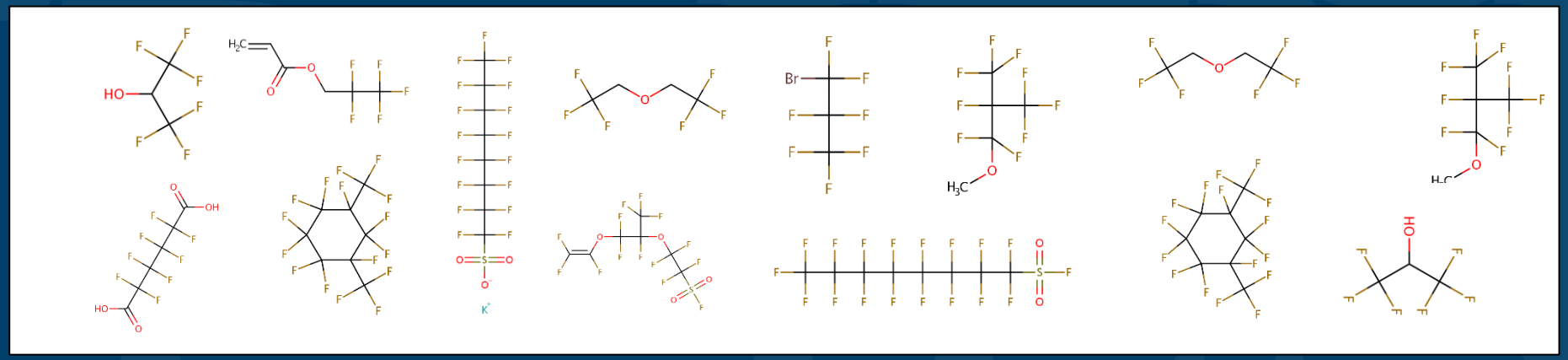
ORD's Tiered Toxicity Testing Strategy for PFAS



EPA Executive BOSC Meeting

September 29, 2021

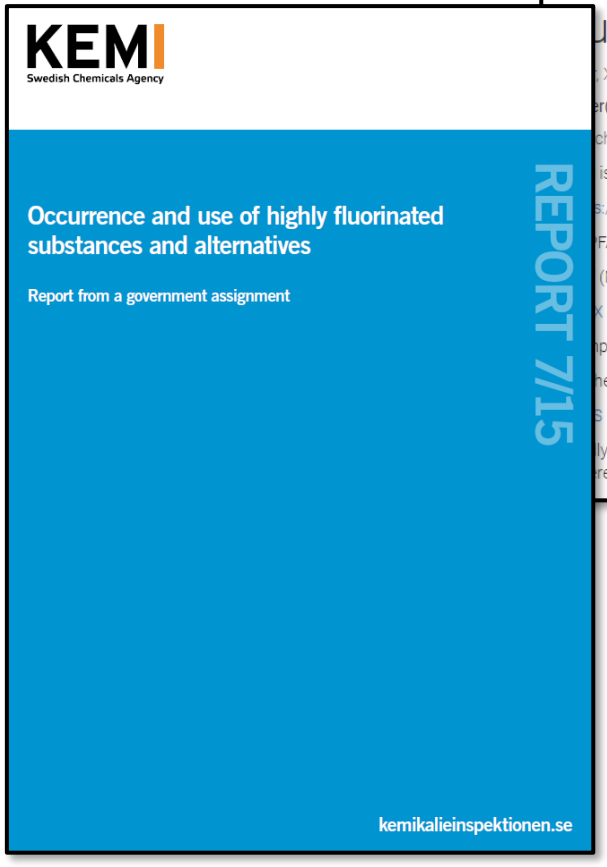
Rusty Thomas
Director, Center for Computational Toxicology and Exposure
Office of Research and Development

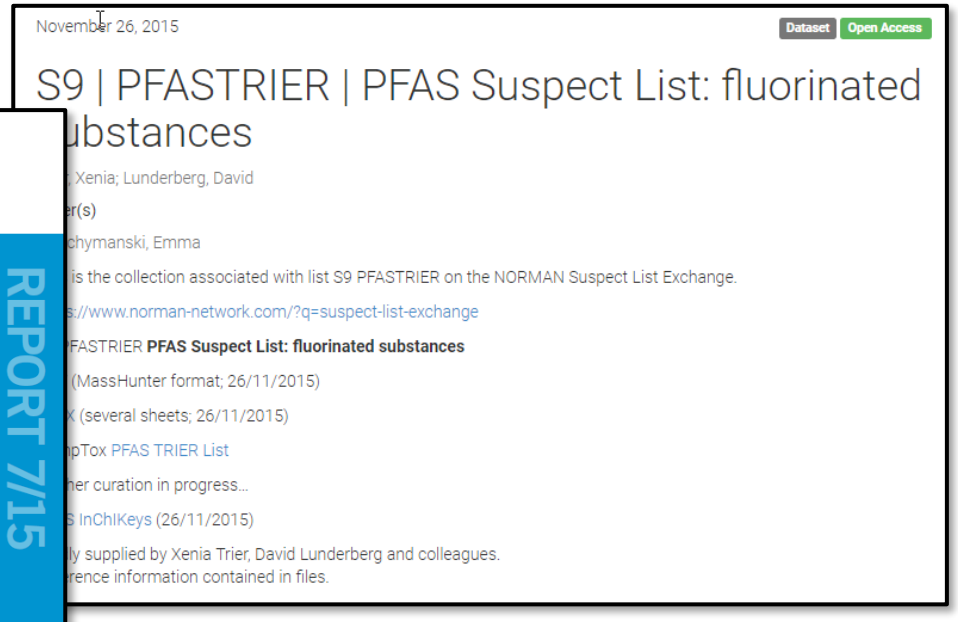
The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. EPA

