Coming to Terms with the State of the Science in Environmental Toxicology and Defining a Path for the Future

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The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA
On Milestone Birthdays, It is a Tradition to Examine Where We Are and Chart a Path for the Future...
Large Numbers of Chemicals in Commerce

- Canadian DSL
- EU REACH Registered
- EU REACH Pre-Registered
- US EPA TSCA Non-Confidential
- US EPA TSCA Active

Substances

(can be expanded for exact numbers)
Lack of Toxicity Data

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

Modified from Judson et al., EHP 2009
Costs of Traditional Toxicity Testing

$1,000
$10,000
$100,000
$1,000,000
$10,000,000

Cost

Acute Tox  In Vivo Skin Sens  In Vivo Genotox  Dev Tox  Sub-chronic Tox  Reproductive Tox  Dev Neurotox  EDSP Tier 1  Cancer
That Is Not a Great Way to Start a Birthday...
Charting a New Path for Toxicology

• Incorporate technological advances to evaluate large numbers of chemicals across toxicological space

• Systematically address limitations of *in vitro* test systems

• Put results in a dose and exposure context

• Characterize variability and relevance of current toxicological test systems

• Delivery of data and models through decision support tools

• Building confidence through regulatory focused case studies
Toxicology is Analogous to Trying to Create a ‘Picture of Everything’

• In 1997 an artist named Howard Hallis started drawing a 'Picture of Everything', it took 13 years to complete, stands at 15 x 14 feet.

• The ideal toxicity testing approach provides comprehensive coverage of relevant toxicological responses

• It should identify the mechanism/mode-of-action (with dose-dependence)

• It should identify responses relevant to the species of interest and include consideration of metabolism (detoxification/bioactivation)

• Responses should ideally be translated into tissue-, organ-, and organism-level effects

• It must be economical and scalable
Application of High-Throughput Assays to Test Thousands of Chemicals

- 96, 384, and 1536-well, laboratory automation compatible
- Relatively expensive (~$20,000 - $30,000 / chemical)
- Coverage of molecular and phenotypic responses
- Multiple assay vendors/labs

ToxCast Assays
- Transcription Factors
- Transporter
- Cytokines
- Kinases
- Nuclear Receptors
- CYP450 / ADME
- Cholinesterase
- Phosphatases
- Proteases
- XME metabolism
- GPCRs
- Ion channels

- ~700 Assay Endpoints

Mode-of-Action Identification
Pathway Coverage
Concentration Response Modeling
Gene Coverage
Incorporating High-Content Technologies to Increase Biological Coverage

- Thousands of Chemicals
- Multiple Cell Types

Concentration Response Screening

Whole Genome Transcriptomics

Mode-of-Action Identification

Multi-Parameter Cellular Phenotypic Profiling

- 384-well, laboratory automation compatible
- Relatively inexpensive ($2.50 - $1,500 per chemical)
- Broad complementary coverage of molecular and phenotypic responses
- Integration of reference materials and controls for performance standards
- Increased portability
High-Throughput Phenotypic Profiling as a Measure of ‘Cellular Pathology’

~1,300 total phenotypic endpoints

<table>
<thead>
<tr>
<th>Cell Compartments</th>
<th>NUCLEUS</th>
<th>RING</th>
<th>CYTOPLASM</th>
<th>MEMBRANE</th>
<th>CELL</th>
</tr>
</thead>
</table>

Shape (M)
Threshold Compactness (C)
Radial distribution (R)
Symmetry (S)
Intensity (I)
Texture (T)
Axial (A)
Profile (P)
Unique Phenotypic Responses Associated with Different MOAs

J. Nyffeler, J. Harrill, Unpublished
Variation in Phenotypic Potencies Across Cell Type and Time

Cell Type Differences (48 hr)

Time Point Differences (U2OS cells)

