



A chemical category-based approach for selecting and screening PFAS for toxicity and toxicokinetic testing

PhD

PFAS Global 2022

March 24, 2022

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- Large numbers of PFAS whose hazard potential needs to be characterized
- Characterize PFAS chemical space
 - Categories and sub-categories
 - Chemical feature-based categories (ToxPrints) vs. OECD categories
- Find PFAS chemicals that can be purchased (limited subset of PFAS Universe)
- Select subset of these chemicals for NAM (New Approach Methods, *in vitro*) testing
- Compile legacy *in vivo* data
- Use categories and data as input for selecting chemicals for *in vivo* testing
- Work in progress



EPA PFAS Strategic Roadmap



PFAS Strategic Roadmap: EPA's Commitments to Action 2021–2024



RESEARCH

Invest in research, development, and innovation to increase understanding of PFAS exposures and toxicities, human health and ecological effects, and effective interventions that incorporate the best available science.

RESTRICT

Pursue a comprehensive approach to proactively prevent PFAS from entering air, land, and water at levels that can adversely impact human health and the environment.

REMEDiate

Broaden and accelerate the cleanup of PFAS contamination to protect human health and ecological systems.

Accelerate public health protections by identifying PFAS categories

Advance the science to assess human health and environmental risks from PFAS



Assemble a PFAS Chemical Library for NAM Work

PFAS[EPA] ToxCast Chemical Inventory

Search EPA/PAAS/NA Chemicals

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/PAFAS7551 list is a prioritized subset of this larger chemical inventory.

The https://comptox.epa.gov/dashboard/chemical_lists/PAFAS7551 list are 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 DTXSID CASRN TOXCAS

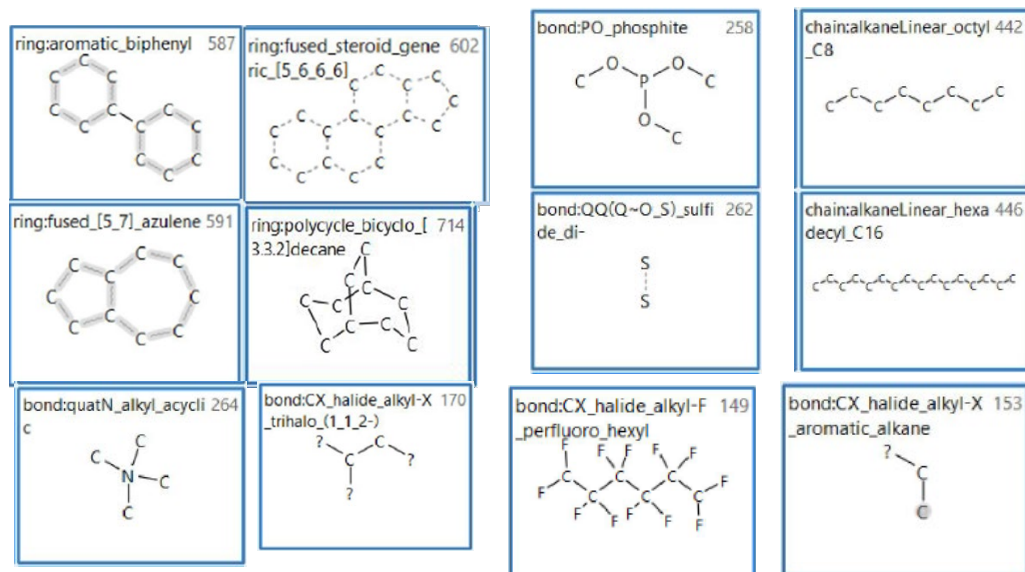
Hide chemicals that are: Filter by Name or CASRN

Chemical Name	DTXSID	CASRN	TOXCAS
2H-Perfluoro-2-propanol	DTXSID:DTXSID0902194	CASRN: 920-66-1	TOXCAS: -
Perfluorooctanesulfonyl fluoride	DTXSID:DTXSID0902740	CASRN: 307-35-7	TOXCAS: -
N-Ethyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide	DTXSID:DTXSID0902748	CASRN: 1891-89-2	TOXCAS: -
N-Methyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide	DTXSID:DTXSID0902751	CASRN: 24448-09-7	TOXCAS: -
8:2 Fluorotelomer alcohol	DTXSID:DTXSID0902904	CASRN: 678-39-7	TOXCAS: 571629
Perfluorobutanesulfonic acid	DTXSID:DTXSID0903030	CASRN: 375-73-5	TOXCAS: -
Perfluorodecanoic acid	DTXSID:DTXSID0903180	CASRN: 335-76-2	TOXCAS: 143793
Perfluorooctanoic acid	DTXSID:DTXSID0903182	CASRN: 307-24-4	TOXCAS: 181668