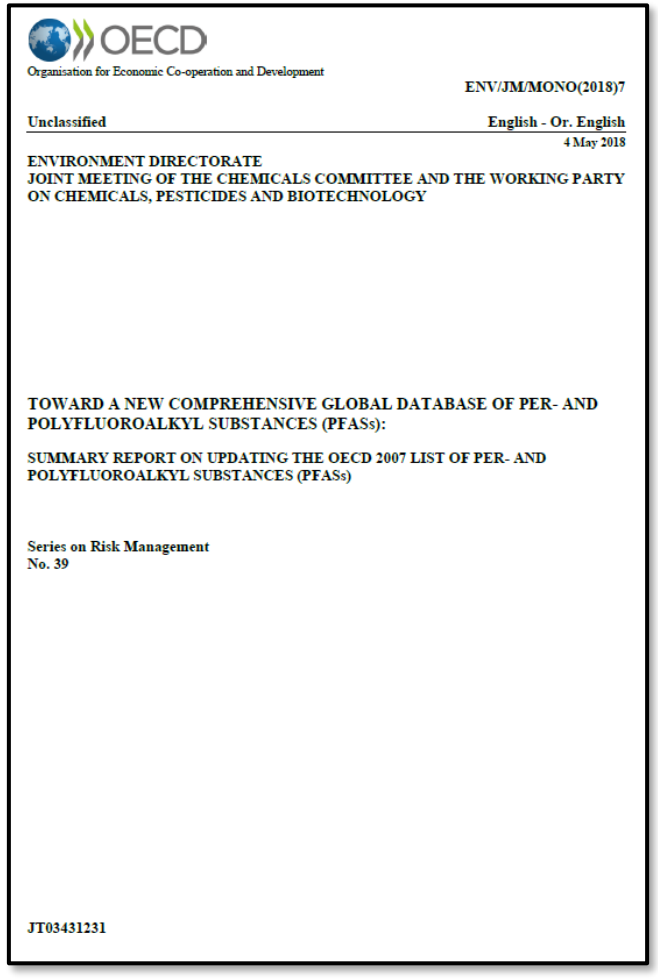
Quick Review of the State of the Science

- There continues to be an evolving definition of what constitutes a PFAS.
- The EPA needs to evaluate a large number of PFAS for potential human and ecological effects.
- Most PFAS have limited or no toxicity data.
- There is emerging consensus on the need to use category/grouping-based approaches to evaluate PFAS for a range of decision contexts.
- In a category/grouping approach, one or more data rich analogs is used to read-across toxicity values for the remaining data poor substances within the group.
- Historically, for human health assessment within EPA, PFAS analogs and/or groups were based on a combination of chain-length and functional groups.

