National PFAS Testing Strategy:
Identification of Candidate Per- and Poly-fluoroalkyl Substances (PFAS) for Testing

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U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Contents

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Overview

The Environmental Protection Agency (EPA) needs to evaluate a large number of PFAS for potential human and ecological effects. Most of the hundreds of PFAS currently in commerce have limited or no toxicity data, and if EPA attempts to research them one at a time, it will be impossible for EPA to expeditiously understand, let alone address, the risks these substances may pose to human health and the environment. To address this data gap and fundamentally advance our understanding of these substances, EPA has developed this National PFAS Testing Strategy (Strategy) to deepen understanding of the impacts of PFAS, including potential hazards to human health and the environment. This Strategy will help EPA identify and select PFAS for which the Agency will require testing using Toxic Substances Control Act (TSCA) authorities. The Strategy develops categories of PFAS based on information about similarities in structure, physical-chemical properties, and existing test data on the toxicity of PFAS (both publicly available and submitted to EPA under TSCA). Consideration of the existing toxicity data prior to requiring further testing also ensures adherence to the TSCA goal of reducing animal testing. EPA will use the Strategy to identify important gaps in existing data and to select one or more candidate chemicals within identified categories for additional study. EPA expects to exercise its TSCA section 4 order authority to require PFAS manufacturers to conduct and fund the studies. EPA plans to issue the first round of test orders on selected PFAS by the end of 2021 with additional phases thereafter.

1. Introduction

PFAS are a large class of man-made chemicals that have been manufactured and used in a variety of industries since the 1940s. PFAS have been or are currently being synthesized for a variety of different uses ranging from adhesives, coatings for clothes and furniture, fire-fighting foams, and many others. PFAS are also used in industrial applications and processes, and in the manufacturing of countless other chemicals and products. PFAS have been released into the environment during manufacturing and use in industrial, commercial, and consumer settings. In addition, PFAS and products that contain them are regularly disposed of in landfills and incinerators, which can also lead to the further release of these compounds into the soil, water, and air.

Although certain PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been studied extensively, most PFAS lack data for robustly characterizing their potential toxicity. The information developed on certain PFAS provides evidence that exposure to such PFAS can lead to acute and chronic adverse human health outcomes.

Studies in laboratory animals indicate some PFAS can cause reproductive, developmental, liver, kidney, and immunological toxicity. In addition, exposure to some PFAS produce tumors in laboratory animals. In humans, the most consistent findings from epidemiology studies are increased cholesterol levels among exposed populations, with more limited findings related to infant birth weights, effects on the

immune system, cancer (for PFOA), and thyroid hormone disruption (for PFOS). Some PFAS can cause adverse effects on the respiratory system following acute inhalation exposures.[1]

To address the many of the data gaps associated with PFAS, in Congress included in the 2020 National Defense Authorization Act direction to EPA to develop a process for prioritizing which PFAS or classes of PFAS should be subject to additional research efforts based on potential for human exposure to, toxicity of, and other available information. The EPA has also initiated several regulatory activities aimed at collecting exposure- and toxicity-related information. For example, 175 PFAS have been added to the Toxics Release Inventory (TRI), which requires facilities that manufacture, process, and/or otherwise use these PFAS to report release and other waste management information to EPA. This information can be used to better understand human exposures to these chemicals. In addition, in June 2021, EPA proposed a TSCA section 8 rule that would require manufacturers and importers to report the identify of any PFAS manufactured since January 1, 2011, as well as byproducts from the manufacturing process, categories of use, production volumes, disposal information, worker exposures, and any information concerning environmental and human health effects.[2] EPA has identified at least 1,364 PFAS that would potentially be subject to the proposed rule. Finally, EPA is taking steps to address PFAS in drinking water. Under the Safe Drinking Water Act (SDWA), EPA is considering comments on the Fifth Unregulated Contaminant Monitoring Rule (UCMR 5) and preparing a final rule to collect new data on PFAS in drinking water. These data would improve EPA's understanding of the frequency that 29 PFAS are found in the nation's drinking water systems and at what levels. It would also expand the number of drinking water systems participating in the program. EPA's PFAS Strategic Roadmap explains additional actions the Agency plans to take to address PFAS through 2024.[3]

2. Purpose

This document describes EPA's Strategy for identifying candidate PFAS for which EPA plans to require companies to perform testing using its TSCA section 4 authority. The information derived from testing will be used by the Agency to evaluate of toxicity and risks associated with this large class of chemicals, and could further inform the Agency's future research, monitoring, and regulatory efforts. Given the large number of PFAS to which exposures may have occurred or that are currently ongoing, the Strategy is based on an approach that groups similar PFAS into categories. The categories serve as the basis for both identifying PFAS chemicals for testing as well as allowing EPA to establish toxicity levels for PFAS within the identified categories. Thus, rather than seeking data about each of the thousands of individual PFAS, which would require extensive resources in terms of time, costs, and animals, the Strategy aims to identify a representative substance(s) for each chemical category where categories have been constructed to span the landscape of PFAS of interest.

[1] EPA website for Basic Information on PFAS (accessed October 2021)
[2] TSCA Section 8(a)(7) Reporting and Recordkeeping Requirements for Perfluoroalkyl and Polyfluoroalkyl Substances, 86 FR 33926 (web link)
[3] EPA PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024 (2021)

3. Starting List of PFAS

The starting list of PFAS used in developing this Strategy was assembled using the process described below and illustrated in the first two elements in Figure 1.