Chemical Name	DTXSID	CASRN	TOXCAS
Fluorotelomer (linear) sulfonic acids	DTXSID:DTXSID0903258	CASRN: NOCAS_893258	TOXCAS: -
Fluorotelomer (linear) alcohols	DTXSID:DTXSID0903581	CASRN: NOCAS_893581	TOXCAS: -
Fluorotelomer (linear) n2 acrylates	DTXSID:DTXSID0903582	CASRN: NOCAS_893582	TOXCAS: -
Fluorotelomer (linear) n2 methacrylates	DTXSID:DTXSID0903583	CASRN: NOCAS_893583	TOXCAS: -
Fluorotelomer symmetric diols	DTXSID:DTXSID0903584	CASRN: NOCAS_893584	TOXCAS: -
Fluorotelomer (linear) amines (secondary)	DTXSID:DTXSID0903585	CASRN: NOCAS_893585	TOXCAS: -
Fluorotelomer (linear) carboxylic acids	DTXSID:DTXSID0903586	CASRN: NOCAS_893586	TOXCAS: -
Fluorotelomer (linear) phosphate esters	DTXSID:DTXSID0903588	CASRN: NOCAS_893588	TOXCAS: -

- 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 initially assigned based on three approaches
 - Buck et al., 2011 categories
 - Markush categories (ToxPrints)
 - OECD categories

- Publicly available tools exist to generate & download ToxPrints e.g. ChemoTyper, CompTox Chemicals Dashboard
- Provides excellent coverage of PFAS chemical space
- Nested, hierarchical nature lends itself to creating flexible categories
- Can augment with computed structure properties (e.g., MW, size, etc.)



ToxPrints:

- 729 chemical features
- Chemically interpretable
- Coverage of diverse chemistry
- Includes scaffolds, functional groups, chains, rings, bonding patterns, atom-types



Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing (NAMs)

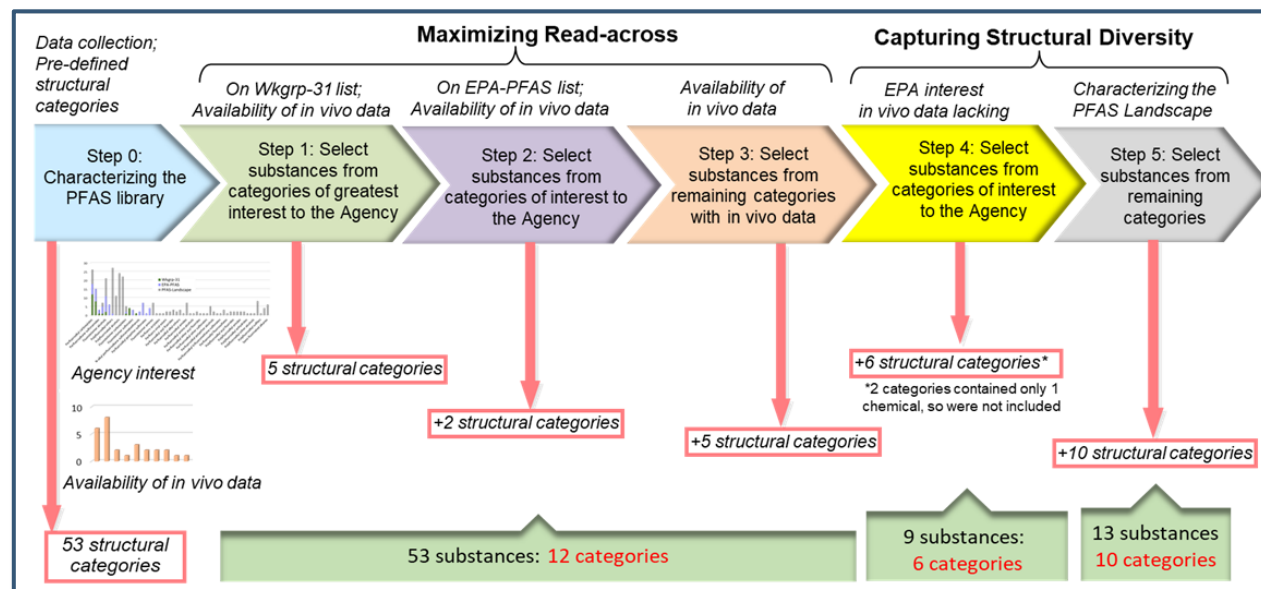


Goals:

- Generate data to support development and refinement of categories for read-across
- 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

