UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

June 16, 2022

Order Under Section 4(a)(2) of the Toxic Substances Control Act

Chemical Substance Subject to this Order:

Chemical Name: 6:2 Fluorotelomer sulfonamide betaine

Chemical Abstracts Service Registry Number (CASRN): 34455-29-3

Docket Identification (ID) Number: EPA-HQ-OPPT-2021-0897

(To access the docket, go to https://www.regulations.gov)

Testing Required by this Order:

1. Physical-Chemical Properties

Tier 1

a. Particle Density (**OECD 109 (2012)**)

b. Aerodynamic Particle Size Distribution (APSD) with Cascade Impactors (**NIOSH NMAM (2017). Chapter BA**)

c. Hydrolysis as a Function of pH (**OECD 111 (2004)**)

2. Health Effects: Inhalation Route

Tier 1

a. Biosolubility Test (Gamble's Solution or Simulated Epithelial Lung Fluid (SELF)) (**ECETOC Technical Report 122, Section 3)**

Tier 2

a. Preliminary Toxicokinetic Study for Development of Information on Absorption, Distribution, Metabolism and Elimination Using Radiolabeling of Test Substance **(OECD 417 (2010), OPP DER, and OECD GD 39 (2018))**

b. Acute Inhalation Toxicity: Concentration × Time Method (**OECD 403 (2009)** and **OECD 424 (1997)**)

c. Inhalation Toxicity Range-Finding Study **(OECD 412 (2018) paragraphs 14-15** and **OECD 424 (1997))**

d. Subacute Inhalation Toxicity: 28-Day Study (**OECD 412 (2018)**)

Recipients of this Order:

Company Name: THE CHEMOURS CO

Company Name: DUPONT DE NEMOURS INC

Company Name: E I DU PONT DE NEMOURS AND COMPANY

Company Name: JOHNSON CONTROLS INC

Company Name: NATIONAL FOAM INC

Dear Recipient:

This Order requires you and the other named manufacturer(s) and/or processor(s) of 6:2 fluorotelomer sulfonamide betaine (CASRN 34455-29-3) to develop and submit certain information for 6:2 fluorotelomer sulfonamide betaine, or otherwise respond to the U.S. Environmental Protection Agency (referred to herein as "EPA" or "the Agency"). Failure to respond to this Order, or failure to otherwise comply with its requirements, is a violation of section 15 of the Toxic Substances Control Act (TSCA), 15 U.S.C. § 2614. Any person who violates TSCA shall be liable to the United States for penalties in accordance with TSCA Section 16, 15 U.S.C. § 2615.

This Order is **effective 5 calendar days after its date of signature by the EPA.** The timeframes and options for responding are described in **Unit IV** (Response Options). Please note that the email transmitting this Order to you will provide the calendar date for the response deadlines as defined in **Unit III** (Deadlines for Responding to this Order). A subsequent email will provide a company specific Order number for you to use in responses and communications about this Order.

This Order is organized as follows:

I. PURPOSE AND AUTHORITY

A. OVERVIEW

This Order is being issued under the authority of the Toxic Substances Control Act (TSCA), 15 U.S.C. § 2601 *et seq*. TSCA section 4 authorizes the EPA to require the development of necessary information related to chemical substances and mixtures.

This Order requires the identified recipients to develop and submit information on 6:2 fluorotelomer sulfonamide betaine. See **Unit II** for a discussion of the scope of this Order.

Information on testing requirements is provided in **Appendix E**. The EPA encourages the formation of industry consortia to jointly conduct testing between the recipients of this Order. See **Unit VIII** for more information on this topic.

The Order provides four response options, listed below. More information on each of these options is provided in **Unit IV**. Timeframes for these options is provided in **Unit III**. Note that the first deadline is to identify as a manufacturer, processor, or both within 30 calendar days after the effective date of this Order.

Option 1: Develop the Information

Use this option to develop information in response to all of the requirements of this Order that apply to you, or use this option in conjunction with other response options identified in this section as appropriate.

Manufacturers who are required to test a chemical substance or mixture pursuant to a TSCA section 4 order are also required to pay a fee (see **Unit VII**).

Option 2: Submit Existing Information

Use this option to submit an existing study and/or other scientifically relevant information that you believe the EPA has not considered, along with supporting rationale that explains how the submittal(s) meets part or all of the information described as necessary in **Unit II**. If the EPA determines that the submitted information satisfies one or more data requirements identified by this Order, the Agency will extinguish any associated test requirement(s).

Option 3: Request an Exemption

Use this option to request an exemption from a testing requirement of this Order. EPA will grant an exemption if:

1. Information on the subject chemical or an equivalent chemical has been submitted in accordance with a rule, order, or consent agreement under TSCA section 4(a), or is being developed in accordance with such a rule, order (including this Order), or consent agreement; and

2. Submission of information by the exemption applicant would be duplicative of information which has been submitted or is being developed in accordance with such rule, order (including this Order), or consent agreement.

Option 4: Claim that You Are Not Subject to this Order

Use this option to claim that you are not subject to this Order. You may claim that you are not subject to this Order if *all* of the following are true:

1. You do not currently manufacture or process the chemical(s) identified by this Order;

2. You do not intend to manufacture or process the chemical(s) within the period of testing provided by the Order; and

3. You have not manufactured or processed the chemical(s) at any time during the ten years preceding the date of this Order.

You must provide an explanation of the basis for your claim, along with appropriate supporting information to substantiate that claim.

B. TERMINOLOGY USED IN THIS ORDER

The term "manufacture" means to import into the customs territory of the United States, to produce, or to manufacture. 15 U.S.C. § 2602(9). Import also includes importing the chemical as an impurity in an article.

The term "process" means the preparation of a chemical substance or mixture, after its manufacture, for distribution in commerce—(A) in the same form or physical state as, or in a different form or physical state from, that in which it was received by the person so preparing such substance or mixture, or (B) as part of an article containing the chemical substance or mixture. 15 U.S.C. § 2602(13).

The term "chemical" or "substance" means a chemical substance or mixture.

C. PERSONS SUBJECT TO THIS ORDER

1. Persons Identified

An order issued under section 4(a) of TSCA may require the development of information by any person who manufactures or processes, or intends to manufacture or process, a chemical substance or mixture subject to the order. The recipients of this Order are listed at the top of the Order.

For purposes of this Order, a recipient of this Order is subject to the Order if it has manufactured or processed the chemical at any time during the ten years preceding the date of this Order. If a recipient of this Order has not manufactured or processed the chemical during the prior ten years, the recipient is nevertheless subject to the Order if they intend to manufacture or process the chemical within the period of testing provided by this Order.

A person who contracts with a producing manufacturer to manufacture or produce a chemical substance is also a manufacturer if (1) the producing manufacturer manufactures or produces the substance exclusively for that person, and (2) that person specifies the identity of the substance and controls the total amount produced and the basic technology for the plant process.

A recipient who is an importer of record of a chemical substance identified by this Order is responsible for the testing requirements of this Order, even if the recipient does not store, handle, use, or otherwise directly deal with the chemical.

The means by which the EPA identified each recipient subject to this Order does not govern whether a recipient is subject to this Order. Ultimately, any recipient that meets the criteria discussed in this section is subject to this Order, regardless of the basis on which the EPA identified the recipient.

2. Corporate Structure of Recipients; Changes of Ownership

EPA has attempted to identify the highest-level U.S. corporate entity for purposes of issuing this Order. The highest-level U.S. corporate entity is ultimately responsible for satisfying the obligations of this Order, although the highest-level U.S. corporate entity may delegate its responsibilities under this Order to a U.S. subsidiary. Where the corporate entity named in this Order is not the highest-level U.S. corporate entity, the EPA nonetheless considers notification of the company named in this Order to constitute notification of the highest-level U.S. corporate entity and holds the highest-level U.S. corporate entity ultimately responsible for satisfying the obligations of this Order.

Should you wish to modify the name of the recipient or identify another U.S. corporate entity in the corporate structure as the point of contact in place of the recipient named in this Order, you must submit a request to the EPA. Submit your request, justification for the change, and contact information for the representatives of the newly named entity to TSCAtestorders@epa.gov. A representative from EPA will contact you and any other representatives regarding this request.

In the event of mergers, acquisitions, or other transactions that create a corporate successor in interest (subsequent to the manufacturing or processing that triggered the reporting obligation, and either before or after receipt of this Order), that successor in interest is responsible for satisfying the obligations of this Order. The successor in interest must notify the EPA of its identity within 14 days following the transaction.

II. SCOPE OF TSCA SECTION 4 TEST ORDER

A. Statutory Standard

Under section 4(a)(1)(A)(i) of TSCA, the EPA shall require testing of a chemical substance or mixture to develop appropriate test data if the Administrator finds that:

(I) The manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or that any combination of such activities, may present an unreasonable risk of injury to health or the environment,

(II) There is insufficient information and experience upon which the effects of such manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and

(III) Testing of such substance or mixture with respect to such effects is necessary to develop such information.

In making section 4(a)(1)(A)(i) findings, the EPA considers, among other things, physical-chemical properties, fate and transport, exposure, and toxicity information to make the finding that the chemical substance or mixture may present an unreasonable risk. For finding (II) above, the EPA examines whether existing information is adequate to reasonably determine or predict the effects on health or the environment from the chemical substance or mixture. In making the third finding that testing is necessary, the EPA considers whether testing which the Agency might require is necessary to develop the needed information.

B. Basis for this Order

As explained above, in **Unit II.A**, to issue an Order under section 4(a)(1)(A)(i) on a chemical substance or mixture, the EPA must make three findings, as provided below.

1. <u>TSCA Section 4(a)(1)(A)(i)(I): The manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or that any combination of such activities, may present an unreasonable risk of injury to health or the environment.</u>

The EPA is basing this Order on the authority of section 4(a)(1)(A)(i) of TSCA. The EPA finds that the manufacture, distribution in commerce, processing, use, or disposal of 6:2 fluorotelomer sulfonamide betaine may present an unreasonable risk of injury to human health or the environment.

6:2 Fluorotelomer sulfonamide betaine is a member of the group of chemicals known as per- and polyfluoroalkyl substances (PFAS). For the purposes of this Order, the EPA's Office of Pollution Prevention and Toxics (OPPT) is using a working structural definition for identifying PFAS. Specifically, this definition includes per- and polyfluorinated substances that structurally contain the unit R-(CF_2)-C(F)(R′)R″. Both the CF_2 and CF moieties are saturated carbons and none of the R groups (R, R′ or R″) can be hydrogen.

<u>Hazard and Exposure for PFAS</u>

PFAS have been used in industry and consumer products since the 1940s because of their useful properties. There are thousands of different PFAS, some of which have been more widely used and studied than others. Studies show that some PFAS may break down very slowly, or break down into other PFAS that break down very slowly, and can build up in people, animals, and the environment over time.

Studies in laboratory animals indicate some PFAS can cause reproductive, developmental, liver, kidney, and immunological toxicity. In addition, exposure to some PFAS produces 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 (*e.g.*, Health Effects Support Document for Perfluorooctanoic Acid (PFOA) (USEPA, 2016b)), and thyroid hormone disruption (*e.g.*, Health Effects Support Document for Perfluorooctane Sulfonate (PFOS) (USEPA, 2016a). Some PFAS can cause adverse effects on the respiratory system following acute inhalation exposures (*e.g.*, Per- and polyfluoroalkyl substances (PFASs) modify lung surfactant function and pro-inflammatory responses in human bronchial epithelial cells (Sørli et al., 2020) and Anionic Surfactants Category in TSCA New Chemicals Program (NCP) Chemical Categories (USEPA/OPPT, 2010). Visit these EPA webpages for more information on general concerns associated with PFAS: PFAS Explained (USEPA, 2022b) and Our Current Understanding of the Human Health and Environmental Risks of PFAS (USEPA, 2022a).

Current research has shown that people can be exposed to PFAS by working in occupations that deal with PFAS and products containing PFAS, drinking water contaminated with PFAS, eating certain foods that may contain PFAS, swallowing contaminated soil or dust, breathing air containing PFAS, and using products made with PFAS or that are packaged in materials containing PFAS. These exposures are compounded when populations are exposed via more than one exposure route.

Hazard and Exposure for 6:2 fluorotelomer sulfonamide betaine

6:2 Fluorotelomer sulfonamide betaine is part of the larger group of chemicals described above as PFAS.

Based on the following estimated physical-chemical properties values for 6:2 fluorotelomer sulfonamide betaine using EPA's model, Open (Quantitative) Structure-activity/property Relationship App (OPERA v2.8.2), EPA concludes it is an insoluble solid substance:

- Vapor pressure: 0.000025 mmHg
- Water solubility: 1.16 mg/L
- Melting point: 77 °C
- Boiling point: 246 °C

The specific health concerns for 6:2 fluorotelomer sulfonamide betaine are based on the physical-chemical properties indicating it is expected to be an insoluble solid substance and therefore may present concern for portal-of-entry effects for inhalation exposures. Manufacturing, processing, and/or transport of solid substances may lead to the formation of respirable particles and inhalation exposures to workers.

In evaluating the exposures to 6:2 fluorotelomer sulfonamide betaine, the Agency considered: (a) its status on the TSCA Inventory, and (b) reporting on the substance under the Chemical Data Reporting

Rule. Section 8(b)(4)(A) of TSCA required the EPA to designate as "active" in commerce any chemical substance manufactured or processed within a specified ten-year period, based on information provided by manufacturers and processors of such chemical substances. As a result of this self-reporting, 6:2 fluorotelomer sulfonamide betaine is listed as "active" on the TSCA Inventory, and currently there are no restrictions (*e.g.*, a significant new use rule) on its use. Based on its active status on the TSCA Inventory, there is potential for exposure to 6:2 fluorotelomer sulfonamide betaine.

Additionally, Chemical Data Reporting (CDR) indicates that there is manufacturing (defined to include importing) of 6:2 fluorotelomer sulfonamide betaine in significant quantities (*e.g.*, more than 25,000 pounds in a given year). CDR reporting indicates that this chemical substance is used as a surface-active agent and as a fire-fighting foam and that a significant number of workers have been exposed to the chemical (see "Type of Process or Use" and "Number of Workers Reasonably Likely to be Exposed" data elements). This reporting supports that there is worker exposure to 6:2 fluorotelomer sulfonamide betaine at levels that may elicit effects of concern, and that this chemical substance is being incorporated into products that may also present exposure concerns beyond the sites reporting to CDR.

Based on this information, there is potential for exposure to 6:2 fluorotelomer sulfonamide betaine. Given the hazard and exposure concerns identified for 6:2 fluorotelomer sulfonamide betaine, the EPA finds that 6:2 fluorotelomer sulfonamide betaine may present an unreasonable risk of injury to health or the environment. The hazard and exposure concerns for PFAS further support this conclusion.

2. TSCA Section 4(a)(1)(A)(i)(II): There are insufficient data and experience upon which the effects of such manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted.

