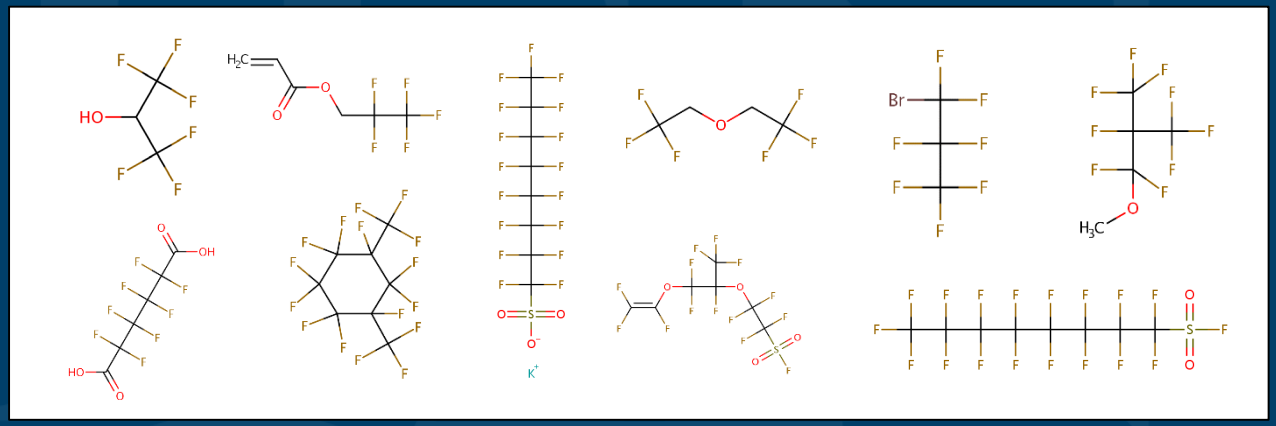
A Chemical Category-Based Approach for Selecting and Screening PFAS for Toxicity and Toxicokinetic Testing



SETAC Environmental Risk Assessment of PFAS Meeting

August 13, 2019

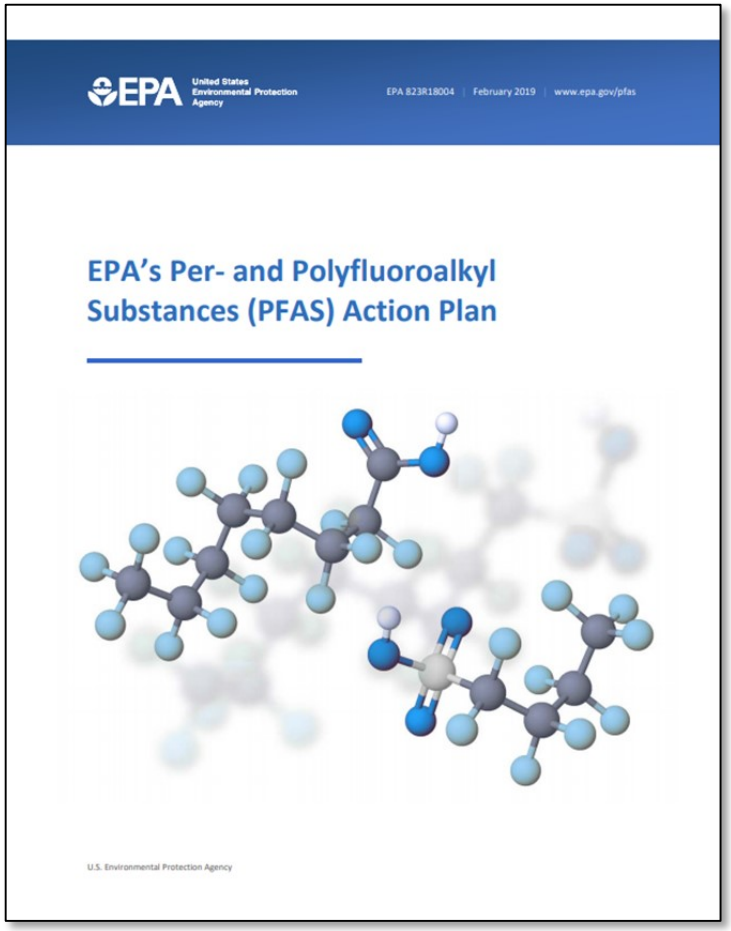
Rusty Thomas
Director
National Center for Computational Toxicology

