NexGen: The Next Generation of Risk Science

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Université d’Ottawa | University of Ottawa
Next generation risk assessment

Advancing the Next Generation of Risk Assessment
Public Dialogue Conference
February 15 & 16, 2011 | Washington, DC
Three Cornerstones

- New paradigm for toxicity testing (TT21C), based on perturbation of toxicity pathways
- Advanced risk assessment methodologies, including those addressed in *Science and Decisions*
- Population health approach: multiple health determinants and multiple interventions
Three Cornerstones

- **New paradigm for toxicity testing (TT21C), based on perturbation of toxicity pathways**

- **Advanced risk assessment methodologies, including those addressed in *Science and Decisions***

- **Population health approach: multiple health determinants and multiple interventions**
Toxicity Testing in the 21st Century

www.nas.edu
# Building the Scientific Toolbox

*(Andersen & Krewski, 2009, Tox. Sci)*

<table>
<thead>
<tr>
<th>Tool</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>High throughput screens</td>
<td>Efficiently identify critical toxicity pathway perturbations across a range of doses and molecular and cellular targets</td>
</tr>
<tr>
<td>Stem cell biology</td>
<td>Develop in vitro toxicity pathway assays using human cells produced from directed stem cell differentiation</td>
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<tr>
<td>Functional genomics</td>
<td>Identify the structure of cellular circuits involved in toxicity pathway responses to assist computational dose response modeling</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>Interpret complex multivariable data from HTS and genomic assays in relation to target identification and effects of sustained perturbations on organs and tissues</td>
</tr>
<tr>
<td>Systems biology</td>
<td>Organize information from multiple cellular response pathways to understand integrated cellular and tissue responses</td>
</tr>
<tr>
<td>Computational systems biology</td>
<td>Describe dose-response relationships based on perturbations of cell circuitry underlying toxicity pathway responses giving rise to thresholds, dose-dependent transitions, and other dose-related biological behaviors</td>
</tr>
<tr>
<td>Physiologically-based pharmacokinetic models</td>
<td>Identify human exposure situations likely to provide tissue concentrations equivalent to in vitro activation of toxicity pathways</td>
</tr>
<tr>
<td>Structure-activity relationships</td>
<td>Predict toxicological responses and metabolic pathways based on the chemical properties of environmental agents and comparison to other active structures</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>Establish biomarkers of biological change representing critical toxicity pathway perturbations</td>
</tr>
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</table>

Krewski et al. (2011), ARPH
Three Cornerstones

1. New paradigm for toxicity testing (TT21C), based on perturbation of toxicity pathways

2. Advanced risk assessment methodologies, including those addressed in Science and Decisions

3. Population health approach: multiple health determinants and multiple interventions
KEY MESSAGES

- Enhanced framework
- Formative focus
- Four steps still core
- Matching analysis to decisions
- Clearer estimates of population risk
- Advancing cumulative assessments
- People and capacity building
Phase I
Formulating and Scoping Problem
For environmental condition:
• What’s the problem?
• What are the options for altering?
• What assessments are needed to evaluate options?

Phase II
Planning and Risk Assessing
Stage 1: Planning for:
• Options Assessment
• Uncertainty and Variability Analysis
Stage 2: Assessing
Stage 3: Confirming Utility of Assessment

Phase III
Risk Management
• Relative benefits of proposed options?
• How are other factors (e.g., costs) affected by options?
• Which option is chosen? What’s the uncertainty and justification?
• How to communicate it?
• Should decision effectiveness be evaluated? If so, how?

Stakeholder involvement at each phase
Methodological Issues

- Adversity
- Variability
- Susceptible populations
- Dose and species extrapolation
- Mixtures and multiple stressors
- Uncertainty analysis
Methodological Issues: (1) Adversity

<table>
<thead>
<tr>
<th>Issue</th>
<th>Current Approach</th>
<th>NexGen Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse outcomes</td>
<td>Apical outcomes in mammalian systems, or precursors to these outcomes, serve as the basis for risk assessment.</td>
<td>In vitro assays identify critical toxicity pathway perturbations, which serve as the basis for risk assessment, even in the absence of a direct link with an apical outcome.</td>
</tr>
</tbody>
</table>
The “Swiss cheese” model of adverse effects