*Data points represent 5th percentile of phenotypic BMDs

Tested range

J. Nyffeler, J. Harrill, Unpublished
Comparing ‘Cellular Pathology’ with *In Vivo* Effects

### Chemicals Where Cellular Effects are Not Protective

<table>
<thead>
<tr>
<th>Chem.Name</th>
<th>MoA / Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylstilbestrol</td>
<td>estrogenic</td>
</tr>
<tr>
<td>Profenofos</td>
<td>inhibition of cholinesterase</td>
</tr>
<tr>
<td>Mevinphos</td>
<td>inhibition of cholinesterase</td>
</tr>
<tr>
<td>Azamethiphos</td>
<td>inhibition of cholinesterase</td>
</tr>
<tr>
<td>Ethoprop</td>
<td>inhibition of cholinesterase</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>inhibition of acetylcholinesterase</td>
</tr>
<tr>
<td>Thiodicarb</td>
<td>potentially inhibition of acetylcholine</td>
</tr>
<tr>
<td>6-Propyl-2-thiouracil</td>
<td>potentially inhibition of acetylcholine</td>
</tr>
<tr>
<td>Parathion</td>
<td>inhibition of acetylcholinesterase</td>
</tr>
<tr>
<td>Fenamiphos</td>
<td>CNS effects</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>inhibition of acetylcholinesterase</td>
</tr>
<tr>
<td>Fosthiazate</td>
<td>inhibition of acetylcholinesterase</td>
</tr>
<tr>
<td>Trichlorfon</td>
<td>inhibition of acetylcholinesterase</td>
</tr>
<tr>
<td>Formetanate hydrochloride</td>
<td>inhibition of acetylcholinesterase</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>inhibition of acetylcholinesterase</td>
</tr>
<tr>
<td>Pymetrozine</td>
<td></td>
</tr>
<tr>
<td>Bromoxynil</td>
<td></td>
</tr>
<tr>
<td>2,6-Dimethylphenol</td>
<td></td>
</tr>
<tr>
<td>Malaoxon</td>
<td>potentially inhibition of acetylcholine</td>
</tr>
<tr>
<td>Methamidophos</td>
<td>potentially inhibition of acetylcholine</td>
</tr>
<tr>
<td>Clodinafop-propargyl</td>
<td>potentially inhibition of acetylcholine</td>
</tr>
</tbody>
</table>

*Results from a single cell type (U2OS), ~50% are within 10-fold.*

Using a single cell type (U2OS), ~50% are within 10-fold.
Incorporating Xenobiotic Metabolism in *In Vitro* Test Systems

**“Extracellular” Approach**
- Chemical metabolism in the media or buffer of cell-based and cell-free assays
- More closely models effects of hepatic metabolism and generation of circulating metabolites

**“Intracellular” Approach**
- Chemical metabolism inside the cell in cell-based assays
- More closely models effects of target tissue metabolism

Integrated strategy to model *in vivo* metabolic bioactivation and detoxification
Application of Extracellular Strategy to Identify Estrogenic Metabolites

AIME Method: S9 Fraction Immobilization in Alginate Microspheres on 96- or 384-well peg lids

Screening Window of VM7 (formerly BG1) ER Transactivation Assay

<table>
<thead>
<tr>
<th>Metabolism</th>
<th>Neg</th>
<th>Pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRS</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>Pos</td>
<td>0.91</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Pilot Screening Results of Pinto et al., 2016

Library Example

Detoxification

Bioactivation

Example

Ethylparaben

trans-Stilbene

D. DeGroot, C. Deisenroth, Unpublished
Application of Intracellular Strategy to Identify Cytotoxic Metabolites

CYP3A4 Metabolism of TST

CYP2D6 Metabolism of DEX

Aflatoxin B1

Amodiaquin dihydrochloride dihydrate

Simmons et al., 2018
Developing Organotypic Culture Models to Identify Tissue/Organ Effects