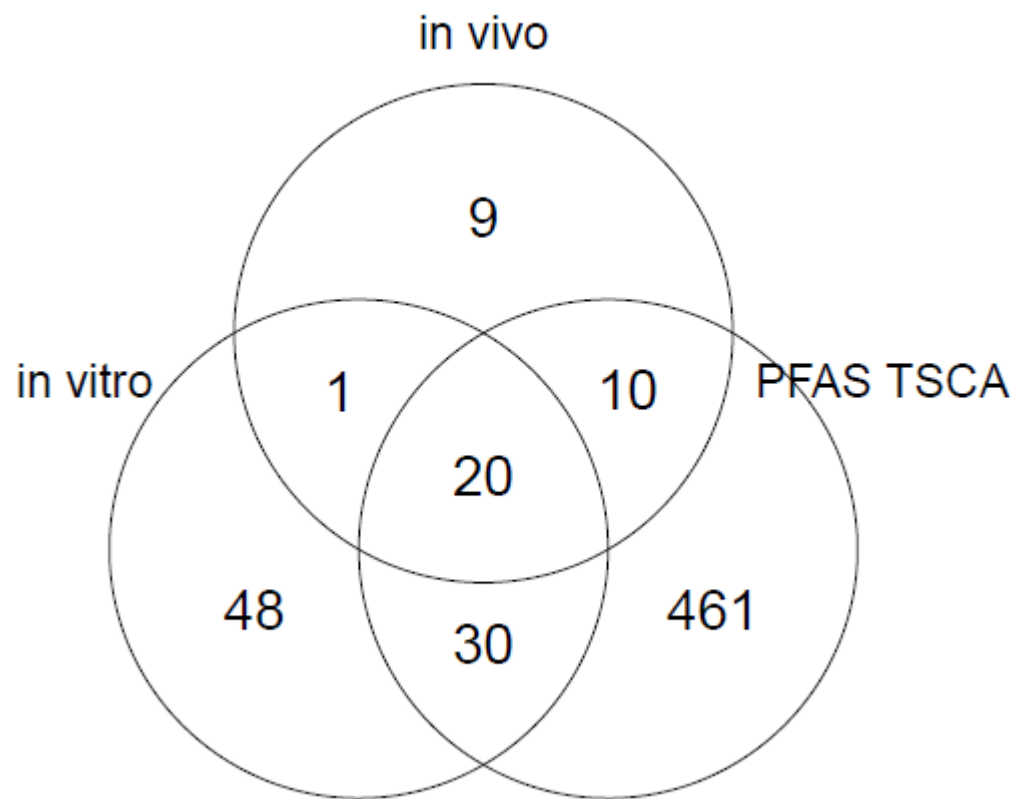




In Vitro Toxicity and Toxicokinetic Testing (NAMs)

Toxicological Response	Assay	Assay Endpoints	Purpose
Developmental Toxicity	Zebrafish embryo assay	Fertilisation, lethality, 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 (HepaRG)	Mitochondrial membrane potential	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

