Advancing the Next Generation of Risk Assessment

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Outline

- Background & Partners
- Risk Context & Approaches
- Specific Examples
- Summary
What is NexGen?
- Program to create a cheaper, faster & more robust system for chemical risk assessment by incorporating new knowledge about system biology

Why is NexGen important?
- Agency must conduct credible, science-based assessments
- New data can improve assessments
- Translates research into application

What are the goals of NexGen?
- Create prototypes
- Develop decision rules for use of new information
- Incorporate advances into risk assessment
Approach to Prototype Development
Reverse Engineering & Proof of Concept

Well-Studied Environmental Public Health Risks

Validate Against Human Disease Knowledge

Molecular Systems Biology Data

New Risk Assessment Methods/Models Value of Information Decision Rules

Validate Against Animal Bioassay Knowledge
NexGen Partners' are providing advice, data & review of NCEA implementation efforts

- EPA's Labs and Centers, & program offices
- National Institutes of Environmental Health Sciences & National Toxicology Program
- Centers for Disease Control & Agency for Toxic Substances and Disease Registry
- National Institutes of Occupational Health & Safety
- NIH Center for Translational Therapeutics
- FDA’s National Center for Toxicological Research
- State of California’s Environmental Protection Agency
- Health Canada
- European Joint Research Commission
Risk Context

**Tier 1**
10,000s of chemicals

**Screening & ranking**
- Greener chemicals & processes
- Assessment queue
- Urgent response
- Research priorities

**Limited decision-making**
- Limited exposures
- Possible water contaminants
- National Air Toxic Assessment
- Urgent response

**Major decision-making**
- National exposures
- High profile assessments
- Community assessments
- Special issues

Increasing Need for Confidence in the Decision
Matching Types of Data to Risk Context

<table>
<thead>
<tr>
<th>Tier 1</th>
<th>Tier 2</th>
<th>Tier 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000s of chemicals</td>
<td>1000s of chemicals</td>
<td>100s of chemicals</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High Throughput Only</th>
<th>High/Med Throughput, High Content</th>
<th>Med/Low Throughput</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Mechanisms</td>
<td>Adds In Vivo/Situ Exposure, Tissue/Organism Level Integration</td>
<td>Adds Most Realistic Scenarios</td>
</tr>
</tbody>
</table>

- **Tier 1**
  - High Throughput Only
  - Molecular Mechanisms
  - QSAR
  - Test system - *in vitro*, robotic only
  - Cytotoxicity
  - Validated assay batteries
  - No traditional data

- **Tier 2**
  - High/Med Throughput, High Content
  - Test systems: *in vivo* exposures – mammalian & alternative species
  - Tissue constructs
  - Improved metabolism
  - Different types of assays
  - Some traditional data

- **Tier 3**
  - Med/Low Throughput
  - Test systems: Molecular epidemiology
  - Molecular clinical
  - Molecular animal
  - All w phenotypic data
  - Often environmental exposures
  - All policy relevant data

Increasing Evidence
• Apply explicit **inclusion/exclusion criteria** for studies & data

• Identify causal molecular **patterns** that make one chemical more likely to produce a specific effect than another
  • Specific phenotypic outcomes depend on both tissues & organism level integration
  • Hence, different phenotypic outcomes can result from the same molecular mechanism based on tissue, species & lifestage

• **Pathway & network important** - knowledge of single events, linear MOAs, or list of genes, in general, is not sufficient

• Apply Bradford-Hill criteria to judge **weight of evidence**

• Can defines **new types** of critical effects for dose-response assessment
• Various approaches exist
  ✓ LOEL(s), LOAEL(s) or BMD
  ✓ Slope(s) in experimental range
  ✓ Integration across results
  ✓ Systems biology modeling
  ✓ Network information flow models

• Biologically, no reason to use different approaches for cancer & noncancer
Other Steps

- Estimate equivalent human exposure &/or dose
  - Reverse dosimetry modeling
  - Monitored exposure & dosimetry/ PK modeling
  - Biomarkers of exposure & effects
- Consider species relevance, if applicable
- Characterize variability among humans as feasible
- Consider background of response/adaptation
- Estimate population risks, including variability & uncertainty

The goal is to move to the extent feasible to replace assumptions with data, thus eliminating the need for extrapolation or uncertainty factors.
Outline

- Background & Partners
- Risk Context & Approaches
- Specific Examples
  - Tier 1: Screening & Ranking
  - Tier 2: Limited Scope Assessments
  - Tier 3: 2 Major Assessments
- Summary
Tier 1: Screening & Ranking
Tier 1: Screening & Ranking

- Tremendous progress has been made in assay development & application
- Interrogates chemical impact on important biologic processes involved in disease
- Coverage of biologic processes is, as yet, incomplete

**ToxCast Assays ~600 Total Endpoints**

**Cellular Assays**
- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
  - Rat hepatocytes
  - Mouse embryonic stem cells

**Biochemical Assays**
- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter

- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

**Alternative Species**
- Zebrafish

**Assay formats**
- Cytotoxicity
- Reporter gene
- Gene expression
- Biomarker production
- High-content imaging for cellular phenotype

Courtesy of David Dix & NCCT folks
Screening & Ranking

- Provides a common relative ranking
- Benchmarks potency against known toxicants
- Can adjust rankings using exposure surrogates & population variability
- Bins into high, medium, low or no toxicity or risk.
- Suggests specific hazards based on adverse outcome pathways