EPA examined whether existing information is adequate to reasonably determine or predict the effects on health or the environment from 6:2 fluorotelomer sulfonamide betaine. The EPA considered information that is reasonably available to the Agency; specifically, human health-related toxicity studies for all relevant potential routes of exposure to 6:2 fluorotelomer sulfonamide betaine including:

- Acute Toxicity
- Subchronic Toxicity
- Chronic Toxicity including Cancer Bioassays
- Developmental Toxicity
- Reproductive Toxicity
- Immunotoxicity
- Neurotoxicity
- Toxicokinetics
- Mutagenicity

- Sensitization/Irritation

The EPA queried for toxicity data from two separate sources – the EPA Toxicity Value Database (ToxValDB) (Judson, 2018) 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 U.S. 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 making data publicly available to the extent possible under current statutory requirements and given resource constraints.

The EPA found that 6:2 fluorotelomer sulfonamide betaine lacks data to determine or predict the effects on human health specifically by the inhalation route. Available data on this chemical includes certain studies for tests done via the oral route, which are not relevant to inhalation exposures due to the concern for portal-of-entry effects. Therefore, EPA finds that the inhalation toxicity information on 6:2 fluorotelomer sulfonamide betaine is insufficient. The tiered testing required in this order begins with generation of data on certain physical-chemical properties (*i.e.*, density, particle size, hydrolysis as a function of pH) necessary to inform whether the higher tiered (Tier 2), *in vivo* testing that EPA has not identified as existing is appropriate.

This Order addresses only the insufficient data that has been identified for purposes of the Order. The EPA may determine the availability of data and experience upon which the effects of such manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted is insufficient for other exposure or hazard endpoints in the future.

3. TSCA Section 4(a)(1)(A)(i)(III): Testing of such substance or mixture with respect to such effects is necessary to develop such information.

The EPA finds that testing of 6:2 fluorotelomer sulfonamide betaine—as described in the **Appendix E** and listed at the beginning of this Order—is necessary to develop physical-chemical properties and human health-related toxicity data that EPA requires to determine or predict the effects discussed in this Order. Further details on the purpose of each required test of this Order is discussed in **Unit V.**

C. Other Uses of This Data: PFAS Terminal Categories

To deepen the understanding of the impacts of PFAS, including potential hazards to human health and the environment, to address variation among effects seen for various endpoints for different PFAS (*e.g.*, Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research (Fenton et al., 2021)), and to aid EPA in identifying and

selecting PFAS for which the Agency will require testing, EPA developed the National PFAS Testing Strategy: Identification of Candidate Per- and Polyfluoroalkyl Substances (PFAS) for Testing (Testing Strategy) (USEPA, 2021a).

The Testing Strategy provides categories of PFAS based on information about similarities in structure, physical-chemical properties, and existing test data on the toxicity of PFAS. The Testing Strategy identifies 70 such categories referred to as "terminal categories."

This Order pertains to 6:2 fluorotelomer sulfonamide betaine (CASRN 34455-29-3), which the EPA determined to be representative for the "unclassified, greater than or equal to 8 carbon atoms" terminal category. As described in the Testing Strategy (USEPA, 2021a), the EPA used computer software developed by Su and Rajan (Su and Rajan, 2021) to systematically analyze the chemical structures from a starting list of 6,504 PFAS into nine primary categories based on their structure. Substances whose structures could not be resolved by the computer software, such as particular salt forms, were labeled as "Unclassified." The 'gte8' label refers to the atomic makeup of the compound, which in this case has greater than or equal to 8 (*i.e.*, gte8, or ≥8) carbon atoms. The chemical proposed for testing actually has 15 carbon atoms (chemical formula $C_{15}H_{19}F_{13}N_2O_4S$). Of the 6,504 PFAS in the starting list, 503 PFAS are members of this category.

EPA's concerns related to 6:2 fluorotelomer sulfonamide betaine, and its decision to issue this Order pursuant to TSCA Section 4(a)(1)(A)(i), also exist for other PFAS in this "unclassified, greater than or equal to 8 carbon atoms" terminal category. This order is the EPA's initial action to collect pertinent toxicity information for the "unclassified, greater than or equal to 8 carbon atoms" terminal category of PFAS. The EPA anticipates that data provided on this chemical substance via this Order will serve to inform understanding of other PFAS within this terminal category. As EPA continues to improve its understanding of PFAS within this terminal category, EPA's understanding of PFAS and how to categorize these chemical substances may evolve. Similarly, EPA may determine that testing is required on other PFAS categorized in the "unclassified, greater than or equal to 8 carbon atoms" terminal category as it is currently defined.

The results of the required testing will inform chemical testing requirements in future iterations of TSCA section 4 Test Orders. Furthermore, the full results of the required testing in this Order taken together with existing information, within a defined chemical safety, regulatory, and hazard characterization context, and uncertainty considerations enable integrated assessment of other substances within the terminal category and potentially PFAS at large.

D. The EPA determined that vertebrate testing is necessary in this Order

The EPA has determined that vertebrate testing is necessary for assessing the effects discussed in this Order (see below for details). TSCA Section 4(h)(1)(A) requires the Agency to take into consideration toxicity information, computational toxicology and bioinformatics, and high-throughput screening methods and the prediction models of those methods prior to adopting a requirement for testing using vertebrate animals. EPA surveyed reasonably available existing information including information from methods not involving vertebrates and found they were insufficient to inform certain endpoints. Further details regarding the reasonably available information and the needs for specific vertebrate testing are provided below. Further information on the EPA review process that led to the inclusion of such testing requirements can be found in **Unit II.B**.

1. Health Effects: Inhalation Toxicity:

All of the Tier 2 testing for human health effects consists of vertebrate testing, which incorporates the following:

- OECD 417: Toxicokinetics (OECD, 2010b)
- Office of Pesticide Programs Data Evaluation Record (OPP DER) on Sulfuryl Fluoride (Shah and Shah, 2014)
- OECD GD 39: Guidance Document on Inhalation Toxicity Studies (OECD, 2018b)
- OECD 403: Acute Inhalation Toxicity (OECD, 2009)
- OECD 424: Neurotoxicity Study in Rodents (OECD, 1997)
- OECD 412: Subacute Inhalation Toxicity: 28-Day Study (OECD, 2018e)

The preliminary inhalation toxicokinetic (TK) study (OECD 417, and OPP DER), combined with results from the acute inhalation study (OECD 403) and range-finding (OECD 412), will be used to inform the study plan and test report requirements for the subacute inhalation study for PFAS Testing Track A (see **Unit V**).

No scientifically valid non-vertebrate test method of equivalent or better scientific quality and relevance currently exists to determine/measure inhalation exposure dosimetry and toxicity for this test substance. As part of this consideration, EPA reviewed OCSPP test methods and data evaluation reports, OECD test guidelines and guidance, and other peer-reviewed and/or publicly available methodology/protocol repositories. EPA considered *in vitro* respiratory toxicity models and found that currently available *in vitro* respiratory tract cell culture models are only relevant to water-soluble and gaseous substances. Therefore, at this time, testing of this type is not envisioned for substances in PFAS Testing Track A.

This Order includes a tiered testing approach, consistent with section 4(h) of TSCA. Certain information (*i.e.*, particle density and size, hydrolysis, and biosolubility) is developed in Tier 1 testing to ensure that the *in vivo* inhalation toxicity tests on vertebrate animals in Tier 2 testing are appropriate. Further, EPA's application of a category approach described in **Unit II.C** reduces the use of vertebrate animals by testing representative PFAS rather than all PFAS in the "unclassified, greater than or equal to 8 carbon atoms" terminal category.

Testing both rats and mice is required in the earlier Tier 2 testing within this Order to select the most appropriate rodent species (*i.e.*, rat or mouse) for later Tier 2 inhalation toxicity testing (see Figure 2 and **Unit V.B** for more details). Existing information on other PFAS (which are not the subject of this Order but which inform the testing required by this Order) has not demonstrated a clear pattern of rodent species' relevance to human health hazard (*in vitro* to *in vivo* extrapolation) (ATSDR, CDC 2021). In the absence of evidence that either rats or mice are more human-relevant for PFAS inhalation exposure, experimental data are needed from both species to account for interspecies differences in accumulation, metabolism, and re-uptake and/or clearance of these substances to inform later Tier 2 required inhalation toxicity testing. Similarly, in the absence of definitive evidence for sex differences, both female and male of each species must be tested for all Tier 2 human health effects testing (OECD (2010b) paragraph 16).

The preliminary toxicokinetic (TK) study (OECD 417, and OPP DER), the first of the Tier 2 required tests, determines the metabolism and elimination potential of the radiolabeled test substance. Testing requires the recovery of as much of the radiolabeled test substance as possible (to achieve mass balance) by serial collection of blood, serum, plasma, and urine samples, and in tissues including but not limited

to, brain, lung (*e.g.*, bronchoalveolar lavage and analysis of BAL fluid), upper and lower respiratory tract, gastrointestinal (GI) tract, kidney, olfactory bulb, and nasal tissues. This TK testing will enable derivation of the peak concentration (C_{max}) and time-to-peak concentration (T_{max}) (maximum blood concentration and the time to reach this maximum, respectively).

Cage-side, clinical observations of animals during this preliminary TK study (OECD 417) will be used by EPA to build the Weight of Scientific Evidence (WoSE) and inform later inhalation toxicity testing study plan and report requirements for potential neurotoxicity and neuropathology (*i.e.*, functional observational battery and motor activity). Taken together with post-exposure blood fluoride concentrations, the study timeline will be determined for potential neurotoxicity/neuropathology, a human health hazard concern indicated in currently available toxicity evidence for PFAS (ATSDR, CDC 2021). This preliminary TK study will also inform which other potential human-relevant health hazards—in addition to existing evidence for other perfluoroalkyls in both humans and rodents—must be examined in later Tier 2 testing. Some potential human health effects include those mediated by peroxisome proliferator-activated receptor-α (PPARα) activation, including liver and immune toxicity, cardiovascular disease (lipid metabolism and pulmonary function), and reproductive and developmental toxicity (ATSDR, CDC 2021). Increased incidence of testicular and kidney cancers was also reported in the currently available epidemiological WoSE in 'highly exposed' humans due to PFOA and PFOS exposures (as reviewed in ATSDR, CDC 2021) and therefore must be addressed in study design and test report rationales. Specific to the test substance subject to this Order, particles pose potential challenges with respect to dosimetry and may also pose human health hazards related to inhalation portal of entry effects in tissues, including but not limited to nasal tissues and upper and lower respiratory tracts. A general half-life estimation will be derived from this preliminary TK study as itemized in the study plan and test report requirements of this Order.

The preliminary TK study (OECD 417) in two rodent species—including the calculated half-life, results from post-exposure blood fluoride concentration measurements and cage-side clinical observations—must be used to plan the acute and subacute inhalation toxicity (OECD 403 and 412) studies, including the inhalation range-finding study. Like the preliminary TK study (OECD 417), both rodent species must also be used for the acute inhalation toxicity study (OECD 403). Inhalation exposure and potential transport of the test substance beyond portal of entry must be examined with the following measurements: blood fluoride levels, bronchoalveolar lavage fluids, histopathology/morphometry of associated tissues including, but not limited to the upper and lower respiratory tract, nasal tissues, and brain. The study plan and test report requirements (*i.e.*, approved and validated severity scoring rubric for histopathology/morphometry) and number of sections examined will be tissue-dependent (see the referenced guidelines, guidance and testing detailed in **Appendix E**). Building on cage-side clinical observations from the preliminary TK study (OECD 417), functional observation battery (FOB) and motor activity observations consistent with the OECD neurotoxicity study (OECD 424) must be performed for this acute inhalation toxicity (OECD 403) study as appropriate and applicable. Results from this acute inhalation toxicity (OECD 403) study must be used to calculate the duration and observational time points (*e.g.*, particularly for FOB and motor activity) for the subacute inhalation toxicity range-finder study that follows.

Selecting the most human-relevant rodent species for the latter two inhalation toxicity studies, including the inhalation toxicity range finding study (OECD 412), is the aim of using two rodent species in the first two Tier 2 studies. As such, the following inhalation toxicity range finding study (OECD 412) will be performed in one rodent species. The primary goal of this inhalation toxicity range finding study is to determine the extent to which FOB and motor activity examinations can be refined as appropriate and applicable for the full subacute inhalation toxicity study. All histopathology/morphometry observations

including, though not limited to, brain, upper and lower respiratory tract, and nasal tissues, must be reported for this subacute range-finder study.

III. DEADLINES FOR RESPONDING TO THIS ORDER

This section describes the deadlines for this Order and possible modifications to such deadlines.

A. Deadlines for Responses to this Order

The table below provides the deadlines for this Order. Deadlines that fall on a weekend or holiday will remain and will not be extended to the next weekday. Descriptions of these response options and the required process associated with each option is provided in **Unit IV.**

Deadlines for Responses, Study Plans, and Test Reports

Order Requirement	**Recipient's Deadline (Days after the effective date of the Order)**	**The EPA Response Deadline* (Days after the effective date of the Order)**
Identify as a Manufacturer, Processor or Both	30	n/a
Submit Request to Modify Corporate Identity Identified (Optional)	30	n/a
Choose to Submit Existing Data (Option 2)	30	45
Claim that You Are Not Subject to this Order (Option 4)	30	45
Choose to Develop the Information - On Own or as Part of a Consortium (Option 1)	65	n/a
Request an Exemption (Option 3)	65	80
Submit Draft Study Plan	80	95
Submit Final Study Plan	110	125
Submit Final Test Report	Deadline varies per Test Requirement (See **Unit V** and **Appendix E**)	

*See **Unit III.B** for potential automatic extensions associated with the EPA responses
Deadlines for submitting final test reports for each required test are provided in **Appendix E**.

B. Automatic Extensions to Deadlines

The EPA will automatically extend deadlines should the Agency fail to meet any EPA response deadline set forth in **Unit III.A**. Specifically, deadlines will be automatically extended should the EPA fail to respond within 15 calendar days of the deadline for a response option if the response was submitted in the CDX application prior to the deadline provided. For each day exceeding the 15-day period following the associated deadline, the EPA will extend subsequent deadlines by one day.

Should a recipient amend their response, at any time, the EPA will not extend any associated or subsequent deadlines. Therefore, the EPA recommends that recipients submit their amendments or extension requests as early as practicable to ensure adequate time to perform any required testing given that the Agency will not automatically extend deadlines for any such amendments to responses.

Deadlines will not be extended for submissions received after the deadline for the given submission. For example, a recipient may submit existing data after the 30-day deadline to submit such data, but the deadline to submit a draft study plan will not be extended due to the submission of the existing data. Further, EPA is not obligated to respond within 15 days to a submission that arrives after the deadline for the given type of submission. Accordingly, the EPA will not automatically extend a deadline for a

response should the EPA respond within 15 days of the deadline for a given response option that was submitted on or before the deadline for that response option. Further, the recipient should plan to meet deadlines for milestones in the Order that follow the missed deadline.

Other than potential automatic extensions to deadlines described here, **Unit III.C** provides the process for requesting an extension to a deadline.