EPA United States Environmental Protection Agency

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…

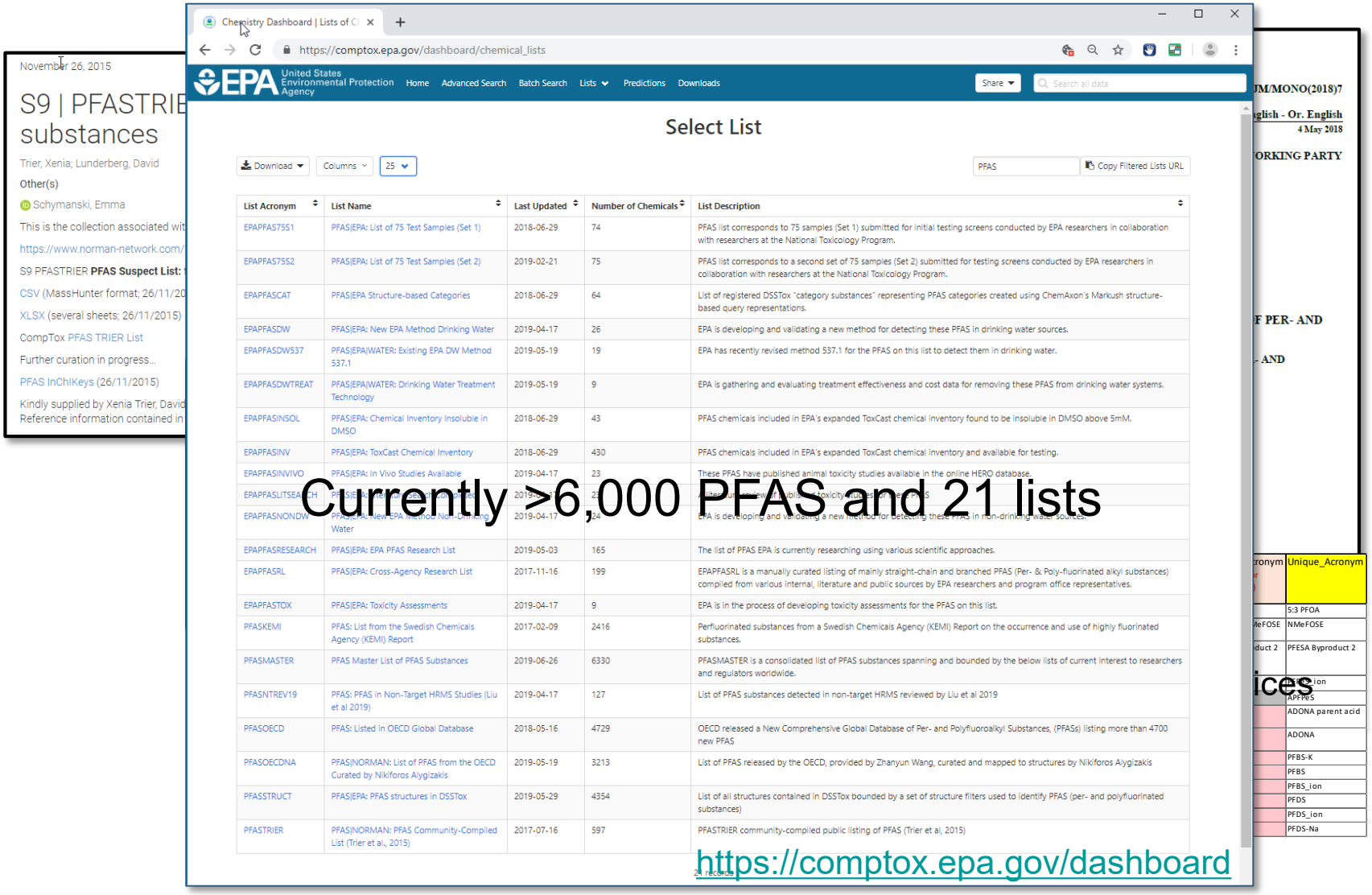
EPA is Using New Approach Methods to Help Fill Information Gaps



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

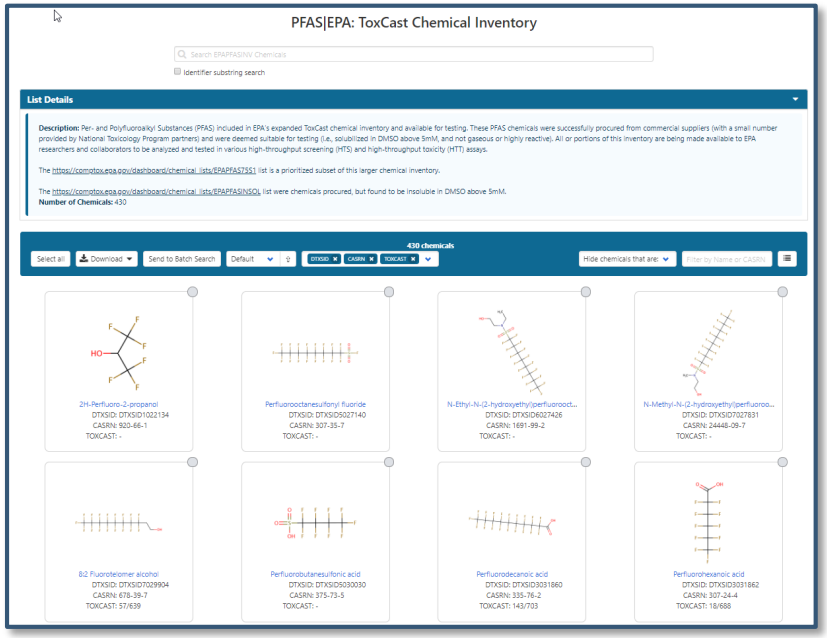
- **Using computational toxicology approaches to fill in gaps.** For the many PFAS for which published peer-reviewed data are not currently available, the EPA plans to use new approaches such as high throughput and computational approaches to explore different chemical categories of PFAS, to inform hazard 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.

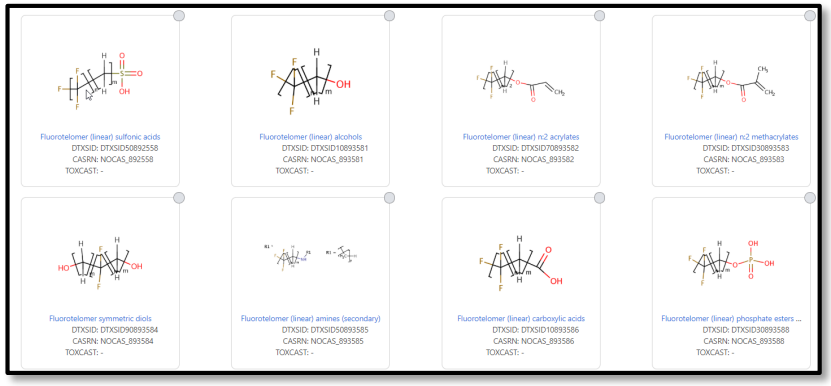
But, It All Starts With Chemistry… Curating Names, Structures, and Identifiers



Antony Williams, Ann Richard, Chris Grulke, and Chemical Curation Team

Assembled a PFAS Chemical Library for Research and Methods Development

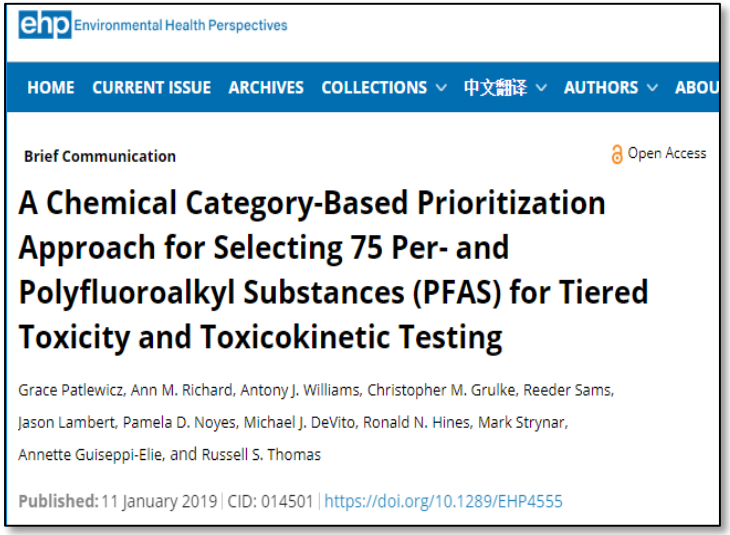




- Attempted to procure ~3,000 based on chemical diversity, Agency priorities, and other considerations
- Obtained 480 total unique chemicals
  - 430/480 soluble in DMSO (90%)
  - 54/75 soluble in water (72%) (incl. only 3 DMSO insolubles)
- Issues with sample stability and volatility
- Categories assigned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

