Applications of Computational Toxicology to the Understanding of Risks of Developmental Toxicity

Robert Kavlock and Tom Knudsen
National Center for Computational Toxicology, US EPA
• Assessment of key exposures (life stages) and toxicity outcomes (neurotoxicity)
• State-of-the-science testing and assessment procedures (genomics, bioinformatics, pharmacokinetics)
• Efficient experimental design and reduced use of laboratory animals
• New and alternative test methods
• Computational and molecular techniques in risk assessment

www.nas.edu
A New Paradigm: Activation of Toxicity Pathways

- Exposure
  - Tissue Dose
  - Biologic Interaction
  - Perturbation

Biologic Inputs

- Early Cellular Changes
- Adaptive Stress Responses
- Normal Biologic Function
- Cell Injury

Higher yet

Morbidity and Mortality

THE NATIONAL ACADEMIES
Advisors to the Nation on Science, Engineering, and Medicine
Design Criteria: Objectives of Toxicity Testing

- Broadest coverage of chemicals, end points, life stages
- Lowest cost; least time
- Detailed mechanistic and dose information for human health risk assessment
- Fewest animals; least suffering per animal
### Options for Future Toxicity Testing Strategies

<table>
<thead>
<tr>
<th>Option I</th>
<th>Option II</th>
<th>Option III</th>
<th>Option IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Vivo</td>
<td>Tiered In Vivo</td>
<td>In Vitro/In Vivo</td>
<td>In vitro</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>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>

- **Option I (In Vivo)**: Primarily uses animals, high doses, low throughput, expensive, time consuming, relative large number of animals, and apical endpoints.
- **Option II (Tiered In Vivo)**: Uses animal biology, high doses, improved throughput, less expensive, less time consuming, fewer animals, and apical endpoints.
- **Option III (In Vitro/In Vivo)**: Primarily human biology, broad range of doses, high and medium throughput, less expensive, less time consuming, substantially fewer animals, and perturbations of toxicity pathways.
- **Option IV (In vitro)**: Primarily human biology, broad range of doses, high throughput, less expensive, less time consuming, virtually no animals, and perturbations of toxicity pathways.
Two Paradigms for Understanding the Effects of Toxic Chemicals

Human Disease Model

Comparisons of genetic profiles of people with and without disease yields fingerprints of disease and susceptibility. Match fingerprints of known disease states with reported chemically-induced effects.

Animal Testing Model

Comparisons of chemical effects with between unknown and known toxicities yields subset of potentially important effects. Develop MOA models chemically induced diseases.

Early Outputs

Library of chemically induced events matched to fingerprints of human disease and susceptibility.

Library of chemically induced events matched to rodent tests results and rodent MOAs.
EPA’s Need for Prioritization

Too Many Chemicals

Too Little Data (%)

EPA Reacts to Challenge of the NRC on the Future of Toxicity Testing

Strategic Goals
- Toxicity Pathway ID and Screening
- Toxicity Based Risk Assessment
- Institutional Transition

Figure 6. Relative (%) Emphasis of the Three Main Components of this Strategic Plan over its Expected 20-year Duration.

http://www.epa.gov/osa/spc/toxicitytesting/index.htm
Future of Toxicity Testing

*in vitro* testing  *in silico* analysis

HTS - *omics*  Bioinformatics/ Machine Learning

- Cancer
- ReproTox
- DevTox
- NeuroTox
- PulmonaryTox
- ImmunoTox
• Post Ames test era push
  – “Smith List” of teratogens
  – Chernoff/Kavlock assay
  – In vitro assays
    • HEPM (Pratt)
    • MOT (Braun)
    • µMass (Flint)
    • CRA (Daston)
    • Hydra (Johnson)
    • Drosophila (Bournias-Vardiabasis)
    • Neuroblastoma (Mummery)
    • FETAX (Sabourin)
    • WEC (New)
So What’s Different This Time?

High Throughput Screening

Information Technology and Management

• Molecular, Cellular and Systems Biology

+ Government Attention and Funding
High Throughput Screening
HTS in Drug Development

1. Identify target, pathway, or cellular phenotype
2. Create testing system (aka, “assay”)
3. HTS tests >100,000 chemicals with no known activity for effect on target
4. Make modifications to active chemicals to make suitable for in vivo testing
5. Test in animals for safety, effectiveness
6. Test in humans for safety, effectiveness
HTS in Toxicology

Test prioritized chemicals in animals

Obtain or create testing systems (assays)

HTS tests chemicals for effect on assays

Obtain or create testing systems (assays)

Identify toxicity pathways, cellular phenotypes

Categorize as inactive subject to further testing

Chemicals with known or suspected toxicity in humans

Computational analysis and synthesis of HTS results
Tox21 Community Development

1.4k Library 2004
2.8k Library 2006
10k Library 2008
5M data points to date 2010
### Tox21 Community