C. Requesting an Extension to a Deadline for responding to this Order

If you believe you cannot submit the required identification as a manufacturer, processor, or both; Order response; draft study plan; final study plan; or final test report to the Agency by the deadline(s) specified in this Order and intend to seek additional time to meet the requirement(s), you must submit a request to the Agency through the EPA's CDX portal as soon as you know you may need an extension. Your request must include: (1) a detailed description of the expected difficulty, including technical and laboratory difficulties, and (2) a proposed schedule including alternative dates for meeting such requirement(s) on a step-by-step basis.

The EPA will grant or deny deadline extension requests at its discretion.

IV. RESPONDING TO THIS ORDER

You are required to respond to this Order, even if you believe your company is not subject to this Order. Failure to provide a response is a violation of section 15 of TSCA.

A. IDENTIFY AS A MANUFACTURER, PROCESSOR, OR BOTH

Within 30 calendar days of the effective date of this Order, you, as a recipient of this Order, are required to respond to this Order through the EPA's Central Data Exchange (CDX) portal, informing the Agency whether you will be responding to this Order as manufacturer or processor (if you manufacture and process the chemical, select manufacturer). To provide your preliminary response to this Order, you will receive an e-mail from the EPA within five days of the Order being signed (*i.e.*, by the effective date of the Order) that provides a CDX Order number for purposes of complying with this Order.

You may claim that you are not subject to this Order if you (1) do not currently manufacture or process the chemical(s) identified by this Order; (2) do not intend to manufacture or process the chemical(s) within the period of testing provided by this Order (see **Unit V**); and (3) have not manufactured or processed the chemical(s) at any time during the ten years preceding the effective date of this Order. See **Unit VI.B.4** for more information on how to claim that you are not subject to this Order.

B. FOUR RESPONSE OPTIONS

A recipient must develop information in response to the Order consistent with Option 1, unless they meet the requirements to respond using Option 2, 3 or 4. See **Unit III** to review the deadlines for this Order.

Option 1: Develop the Information

If you respond to this Order by the develop the information response, you must select this option in the CDX portal form.

For details on the steps of this response option, see **Unit VI.**

For more information on this Order's required tests, required protocols/methodologies, and deadlines for submission of test reports see **Unit V and Appendix E**.

Option 2: Submit Existing Information

If you respond to this Order by submitting an existing study and/or other scientifically relevant information that you believe the EPA has not considered, your response in the EPA's CDX portal must be submitted to the EPA within 30 days of the effective date of the Order and include the study(ies) and/or other scientifically relevant information, along with supporting rationale that explains how the study and/or other scientifically relevant information meets part or all of the information or obviates the need for the information described as necessary in **Unit II**.

The EPA's determination regarding whether the study and/or other relevant information satisfies part or all of the information or obviates the need for the information described as necessary in **Unit II** will be based on the weight of the scientific evidence from all relevant information reasonably available to the Agency. Further, for human health animal toxicity studies all submitted existing information will be evaluated for study quality using the TSCA systematic review method (EPA-HQ-OPPT-2021-0414-0005, Appendix Q.4.2).

The Agency will notify you of its determination through CDX. If the Agency determines that the study and/or other scientifically relevant information satisfies the need in lieu of the testing required in this Order, and the original testing requirement is no longer needed, the EPA will extinguish those testing obligations from this Order that are no longer necessary, with respect to the appropriate recipients of this Order. If the study was your only testing obligation under the Order, all your obligations under this Order will be extinguished upon notification by the Agency.

If the EPA determines that the study and/or other scientifically relevant information does not satisfy that need, you must modify your response in the EPA's CDX portal to choose one of the other response options in **Unit IV** within 10 calendar days of being notified by the EPA

Note that the submission of existing information will not extend the deadline for the draft study plan submission for that testing requirement unless the existing information is submitted within 30 days of the effective date of the Order and the EPA does not respond within 45 days of the effective date of the Order. Thus, failure to submit existing information prior to the 30-day deadline will result in a need to submit a draft study plan by the 80-day deadline. See Unit **III.B** for information on the potential automatic extension of deadlines.

Option 3: Request an Exemption

Any person required by this Order to conduct tests and submit information on a chemical may apply for an exemption from such requirement (TSCA section 4(c)(1)).

The EPA will grant a request for exemption from the requirement to conduct tests and submit information on a chemical substance if:

1. Information on the subject chemical or an equivalent chemical has been submitted in accordance with a rule, order, or consent agreement under TSCA section 4(a), or is being

developed in accordance with such a rule, order (including this Order), or consent agreement, and

2. Submission of information by the exemption applicant would be duplicative of information which has been submitted or is being developed in accordance with such rule, order (including this Order), or consent agreement.

An exemption request must be submitted through the CDX portal and contain the following:

1. This Order number, the chemical identity, and the CAS Registry No. of the test substance subject to this Order on which the application is based.

2. The specific testing requirement(s) from which an exemption is sought.

3. The basis for the exemption request when another company(ies) has/have submitted the information or is/are developing information for the subject chemical or an equivalent chemical pursuant to a TSCA section 4(a) rule, order, or consent agreement. Your request must identify the company(ies) that submitted or is/are developing the information.

4. The chemical identity of the equivalent chemical (the test substance in the information submitted or being developed) on which the application is based.

5. The equivalence data ("chemical data or biological test data intended to show that two substances or mixtures are equivalent" (see **Appendix A**)) if data on an equivalent chemical is being submitted.

6. The name, mailing address, telephone number, and e-mail address of applicant.

7. The name, mailing address, telephone number, and e-mail address of appropriate individual to contact for further information.

8. A Statement of Financial Responsibility: The following sworn statement (*i.e.*, signed and notarized) must accompany each request for an exemption:

    a. "I understand that if this application is granted, I must pay fair and equitable reimbursement to the person or persons who incurred or shared in the costs of complying with the requirement to submit information and upon whose information the granting of my application was based."

The EPA's grant of an exemption is conditional upon the completion of the required tests according to the specifications of this Order (or other applicable rule, order, or consent agreement), including any modifications approved by the EPA. If the Agency subsequently determines that equivalent data has not been submitted in accordance with the applicable rule, order, or consent agreement, the Agency will provide notice through CDX of its preliminary decision to terminate the exemption. Within 30 days after receipt of such notice, the exemption holder may submit information in the CDX portal either to rebut the EPA's preliminary decision to terminate the exemption or notify the EPA of its intent to develop the required information pursuant to the specifications established in this Order and any modifications approved by the EPA. If the exemption holder submits information to rebut the EPA's preliminary decision to terminate the exemption, then the EPA will provide the exemption holder an opportunity to request a hearing prior to issuing a final decision to terminate the exemption. Following the receipt of

information to rebut the EPA's preliminary decision and any subsequent hearing, the EPA will render a final decision on whether to terminate the exemption, taking into account information submitted to rebut the EPA's preliminary decision and information presented at any hearing, as applicable.

If you receive the Agency's preliminary decision to terminate the exemption and do not submit information to rebut that preliminary decision or request a hearing, or if you receive the Agency's final decision to terminate the exemption following the submission of information to rebut that preliminary decision or a hearing, you must resubmit a response in accordance with one of the options described in **Unit IV.B** of this Order within 30 calendar days of receipt of the Agency's decision to terminate the exemption, including as applicable the information required under **Unit V** of this Order. Failure to timely resubmit the response will constitute a violation of this Order and of TSCA section 15(1). Should the EPA terminate the exemption, a draft study plan will be due 30 days from the termination, with the final study plan being due 60 days from the termination.

If the EPA extinguishes a testing obligation pursuant to **Unit IV.B.2** of this Order, the corresponding exemption will be extinguished, as the exemption will no longer be necessary. In such a situation, companies who requested an exemption from that specific testing obligation are not required to reimburse the company that submitted existing data.

As explained in **Appendix B** on Cost Sharing, persons who receive exemptions from testing have an obligation to reimburse the person(s) who perform the required testing and submit the required information for a portion of the costs incurred in complying with the requirement to submit such information, and any other person required to contribute to a portion of such costs. Normally, this is worked out by the parties involved, without the involvement of the EPA. However, if agreement cannot be reached on the amount or method of reimbursement, and the company who is entitled to reimbursement requests in accordance with the procedures in **Appendix B** that the EPA order reimbursement, the Administrator shall order the person granted the exemption to provide fair and equitable reimbursement. See TSCA section 4(c).

Option 4: Claim that You Are Not Subject to this Order

You may claim that you are not subject to this Order if you do not manufacture or process the chemical(s) identified by this Order; do not intend to manufacture or process the chemical(s) within the period of testing provided by this Order (see **Unit V**); and have not manufactured or processed the chemical(s) at any time during the ten years preceding the effective date of this Order.

An explanation of the basis for your claim, along with appropriate supporting information to substantiate that claim, must accompany your response in the CDX portal so that the EPA can evaluate the claim.

Note that if your company ceased manufacturing (including import) or processing of the chemical substance(s) subject to this Order more than ten years prior to the effective date of this Order, you can claim that you are not subject to this Order.

In the instance that you claim you are Not Subject to this Order, your claim must include (1) a statement explaining why your company is not subject to this Order, such as no longer importing, manufacturing, or processing the subject chemical substance (intentionally or unintentionally) within the ten years prior to the effective date of this Order and not intending to manufacture (including import) or process the chemical within the period of testing provided by this Order (see **Unit V**), and (2) the certifying statement "I certify that the statements made in this letter are true, accurate, and complete. I

acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law."

If based on the evidence you provide and other evidence available to the EPA, the Agency deems your claim to be inadequately substantiated, the EPA will deny your claim, and the original requirements and deadlines in this Order will remain. If your claim is approved, the EPA will notify you that you are not subject to this Order through CDX correspondence. The EPA expects to provide such notification within 45 days of the effective date of this Order.

To assert a claim using this option, you must do so within 30 days of the effective date of this Order.

V. OVERVIEW OF TESTING REQUIRED BY THIS ORDER

This unit applies to Option 1: Develop the Information and Option 2: Submit Existing Information (**Units IV.B.1** and **IV.B.2**).

Where the required protocol is an EPA guideline, the guideline is available on the EPA OCSPP Test Guideline website (USEPA, 2015) or from the National Technical Information Service (NTIS), Attn: Order Desk, 5285 Port Royal Road, Springfield, VA 22161 (tel: 703-605-6000). This EPA website also provides information on OECD guidelines, alternatively available via OECD Guidelines for the Testing of Chemicals (OECD, 2018c). **Appendix E** provides additional sources for guidelines associated with specific testing.

The EPA reserves the right to revise this Order to extinguish specific testing obligations where existing information subsequently comes to the Agency's attention that in the EPA's scientific judgment obviates the need for specific test data required under this Order. Specific information for ordered test(s) are provided in **Appendix E**.

See **Appendix E** for details on the required test protocols.

A. Overview of PFAS Testing Track A Tier 1 Requirements

As explained in **Unit II.C**, pursuant to the PFAS Testing Strategy, the EPA ultimately divided PFAS included in the testing strategy into 70 terminal categories based on information about similarities in structure and physical-chemical properties. EPA began the work to identify unique sets of testing requirements for PFAS based on their physical-chemical properties (estimated or experimental, as applicable). Collecting information pursuant to this Order will help the Agency refine and/or create additional PFAS testing tracks (PTT), based on physical-chemical and potentially other properties, as appropriate. Note that within a terminal category, PFAS members may have varying physical-chemical properties, which can result in PFAS in a given category fitting into different PTT. For 6:2 Fluorotelomer sulfonamide betaine, the EPA is applying the first established PFAS Testing Track, PTT-A, which applies to insoluble solid substances.

Per TSCA section 4(a)(4), when requiring the development of new information, the EPA shall employ a tiered screening and testing process under which the results of screening-level tests or assessments of available information inform the decision as to whether additional tests are necessary. This use of earlier testing results to inform the need for and scope of later testing requirements supports EPA's goal to use weight of scientific evidence (WoSE) and Integrated Approaches to Testing and Assessment (IATA) frameworks when possible. Accordingly, Tier 1 testing will confirm the applicability of PTT-A before Order recipients proceed to Tier 2 testing. If substances assigned to PTT-A are determined to be 'not

biosoluble' based on Tier 1 testing for *in vitro* biosolubility, then the substance will remain in PTT-A, progressing to Tier 2 *in vivo* health effects testing (see Figure 1, blue box, bottom). If Tier 1 testing indicates that the substances assigned to PTT-A are biosoluble, an alternate PTT will be applied based on the additional physical-chemical properties pursuant to a separate Order. Likewise, if Tier 1 testing of particle size indicates the substance meets nanomaterial criteria (described in 40 CFR Part 704; particle size data indicates one dimension <100 nm), alternate testing will be determined separately by EPA pursuant to a separate Order.

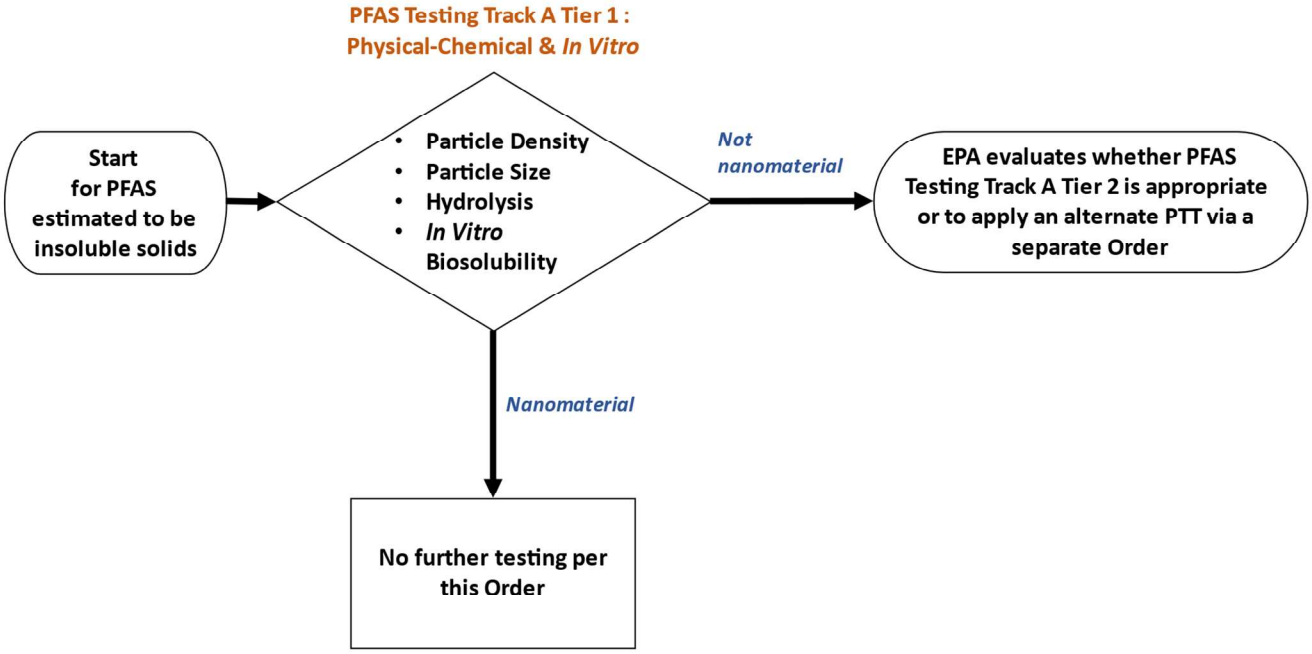

Figure 1. PFAS Testing Track A Tier 1

1. Physical-Chemical Properties

Physical-chemical property testing for PTT-A will help indicate whether additional testing is appropriate. Tier 1 testing specifically includes particle density (OECD 109), particle size distribution (NIOSH NMAM), and hydrolysis as a function of pH (OECD 111). Hydrolysis as a function of pH influences stability in the respiratory tract and environmental media, as well as other parameters important for determining inhalation dosimetry and toxicity.