Kathy Coutros, Chris Grulke, and Ann Richard

Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing



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Brief Communication

Open Access

A Chemical Category-Based Prioritization Approach for Selecting 75 Per- and Polyfluoroalkyl Substances (PFAS) for Tiered Toxicity and Toxicokinetic Testing

Grace Patlewicz, Ann M. Richard, Antony J. Williams, Christopher M. Grulke, Reeder Sams, Jason Lambert, Pamela D. Noyes, Michael J. DeVito, Ronald N. Hines, Mark Strynar, Annette Guiseppi-Elie, and Russell S. Thomas

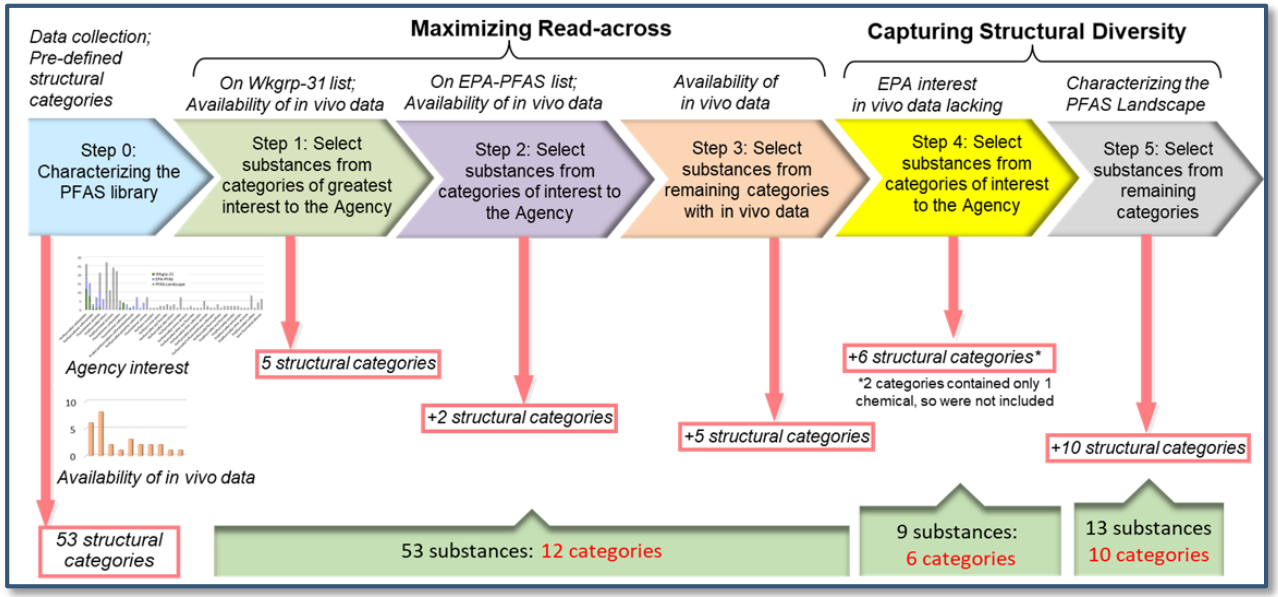
Published: 11 January 2019 | CID: 014501 | https://doi.org/10.1289/EHP4555

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



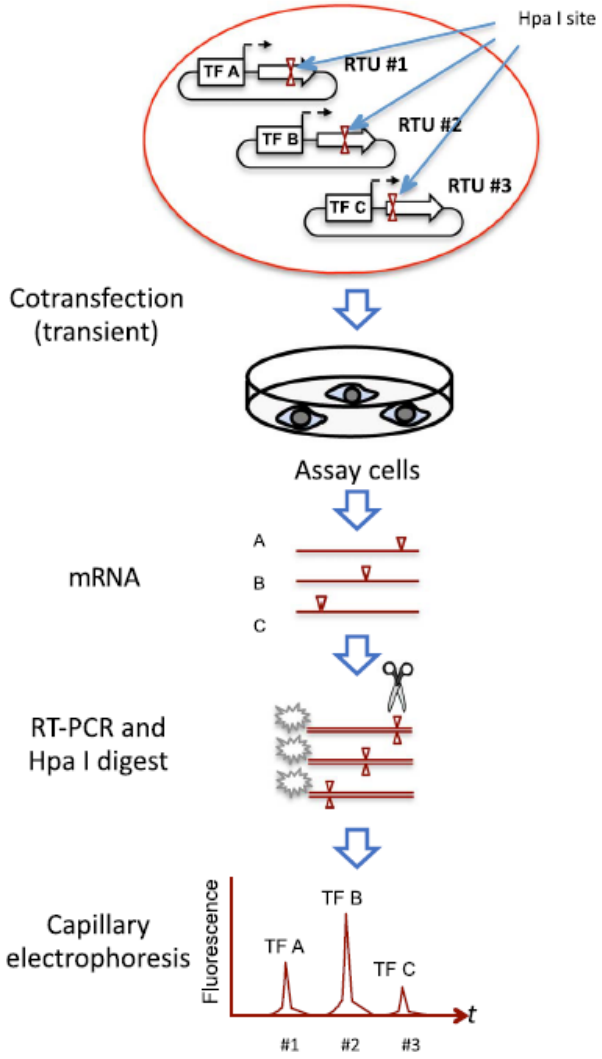
EPA United States Environmental Protection Agency