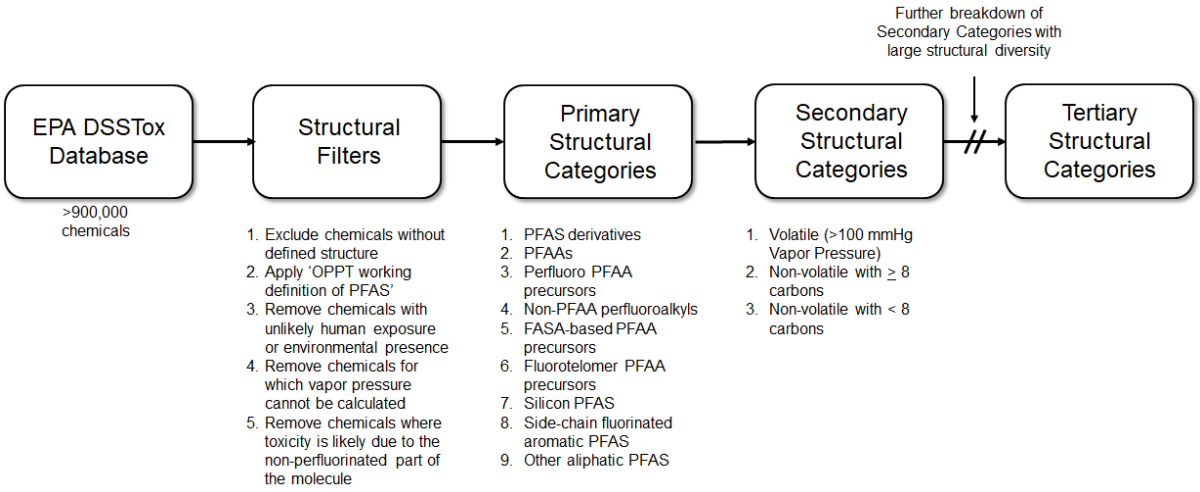

Figure 1: Schematic of Process Used to Create PFAS Categories

In the first step of the process, the EPA DSSTox database was used as the inventory of chemical substances from which the list was drawn (Version – April 2021).[4] The version of the EPA DSSTox database used to assemble the list contains over 900,000 chemical substances.

In the second step of the process, "Structural Filters," EPA used a series of five filters to generate the "starting list" of PFAS considered for the Strategy. First, chemical substances in the database without a defined structure were excluded from consideration because they did not have sufficient information to determine whether they should be considered a PFAS. Second, the resulting chemical substances were filtered for those that met the working definition of a PFAS used by EPA's Office of Pollution Prevention and Toxics (OPPT), which administers TSCA:

> "a structure that contains the unit R-CF_2-CF(R')(R''), where R, R', and R'' do not equal "H" and the carbon-carbon bond is saturated (note: branching, heteroatoms, and cyclic structures are included)."[5]

The working definition identifies chemicals with at least two adjacent carbon atoms, where one carbon is fully fluorinated and the other is at least partially fluorinated. This working definition provides focus on PFAS of concern based on their persistence and potential for presence in the environment and human exposure. For example, chemicals with (-CF2-) that are not (-CF3) are expected to degrade in the environment and most substances with only one terminal carbon (-CF3) are expected to degrade to trifluoroacetic acid, which is a well-studied non-PFAS. Chemicals with such degradation potential and for which vapor pressure could not be calculated were also excluded from the starting list.

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[4] Grulke CM, Williams AJ, Thillanadarajah I, Richard AM. EPA's DSSTox database: History of development of a curated chemistry resource supporting computational toxicology research. *Comput Toxicol*. 12:10.1016, 2019.

[5] Ibid TSCA Section 8(a)(7)

In addition, the Strategy focuses on PFAS where the toxicity of the substance is expected to primarily arise from the perfluorinated nature of the compound. As a result, additional filters were applied to develop the starting list. These filters eliminated free radicals and bare anions, while other filters eliminated salt forms where the counterion is expected to exert significant toxicity (e.g., transition metal salts/organometallics) and a variety of ringed structures. Many of the substances removed by the final filter were large multicyclic or macrocyclic structures with a small, fluorinated tail attached at some point.

The five sets of structural filters identified a starting list of 6,504 PFAS used in the development of the Strategy.

4. Dividing PFAS into Categories

Due to the large number and diverse types of PFAS, there have been several efforts to develop systematic terminology for their description and categorization.[6,7] However, the terminology and categories used in these efforts rely on manual assignment by trained chemists using standard criteria, which can be both subjective and time consuming when applied to thousands of chemicals. To overcome these issues, EPA used computer software developed by Su and Rajan[8] to systematically assign the starting list of 6,504 PFAS into the following nine primary categories based on their structure as illustrated in the third element ("Primary Structural Categories") of Figure 1 above:

- PFAS derivatives
- Perfluoroalkyl acids (PFAAs)
- Perfluoro PFAA precursors
- Non-PFAA perfluoroalkyls
- Perfluoroalkane sulfonamide (FASA)-based PFAA precursors
- Fluorotelomer-based PFAA precursors
- Silicon PFAS
- Side-chain Fluorinated Aromatic PFAS
- Other Aliphatic PFAS

PFAS that did not meet the conditions of membership for one of the primary categories listed above based on the structural rules were placed into an additional category denoted as "Others". Substances whose structures could not be resolved by the computer software, such as particular salt forms, were labelled as "Unclassified".

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[6] Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P, Jensen, A.A., Kannan, K., Mabury, S.A., and van Leeuwen S.P.J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integr Environ Manag.* 7(4):513-541, 2011.

[7] Organization for Economic Cooperation and Development (OECD). 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). 2018. Series on Risk Management, No. 39. ENV/JM/MONO(2018)7.

