Genomic Dose Response: The BIG Picture

NTP Genomic Dose Response Modeling Expert Panel Meeting
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The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA
Scott May Want To Rethink Asking Me To Be The “Big Picture Guy”…

I’ve decided to become more of a big picture guy.

Lesser minds can do the managing and implementing while I criticize them for not “getting it.”

So... you want to get paid to be a jerk? Said the implementer.
It is a Well Known Fact that Toxicology Continues to Have a Data Problem

<table>
<thead>
<tr>
<th>Category</th>
<th>Size of Category</th>
<th>Estimate Mean Percent In the Select Universe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pesticides and Inert Ingredients of Pesticides Formulations</strong></td>
<td>3,350</td>
<td><img src="chart1" alt="Symbol" /></td>
</tr>
<tr>
<td><strong>Cosmetic Ingredients</strong></td>
<td>3,410</td>
<td><img src="chart2" alt="Symbol" /></td>
</tr>
<tr>
<td><strong>Drugs and Excipients Used in Drug Formulations</strong></td>
<td>1,815</td>
<td><img src="chart3" alt="Symbol" /></td>
</tr>
<tr>
<td><strong>Food Additives</strong></td>
<td>8,627</td>
<td><img src="chart4" alt="Symbol" /></td>
</tr>
<tr>
<td><strong>Chemicals in Commerce: At Least 1 Million Pounds/Year</strong></td>
<td>12,860</td>
<td><img src="chart5" alt="Symbol" /></td>
</tr>
<tr>
<td><strong>Chemicals in Commerce: Less than 1 Million Pounds/Year</strong></td>
<td>13,911</td>
<td><img src="chart6" alt="Symbol" /></td>
</tr>
<tr>
<td><strong>Chemicals in Commerce: Production Unknown or Inaccessible</strong></td>
<td>21,752</td>
<td><img src="chart7" alt="Symbol" /></td>
</tr>
</tbody>
</table>

- Major challenge is too many chemicals and not enough data
- Total # chemicals = 65,725
- Chemicals with no toxicity data of any kind = ~46,000

US National Research Council, 1984

- Complete Health Hazard Assessment Possible
- Partial Health Hazard Assessment Possible
- Minimal Toxicity Information Available
- Some Toxicity Information Available (But Below Minimal)
- No Toxicity Information Available
It is a Well Known Fact that Toxicology Continues to Have a Data Problem

Modified from Judson et al., EHP 2009
…data compiled from 150 compounds with 221 human toxicity events reported. The results showed the true positive human toxicity concordance rate of 71% for rodent and nonrodent species, with nonrodents alone being predictive for 63% of human toxicity and rodents alone for 43%.
Budding Field of Toxicogenomics Promised to Change All That...
Initial Focus of Toxicogenomics Was on Inferring MOA

- Initial applications of toxicogenomics to identify MOA have generally lacked a systematic approach
- More art than science
- Development of large reference databases difficult due to cost constraints
- Most expert committees/reports defaulted to using it as part of an overall weight-of-evidence
- Not very satisfying
Focus Shifted Towards Supervised Classification Approaches

Most studies show 60-85% accuracy for predicting cancer-related endpoints.

Only a limited number of tissues have been evaluated.

Requires >20 compounds with adequate redundancy and diversity in mechanisms to have a robust training set (Thomas et al., 2009).

>30 organs show tumor responses in NTP database with ~50% having >10 chemicals in at least one species/sex.

Difficult to justify as a comprehensive screen for rodent carcinogenicity.
**In Vivo Study to Assess Transcriptional and Apical Correlation**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Route</th>
<th>Doses(^c)</th>
<th>Rodent Model</th>
<th>Time Point</th>
<th>Target Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,4-Tribromobenzene(^a)</td>
<td>Gavage</td>
<td>2.5, 5, 10, 25, 75 mg/kg</td>
<td>Male Sprague Dawley rats</td>
<td>5 d, 2, 4, 13 wks</td>
<td>Liver</td>
</tr>
<tr>
<td>Bromobenzene(^a)</td>
<td>Gavage</td>
<td>25, (50), 100, 200, 300, 400 mg/kg</td>
<td>Male F344 rats</td>
<td>5 d, 2, 4, 13 wks</td>
<td>Liver</td>
</tr>
<tr>
<td>2,3,4,6-Tetrachlorophenol(^a)</td>
<td>Gavage</td>
<td>10, 25, 50, 100, 200 mg/kg</td>
<td>Male Sprague Dawley rats</td>
<td>5 d, 2, 4, 13 wks</td>
<td>Liver</td>
</tr>
<tr>
<td>4,4'-Methylenebis (N,N-dimethyl) benzenamine(^b)</td>
<td>Feed</td>
<td>50, 200, 375, 500, 750 ppm</td>
<td>Male F344 rats</td>
<td>5 d, 2, 4, 13 wks</td>
<td>Thyroid(^b)</td>
</tr>
<tr>
<td>N-Nitrosodiphenylamine(^b)</td>
<td>Feed</td>
<td>250, 1000, 2000, 3000, 4000 ppm</td>
<td>Female F344 rats</td>
<td>5 d, 2, 4, 13 wks</td>
<td>Bladder(^b)</td>
</tr>
<tr>
<td>Hydrazobenzene(^b)</td>
<td>Feed</td>
<td>5, 20, 80, 200, 300 ppm</td>
<td>Male F344 rats</td>
<td>5 d, 2, 4, 13 wks</td>
<td>Liver</td>
</tr>
<tr>
<td>1,4-Dichlorobenzene(^b)</td>
<td>Gavage</td>
<td>100, 300, 400, 500, 600 mg/kg</td>
<td>Female B6C3F1 mice</td>
<td>13 wks</td>
<td>Liver</td>
</tr>
<tr>
<td>1,4-Dichlorobenzene(^b)</td>
<td>Gavage</td>
<td>2, 6, 20, 40, 60 mg/kg</td>
<td>Female B6C3F1 mice</td>
<td>13 wks</td>
<td>Liver</td>
</tr>
<tr>
<td>1,2,3-Trichloropropane(^b)</td>
<td>Gavage</td>
<td>2, 6, 20, 40, 60 mg/kg</td>
<td>Female B6C3F1 mice</td>
<td>13 wks</td>
<td>Liver</td>
</tr>
<tr>
<td>Methylene Chloride(^b)</td>
<td>Inhalation</td>
<td>100, 500, 2000, 3000, 4000 ppm</td>
<td>Female B6C3F1 mice</td>
<td>13 wks</td>
<td>Liver, Lung</td>
</tr>
<tr>
<td>Naphthalene(^b)</td>
<td>Inhalation</td>
<td>0.5, 3, 10, 20, 30 ppm</td>
<td>Female B6C3F1 mice</td>
<td>13 wks</td>
<td>Lung</td>
</tr>
<tr>
<td>1,2,4-Tribromobenzene(^a)</td>
<td>Gavage</td>
<td>2.5, 5, 10, 25, 75 mg/kg</td>
<td>Female B6C3F1 mice</td>
<td>13 wks</td>
<td>Liver</td>
</tr>
</tbody>
</table>