Tier 1 testing is on the following physical-chemical properties:

- OECD 109: Density of Liquids and Solids (OECD, 2012)
- Aerodynamic Particle Size Distribution (APSD) with Cascade Impactors (NIOSH, 2017) (Chapter BA)
- OECD 111: Hydrolysis as a Function of pH (OECD, 2004)

EPA lists additional physical-chemical property testing —which, if conducted, could replace the EPA-estimated *in silico* values (*i.e.*, modeled values)— as optional in Appendix E. Depending on recipients

providing experimental values for vapor pressure (OECD TG 104, 2006), water solubility (OECD 105, 1995), melting point/melting range (OECD 102, 1995), and/or boiling point (OECD 103, 1995), the PTT may change.

2. *In vitro* Biosolubility Test (Gamble's Solution or Simulated Epithelial Lung Fluid (SELF)) (ECETOC Technical Report 122, Section 3)

Biosolubility is a key parameter that influences the rate of clearance from the respiratory tract, thereby influencing whether 6:2 fluorotelomer sulfonamide betaine will be subject to dissolution and subsequent absorption or elimination or will instead accumulate in the lung leading to lung overload. *In vitro* biosolubility testing using either Gamble's solution or simulated epithelial lung fluid (SELF) will provide this important information regarding the balance between accumulation and elimination (ECETOC, 2013).

Insoluble substances subject to Tier 2 Testing must be tested via the methods set forth in PTT-A; upon evaluation of biosolubility test results, an alternate PTT may need to be applied to biosoluble substances (solubility greater than 100 mg/L in Gamble's Solution or Simulated Epithelial Lung Fluid (SELF)) pursuant to a separate Order, or in the unlikely event the substance meets criteria for nanomaterials (described in 40 CFR 704.20(a) for the definition of "reportable chemical substance"; particle size data indicates one dimension <100 nm) pursuant to a separate Order.

B. PFAS Testing Track A Tier 2 Requirements

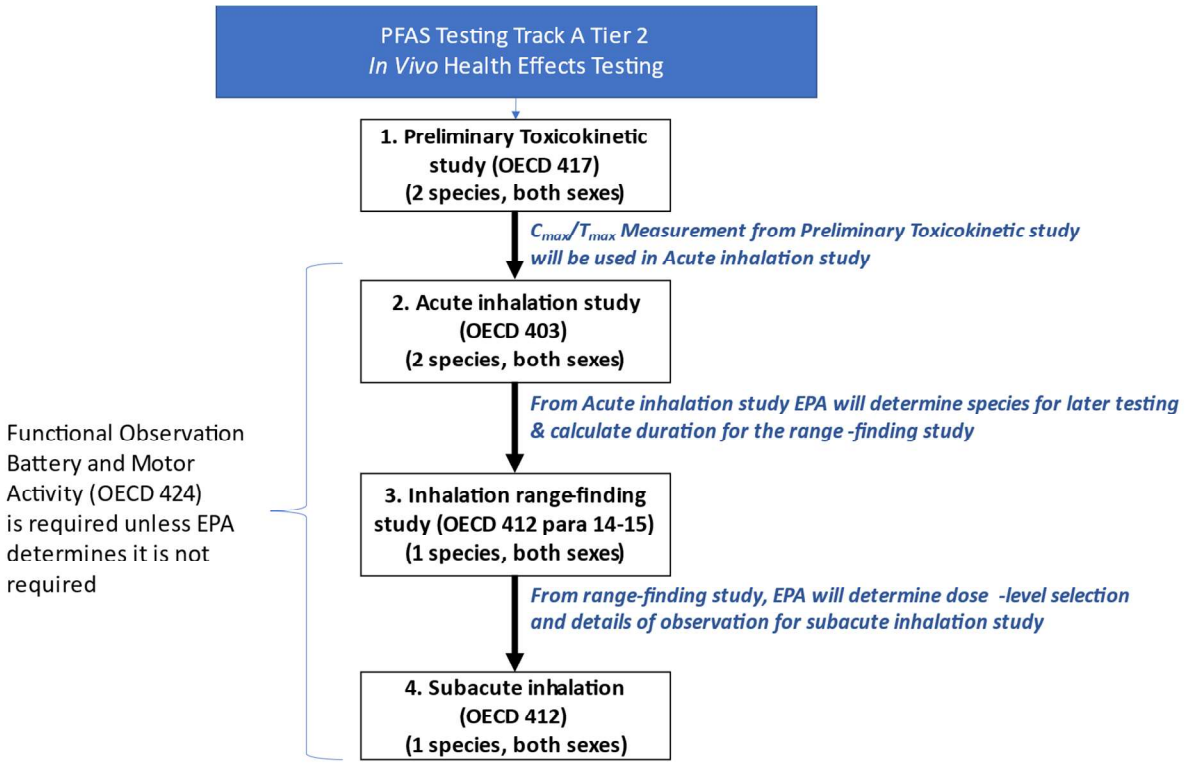

Figure 2. PFAS Testing Track A Tier 2

1. Preliminary Toxicokinetic Study for Development of Information on Absorption, Distribution, Metabolism and Elimination Using Radiolabeling of Test Substance (OECD 417 (2010), OECD GD 39 (2018))

Tier 2 begins with a modified version of OECD 417: Toxicokinetics via Inhalation. This short-term preliminary study in two species (rats and mice) will determine pharmacokinetic parameters such as bioavailability, maximum blood concentration and the time to reach this maximum (C_{max} and T_{max}), and biomarkers of metabolism (particularly free fluoride). C_{max} and T_{max} will be used to guide the design of latter Tier 2 testing required by the Order (*i.e.,* acute inhalation studies in rats and mice with neurotoxicity endpoints, specifically the timing of functional observation battery (FOB) and motor activity testing relative to the exposure period).

The short-term preliminary study based on OECD 417 provides a detailed description of the toxicokinetics (TK) in two rodent species and information needed to plan and execute the acute inhalation toxicity (OECD 403) study that will be useful in dose setting and decisions about what endpoints to measure for the subacute inhalation toxicity (OECD 412) testing that follows. The preliminary TK study (OECD 417) also determines whether the subject test substance of this Order is transported beyond the portal-of-entry and/or any potential human-relevant systemic effects. The preliminary TK study (OECD 417) is not designed to assess hazard. The acute inhalation toxicity (OECD 403) study provides more context and scaling, and from these studies considered together, a single species will be selected for later subacute testing.

2. Acute Inhalation Toxicity: Concentration × Time Method (OECD 403 (2009) and OECD 424 (1997))

The acute inhalation toxicity study is a combination of the OECD 403: Acute Inhalation Toxicity and the OECD 424: Neurotoxicity Study in Rodents. The preliminary TK study (OECD 417) described above will be used by EPA to inform selection of exposure duration (Concentration × Time combinations) for this study. This acute inhalation toxicity (OECD 403) study is necessary to scale study design parameters from effects observed in the preliminary TK (OECD 417) study to the *in vivo* subacute inhalation toxicity (OECD 412) testing that follows. Specifically, the FOB and motor activity observations from OECD 424 (1997) are required study plan and test report criteria to account for any potential WoSE for neurotoxicity and neuropathology that are not explicitly itemized in OECD 403 (2009).

The short-term preliminary TK study (OECD 417) and acute inhalation toxicity (OECD 403) study together will be used by EPA to select the species for subsequent Tier 2 *in vivo* tests, as well as to determine the number of doses needed to achieve pseudo-steady state in an inhalation toxicity range-finding study (OECD 412) used to design a Subacute Inhalation Toxicity study (OECD 412).

3. Inhalation Toxicity Range Finding Study (OECD 412 (2018) paragraphs 14-15 and OECD 424 (1997))

Generally, among other goals, as discussed in paragraphs 14 and 15 of the OECD 412 test guideline, a range-finding study informs what concentration levels to use for a main study. The primary goal of this subacute inhalation toxicity (OECD 412) range-finding study is to determine time-to-peak effects, particularly for neurotoxicity. A neurotoxicity study (OECD 424) is being added to this range-finding inhalation toxicity study to conduct FOB and motor activity observation that will inform the study plan and test report criteria for the full subacute inhalation toxicity (OECD 412) study.

Results from this range-finding study including time-dependent, post-exposure lung burden (see OECD GD 39, *Particle-size Distribution* section, paragraphs 74 and 75), taken together with test substance water solubility (*e.g.*, estimated or experimental), will determine the full subacute inhalation toxicity (OECD 412) study duration post-exposure period and spacing of post-exposure observation (PEO) time/time-to-peak effects points (OECD GD 39).

4. Subacute Inhalation Toxicity: 28-Day Study (OECD 412 (2018))

All prior PTT-A testing results will be used by EPA to inform the study plan and test report for this subacute inhalation toxicity (OECD 412) study. This subacute inhalation toxicity (OECD 412) study must be combined with the neurotoxicity study in rodents (OECD 424), as also required in the acute inhalation toxicity (OECD 403) and inhalation toxicity range-finding (OECD 412) studies. Specifically, the FOB and motor activity observations from OECD 424 (1997) must be included in the study plan and test report criteria to account for any potential WoSE for neurotoxicity and neuropathology that are not explicitly itemized in OECD 412 (2018). However, if earlier *in vivo* Tier 2 testing (*e.g.*, preliminary TK (OECD 417), acute inhalation toxicity (OECD 403) and inhalation toxicity range-finding (OECD 412) studies) demonstrate no evidence of neurotoxicity/neuropathology (*i.e.*, if adverse effects are observed at one of the exposure levels but no significant changes are observed in the FOB or motor activity measurements), FOB and motor activity requirements may be determined by EPA to be unnecessary supplements to this OECD 412; as applicable, EPA will provide confirmation to the Order recipient of the removal of this testing requirement. Translocation of test substance particles to the brain and other target organs (*e.g.*, upper and lower respiratory tract, lymph nodes, thyroid, etc.) will remain in the study plan and test report requirements, including—and not limited to—histopathology/morphometry of the target organs and/or measurement of the metabolism that liberates fluoride into the bloodstream.

Based on the WoSE for all PTT-A testing, reproductive and developmental testing may be required later.

C. Deadlines for Required Testing Protocol(s)/Methodology(ies)

For Tier 1 testing, as discussed in the table in **Unit III.A**, draft study plans and final study plans are due 80 and 110 days after the effective date of the Order, respectively. The final test reports for Tier 1 are provided in the table below. Following receipt of the Tier 1 final test reports, the EPA will provide notification of whether the Tier 2 health effects testing are or are not required. Deadlines associated with developing the associated Tier 2 draft study plans, final study plans, and test reports are based on when EPA concludes review of the prior study and are as follows: draft study plans are due within 80 days and the final study plans are due within 110 days of EPA's notification of the conclusion of the review of the *prior* study. See the table below for more information on the deadlines to submit final reports.

Deadlines that fall on a weekend or holiday will remain and will not be extended to the next weekday.

Test Names	Protocols/Methodologies	Deadlines to Submit Final Reports to the EPA
Required Physical/Chemical Properties		
Particle Density	OECD 109 (2012)	305 days after effective date of the Order
Aerodynamic Particle Size Distribution (APSD) with Cascade Impactors	NIOSH Manual of Analytical Methods (NMAM), Fifth Edition (2017). Chapter BA: "Sampling and Characterization of Bioaerosols."	305 days after effective date of the Order
Hydrolysis as a Function of pH	OECD 111 (2004)	400 days after effective date of the Order

Required Health Effects (Tiered)		
Tier 1		
Biosolubility Test	Gamble's Solution or Simulated Epithelial Lung Fluid (SELF) ECETOC Technical Report 122, Section 3 and *Development and application of an inhalation bioaccessibility method (IBM) for lead in the PM10 size fraction of soil*, Section 2	230 days after effective date of the Order
Tier 2		
Preliminary Toxicokinetic Study for Development of Information on Absorption, Distribution, Metabolism and Elimination Using Radiolabeling of Test Substance	OECD TG 417 (2010), OECD GD 39 (2018)	Once the EPA confirms with the Order recipient that *Tier 2 testing* is required based on EPA's conclusion of the review of the results from the *Tier 1 testing:* the draft study plan is due within 80 days, the final study plan is due within 110 days, and the final test report is due within 665 days
Acute Inhalation Toxicity: Concentration × Time Method	OECD 403 (2009) and OECD 424 (1997)	Once the EPA confirms with the Order recipient that EPA's review of the *Preliminary Toxicokinetic Study* has concluded: the draft study plan is due within 80 days, the final study plan is due within 110 days, and the final test report is due within 305 days
Inhalation Toxicity Range Finding Study	OECD 412 (2018) paragraphs 14-15 and OECD 424 (1997)	Once the EPA confirms with the Order recipient that EPA's review of the *Acute Inhalation Toxicity Study* has concluded: the draft study plan is due within 80 days, the final study plan is due within 110 days, and the final test report is due within 665 days
Subacute Inhalation Toxicity: 28-Day Study	OECD 412 (2018) and OECD 424 (1997) (EPA may determine the OECD 424 is not required)	Once the EPA confirms with the Order recipient that EPA's review of the *Inhalation Toxicity Range Finding Study* has concluded: the draft study plan is due within 80 days, the final study plan is due within 110 days, and the final test report is due within 665 days

VI. REQUIREMENTS OF RESPONSE OPTION 1: DEVELOP THE INFORMATION REQUIRED BY THIS ORDER

A. OVERVIEW

The draft study plan for Tier 1 testing is due to the EPA **80 days** after the effective date of this Order. The EPA will then review the draft study plan and provide input to ensure adequacy of the final study plan. For the final study plans and the final test reports, see the Deadlines for Responses, Study Plans, and Test Reports table in **Unit III.A**.

All testing described in **Unit V** must be conducted in accordance with the Good Laboratory Practice (GLP) standards in 40 CFR part 792, as specified in the CFR on the Effective Date of this Order. You must provide a statement of compliance with these GLP standards when submitting information to the EPA pursuant to this Order.

Deviations from the test guideline or specific GLP standards are allowed provided justifications for such deviations are approved by the EPA. A justification is required for each deviation. Justifications should demonstrate that, despite the deviation from the given test guideline or GLP standard, that data integrity,

control of bias, and study quality will be maintained with similar effectiveness. Any requested deviations and corresponding justifications must be included in the draft study plan for the EPA's consideration and, if approved, described in the test report.