[8] Su, A., Rajan, K. A database framework for rapid screening of structure-function relationships in PFAS chemistry. *Sci Data* 8:14, 2021.

Each of the primary structural categories were further broken down into one of three secondary categories as illustrated in the fourth element ("Secondary Structural Categories") in Figure 1. The secondary categories include volatiles (>100 mmHg vapor pressure), non-volatiles with ≥8 carbons, and non-volatiles with <8 carbons. These secondary structural categories were employed because historically, changes in the length of the carbon chain have resulted in differences in toxicity and the length of time the chemicals spend in the body. The use of volatility to break down the primary structural categories was important when considering the route of exposure for testing.

Figure 2 below shows a bar graph depicting the number of PFAS within each secondary category that were identified as result of this process. Over 30 percent of the substances in the filtered starting list were assigned to the "Others, gte8" secondary category (gte8 = greater than or equal to 8 carbons). Of the 1,927 PFAS in the "Others, gte8" secondary category, only 29 are "active" in commerce in the United States as determined in recent Active/Inactive reporting required under TSCA at 40 CFR Part 710 (82 FR 37520) (FRL-9964-22).

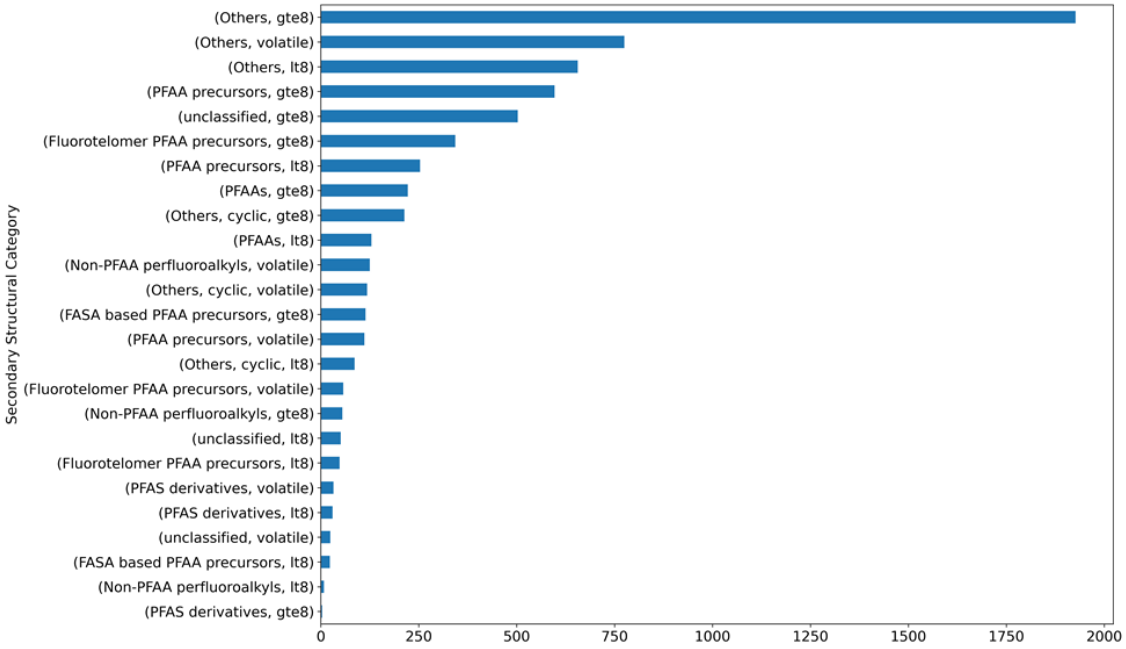

Figure 2: Frequency Plot of Number of Substances by Secondary Category
Key: lt8 = less than 8 carbons; gte8 = greater than or equal to 8 carbons

Since the Strategy is based on an approach that groups similar PFAS into categories based on structure, it is important to evaluate the degree of structural similarity within each category and compare that to similarity across the larger set of PFAS. To achieve this, each PFAS was characterized by a chemical fingerprint[9] [10] that is composed of the various structural features of the molecule. These structural features include the different types and arrangement of elements in the molecule, the bonds that hold

[9] Morgan, H.L. The generation of a unique machine description for chemical structures - A technique developed at Chemical Abstracts Service. *J. Chem. Doc*. 5:107-112, 1965.

[10] Morgan fingerprints are a type of hashed fingerprints. Hashed fingerprints do not require a pre-defined fragment library. Instead, they are generated by enumerating the molecule through all possible fragments that are not larger than a certain size and then converting the fragments into numeric values using a hash function. These numeric values can then be used to indicate bit positions in the hashed fingerprint. Circular fingerprints are generated by considering the 'circular' environment of each atom up to a given radius. The Morgan fingerprints calculated in this study were of length 1024 using a radius of 3.

those elements together, and other features of the chemical. The use of chemical fingerprints allowed for an objective comparison of how similar or different each PFAS is relative to another. When looking at chemical structures chemists often refer to similarity with the concept of structural distance. The smaller the structural distance between two chemicals, the more structurally similar they are. Using the chemical fingerprints, EPA calculated the structural similarity[11] [12] for each possible pair of PFAS on the starting list. This produced a large matrix where the similarity between all PFAS on the starting list could be examined.

To determine which secondary categories needed to be further divided, the structural distances (i.e., the degree of similarity) were calculated both within each secondary category and between categories as illustrated above in the fifth element ("Tertiary Structural Categories") of Figure 1. The rationale behind this approach is that the structural similarity within a category should be greater than the structural similarity between categories. A conceptual schematic of "within" and "between" category distances is provided in Figure 3.

