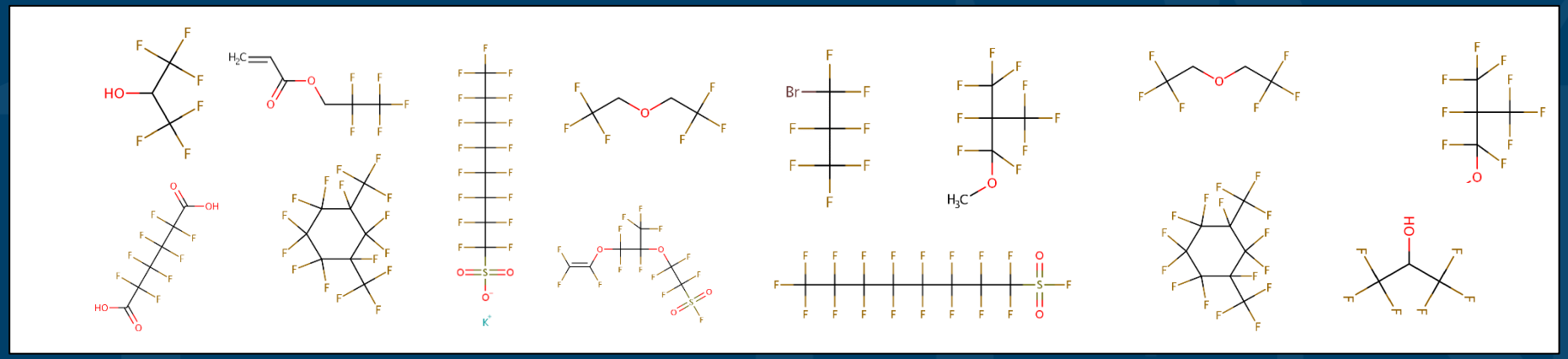
Overview of EPA National PFAS Testing Strategy and Related Studies



Presentation to JSC EIPH PFAS Strategy Team
July 25, 2022

Tala Henry, Deputy Director
Office of Pollution Prevention & Toxics
Office of Chemical Safety & Pollution Prevention

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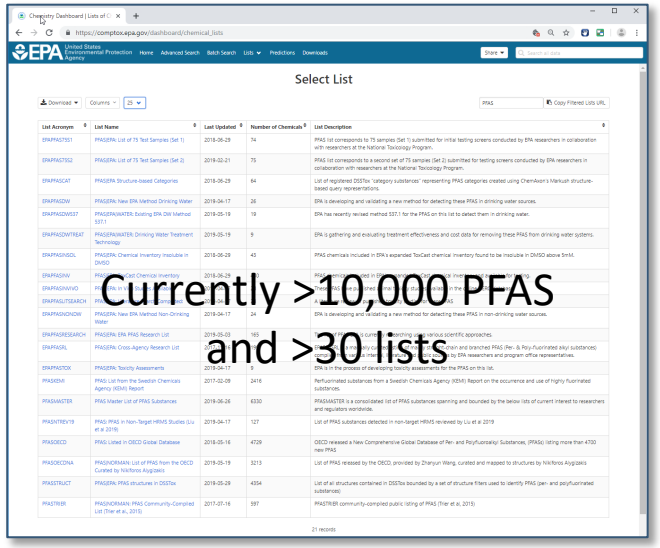
Center for Computational Toxicology & Exposure
Office of Research & Development

Jeff Dawson, Sr. Science Advisor
Office of Chemical Safety & Pollution Prevention

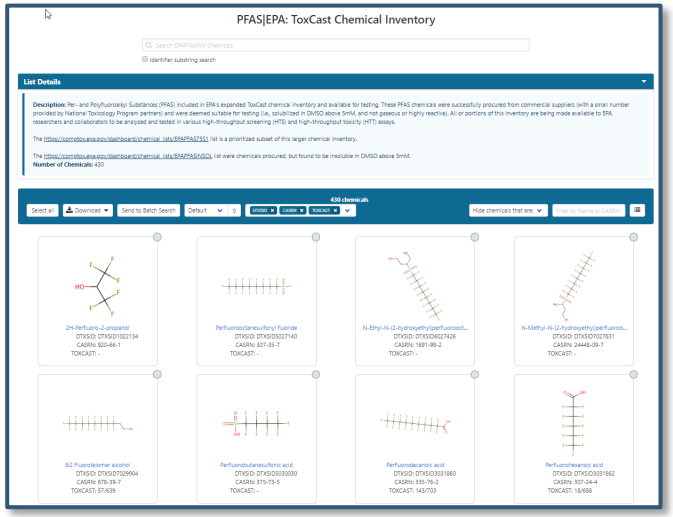
Quick Review of the State of the Science

- 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 (e.g., Toxic Substances Control Act, EPA and OECD High Production Volume Programs, Congressional direction).
- 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.