In Vitro Toxicity and Toxicokinetic Testing

Toxicological Response	Assay	Assay Endpoints	Purpose
Hepatotoxicity	3D HepaRG assay	Cell death and transcriptomics	Measure cell death and changes in important biological pathways
Developmental Toxicity	Zebrafish embryo assay	Lethality, hatching status and structural defects	Assess potential teratogenicity
Immunotoxicity	Bioseek Diversity Plus	Protein biomarkers across multiple primary cell types	Measure potential disease and immune responses
Mitochondrial Toxicity	Mitochondrial membrane potential and respiration (HepaRG)	Mitochondrial membrane potential and oxygen consumption	Measure mitochondrial health and function
Developmental Neurotoxicity	Microelectrode array assay (rat primary neurons)	Neuronal electrical activity	Impacts on neuron function
Endocrine Disruption	ACEA real-time cell proliferation assay (T47D)	Cell proliferation	Measure ER activity
General Toxicity	Attagene cis- and trans- Factorial assay (HepG2)	Nuclear receptor and transcription factor activation	Activation of key receptors and transcription factors involved in hepatotoxicity
	High-throughput transcriptomic assay (multiple cell types)	Cellular mRNA	Measures changes in important biological pathways
	High-throughput phenotypic profiling (multiple cell types)	Nuclear, endoplasmic reticulum, nucleoli, golgi, plasma membrane, cytoskeleton, and mitochondria morphology	Changes in cellular organelles and general morphology
Toxicokinetic Parameter	**Assay**	**Assay Endpoints**	**Purpose**
Intrinsic hepatic clearance	Hepatocyte stability assay (primary human hepatocytes)	Time course metabolism of parent chemical	Measure metabolic breakdown by the liver
Plasma protein binding	Ultracentrifugation assay	Fraction of chemical not bound to plasma protein	Measure amount of free chemical in the blood

*Assays being performed by NTP and EPA

Attagene cis- and trans- Factorial Assay



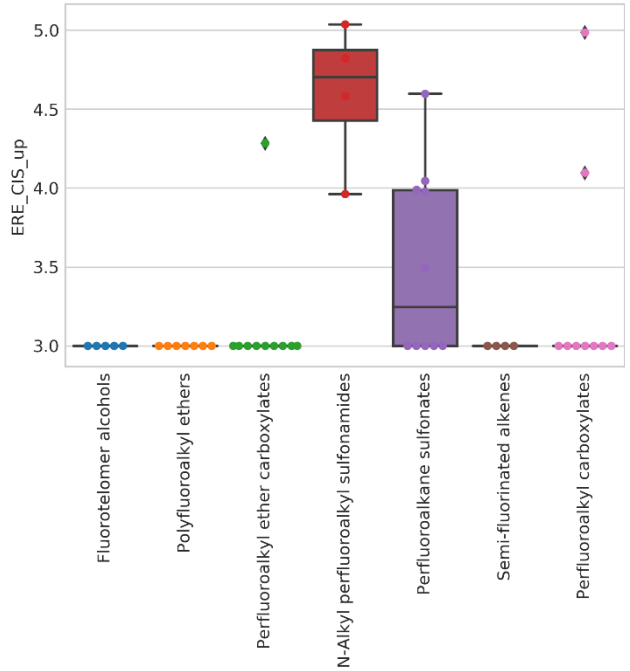
- *CIS* Assay
  - 47 Endogenous Transcription Factors
    - *Xenobiotic pathways*
    - *Cell growth/differentiation*
    - *Endocrine pathways*
    - *Stress response*
- TRANS Assay
  - 24 human nuclear receptors
  - GAL-4 formats (NR ligand-binding domains)
- HepG2 cells
  - Concentration-response testing
  - 24-hour exposure

Keith Houck and Grace Patlewicz

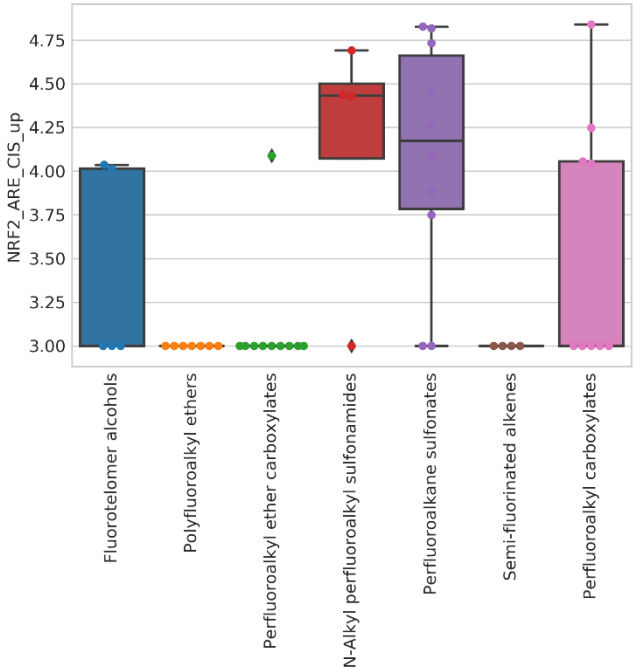
Preliminary Category-Based Analysis of the Attagene Transcription Factor Assay

United States Environmental Protection Agency

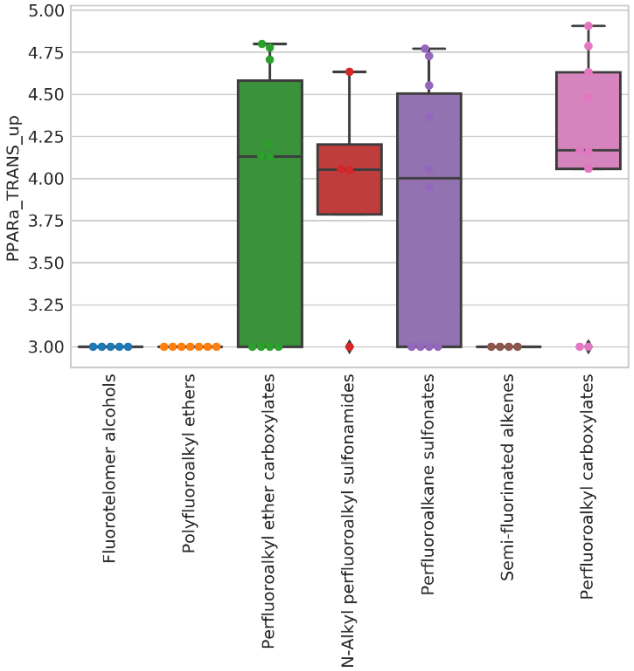
Estrogen Receptor Activity



NRF2 Activity



PPARa Receptor Activity



*Results are preliminary. Chemicals still undergoing analytical QC.