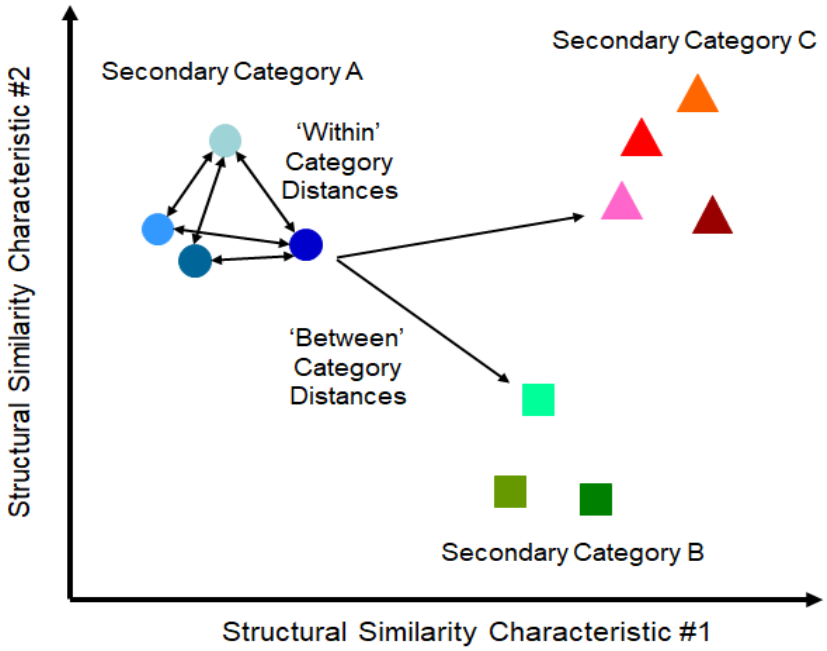

Figure 3. Conceptual Schematic to Illustrate the Within and Between Category Distances for the Secondary Categories

The distributions of the "within" and "between" structural distances among the secondary categories are provided below in Figure 4. A distance threshold for secondary categories that lack adequate structural similarity was set at the lower 5th percentile of the "between" category distribution. Secondary categories exceeding this median distance were further divided into tertiary categories to obtain greater structural similarity. A total of 70 terminal categories were identified (i.e., secondary or tertiary categories with adequate similarity).

[11] Jaccard, P. The distribution of the flora in the alpine zone. *New Phytologist*. 11(2):37–50, 1912.

[12] The Jaccard distance is a unitless number between zero and one that measures how dissimilar two sets (in this case two chemicals) are from one another. A Jaccard distance of zero means the two chemicals are identical, a Jaccard distance of one means the chemicals share nothing in common. In the context of Morgan fingerprints, a Jaccard distance of 0.5 means that half the fingerprint matches between two chemicals while the other half does not match.

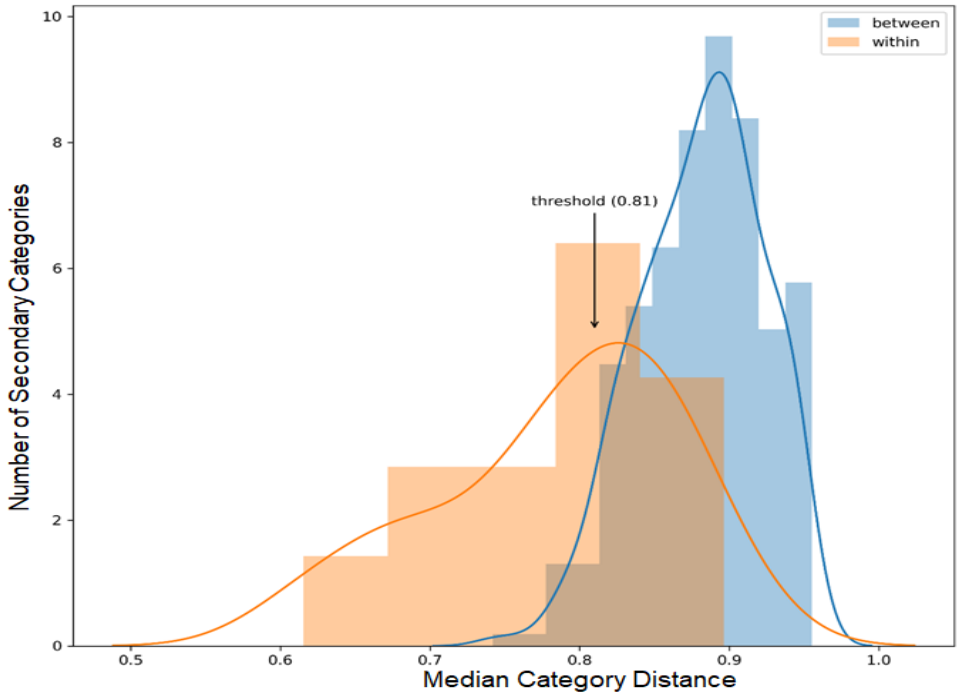

Figure 4: Probability Density Function Plot & Histogram - Within & Between Primary-Secondary Combinations

For each terminal category, EPA calculated the average or "centroid" of all the chemical structural features. The centroid depicts the most representative virtual chemical structure in that category as illustrated below in Figure 5. It may or may not depict an actual PFAS structure. EPA then used the centroids as the conceptual anchor within each terminal category to define a candidate PFAS for testing.

