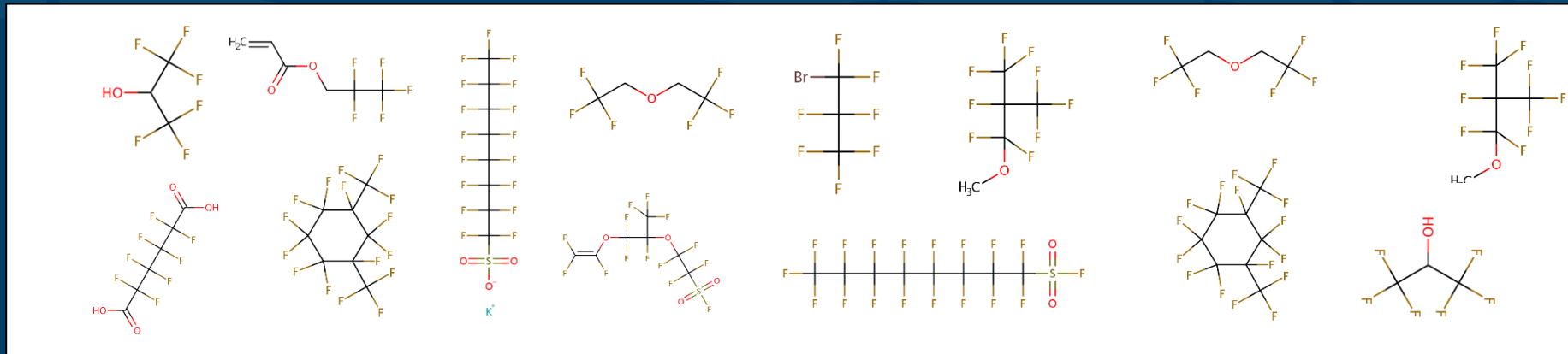


# ORD's Tiered Toxicity Testing Strategy for PFAS



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

**KEMI**  
Swedish Chemicals Agency

Occurrence and use of highly fluorinated substances and alternatives

Report from a government assignment

REPORT 7/15

kemikalieinspektionen.se

November 26, 2015

Dataset Open Access

## S9 | PFASTRIER | PFAS Suspect List: fluorinated substances

Xenia; Lunderberg, David

er(s)

chymanski, Emma

is the collection associated with list S9 PFASTRIER on the NORMAN Suspect List Exchange.

s://www.norman-network.com/?q=suspect-list-exchange

FASTRIER PFAS Suspect List: fluorinated substances

(MassHunter format; 26/11/2015)

X (several sheets; 26/11/2015)

pTox PFAS TRIER List


her curation in progress...

S InChIKeys (26/11/2015)

ly supplied by Xenia Trier, David Lunderberg and colleagues.

reference information contained in files.

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 PFDA
DTXSID7027831	N-Methyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide	24448-09-7	N-Methylperfluorooctanesulfonamide:ethanol		NMeFOSE, MeFOSE	NMeFOSE
DTXSID10892352	Perfluoro-2-[(perfluoro-3-(perfluoroethoxy)propyl)oxy]ethanesulfonic acid	749836-20-2	tetrafluoroethoxymethyl)-1,2,2,2-tetrafluoroethyl-1,1,2,2-tetrafluoroethanesulfonic acid	749836-20-2	PFESA Byproduct 2	PFESA Byproduct 2
DTXSID70892479	Perfluorobutanesulfonic acid	375-73-5	Perfluorobutanesulfonic acid	375-73-5	PFBS	PFBS
DTXSID8071354	Ammonium perfluoropentanesulfonate	55829-09-6	Ammonium perfluoropentanesulfonate	5829-09-6	APPF5s	APPF5s
DTXSID40881350	4,8-Dioxo-3H-perfluorononanoic acid	919005-14-4	2,2,2-Trifluoro-3-(1,1,2,2,3,3-hexafluoro-3-oxopropoxy)butanoic acid	919005-14-4	ADONA	ADONA parent acid
DTXSID00874026	Ammonium 4,8-dioxo-3H-perfluorononanoate	958445-44-8	Ammonium 4,8-dioxo-3H-perfluorononanoate	958445-44-8	ADONA	ADONA
DTXSID3037707	Potassium perfluorobutanesulfonate	29420-49-3	Potassium perfluoro-1-butanesulfonate		PFBS	PFBS-K
DTXSID05030300	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



ENV/JM/MONO(2018)7

Unclassified English - Or. English 4 May 2018

ENVIRONMENT DIRECTORATE  
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY  
ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

TOWARD A NEW COMPREHENSIVE GLOBAL DATABASE OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFASs):  
SUMMARY REPORT ON UPDATING THE OECD 2007 LIST OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFASs)

