EPA National Center for Computational Toxicology

UPDATE

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ICCVAM Public Forum
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A couple of new and exciting activities at NCCT

1. Chemical library update
2. Chemistry Dashboard
3. Retrofitting in vitro assays with metabolic competence
4. In vitro PK
5. Summer Surprise
ToxCast & Tox21
Chemicals, Data and Release Timelines

<table>
<thead>
<tr>
<th>Set</th>
<th>Chemicals</th>
<th>Assays</th>
<th>Endpoints</th>
<th>Completion</th>
<th>Available</th>
</tr>
</thead>
<tbody>
<tr>
<td>ToxCast Phase I</td>
<td>293</td>
<td>~600</td>
<td>~700</td>
<td>2011</td>
<td>Now</td>
</tr>
<tr>
<td>ToxCast Phase II</td>
<td>767</td>
<td>~600</td>
<td>~700</td>
<td>03/2013</td>
<td>Now</td>
</tr>
<tr>
<td>ToxCast E1K</td>
<td>800</td>
<td>~50</td>
<td>~120</td>
<td>03/2013</td>
<td>Now</td>
</tr>
<tr>
<td>ToxCast Phase III</td>
<td>~900</td>
<td>~300</td>
<td>~300</td>
<td>In progress</td>
<td>2016</td>
</tr>
<tr>
<td>Tox21</td>
<td>~9000</td>
<td>~80</td>
<td>~150</td>
<td>In progress</td>
<td>ongoing</td>
</tr>
</tbody>
</table>

Pesticides, antimicrobials, food additives, green alternatives, HPV, MPV, endocrine reference cmpds, tox reference cmpds, NTP in vivo, FDA GRAS, FDA PAFA, EDSP, water contaminants, exposure data, industrial, failed drugs, marketed drugs, fragrances, flame retardants, etc.
Chemical Library Update

1) **Filling in holes**
   - Completing testing of all Phase 1-3 chemicals in Attagene assays

2) **Water Soluble Chemicals**
   - About 650 chemicals were not tested as part of ToxCast due to lack of solubility in DMSO (e.g., glyphosate)
   - Currently developing a ‘water-soluble’ chemical library

3) **Volatile Chemicals**
   - Current assays do not allow for accurate testing of many VOCs/SVOCs
   - Working with NHEERL to develop medium throughput assay methods for volatile chemicals.
iCSS Chemistry Dashboard

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

Web access ~720,000 chemicals

Recently Released:
https://comptox.epa.gov/dashboard
Chemistry Dashboard
DSSTox Chemistry Content

Establishing confidence levels for content

**QC Level Totals**
- DSSTox_High: 4535
- DSSTox_Low: 16K
- Public_High: 33K
- Public_Medium: 101K
- Public_Low: 584K
- Public_Untrusted: ~150K pending
- ~310K pending

**QC Levels**
- DSSTox_High: Hand curated and validated
- DSSTox_Low: Hand curated and confirmed using multiple public sources
- Public_High: Extracted from EPA SRS and confirmed to have no conflicts in ChemID and PubChem
- Public_Medium: Extracted from ChemID and confirmed to have no conflicts in PubChem
- Public_Low: Extracted from ACToR or PubChem
- Public_Untrusted: Postulated, but found to have conflicts in public sources

Constantly under curation
Expt’l and Predicted PhysChem Data

Search: atrazine

**Molecular Formula:** C8H14ClN5

**Average Mass:** 215.890002 g/mol

**Monoisotopic Mass:** 215.093773 g/mol

<table>
<thead>
<tr>
<th>Property</th>
<th>Average (Exp.)</th>
<th>Range (Exp.)</th>
<th>Average (Pred.)</th>
<th>Range (Pred.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility</td>
<td>0.0 (1)</td>
<td>0.0001296 to 0.0001298</td>
<td>0.525 (2)</td>
<td>0.05716 to 0.9626</td>
</tr>
<tr>
<td>Melting Point</td>
<td>174.25 (6)</td>
<td>172.5 to 177.0</td>
<td>150.55 (2)</td>
<td>113.9 to 187.2</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>N/A</td>
<td>N/A</td>
<td>326.0 (2)</td>
<td>313.0 to 339.0</td>
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<tr>
<td>LogP</td>
<td>2.617 (3)</td>
<td>2.61 to 2.632</td>
<td>2.721 (3)</td>
<td>2.67 to 2.82</td>
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<tr>
<td>Atmospheric Hydroxylation Rate</td>
<td>N/A</td>
<td>N/A</td>
<td>0.0 (1)</td>
<td>1.711e-11 to 1.711e-11</td>
</tr>
<tr>
<td>LogBCF</td>
<td>0.9 (1)</td>
<td>0.9 to 0.9</td>
<td>0.598 (1)</td>
<td>0.938 to 0.938</td>
</tr>
<tr>
<td>Biodegradation Half-life</td>
<td>N/A</td>
<td>N/A</td>
<td>4.921 (1)</td>
<td>4.021 to 4.021</td>
</tr>
<tr>
<td>Henry’s Law Constant</td>
<td>N/A</td>
<td>N/A</td>
<td>4.0 (1)</td>
<td>4.2e-10 to 4.2e-10</td>
</tr>
<tr>
<td>Fish Biotransformation Half-life</td>
<td>0.089 (1)</td>
<td>0.08913 to 0.08913</td>
<td>0.136 (1)</td>
<td>0.1359 to 0.1359</td>
</tr>
<tr>
<td>LogKOA</td>
<td>N/A</td>
<td>N/A</td>
<td>8.395 (1)</td>
<td>8.395 to 8.395</td>
</tr>
<tr>
<td>LogKOC</td>
<td>2.24 (1)</td>
<td>2.24 to 2.24</td>
<td>2.305 (1)</td>
<td>2.305 to 2.305</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>0.0 (1)</td>
<td>7.209e-11 to 7.209e-11</td>
<td>0.0 (1)</td>
<td>2.025e-07 to 2.025e-07</td>
</tr>
</tbody>
</table>
Strategy for Retrofitting *In Vitro* Assays with Metabolic Competence