PFAS Chemical Curation and Library Development Efforts



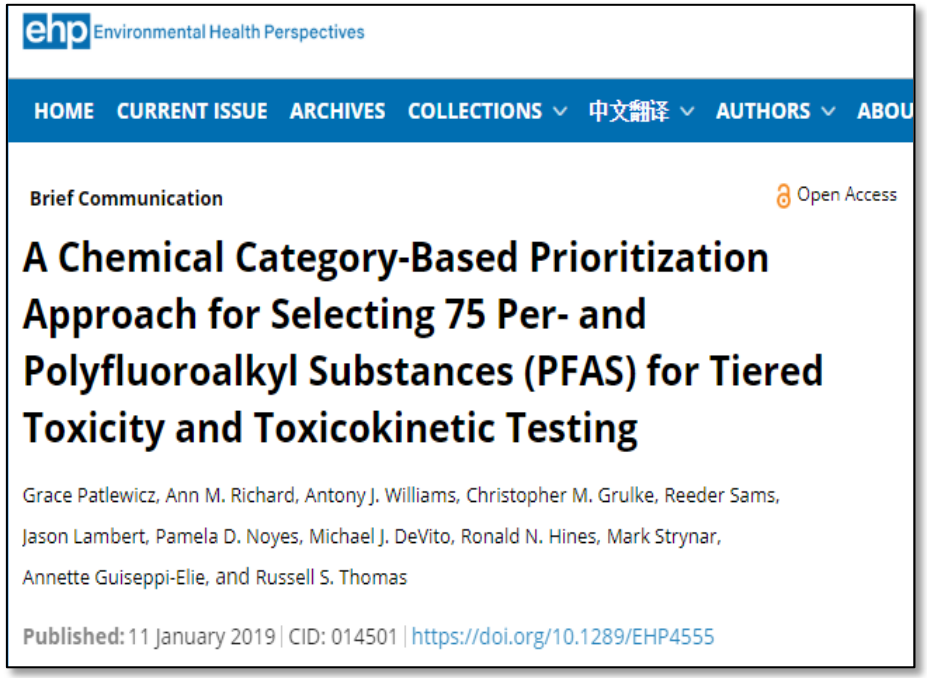
https://comptox.epa.gov/dashboard



- EPA has been curating PFAS structures and reference lists for multiple years to help standardize identifiers and provide a foundational resource for PFAS chemistry, toxicity, exposure, and environmental information.
- EPA attempted to procure ~3,000 PFAS for a physical library to provide substances for reference analytical standards, toxicity testing, mixture evaluation, and method development.
- A total of 480 unique PFAS were obtained, solubilized, and stored
  - ~90% of those tested were soluble in DMSO
  - ~70% of those tested were soluble in water
  - ~50% of those evaluated had issues with degradation, volatility, etc.
- PFAS library has been a community resource and shared with multiple federal, state, commercial, and academic partners

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

Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing



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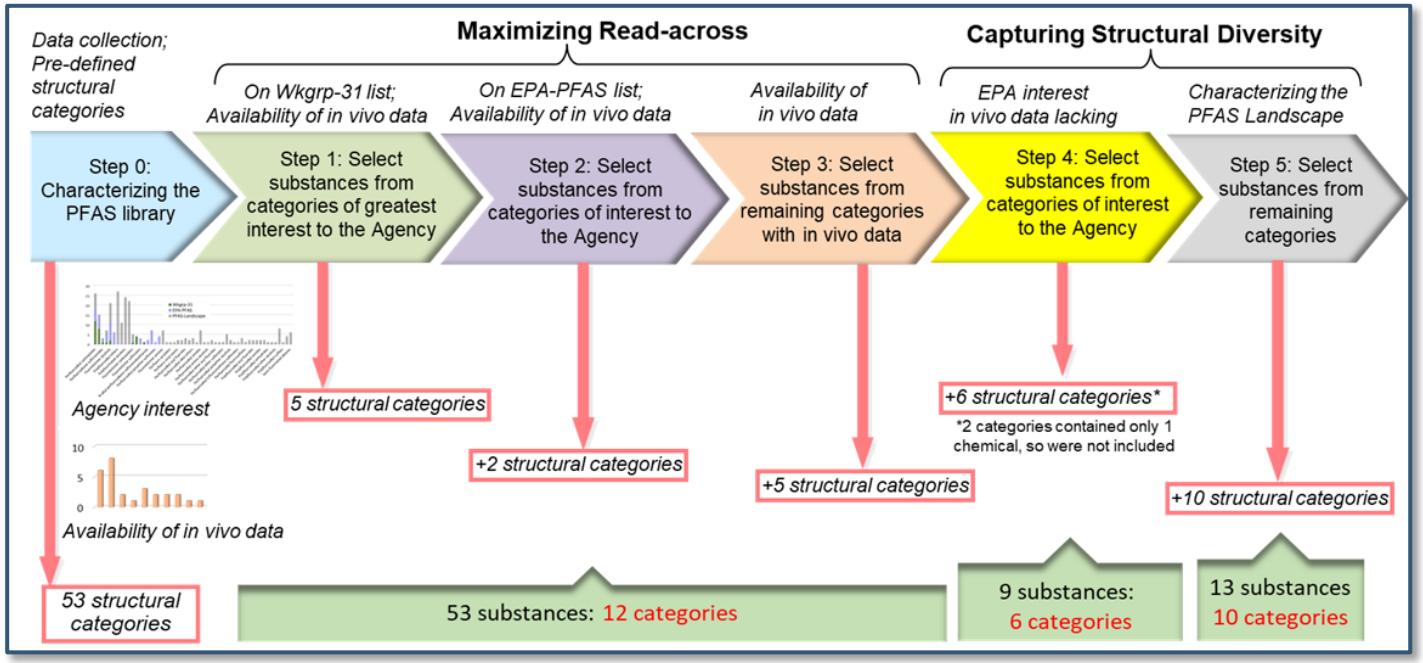
Brief Communication

