Overview of NCCT Activities in the Chemical Safety for Sustainability National Program

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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
NCCT Activities in CSS Projects

- Chemical Evaluation:
  Project areas: **High-throughput Toxicology (HTT), Rapid Exposure and Dosimetry (RED).**

- Lifecycle Analytics:
  Project areas: Lifecycle-Human Exposure Modeling (LCHEM), **Sustainable Chemistry, Emerging Materials (Nanomaterials), Ecological Modeling.**

- Complex Systems Science:
  Project areas: **Adverse Outcome Pathways Discovery and Development (AOPDD), Virtual Tissue Modeling (VTM).**

- Solutions-based Translation and Knowledge Delivery:
  Project area: **Demonstration and Evaluation for Risk-Based Decisions.**
Research Focus Areas

- Increasing biological coverage in high-throughput *in vitro* test systems
- Systematically addressing technical limitations of *in vitro* test systems
- Continued integration of high-throughput results into tiered testing
- Characterization of uncertainty and variability
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High-Throughput Screening in ToxCast

Thousands of Chemicals

Concentration Response Screening

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

Concentration Response Modeling

- 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
Efforts to Expand Biological Coverage Using High Content Technologies

- **Concentration Response Screening**
- **Whole Genome Transcriptomics**
- **Mode-of-Action Identification**
- **Pathway Coverage**

- Thousands of Chemicals
- Multiple Cell Types

- 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
Initial Application of High-Throughput Transcriptomic Screening

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Type(s)</td>
<td>MCF7</td>
</tr>
<tr>
<td>Chemicals</td>
<td>2,112</td>
</tr>
<tr>
<td>Time Points:</td>
<td>6 hours</td>
</tr>
<tr>
<td>Concentrations:</td>
<td>8</td>
</tr>
<tr>
<td>Biological Replicates:</td>
<td>3</td>
</tr>
</tbody>
</table>

- **Number of samples:** 54,432
- **Number of endpoints:** $1.15 \times 10^9$
- **Total amount of data:** ~50 TB

Mode-of-Action Identification

Currently comparing a range of approaches...
Cmap, ML, Pathway

Concentration Response Modeling

Currently comparing a range of approaches...
BMDExpress, Proast, tcpl, and new NB model
Identifying Potential Biological Targets

Annotated Targets in CMap v2 and RefChem

Example Signature from CMap v2

<table>
<thead>
<tr>
<th>Target</th>
<th>Signature size</th>
<th>Sensitivity</th>
<th>Positives</th>
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<tbody>
<tr>
<td>CYP2C9</td>
<td>131</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ESR1</td>
<td>257</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>HDAC1</td>
<td>124</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DHFR</td>
<td>215</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NR1I2</td>
<td>139</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>PGR</td>
<td>115</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HMGCR</td>
<td>236</td>
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<td>1</td>
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<tr>
<td>ABCC2</td>
<td>357</td>
<td>1</td>
<td>1</td>
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<tr>
<td>TYMS</td>
<td>329</td>
<td>1</td>
<td>1</td>
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<tr>
<td>ESR2</td>
<td>281</td>
<td>0.86</td>
<td>7</td>
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<tr>
<td>AR</td>
<td>261</td>
<td>0.78</td>
<td>9</td>
</tr>
<tr>
<td>NR3C2</td>
<td>352</td>
<td>0.5</td>
<td>2</td>
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<tr>
<td>ABCB1</td>
<td>117</td>
<td>0.5</td>
<td>2</td>
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<tr>
<td>NR3C1</td>
<td>148</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>CA1</td>
<td>176</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>CA2</td>
<td>341</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>PTGS1</td>
<td>307</td>
<td>0.25</td>
<td>4</td>
</tr>
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</table>

*In process of curating/testing hits to determine specificity*
Characterizing Concentration Response

Most chemicals affect only a small number of pathways.

Majority of pathways affected by small numbers of chemicals.

