>NFAT5</td>
<td>None</td>
<td>p38, ATM, PKA</td>
</tr>
</tbody>
</table>
A second proposal for using information in risk assessment

- Collect in vitro results to ascertain adversity

- Utilize mechanistic models – computational systems biology models - of pathways to understand dose-dependent transitions and low dose risks

- Apply in vitro-in vivo adjustments using PK models for extrapolations
Using in vitro results and computational tools for risk assessment

(1) Defining adversity

Panel of pathway assays → Assessing adversity in vitro → Point of Departure (concentration) → Mechanistic pathway modeling → Acceptable concentration in vitro (ug/l)

(2) Goal is to define exposures that will be without significant perturbation not to predict apical toxicity

in vivo human exposure ‘standard’ mg/kg/day

(3) Regulatory standard

Pharmacokinetic modeling
** in vitro - in vivo extrapolation **

EC XX**

a. conc 1
b. conc 2
c. conc 3
d. conc 4

\[ \text{Adversity}_{xx} = f(\text{conc}_i) \]

Systems model for DNA repair

\[ \text{Exp}_c = \text{mg/kg/day} \]
What is validation in this process?

Understanding the circuitry of the assay with respect to ‘read-outs’

Showing the behavior of the assay for ‘positive controls’

Developing mechanistic computational models for expected dose response behaviors in the assay
Computational Systems Biology and Dose Response Modeling Workshop

September 22-26, 2008
Division of Computational Biology

With support from the Superfund Basic Research Program at Michigan State University and the Exxon-Mobil Foundation.

2008 Computational Systems Biology and Dose Response Modeling Workshop
Basal Dynamics of p53 Reveal Transcriptionally Attenuated Pulses in Cycling Cells

Alexander Loewer,¹ Eric Batchelor,¹ Giorgio Gaglia,¹ and Galit Lahav¹,*
¹Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: galit@hms.harvard.edu
DOI 10.1016/j.cell.2010.05.031

Coherent feed forward loop
Examine individual cells
Stress Pathway Dynamics

Phase plane trajectories of oscillators and pulse generators

(green = mdm2-gfp ; red = p53-rfp)
www.pnas.org/cgi/doi/10.1073/pnas.1001107107
Map and apply to various chemicals that target DNA to assess dose response & pathway activation on the basis of pathway activation and subsequent alterations in mutational frequency.
The Sho1 branch is an inducible non-basal system, whereas the Sln1 branch shows high basal signaling that is restricted by a MAPK-mediated feedback mechanism.

A Systems-Level Analysis of Perfect Adaptation in Yeast Osmoregulation

Ongoing Discussions about these various topics

- Full citable text of NRC report - Krewski et al. (2010)
- US EPA Strategic Plan for Evaluating the Toxicity of Chemicals
- 14 invited articles on aspects of the toxicity testing in the 21st century
**Point 4: How can such a change be implemented?**

The First Idea --- The NRC Report

**Phase I**
- Elucidate toxicity pathways.
- Establish data-storing and -management systems.
- Establish practices for assay conduct and reporting.
- Plan human-surveillance and biomonitering strategy.

**Phase II**
- Develop suite of representative human cell lines and cultures.
- Develop and validate high- and medium-throughput assays.
- Develop biomarkers for exposure, susceptibility, effect for human surveillance and biomonitering.

**Phase III**
- Gain experience through 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.

**Phase IV**
- Propose then validate suites of assays for use in place of identified apical tests.

Program Time Line – NRC Report – 1 -2 decades; ~$ 1-2 billion
A Second: Incrementally bring tools to bear - *Tox21*

EPA, NTP, NCGC, FDA Collaboration

- Predict results of animal studies
- Prioritize for in vivo testing
- Assist in risk assessment

**High Throughput Screening and Computational Toxicology**

Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.
1. Select a group of well-studied prototype compounds/pathways (~ 3 to 10)

2. Design human/rodent cell-based toxicity pathway assays with read-out over varying levels of perturbation

3. Examine relationships of perturbations with “adversity” for the prototypes
4. Refine the next generation quantitative risk assessment tools, i.e., computational systems biology of pathways and in vitro-in vivo extrapolation.

5. Integrate results into proposed health safety/risk assessments. Show how it will work.
A Fourth: Global Opportunities

- Developing economies not as wedded to risk assessment/toxicity testing approaches tradition as the West.

- Global economies are increasingly concerned about international safety issues in products, foods, etc.

- Toxicity testing needs provide an opportunity for entrepreneurs for implementing a 21st century, modern biology-based infrastructure for toxicity testing.

- The pay-off would be improved decision-making, enhanced consumer confidence, and world-leadership in safety testing.
**Value of Using Prototypes**

Exposure → tissue dose → intermediate responses → Apical Endpoint

- concentration
- In vitro

Evaluation of ‘adverse’ degree of system perturbation from panel of assays and computational systems for d-r modeling

"likely to be a high dose toxicant"
Overarching Issues – Summary

1. Define adversity from appropriately designed panels of assays

2. Don’t predict high dose responses but assess regions of safety

3. Use new biology, mechanistic modeling and PK models for risk assessment

4. Just get moving with prototypes to show the process in operation, think globally
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