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



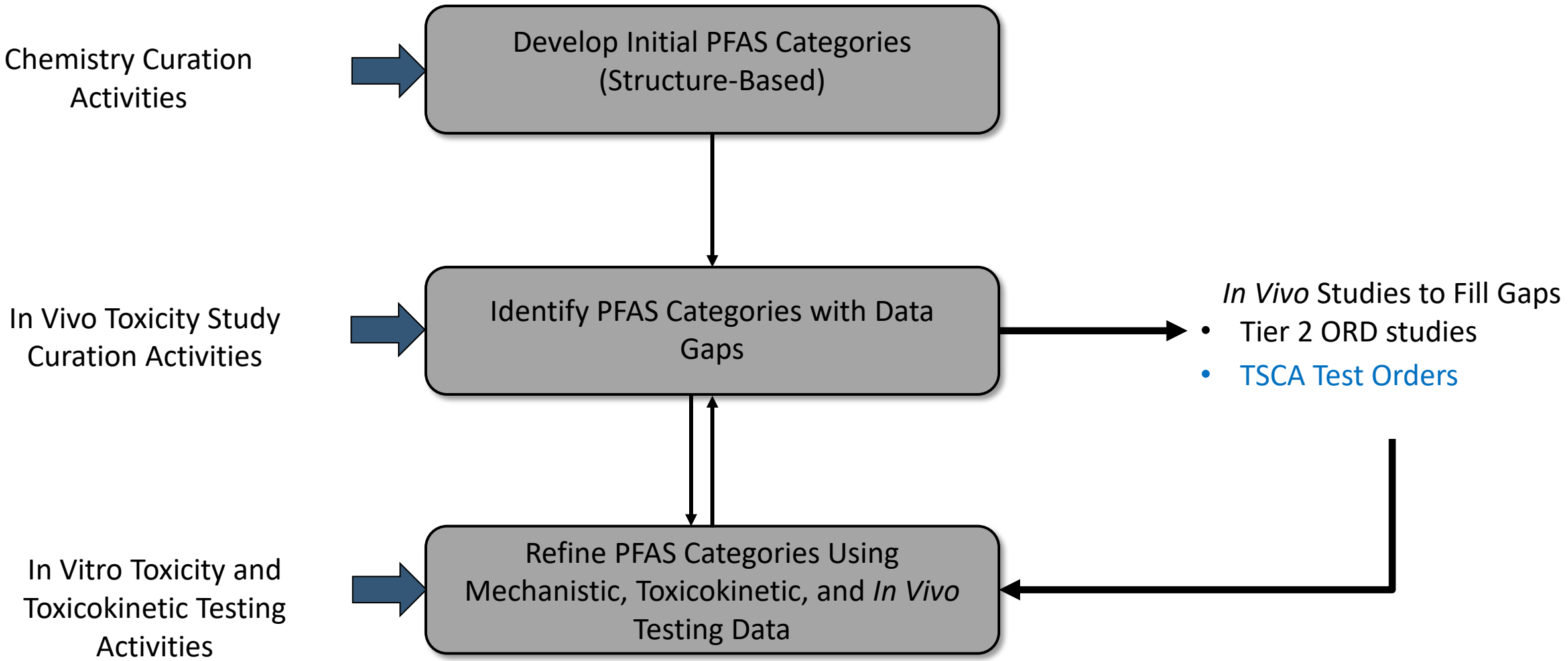
- Developed initial structural categories based on Buck et al., 2011
- A total of 150 PFAS were selected for Tier 1 *in vitro* mechanistic and toxicokinetic testing to support refinement of categories and read-across evaluation and other Agency priorities.