Series on Risk Management  
No. 39

JT0431231

# It Starts With Chemistry... Curating Names, Structures, and Identifiers

**KEMI**  
Swedish Chemicals Agency

Occurrence and use of highly fluorinated substances and alternatives

Report from a government assignment

kemikalieinspektio

Chemistry Dashboard | Lists of Chemicals

https://comptox.epa.gov/dashboard/chemical\_lists

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.
EPAPFASDWTRTAT	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.
EPAPFASINDB	PFAS(EPA): Chemical Inventory Database	2019-04-17	23	The list may be used to identify PFAS in the EPA's chemical inventory database.
EPAPFASLITER	PFAS(EPA): Literature Search Completed	2019-04-17	22	A literature review of published toxicity studies for these PFAS.
EPAPFASNONDNDW	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 Alygizakis	2019-05-19	3213	List of PFAS released by the OECD, provided by Zhanyun Wang, curated and mapped to structures by Nikiforos Alygizakis
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)
PFASRIER	PFAS(NORMAN): PFAS Community-Compiled List (Trier et al, 2015)	2017-07-16	597	PFASRIER community-compiled public listing of PFAS (Trier et al, 2015)

Currently >10,000 PFAS and >30 lists

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

Directorate of Chemicals and Pesticides

ENV/JM/MONO(2018)7

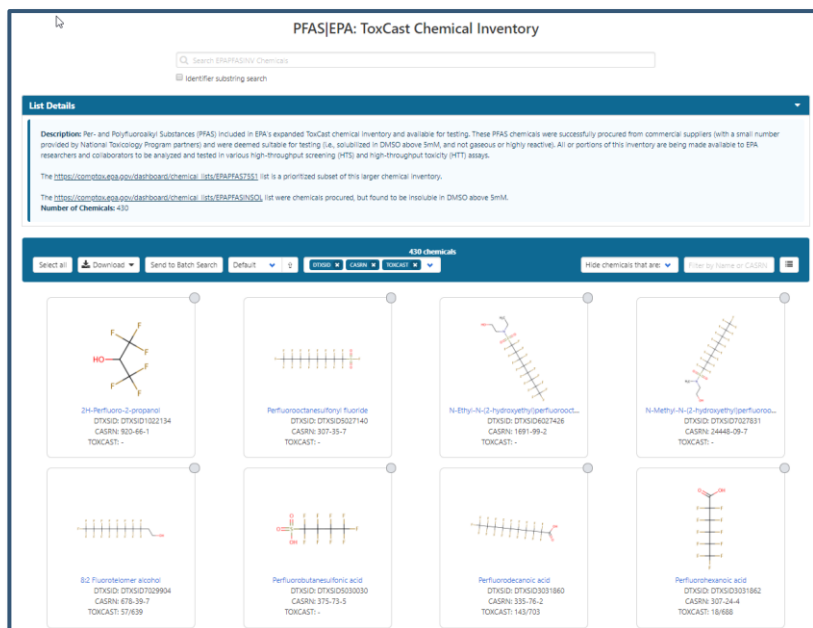
English - Or. English  
4 May 2018

SECRETORATE OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON PESTICIDES AND BIOTECHNOLOGY

COMPREHENSIVE GLOBAL DATABASE OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFASs):

WORKING PARTY ON UPDATING THE OECD 2007 LIST OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFASs)

# It Starts With Chemistry... Curating Names, Structures, and Identifiers



**PFAS|EPA: ToxCast Chemical Inventory**

Search:

Identify:  Identifier:  Substring:  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.

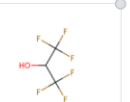


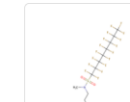


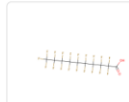

The [https://comptox.epa.gov/dashboard/chemical\\_lists/PFAS2021](https://comptox.epa.gov/dashboard/chemical_lists/PFAS2021) list is a prioritized subset of this larger chemical inventory.

The [https://comptox.epa.gov/dashboard/chemical\\_lists/PFAS2020](https://comptox.epa.gov/dashboard/chemical_lists/PFAS2020) list were chemicals procured, but found to be insoluble in DMSO above 5mM.

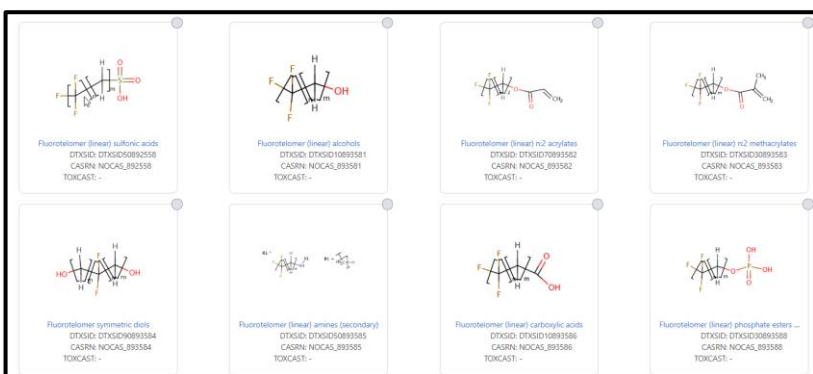
**Number of Chemicals:** 430





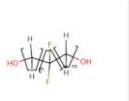
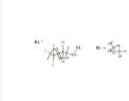
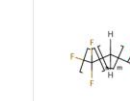