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





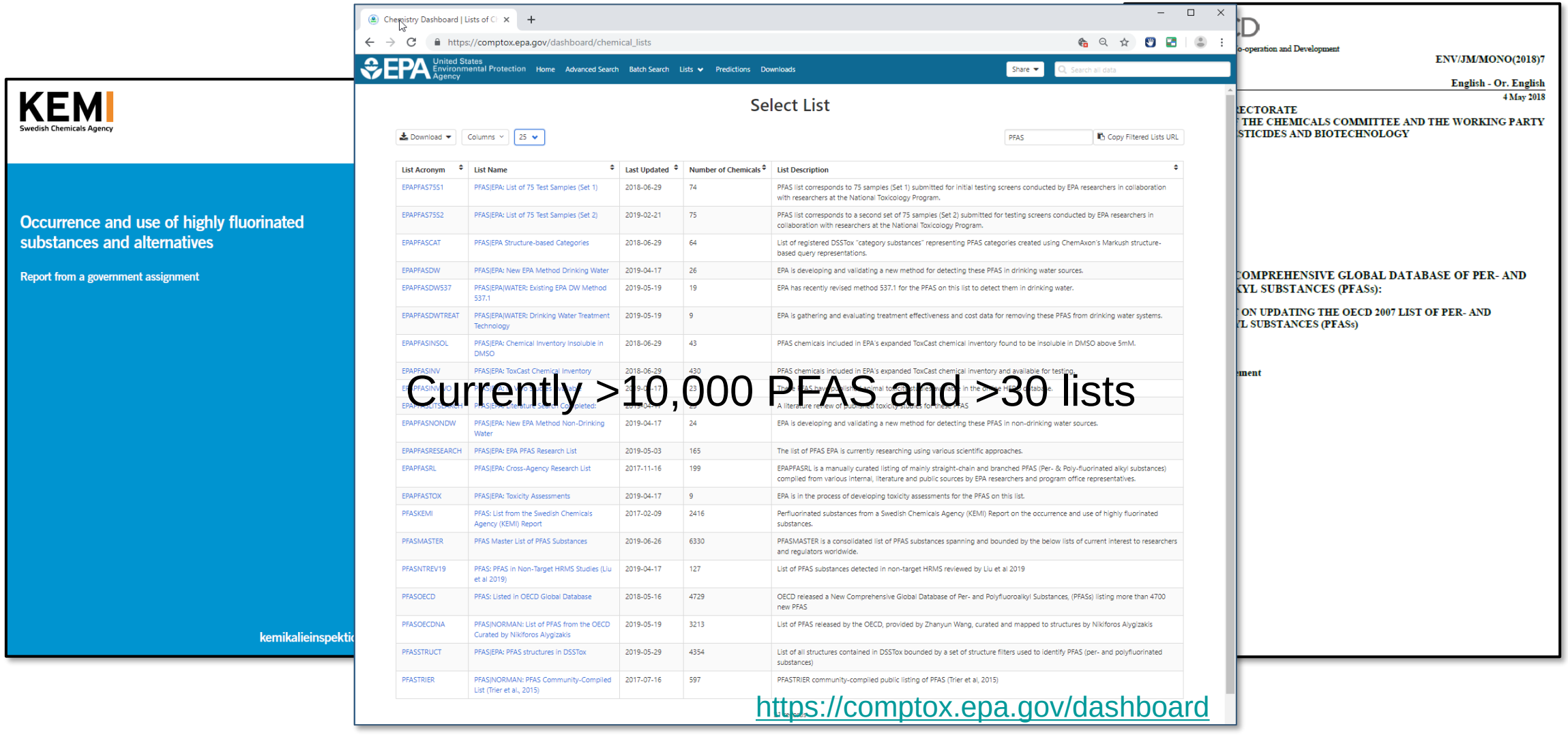


Lists from EPA Regulatory Offices and Regions

DTXSID	Substance_Name	Substance_CASRN	Source_Name (incorrect or ambiguous)	Source_CASRN (incorrect or invalid)	Source_Acronym (incorrect or ambiguous)	Unique_Acronym
DTXSID20874028	2H,2H,3H,3H-Perfluorooctanoic acid	914637-49-3	5:3 Polyfluorinated acid	914637-49-3	5:3 acid	5:3 PFOA
DTXSID7027831	N-Methyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide	24448-09-7	N-Methyl perfluorooctanesulfonamideoethanol		NMeFOSE, MeFOSE	NMeFOSE
DTXSID10892352	Perfluoro-2-([perfluoro-3-(perfluoroethoxy)-2-propanyl]oxy)ethanesulfonic acid	749836-20-2	Ethanesulfonic acid, 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro	749836-20-2	PFESA Byproduct 2	PFESA Byproduct 2
DTXSID70892479	Perfluoropentanesulfonate	17[illegible]	Perfluoropentanesulfonate	270[illegible]-91-[illegible]	PFPeS	PFPeS_ion
DTXSID8071354	Ammonium perfluoropentanesulfonate	68259-09-6	Ammonium perfluoropentansulfonate	68259-09-6		APFPeS
DTXSID40881350	4,8-Dioxa-3H-perfluorononanoic acid	919005-14-4	2,2,3-Trifluoro-3-(1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy)propanoic acid	919005-14-4	ADONA	ADONA parent acid
DTXSID00874026	Ammonium 4,8-dioxa-3H-perfluorononanoate	958445-44-8	Ammonium 4,8-dioxa-3H-perfluorononanoate	958445-44-8	ADONA	ADONA
DTXSID3037707	Potassium perfluorobutanesulfonate	29420-49-3	Potassium perfluoro-1-butanesulfonate		PFBS	PFBS-K
DTXSID5030030	Perfluorobutanesulfonic acid	375-73-5	Perfluorobutanesulfonic acid	375-73-5	PFBS	PFBS
DTXSID60873015	Perfluorobutanesulfonate	45187-15-3	Perfluorobutanesulfonate	375-73-5	PFBS	PFBS_ion
DTXSID3040148	Perfluorodecanesulfonic acid	335-77-3	Perfluorodecanesulfonic acid		PFDS	PFDS
DTXSID00873014	Perfluorodecanesulfonate	126105-34-8	Perfluorodecanesulfonate	335-77-3	PFDS	PFDS_ion
DTXSID60892443	Sodium perfluorodecanesulfonate	2806-15-7	Sodium perfluoro-1-decanesulfonate		PFDS	PFDS-Na

Antony Williams, Ann Richard, and Chemical Curation Team

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



Currently >10,000 PFAS and >30 lists

https://comptox.epa.gov/dashboard

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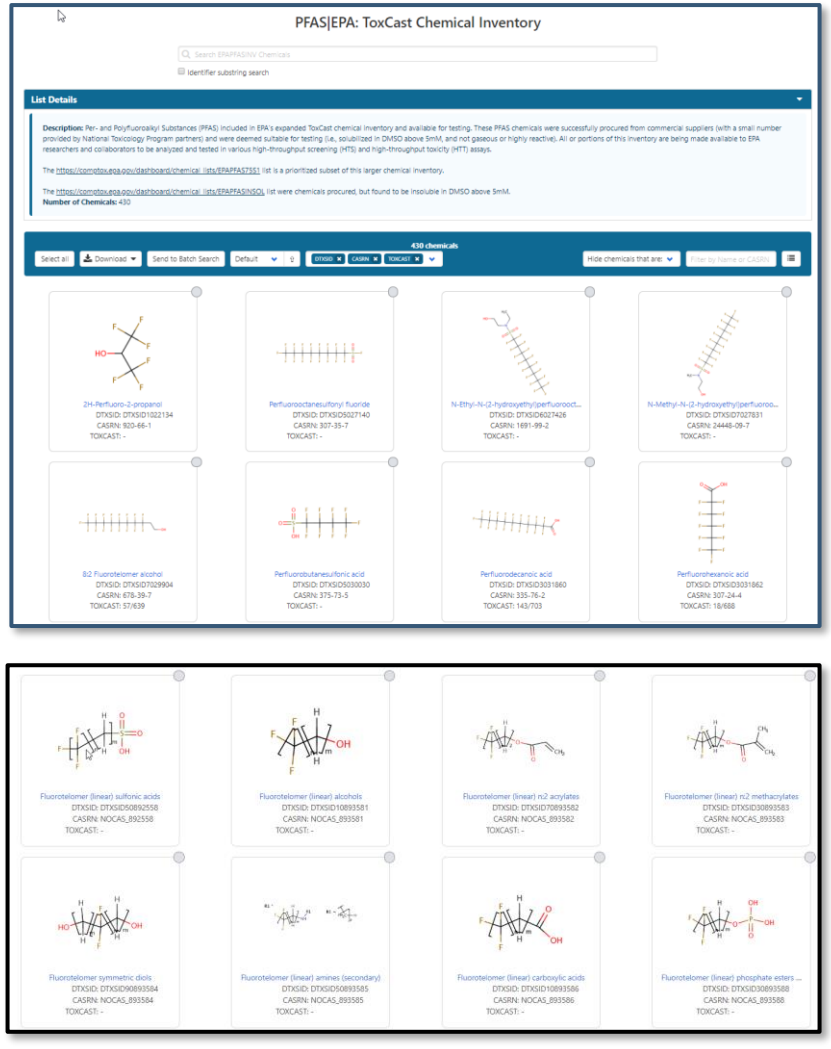
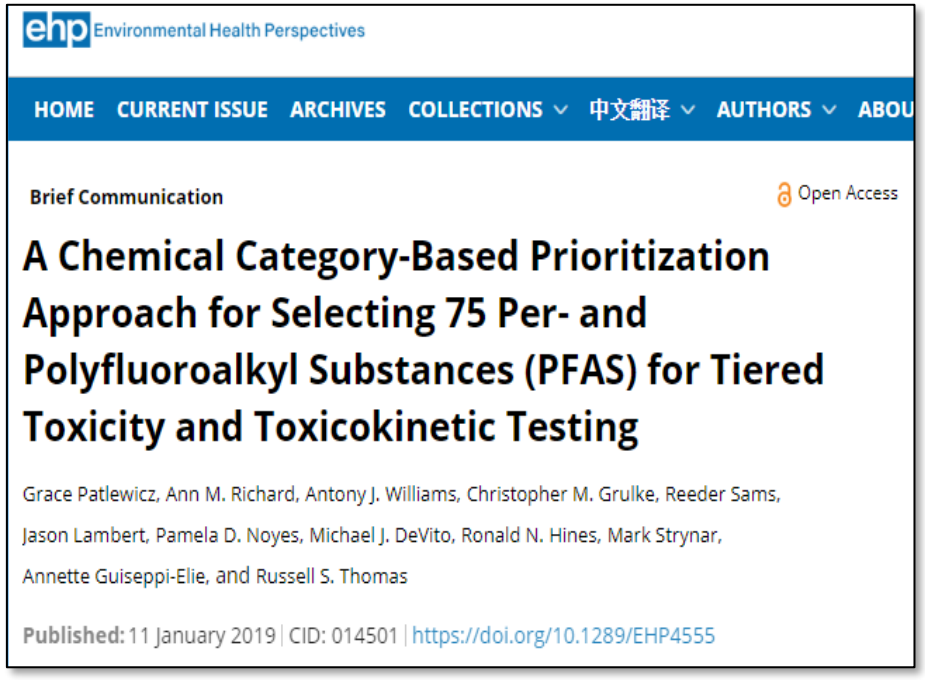
It Starts With Chemistry… Curating Names, Structures, and Identifiers