Once the EPA has completed its review of the submitted test reports and accepts the information as fully complying with your testing obligations under this Order, the EPA will notify you.

B. DRAFT STUDY PLAN REQUIREMENTS

1. Study Plan Requirements for All Categories of Tests

If you choose to develop the required information to comply with this Order, you must obtain and review the required protocols/methodologies. **Unit V and Appendix E** provide the protocols/methodologies that must be followed to perform each required test.

If questions and/or issues arise during Study Plan development, the EPA encourages questions/comments be submitted along with the Study Plan submission in accordance with the draft study plan deadline. If the EPA's review of the draft study plan that includes the questions/comments is delayed, the procedure outlined in **Unit III.B** will be followed for automatic extensions of the study plan.

In addition to requirements provided in **Appendix E** for a given test required by this Order, the Study Plans must contain the following information:

1. This Order number, excluding the unique 6-digit company number using X's in place of the unique company number so as to protect each company's private access to the reporting module via Central Data Exchange (CDX). For example, if your Order number is TO-2020-0000-438435-00-0 then provide this number in the Study Plan: TO-2020-0000-XXXXXX-00-0.

2. Name of test to be covered by the test protocol/methodology.

3. The name/number of the protocol/methodology identified in this Order which you intend to follow, a copy of the identified protocol/methodology with your proposed modifications, or a copy of the alternate protocol/methodology you propose to use. Justification(s) must be provided for any deviation from the protocol/methodology identified in this Order.

4. The identity of and supporting data on the chemical substance to be tested including physical constants, spectral and chromatographic data, chemical analysis, and stability under test and storage, and test conditions required by the protocol. A Certificate of Analysis of the test substance must be provided.

5. The sampling and analytical method that will be used.

6. A description of the preparation and processing of samples that will be done before sampling and during sampling, including equilibration, weighing, calibration, test conditions (temperature, humidity), number and type of samples, and identification of equipment and accessories used (make, model, size/capacity, and operating conditions), including the specific sampling media and sampling instruments that will be used.

7. A description of all quality assurance and quality control protocols used.

8. The name(s) and address(es) of the company(ies) sponsoring the test and whether they comprise a testing consortium.

9. The name(s), mailing address(es), phone number(s), and e-mail address(es) of the appropriate individual(s) for the EPA to contact concerning the planned test.

10. The name of the testing facility and the names, mailing addresses, telephone numbers, and email addresses of the testing facility's administrative officials, study director/project managers and quality control officer responsible for ensuring the testing protocol follows appropriate quality assurance and quality control procedures.

2. Modifying a Required Protocol/Methodology in a Draft Study Plan

The draft study plan must include the required protocols/methodologies outlined in **Unit VI.A.1** and **Appendix E**. If you believe modifications of these required protocols/methodologies are necessary, you should propose the modification in the draft study plan and submit to the Agency with request for the Agency to consider the modifications. Any consultation regarding modifications to the required protocols/methodologies will not extend the deadline for submission of the draft study plan.

Any submitted requests for modifications of the required protocols/methodologies must include a detailed description of the proposed modification as well as a detailed description of the justification and reasoning for such modifications. Requests for modifications of protocol/methodology or the use of an alternate protocol/methodology must discuss why such changes are appropriate and whether they could alter the validity of the study. The rationales do not have to be listed in a separate document in the study plan if they are included and clearly identified in the relevant section of the study plan describing the protocols/methodologies.

If the EPA has concerns about the requested protocol/methodology or your requested modifications of the required protocol/methodology, the Agency will inform you of concerns that must be addressed before the EPA will approve your study plan. The EPA has 15 days from the deadline for the study plan to respond. For each day following this period that the EPA does not respond, the EPA will extend the deadline for the final study plan by one day (see **Unit III**).

3. The EPA Review of Study Plans and Final Test Reports

The EPA will not conduct a substantive review of any draft study plan that does not meet the requirements as provided in **Unit VI.B.1** and **Appendix E**. Such a submission does not constitute meeting the deadline for the draft study plan submission. **Unit III** provides information on deadlines and the EPA response timelines.

Failure to submit a draft study plan, final study plan, and final test report which do not fully comply with the terms of this Order and by the deadlines provided in **Unit III** may result in a violation of TSCA section 15.

a. Study Plans

Following review of a draft study plan submission, EPA will indicate what modifications, if any, are required and must be incorporated into the final study plan. Accompanying a proposed final study plan

submission, the submitter must provide a clean and red-lined version. The red-lined version will indicate the changes incorporated into the final study plan as compared with the prior study plan submission.

If the EPA requires modifications to a submitted draft study plan, the Agency may elect to provide a line-by-line list of comments that must be addressed and corrected before the final study plan will be approved. If the submitter receives a line-by-line list of comments, the submitter must address each individual comment and include this in their response to the Agency along with the proposed final study plan.

Prior to initiating any test, the Company/Consortium must first address the EPA's input on the study plan and receive the EPA's acceptance of the final study plan.

The EPA's acceptance of a final study plan does not constitute pre-acceptance of any future test results. If testing conducted according to a requested protocol/methodology or requested modifications of the required protocol/methodology is initiated prior to EPA approval, that testing will not satisfy the requirements of the Company under this Order.

If, after the final study plan has been approved or after testing is underway, you wish to make a modification to an identified protocol/methodology or use a different protocol/methodology, you must submit a request to the EPA to make these changes in your study and you must still meet the deadlines set out in **Unit V** and **Appendix E** for the relevant test or request an extension (see also **Unit III.C**), if needed.

Note that submitting questions to the EPA regarding study plan requirements will not extend the deadline for a study plan submission.

b. Final Test Reports

Once the EPA has completed its initial review and accepted data for all test reports subject to this Order for a given testing requirement, the EPA will notify the designated contact for the company or consortium subject to this Order that this testing requirement has been satisfied, which in turn will close out the testing requirement of this Order for the companies and participants in any consortium subject to this Order. For human health animal toxicity test reports, all submitted existing information will be evaluated for study quality using the TSCA systematic review method (EPA-HQ-OPPT-2021-0414-0005, Appendix Q.4.2).

Failure to file a final test report meeting all the requirements in this Order by the deadline in **Unit III** is a violation of TSCA. Your final test report must be submitted along with the data in the associated OECD harmonized template format, if available. OECD harmonized templates can be located at the OECD Harmonised Templates webpage (OECD, 2018d):

a. Particle Density OECD 109 (2012)

- *Harmonized Template Identifier:* OHT 4 (Density)

b. Aerodynamic Particle Size Distribution (APSD) with Cascade Impactors ((NIOSH NMAM (2017). Chapter BA))

- *Harmonized Template Identifier:* OHT 5 (Particle size distribution (Granulometry)/Fiber length and diameter distribution)

c. *Hydrolysis as a Function of pH OECD 111 (2004)*

- *Harmonized Template Identifier:* OHT 25 (Hydrolysis)

d. *Preliminary Toxicokinetic Study for Development of Information on Absorption, Distribution, Metabolism and Elimination Using Radiolabeling of Test Substance (OECD TG 417 (2010), OECD GD 39 (2018))*

- *Harmonized Template Identifier: OHT 61 (Acute toxicity: inhalation)*

e. *Acute Inhalation Toxicity: Concentration × Time protocol (OECD 403 (2009))*

- *Harmonized Template Identifier:* OHT 61 (Acute toxicity: inhalation)

f. *Inhalation Toxicity Range finding study*

- Harmonized Template Identifier: OHT 61 (Acute toxicity: inhalation)

g. *Subacute Inhalation Toxicity: 28-Day Study OECD 412 (2018)*

- *Harmonized Template Identifier:* OHT 68 (Repeated dose toxicity: inhalation)

VII. FEES FOR SUBMITTING INFORMATION

Per 40 CFR § 700.45, and taking into account the inflation adjustment that went into effect on January 1, 2022, the Test Order fee is $11,650 to be split evenly among the manufacturers who are required to test a chemical substance or mixture subject to the Test Order (accounting for small business considerations). Processors are not subject to this fee, nor are manufacturers who submit existing information or receive an exemption in compliance with this Order.

Small businesses may be subject to no more than 20% of the amount of the applicable fee. A company may qualify for a "small business concern" discount if their total number of employees is at or below the maximum allowed in the final rule for that company's North American Industry Classification System (NAICS) code (see 40 CFR 700.43). In order for an entity to qualify as a "small business concern," its number of employees shall not exceed the size standard for the applicable industry. When calculating the number of employees, the company must include the employees of all parent and subsidiary companies within the corporate chain. Please note that small business fees are only applicable to qualifying small businesses who are either not associated with a consortium or associated with an all-small business consortium. See the TSCA User Fees webpage (USEPA, 2021b) for more information.

A company can identify itself as a small business when responding to this Order via the CDX application. The "small business concern" discount will be included in the determination of company-specific invoices for the distribution of the $11,650 fee across all manufacturers conducting testing for the given Test Order. Where a consortium is responsible for the fee for its members for purposes of this Order, and at least one of the members is not a small business, the EPA does not apply a "small business concern" discount to the portion of the $11,650 distributed to the consortium.

Fees for Test Orders under TSCA section 4 will be invoiced electronically by the EPA. Invoice notices will be populated into the specific user's "Copy of Record" screen in CDX and will contain a button that will initiate the payment process. When an invoice is generated, notification e-mails will be sent to the

user's CDX inbox and the e-mail address associated with the relevant CDX account. Payment information will be collected in CDX and then submitted to Pay.gov for processing.

Note that there are many fees associated with TSCA-related activities. See the TSCA Fees table webpage (USEPA, 2021c) for more information. The TSCA section 4 Test Order fee is separate from these fees. A company's inclusion in or exclusion from other TSCA fees is unrelated to that company's status with regards to TSCA section 4 Test Order fees.

Pursuant to 40 CFR § 700.45, the applicable fee shall be paid in full no later than 120 days after the effective date of the Order. Should the EPA invoice the fee more than 90 days after the effective date of the Order, payment will be due within 30 days of such invoicing.

VIII. INSTRUCTIONS IF YOU CHOOSE TO PARTICIPATE IN A CONSORTIUM

If you choose to form or join a consortium to share in the cost of developing the required information, you (as well as the other Order recipients who are participants in the consortium) must, individually in the CDX portal, state your intention to participate in a testing consortium for each specific chemical and specific test. Consortium participants must individually respond in the CDX portal with their intent to participate before designated leads are able to add them to the consortium.

In addition, the designated lead for the consortium must submit a consortium response to the EPA in the CDX portal. The response must confirm the formation of the consortium, identify its member companies, and list the testing obligations that the consortium plans to fulfill on behalf of each company by indicating each specific test. The response must also include contact information for the designated lead of the consortium, who must be domiciled in the United States. The designated lead for the consortium must submit the response and required information on behalf of the consortium and its member companies by the deadlines listed in **Unit III.A**. Submissions made on behalf of the consortium must be in accordance with instructions in **Appendix C**. Note that a consortium lead need not be a recipient of an Order; other entities (such as trade organizations) may act as a lead and submit the information required under this Order. After the results of the last required test of this Order are submitted and the EPA accepts the information as complying with this Order, or the EPA accepts existing information submitted by the Consortium, the EPA will provide notification of compliance with this Order to this Order's recipients and the designated lead of the consortium.

Even if you agree to jointly submit the information as part of a consortium, each Order Recipient is still required to comply with this Order (with the study plan and results being submitted by the consortium) and is individually liable in the event of any failure to comply with this Order. If the consortium fails to submit the information or meet any of the requirements of this Order on your behalf, you will be in violation of this Order unless you submit the required information or meet the requirement individually.

The Agency has provided a list of the manufacturers and processors that have received this Order at the top of this Order in the Summary Information section. This list of manufacturers and processors can be used to help Order Recipients form a consortium to jointly develop information, consolidate testing and share the cost of testing. Information on cost sharing is provided in **Appendix B**.

IX. CONFIDENTIALITY

Under TSCA section 14(b)(2), health and safety studies submitted under TSCA and data reported to or otherwise obtained by the Administrator from health and safety studies are not protected from disclosure if the studies and data concern a chemical that is offered for commercial distribution, or for which

testing is required under TSCA section 4 or notification is required under TSCA section 5. However, TSCA section 14(b)(2) does not apply to information that discloses processes used in the manufacturing or processing of a chemical substance or mixture or, in the case of a mixture, the portion of the mixture comprised of the chemical subject to this Order. Therefore, some or all of the information in the studies required to be submitted under this Order might not be eligible for TSCA confidential business information (CBI) protections.

Information submitted under TSCA that you wish to have the EPA protect as confidential business information (CBI) must be clearly identified as such when submitted (see **Appendix C** for instructions for submitting information claimed as CBI). For sections of the report that are claimed as CBI, the report must be accompanied by a sanitized version of the report only removing the specific information claimed as CBI. A sanitized test report that redacts all or most of the study may be rejected by the EPA as not satisfying the requirements of this Order.

When claiming information as CBI, you must certify to the following:

> "I hereby certify to the best of my knowledge and belief that all information entered on this form is complete and accurate.
>
> I further certify that, pursuant to 15 U.S.C. § 2613(c), for all claims for confidentiality made with this submission, all information submitted to substantiate such claims is true and correct, and that it is true and correct that
>
> (i) My company has taken reasonable measures to protect the confidentiality of the information;
>
> (ii) I have determined that the information is not required to be disclosed or otherwise made available to the public under any other Federal law;
>
> (iii) I have a reasonable basis to conclude that disclosure of the information is likely to cause substantial harm to the competitive position of my company; and
>
> (iv) I have a reasonable basis to believe that the information is not readily discoverable through reverse engineering.
>
> Any knowing and willful misrepresentation is subject to criminal penalty pursuant to 18 U.S.C. § 1001."

In addition, information claimed as CBI must be substantiated upon submission, with the exception of information described in TSCA Section 14(c)(2). See guidance for substantiating CBI claims (USEPA, 2021d).

Failure to follow the statutory requirements for asserting and substantiating a CBI claim may result in the information being made available to the public without further notice to the submitter.

When a claim of CBI is asserted for certain information under TSCA section 14, the Administrator will generally protect that information from disclosure for 10 years (*e.g.*, unless the protection from disclosure is withdrawn by the person that asserted the claim), whereupon the claim must be reasserted and re-substantiated if the submitter wishes to maintain the CBI claim. In certain cases, the EPA may review claims prior to the expiration of the 10-year period.

Under circumstances stated in TSCA section 14(d), the EPA may disclose information claimed as CBI to other persons including, for example, Federal and State authorities, health and environmental professionals, poison control centers, and emergency responders.

X. CONSEQUENCES OF FAILURE TO COMPLY WITH THIS ORDER

Failure to comply with any of the requirements in this Order is a violation of TSCA section 15 and could subject you to civil and/or criminal penalties under TSCA section 16, 15 U.S.C. § 2615 as modified by the Federal Civil Penalties Inflation Adjustment Act. Each day that failure to meet the requirements continues constitutes a separate violation.

