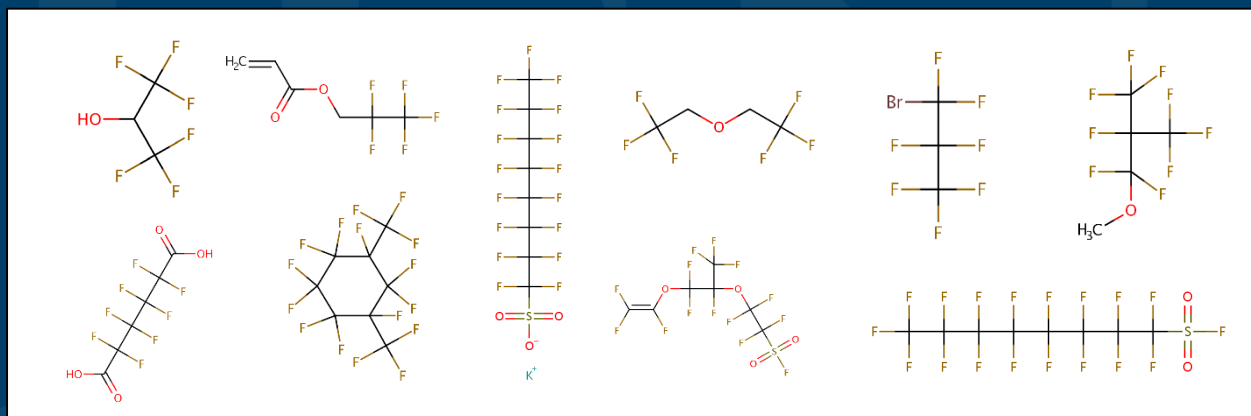


A Chemical Category-Based Approach for Selecting and Screening PFAS for Toxicity and Toxicokinetic Testing



SETAC Environmental Risk Assessment of PFAS Meeting

August 13, 2019

Rusty Thomas

Director

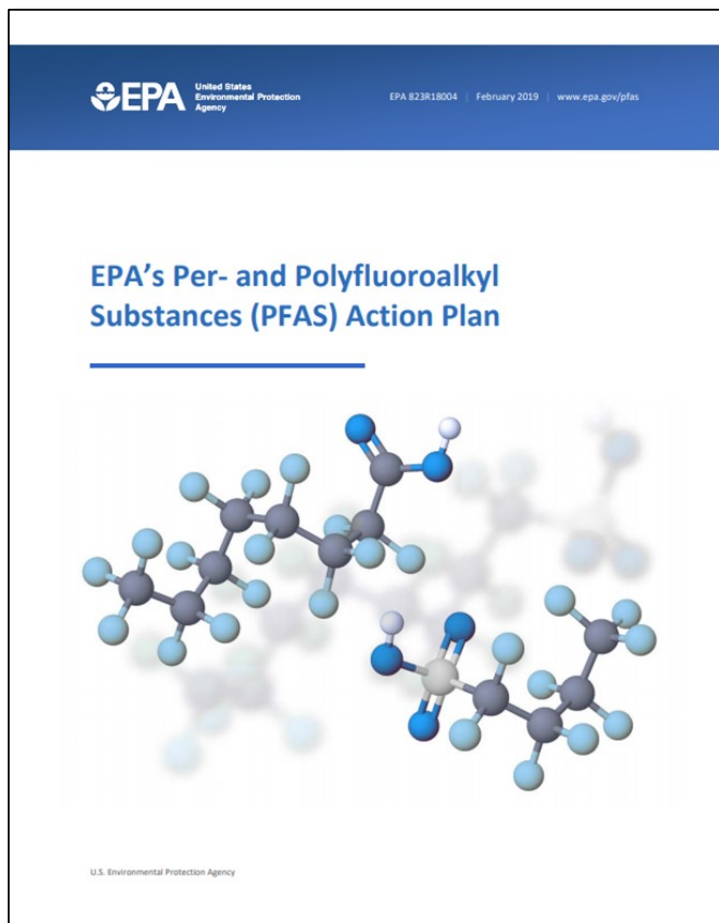
National Center for Computational Toxicology

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

November 26, 2015

S9 | PFASTRIER substances

Trier, Xenia; Lunderberg, David
Other(s)

Schymanski, Emma

This is the collection associated with
<https://www.norman-network.com/>

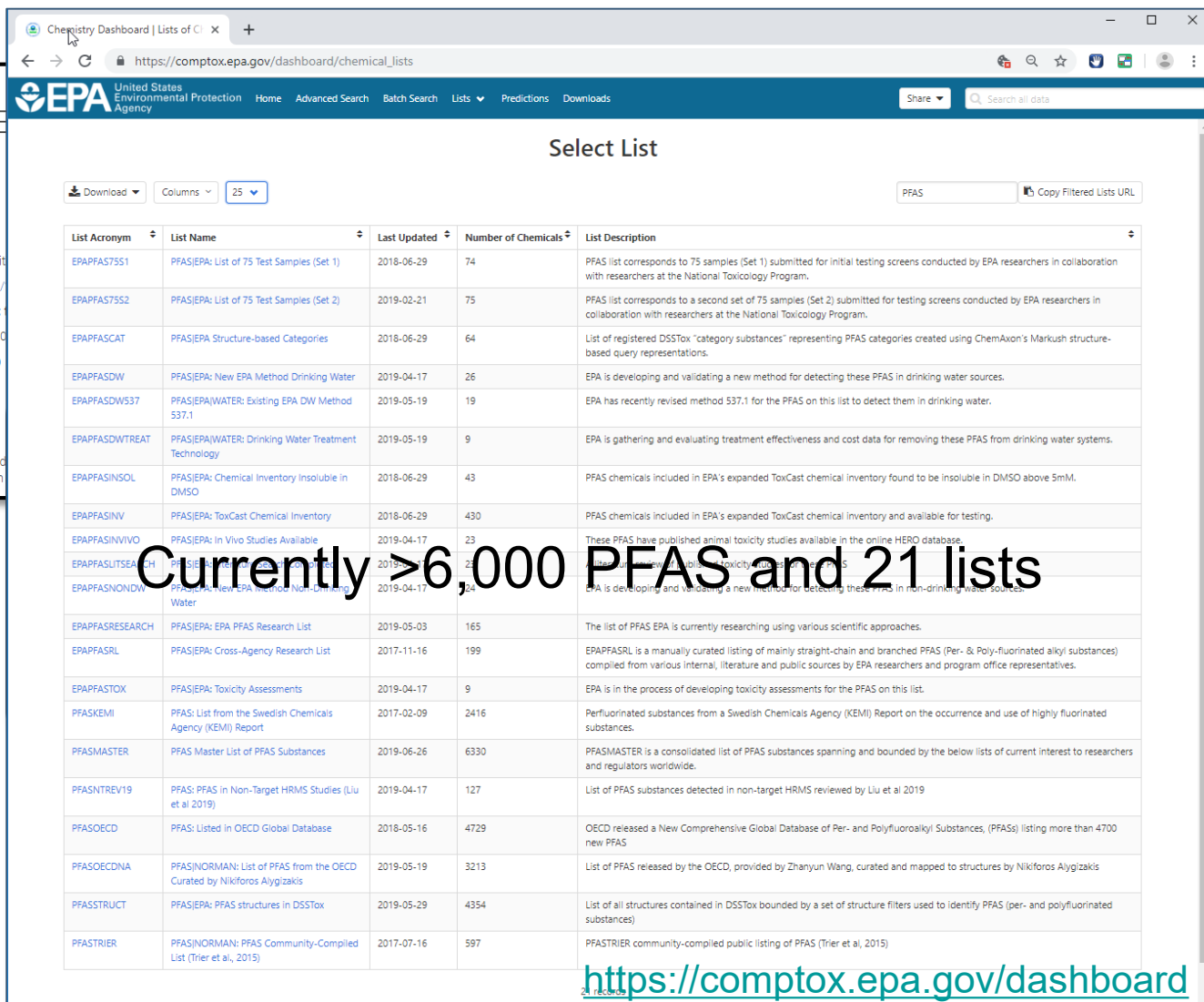
S9 PFASTRIER PFAS Suspect List:
CSV (MassHunter format; 26/11/2015)
XLSX (several sheets; 26/11/2015)