- Attempted to procure ~3,000 based on chemical diversity, Agency priorities, and other considerations
- Obtained 480 unique chemicals
  - 430/480 soluble in DMSO (90%)
- Initially selected 150 PFAS in two phases for testing
- Currently, over 100 have passed QC and are undergoing Tier 1 testing and available for Tier 2 and other testing

Kathy Coutros, Ann Richard, Barbara Wetmore, and Analytical Chemistry Team

Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing



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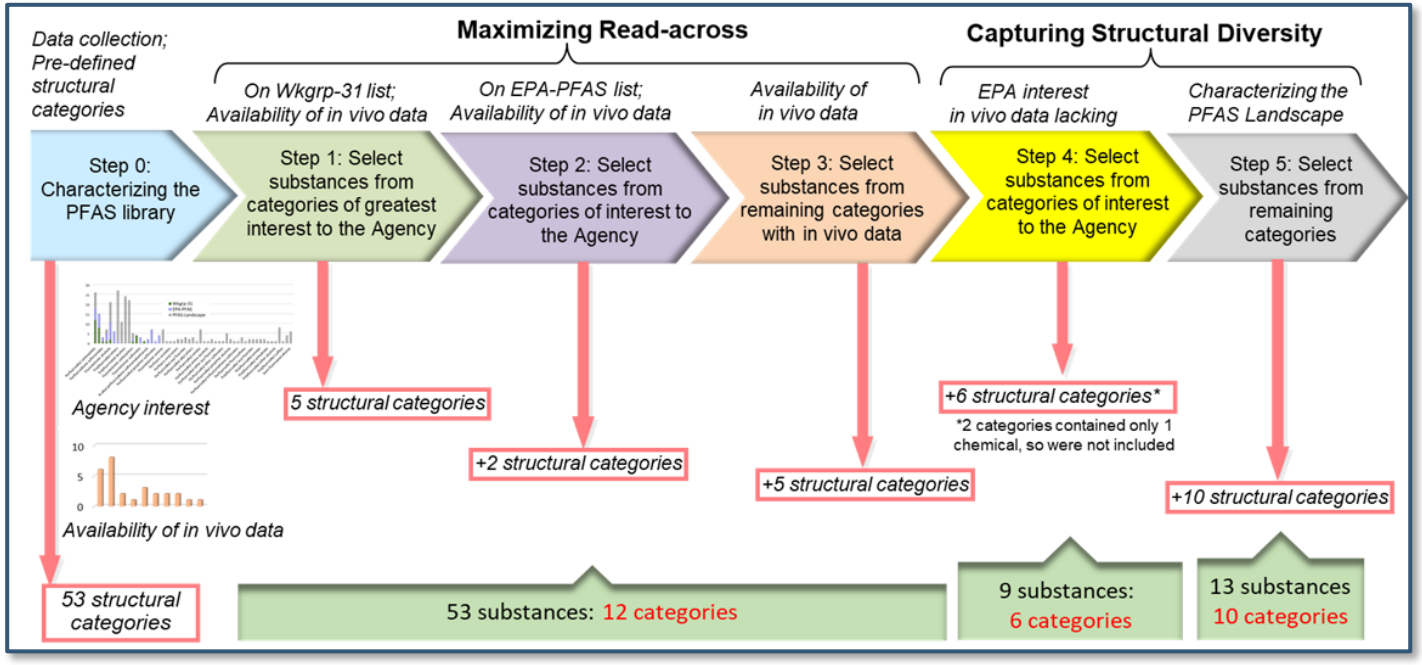
Brief Communication

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



- Developed initial structural categories based on Buck et al., 2011
- Selected 150 substances to support refinement of categories and read-across evaluation.
- Testing substances in a range of assays to characterize mechanistic and toxicokinetic properties.