Select all:  Download:  Send to Batch Search:     Hide chemicals that are:

**430 Chemicals**

 <b>2H-Perfluoro-2-propanol</b> DTXSID: DTXSID1821134 CASRN: 933-66-1 TOXCASID: -	 <b>Perfluorooctanesulfonyl fluoride</b> DTXSID: DTXSID0207140 CASRN: 337-35-7 TOXCASID: -	 <b>N-Ethyl-N-(2-hydroxyethyl)perfluoro...</b> DTXSID: DTXSID0219426 CASRN: 1891-99-2 TOXCASID: -	 <b>N-Methyl-N-(2-hydroxyethyl)perfluoro...</b> DTXSID: DTXSID0219381 CASRN: 24468-09-7 TOXCASID: -
 <b>8:3 Fluorotelomer alcohol</b> DTXSID: DTXSID7029904 CASRN: 878-39-7 TOXCASID: 874939	 <b>Perfluorobutanesulfonic acid</b> DTXSID: DTXSID0303030 CASRN: 375-73-5 TOXCASID: -	 <b>Perfluorodecanoic acid</b> DTXSID: DTXSID03031860 CASRN: 335-76-2 TOXCASID: 140703	 <b>Perfluorohexanoic acid</b> DTXSID: DTXSID03031862 CASRN: 337-24-4 TOXCASID: 181668

- 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



 <b>Fluorotelomer (linear) sulfonic acids</b> DTXSID: DTXSID0892558 CASRN: NOCAS_892558 TOXCASID: -	 <b>Fluorotelomer (linear) alcohols</b> DTXSID: DTXSID10893581 CASRN: NOCAS_893581 TOXCASID: -	 <b>Fluorotelomer (linear) n2 acrylates</b> DTXSID: DTXSID70893582 CASRN: NOCAS_893582 TOXCASID: -	 <b>Fluorotelomer (linear) n2 methacrylates</b> DTXSID: DTXSID30893583 CASRN: NOCAS_893583 TOXCASID: -
 <b>Fluorotelomer symmetric diols</b> DTXSID: DTXSID90893584 CASRN: NOCAS_893584 TOXCASID: -	 <b>Fluorotelomer (linear) amines (secondary)</b> DTXSID: DTXSID0893585 CASRN: NOCAS_893585 TOXCASID: -	 <b>Fluorotelomer (linear) carboxylic acids</b> DTXSID: DTXSID10893586 CASRN: NOCAS_893586 TOXCASID: -	 <b>Fluorotelomer (linear) phosphate esters</b> DTXSID: DTXSID30893588 CASRN: NOCAS_893588 TOXCASID: -

# Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing

ehp Environmental Health Perspectives

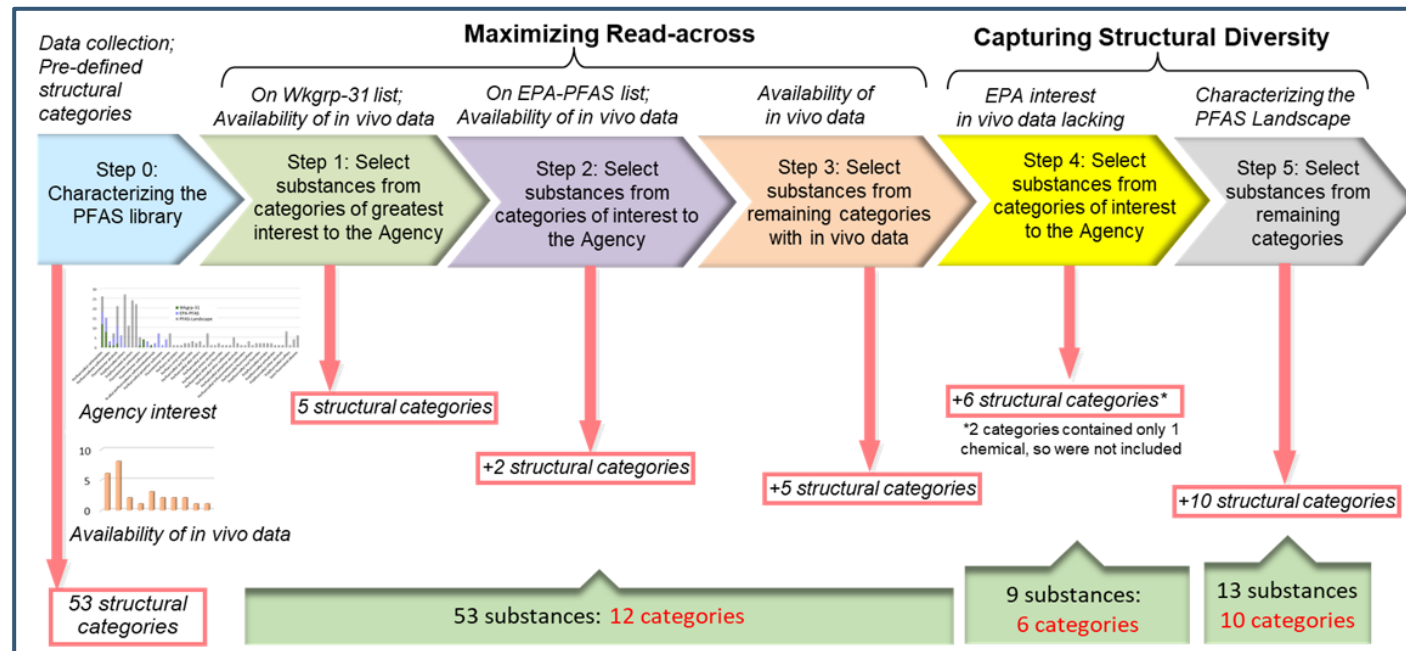
HOME CURRENT ISSUE ARCHIVES COLLECTIONS 中文翻译 AUTHORS ABOUT