XI. REFERENCES

The following is a listing of the documents that are generally applicable to this Order. Please note that references, guidance, and information from additional sources could be considered, with EPA approval, during the development of study plans.

The docket includes these documents and other information considered by the EPA, including documents that are referenced within the documents that are included in the docket, even if the referenced document is not physically located in the docket. For assistance in locating these other documents, please consult the technical person listed under **FOR FURTHER INFORMATION CONTACT**.

General References for this Test Order

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XII. PAPERWORK REDUCTION ACT NOTICE

This collection of information is approved by the Office of Management and Budget (OMB) under the Paperwork Reduction Act, 44 U.S.C. § 3501 et seq. (OMB Control No. 2070-0033). Responses to this collection of information are mandatory under the Toxic Substances Control Act (TSCA), 15 U.S.C. § 2601 et seq. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. The public reporting and recordkeeping burden for this collection of information is estimated to be 137 hours for the average response on a per-chemical basis. Under the PRA, burden is defined at 5 CFR 1320.3(b). Send comments on the Agency's need for this information, the accuracy of the provided burden estimates and any suggested methods for minimizing respondent burden to the Regulatory Support Division Director, U.S. Environmental Protection Agency (2821T), 1200 Pennsylvania Ave., NW, Washington, D.C. 20460. Include the OMB control number in any correspondence. Do not send the completed form to this address.

XIII. FOR FURTHER INFORMATION CONTACT

For technical information contact: TSCATestOrders@epa.gov.

For general information contact: The TSCA Hotline, ABVI-Goodwill, 422 South Clinton Ave., Rochester, NY 14620; telephone number: (202) 554-1404; email address: TSCA-Hotline@epa.gov.

XIV. SIGNATURE

Under the authority in TSCA Section 4(a)(1), the United States Environmental Protection Agency hereby issues this Order.

MICHAL FREEDHOFF

Digitally signed by MICHAL FREEDHOFF
Date: 2022.06.16 14:20:07 -04'00'

Michal Freedhoff,

Assistant Administrator, Office of Chemical Safety and Pollution Prevention.

APPENDIX A - EQUIVALENCE DATA

For purposes of this Order, "equivalence data" means "chemical data or biological test data intended to show that two substances or mixtures are equivalent." 40 CFR § 709.3. Also, when a chemical substance is "equivalent," it means "that a chemical substance is able to represent or substitute for another in a test or series of tests, and that the data from one substance can be used to make scientific and regulatory decisions concerning the other substance," as defined in 40 CFR § 790.3.

If testing under TSCA section 4(a) is required of an equivalent chemical substance, the EPA may grant an exemption from testing to the manufacturer or processor of one substance if the information required under TSCA section 4(a) is submitted or is being developed on the other, and the manufacturer or processor submits the following information to support equivalence with its exemption application:

1. The chemical identity of each chemical substance or mixture manufactured or processed by the applicant for which the exemption is sought. The exact type of identifying data required may be specified in this Order and may include all characteristics and properties of the applicant's substance or mixture, such as boiling point, melting point, chemical analysis (including identification and amount of impurities), additives, spectral data, and other physical or chemical information that may be relevant in determining whether the applicant's substance or mixture is equivalent to the specific test substance.

2. The basis for the applicant's belief that the substance or mixture for which the exemption is sought is equivalent to the test substance or mixture.

3. Any other data which exemption applicants are directed to submit in this Order which may have bearing on a determination of equivalence. This may include a description of the process by which each chemical substance or mixture for which an exemption is sought is manufactured or processed prior to use or distribution in commerce by the applicant.

APPENDIX B – COST SHARING

The EPA encourages Order recipients that are responsible for developing the same information on the same chemical(s) to avoid duplicative testing and share the cost of information development. If a test is conducted according to a final, approved protocol, it is sufficient that the test is conducted once. Two ways to avoid duplicative testing are discussed in this Order. They are forming or joining a consortium, discussed in **Unit VIII**, or requesting an exemption, discussed in **Unit IV.B.3**.

Consortia

Persons that form or join a consortium typically execute an agreement with the other members of the consortium concerning how costs will be shared and how the consortium will operate.

Exemptions

Persons that receive exemptions from testing have an obligation to reimburse the person(s) who perform the testing and submit the required information that is the basis for the exemption for a portion of the costs incurred in complying with the requirement to submit such information, and any other person required to contribute to a portion of such costs. Apportionment of costs between persons receiving exemptions and the person who actually conducts the test(s) is ideally negotiated between the companies involved, without the EPA participation. The EPA has promulgated regulations that explain how the EPA views fair and equitable reimbursement in the context of TSCA Section 4(a) test rules. In general, those regulations (40 CFR § 791.40 through § 791.52) make a presumption that a person's fair share of the test costs is in proportion to their share of the total production volume of the test chemical over a specified period of time that begins one calendar year before the effective date of the rule and continues up to the latest data available upon resolution of a dispute. While those regulations do not apply to TSCA Section 4 orders, you may wish to consider them as you decide how to share the costs.

If persons subject to an order include a person that has been granted an exemption and agreement cannot be reached on the amount and method of sharing the cost of developing the information, the person whose information is the basis for the exemption may request that the Administrator order the person(s) granted the exemption to provide fair and equitable reimbursement after considering all relevant factors, including the share of the market and the effect on the competitive position of the person required to provide reimbursement in relation to the person to be reimbursed. See TSCA Section 4(c)(3)(A). Upon receipt of such a request, the EPA will determine fair and equitable reimbursement and issue an order accordingly. The Agency may, at its discretion, make use of procedures and standards applicable to data reimbursement regarding TSCA Section 4 rules, contained in 40 CFR part 791.

APPENDIX C - How to Access the CDX Application and Recordkeeping Requirements

How to Access the CDX Application

The initial response, draft and final study plans, final test reports with underlying data, existing studies, any testing related requests, and all related correspondence must be submitted electronically to the EPA as follows:

1. Submit to the EPA's CDX system. CDX is the point of entry on the Environmental Information Exchange Network (Exchange Network) for submissions to the Agency.

2. The URL for the CDX website is *https://cdx.epa.gov/* which takes you to the CDX homepage.

3. On the homepage you may select "Log in" or, if you haven't already registered, select "Register with CDX."

4. Once you have logged on to CDX, follow the instructions for submitting TSCA Section 4 Order information. To access the instructions, select "Report electronically" on the EPA Assessing and Managing Chemicals under TSCA webpage.

5. The CDX Help Desk is available for data submission technical support between the hours of 8:00 am and 6:00 pm (EST) at 1-888-890-1995 or helpdesk@epacdx.net. The CDX Help Desk can also be reached at 970-494-5500 for international callers.

The EPA may revise these submission instructions with advance notice.

Recordkeeping

You must retain copies of all information documenting your compliance with this Order for ten years. This includes your response and other documents and correspondence submitted to comply with this Order, such as test protocols, testing related requests, final test reports with their underlying data, and any penalties remitted.

APPENDIX D - Order Recipient Selection

This Appendix describes the process by which the EPA identified recipients of this Order. This information is for your use and does not govern the obligations under this Order or the identities of the companies subject to this Order. A recipient of this Order that manufactures or processes the chemical as per the definitions provided in **Unit I.B** is subject to this Order, regardless of the basis on which the EPA identified the recipient.

The EPA queried for companies with known associations with 6:2 fluorotelomer sulfonamide betaine from the EPA Chemical Information System (CIS) within the past 15 years. The EPA CIS is an internal platform for managing data and reporting submissions under TSCA. Some submission types that are housed in CIS include Chemical Data Reporting (CDR), Pre-manufacture Notifications, and Notice of Activity forms. Based on these such submissions, the EPA has included entities associated with this chemical substance.

EPA also searched publicly available records, such as Safety Data Sheets, for companies associated with 6:2 fluorotelomer sulfonamide betaine and included such companies.

APPENDIX E - Specific Requirements and Guidance for This Order

This appendix provides requirements of study plans and test reports for specific testing requirements of this Order.

For information on how the EPA determined the need for testing in this Order, refer to **Unit II.B**.

I. Physical-Chemical Properties

a. Particle Density (OECD 109 (2012)) OECD (2012)

i. Study Plans

See **Unit VI.B** of the Order for overall requirements for study plans. Additional requirements specific to OECD (2012) include:

1. The density measurements must be for the particles that make up the relevant process stream in contrast to the bulk density. This can be done with a pycnometer, hydrometer, porosimeter, or other suitable method.

ii. Test Reports

In addition to the requirements provided by **Unit VI**, test reports submitted to the EPA for this test are due 305 days after the effective date of the Order and must include the following, as applicable:

1. Harmonized Template Identifier: OHT 4 (Density)

2. Harmonized Template URL: https://www.oecd.org/env/ehs/testing/OHT%204%20-%20ENDPOINT_STUDY_RECORD.Density_v4.2%20-Dec%202018.doc

b. Aerodynamic Particle Size Distribution (APSD) with Cascade Impactors ((NIOSH Manual of Analytical Methods (NMAM) (2017). Chapter BA: "Sampling and Characterization of Bioaerosols") NIOSH (2017)

i. Study Plans

See **Unit VI.B** of the Order for overall requirements for study plans. Additional requirements specific to Procedures for Cascade Impactor Calibration and Operation in Process Streams (USEPA, 1977) include:

1. The particle size measurements must be performed on the airborne particles as part of the process stream.

2. The particle size measurements must be performed using a 6-stage Andersen cascade impactor or similar device suitable for the measurement of the test substance.

ii. Test Reports

In addition to the requirements provided by **Unit VI**, test reports submitted to the EPA for this test are due 305 days after the effective date of the Order and must include the following, as applicable:

1. Harmonized Template Identifier: OHT 5 (Particle size distribution (Granulometry)/Fiber length and diameter distribution)

2. Harmonized Template URL: https://www.oecd.org/env/ehs/testing/OHT%205%20-%20ENDPOINT_STUDY_RECORD.Granulometry%20(Fibre%20length%20and%20diameter%20distribution)%20_v4.2%20-Dec%202018.doc.

c. Hydrolysis as a Function of pH (OECD 111 (2004)) OECD (2004)

i. Study Plans

See **Unit VI.B** of the Order for overall requirements for study plans.

No additional requirements.

ii. Test Reports

In addition to the requirements provided by **Unit VI**, test reports submitted to the EPA for this test are due 400 days after the effective date of the Order and must include the following, as applicable:

1. Harmonized Template OHT 25 (Hydrolysis)

2. Harmonized Template URL: https://www.oecd.org/env/ehs/testing/OHT%2025%20-%20ENDPOINT_STUDY_RECORD.Hydrolysis_v4.3%20-Dec%202018.doc

II. Health Effects

a. Tier 1: Biosolubility (Gamble's Solution or Simulated Epithelial Lung Fluid (SELF)) Solubility Test (ECETOC Technical Report 122, Section 3) (ECETOC, 2013))

i. Study Plans

Please see **Unit VI.B** of the Order for overall requirements for study plans. Solubility should be determined with Gamble's solution or SELF to represent initial dissolution for characterization of the potential for clearance and support for retained dose predictions ECETOC (2013).

If the SELF method is selected to assess biosolubility, use the following publication to develop a draft protocol for measuring simulated lung fluid solubility for EPA review: Section 2 of

Development and application of an inhalation bioaccessibility method (IBM) for lead in the PM10 size fraction of soil.

Requirements and recommendations specific to the *Biosolubility* test include:

1. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, non-test guidelines, and inhalation applicability, including, and not limited to: all methodological and reporting quality details (*e.g.*, general test information, method definitions, test performance and lab proficiency, results interpretations, and risk of bias considerations), sources of materials, and relevance of the model system (Hartung et al., 2019) see 'Box 1 Summary of features to report according to OECD (2014) and summarized in Samuel et al. (2016), Emmerich and Harris (2019), Clippinger et al. (2018), OECD (2017), OECD (2014), OECD (2005).

2. Must include all relevant information required to assess the relevance, quality, validation, and applicability of the data (Kolle et al., 2019; Clippinger et al., 2018; OECD, 2018a, 2005) and specifically for biosolubility testing, including composition and concentrations of *in vitro* lung bioaccessibility fluid (*e.g.*, salts, water, proteins, and lipids), preparation conditions and stability of the fluid, procedural blanks, and appropriate bioaccessibility calculations for the substance with referenced values (Boisa et al., 2014).

3. The EPA is relying on estimated values for the following physical-chemical properties as the best available science. If you have additional information/measurement of the following properties, include it in the study plan, indicating what deviations from the required testing are appropriate, and including justifications for such deviations. Alternatively, you may elect to develop information on these properties using the methodologies listed below to help inform this testing requirement:

    a. Vapor Pressure

        i. Protocol/Methodology: OECD 104 (2006) OECD (2006) or OCSPP 830.7950

        ii. Harmonized Template OHT 6 (Vapor pressure)

        iii. Harmonized Template URL: https://www.oecd.org/env/ehs/testing/OHT%206%20-%20ENDPOINT_STUDY_RECORD.Vapour_v4.2%20-Dec%202018.doc

    b. Water Solubility

        i. Protocol/Methodology: OECD 105 (1995) OECD (1995a) or OCSPP 83.7840

ii. Harmonized Template OHT 8 (Water solubility)

iii. Harmonized Template URL: https://www.oecd.org/env/ehs/testing/OHTx%208%20-%20ENDPOINT_STUDY_RECORD.WaterSolubility_v4.2%20-Dec%202018.doc

c. Melting Point/Melting Range

i. Protocol/Methodology: OECD 102 (1995) OECD (1995b) or OCSPP 830.7200 USEPA (1998)

ii. Harmonized Template OHT 2 (Melting point/freezing point)

iii. Harmonized Template URL: https://www.oecd.org/env/ehs/testing/OHT%202%20-%20ENDPOINT_STUDY_RECORD.Melting_v5.2%20-Dec%202018.doc

d. Boiling Point

i. OECD 103 (1995) OECD (1995cTG 102) or OCSPP 830.7220 (1996) USEPA (1996)

ii. Harmonized Template OHT 3 (Boiling point)

iii. Harmonized Template URL: https://www.oecd.org/ehs/templates/OHT-3-endpoint-study-record-BoilingPoint-v6.3-Sept-2020.doc

ii. Test Reports

In addition to the requirements provided by **Unit VI**, test reports submitted to the EPA for this test are due 230 days after the effective date of the Order and must include the following, as applicable:

1. Must include relevant information considerations that can strengthen and enable data quality assessment and relevance to the *in vivo* scenario, especially for inhalation: methodological and reporting quality details, sources of materials and relevance of the model system (Emmerich and Harris, 2019; Hartung et al., 2019; OECD, 2014). Must include the metadata described in study plan requirements and fully describe SELF composition and concentrations (*e.g.*, salts, water, proteins, and lipids) (Emmerich and Harris, 2019; Hartung et al., 2019; Clippinger et al., 2018; Samuel et al., 2016; Boisa et al., 2014).