CompTox PFAS TRIER List

Further curation in progress...

PFAS InChIKeys (26/11/2015)

Kindly supplied by Xenia Trier, David
Reference information contained in



Chemistry Dashboard | Lists of Chemicals

United States Environmental Protection Agency

Home Advanced Search Batch Search Lists Predictions Downloads

Search all data

Select List

Download Columns 25 PFAS Copy Filtered Lists URL

List Acronym	List Name	Last Updated	Number of Chemicals	List Description
EPAPFAS75S1	PFAS(EPA): List of 75 Test Samples (Set 1)	2018-06-29	74	PFAS list corresponds to 75 samples (Set 1) submitted for initial testing screens conducted by EPA researchers in collaboration with researchers at the National Toxicology Program.
EPAPFAS75S2	PFAS(EPA): List of 75 Test Samples (Set 2)	2019-02-21	75	PFAS list corresponds to a second set of 75 samples (Set 2) submitted for testing screens conducted by EPA researchers in collaboration with researchers at the National Toxicology Program.
EPAPFASCAT	PFAS(EPA) Structure-based Categories	2018-06-29	64	List of registered DSSTox "category substances" representing PFAS categories created using ChemAxon's Markush structure-based query representations.
EPAPFASDW	PFAS(EPA): New EPA Method Drinking Water	2019-04-17	26	EPA is developing and validating a new method for detecting these PFAS in drinking water sources.
EPAPFASDW537	PFAS(EPA)(WATER): Existing EPA DW Method 537.1	2019-05-19	19	EPA has recently revised method 537.1 for the PFAS on this list to detect them in drinking water.
EPAPFASDWTREAT	PFAS(EPA)(WATER): Drinking Water Treatment Technology	2019-05-19	9	EPA is gathering and evaluating treatment effectiveness and cost data for removing these PFAS from drinking water systems.
EPAPFASINSOL	PFAS(EPA): Chemical Inventory Insoluble in DMSO	2018-06-29	43	PFAS chemicals included in EPA's expanded ToxCast chemical inventory found to be insoluble in DMSO above 5mM.
EPAPFASINV	PFAS(EPA): ToxCast Chemical Inventory	2018-06-29	430	PFAS chemicals included in EPA's expanded ToxCast chemical inventory and available for testing.
EPAPFASINVIVO	PFAS(EPA): In Vivo Studies Available	2019-04-17	23	These PFAS have published animal toxicity studies available in the online HERO database.
EPAPFASUTSEARCH	PFAS(EPA): New EPA Method Non-Drinking Water	2019-04-17	24	EPA is developing and validating a new method for detecting these PFAS in non-drinking water sources.
EPAPFASNONDW	PFAS(EPA): New EPA Method Non-Drinking Water	2019-04-17	24	EPA is developing and validating a new method for detecting these PFAS in non-drinking water sources.
EPAPFASRESEARCH	PFAS(EPA): EPA PFAS Research List	2019-05-03	165	The list of PFAS EPA is currently researching using various scientific approaches.
EPAPFASRL	PFAS(EPA): Cross-Agency Research List	2017-11-16	199	EPAPFASRL is a manually curated listing of mainly straight-chain and branched PFAS (Per- & Poly-fluorinated alkyl substances) compiled from various internal, literature and public sources by EPA researchers and program office representatives.
EPAPFASTOX	PFAS(EPA): Toxicity Assessments	2019-04-17	9	EPA is in the process of developing toxicity assessments for the PFAS on this list.
PFASKEMI	PFAS: List from the Swedish Chemicals Agency (KEMI) Report	2017-02-09	2416	Perfluorinated substances from a Swedish Chemicals Agency (KEMI) Report on the occurrence and use of highly fluorinated substances.
PFASMASTER	PFAS Master List of PFAS Substances	2019-06-26	6330	PFASMASTER is a consolidated list of PFAS substances spanning and bounded by the below lists of current interest to researchers and regulators worldwide.
PFASNTREV19	PFAS: PFAS in Non-Target HRMS Studies (Liu et al 2019)	2019-04-17	127	List of PFAS substances detected in non-target HRMS reviewed by Liu et al 2019
PFASOECD	PFAS: Listed in OECD Global Database	2018-05-16	4729	OECD released a New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs) listing more than 4700 new PFAS
PFASOECDNA	PFAS(NORMAN): List of PFAS from the OECD Curated by Nikiforos Aiygizakis	2019-05-19	3213	List of PFAS released by the OECD, provided by Zhanyun Wang, curated and mapped to structures by Nikiforos Aiygizakis
PFASSTRUCT	PFAS(EPA): PFAS structures in DSSTox	2019-05-29	4354	List of all structures contained in DSSTox bounded by a set of structure filters used to identify PFAS (per- and polyfluorinated substances)
PFASTRIER	PFAS(NORMAN): PFAS Community-Compiled List (Trier et al, 2015)	2017-07-16	597	PFASTRIER community-compiled public listing of PFAS (Trier et al, 2015)