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>



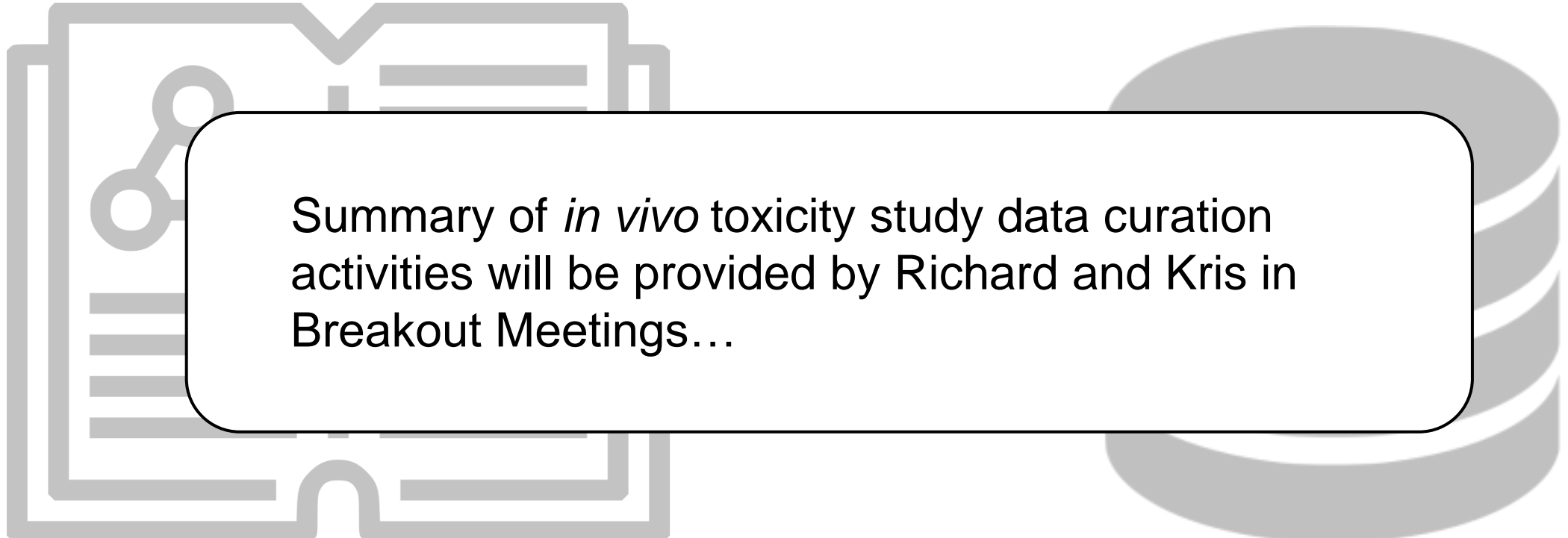
- Developed initial structural categories based on Buck et al., 2011
- Selected 150 substances to support refinement of categories and read-across evaluation.
- Testing substances in a range of assays to characterize mechanistic and toxicokinetic properties.

# 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	ACEA	Cell	Measure ER activity
Receptor-Mediated			Receptors and is involved in al mechanisms
General Toxicity			in important
General Toxicity			organelles and y
		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