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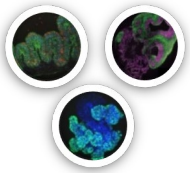
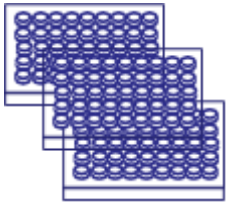
High-Throughput Phenotypic Profiling (aka 'Cellular Pathology')

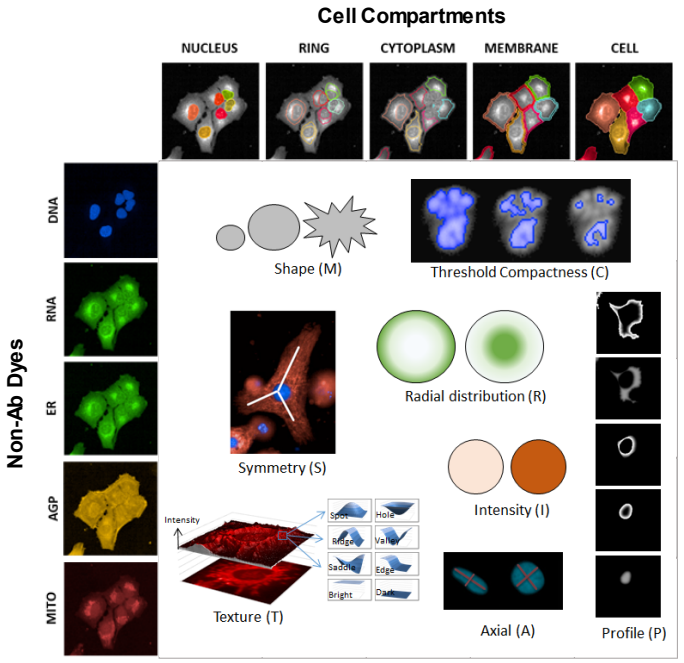
Multiple Cell Types



Concentration Response Screening

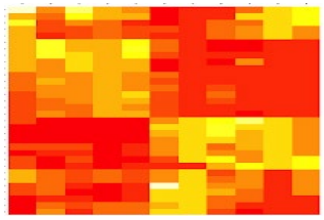


Multi-Parameter Cellular Phenotypic Profiling

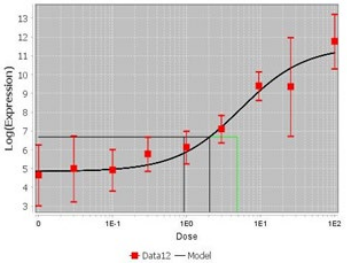


~1,300 endpoints

Mode-of-Action Grouping



Concentration Response Modeling

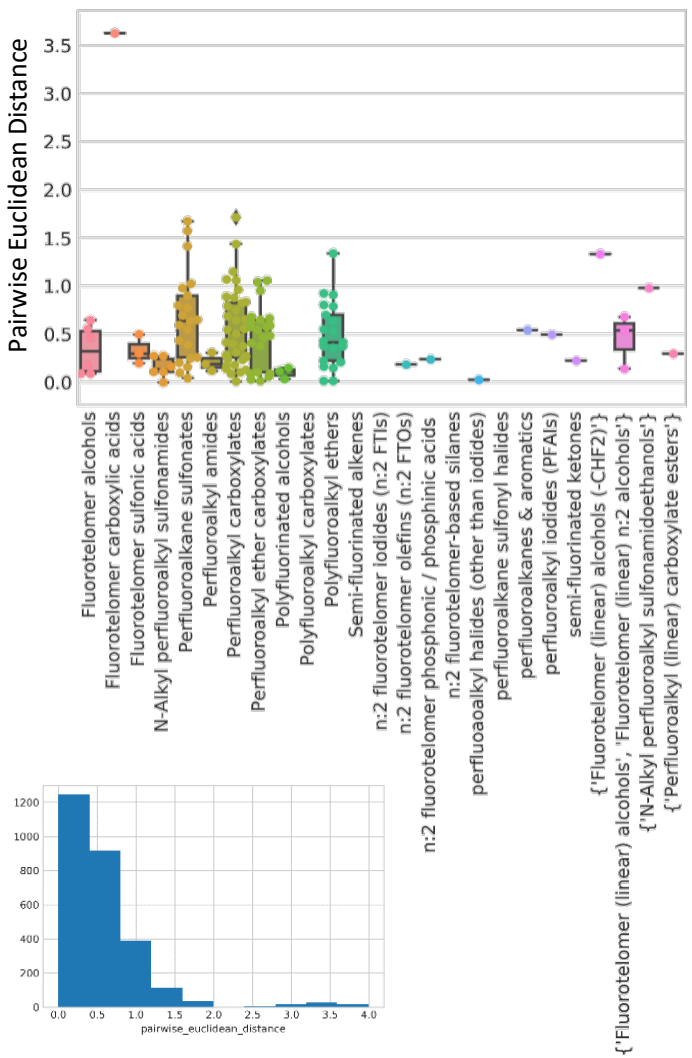


Joshua Harrill and Johanna Nyffeler

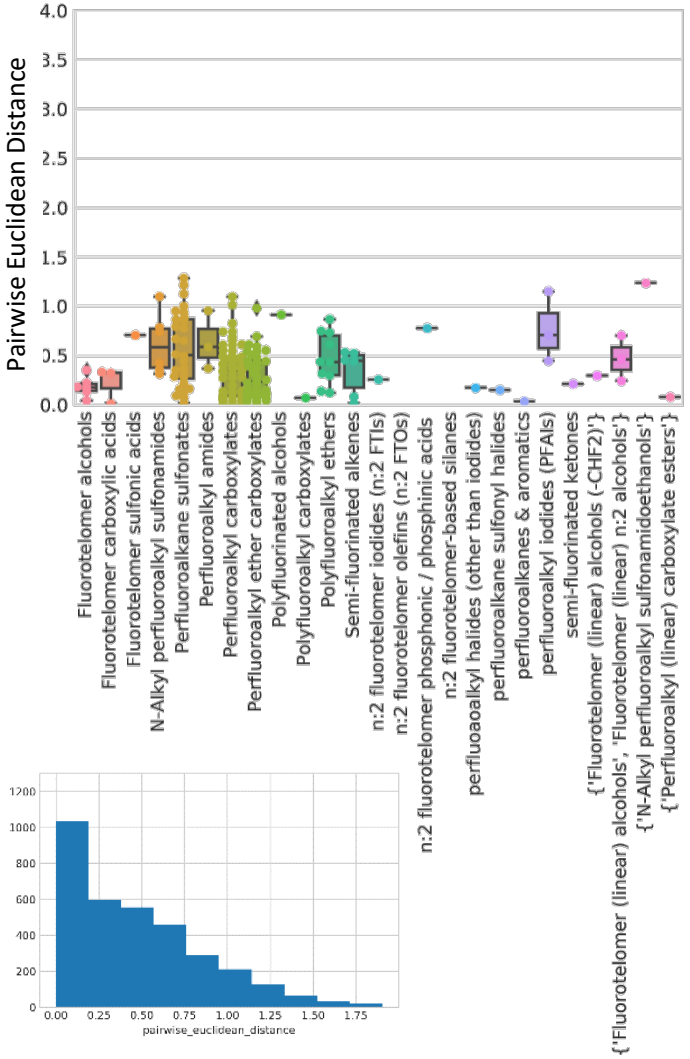
Preliminary Category-Based Analysis of the Phenotypic Profiling Assay