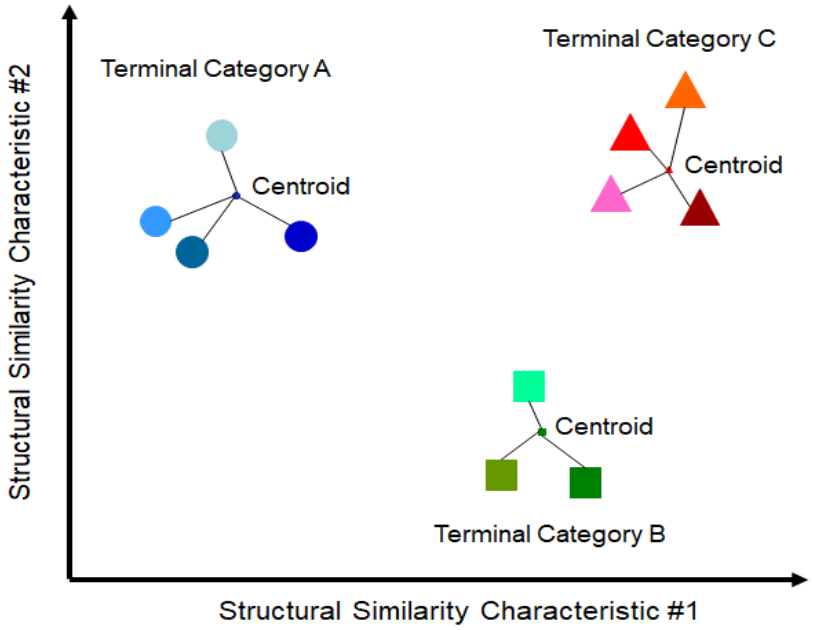

Figure 5: Graphical Illustration of Centroid Concept

5. Assembling Existing Toxicity Data

For each substance on the starting list of PFAS, EPA identified all available, human health-related toxicity studies and divided them into the following study types:

a. Acute
b. Subchronic
c. Chronic including Cancer Bioassays
d. Developmental
e. Reproductive
f. Immunotoxicity
g. Neurotoxicity
h. Toxicokinetics
i. Mutagenicity
j. Sensitization/Irritation

EPA identified toxicity data from two separate sources – the EPA Toxicity Value Database (ToxValDB) and the EPA Chemical Information System (CIS).

The EPA ToxValDB is a compilation of publicly-derived experimental toxicity data on ~34,000 chemicals from 43 distinct sources including US EPA, U.S. Food and Drug Administration (FDA), California Office of Environmental Health Hazard Assessment (OEHHA), Agency for Toxic Substances and Disease Registry (ATSDR), Department of Energy (DOE), California Department of Public Health (DPH), the World Health Organization (WHO), Health Canada, the European Chemicals Agency (ECHA), European Food Standards Agency (EFSA), and the European Commission's Cluster of Systems of Metadata for Official Statistics (COSMOS) database. These sources include toxicity data from the scientific literature, reports, regulatory toxicology study submissions, or government-sponsored studies (e.g., U.S. National Toxicology Program).

The EPA CIS is an internal platform for managing data submissions under TSCA, including toxicity studies. Most of the data within CIS has been provided by industry in conjunction with TSCA submissions and are not publicly available. EPA is working on to make data publicly available to the extent possible under current statutory requirements and given resource constraints.

6. Initial Test Candidate Identification

To identify the initial PFAS candidates for testing, EPA mapped the existing toxicity data from ToxValDB and CIS onto each of the 70 terminal categories. Through this mapping process, EPA identified a total of 56 terminal categories that lack any data about the toxicity of the PFAS in that category. EPA identified PFAS candidates for testing from each of those 56 terminal categories based on the following considerations:

- Whether EPA can identify one or more manufacturer(s) of the PFAS candidate at this time (i.e., EPA can readily and confidently identify recipient(s) for TSCA test orders).[13]

[13] EPA consulted a variety of submissions received pursuant to TSCA (e.g., sections 4, 5 and 8) to identify potential section 4 test order recipients.

- The candidate's structural distance from the centroid of the terminal category (i.e., the closer to the centroid the greater preference for testing).

Of the 56 terminal categories lacking toxicity data, only 24 contained PFAS with an identifiable manufacturer(s) to whom EPA could issue a test order (Appendix A). As a result, EPA will consider the distance from the centroid in selecting PFAS for testing for 24 terminal categories. However, this Strategy is an iterative process and as EPA identifies additional PFAS manufacturers (e.g., through reporting under the future TSCA section 8(a)(7) rule) EPA may expand this initial list of candidate PFAS. Figure 6 below provides an overview of the steps of the process involved in the identification of initial testing candidates.