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

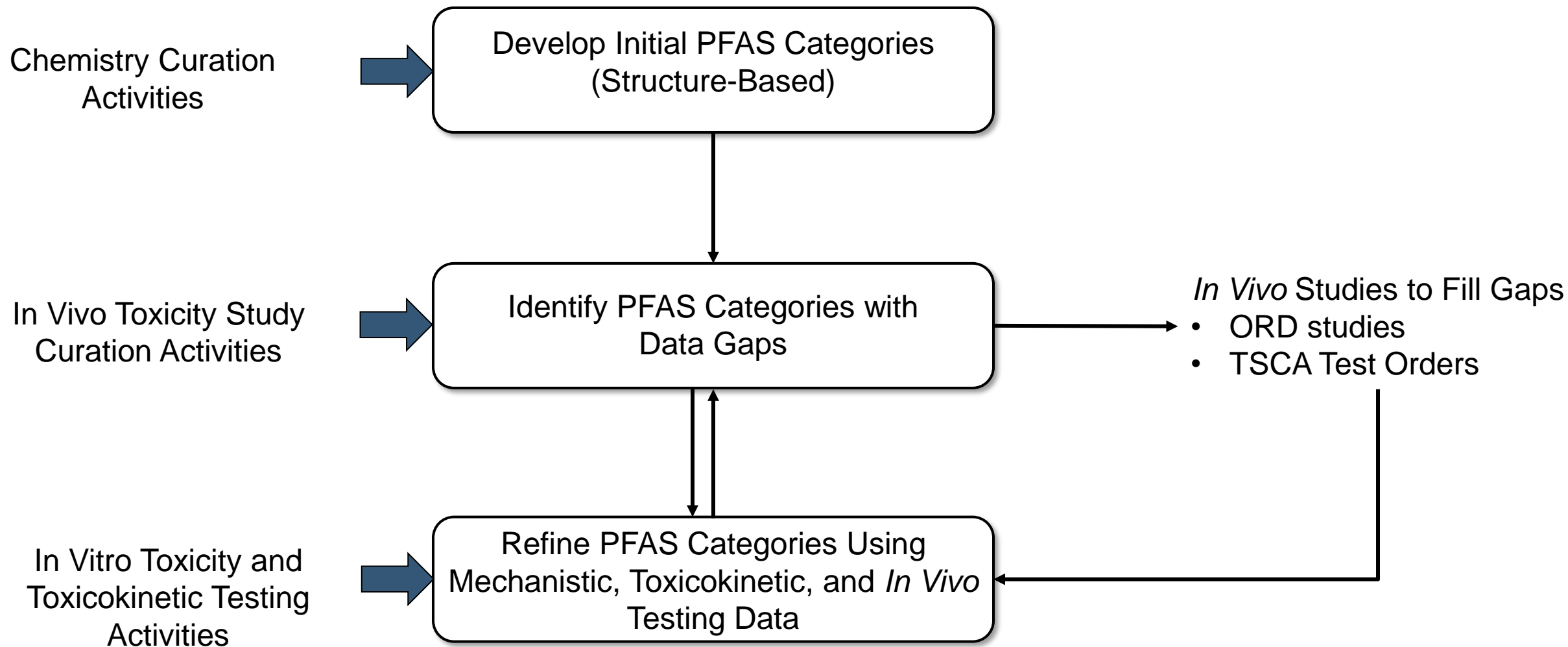
# Legacy *In Vivo* Toxicity Study Data Curation



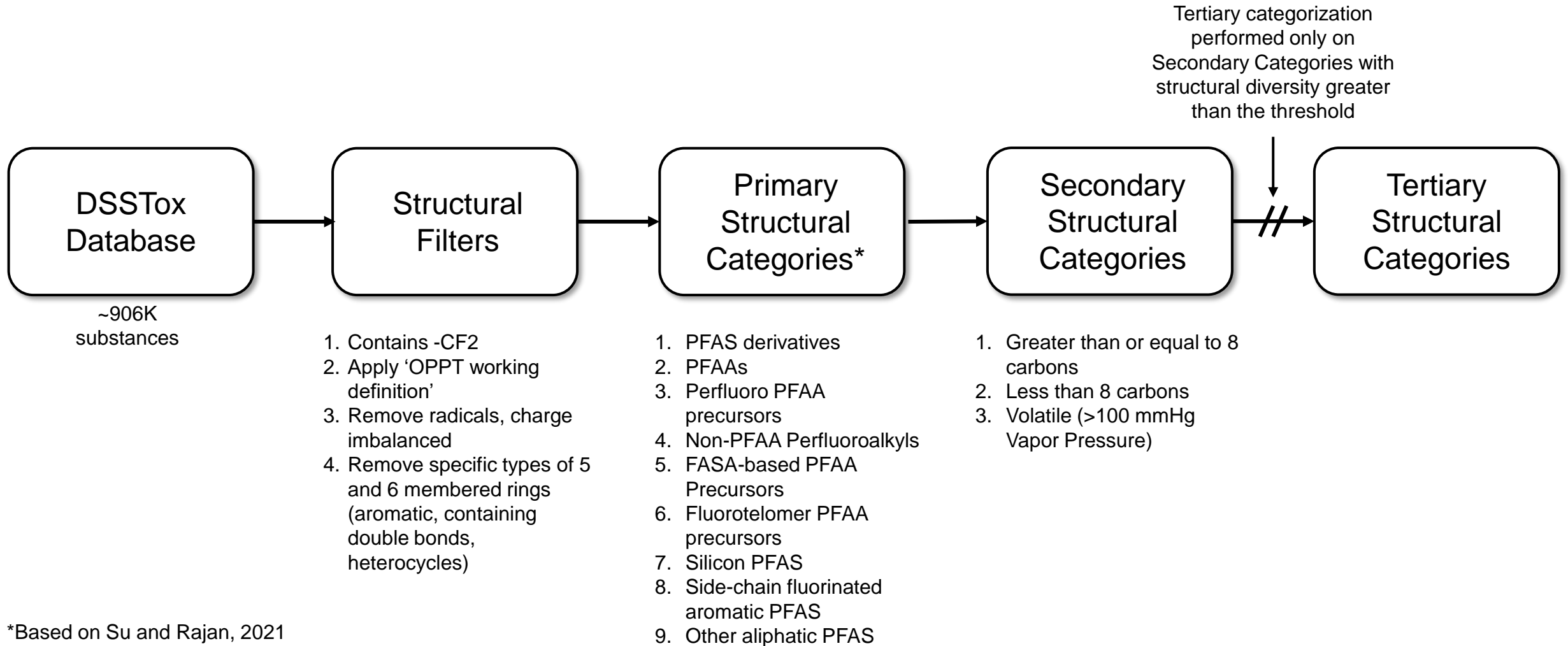
Summary of *in vivo* toxicity study data curation activities will be provided by Richard and Kris in Breakout Meetings...