2. Must use (OECD, 2014) to report/describe non-guideline *in vitro* test methods (see also, Kolle et al., 2019).

3. Must additionally use (OECD, 2005) and (OECD, 2018a) GIVIMP to report the relevance, applicability and validity of the test system, performance, and results.

4. Must use OECD (2017) and OECD (2016), to the extent possible, to report the test results in the context of this tiered testing track and IATA, and to inform refinement of subsequent tiered testing of this Order.

5. IMPORTANT NOTE: This testing track was prepared based on the assumption that the estimated physical-chemical properties of the test materials are accurate. If biosolubility is determined to be >100 mg/L in Gamble's solution or SELF, the required Tier 2 testing may need to change from what is articulated in this test order. Therefore, submitters must contact the Agency as soon as possible if solubility as defined above is determined, as the next set of testing that would be required for this substance will need to be evaluated.

6. Similarly, this track was not prepared for nanomaterials. Nanomaterials for the purposes of this testing track are those materials that are reportable nanoscale chemical substances as described at 40 CFR Part 704 (CFR, 1987). If the test data from Tier 1 indicate that this substance is a nanomaterial, submitters should contact the Agency to determine how Tier 2 testing requirements may be modified to capture the particular toxicity concerns for nanomaterials.

b. Tier 2: Preliminary Toxicokinetic Study for Development of Information on Absorption, Distribution, Metabolism and Elimination Using Radiolabeling of Test Substance (OECD TG 417 (2010), OECD GD 39 (2018))

This testing is required unless particle size data indicates the substance is a nanomaterial (one dimension <100 nm).

EPA will evaluate whether this testing, as provided, is appropriate based on the biosolubility test showing solubility >100 mg/L in Gamble's solution or simulated epithelial lung fluid (SELF) (Boisa et al., 2014).

i. Study Plans

Please see **Unit VI.B** of the Order for overall requirements for study plans.

1. This preliminary TK study is modified to be consistent with a 'sighting study' as a best practice for acute inhalation toxicity testing, OECD GD 39 (2018)(OECD, 2018b). A science-based section and rationale for inhalation exposure characterization is particularly critical for PTT-A testing due to potential dosimetry challenges from test substance particles and portal-of-entry effects (*e.g.*, lung burden). Exposure characterization must account for effects of portal-of-entry physiological responses that may alter test substance uptake (*i.e.*, hyper- or hyperventilation) and/or inter-animal variability.

2. Generally, this preliminary study for toxicokinetic characterization must include the following:

    - exposure particle size distribution (which must be consistent with the requirements for inhalation exposure characterization in the 4th bullet of paragraph 2 of the OECD (2018e)); density; measurements for radioactivity mass balance; absorption; bioavailability; serial sampling of blood, serum, plasma, and urine;

    - tissue distribution and accounting for mass balance including, and not limited to, liver, fat, kidney, spleen, whole blood, residual carcass, heart, lung, thyroid, upper and lower respiratory tract, gastrointestinal (GI) tract, brain, nasal and mucosal tissues and any other tissues and organs that may be indicative of an adverse outcome for human health;

    - accounting for radioactive mass balance; metabolism and excretion (OECD, 2010b); analysis of bronchoalveolar lavage (BAL), bronchoalveolar lavage fluid (BALF;lactate dehydrogenase release, blood oxygen content) and lung-associated lymph node burdens (LALN); blood fluoride levels; absolute and relative organ weights for lung, heart, brain, kidney, liver, and thyroid.

    - Additional organs and tissues will be required by EPA, as appropriate, to determine the potential transport and/or accumulation/deposition of particles (OECD (2010b), 'Other tissue kinetics').

3. The preliminary TK study must be performed in both rats and mice, of both sexes.

4. The exposure particle size distribution must be characterized and consistent with the requirements in the 4th bullet of paragraph 2 of the OECD (2018e): "The 2009 version of TG 412 required particulate aerosols to have a mass median aerodynamic diameter (MMAD) of 1-3 µm with a geometric standard deviation (σ_g or GSD) of 1.5-3.0. Justification should be provided in the study report if this standard cannot be met, including a description of efforts taken to meet it, such as milling (refer to GD 39)."

5. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, non-test guidelines, and inhalation applicability, including, and not limited to: all methodological and reporting quality details (*e.g.*, general test information including rationale, method definitions, test performance and lab proficiency, results interpretations, and risk of bias considerations), sources of

materials, and relevance of the model system (Samuel et al., 2016; Beronius et al., 2014; OECD, 2005).

6. Per OECD TG 417 (2010)(OECD, 2010b), the dose must be non-lethal/non-toxic, but? high enough to allow for potential metabolite identification in excreta and other biofluids, tissues and organs as appropriate, to sufficiently meet the stated purpose of this preliminary study.

7. Consistent with OECD (2010b) (see paragraph 18), the radiolabel must be located in a '… core portion of the molecule, which is metabolically stable.' Further, labeling of multiple sites may be necessary to track the metabolic fate of the test substance. The parent and potential metabolites must be traceable by radiolabel and able to be accounted for in the total mass.

8. Testing must include at least six animals per sex, species, and dose group. Three animals will be needed for immediate sacrifice following exposure, to determine peak total test substance levels, and detectable metabolites in tissues, including and not limited to: brain, lungs, kidneys, olfactory bulb, nasal mucosa, and other nasal tissues (mucosa plus underlying bone and cartilage), eyes, thyroid, and liver. The other three animals will be used for serial sampling post exposure of blood, plasma, serum, urine and lung burden measurements like bronchoalveolar lavage (BAL), BALF and LALN at appropriate intervals, suited for each biofluid, and including cage washes (OECD, 2018b, 2010b). Whenever possible, must use the time course lung burden measurements to calculate relative aerosol deposition percentages in the respiratory tract. Software to perform these calculations are available for free (OECD (2018b), see paragraph 75; 86 FR 15476).

9. The toxicity profile (ToxProfile) developed by Agency for Toxic Substances and Disease Registry (ATSDR), the Centers for Disease Control and Prevention (CDC), for perfluoroalkyl substances (ATSDR, 2021) may inform suitable biofluid sampling time intervals. The perfluoroalkyl ToxProfile used information from 10 perfluoroalkyls previously measured in the serum collected from a representative U.S. population 12 years of age and older in the National Health and Nutrition Examination Survey (NHANES) 2003–2004 (Calafat et al., 2007). There was limited information available for perfluoroalkyls inhalation exposure and TK (or elimination half-lives; most available information was in rodents and are from oral exposures, *e.g.*, see tables 1-1, and 3-5 for examples). In rats, PFOA was detectable in plasma within 30 minutes of initiating nose-only exposure to aerosols, increasing in plasma concentration through the 6-hour exposure duration, and peaked at 9 hours in male rats, and 7-hours in female rats. As an insoluble solid substance, systemic absorption of the test article may be limited, and accumulation is likely to occur in the respiratory

tract; the preliminary study will inform the extent to which the substances reach tissues other than those at the portal of entry.

10. Consistent with inhalation route of exposure, and the EPA associated data evaluation report (DER) TXR 0056507 for study MRID 48549211: Hotchkiss et al. (2011), detection of pulmonary, upper and lower respiratory tract and nasal inflammation and/or hyperplastic changes in other organs/tissues may also be required.

11. The mandatory BALF parameters are (OECD, 2018b):

    - lactate dehydrogenase (LDH)
    - total protein or albumin
    - total leukocyte count, absolute cell counts, and calculated differentials for alveolar macrophages, lymphocytes, neutrophils, and eosinophils.

12. Measure blood fluoride levels to determine the potential of test substance to transport to the brain as a mechanistic justification for neurotoxicity/neuropathology.

    - To fulfill previous EPA requests to measure total fluoride and fluoride cleavage, gas chromatography with electron capture detection (GC/EC) has been used in tandem with ion chromatography/negative ion electrospray ionization/mass spectrometry (IC/NESI/MS). Total fluoride content has been analyzed by micro-diffusion/ion-specific electrode detection (MD/ISE). See EPA associated data evaluation report (DER) TXR 0056507 for study MRID 48549211: Sulfuryl fluoride: Probe study to evaluate absorption and limited pharmacokinetics following a single, 6-hour, 600 ppm exposure in New Zealand white rabbits (Hotchkiss et al., 2011).

13. This preliminary study must evaluate whether radiolabeled substance is in expired air (OECD (2010b), paragraph 45) to determine if that is a concern for later testing.

14. Cage-side examinations must be recorded daily (at the same time each day) for informing neurotoxicity concerns to be explored in subsequent testing. These should exclude handling due to radiolabeling and to minimize undue stress to the animal and any disruption to study measurements, and should detect and/or focus on the following: significant clinical abnormalities, decreased/increased activity, repetitive behavior, vocalization, incoordination/limping, injury, neuromuscular function (convulsion, fasciculation, tremor, twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture),

alterations in fecal consistency, and fecal/urinary quantity. Further, all animals should be observed for morbidity, mortality, and the availability of feed and water at least twice daily. All animals should be weighed and examined prior to exposure to the test material and observed at least every 30 minutes during the exposure period.

15. Consistent with OECD inhalation test guidelines, "nose-cone" or "head-only" exposures must be used to prevent absorption by alternate routes of exposure. "Nose-only" exposure is required unless it is determined to be infeasible, in which case the study plan should indicate why nose-only inhalation is infeasible.

16. Use OECD (2002) for humane checkpoints for experimental animals.

17. Must calculate a general half-life from the results of this pre-liminary TK study.

ii. Test Reports

In addition to the requirements provided by **Unit VI** and all study plan requirements should be addressed in the final test report including all non-significant and negative results and/or deviations from the protocol. Test reports submitted to EPA for this test are due 665 days after EPA confirms with the Order recipient that *Tier 2 testing* is required based on EPA's conclusion of the review of the results from the *Tier 1 testing* and must include the following, as applicable:

1. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, non-test guidelines, and inhalation applicability, including, and not limited to methodological and reporting quality details, sources of materials, relevance, applicability and validity of the test and model systems, performance and results (Hartung et al., 2019; Samuel et al., 2016; Beronius et al., 2014; OECD, 2005).

2. Must identify any rationale from this preliminary study as a basis for dose selections for the full toxicokinetic, acute and rangefinder, and subacute studies and possible reproductive and developmental study.

3. Must identify any rationale from this preliminary study as a basis for selecting time points for collection of biofluids, organs or other tissues.

4. Must report the general half-life calculated from the results of this pre-liminary TK study.

5. Must report and use the calculated relative aerosol deposition percentages in the respiratory tract to inform subsequent toxicity testing dosimetry.

6. Harmonized Template OHT 61 (Acute toxicity: inhalation)

7. Harmonized Template URL: https://www.oecd.org/ehs/templates/OHT%2061%20-

%20ENDPOINT_STUDY_RECORD.AcuteToxicityInhalation_v10.1%20-Nov%202021.docx

c. Tier 2: Acute Inhalation Toxicity: Concentration × Time Method (OECD 403)

This testing is required unless particle size data indicates the substance is a nanomaterial (one dimension <100 nm).

EPA will evaluate whether this testing, as provided, is appropriate based on the biosolubility test showing solubility >100 mg/L in Gamble's solution or simulated epithelial lung fluid (SELF) (Boisa et al., 2014).

ii. Study Plans

Please see **Unit VI.B** of the Order for overall requirements for study plans. Additional requirements specific to OECD 403 (2009)(OECD, 2009) include:

1. Use the concentration × time protocol described on pg. 8-9 of the test guideline. Must be performed in both rats and mice.

2. The exposure particle size distribution must be characterized and consistent with the requirements in the 4th bullet of paragraph 2 of the OECD (2018e): "The 2009 version of TG 412 required particulate aerosols to have a mass median aerodynamic diameter (MMAD) of 1-3 µm with a geometric standard deviation (σ_g or GSD) of 1.5-3.0. Justification should be provided in the study report if this standard cannot be met, including a description of efforts taken to meet it, such as milling (refer to GD 39)."

3. Must use the calculated relative aerosol deposition percentages in the respiratory tract from the preliminary TK study data, to inform dosimetry.

4. Inhalation exposure must be nose-only if feasible

5. Exposure particle size distribution and density

6. Include pulmonary function testing, analysis of bronchoalveolar lavage fluid, lactate dehydrogenase release, blood oxygen content, blood fluoride levels, absolute and relative organ weights for lung, heart, and brain, histopathology/morphometry of respiratory tract (upper and lower), heart, and brain, and a satellite reversibility group. Histopathology/morphometry requirements are described in greater detail below.

7. At least 5 animals per sex, species, and exposure group.

8. Rationale for dose selections should be justified and supported by the use of a sighting study as described in paragraph 35 of the test guideline.

9. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, non-test guidelines, and inhalation applicability, including, and not limited to: all methodological

and reporting quality details (*e.g.*, general test information, method definitions, test performance and lab proficiency, results interpretations, and risk of bias considerations), sources of materials, and relevance of the model system (Hartung et al. (2019) see 'Box 1 Summary of features to report according to OECD (2014), specifically see heading 1. General information,' and summarized in Samuel et al. (2016); Beronius et al. (2014); OECD (2005).

10. Use OECD (2002) for humane checkpoints for experimental animals.

11. Measure blood fluoride levels to determine the potential of test substance to transport to the brain.

12. Must measure BAL and analyze F. The mandatory BALF parameters are (OECD, 2018b):

    a. LDH

    b. total protein or albumin

    c. total leukocyte count, absolute cell counts, and calculated differentials for alveolar macrophages, lymphocytes, neutrophils, and eosinophils.

13. Perform histopathology and morphometry of the respiratory tract and brain to evaluate portal of entry effects and potential transport and toxicity to the brain (Corps et al., 2010; OECD, 1997; Harkema et al., 1987),`, see 'Histopathology,' beginning at paragraph 40}.

    a. Histopathology/morphometry of the brain should include seven levels including examination of the hippocampus (using Rao et al. (2011), level 3).

    b. For consistent and informative pathology and morphometry observations, refer to the consensus reached by Kaufmann et al. (2009) as a model for using descriptive terms and objective details, and devising severity scoring criteria (Kaufmann et al., 2009). Severity scoring criteria will have to be submitted and reviewed by EPA prior to beginning any study activities. Informative criteria should clearly define what morphologic features the pathologist observed to categorize a given change as minimal, mild, moderate, or severe (*e.g.*, number of cell layers, approximate percent area affected, etc.). There is concern that there might be difficulty interpreting severity if only vague statements are provided for each grade, which would hinder the ability to differentiate between adverse and non-adverse lesions.

    c. Sections must be examined from every level of the entire respiratory tract to ensure that all regions are adequately evaluated by histopathology/morphometry for portal-of-entry effects. This

should include, and potentially not limited to, at least three sections from the lungs and four from the nasal cavity, and collected from each animal.

d. Must measure lung burden, if the range-finding study indicated the test substance/poorly soluble aerosol is deposited/retained in the lung. Perform Functional Observation Battery (FOB) and motor activity to inform the need for neurotoxicity testing in later inhalation toxicity testing. FOB and motor activity is favored over ECOs and standard clinical observations, since FOB has been widely used and validated across laboratories (Gauvin et al., 2016; Moser, 2011, 2000). These observations are required to inform and refine testing need for tier 3 testing for neurotoxicity, including developmental, testing.