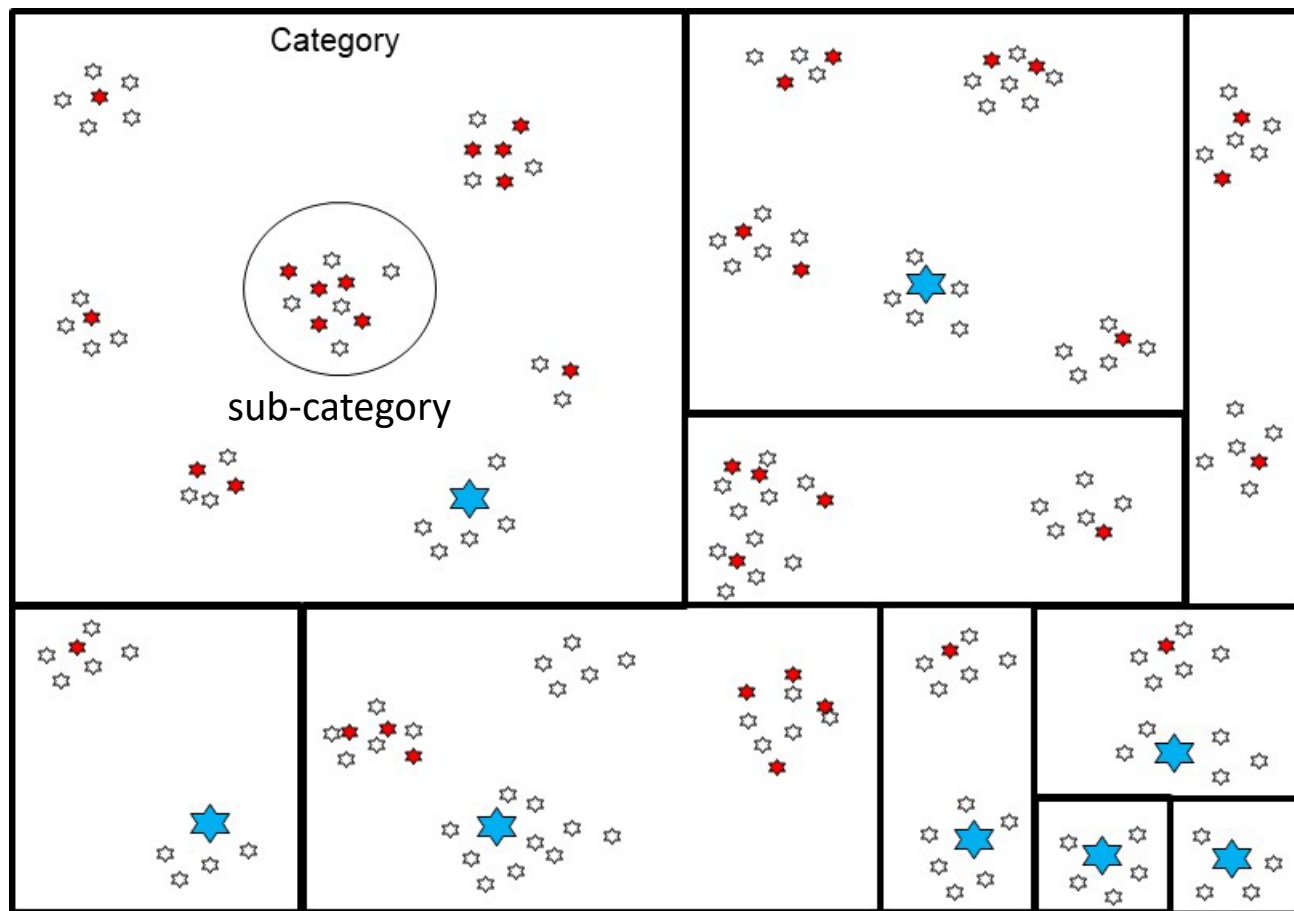


In Vitro = 99 NAM chemical passing QC & VP criteria

In Vivo = chemicals with PODs in ToxValDB

PFAS TSCA = non-CBI TSCA Active Inventory with structure

Category / Sub-Category Approach

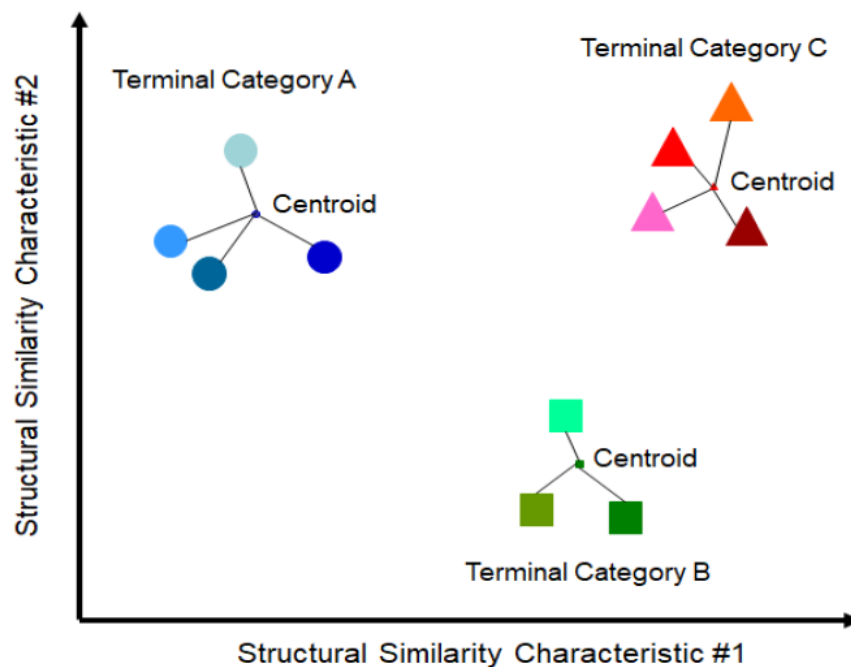
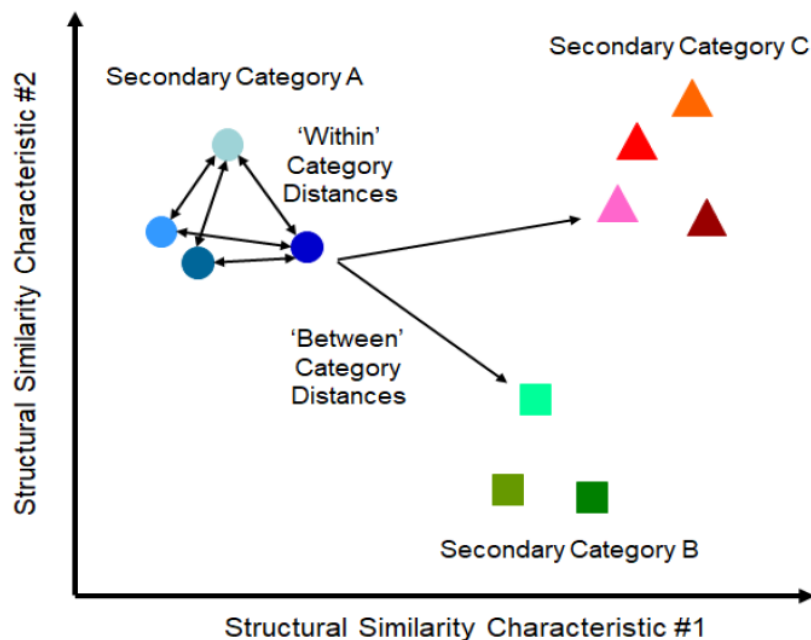