United States Environmental Protection Agency

MCF7 Cells



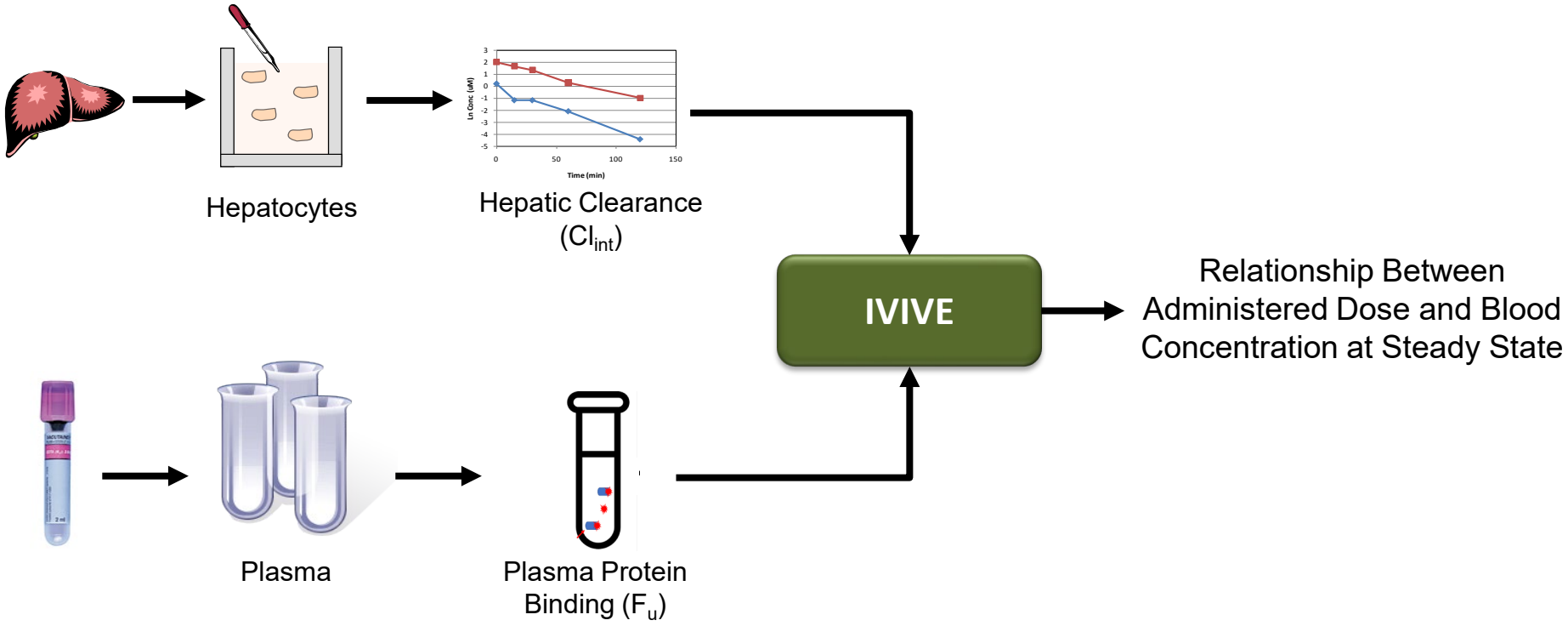
U2OS Cells



*Results are preliminary. Chemicals still undergoing analytical QC.

Joshua Harrill, Johanna Nyffeler, and Grace Patlewicz

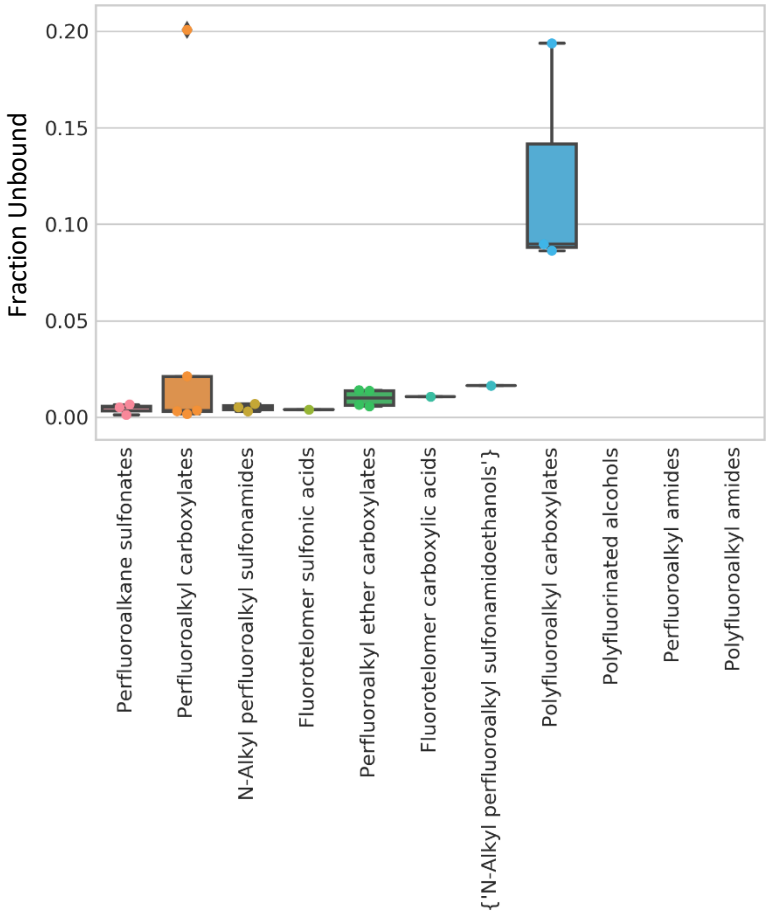
In Vitro Toxicokinetic Assays and *In Vitro*-to-*In Vivo* Extrapolation