Rank All Chemical

- High
- Med
- Low
- No Data

Sort by MOA

Final Product

- Binned Chemicals
- Supports Some Decisions
- Queues up additional assessment or targeted testing
Work Continues to Automate Integration of HT Data Streams

**Figure 1. A snapshot of the data matrix.**

<table>
<thead>
<tr>
<th>Inherent Chemical Properties</th>
<th>Experimental Data</th>
<th>Points of Departure</th>
<th>Toxicity Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD50</td>
<td>TD50</td>
<td>TD50</td>
</tr>
<tr>
<td>Count</td>
<td>CAS</td>
<td>SMILES</td>
<td>[Rat]</td>
</tr>
<tr>
<td>1</td>
<td>Acenaphthene</td>
<td>83-32-9</td>
<td>C10H8 (123)</td>
</tr>
<tr>
<td>2</td>
<td>Acetophenone</td>
<td>90-67-6</td>
<td>C6H5COCH3</td>
</tr>
<tr>
<td>3</td>
<td>Benzo(a)pyrene</td>
<td>50-32-8</td>
<td>C20H12 (123)</td>
</tr>
<tr>
<td>7</td>
<td>Dichlorobenzene, 1,2-</td>
<td>80-03-2</td>
<td>C6H5Cl2</td>
</tr>
</tbody>
</table>

**A.** Inherent chemical properties (i.e., descriptors)

**B.** Experimental data (e.g., LD₅₀, TD₅₀, ...)

**C.** Combined matrix of inherent chemical properties and experimental data

- **QSAR model**
- **Biological (e.g., experimental data-based) model**
- “Hybrid” model

_**Courtesy of Ivan Rusyn & Kate Guyton, Rusyn 2012**_
Tier 2: Limited Scope Assessments
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- Aim is to generate a reference value as opposed to binning
- More data rich, highest confidence ranking & screening chemicals advance to limited scope assessments
- The hallmark of these assessments:
  - more intact metabolism
  - a higher level of biologic integration
  - additional endpoints e.g. neurobehavioral
  - often reflects omics of various cell types
Limited Scope Assessments
Short Duration In Vivo Exposures - *Rodent*

Correlation Between Cancer & Transcriptional Endpoints

BMDs averaged across genes to develop the lowest BMD for the pathway (Thomas et al. 2011)
Limited Scope Assessments
Short Duration In Vivo Exposures – Alternative Species

- Alternative species data can be used to determine hazard & dose response.
- Species differences need to be characterized.
- Understanding dose equivalents in various test systems is a challenge.

Zebrafish eleuthroembryos in comparison to mammals

![Image of zebrafish and mammalian embryos]

Short-term exposure to direct TGFD induced a strong decrease in IT4C thyroid follicles of zebrafish eleuthroembryos.

Courtesy of Ed Perkins
Tier 3: Major Assessments

Aim of the major decision-making prototypes is three fold:
1. Robust proofs of concept
2. Informing difficult issues not well resolved by traditional data
3. Extending what is learned to chemicals with less data
The major decision-making prototypes (ozone, benzene & PAHs) involve:

- Humans & well understood, environmental exposures
- Known causal associations among exposure, traditional upstream events, & phenotypic outcomes
- Omics data from primary cells of phenotypically affected tissues
Ozone & Lung Inflammation/Injury

Benzene & Hematotoxicity/Leukemia

PAHs & Lung Cancer

Top Functions:
- Inflammatory Response
- Cell-to-Cell Signaling and Interactions
- Cell Stress

Courtesy of Kelly Duncan, David Miller & Bob Devlin

Courtesy of Ruben Thomas, Martyn Smith et al.

Courtesy of Lyle Burgoon & Ken Ramos
Tier 3: Major Assessments

• Proof of Concept
  • Specific alterations in gene expression profiles can be consistent, coherent & biologically plausible indicators of both traditional upstream & phenotypic events.
  • Induced alterations in gene transcription profiles appear both dose & time dependent.
  • Can be demonstrated experimentally

• Difficult issues
  • Relevance of animal data can be better addressed
  • Human susceptibility better described

• Argues that data limited chemicals with the same signatures maybe of concern for the same effects
Hazard Id & DR are about both about pattern recognition.

Patterns at the pathway & network level can be used to:

- Identify the likely hazards & estimate potencies
- Better characterize human variability, relevance of nonhuman models & mixtures interactions.

The number of chemical with sufficient data are still limited.

Weight of evidence varies depending on the type of data & test system.

Significant progress is being made modernizing risk assessment, but more needs to be done.
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Basic Steps in Assessment

1. Apply selection criteria for studies & data
2. Identify critical effects & evaluate overall causal weight of evidence
3. Apply optimal approach for dose-response evaluation
4. Estimate equivalent human exposure &/or dose
5. Consider species relevance, if applicable
6. Characterize variability among humans to the extent possible
7. Consider background of response/adaptation
8. Estimate population risks, including variability & uncertainty
Dose-Response

Proposed Criteria & Principles

1. Key drivers within pathways must be identified & dose-response modeling performed on these key drivers whenever possible.

2. These key drivers are the criteria genes, proteins, & metabolites that are associated with the key pathway.

3. The criteria genes, proteins, metabolites, or pathways must demonstrate a statistically significant difference compared to control.

4. The point of departure & ED$_{50}$ for criteria genes, proteins, metabolites, or pathways must not be greater than that for the key end-point.

5. The criteria pathway must be consistent across multiple studies (when multiple studies are present). Specific genes, proteins, or metabolites do not need to be consistent across multiple studies.

6. The criteria pathway must be involved in the key end-point, & must be part of the MOA.