<table>
<thead>
<tr>
<th>Activities</th>
<th>NTP</th>
<th>NCGC</th>
<th>EPA</th>
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<tbody>
<tr>
<td>Historical Toxicology Data</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Experimental Toxicology</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Ultra High-Throughput Testing</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Mid- to High Throughput Systems</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Lower Organism Model System</td>
<td>✓ C. elegans</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>In Vitro 3-D Model Systems</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Effect of Human/Rodent Genetic Background on Toxic Effects</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Computational Toxicology</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Validation Experience</td>
<td>✓ (NICEATM-ICCVAM)</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Tox21 assays screened at NCGC to date

- Cell viability
  - ATP (Cell titer glo)
  - LDH
  - Protease release
- Caspases
  - 3/7
  - 8
  - 9
- Pathways
  - AP1
  - ARE
  - CRE
  - HRE
  - NFκB
- DNA damage
  - p53
  - Multiple repair gene-deficient cell lines
- Nuclear receptors
  - AR
  - ERα
  - FXR
  - GR
  - LXRβ
  - PPARα
  - PPARγ
  - PPARδ
  - PXR
  - RXR
  - TRβ
  - VDR
- Inter-individual variation in chemical response
  - 20 sets of identical twins
Molecular Tools for Endocrine Profiling
Information Technology and Management
Text Mining Workflow

Keyword List → Perl Script
Pubmed Search → Abstract
Full Text (if available) → IS RELEVANT (Manual Process) (TP FP)

Library: GENES, DEFECTS, PATHWAYS, GO ONTOLOGY, CHEMICALS, Etc….

Perl Script
Break Abstracts into Sentences

Extract and Tag with library Words for each sentences*

Flag the record → Summarization Co-occurrences Statistics

Knowledge Base Entry Tool → KB Web Tool

Will be displayed
Manual Process
Will be stored in database

* All the sentences with genes - +/- 2 words will be captured

Source: Amar Singh, Poster #9
Building a gene regulatory network

Computing relevance scores for association of gene-malformation in the eye based on co-occurrence of DevTox terms in the abstracts

Profiling developmental toxicity

**in vivo endpoints** (target, description)

[www.epa.gov/ncct/toxrefdb](http://www.epa.gov/ncct/toxrefdb)

**ToxRefDB** 387 chemicals, 751 prenatal studies, 988 effects annotated (enhanced DevTox.org)

283 chemicals x 293 effects → 19 target systems from rat (■) and rabbit (■) studies

**SOURCE:** Knudsen et al. (2009) Reproductive Toxicology (in press) DOI 10.1016/j.reprotox.2009.03.016

Also see Abstract #16
Pathway Analysis – PPARα Assays in ToxCast
ToxCast™ activators
Src-Fyn-Lyn-Btk-Abl-InsR

Developmental predictors
appendicular skeleton
orofacial defects
urogenital defects
brain-eye (Lyn)

Chemicals
ethylenethiourea
mancozeb
maneb
metam-sodium hydrate
metiram-zinc

SOURCE: human kinome map www.cellsignal.com
Pathway Targets for Chlorpyrifos
Molecular, Cellular and Systems Biology
Predicting Human Toxicity: The Grand Challenge in Toxicology

- Biochemical HTS
- Cell-Based HTS
- Model Organism MTS
- Cellular Systems
- Cellular Networks
- Toxicity

Virtual Tissues

Complex Cellular and HCS HTS

Tissues

Molecular Targets

Cell-Based HTS

ToxRefDB

Virtual Tissues
Core developmental processes
- patterning (sets up future events)
- timing (clocks and oscillators)
- differentiation (cell diversification)
- morphogenesis (tissue organization)

Cellular primitives
- growth (proliferation)
- death (apoptosis)
- differentiation (function)
- adhesion (DAH)
- shape (geometry)
- motility (cell migration)
- ECM (remodeling)

Morphogenetic movement
- folding
- epiboly
- convergent extension
- branching morphogenesis
- cell condensation
- cell sorting
- trans-differentiation
- cavitation
- involution
- tractional forces

Directed cell movement
- contact guidance (boundaries)
- haptotaxis (ECM tracks)
- chemotaxis (chemical signals)

A Building Block of a Virtual Embryo

Zebrafish embryo
SOURCE: CB Chien lab (2009)
http://chien.neuro.utah.edu/

FGF8 – BMP4 signaling network, modeled with VT-KB algorithms
SOURCE: A Singh (2009), NCCT
CC3D: Cells as autonomous agents
Example: reciprocal signaling - lens induction
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