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

MMONO(2018)7

English - Or. English
4 May 2018

WORKING PARTY

F PER- AND

- AND

onym Unique_Acronym

S-3 PFOA

MeFOSE NMeFOSE

duct 2 PFESA Byproduct 2

ices

ADONA parent acid

ADONA

PFBS-K

PFBS

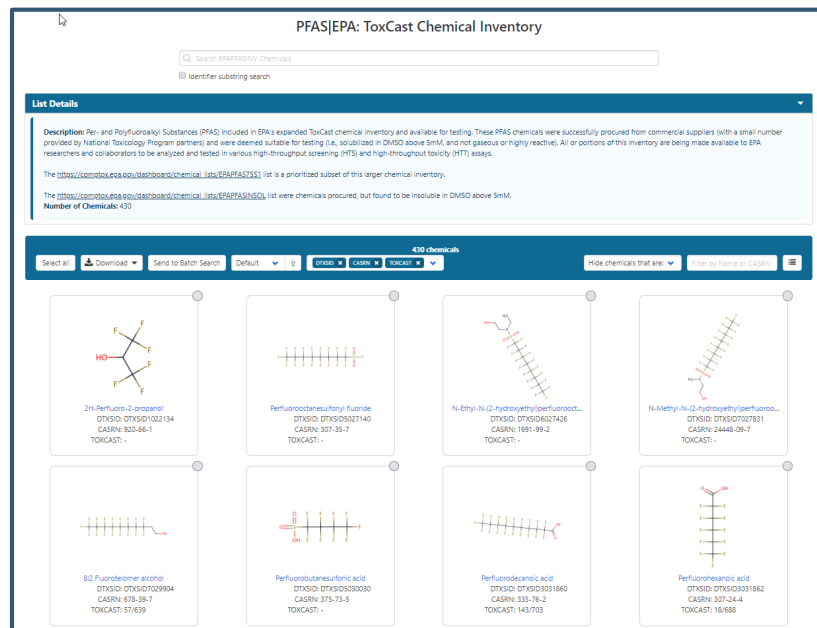
PFBS_ion

PFDS

PFDS_ion

PFDS-Na

Assembled a PFAS Chemical Library for Research and Methods Development



PFAS|EPA: ToxCast Chemical Inventory

Search: PFAS|EPA: ToxCast Chemicals

Identifier: substructure search

List Details

Description: Per- and Polyfluoroalkyl Substances (PFAS) included in EPA's expanded ToxCast chemical inventory and available for testing. These PFAS chemicals were successfully procured from commercial suppliers (with a small number provided by National Toxicology Program partners) and were deemed suitable for testing (i.e., solubilized in DMSO above 5mM, and not gaseous or highly reactive). All or portions of this inventory are being made available to EPA researchers and collaborators to be analyzed and tested in various high-throughput screening (HTS) and high-throughput toxicity (HTT) assays.

The https://comptox.epa.gov/dashboard/chemical_lists/PFAS57351 list is a prioritized subset of this larger chemical inventory.

The https://comptox.epa.gov/dashboard/chemical_lists/PFAS57351 list were chemicals procured, but found to be insoluble in DMSO above 5mM.

Number of Chemicals: 430

430 chemicals

Select all Download Send to Batch Search Default 0 DTXSID CASRN TOXCAST

Hide chemicals that are: Filter by Name or CASRN

2H-Perfluoro-2-propanol
DTXSID: DTXSID1022134
CASRN: 302-46-1
TOXCAST: -

Perfluorooctanesulfonyl fluoride
DTXSID: DTXSID0527140
CASRN: 307-25-7
TOXCAST: -

N-Ethyl-N-(2-hydroxyethyl)perfluoro...
DTXSID: DTXSID0527426
CASRN: 1891-89-2
TOXCAST: -

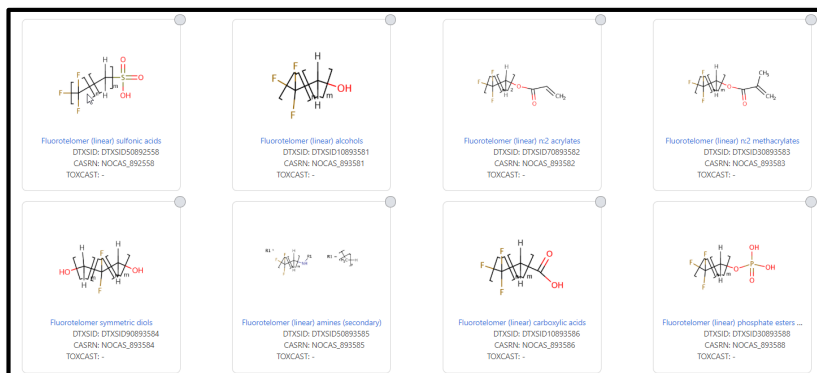
N-Methyl-N-(2-hydroxyethyl)perfluoro...
DTXSID: DTXSID0527831
CASRN: 2446-88-7
TOXCAST: -

8:2 Fluorotelomer alcohol
DTXSID: DTXSID0709904
CASRN: 678-39-7
TOXCAST: 57439

Perfluorobutanesulfonic acid
DTXSID: DTXSID0303090
CASRN: 375-75-5
TOXCAST: -

Perfluorodecanoic acid
DTXSID: DTXSID03031860
CASRN: 307-24-4
TOXCAST: 143703

Perfluorohexanoic acid
DTXSID: DTXSID03031862
CASRN: 307-24-4
TOXCAST: 18468



Fluorotelomer (linear) sulfonic acids
DTXSID: DTXSID05092558
CASRN: NOCAS_892558
TOXCAST: -

Fluorotelomer (linear) alcohols
DTXSID: DTXSID10893581
CASRN: NOCAS_893581
TOXCAST: -

Fluorotelomer (linear) n:2 acrylates
DTXSID: DTXSID07093582
CASRN: NOCAS_893582
TOXCAST: -

Fluorotelomer (linear) n:2 methacrylates
DTXSID: DTXSID07093583
CASRN: NOCAS_893583
TOXCAST: -

Fluorotelomer symmetric diols
DTXSID: DTXSID09093584
CASRN: NOCAS_893584
TOXCAST: -

Fluorotelomer (linear) amines (secondary)
DTXSID: DTXSID09093585
CASRN: NOCAS_893585
TOXCAST: -

Fluorotelomer (linear) carboxylic acids
DTXSID: DTXSID10893586
CASRN: NOCAS_893586
TOXCAST: -

Fluorotelomer (linear) phosphate esters ...
DTXSID: DTXSID09093588
CASRN: NOCAS_893588
TOXCAST: -