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

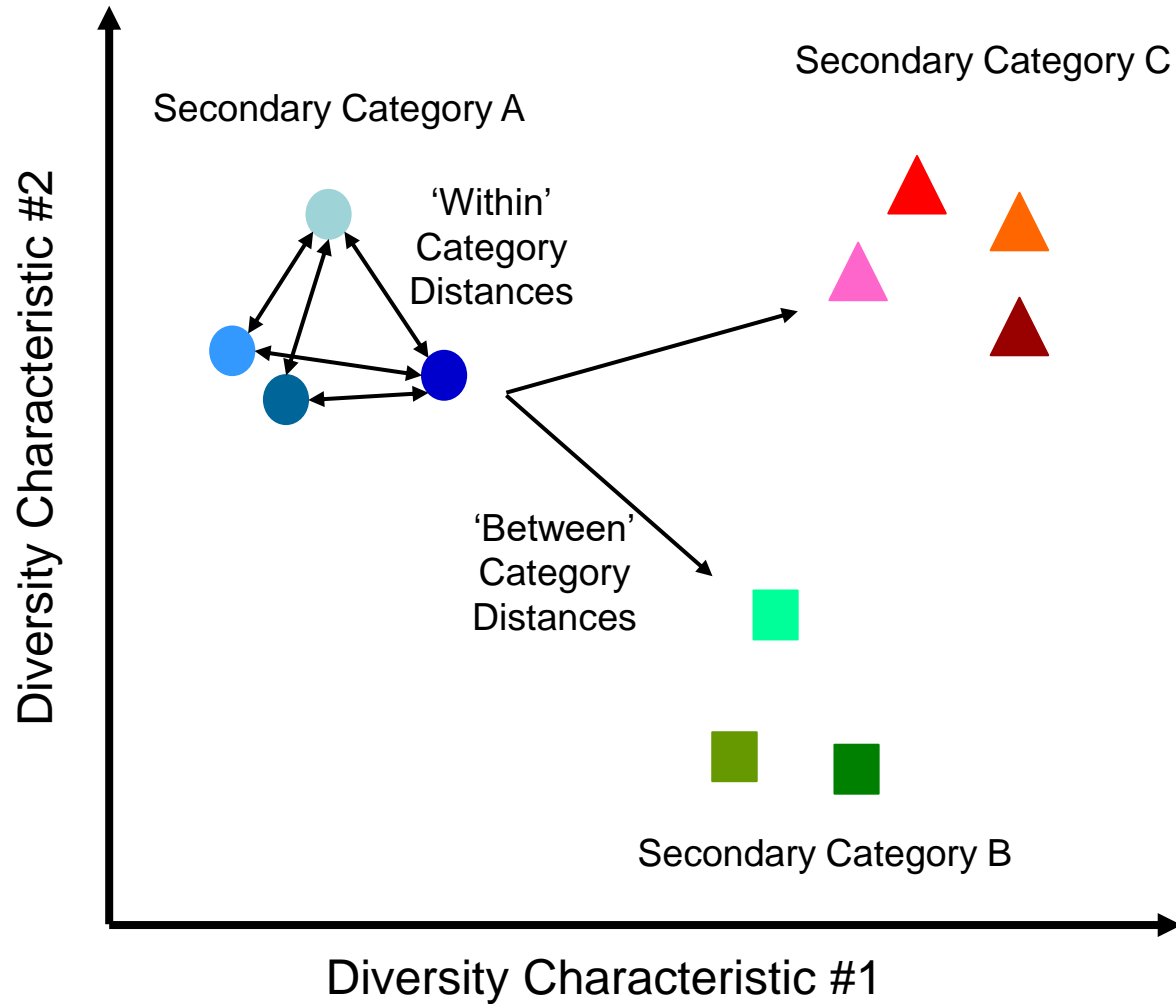


# Develop Initial PFAS Structural Categories for Hazard Assessment in Collaboration with OCSPP

