PFAS Tiered Testing Strategy

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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.
Problem formulation:
- Hundreds of environmentally-relevant PFAS without data
- Traditional single-chemical toxicity testing not practical
- Toxicity & toxicokinetic information needed to inform decision-making

How do we generate informative toxicity and kinetic information quickly?
- ORD, NTP, and partners have the capacity to generate toxicity and toxicokinetic data using high throughput screening (HTS) assays
Tiered Testing Overview

- Procure a library of ~300-400 PFAS (PFAS Screening Library) to support development of analytical methods, environmental monitoring, and toxicity testing
- Define a reference subset of PFAS
- Conduct tiered toxicity and toxicokinetic testing chemicals within the PFAS landscape
- Data will support multiple outputs, making use of the integrated dataset (in vivo, in vitro, etc.) to inform toxicity and kinetics across the PFAS landscape
Tiered Toxicity and Toxicokinetic Testing Strategy

Tier 0 & 1
*In Vitro* Toxicity and Toxicokinetic Assays

Human Exposure Characterization

~75 Reference PFAS

- Read-Across Analyses
- *In Vitro* POD Derivations
- Inform Risk Assessments
- Derive BERs

High Potential Risk

Tier 2
*In Vivo* Toxicological and Toxicokinetic Assays

*Collaboration between EPA and NTP*
PFAS Library and Chemical Selection

**EPA DSSTox Database:** > 758K chemicals

- Chemical structures, downloadable files
- Predicted phys-chem properties
- External links & list overlaps

**PFAS Chemical Landscape:**

DSSTox-registered >5000 PFAS substances (>3 F, alkyl)

- Spanning public lists of interest to EPA

- Attempt to procure (chemicals w/ structures)

- PFAS Standards library
  (current library 430 PFAS)

- EPA’s prioritized list for PFAS research
  (exposure, occurrence, health data)

- PFAS Reference subset
  (tiered toxicity & toxicokinetic testing)

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Per(poly)-fluorinated substances (PFAS)

- PFAS chemical names, acronyms, synonyms
- PFAS chemical structure categories
Selecting the Subset of 75 PFAS

Workflow for selection of structural categories containing the reference subset of 75 PFAS for tiered toxicity and toxicokinetic testing.
## Tiered Testing: *In Vitro* Toxicity Assays

<table>
<thead>
<tr>
<th>Endpoint of Interest</th>
<th>NTP</th>
<th>EPA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genome-Wide Screening (Tier 0)</strong></td>
<td></td>
<td>High Throughput Transcriptomics (<em>Httr</em>) in 2 cell types and Phenotypic Profiling</td>
</tr>
<tr>
<td><strong>Hepatotoxicity</strong></td>
<td>2D HepaRG cytotoxicity; 3D HepaRG (spheroid) transcriptomics</td>
<td>Attagene cis- and trans-Factorial assay (HepG2)</td>
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<tr>
<td><strong>Developmental Toxicity</strong></td>
<td></td>
<td>[Zebrafish embryo assay (ZEA)]</td>
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<tr>
<td><strong>Immunotoxicity</strong></td>
<td>Bioseek Immune panel</td>
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<tr>
<td><strong>Mitochondrial Toxicity</strong></td>
<td>2D HepaRG mitochondrial dye and Seahorse assay</td>
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<tr>
<td><strong>Developmental Neurotoxicity</strong></td>
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<td>Neurodevelopmental microelectrode assay (MEA)</td>
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</table>
## Tiered Testing: *In Vitro* Toxicokinetic Assays

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<tr>
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<tr>
<td>Hepatic Clearance</td>
<td>Human primary hepatocyte clearance</td>
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<td>Plasma Protein Binding</td>
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<td>Renal Transport</td>
<td>Renal proximal tubule permeability assay</td>
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<td>Enterohepatic Recirculation</td>
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<td>Piloting hepatic transporter assay</td>
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<tr>
<td><em>In Vitro</em> Disposition</td>
<td>Cell vs. nominal concentration (Tox21 joint project)</td>
<td>Cell vs. nominal concentration (Tox21 joint project)</td>
</tr>
</tbody>
</table>
PFAS MASTER LIST

- 7 component lists (EPA, EU, International)
- 5070 total unique substances
  - 5057 with CAS
  - 3992 with chemical structures
- EPA research & test landscape
  - 430 in EPA ToxCast Inventory (soluble in DMSO)
  - 43 in EPA ToxCast Inventory (insoluble in DMSO)
  - 126 different form (salt, anion) or unable to procure

<table>
<thead>
<tr>
<th>PFASMASTER LISTS</th>
<th>PFASOECD</th>
<th>PFKEMI</th>
<th>PFASTRIER</th>
<th>EPAPFASRL</th>
<th>EPAPFASINV</th>
<th>EPAPFASINSOL</th>
<th>EPAPFAS75S1</th>
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OECD PFAS List
KEMI (Swedish Chem Agency) PFAS List
Community PFAS List (2015)
EPA PFAS Research List
EPA PFAS Inventory (DMSO Soluble)
EPA PFAS Inventory (DMSO Insoluble)
EPA PFAS 75 Test Sample (Set 1)
• Procured and established PFAS Chemical Screening Library – 430 compounds – Complete
  ✓
• Established process to optimize future read-across efforts and selected subset of 75 PFAS
  ✓
• Developed commentary on selection process and tiered testing for submission to Environmental Health Perspectives- Accepted ✓
• Plated and shipped PFAS to all labs conducting testing ✓
• Multiple PFAS lists processed through data curation and added to Comptox Chemistry Dashboard (5070 unique substances) ✓
• High throughput testing underway in most labs and anticipate raw data Winter 2018/19
• Selecting an additional 75 PFAS with the addition of HTTr and phenotypic profiling for the complete set (total of 150)- in process
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