- 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

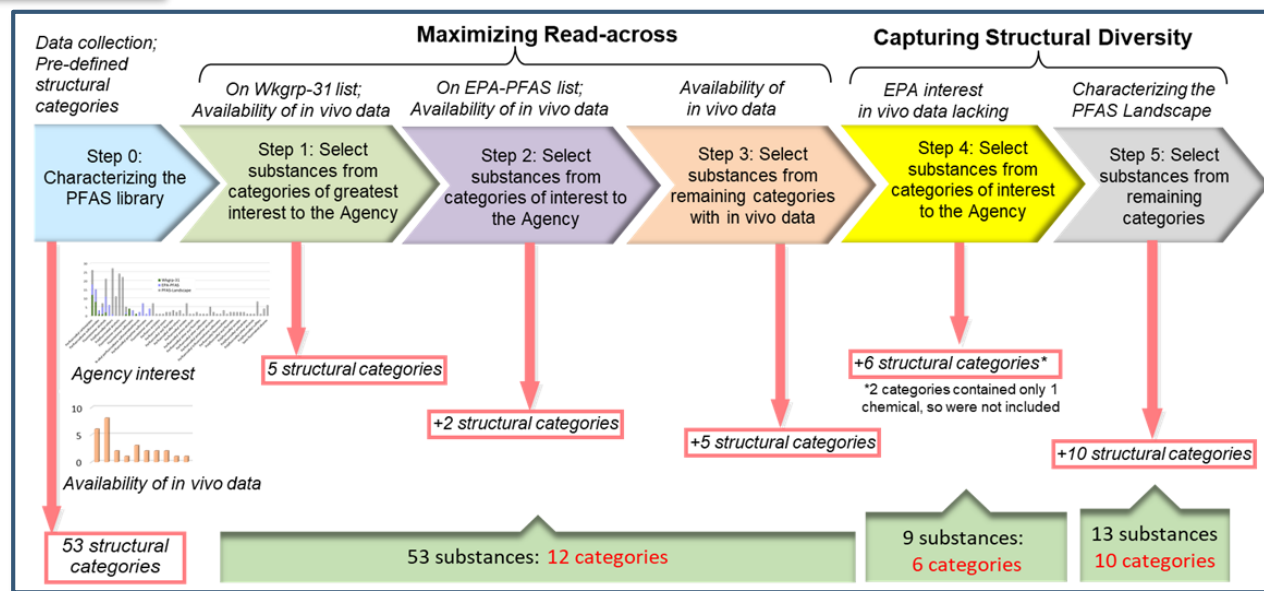


Goals:

- 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

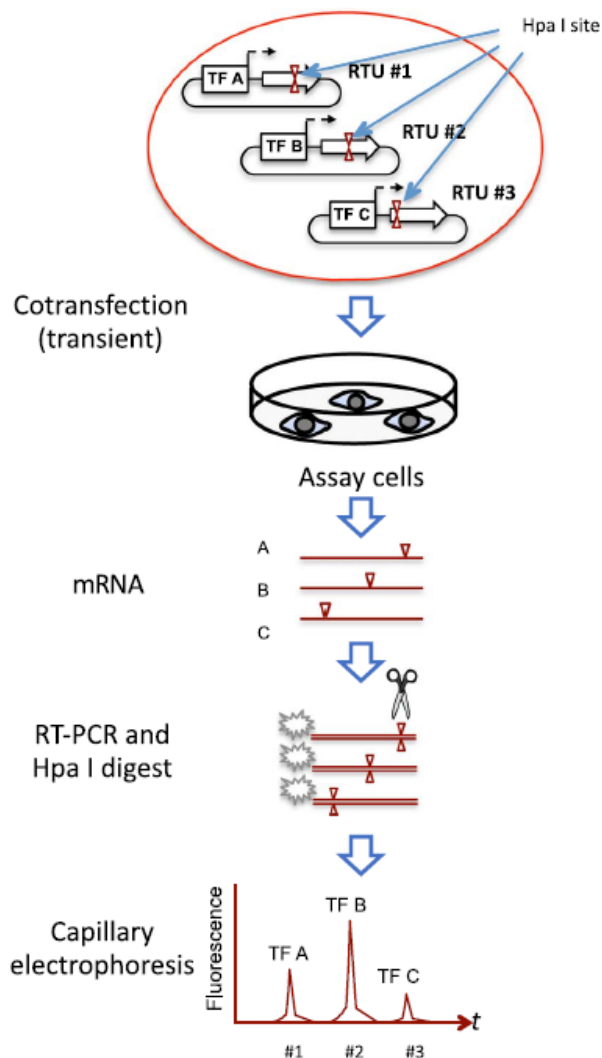


In Vitro Toxicity and Toxicokinetic Testing

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

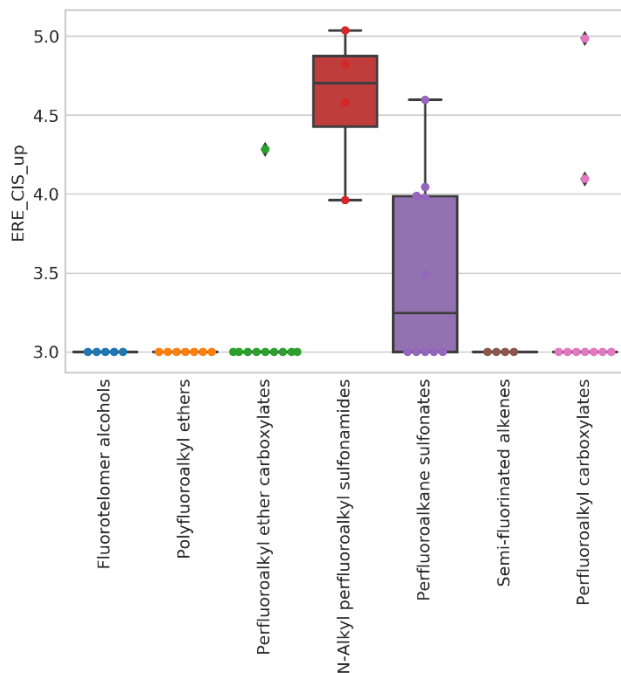
Attagene cis- and trans- Factorial Assay



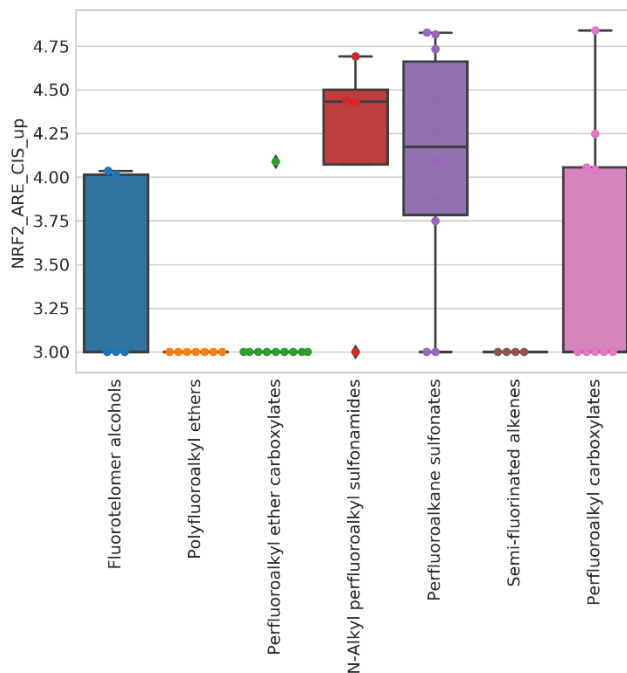
- **CIS Assay**
 - 47 Endogenous Transcription Factors
 - *Xenobiotic pathways*
 - *Cell growth/differentiation*
 - *Endocrine pathways*
 - *Stress response*
- **TRANS Assay**
 - 24 human nuclear receptors
 - GAL-4 formats (NR ligand-binding domains)
- **HepG2 cells**
 - Concentration-response testing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