J. Harrill, I. Shah, W. Setzer, Judson Unpublished
Development of High-Throughput Phenotypic Profiling

<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>

~1,300 total phenotypic endpoints
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
Research Focus Areas

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Expanding Chemical Coverage of High Throughput \textit{In Vitro} Systems

Pilot Scale Water Soluble Library

\textit{VOC In Vitro} Exposure System

M. Higuchi (EPA-NHEERL)

Transcriptional BMDs from HTTR Analysis

Acrolein

- Reactome
- BioPlanet
- GO-BP

Pathway/Process Accumulation

TLV

BMD Median

J. Harrill, M. Higuchi, and J. Zavala-Mendez, Unpublished
Addressing Limitations in Xenobiotic Metabolism

“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

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>
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</tbody>
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Pilot Screening Results of Pinto et al., 2016

Library Example Detoxification

Example Bioactivation

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

S. Simmons, Unpublished
Developing Targeted Organotypic Models that Predict Tissue Effects

3D Microtissue Model of Primary Human Thyrocytes

Thyroglobulin secretion is enhanced over time in a 3D culture model

Thyroid hormone is synthesized and secreted over time in a 3D culture model

C. Deisenroth, Unpublished
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Characterization of Uncertainty and Variability

Pharmacodynamic

Experimental

In Vivo

Pharmacokinetic

Experimental and Inter-Individual

John will cover some of this…

In Vitro-to-In Vivo
Characterizing Uncertainty for In Vitro Testing Systems and Computational Modeling

Bootstrap Uncertainty in In Vitro Potency Values

Computational Modeling

Propagation of Uncertainty in Modeling Output

18 ER In Vitro Assays

ER Pathway Model

Chemical Rank

Watt and Judson, PLOS One 2018
Characterizing Uncertainty for *In Vivo* Toxicity Studies

Qualitative 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>
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</thead>
<tbody>
<tr>
<td>Liver</td>
<td>dog</td>
<td>20</td>
<td>26</td>
<td>46</td>
<td>71.7</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>dog</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>mouse</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>rat</td>
<td></td>
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</tr>
<tr>
<td>Spleen</td>
<td>dog</td>
<td></td>
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<tr>
<td></td>
<td>mouse</td>
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<tr>
<td></td>
<td>rat</td>
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<tr>
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<td>rat</td>
<td>135</td>
<td>87</td>
<td>23</td>
<td>64.5</td>
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<tr>
<td>Adrenal gland</td>
<td>dog</td>
<td>76</td>
<td>12</td>
<td>4</td>
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<td>mouse</td>
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<td>23</td>
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<td>83.5</td>
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<td></td>
<td>rat</td>
<td>142</td>
<td>83</td>
<td>20</td>
<td>66.1</td>
</tr>
</tbody>
</table>

Quantitative Variability in Effect Levels from *In Vivo* Repeat Dose Toxicity Studies

Using an RMSE=0.59, the 95% CI of an LEL/LOAEL is:
1 mg/kg/day $\rightarrow$ 0.07 – 14 mg/kg/day.
10 mg/kg/day $\rightarrow$ 0.7 – 143 mg/kg/day.

Section 4(h) in the new TSCA legislation requires that –

NAMs need to provide “information of equivalent or better scientific quality and relevance…” than the traditional animal models.
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Enable Translation Through Data Visualization and Decision Support Tools

Comptox Chemicals Dashboard

https://comptox.epa.gov/dashboard/

RapidTox Dashboard

WORK IN PROGRESS
Significantly Enhanced Functionality in New Release of CompTox Chemicals Dashboard
Similar to Financial Tools, RapidTox will Have Multiple Workflows to Address Different Decision Contexts

- Semi-automated decision support workflows
- Flexible integration of information related to chemical properties, fate and transport, hazard, and exposure
- Enable expert users to review the assumptions made and refine the results
- Presents alternative data together with traditional toxicology data

Workflow to Calculate Your Taxes

Workflows to Integrate Safety Data for Regulatory Decisions

https://turbotax.intuit.com/
Beginning to Incorporate RapidTox Workflows into Regulatory Decision Making

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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.
Bioactivity Provides a Conservative Estimate of a NOAEL/LOAEL

For ~92% of the chemicals, POD_{ToxCast} was conservative. (~100-fold with human HTTK, ~50-fold with rat HTTK)

Less conservative than TTC

Total = 448 chemicals

httk, ToxCast data, and POD value(s) currently available

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA
• Applying and refining new technologies for comprehensively evaluating toxicological space at significantly less cost
• Systematically addressing previous technical limitations such as a lack of metabolism, limited chemical space, and organ/tissue effects
• Making progress in integrating new technologies into tiered toxicity testing framework
• Rigorously characterizing uncertainties and variability in both *in vitro* test systems and traditional *in vivo* models
• Enabling application of new technologies to chemical safety decisions through delivery and integration using a broad range of IT tools
• Partnering with regulators on case studies to increase confidence and accelerate application to chemical risk assessment
Acknowledgements and Questions

Tox21 Colleagues:
- NTP
- FDA
- NCATS

EPA Colleagues:
- NERL
- NHEERL
- NCEA

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