- Chemical Exposure
  - Chemical is electrophilic
  - Irreversible changes
  - Abrupt dose-response transition
  - Mitochondrial dysfunction

- Latent Failures
  - Toxicity Pathways Targeted Testing
  - Dose Response Extrapolation Modeling
  - Apical Event

- Active Failures
  - Adverse Effect

Adapted from Boekelheide and Campion, Toxicol. Sci., 2010.
## Methodological Issues: (2) Dose-response

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<tr>
<th>Issue</th>
<th>Current Approach</th>
<th>NexGen Approach</th>
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<tbody>
<tr>
<td>Dose-response assessment</td>
<td>Empirical or biologically-based models describe apical endpoints, and determine an appropriate point of departure (such as the benchmark dose) for establishing a reference dose.</td>
<td>Computational systems biology pathway models describe dose-response relationships for pathway perturbations, reflecting dose-dependent transitions throughout the dose range of interest.</td>
</tr>
</tbody>
</table>
Signal-to-Noise Crossover Dose (SNCD)

Sand, Portier & Krewski (2011), EHP
# Methodological Issues: (3) Variability

<table>
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<tr>
<th>Issue</th>
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</thead>
<tbody>
<tr>
<td>Inter-individual variability</td>
<td>Adjustment factors used in establishing reference doses account for inter-individual variability in PK and PD.</td>
<td>Variability in biological response is characterized through the use of a diverse range of human cell lines.</td>
</tr>
<tr>
<td></td>
<td>Variability in exposure is also taken into account.</td>
<td>Dosimetry models link variation in human exposure with corresponding in vitro doses.</td>
</tr>
</tbody>
</table>
From Zeise et al. (2012): “Assessing Human Variability in the Next Generation Health Assessments of Environmental Chemicals”
### Methodological Issues: (4) Susceptibility

<table>
<thead>
<tr>
<th>Issue</th>
<th>Current Approach</th>
<th>NexGen Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>Life-stage, genetics, and socioeconomic and lifestyle factors determine susceptible population groups.</td>
<td>Molecular and genetic epidemiology defines susceptible populations in terms of critical pathway perturbations.</td>
</tr>
</tbody>
</table>
How susceptibility arises from variability
(from Zeise et al., 2012)
<table>
<thead>
<tr>
<th>Issue</th>
<th>Current Approach</th>
<th>NexGen Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose and species extrapolation</td>
<td>Dose and species extrapolation translate animal test results to humans.</td>
<td>Cellular assays provide direct measures of toxicity pathway perturbations in humans. IVIVE techniques and pathway modeling calibrate in vitro and in vivo exposures. Sensitive in vitro tests are used to evaluate risk directly at environmental exposure levels.</td>
</tr>
</tbody>
</table>
## Methodological Issues: (6) Mixtures

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<th>Current Approach</th>
<th>NexGen Approach</th>
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</thead>
<tbody>
<tr>
<td>Mixtures and multiple stressors</td>
<td>Common experimental protocols include testing of mixtures and factorial experiments with joint exposures. However, there are only a limited number of such studies because of cost and complexity of experimental design.</td>
<td>Cost-effective high throughput technologies permit expanded testing of mixtures and multiple stressors.</td>
</tr>
</tbody>
</table>
## Methodological Issues: (7) Uncertainty

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<tbody>
<tr>
<td>Uncertainty analysis</td>
<td>Uncertainty considerations include species differences in susceptibility, low-dose and route-to-route extrapolation, and exposure ascertainment.</td>
<td>Probabilistic risk assessments characterize overall uncertainty, and identify the most important sources of uncertainty that guide value-of-information decisions.</td>
</tr>
</tbody>
</table>
Reverse Toxicokinetics (rTK): *in vitro concentration to in vivo dose*

**Pharmacodynamics**
- MOA
- Key Events
- Toxicity Pathway
- HTS Assays
- Biological Pathway Activating Concentration (BPAC)
- Probability Distribution

**Pharmacokinetics**
- Dose-to-Concentration Scaling Function ($C_{ss}/DR$)
- Probability Distribution
- PK Model
- Populations
- Intrinsic Clearance
- Plasma Protein Binding
Three Cornerstones