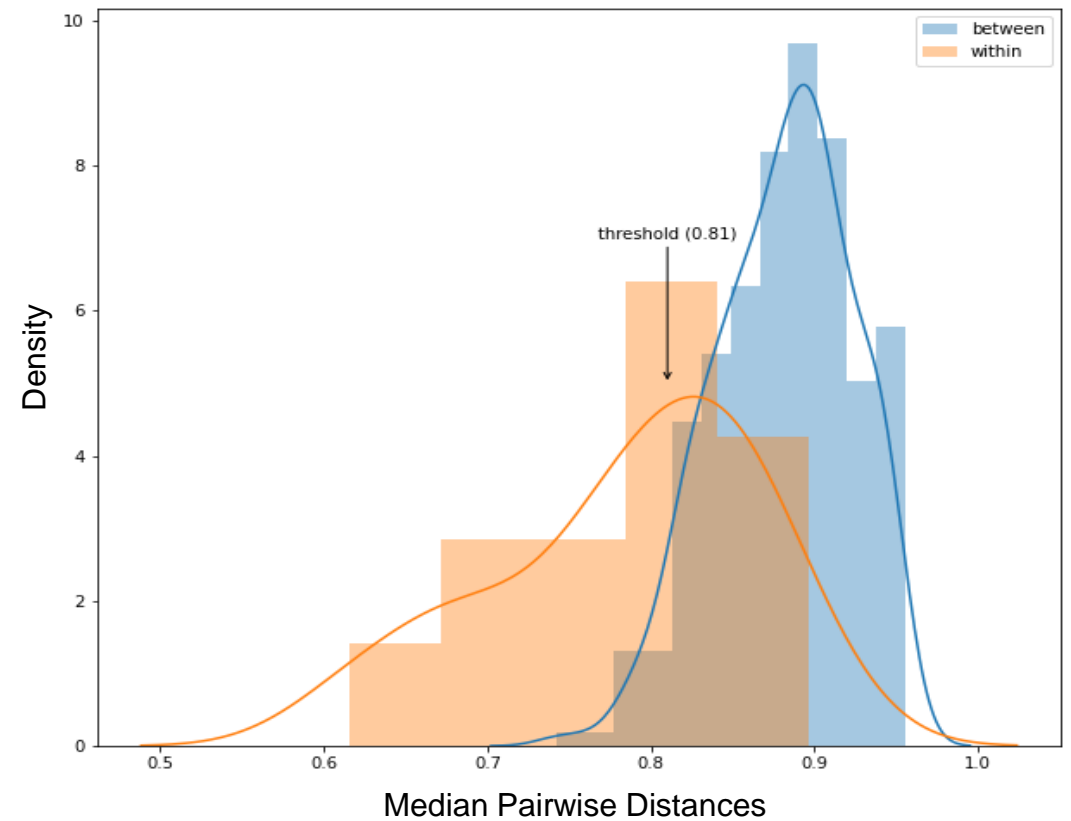


\*Based on Su and Rajan, 2021

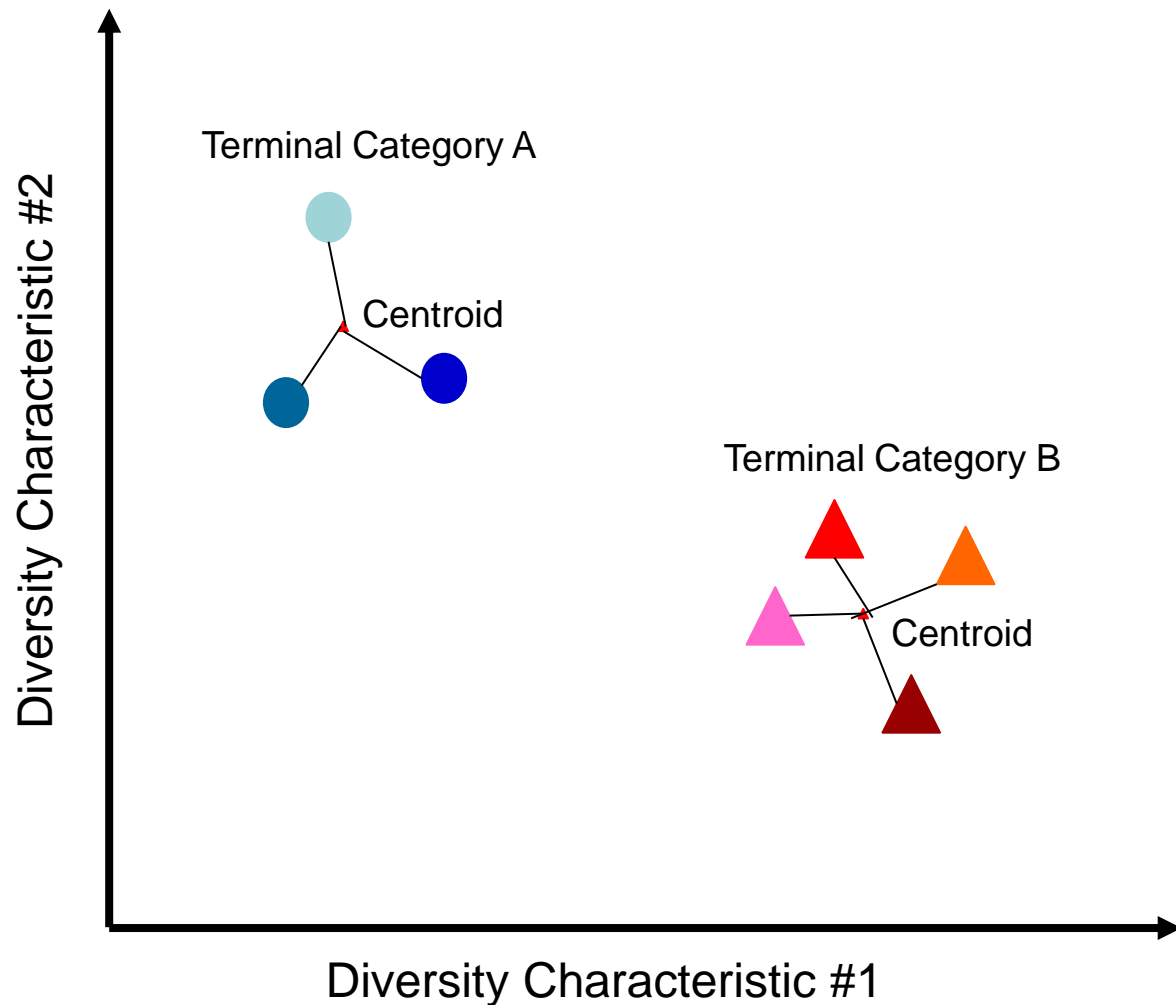
# Structural Diversity 'Within' and 'Between' Secondary Categories Used to Set Diversity Threshold