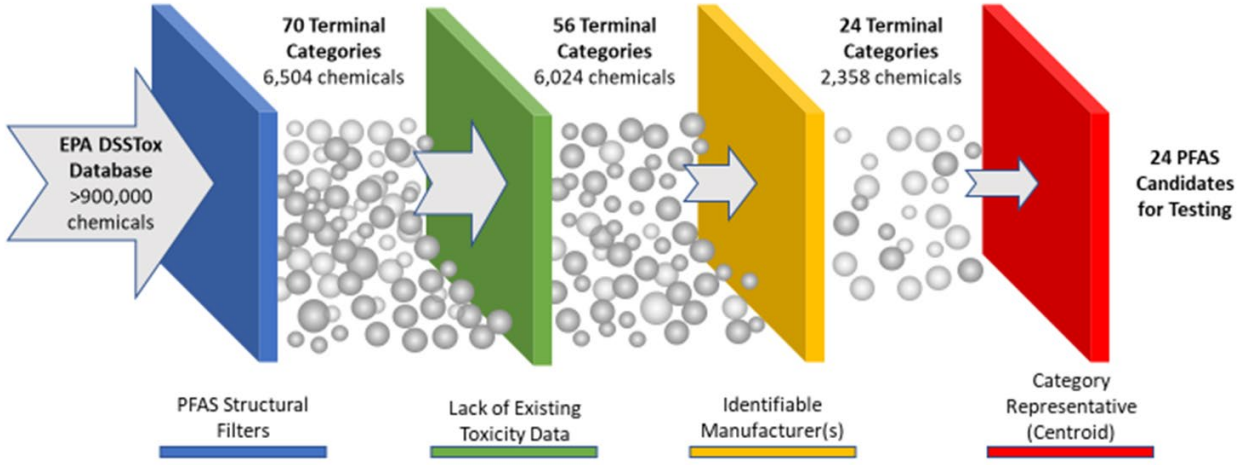

Figure 6: Overview of the Process for Identifying Initial Testing Candidates

7. Potential Tests

EPA's application of the category approach described above is consistent with the statutory mandate to reduce and replace the use of vertebrate animals in the testing of chemicals under section 4(h) of TSCA. The use of a tiered approach to identify specific testing for the candidate PFAS is also consistent with section 4(h) of TSCA.

EPA's Office of Chemical Safety and Pollution Prevention (OCSPP) has developed and uses a variety of test guidelines to support regulatory actions for chemicals under various statutes, including TSCA.[14] These guidelines are extensive and cover a wide array of test endpoints. Other organizations have also developed and utilize similar testing approaches, including the Organization of Economic Cooperation and Development (OECD), which maintains published testing guidelines for evaluating health effects.[15] OECD guidelines are considered routinely by EPA under the OECD mutual acceptance of data (MAD) system.[16] EPA has developed a crosswalk for the OECD guidelines with its own, which also provides a summary of all study types and the organizational codes associated with them.[17] EPA also

[14] EPA web site on Test Guidelines for Pesticides and Toxic Substances (accessed October 2021)

[15] OECD web site on Test Guidelines for Chemicals (accessed October 2021)

[16] OECD web site on Mutual Acceptance of Data (accessed October 2021)

[17] OCSPP list of harmonized test guidelines (last updated September 2019, accessed October 2021)

routinely considers other scientifically relevant information (OSRI) in lieu of testing that is conducted strictly in accordance with test guidelines. OSRI would have to be evaluated by EPA and considered adequate in addressing data needs.

A general overview of the tiered approach is presented below.

Tier I: consists of physical-chemical properties and *in vitro* testing to inform and guide whether additional short-term *in vivo* toxicity and/or toxicokinetic tests should be considered. For instance, PFAS that are gases will generally not be subject to Tier I *in vitro* testing due to methodological limitations and therefore higher tier *in vivo* toxicity testing may be the most logical initial testing approach.

- Physical-chemical property tests: vapor pressure, water solubility, log K_{ow}, particle size and surface tension (measures surfactant properties) to inform the conduct of test guideline protocols (e.g., closed systems for volatile PFAS, relevant route(s) of exposure, etc.).

- *In vitro* metabolism and protein binding studies (e.g., liver metabolism, protein binding and kidney transport protein binding) to inform the need for *in vivo* toxicokinetic studies.

- Some PFAS show positive results for genotoxicity.[18] Therefore, EPA is considering *in vitro* genotoxicity for chromosomal aberrations/gene mutations (e.g., OECD TG 471 and OECD TG 473 or 487) to inform the need for higher-tier *in vivo* toxicity testing for adverse outcomes related to genotoxicity.

- *In vitro* nuclear receptor/activation assays may also be considered because PFAS have been shown to activate multiple nuclear receptors.[19,20] These data can provide insights regarding human relevance (e.g., whether the chemical is active only in the PPARα assay) and inform the need for higher tier *in vivo* toxicity testing (e.g., for cancer and non-cancer endpoints).

Tier II: consists of testing to inform which species and doses to use in Tier III testing. Depending on results of Tier I, and types of toxicities identified for the PFAS categories based on existing available data, Tier II tests may include:

- *In vitro* skin absorption testing (e.g., OECD TG 428) for PFAS that have conditions of use with potential for dermal exposures. Results may also be useful for route-to-route extrapolation, thereby expanding applicability of existing or new higher tier tests.

- *In vivo* genotoxicity testing (e.g., OECD TG 474), depending on the results of Tier I *in vitro* genotoxicity testing.

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[18] ATSDR (Agency for Toxic Substances and Disease Registry). 2021. Toxicological Profile for Perfluoroalkyls. U.S. Department of Health and Human Services. May 2021.

[19] Houck, K.A., Patlewicz, G., Richard, A.M., Williams, A.J., Shobair, M.A., Smeltz, M., Clifton, M.S., Wetmore, B.A., Medvedev, A., Makarov, S. Bioactivity profiling of per-and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure. *Toxicology.* 457:152789, 2021.

[20] Ibid, ATSDR.

- Acute *in vivo* inhalation toxicity testing (OECD TG 403), based on Tier I physical-chemical properties testing that indicate potential for surfactant effects.

- *In vivo* toxicokinetic testing in rats and/or mice (OECD TG 417) with evaluation of metabolites. Existing data indicate half-lives and clearance rates may differ significantly among PFAS and species.[21,22] Therefore, this data will inform which species and dosing regimes are most appropriate for higher tier toxicity testing. *In vivo* toxicokinetic testing will be informed by Tier I *in vitro* metabolism and protein binding studies when feasible.