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 real-time cell proliferation assay (T47D)	Cell proliferation	Measure ER activity
Receptor-Mediated Toxicity	Attagene cis- and trans- Factorial assay (HepG2)	Nuclear receptor and transcription factor activation	Activation of key receptors and transcription factors involved in multiple toxicological mechanisms
General Toxicity	High-throughput transcriptomic assay (multiple cell types)	Cellular mRNA	Measures changes in important biological pathways
General Toxicity	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

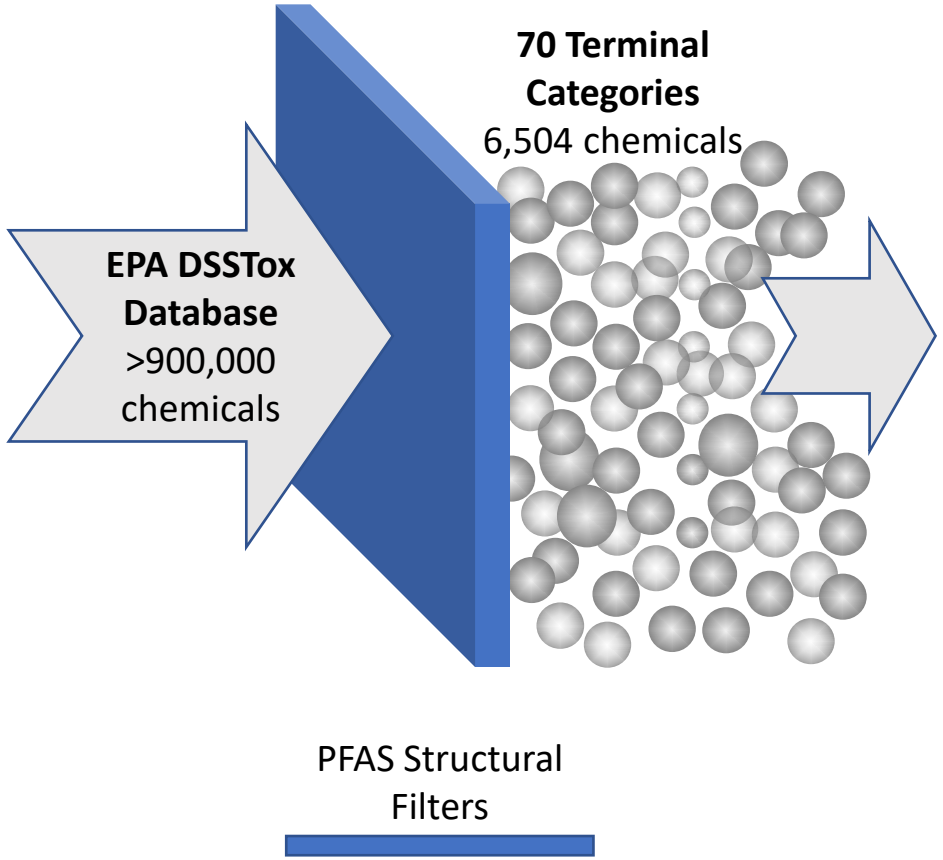
Toxicokinetic Parameter	Assay	Assay Endpoints	Purpose
Intrinsic hepatic clearance	Hepatocyte stability assay (primary human hepatocytes)	Time course metabolism of parent chemical	Measure metabolic breakdown by the liver
Plasma protein binding	Ultracentrifugation assay	Fraction of chemical not bound to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

Develop and Refine PFAS Categories for to Strategically Identify PFAS Candidates for Testing



Antony Williams, Grace Patlewicz, Richard Judson, Stan Barone, Martin Phillips, Kellie Fay, Tristan Butler

Testing Candidate Identification: Develop Initial PFAS Structural Categories



Structural Filters

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)