- Chemical substances are placed into high-level categories based on structure
- Sub-categories are derived using NAM, chemical property and existing *in vivo* data
- At least one chemical substance needs *in vivo* data per sub-category for read-across

Chemical Types

- ☆ Untested
- ★ Existing in vivo data
- ★ Proposed for new testing

Sub-Category Approach

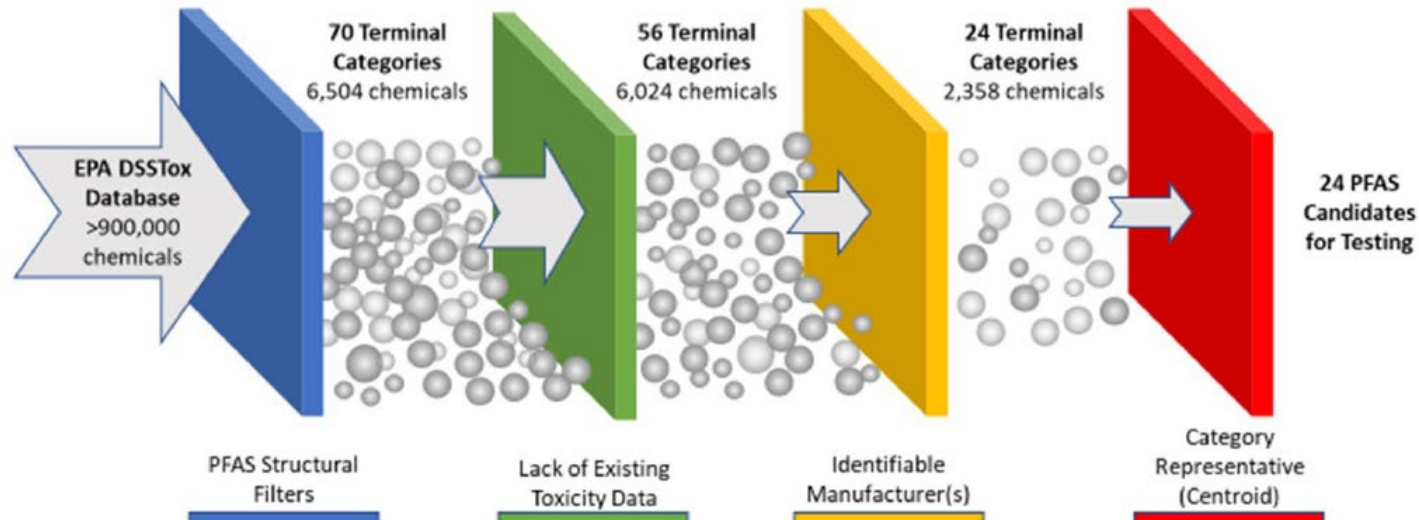


Similarity metric combines

- Chemical structures
- Chemical properties
- NAM data
- *In vivo* data

Select chemical nearest centroid for testing

- NAM data generation for PFAS 150 close to complete
- Initial categorization approach developed
- Used as input to the National PFAS Testing Strategy (October 2021)
 - <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/national-pfas-testing-strategy>





Acknowledgements