Tier III: consists of testing to identify dose levels (i.e., points of departure) for risk evaluation. Existing data on tested PFAS provide evidence for probable links between PFOA and both kidney and testicular cancer in humans.[23] Other epidemiological studies have identified some associations between PFAS and certain cancers including prostate and breast cancer.[24] Both PFOA and GenX are known to cause tumors in animal studies.[25,26] Based on existing data, PFAS may also cause cancer via a non-genotoxic mechanism. Therefore, EPA will consider systemic toxicity testing that measures adverse endpoints such as liver and kidney disease, immunotoxicity, thyroid function, lipid dysregulation and reproductive and developmental toxicity.[27] The types of effects identified for additional testing may include:

- Testing for cardiac sensitization. Certain terminal categories consisting of short-chain volatile PFAS may be considered for testing for cardiac sensitization[28] because existing data for halogenated hydrocarbons indicate these compounds may lead to cardiac arrythmias and occasionally to sudden death resulting from sensitization of the heart muscle to endogenous compounds in the body (e.g., adrenaline).[29,30]

- 28-day inhalation toxicity test (OECD TG 412). If the Tier II acute inhalation toxicity test shows a toxic dose level (i.e., low observable adverse effect concentration) below the limit dose (< 2,000 mg/m^3), longer duration testing via inhalation route may be considered.

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[21] ibid, ATSDR.

[22] Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M. Per- and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategy for informing future research. *Environ Toxicol Chem*. 40(3):606-630, 2021.

[23] C8 Science Panel web site on the Probable Link Evaluation of Cancer (Created April 2012, accessed October 2021).

[24] ibid ATSDR

[25]United Stated Environmental Protection Agency (EPA). 2016. Health Effects Support Document for Perfluorooctanoic Acid (PFOA). Office of Water. EPA 822-R-16-003. 2016.

[26] United States Environmental Protection Agency (EPA). Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3). Public Comment Draft. Office of Water. EPA-823-P-18-001. 2018.

[27] ibid, ATSDR & ibid Fenton

[28] European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Evaluation of cardiac sensitization test methods. Technical Report No. 105, 2009.

[29] Brock, W.J., Rusch, G.M., Trochimowicz, H.J. Cardiac sensitization: Methodology and interpretation in risk assessment. *Regul Toxicol Pharmacol.* 38(1):78-90, 2003.

[30] Himmel, H.M. Mechanisms involved in cardiac sensitization of volatile anaesthetics: General applicability to halogenated hydrocarbons? *Crit Rev Toxicol.* 38(9):773-803, 2008.

- 28- or 90-day toxicity testing (OECD TG 407 or 408) may be included in Tier III because some PFAS have shown immunotoxicity, liver and kidney disease, thyroid function, lipid dysregulation in previous studies.[31]

- Prenatal developmental toxicity testing (OECD TG 414) may be included in Tier III because some PFAS have shown delayed ossification and other developmental effects in previous studies.[32]

- Extended one-generation reproductive toxicity testing (OECD TG 443) may be included in Tier III because some PFAS have shown postnatal toxicological effects, including delays in sexual maturation and growth, other developmental delays, and mortality.[33] The extended one-generation reproductive toxicity test can also address concerns related to potential maternal, fetal, and postnatal thyroid hormone disruption as well as includes options for evaluating developmental toxicity and developmental immunotoxicity, which are effects identified in animal and epidemiological studies for some PFAS.[34]

- Carcinogenicity testing (OECD TG 451) may be included in Tier III because some PFAS have produced tumors in animals and have been associated with cancer in humans. The need for carcinogenicity testing will informed by physical-chemical properties, Tier I testing, and existing data. For example, the reactivity, ability to cause glutathione depletion, genotoxicity, *in vitro* nuclear receptor assays, and the results from shorter-duration *in vivo* toxicity studies will be considered holistically in a weight of evidence to inform the need for carcinogenicity testing.

The tiered-testing approach of this Strategy aims to first and foremost collect information for each candidate PFAS that is sufficient to estimate or predict the physical-chemical properties and toxicity of other PFAS in the associated category. EPA anticipates that collecting this information will inform whether refinements to the category may be needed and determine whether testing additional PFAS within a category may be necessary. For example, similarities, differences, or trends in testing results across categories may indicate that further dividing the terminal categories is justified. As EPA obtains data for the candidate PFAS throughout the testing process, the agency may use those results to revise the testing strategy.

8. Phased Implementation

EPA intends to implement the Strategy in Phases (Figure 7). Phase IA is focused on human health data collection. EPA will be initiate Phase IA by the end of 2021 using TSCA Section 4 authorities. Then, in Phase IB, EPA will refine the initial structural categories using mechanistic and toxicokinetic data from EPA Office of Research and Development (ORD) as well as further evaluation of degradation products and exposure data (e.g., environmental monitoring, biomonitoring). The EPA expects to issue further TSCA Test Orders after the categories are refined. The process for refining and issuing Test Orders will be an iterative process as testing data is submitted to the Agency. In the second Phase of the Strategy (Phases IIA and IIB), EPA expects to use the category approach described above to inform ecological toxicity testing needs.