\(^a\)Chemicals in IRIS database for non-cancer endpoints only

\(^b\)Chemicals previously tested by the U.S. National Toxicology Program

\(^c\)Underlined doses used in NTP two-year rodent bioassay or IRIS database

Measured apical (histological and organ weight; n = 10) and gene expression changes (n = 5) at each dose and time point in the target tissue.
Temporal Changes Between Transcriptional and Non-Cancer PODs

Thomas et al., Toxicol Sci, 2013
Combined Correlation Between Non-Cancer and Transcriptional PODs

Median Log$_2$ Ratio Apical:Transcriptional BMD = 0.068 (1.05)

$r = 0.827 \ (p = 0.0031)$

Thomas et al., *Toxicol Sci*, 2013
Temporal Changes Between Transcriptional and Cancer PODs

Thomas et al., Toxicol Sci, 2013
Combined Correlation Between Cancer and Transcriptional PODs

Median Log$_2$ Ratio Apical: Transcriptional BMD = 0.524 (1.44)

$r = 0.940 (p = 0.0002)$

Thomas et al., Toxicol Sci, 2013
Why Could this Be True?

• Most histological changes do not occur without upstream or downstream changes in the transcriptome

• Most environmental chemicals are highly non-selective in their interactions with biological systems

The Next Frontier for Toxicogenomics

ToxCast
~600 assays

Tox21
~50 assays

~1,000 chemicals

~10,000 chemicals

Response

Concentration

Gene Coverage

Pathway Coverage*

*At least one gene from pathway represented
Developing a Portfolio of High-Throughput Toxicogenomic Tools

High-Throughput Transcriptomic Screen
- Low cost, 384-well, cell lysate compatible
- Whole transcriptome (EPA), S1500+ (NTP)
- Workflow integration of reference materials and controls, development of performance standards
- Portable platform/workflow for collaborative data generation

Mode of Action/MIE Analysis
- Refined CMAP tool and machine learning approaches
- Curating reference chemical database for MIE and directional response
- >60 MIEs and growing

Concentration Response Analysis
- BMDExpress 2.0
- Tcpl

Karmaus, Unpublished
Expanding the Tiered 21st Century Toxicity Testing Framework

Incorporating New Tools and Risk Assessment: Modeling

Russell S. Thomas, MD, J. Craig Rowlands, PhD, Maurice Jason C. Lambert, MD, Harvey Edward W. Cann, and Marc Reiter

ERα Antagonist

EC50 = 1.5 – 2.5 μM

BMD ~ 1-2 μM

Refined Testing
- Refined Pharmacokinetic Estimates
- Refined Second Order Human Exposure Characterization

Tier 2 Testing

Protective
- In Vitro Assays for Biodiversity
- Define First Order Margin of Exposure

Predictive
- Weigh Non-Steroid Interacting Chemicals
- Define Tentative Mode-of-Action

Human In Vitro Pharmacokinetic Assays and IVIVE Modeling
- Conservative First Order Human Exposure Characterization

Antagonist

Agonist
International Case Study Evaluating Bioactivity as a Conservative POD

For ~87% of the chemicals, POD_{ToxCast} was conservative.

Missing an important component of biology?
The Time is Now for Potential Regulatory Applications

“1) PRIORITIZATION FOR RISK EVALUATIONS.—

“(A) ESTABLISHMENT OF PROCESS.—Not later than 1 year after the date of enactment of the Frank R. Launenberg Chemical Safety for the 21st Century Act, the Administrator shall establish, by rule, a risk-based screening process, including criteria for designating chemical substances as high-priority substances for risk evaluations or low-priority substances for which risk evaluations are not warranted at the time. The process to designate the priority of chemical substances shall include a consideration of the hazard and exposure potential of a chemical substance or a category of chemical substances (including consideration of persistence and bioaccumulation, potentially exposed or susceptible subpopulations and storage near significant sources of drinking water), the conditions of use or significant changes in the conditions of use of the chemical substance, and the volume or significant changes in the volume of the chemical substance manufactured or processed.
But, A Necessary Piece Is Convergence and Acceptance of Analysis Approaches
Acknowledgements and Questions

Tox21 Colleagues:
- NTP
- FDA
- NCATS

EPA Colleagues:
- NERL
- NHEERL
- NCEA

Collaborative Partners:
- Unilever
- A*STAR
- ECHA
- EFSA
- Health Canada