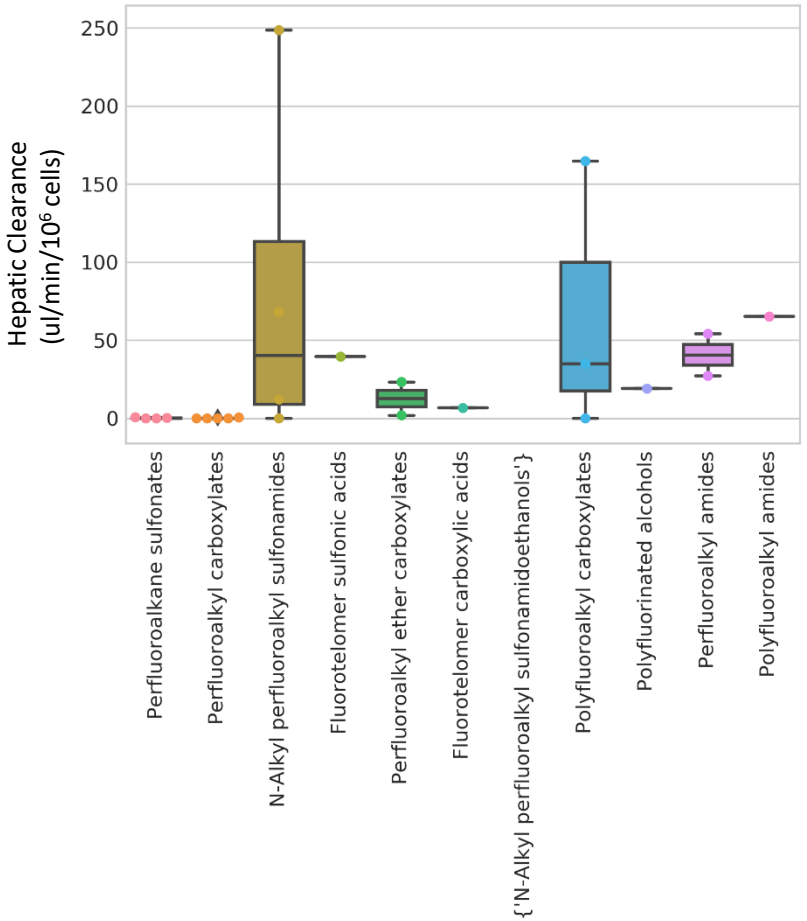


Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012
Wetmore *et al., Tox Sci.,* 2015

David Crizer, See Poster TP050
Barbara Wetmore, See Poster WP011

Preliminary Analysis of the Toxicokinetic Assays

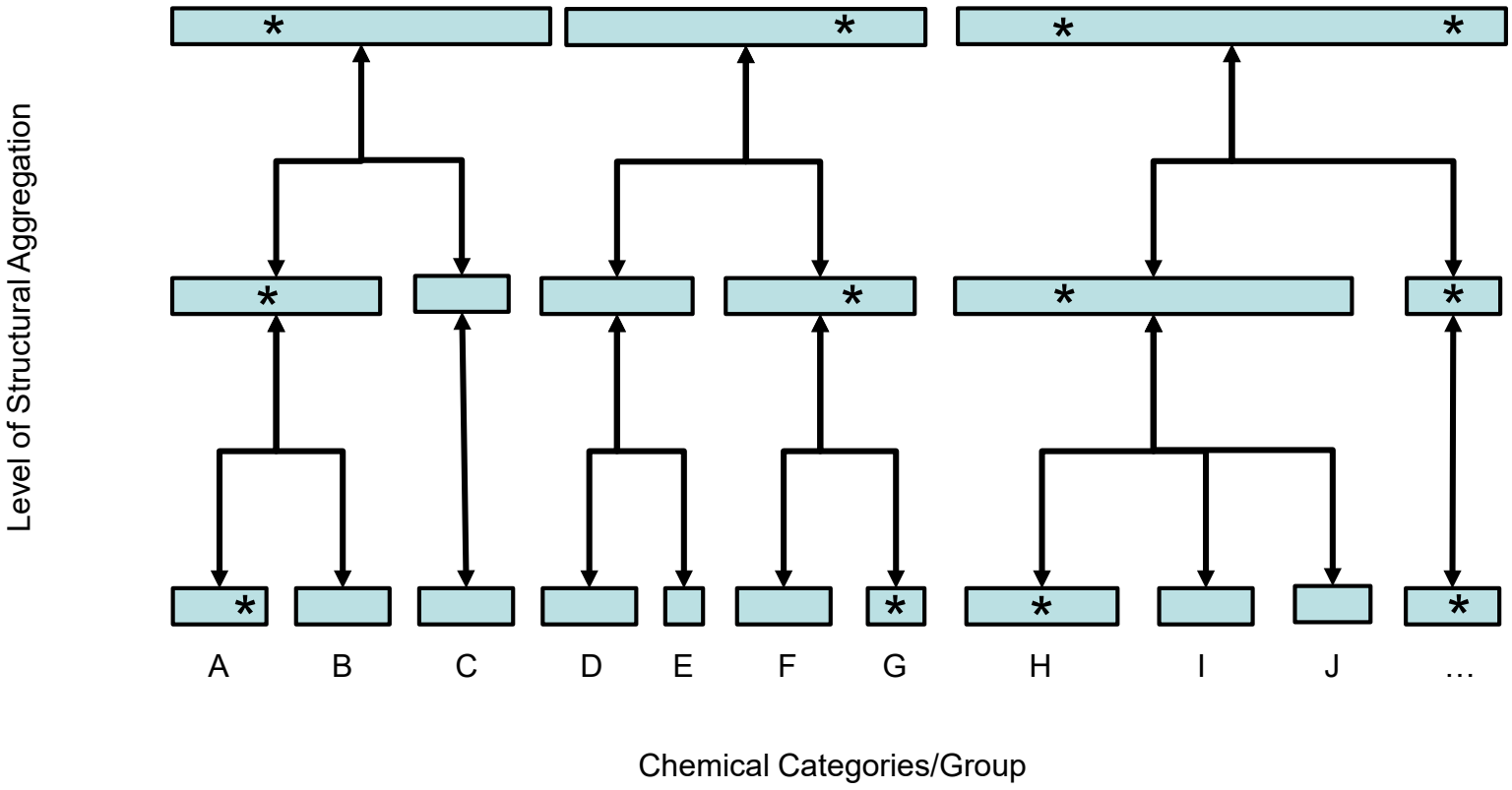




*Results are preliminary. Chemicals still undergoing analytical QC.