Preliminary Category-Based Analysis of the Attagene Transcription Factor Assay

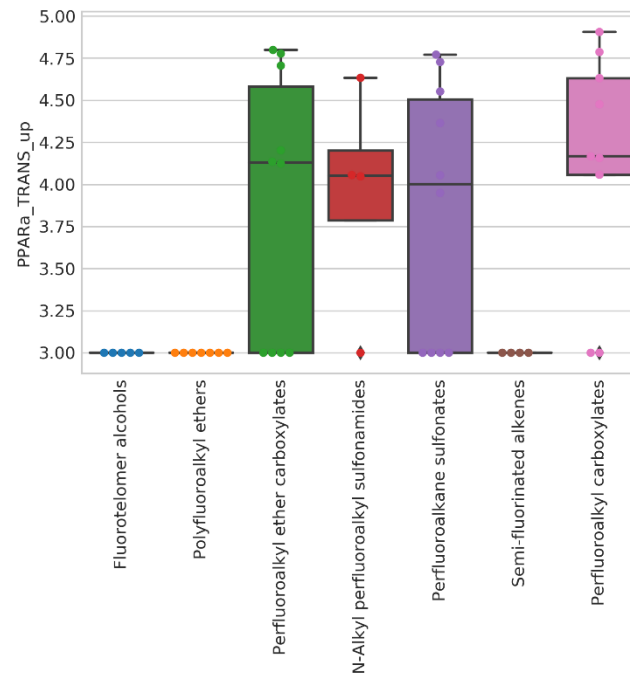
Estrogen Receptor Activity



NRF2 Activity

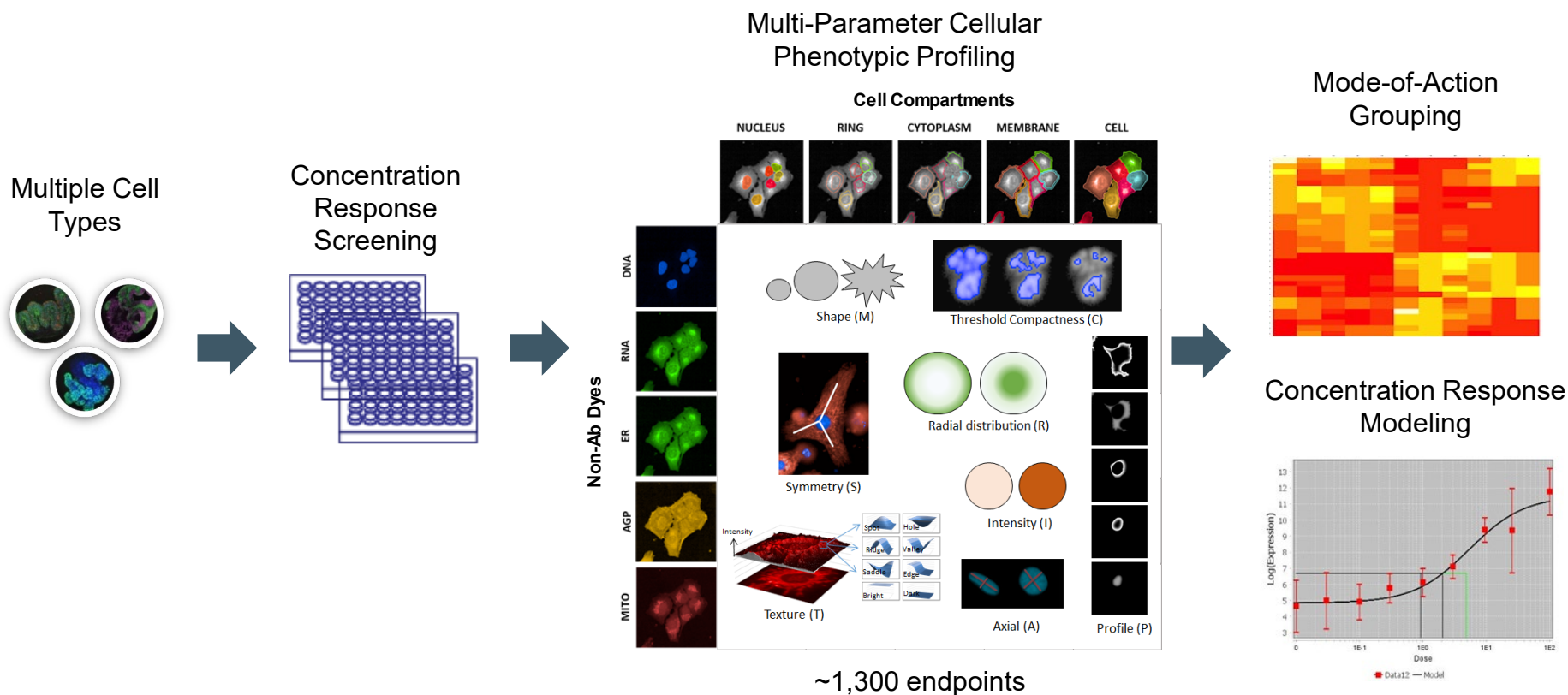


PPARα Receptor Activity



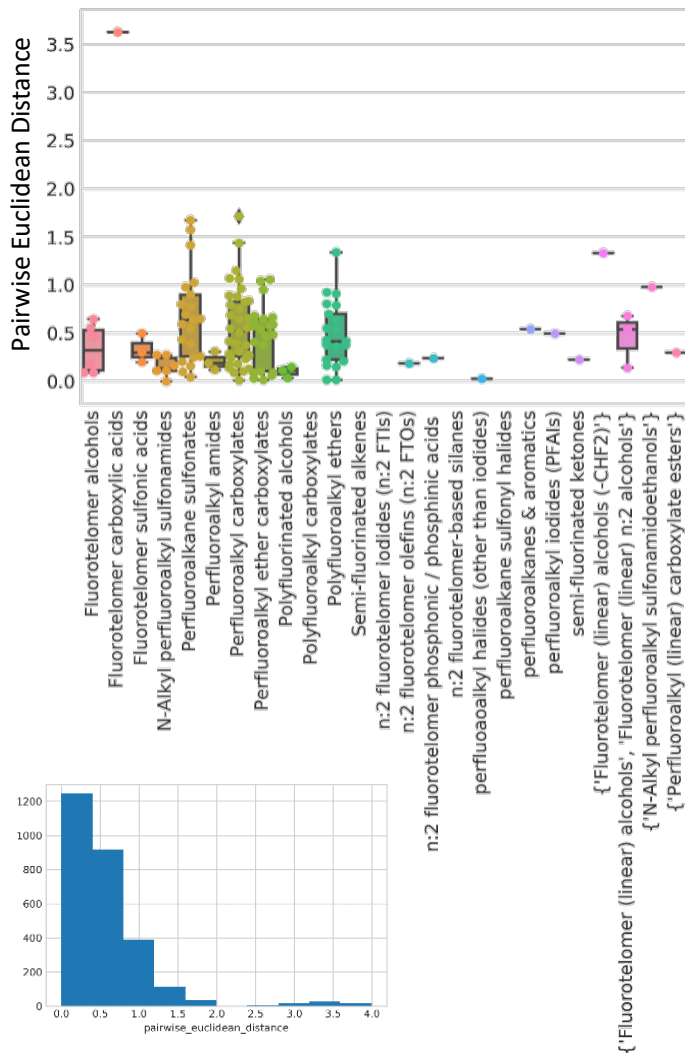
*Results are preliminary. Chemicals still undergoing analytical QC.