Primary Structural Categories*

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

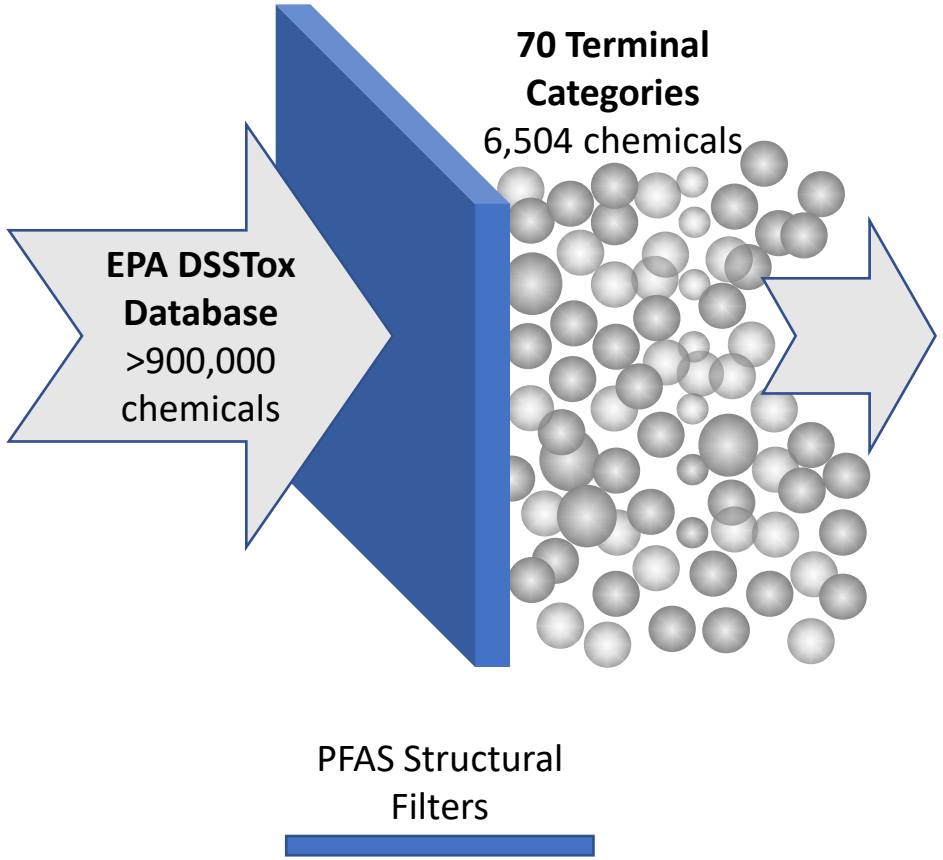
Secondary Structural Categories

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

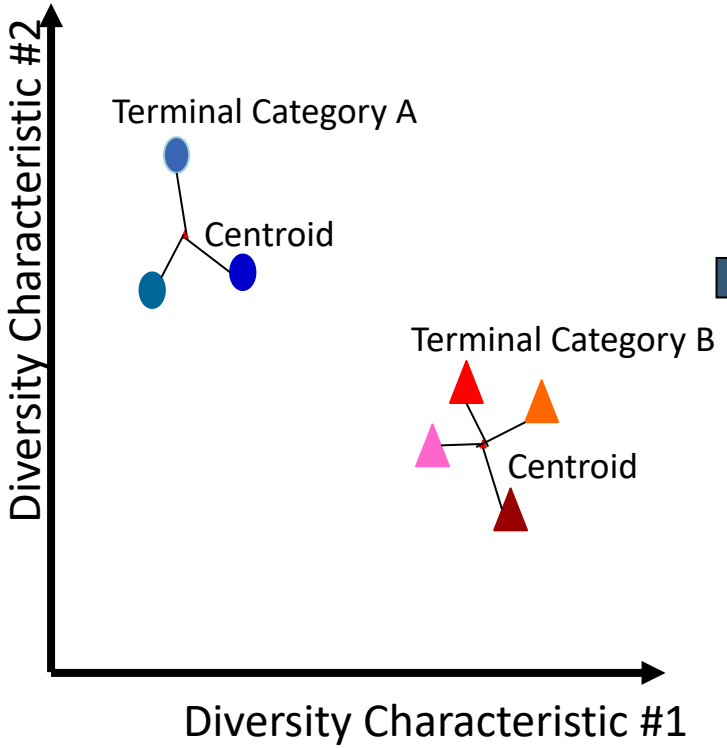
Tertiary Structural Categories

Tertiary categorization performed only on Secondary Categories with structural diversity greater than a defined threshold