**“Extracellular”**
- Strategy
- Chemicals 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”**
- Strategy
- Capable of metabolizing chemicals inside the cell in cell-based assays
- More closely models effects of target tissue metabolism

Integrated approach to model *in vivo* metabolic bioactivation and detoxification

NCCT Metabolic Competence Project Group:
- Steve Simmons (PI)
- Danica DeGroot (Postdoc)
1. Retrofitting Assays for Metabolic Competence – Extracellular Approach

Alginate Immobilization of Metabolic Enzymes (AIME)

Amount of XME Activity in Microspheres

DeGroot et al. 2016 SOT poster #3757
2. Retrofitting Assays for Metabolic Competence – mRNA Intracellular Strategy

Pool in vitro transcribed mRNAs chemically modified with pseudouridine and 5-methylcytidine to reduce immune stimulation.

293T cells 21.5 h post transfection with 90 ng of EGFP mRNA using TransIT reagent.

Advantage of transfecting with mRNA: Titrate different CYPs to match different ratios in different tissues.
NCCT, NTP, NCATS joint sponsored challenge to retrofit HTS assays with xenobiotic metabolic competence

Stage 1
- Deadline was 4/8/16
- ~25 proposals – 10 were selected for Phase 2
- Each gets $10k to develop method and provide proof of principal

Stage 2
- Semi-finalists start-up workshop RTP 7/8/16
- Up to five applicants selected as finalists, awarded a prize of up to $100,000 each, and invited to participate in the final stage of the competition.

Stage 3
- Based on results one winner may be selected and awarded $400k

http://www.transformtoxtesting.com/
Toxicokinetics (TK) provides a bridge between toxicity and exposure assessment by predicting tissue concentrations due to exposure
• Traditional TK methods are very resource intensive

NCCT recently released an R package called “httk”
• Uses “reverse dosimetry” (Reverse TK or RTK) converts in vitro HTS conc to human daily dose
• Freely available on CRAN
• Allows RTK for 543 chemicals (more coming)

New R package called ‘httk-pop” package
• estimates exposures for susceptible populations
• Ring et al. Refining high-throughput prioritization of environmental chemicals to include inter-individual variability across subpopulations. (submitted)

Access httk from the R GUI: “Packages” then “Install Packages”
ToxCast *in vitro*

- ToxCast/Tox21 bioactive concentrations alone make it hard to prioritize chemicals

Wetmore et al. (2012)
Translation from *in vitro* to steady-state oral equivalent doses allow greater discrimination between effective chemical potencies

Wetmore *et al.* (2012)
Coming Soon
RapidTox Dashboard

Provides a place to find and integrate all available chemistry, exposure, and bioactivity information
Goals of the Project

- Development of a screening level decision support tool for hundreds to thousands of data poor chemicals
- Integrate a range of information related to chemical properties, fate and transport, hazard, and exposure through an interactive on-line dashboard, including...
  - Traditional data (as available)
  - New ORD data streams such as automated read-across methods, ToxCast data, AOPs, ExpoCast, and high-throughput toxicokinetic models
- Deliver quantitative toxicity values with associated estimates of uncertainty
- Initial prototype expected end of FY16
Thanks for Listening

EPA’s National Center for Computational Toxicology
EXTRAS
Tox21 & ToxCast Analytical QC

- Process, summarize & store results in database for surfacing in dashboards
- For what types of chemicals do methods (LC, GC) work? Not work?
- Can chemicals in different QC categories be characterized structurally?
- Why larger failure rate in Tox21 All library?

**EPA Tox21 QC (3729)**
- Pass: 52%
- Fail: 39%
- Degrade: 3%
- ND: 6%

**Tox21 All QC (8593)**
- Pass: 50%
- Fail: 34%
- Degrade: 14%
- ND: 2%

Pass = C (75%) or greater
Fail = D, F, Ac, Bc, Cc
Two Case Studies

**OSWER-Region Case Study**

Decision Context: Estimate toxicity values with associated uncertainty for data poor chemicals at Superfund sites

Desired Components:
- Phys-Chem properties with environ fate and transport
- Hazard profile – GL and GL-like studies, RA, and QSAR
  - Acute and chronic tox endpoints
- ToxCast data in AOP context
- Toxicokinetic data (*in vivo* and *in vitro*)
- Bioavailability (sediment and Caco-2)
- Consumer and industrial use
- Screening level estimates with defined exposure scenarios
- Available analytical chemistry methods

**OPP Case Study**

Decision Context: Prioritize non-food use inert ingredients for additional study

Desired Components:
- Phys-Chem properties with environ fate and transport
- Hazard profile – GL and GL-like studies, RA, and QSAR
  - Chronic tox endpoints
- ToxCast data in AOP context
- Toxicokinetic data (*in vivo* and *in vitro*)
- Consumer and industrial use