14. FOB and motor activity in at least 5 animals, per sex, per species, and per dose group should be evaluated, consistent with OECD (1997). Minimal list for FOB include and potentially not limited to: a) any unusual bodily responses, e.g., *p*osition, activity level, movement and coordination and gait; b) any unusual behavior including but not limited to head flicking, head searching, compulsive biting or licking, self-mutilation, circling, and walking backwards; c) presence of (1) convulsions, (2) tremors, (3) increased levels of lacrimation and/or red-colored tears, (4) increased levels of salivation, (5) piloerection, (6) pupillary dilation or constriction, (7) unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing, (8) diarrhea, (9) excessive or diminished urination, and (10) vocalization; d) forelimb/hindlimb grip strength (Meyer et al., 1979); e) sensory function including reflex and pain perception (Deuel, 1977), paragraph (f) of CFR (1987).

ii. Test Reports

In addition to the requirements provided by **Unit VI** and all study plan requirements including all non-significant and negative results and/or deviations from the protocol, test reports submitted to EPA for this test are due 305 days after the EPA confirms with the Order recipient that EPA's review of the *Preliminary Toxicokinetic Study* has concluded, and must include the following, as applicable:

1. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, non-test guidelines, and inhalation applicability, including, and not limited to methodological and reporting quality details, sources of materials, and relevance of the model system (Hartung et al., 2019; Samuel et al., 2016; Beronius et al., 2014; OECD, 2014).

2. Must additionally use OECD (2005) to report the relevance, applicability and validity of the test system, performance, and results

3. Harmonized Template OHT 61 (Acute toxicity: inhalation)

4. Harmonized Template URL: https://www.oecd.org/ehs/templates/OHT%2061%20-%20ENDPOINT_STUDY_RECORD.AcuteToxicityInhalation_v10.1%20-Nov%202021.docx

d. Tier 2: Inhalation Toxicity Range finding Study (OECD TG 412 (2018) OECD (2018e) **paragraphs 14-15 and OECD TG 424 (1997)** OECD (1997)**)**

This testing is required unless particle size data indicates the substance is a nanomaterial (one dimension <100 nm).

EPA will evaluate whether this testing, as provided, is appropriate based on the biosolubility test showing solubility >100 mg/L in Gamble's solution or simulated epithelial lung fluid (SELF) (Boisa et al., 2014).

i. Study Plans

Please see **Unit VI.B** of the Order for overall requirements for study plans. Additional requirements specific to the Inhalation Toxicity Range finding Study include:

1. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, inhalation exposure and rationale for test guideline modifications including, and not limited to, all methodological and reporting quality details (*e.g.*, general test information including rationale, method definitions, test performance and lab proficiency, results interpretations, and risk of bias considerations), and sources of materials.

2. Must be performed in *either* rats or mice, as directed by EPA and based on results of prior Tier 2 testing.

3. The exposure particle size distribution must be characterized and consistent with the requirements in the 4th bullet of paragraph 2 of the OECD (2018e): "The 2009 version of TG 412 required particulate aerosols to have a mass median aerodynamic diameter (MMAD) of 1-3 µm with a geometric standard deviation (σ_g or GSD) of 1.5-3.0. Justification should be provided in the study report if this standard cannot be met, including a description of efforts taken to meet it, such as milling (refer to GD 39)."

4. Must use the calculated relative aerosol deposition percentages in the respiratory tract from the preliminary TK study data refined with acute testing data, to inform dosimetry.

5. Inhalation exposure must be nose-cone or head-only if feasible.

6. Histopathology/morphometry of the brain must include seven levels including examination of the hippocampus (using, Rao et al., 2011, level 3).

7. For consistent and informative pathology and morphometry observations, refer to the consensus reached by Kaufmann et al. (2009) as a model for using descriptive terms and objective details, and devising severity scoring criteria (Kaufmann et al., 2009). Severity scoring criteria will have to be submitted and reviewed by EPA prior to beginning any study activities. Informative criteria should clearly define what morphologic features the pathologist observed to categorize a given change as minimal, mild, moderate, or severe (*e.g.*, number of cell layers, approximate % area affected, etc.). There is concern that there might be difficulty interpreting severity if only vague statements are provided for each grade, which would hinder the ability to differentiate between adverse and non-adverse lesions.

8. Sections must be examined from every level of the entire respiratory tract to ensure that all regions are adequately evaluated by histopathology/morphometry for portal-of-entry effects. This should include, and potentially not be limited to, at least three sections from the lungs and four from the nasal cavity and collected from each animal.

9. To determine translocation potential, measure lung burden and local area lymph nodes (LALN).

10. When testing an aerosol of a solid material, an assessment of the test chemical solubility in water and post-exposure lung burden must inform a decision on the duration of the main study post-exposure period and the spacing of post-exposure observation (PEO) time/time-to-peak effect points (OECD, 2018b).

11. Perform FOB and motor activity to inform the need for potential neurotoxicity in later inhalation toxicity testing (OECD, 1997). FOB and motor activity is favored over expanded clinical observations (ECOs) and standard clinical observations, since FOB has been widely used and validated across laboratories (Gauvin et al., 2016; Moser, 2011, 2000).

12. FOB and motor activity in at least 10 animals per sex, per species, and per dose group should be evaluated, consistent with OECD (1997). Minimal list for FOB include and potentially not limited to: (a) any unusual bodily responses, e.g., *p*osition, activity level, movement and coordination and gait; (b) any unusual behavior including but not limited to head flicking, head searching, compulsive biting or licking, self-mutilation, circling, and walking backwards; (c) presence of (1) convulsions, (2) tremors, (3) increased levels of lacrimation and/or red-colored tears, (4) increased levels of salivation, (5) piloerection, (6) pupillary dilation or constriction, (7) unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing, (8) diarrhea, (9) excessive or diminished urination, and (10) vocalization; (d) forelimb/hindlimb grip strength (Meyer et al., 1979); e) sensory function including reflex and pain perception (Deuel, 1977), paragraph (f) of CFR (1987).

ii. Test Reports

In addition to the requirements provided by **Unit VI** and all study plan requirements including all non-significant and negative results and/or deviations from the protocol, test reports submitted to EPA for this test are due 665 days after the EPA confirms with the Order recipient that EPA's review of the *Acute Inhalation Toxicity Study* has concluded and must include the following, as applicable:

1. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, inhalation exposure and rationale for test guideline modifications including, and not limited to, methodological and reporting quality details, sources of materials, and relevance of the model system (Hartung et al., 2019; Samuel et al., 2016; Beronius et al., 2014; OECD, 2014).

2. Must use OECD (2017) and OECD (2016), to the extent possible, to report the test results in the context of this tiered testing track and IATA, and to inform refinement of subsequent tiered testing of this Order (OECD, 2017, 2016).

3. Harmonized Template OHT 61 (Acute toxicity: inhalation)

4. Harmonized Template URL: https://www.oecd.org/ehs/templates/OHT%2061%20-%20ENDPOINT_STUDY_RECORD.AcuteToxicityInhalation_v10.1%20-Nov%202021.docx

e. Tier 2: Subacute Inhalation Toxicity: 28-Day Study (OECD 412 (2018) OECD (2018e)**)**

This testing is required unless particle size data indicates the substance is a nanomaterial (one dimension <100 nm).

EPA will evaluate whether this testing, as provided, is appropriate based on the biosolubility test showing solubility >100 mg/L in Gamble's solution or simulated epithelial lung fluid (SELF) (Boisa et al., 2014).

i. Study Plans

Please see **Unit VI.B** of the Order for overall requirements for study plans. Additional requirements specific to OECD (2018e) include:

1. Must include the following: exposure particle size distribution, density and using the concentration × time protocol to measure pulmonary function testing, analysis of bronchoalveolar lavage fluid, lactate dehydrogenase release, blood oxygen content, blood fluoride levels, absolute and relative organ weights for lung, heart, and brain, histopathology/morphometry of respiratory tract (upper and lower), heart, and brain, and a satellite reversibility group.

2. Must be performed in *either* rats or mice, as directed by EPA and based on results of prior Tier 2 testing.

3. The exposure particle size distribution must be consistent with the requirements in the 4th bullet of paragraph 2 of the OECD (2018e): "The 2009 version of TG 412 required particulate aerosols to have a mass median aerodynamic diameter (MMAD) of 1-3 µm with a geometric standard deviation (σ_g or GSD) of 1.5-3.0. Justification should be provided in the study report if this standard cannot be met, including a description of efforts taken to meet it, such as milling (refer to GD 39)."

4. Must use the calculated relative aerosol deposition percentages in the respiratory tract from previous testing data, to inform dosimetry.

5. Dose levels and timing of FOB and motor activity measurements must be informed by the results of the preliminary TK study and acute inhalation toxicity study (OECD, 2009).

6. Rationale for dose selections must be justified and supported by the range-finding study results. The upper concentration should be tolerated by the animals without undue stress, and the lower limit should ideally be a no observed adverse effect level.

7. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, non-test guidelines, and inhalation applicability, including, and not limited to: all methodological and reporting quality details (*e.g.*, general test information, method definitions, test performance and lab proficiency, results interpretations, and risk of bias considerations), sources of materials, and relevance of the model system (Hartung et al. (2019), see 'Box 1 Summary of features to report according to OECD (2014)' and summarized in Samuel et al. (2016), Beronius et al. (2014), OECD (2005)).

8. Inhalation exposure must be nose-cone or head-only if feasible.

9. Use OECD (2002) for humane checkpoints for experimental animals.

10. Measure blood fluoride levels to determine the potential of test substance to transport to the brain, a mechanistic justification for neurotoxicity.

11. Must measure BAL and analyze BALF at various time intervals to determine time-to-peak effects. The mandatory BALF parameters are:

    a. LDH

    b. total protein or albumin

    c. total leukocyte count, absolute cell counts, and calculated differentials for alveolar macrophages, lymphocytes, neutrophils, and eosinophils.

12. Based on the range-finding study, the study plan and test report requirements may need to address the following endpoints for portal of entry effects, translocation potential and neurotoxicity:

    a. Perform histopathology/morphometry of the respiratory tract and brain to evaluate portal of entry effects and potential transport and toxicity to the brain (Corps et al., 2010; OECD, 1997, see 'Histopathology, beginning at paragraph 40; Harkema et al., 1987).

        i. Histopathology/morphometry of the brain must include seven levels including examination of the hippocampus (using Rao et al. (2011), level 3).

        ii. For consistent and informative pathology and morphometry observations, refer to the consensus reached by Kaufmann et al. (2009) as a model for using descriptive terms and objective details, and devising severity scoring criteria (Kaufmann et al., 2009). Severity scoring criteria will have to be submitted and reviewed by EPA prior to beginning any study activities. Informative criteria should clearly define what morphologic features the pathologist observed to categorize a given change as minimal, mild, moderate, or severe (*e.g.*, number of cell layers, approximate percent area affected, etc.). There is concern that there might be difficulty interpreting severity if only vague statements are provided for each grade, which would hinder the ability to differentiate between adverse and non-adverse lesions.

        iii. Sections must be examined from every level of the respiratory tract to ensure that all regions are adequately evaluated by histopathology/morphometry for portal-of-entry effects. This should include, and potentially not limited to, at least 6 sections each from the lungs and the nasopharyngeal tissue collected from each animal. Unless shown otherwise, the test substance has potential to have poorly soluble particles, resulting in deposition and/or other retention in the lungs and within the respiratory tract. The experimental plan must include lymph nodes from the hilar region of the lung. A range of post-exposure timepoints, based on the time-to-peak effects and range-finding studies, should sample nasopharyngeal and lymphoid tissues, and distal lymph nodes to account for anticipated particle deposition and retention in the lungs and respiratory tract, also immunological effects (see OECD (2018e), Table 2. Organs and tissues preserved during gross necropsy and relevant references; and OECD (2010a)).

        iv. Weigh (prior to fixation) and perform histopathology/morphometry for thyroid gland. Retain

plasma and serum samples for the possibility of effects on the hypothalamus-pituitary-thyroid axis. Plasma and serum samples can be affected by time of sampling (diurnal variation), method of sacrifice (undue stress), and/or differences among hormone testing kits by standard curves (OECD, 2008).

13. Must measure lung burden, if the range-finding study indicated the test substance/poorly soluble aerosol is deposited/retained in the lung.

14. Perform FOB and motor activity to inform potential for neurotoxicity. FOB and motor activity is favored over ECOs since FOB has been widely used and validated across laboratories (Gauvin et al., 2016; Moser, 2011, 2000). These observations are required to inform and refine testing need for tier 3 testing for neurotoxicity, including developmental, testing.

    FOB and motor activity in at least 10 animals per sex, per species, and per dose group should be evaluated, consistent with OECD (1997). Minimal list for FOB include and potentially not limited to: a) any unusual bodily responses, e.g., *p*osition, activity level, movement and coordination and gait; b) any unusual behavior including but not limited to head flicking, head searching, compulsive biting or licking, self-mutilation, circling, and walking backwards; c) presence of (1) convulsions, (2) tremors, (3) increased levels of lacrimation and/or red-colored tears, (4) increased levels of salivation, (5) piloerection, (6) pupillary dilation or constriction, (7) unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing, (8) diarrhea, (9) excessive or diminished urination, and (10) vocalization; d) forelimb/hindlimb grip strength (Meyer et al., 1979); e) sensory function including reflex and pain perception (Deuel, 1977), paragraph (f) of CFR (1987)).

ii. Test Reports

In addition to the requirements provided by **Unit VI**, test reports submitted to EPA for this test are due 665 days after the EPA confirms with the Order recipient that EPA's review of the *Inhalation Toxicity Range Finding Study* has concluded and must include the following, as applicable:

1. The study plan requirements should be reflected in the final test report including all non-significant and negative results.

2. Mass median aerodynamic diameter (MMAD) which should be ≤2 µm with a σg of 1-3 in line with (OECD, 2018b).

3. Must include all relevant information required to assess the quality and applicability of the data for the *in vivo* scenario, inhalation exposure, and test guideline modifications, including, and not limited to: methodological and reporting quality details, sources of materials, and relevance of the

model system (Hartung et al., 2019; Samuel et al., 2016; Beronius et al., 2014; OECD, 2014).

4. Must use OECD (2017) and OECD (2016), to the extent possible, to report the test results in the context of this tiered testing track and IATA, and to inform refinement of subsequent tiered testing of this Order (OECD, 2017, 2016).

5. Harmonized Template OHT 68 (Repeated dose toxicity: inhalation) Harmonized Template URL: https://www.oecd.org/env/ehs/testing/OHT%2068%20-%20ENDPOINT_STUDY_RECORD.RepeatedDoseToxicityInhalation_v6.3%20-Dec%202018.doc

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