Testing Candidate Identification: Identifying Most Representative Substance



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

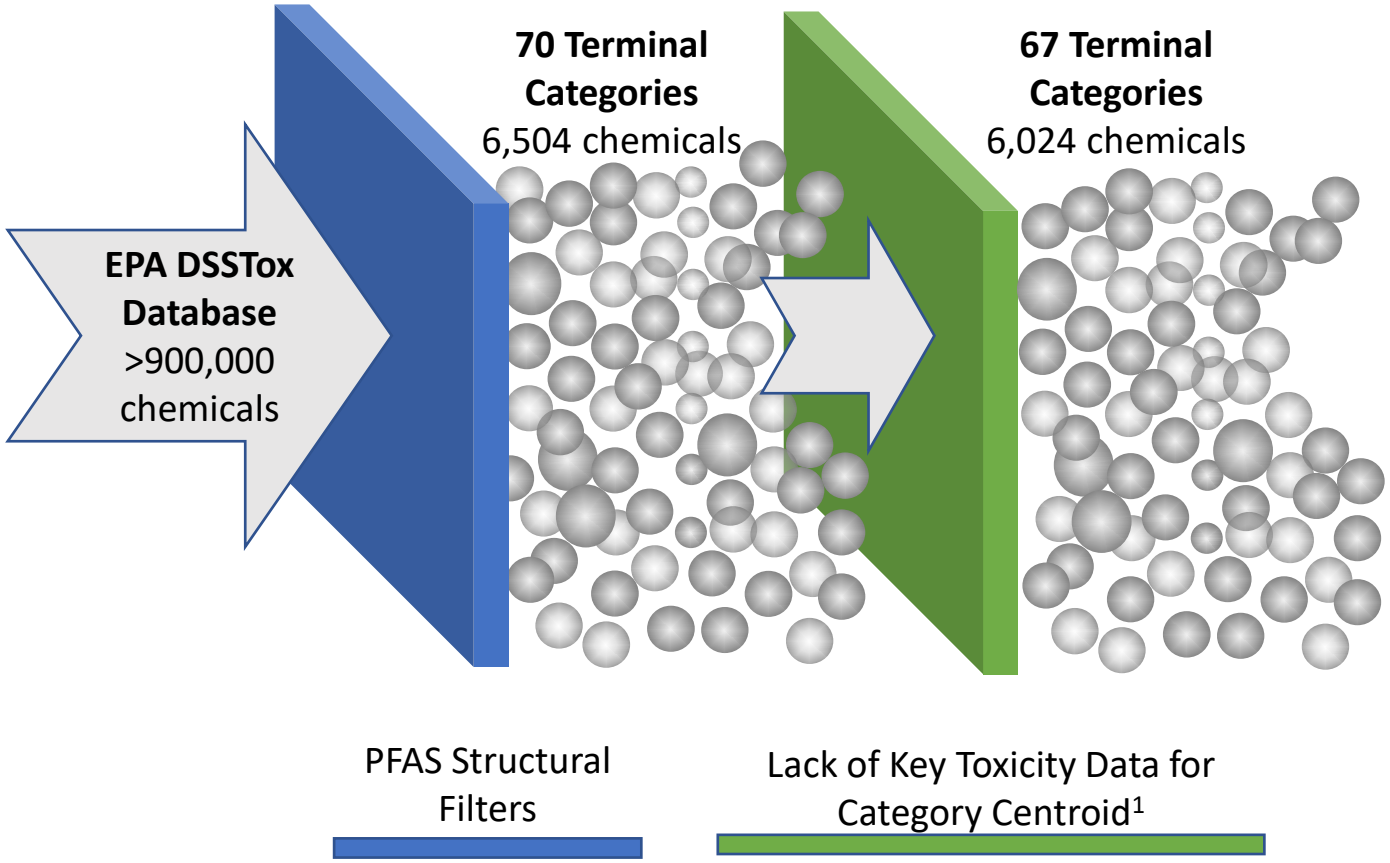


Mean of the chemical fingerprint matrix used to identify the 'centroid'.

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

Testing Candidate Identification: Existing Toxicity Data

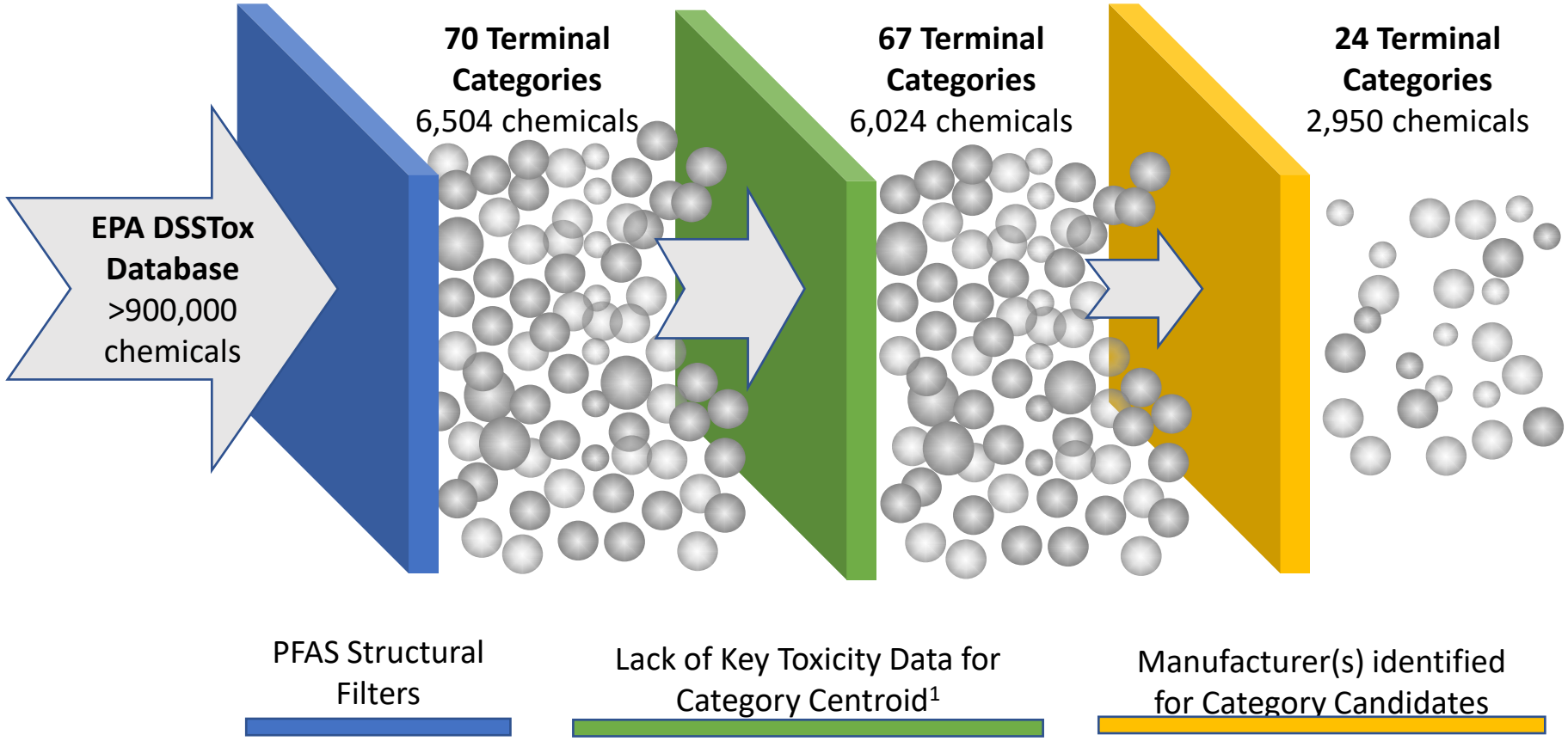
Prior to ordering testing using vertebrate animals, TSCA requires that available existing information be considered