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Health Risk Science
Determinants and Interactions

Health Risk Policy Analysis
Evidence Based Policy

Population Health

Regulatory
Economic
Advisory
Community
Technological

Multiple Interventions

Biology
and
Genetics

Environment
and
Occupation

Social
and
Behavioural

Biology-environment interactions
Environment-social interactions
Biology-social interactions
Social-Environment Interaction

Social-Genetic Interaction

The Breast 19 (2010) 479–483

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Alcohol consumption and the risk of breast cancer among BRCA1 and BRCA2 mutation carriers

Jessica Dennis\textsuperscript{a,b}, Parviz Ghadirian\textsuperscript{b,c}, Julian Little\textsuperscript{a,b}, Jan Lubinski\textsuperscript{d}, Jacek Gronwald\textsuperscript{d}, Charmaine Kim-Sing\textsuperscript{e}, William Foulkes\textsuperscript{f}, Pal Moller\textsuperscript{g}, Henry T. Lynch\textsuperscript{h}, Susan L. Neuhausen\textsuperscript{i}, Susan Domchek\textsuperscript{j}, Susan Armel\textsuperscript{k}, Claudine Isaacs\textsuperscript{l}, Nadine Tung\textsuperscript{m}, Kevin Sweet\textsuperscript{n}, Peter Ainsworth\textsuperscript{o}, Ping Sun\textsuperscript{p}, Daniel Krewski\textsuperscript{a,b}, Steven Narod\textsuperscript{p,*} the Hereditary Breast Cancer Clinical Study Group\textsuperscript{q}
Risk Management
Risk Management Principles (1/2)

- Beneficence and non-maleficence (*do more good than harm*)
- Natural justice (*a fair process of decision making*)
- Equity (*ensure an equitable distribution of risk*)
- Utility (*seek optimal use of limited risk management resources*)
- Honesty (*be clear on what can and cannot be done to reduce risk*)
Risk Management Principles (2/2)

- Acceptability of risk (*do not impose risks that are unacceptable to society*)
- Precaution (*be cautious in the face of uncertainty*)
- Autonomy (*foster informed risk decision-making for all stakeholders*)
- Flexibility (*continually adapt to new knowledge and understanding*)
- Practicality (*the complete elimination of risk is not possible*)
Case Study
Prototypes
NexGen Tiered Approach to Risk Assessment

Tier 1 Assessments
- Screening and prioritization (thousands of chemicals)
- High throughput and QSAR driven
- Virtual tissue models (under development)
- Surrogates for or modeling of exposure/dose
- Minimize false negatives

Tier 2 Assessments
- More in depth evaluation of many chemicals (hundreds)
- Tier 1 approaches plus:
  - High-content assays
  - Some short-duration in vivo exposures
  - Expanded modeling of exposure/dose
  - Limited traditional data
  - Science-based defaults and upper confidence limits

Tier 3 Assessments
- Extensive evaluation of few chemicals (dozens) – only highest hazard and exposure chemicals
- All feasible, policy-relevant, emerging and traditional data
- Omics supported epidemiology
- Best estimates of risk and uncertainty analyses

LEGEND:
- Decision-making/Policy Input
- Decision-making-Testing - Research Loop

Research
New Test Data
Decision-making

NexGen Tiered Approach to Risk Assessment
www.epa.gov/risk/nexgen/docs/NexGen-Program-Synopsis.pdf
Designing Science in a Crisis: The Deepwater Horizon Oil Spill

PAUL T. ANASTAS
CYNTHIA SONICH-MULLIN
BECKY FRIED
Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Tier 1: Screening and Ranking
Summary plot of results for all Attagene assays and the dispersants. Each horizontal band displays EC50 values for a single dispersant. Points are staggered in the y-direction to make overlapping points visible. Multiple assays for a given gene target (e.g. PPARα, PPARδ, PPARγ) are represented by a single symbol, plotted repeatedly. 95% confidence intervals are shown on assays for the NRF2 as an example. The dispersant-specific vertical red lines indicate the LC50 for cytotoxicity in the Attagene assays (HepG2 cells).
Tier 2: Limited Scope Assessment