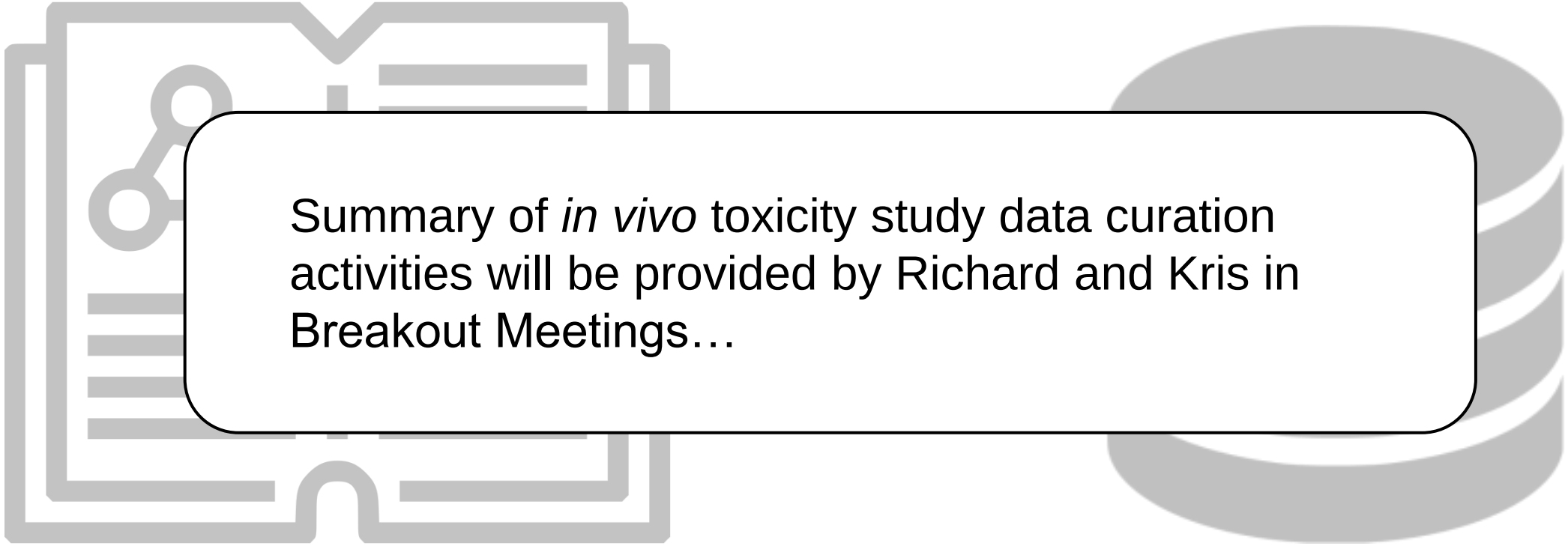
Tier 1 *In Vitro* Toxicity and Toxicokinetic Testing

Toxicological Response	Assay	Assay Endpoints	Purpose
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
Developmental Neurotoxicity	Microelectrode array assay (rat primary neurons)	Neuronal electrical activity	Impacts on neuron function
Endocrine Disruption			tivity
Receptor-Mediated			eceptors and s involved in al mechanisms
General Toxicity			in important
General Toxicity		morphology	organelles and

Summary of Tier 1 toxicity and toxicokinetic testing will be provided by Richard and Barbara in Breakout Meetings…

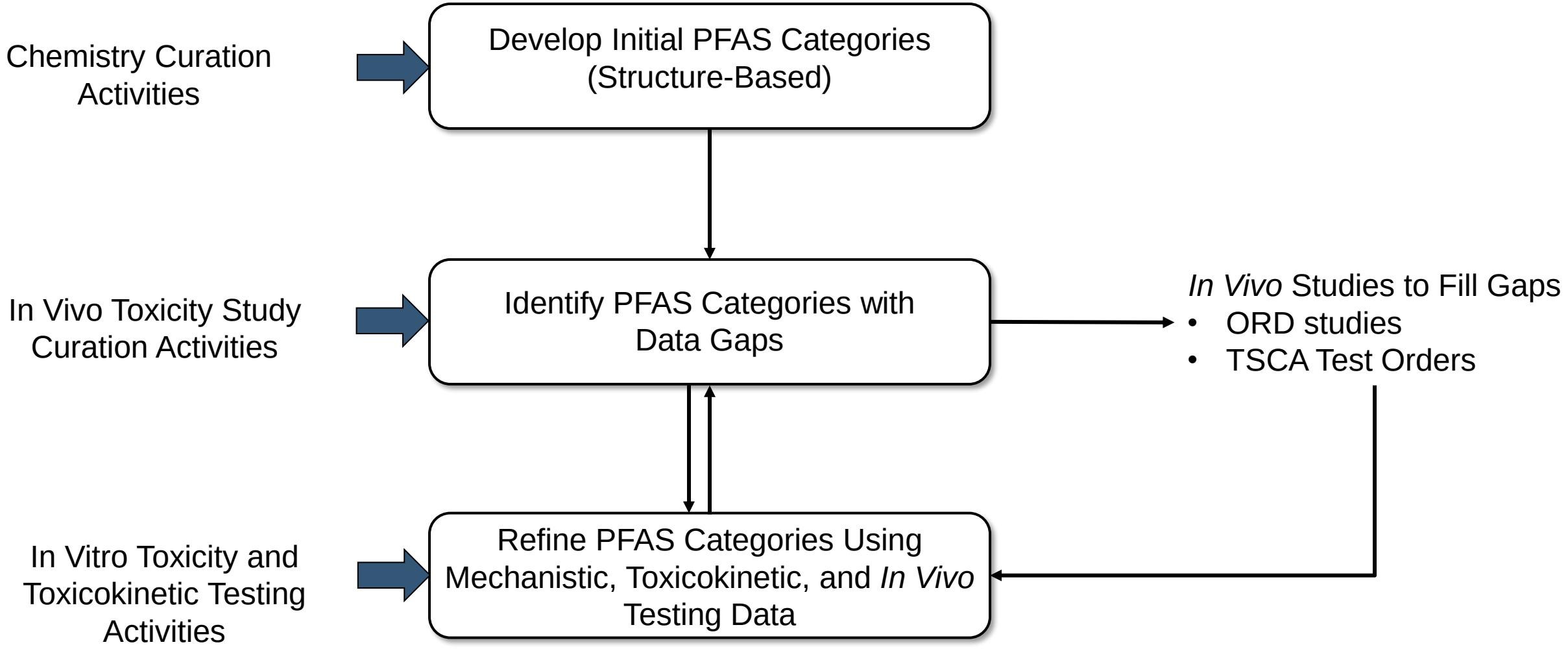
Toxicokinetic Parameter	Assay	Assay Endpoints	Purpose
Intrinsic hepatic clearance	Hepatocyte stability assay (primary human hepatocytes)	Time course metabolism of parent chemical	Measure metabolic breakdown by the liver
Plasma protein binding	Ultracentrifugation assay	Fraction of chemical not bound to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