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

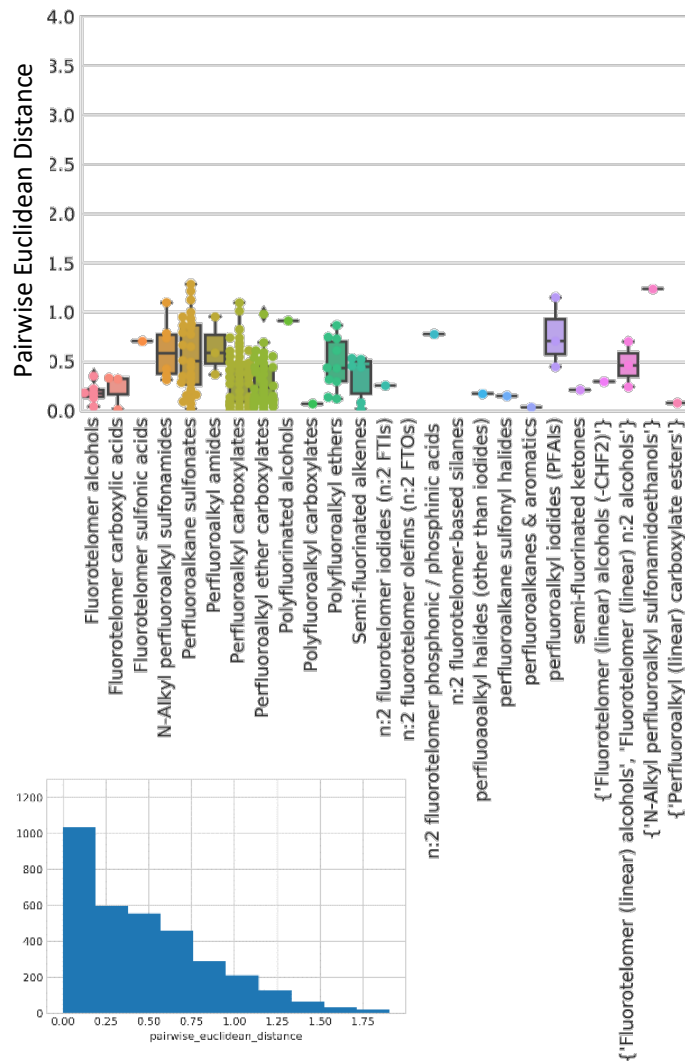


Preliminary Category-Based Analysis of the Phenotypic Profiling Assay

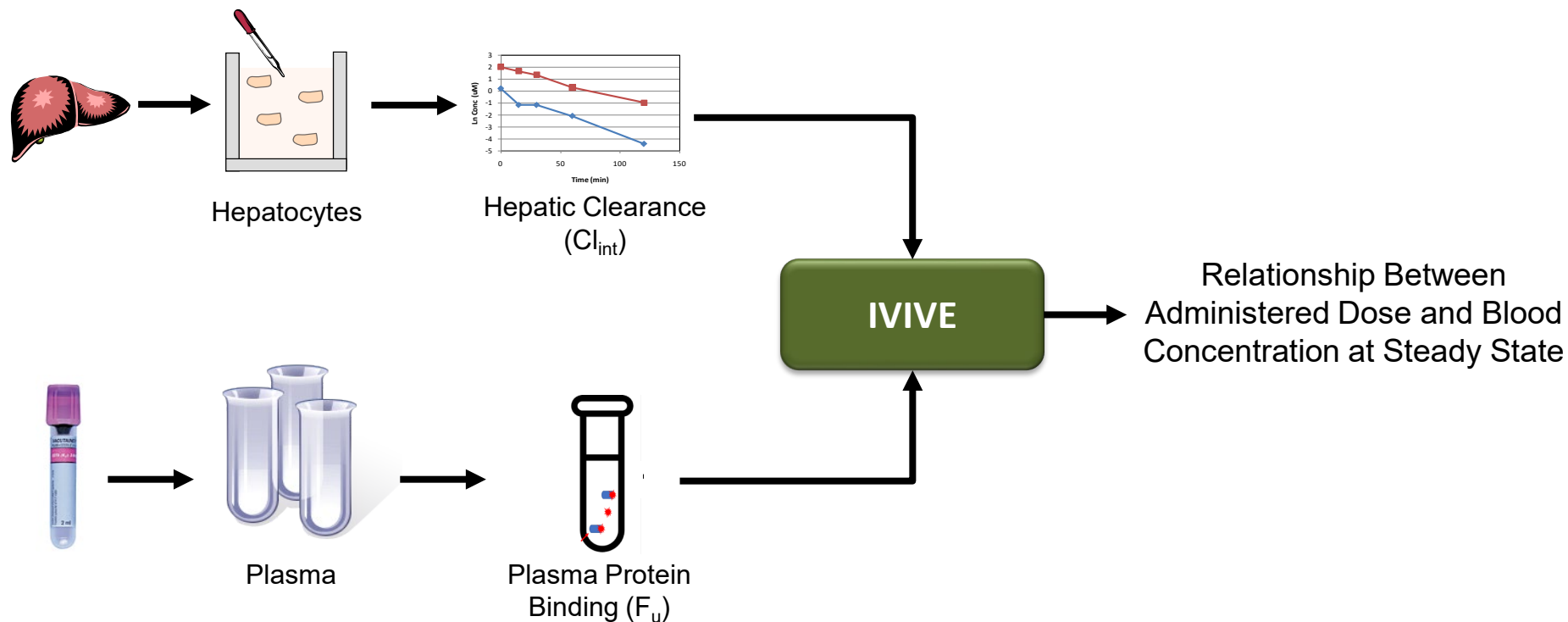
MCF7 Cells



U2OS Cells

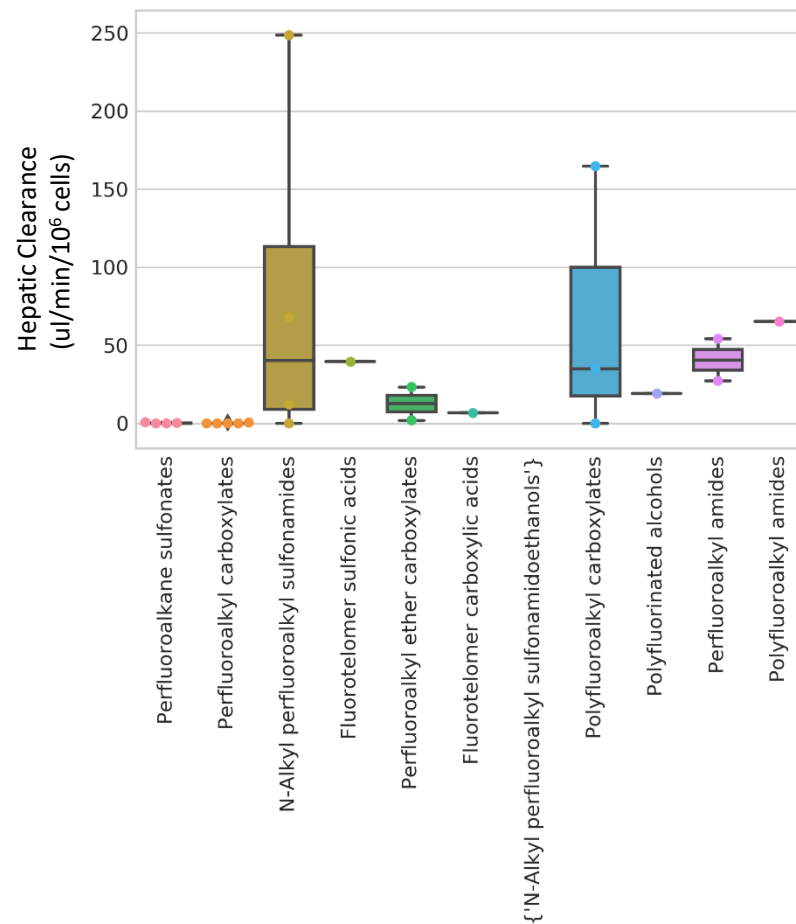