Normal Human Thyroid Gland

2D Cell Expansion

Donor Cryopreservation Bank

QC Validation

2D Monolayer Culture

3D Sandwich Culture

Harvest Follicle Fragments
Attachment and Outgrowth of Cells

C. Deisenroth, Unpublished
3D Thyroid Model Shows More Relevant Structure and Gene Expression

Blue, Hoechst 33342 /DNA
Green, Phalloidin/Actin

C. Deisenroth, Unpublished
Inhibition of Thyroid Hormone Synthesis by Reference Chemicals

T4 Hormone: Control

T4 Hormone: Methimazole

T4 Hormone: 6-Propyl-2-thiouracil

T4 Hormone: Sodium Perchlorate

T4 Hormone: VA-K-14

T4 Hormone: Benzophenone 3

C. Deisenroth, Unpublished
Putting Alternative Test Results in a Dose and Exposure Context

**Liver Metabolism**

**Tissue Partitioning**

**Plasma Protein Binding**

**Population-Based IVIVE Model**

Oral Dose Required to Achieve Concentrations Equivalent to *In Vitro* Bioactivity

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**R package “httk”**

- Open source, transparent, and peer-reviewed tools and data for **high throughput toxicokinetics (httk)**
- Allows *in vitro-in vivo* extrapolation (IVIVE) and physiologically-based toxicokinetics (PBTK)
- v1.10 features **942 total chemicals**
- Now allows propagation of uncertainty

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Rotroff et al., *Tox Sci.*, 2010
Wetmore et al., *Tox Sci.*, 2012
Wetmore et al., *Tox Sci.*, 2015
Incorporating Measurements and Predictions of Bioavailability

Assume 100% Bioavailability

Using CaCo2 Bioavailability

Using New QSAR Model

<table>
<thead>
<tr>
<th>Value</th>
<th>Route</th>
<th>Stat.</th>
<th>$F_{DIO} = 1$</th>
<th>Meas. $F_{bio}$</th>
<th>Meas. $P_{AB}$</th>
<th>QSAR $P_{AB}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>All</td>
<td>RMSE</td>
<td>0.96</td>
<td>0.98</td>
<td>0.99</td>
<td>1.05</td>
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<tr>
<td></td>
<td></td>
<td>COR</td>
<td>0.75</td>
<td>0.86</td>
<td>0.86</td>
<td>0.79</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>All</td>
<td>RMSE</td>
<td>1.14</td>
<td>0.76</td>
<td>0.76</td>
<td>0.90</td>
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<tr>
<td></td>
<td></td>
<td>COR</td>
<td>0.66</td>
<td>0.86</td>
<td>0.83</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Wambaugh et al., 2018; Honda, unpublished
Adding Inhalation Route of Exposure

Wambaugh et al., 2018; Honda, unpublished
Drive to Characterize Variability and Relevance of Current Toxicity Models

In US, Section 4(h) in amended TSCA says –

- New approach methods (NAMs) need to provide “information of equivalent or better scientific quality and relevance…” than the traditional animal models

In Europe, REACH says –

- Annex XI: “Results obtained from suitable in vitro methods may indicate the presence of a certain dangerous property or may be important in relation to a mechanistic understanding, which may be important for the assessment…” BUT confirmation using standard in vivo tests are still required unless:
  - Results are derived from an in vitro method whose scientific validity has been established by a validation study, according to internationally agreed validation principles; AND
  - Results are adequate for the purpose of classification and labelling and/or risk assessment; AND
  - Adequate and reliable documentation of the applied method is provided.
Building a Database of Legacy *In Vivo* Toxicity Studies