Legacy *In Vivo* Toxicity Study Data Curation



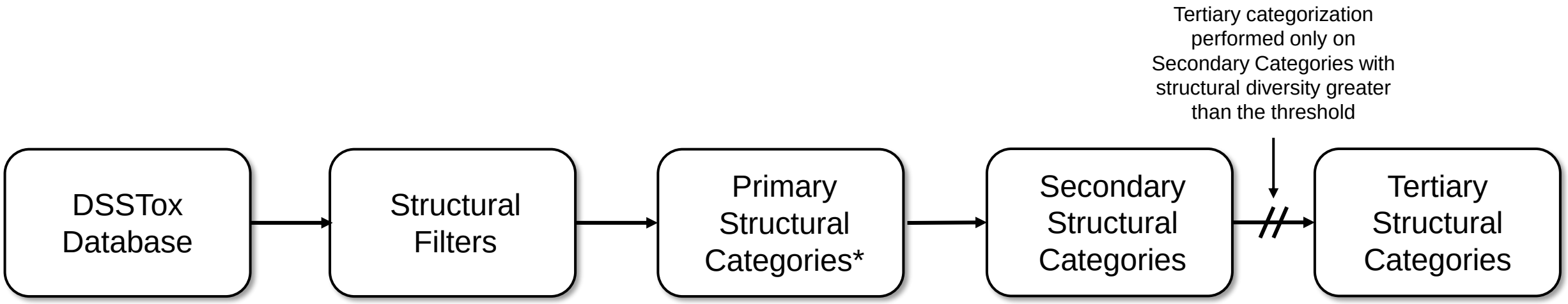
Summary of *in vivo* toxicity study data curation activities will be provided by Richard and Kris in Breakout Meetings…

Develop and Refine PFAS Categories for Hazard Assessment in Collaboration with OCSPP



Antony Williams, Grace Patlewicz, Richard Judson, and OPPT Collaborators

Develop Initial PFAS Structural Categories for Hazard Assessment in Collaboration with OCSPP



~906K substances

1. Contains -CF2
2. Apply 'OPPT working definition'
3. Remove radicals, charge imbalanced
4. Remove specific types of 5 and 6 membered rings (aromatic, containing double bonds, heterocycles)

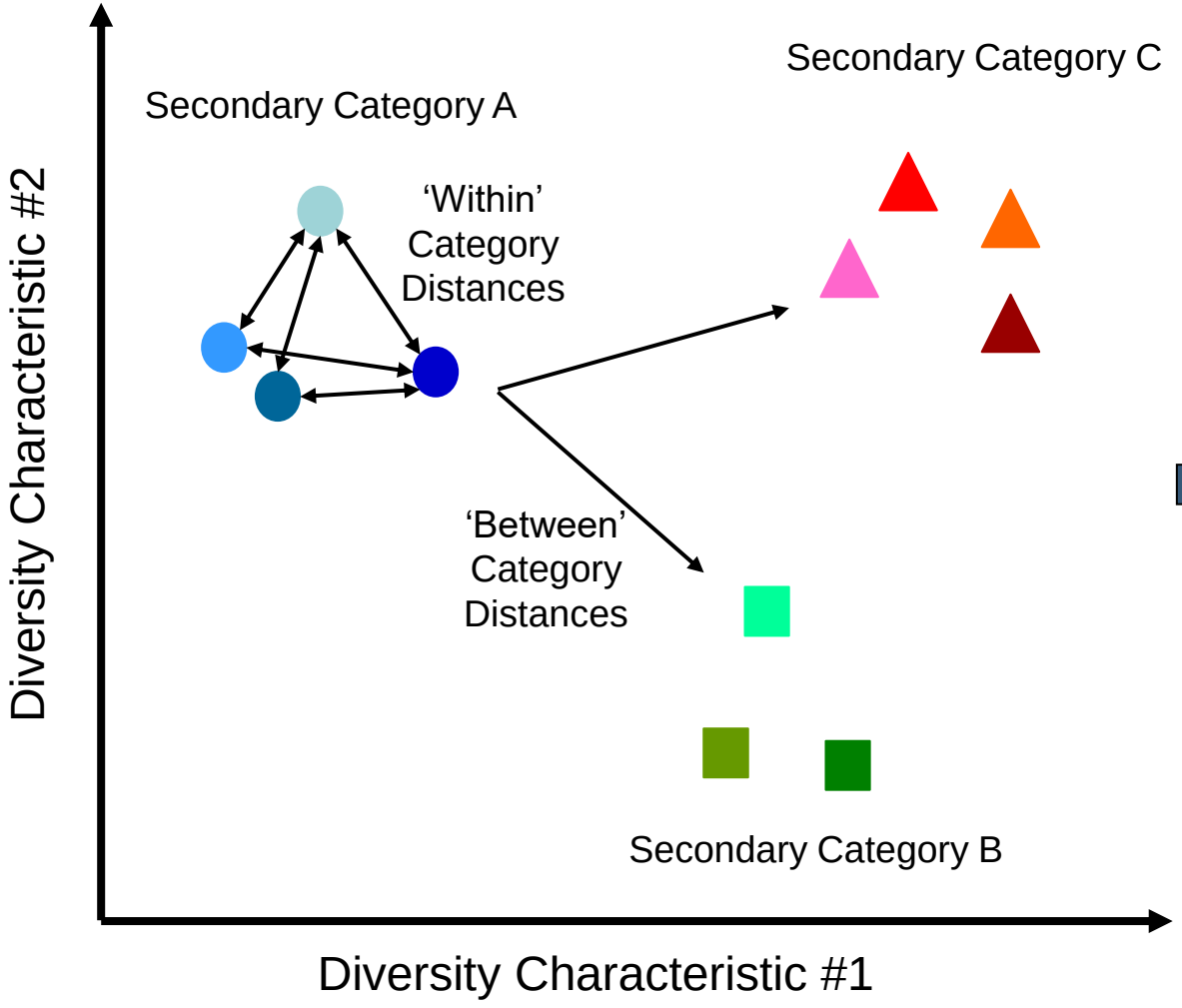
1. PFAS derivatives
2. PFAAs
3. Perfluoro PFAA precursors
4. Non-PFAA Perfluoroalkyls
5. FASA-based PFAA Precursors
6. Fluorotelomer PFAA precursors
7. Silicon PFAS
8. Side-chain fluorinated aromatic PFAS
9. Other aliphatic PFAS