Legacy *In Vivo* Toxicity Study Data Curation
Publicly Available (ORD) & TSCA Holdings (OPPT)

Testing Candidate Identification: Identify Manufacturers

To compel testing, EPA must identify manufacturer(s) of the PFAS to which TSCA Section 4 Order(s) are issued



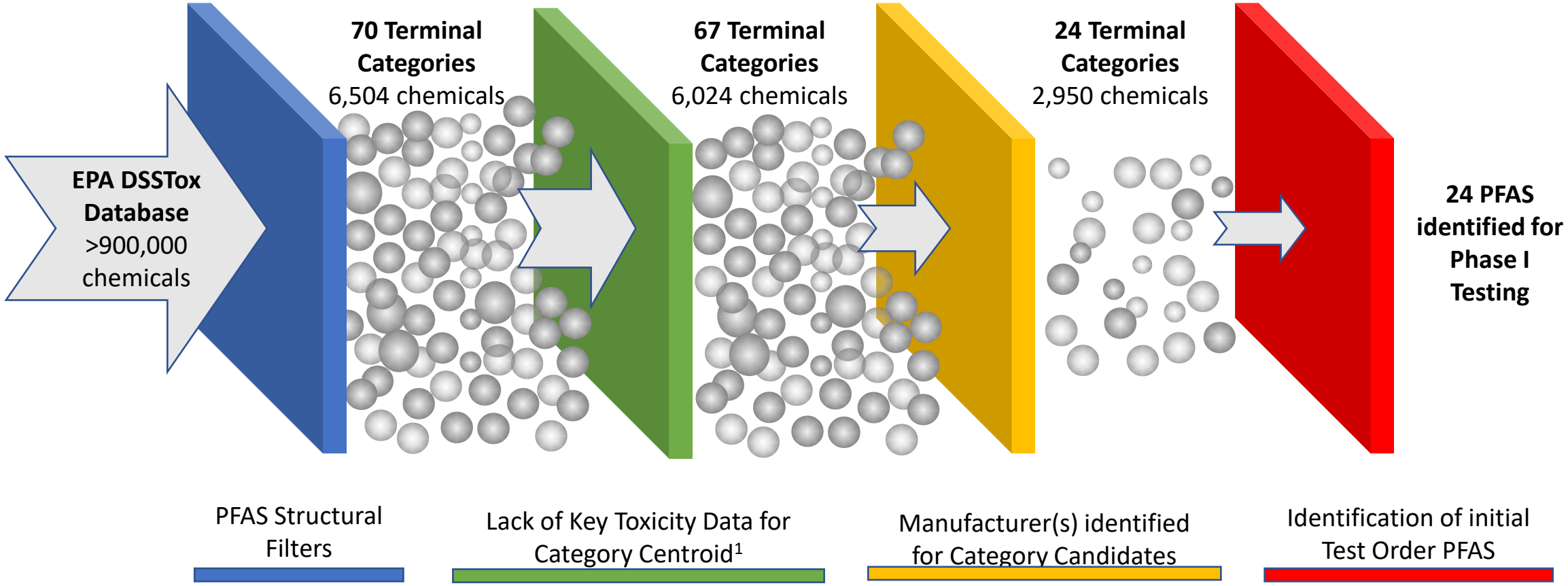
40 CFR § 711.3

Manufacturer Means a person who manufactures a chemical substance.