Mutation Research 746 (2012) 135–143

Contents lists available at SciVerse ScienceDirect
Mutation Research/Genetic Toxicology and Environmental Mutagenesis
journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: A five chemical case study

Russell S. Thomas a,*, Harvey J. Clewell III a, Bruce C. Allen b, Longlong Yang a, Eric Healy a, Melvin E. Andersen a

a The Hamner Institutes for Health Sciences, 6 Davis Drive, Research Triangle Park, NC 27709, United States
b Bruce Allen Consulting, 101 Corbin Hill Circle, Chapel Hill, NC 27514, United States
Traditional versus Toxicogenomics Determination of BMD

Transcriptional benchmark dose estimates for the most sensitive pathway.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>GeneGo pathway map</th>
<th>Total genes in category/genes with BMD</th>
<th>Median BMD (mg/kg-d or mg/m³)b</th>
<th>Median BMDL (mg/kg-d or mg/m³)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCBZ</td>
<td>Neolacto-series GSL metabolism p.2 (ID: 905)</td>
<td>17/6</td>
<td>90.7</td>
<td>61.2</td>
</tr>
<tr>
<td>PGBE</td>
<td>Beta-alanine metabolism (ID:2313)</td>
<td>10/5</td>
<td>630.0</td>
<td>368.9</td>
</tr>
<tr>
<td>TCPN</td>
<td>Galactose metabolism (ID: 821)</td>
<td>21/6</td>
<td>9.5</td>
<td>4.5</td>
</tr>
<tr>
<td>MECL (liver)</td>
<td>CFTR translational fidelity (class I mutations) (ID: 2939)</td>
<td>77/59</td>
<td>1460.5</td>
<td>945.9</td>
</tr>
<tr>
<td>MECL (lung)</td>
<td>Folic acid metabolism (ID: 879)</td>
<td>14/5</td>
<td>1074.0</td>
<td>659.8</td>
</tr>
<tr>
<td>NPTH</td>
<td>Acetaminophen metabolism (ID: 2377)c</td>
<td>14/9</td>
<td>7.5</td>
<td>5.4</td>
</tr>
</tbody>
</table>

a DCBZ,1,4-dichlorobenzene; PGBE, propylene glycol mono-t-butyl ether; TCPN, 1,2,3-trichloropropane; MECL, methylene chloride; NPTH, naphthalene.  
b Median transcriptional BMD and BMDL values for the associated GeneGo pathway map.  
c GeneGo pathway map for acetaminophen metabolism contains five genes associated with Ug1α isoforms that map to the same probe sets which skews the median value. This is due to the mouse Ug1 locus that produces nine different genes through the alternative splicing of 14 variable exons to four constant exons [62].

Thomas et al. (2012), Mutation Research
Thomas et al. (2012) found a strong correlation between transcriptional BMDs for specific pathways and traditional BMDs.
Tier 3: Major Assessment

Lung Injury and Ozone

- To identify toxicity pathways using ‘omics’ data
- To determine accuracy of predicting in vivo responses from in vitro toxicity pathway induction from toxicants
- To develop a biologically-based dose-response (BBDR) based on the integration of diverse data sets
Response time course of IL8 RNA expression, relative to mean air value. Peak response occurs 3 hours post exposure cessation.
Conclusion

NF-κB signaling is seen early during ozone exposure, and there is a clear dose-response with the cytokine IL-8.
<table>
<thead>
<tr>
<th>Scientific Tools</th>
<th>Tier 1: Ranking and Screening</th>
<th>Tier 2: Limited Scope Assessment</th>
<th>Tier 3: Major Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DWH Oil Spill Dispersants</td>
<td>Type-2 Diabetes</td>
<td>Short-term In Vivo Assay</td>
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<tr>
<td>Structure-activity relationships</td>
<td>✓</td>
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<td>✓</td>
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<tr>
<td>MOA based in vitro toxicity pathway assays</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>High throughput screening</td>
<td>✓</td>
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<tr>
<td>PBPK and dosimetry</td>
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<tr>
<td>Toxicity-related biological pathway altering dose</td>
<td>✓</td>
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<td>Molecular and genetic epidemiology</td>
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