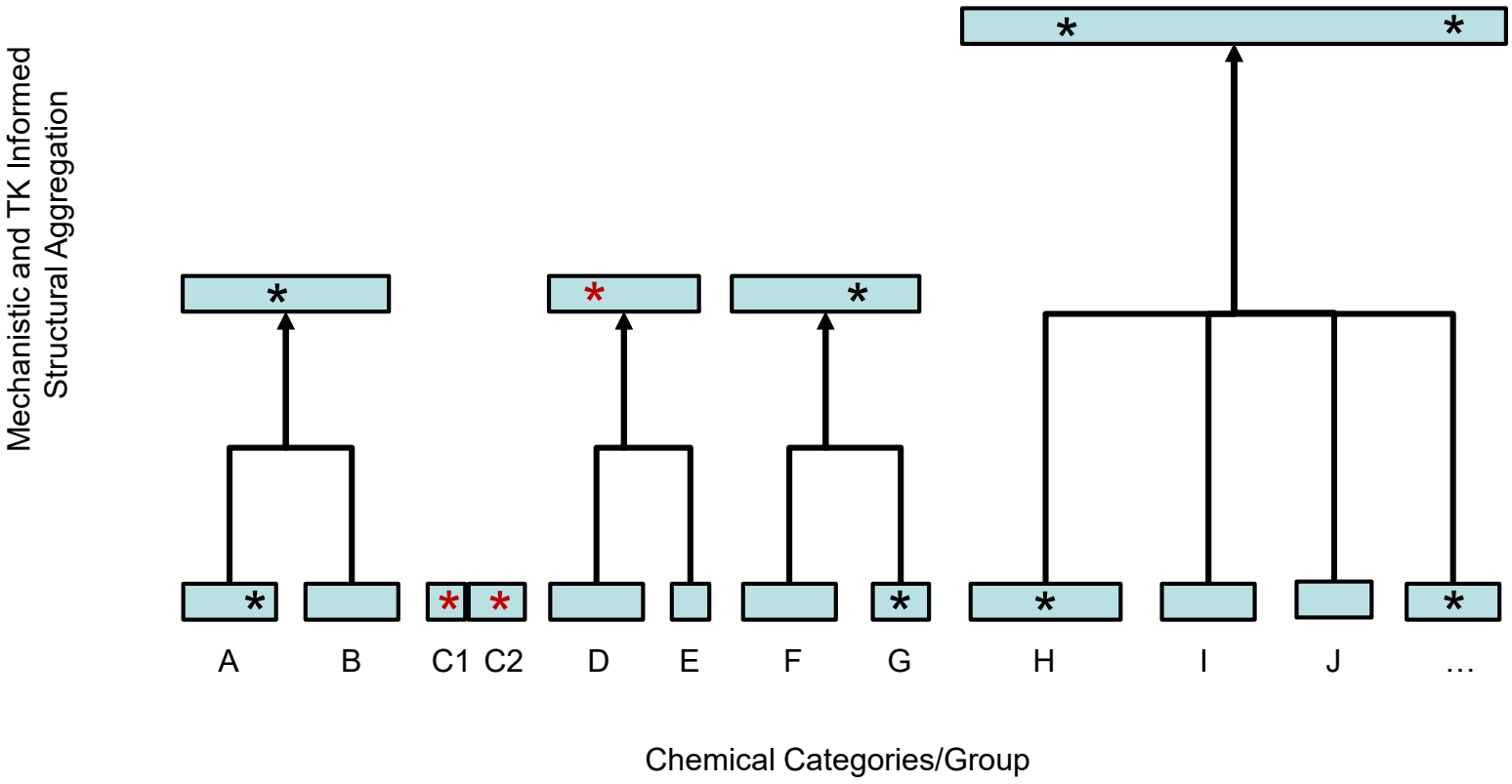
David Crizer, Barbara Wetmore, and Grace Patlewicz

Current PFAS Grouping Approaches Use Different Levels of Aggregation



* Available source *in vivo* tox study

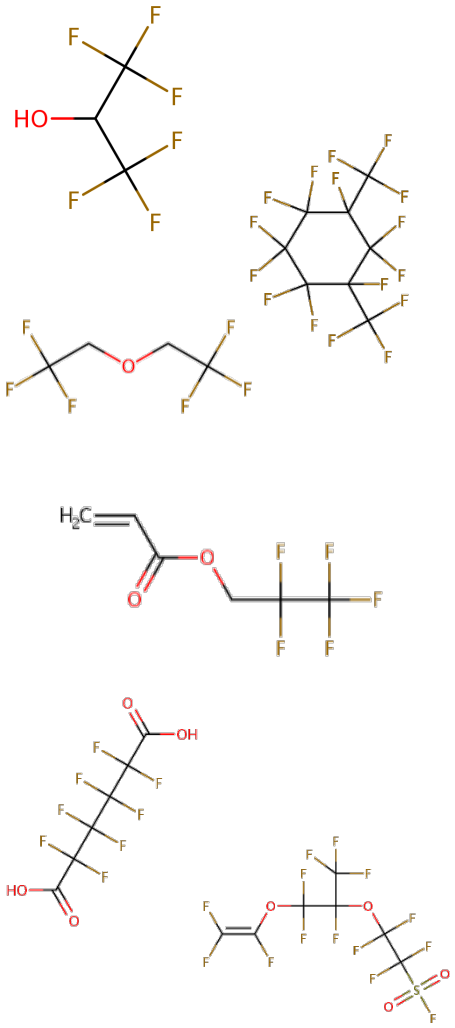
Incorporating Mechanistic and Toxicokinetic Data to Inform PFAS Category Aggregation



* 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

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