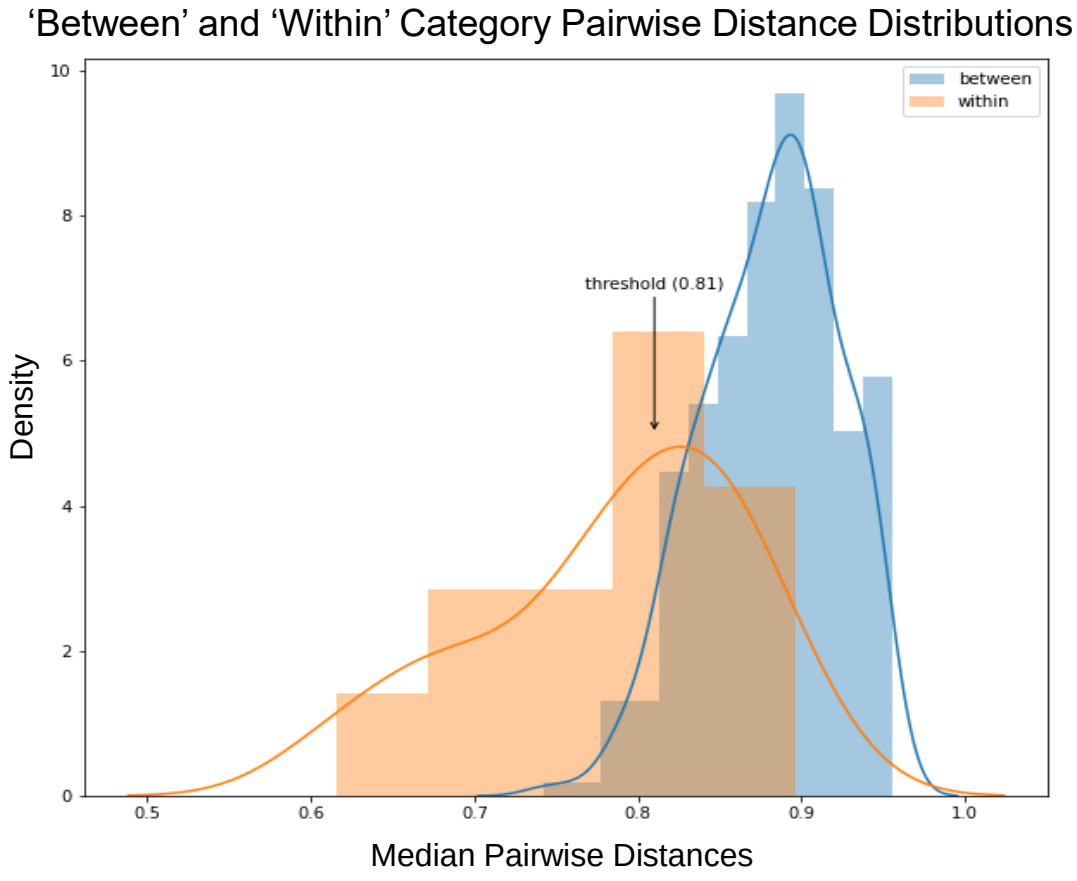
1. Greater than or equal to 8 carbons
2. Less than 8 carbons
3. Volatile (>100 mmHg Vapor Pressure)

*Based on Su and Rajan, 2021

Antony Williams, Grace Patlewicz, Richard Judson, and OPPT Collaborators

Structural Diversity ‘Within’ and ‘Between’ Secondary Categories Used to Set Diversity Threshold





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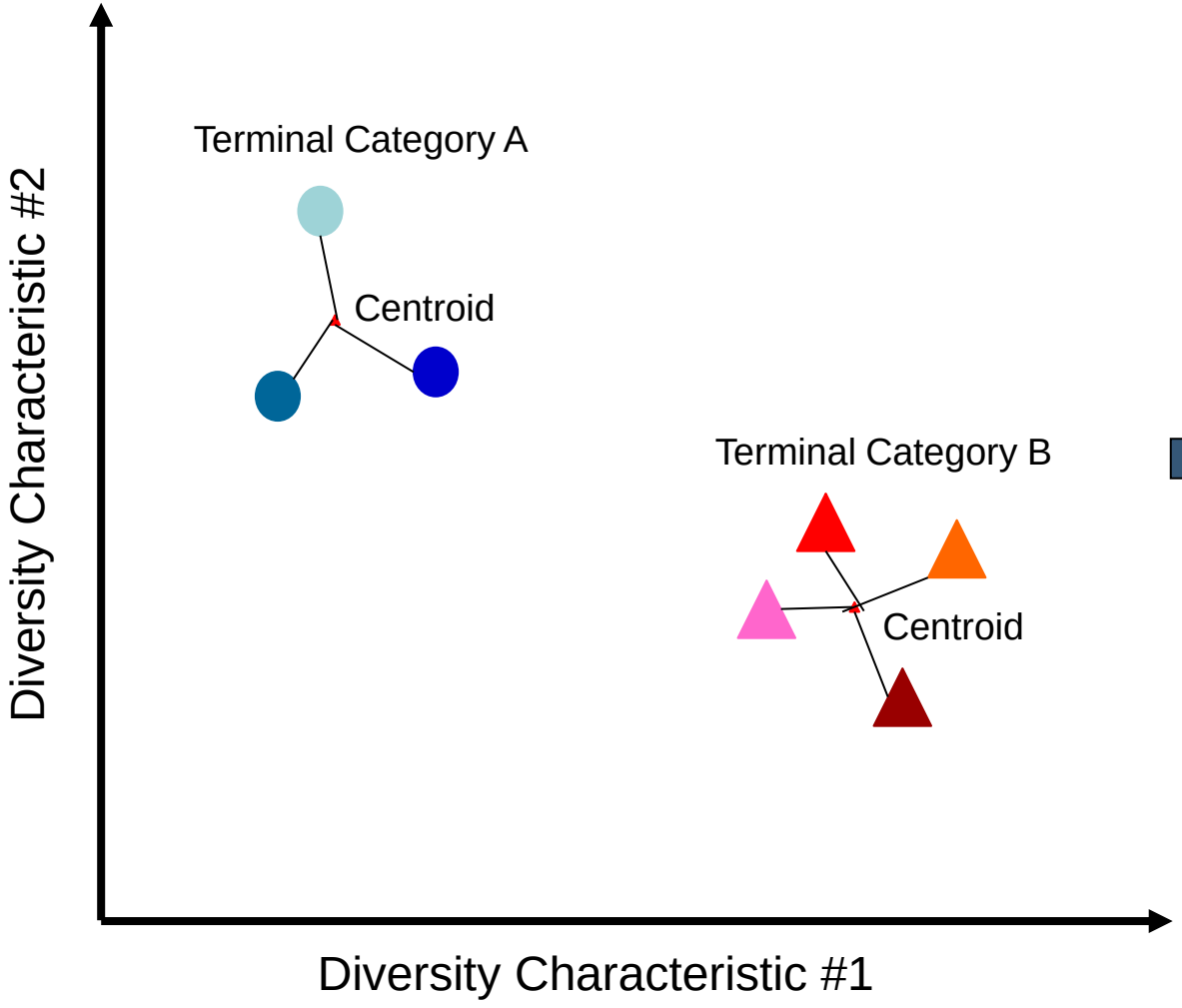
Centroids Calculated for Each Terminal Category to Help Select Most Representative PFAS for Testing