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[31] ibid ATSDR & ibid Fenton

[32] ibid ATSDR

[33] ibid ATSDR

[34] ibid ATSDR & ibid Fenton

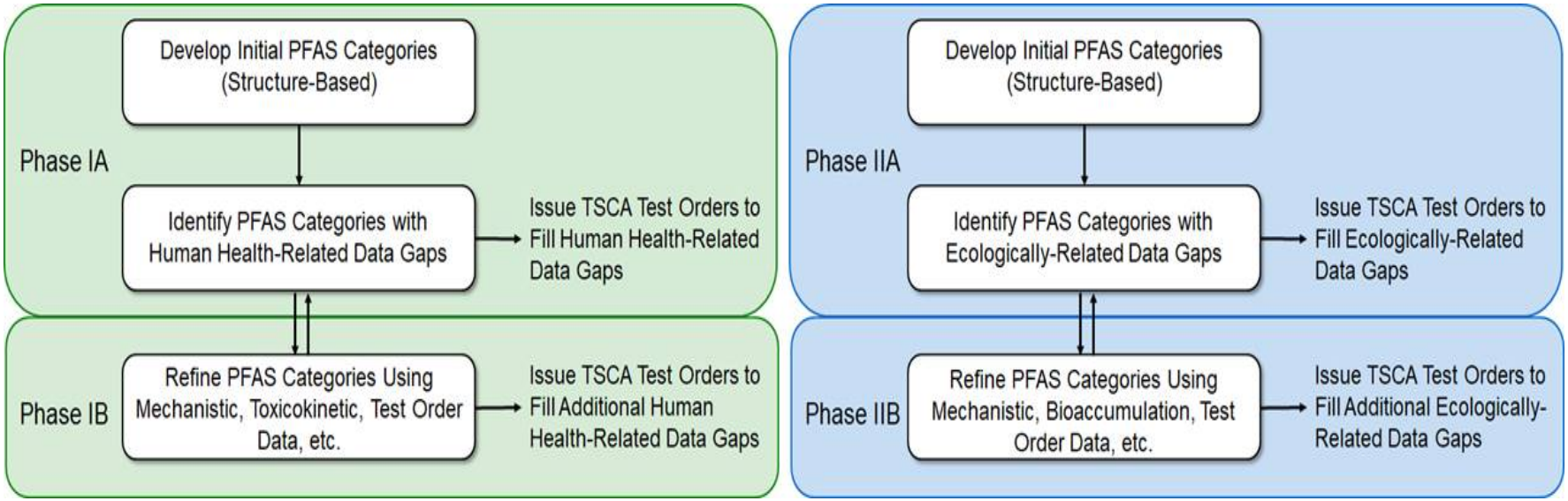

Figure 7: Multi-phase Testing Strategy for Filling Human Health and Ecological Data Gaps. Phases IA and IB are highlighted in green and are focused on human health-related data. Phases IIA and IIB are highlighted in blue and are focused on ecologically-related data.

Appendix A: List of PFAS Candidates for Testing

DTXSID_hyperlink	CASRN	Terminal Category	Candidate PFAS Name
DTXSID4059966	422-05-9	('Fluorotelomer PFAA precursors', 'lt8')	2:1 Fluorotelomer alcohol
DTXSID0046511	306-94-5	('Non-PFAA perfluoroalkyls', 'gte8')	Perflunafene
DTXSID9041811	115-25-3	('Non-PFAA perfluoroalkyls', 'volatile')	Octafluorocyclobutane
DTXSID7046548	355-42-0	('Non-PFAA perfluoroalkyls', 'volatile')	Perfluorohexane
DTXSID50880192	3330-14-1	('Others', 'gte8')	2H-Perfluoro-5-methyl-3,6-dioxanonane
DTXSID60862823	2062-98-8	('Others', 'lt8')	Perfluoro(2-methyl-3-oxahexanoyl) fluoride
DTXSID0059879	355-80-6	('Others', 'lt8')	1H,1H,5H-Perfluoropentanol
DTXSID2067327	27619-88-1	('Others', 'lt8')	3,3,4,4,5,5,6,6,6-Nonafluorohexane-1-sulphonyl chloride
DTXSID3059927	376-90-9	('Others', 'lt8')	Hexafluoroamylene glycol
DTXSID50862736	1682-78-6	('Others', 'volatile')	2,3,3,3-Tetrafluoro-2-(perfluoroethoxy)propanoyl fluoride
DTXSID0061826	1623-05-8	('Others', 'volatile')	Perfluoropropyl trifluorovinyl ether
DTXSID90505110	42532-60-5	('Others', 'volatile')	2,3,3,3-Tetrafluoro-2-(trifluoromethyl)propanenitrile
DTXSID30889183	475678-78-5	('Others, cyclic', 'gte8')	3-Methyl-3-[[(3,3,4,4,5,5,6,6,6-nonafluorohexyl)oxy]methyl]-oxetane
DTXSID30880413	38565-52-5	('Others, cyclic', 'gte8')	3-(Perfluorohexyl)-1,2-epoxypropane
DTXSID7059933	382-28-5	('Others, cyclic', 'lt8')	Perfluoro(N-methylmorpholine)
DTXSID6029177	428-59-1	('Others, cyclic', 'volatile')	Trifluoro(trifluoromethyl)oxirane
DTXSID50880218	15290-77-4	('Others, cyclic', 'volatile')	1H,1H,2H-Perfluorocyclopentane
DTXSID5027140	307-35-7	('PFAA precursors', 'gte8')	Perfluorooctanesulfonyl fluoride
DTXSID70887648	69116-72-9	('PFAA precursors', 'lt8')	Methyl perfluoro-3-[(perfluoro-3-oxopropan-2-yl)oxy]propanoate
DTXSID3044596	16090-14-5	('PFAA precursors', 'lt8')	Perfluoro(4-methyl-3,6-dioxaoct-7-ene)sulfonyl fluoride
DTXSID0047583	423-39-2	('PFAA precursors', 'volatile')	Nonafluoro-1-iodobutane
DTXSID20861913	375-72-4	('PFAA precursors', 'volatile')	Perfluorobutanesulfonyl fluoride
DTXSID6021377	76-13-1	('PFAS derivatives', 'volatile')	1,1,2-Trichloro-1,2,2-trifluoroethane
DTXSID4041284	34455-29-3	('unclassified', 'gte8')	6:2 Fluorotelomer sulfonamide betaine