ToxRefDB Version 2.0

>1,200 chemicals
## Qualitative Reproducibility of Traditional Toxicity Studies

Reproducibility in Target Organ Effects in Repeat Dose Toxicity Studies

<table>
<thead>
<tr>
<th>Organ</th>
<th>Species</th>
<th>Repeated negative</th>
<th>Mixed effects</th>
<th>Repeated positive</th>
<th>% Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>dog</td>
<td>20</td>
<td></td>
<td></td>
<td>71.7</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>30</td>
<td></td>
<td></td>
<td>71.2</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>42</td>
<td></td>
<td></td>
<td>71.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>dog</td>
<td>49</td>
<td></td>
<td></td>
<td>64.1</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>61</td>
<td></td>
<td></td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>60</td>
<td></td>
<td></td>
<td>57.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>dog</td>
<td>64</td>
<td>21</td>
<td>7</td>
<td>77.2</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>93</td>
<td>31</td>
<td>15</td>
<td>77.7</td>
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<tr>
<td></td>
<td>rat</td>
<td>132</td>
<td>84</td>
<td>29</td>
<td>65.7</td>
</tr>
<tr>
<td>Testes</td>
<td>dog</td>
<td>65</td>
<td>20</td>
<td>7</td>
<td>78.3</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>110</td>
<td>20</td>
<td>9</td>
<td>85.6</td>
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<tr>
<td></td>
<td>rat</td>
<td>135</td>
<td>87</td>
<td>23</td>
<td>64.5</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>dog</td>
<td>76</td>
<td>12</td>
<td>4</td>
<td>87.0</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>109</td>
<td>23</td>
<td>7</td>
<td>83.5</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>142</td>
<td>83</td>
<td>20</td>
<td>66.1</td>
</tr>
</tbody>
</table>

56% concordance across species

39% concordance across species
Variability in Quantitative Effect Levels from *In Vivo* Repeat Dose Toxicity Studies

Two ways to statistically model the data across multiple study types

Variability within a specific study type

RMSE ranged from 0.41 to 0.59 log10-mg/kg/day, depending on model and dataset

Using an RMSE=0.59, the 95% CI of an LEL/LOAEL is:

- **1 mg/kg/day**: 0.07 – 14 mg/kg/day
- **10 mg/kg/day**: 0.7 – 143 mg/kg/day.

This confidence interval spans the difference between GHS STOT Category 1 (<10 mg/kg/d) and Category 2 (<100 mg/kg/d)

LyLy Pham and Katie Paul-Friedman, Unpublished
...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 non-rodent species, with non-rodents alone being predictive for 63% of human toxicity and rodents alone for 43%.
Enabling Translation Through Data Consolidation and Visualization

Data
- Now with 875,000 chemicals (up from ~760,000).
- High throughput *in vitro* assay information including new assays and more detailed descriptions
- *In vivo* toxicity values for human health and eco
- QSAR predictions for chemical properties
- Important lists (e.g., PFAS)
- ADME and exposure
- Functional use
- Literature search interface
- Read across workflow
Integrating Data for Regulatory Application with Decision Support Tools

- **RapidTox** is a suite of workflows that facilitate the application of data surfaced in the CompTox dashboard in diverse assessment decision contexts.

- Flexible integration of information related to chemical properties, fate and transport, hazard, exposure, and risk assessment.

- Enable expert users to review the assumptions made, refine results, and record the decisions.

- Presents data from new approach methods together with traditional toxicology data.

- Three workflows currently under development:
  - Chemical binning for TSCA (OCSPP)
  - Emergency response (OLEM)
  - Site-specific screening assessments (OLEM)
Translation of Results Through Regulatory Focused Case Studies

- Multiple international case studies stemming from 2016 inter-governmental workshop

- Example: *In Vitro* Bioactivity as a Conservative Point of Departure

- Participants include EPA, Health Canada, ECHA, EFSA, JRC, and A*STAR

- Goal: Determine whether *in vitro* bioactivity from broad high-throughput screening studies (e.g., ToxCast) can be used as a conservative point-of-departure and when compared with exposure estimates serve to prioritize chemicals for future study or as lower tier risk assessment.
Case Study Evaluating Bioactivity as a Protective Point-of-Departure

For ~92% of the chemicals, POD_{NAM} was conservative. (~100-fold on average)

Chemicals where POD_{NAM} was not conservative enriched in OPs/carbamates

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA
Take Home Messages…

• Charting a new path in toxicology will require a continued commitment to a different future
• New technologies exist for rapidly and comprehensively covering toxicological space at significantly less cost
• Addressing previous technical limitations such as a lack of metabolism and organ/tissue effects are within reach
• New methods should be evaluated in the context of the variability and relevance of existing models
• Enabling application of new technologies to regulatory with require delivery and integration using a broad range of IT tools
• Partnering with regulators on case studies will increase confidence and acceleration application to chemical risk assessment
Acknowledgements and Questions

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- ECHA
- EFSA
- Health Canada

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