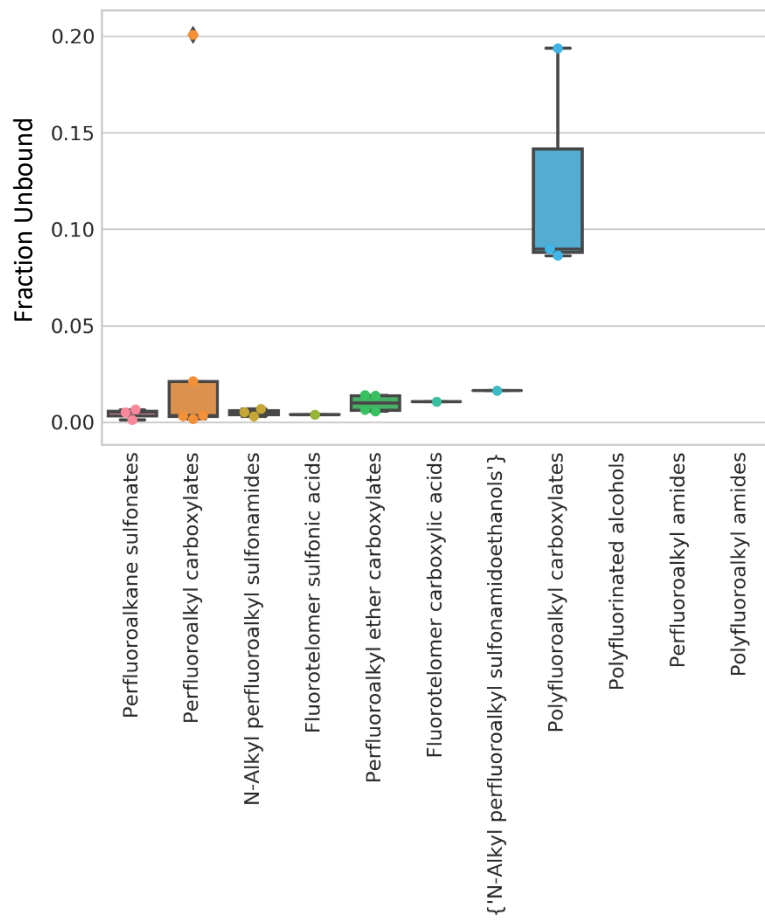


In Vitro Toxicokinetic Assays and *In Vitro*-to-*In Vivo* Extrapolation



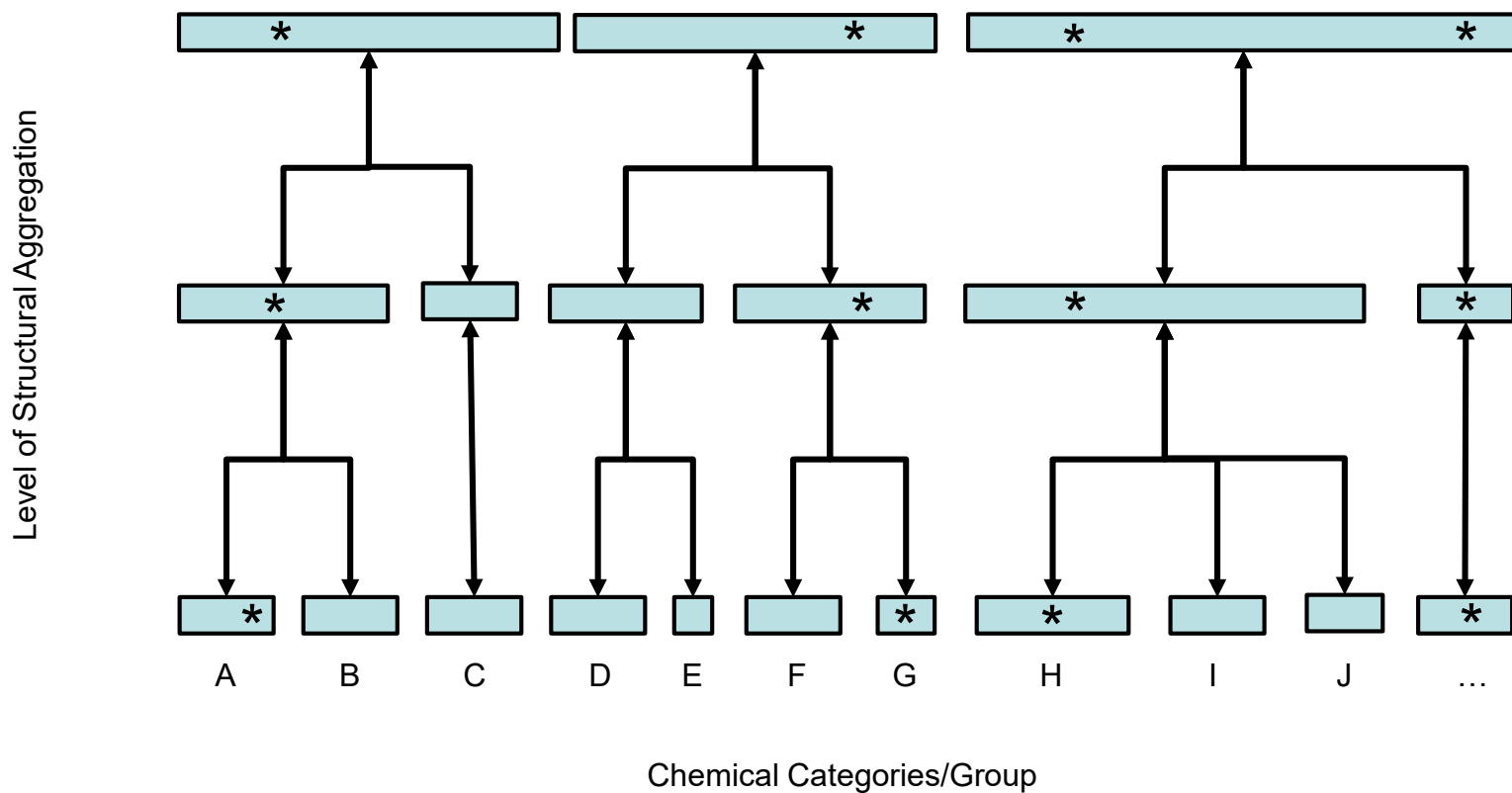
Rotroff *et al.*, *Tox Sci.*, 2010
Wetmore *et al.*, *Tox Sci.*, 2012
Wetmore *et al.*, *Tox Sci.*, 2015