'Between' and 'Within' Category Pairwise Distance Distributions



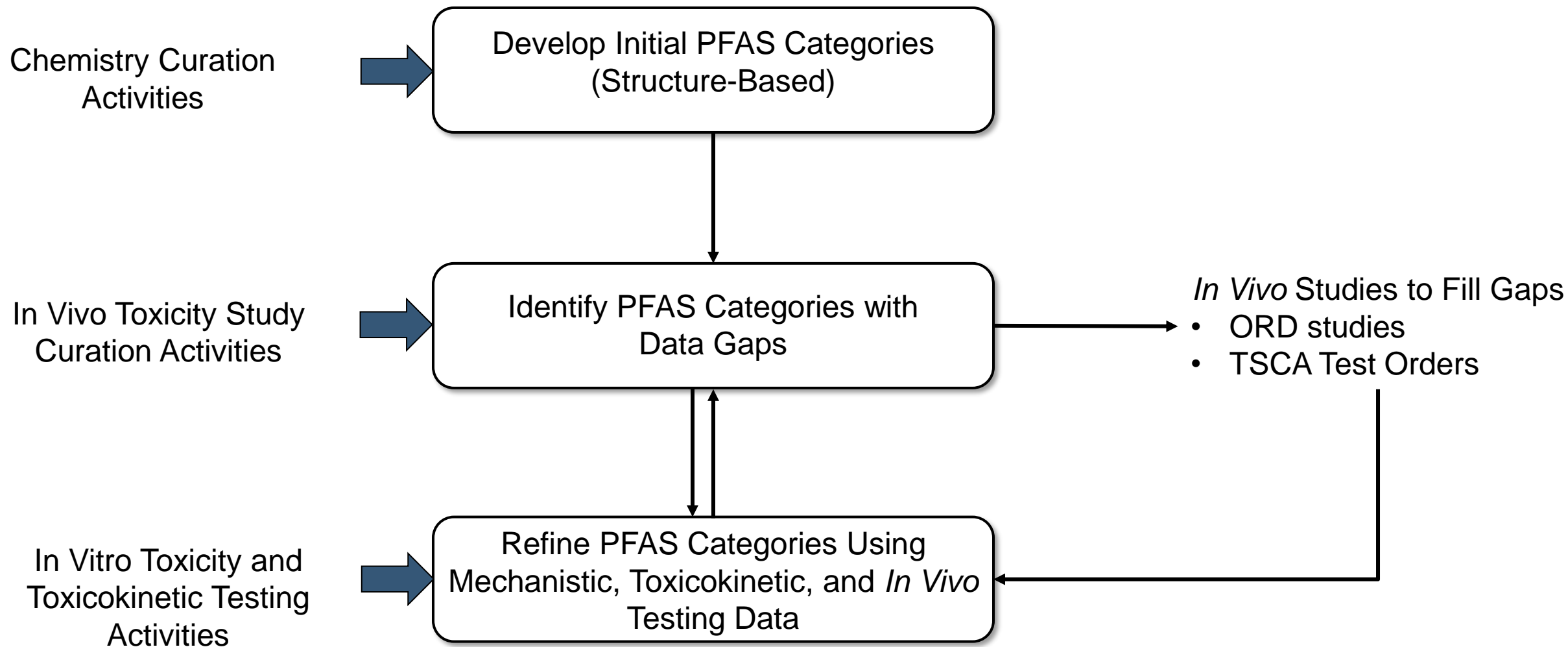
# Centroids Calculated for Each Terminal Category to Help Select Most Representative PFAS for Testing



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

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

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



# 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.

# Questions?