A Chemical Category-Based Approach for Selecting and Screening PFAS for Toxicity and Toxicokinetic Testing

SETAC Environmental Risk Assessment of PFAS Meeting
August 13, 2019
Rusty Thomas
Director
National Center for Computational Toxicology

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
Background and Importance of the Problem Already Covered By Others...

Bottom line is that we cannot readily dig our way out of this hole using only traditional testing approaches...
EPA is Using New Approach Methods to Help Fill Information Gaps

Research Area 1: What are the human health and ecological effects of exposure to PFAS?

- Using computational toxicology approaches to fill in gaps. For the many PFAS for which published peer-reviewed data are not currently available, the EPA plans to use new approaches such as high throughput and computational approaches to explore different chemical categories of PFAS, to inform hazard effects characterization, and to promote prioritization of chemicals for further testing. These data will be useful for filling gaps in understanding the toxicity of those PFAS with little to no available data. In the near term, the EPA intends to complete assays for a representative set of 150 PFAS chemicals, load the data into the CompTox Chemicals Dashboard for access, and provide peer-reviewed guidance for stakeholders on the use and application of the information. In the long term, the EPA will continue research on methods for using these data to support risk assessments using New Approach Methods (NAMs) such as read-across and transcriptomics, and to make inferences about the toxicity of PFAS mixtures which commonly occur in real world exposures. The EPA plans to collaborate with NIEHS and universities to lead the science in this area and work with universities, industry, and other government agencies to develop the technology and chemical standards needed to conduct this research.
But, It All Starts With Chemistry... Curating Names, Structures, and Identifiers

<table>
<thead>
<tr>
<th>Substance Name</th>
<th>CASRN</th>
<th>Source Name (incorrect or ambiguous)</th>
<th>Source_CASRN (incorrect or invalid)</th>
<th>Source_Acronym (incorrect or ambiguous)</th>
<th>Unique_Acronym</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2,3-trifluoro-3-(1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy)propanoic acid</td>
<td>919005-14-4</td>
<td>ADONA parent acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium 4,8-diola-3H-perfluorononanoate</td>
<td>958445-44-8</td>
<td>ADONA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium perfluoropentanesulfonate</td>
<td>68259-09-6</td>
<td>APFPeS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Propenoic acid, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heneicosafluorododecyl ester, polymer with 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl 2-propenoate, ╬▒-(2-methyl-1-oxo-2-propenyl) ¤ë-(2-methyl-1-oxo-2-propenyl)oxypoly(oxy-1,2-ethanediyl), 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,14-nonacosafluorohexadecyl 2-propenoate, octadecyl 2-propenoate and 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-pentacosafluorotetradecyl 2-propenoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Currently >6,000 PFAS and 21 lists

https://comptox.epa.gov/dashboard

Antony Williams, Ann Richard, Chris Grulke, and Chemical Curation Team
Assembled a PFAS Chemical Library for Research and Methods Development

- Attempted to procure ~3,000 based on chemical diversity, Agency priorities, and other considerations
- Obtained 480 total unique chemicals
  - 430/480 soluble in DMSO (90%)
  - 54/75 soluble in water (72%)
    (incl. only 3 DMSO insolubles)
- Issues with sample stability and volatility
- Categories assigned based on three approaches
  - Buck et al., 2011 categories
  - Markush categories
  - OECD categories
  - Manual assignment
Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing

Goals:
- Generate data to support development and refinement of categories and read-across evaluation
- Incorporate substances of interest to Agency
- Characterize mechanistic and toxicokinetic properties of the broader PFAS landscape

Selected 150 PFAS in two phases representing 83 different categories
- 9 categories with > 3 members
- Lots of singletons
# In Vitro Toxicity and Toxicokinetic Testing