Preliminary Analysis of the Toxicokinetic Assays



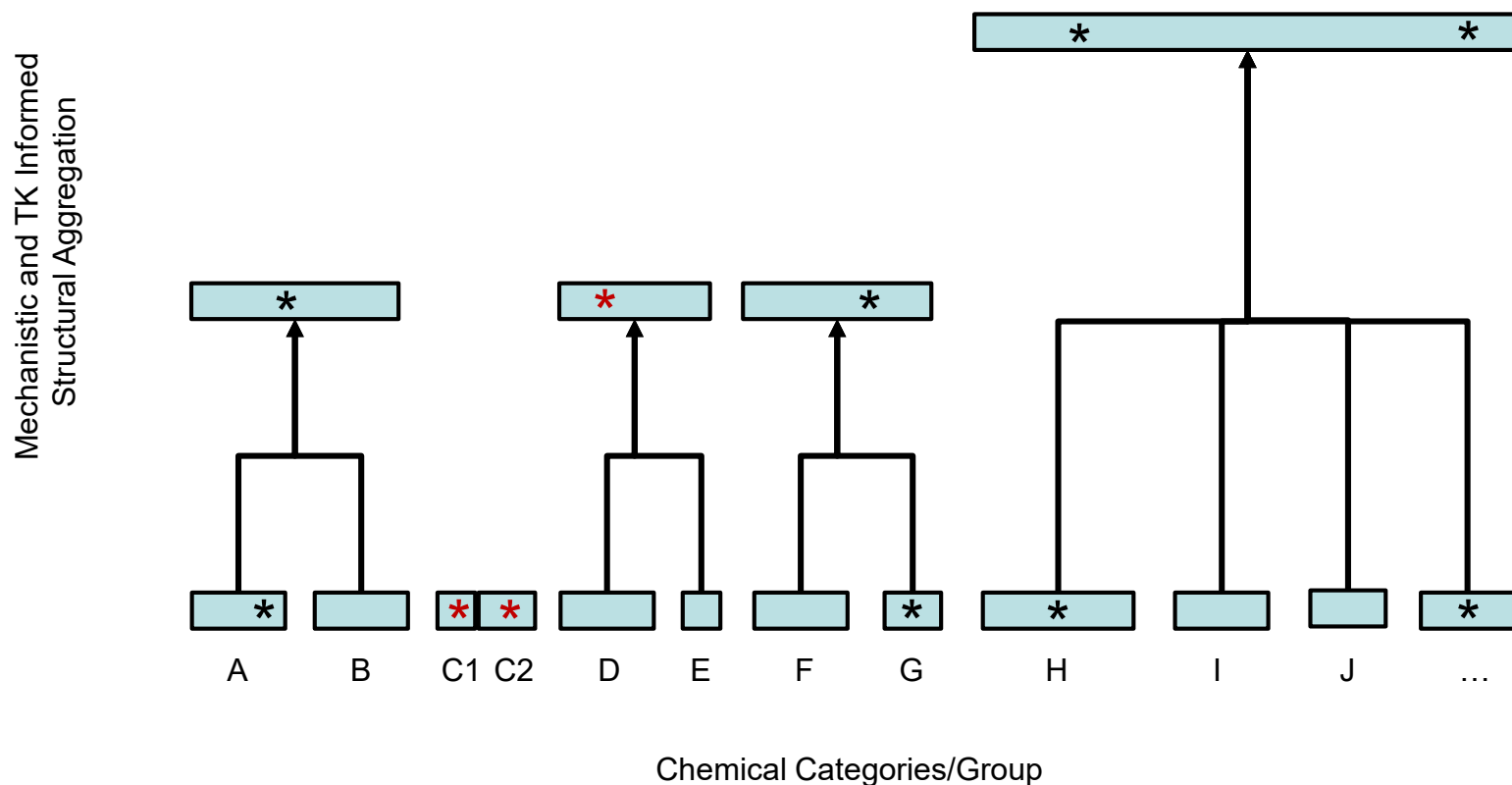
*Results are preliminary. Chemicals still undergoing analytical QC.

Current PFAS Grouping Approaches Use Different Levels of Aggregation



* Available source *in vivo* tox study

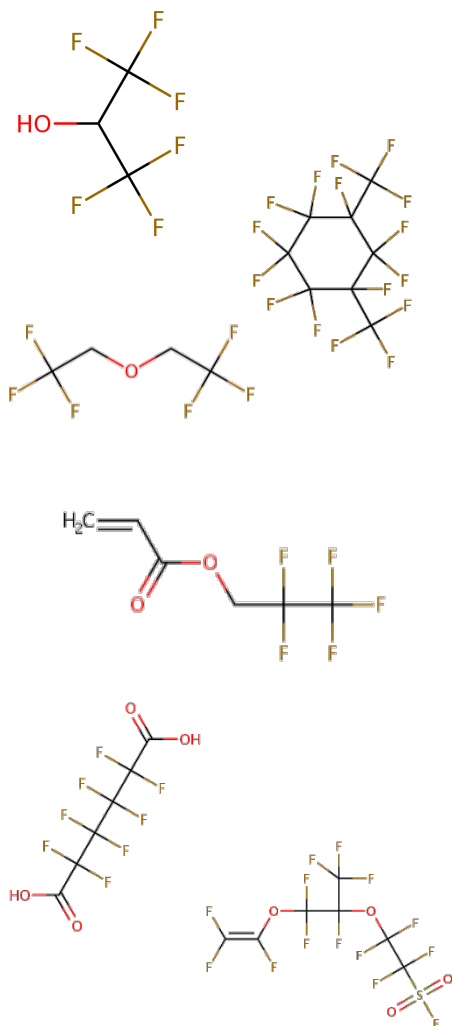
Incorporating Mechanistic and Toxicokinetic Data to Inform PFAS Category Aggregation



* 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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