Manufacture means to manufacture, produce, or import, for commercial purposes.

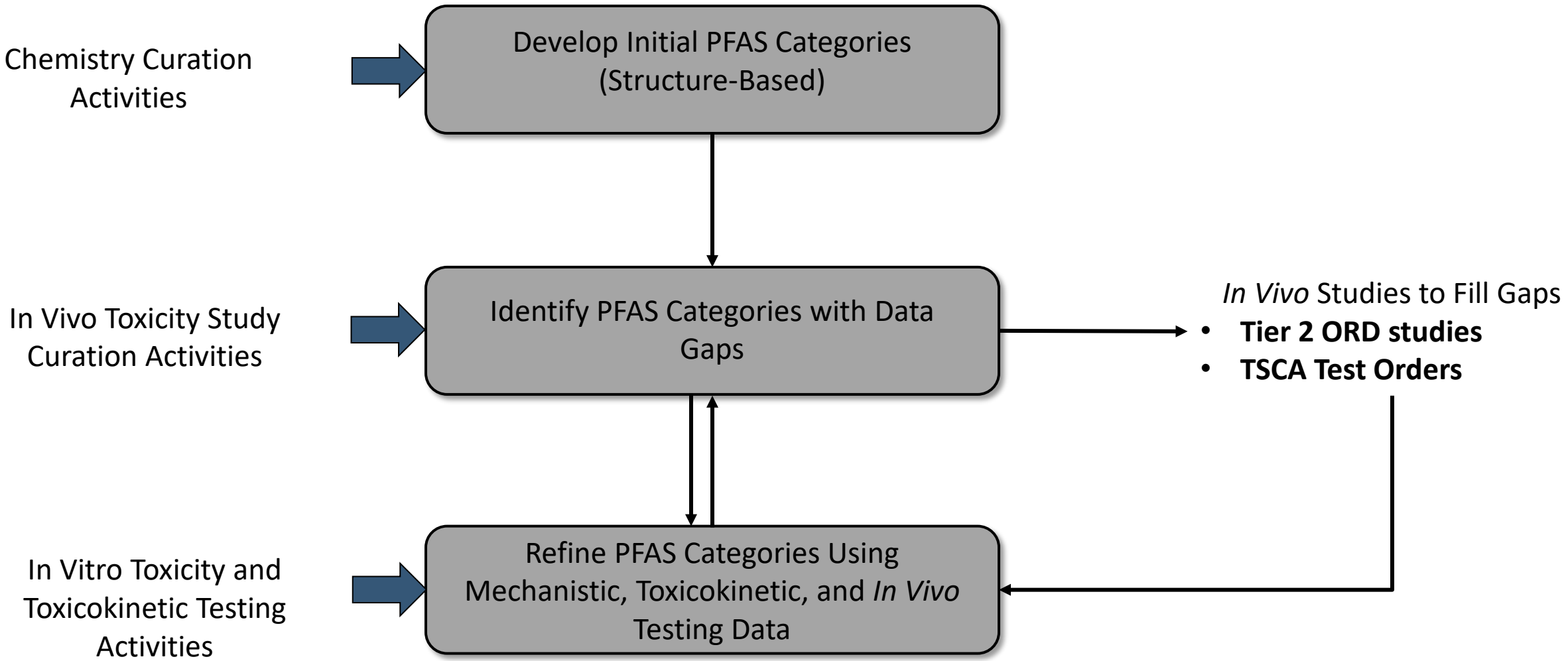
Testing Candidate Identification: 24 Candidates for Testing

24 PFAS from 24 terminal categories were identified for Phase I testing, which covers ~2,950 substances



*In some cases, a PFAS within the category with close structural distance to the category's centroid was selected as the candidate

Develop and Refine PFAS Categories for to Strategically Identify PFAS Candidates for Testing



Take Home Messages

- EPA ORD undertaking a multi-pronged strategy to characterize the chemistry, toxicity, and toxicokinetic properties of the broad class of PFAS.
- The testing to be conducted under the National PFAS Testing Strategy, and the category approach it employs, is strategic—to fill data gaps in a manner that will allow regulatory agencies to identify and focus on the highest potential risk PFAS soonest – and is also consistent with statutory direction to utilize a tiered testing approach and reduce testing in vertebrate animals.
- Initial structural categories will be refined using the mechanistic and toxicokinetic data.
- Link to EPA National PFAS Testing Strategy https://www.epa.gov/system/files/documents/2021-10/pfas-natl-test-strategy.pdf