<table>
<thead>
<tr>
<th>Toxicological Response</th>
<th>Assay</th>
<th>Assay Endpoints</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatotoxicity</td>
<td>3D HepaRG assay</td>
<td>Cell death and transcriptomics</td>
<td>Measure cell death and changes in important biological pathways</td>
</tr>
<tr>
<td>Developmental Toxicity</td>
<td>Zebrafish embryo assay</td>
<td>Lethality, hatching status and structural defects</td>
<td>Assess potential teratogenicity</td>
</tr>
<tr>
<td>Immunotoxicity</td>
<td>Bioseek Diversity Plus</td>
<td>Protein biomarkers across multiple primary cell types</td>
<td>Measure potential disease and immune responses</td>
</tr>
<tr>
<td>Mitochondrial Toxicity</td>
<td>Mitochondrial membrane potential and respiration (HepaRG)</td>
<td>Mitochondrial membrane potential and oxygen consumption</td>
<td>Measure mitochondrial health and function</td>
</tr>
<tr>
<td>Developmental Neurotoxicity</td>
<td>Microelectrode array assay (rat primary neurons)</td>
<td>Neuronal electrical activity</td>
<td>Impacts on neuron function</td>
</tr>
<tr>
<td>Endocrine Disruption</td>
<td>ACEA real-time cell proliferation assay (T47D)</td>
<td>Cell proliferation</td>
<td>Measure ER activity</td>
</tr>
<tr>
<td>General Toxicity</td>
<td>Attagene cis- and trans- Factorial assay (HepG2)</td>
<td>Nuclear receptor and transcription factor activation</td>
<td>Activation of key receptors and transcription factors involved in hepatotoxicity</td>
</tr>
<tr>
<td></td>
<td>High-throughput transcriptomic assay (multiple cell types)</td>
<td>Cellular mRNA</td>
<td>Measures changes in important biological pathways</td>
</tr>
<tr>
<td></td>
<td>High-throughput phenotypic profiling (multiple cell types)</td>
<td>Nuclear, endoplasmic reticulum, nucleoli, golgi, plasma membrane, cytoskeleton, and mitochondria morphology</td>
<td>Changes in cellular organelles and general morphology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicokinetic Parameter</th>
<th>Assay</th>
<th>Assay Endpoints</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic hepatic clearance</td>
<td>Hepatocyte stability assay (primary human hepatocytes)</td>
<td>Time course metabolism of parent chemical</td>
<td>Measure metabolic breakdown by the liver</td>
</tr>
<tr>
<td>Plasma protein binding</td>
<td>Ultracentrifugation assay</td>
<td>Fraction of chemical not bound to plasma protein</td>
<td>Measure amount of free chemical in the blood</td>
</tr>
</tbody>
</table>

*Assays being performed by NTP and EPA*
Attagene cis- and trans- Factorial Assay

- **C/S Assay**
  - 47 Endogenous Transcription Factors
    - Xenobiotic pathways
    - Cell growth/differentiation
    - Endocrine pathways
    - Stress response

- **TRANS Assay**
  - 24 human nuclear receptors
  - GAL-4 formats (NR ligand-binding domains)

- HepG2 cells
  - Concentration-response testing
  - 24-hour exposure
Preliminary Category-Based Analysis of the Attagene Transcription Factor Assay

*Results are preliminary. Chemicals still undergoing analytical QC.*

Keith Houck and Grace Patlewicz
High-Throughput Phenotypic Profiling (aka ‘Cellular Pathology’)

- Multiple Cell Types
- Concentration Response Screening
- Multi-Parameter Cellular Phenotypic Profiling
- Mode-of-Action Grouping
- Concentration Response Modeling

~1,300 endpoints

Joshua Harrill and Johanna Nyffeler
Preliminary Category-Based Analysis of the Phenotypic Profiling Assay

*Results are preliminary. Chemicals still undergoing analytical QC.*

Joshua Harrill, Johanna Nyffeler, and Grace Patlewicz
In Vitro Toxicokinetic Assays and In Vitro-to-In Vivo Extrapolation

Rotroff et al., Tox Sci., 2010
Wetmore et al., Tox Sci., 2012
Wetmore et al., Tox Sci., 2015

David Crizer, See Poster TP050
Barbara Wetmore, See Poster WP011
Preliminary Analysis of the Toxicokinetic Assays

*Results are preliminary. Chemicals still undergoing analytical QC.

David Crizer, Barbara Wetmore, and Grace Patlewicz
Current PFAS Grouping Approaches Use Different Levels of Aggregation

![Diagram showing different levels of structural aggregation for chemical categories/group.]

* Available source $in vivo$ tox study
Incorporating Mechanistic and Toxicokinetic Data to Inform PFAS Category Aggregation

Mechanistic and TK Informed Structural Aggregation

Chemical Categories/Group

* Needed in vivo tox study
* Available source in vivo tox study
Take Home Messages…

• Chemical curation efforts are important to harmonize structure, naming, and identifiers across the PFAS space

• A chemical library of 430 PFAS has been assembled for chemical screening, analytical method development, and other research needs

• A subset of 150 PFAS selected for in vitro toxicity and toxicokinetic testing to refine/support read across categories

• In vitro toxicity and toxicokinetic testing and analysis are underway and demonstrate the diverse biological activities and toxicokinetic properties of PFAS
Acknowledgements and Questions

**EPA ORD**
- Grace Patlewicz
- Josh Harrill
- Barbara Wetmore
- Monica Linnenbrink
- Reeder Sams
- Johanna Nyffeler
- Antony Williams
- Ann Richard
- Chris Grulke
- Kathy Coutros
- Stephanie Padilla
- Tim Shafer
- Jason Lambert
- Mark Strynar
- Ron Hines
- Annette Guiseppi-Elie

**National Toxicology Program**
- Michael Devito
- David Crizer
- Suramya Waidyananatha
- Steve Ferguson
- Nisha Sipes

- Marci Smeltz
- Richard Judson
- Indira Thillainadarajah
- Brian Meyer
- Andy Gillespie
- Scott Clifton
- Matt Henderson
- Anna Kreutz
- Evgenia Korol-Bexell
- Larry McMillan
- Chris Mazur