Mean of the chemical fingerprint matrix used to identify the 'centroid'.

'Centroids' used to identify the most representative substance for the group

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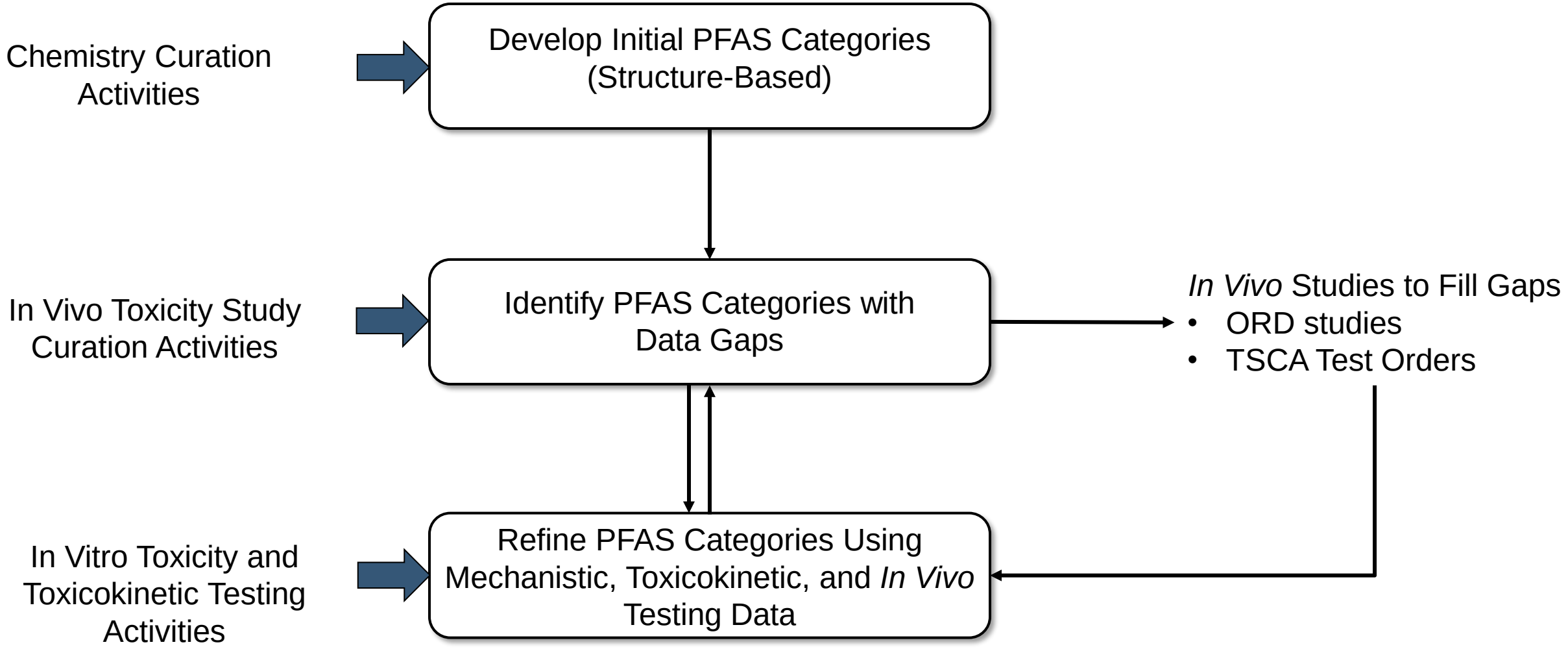
Develop and Refine PFAS Categories for Hazard Assessment in Collaboration with OCSPP



Antony Williams, Grace Patlewicz, Richard Judson, and OPPT Collaborators

EPA United States Environmental Protection Agency

Take Home Messages

- ORD undertaking a multi-pronged strategy to characterize the chemistry, toxicity, and toxicokinetic properties of the broader class of PFAS.
- A category-based strategy is being used to identify candidate PFAS for further *in vivo* testing and provide toxicity values for the constituent substances.
- Initial structural categories will be refined using the mechanistic and toxicokinetic data.
- ORD is working closely with OCSPP to develop the strategy for regulatory application.

EPA United States Environmental Protection Agency

Questions?