PFAS TSCA Workshop: Testing Overview

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Overview

Category Formation
- Starts with PFAS Universe (DSSTox/CompTox Dashboard)
- Analysis of structural features
- Ends with categories

Candidate Selection
- Incorporates data availability, TSCA Inventory status, exposure potential
- Ends with candidates

Testing Track
- Incorporates predicted exposure pathways based on chemical characteristics (e.g., physical state)
- Ends with testing requirements

Tier 1* Tests
- Non-vertebrate methods + screening
- Informs hazard identification, Mode of Action, category cohesiveness (read across), can affect Tiers 2-3 testing requirements

Tier 2* Tests
- Vertebrate methods (low animal use) including toxicokinetics
- Informs hazard identification, category cohesiveness (read across), refines testing needs (e.g., species selection), supports route-to-route extrapolation

Tier 3* Tests
- Vertebrate methods (high animal use)
- Data driven—not all chemicals will require Tier 3 testing
- Dose-response assessment, chronic effects, PESS†, carcinogenicity

* Tiers are flexible; this is the general approach but each Order is fit-for-purpose

† PESS: Potentially Exposed and Susceptible Subpopulations

Test Tracking

- Four PFAS testing tracks
  - A
    - Insoluble solid substances
  - B
    - Soluble solid substances
    - Soluble, low volatility liquid substances
  - C
    - Insoluble liquids (regardless of volatility)
    - Volatile liquids (regardless of solubility)
    - Highly soluble gases with low Henry's Law Constants
  - D
    - Insoluble gases
    - Soluble gases with high Henry's Law Constants

Exposure Routes

- Inhalation is of concern for all chemicals
- Oral is of concern for all chemicals *except* group D
- Dermal is of concern for groups A and B, and sometimes C
- Ocular is of concern for groups B and C
- Generally, except for gases, repeated dose toxicity testing will be via the oral route, with TK and skin absorption testing being used as a basis for route-to-route extrapolation

Testing Practicalities

- Insoluble and volatile substances are difficult to test *in vitro*
- Many standardized test guidelines are not applicable to gases
- Insoluble solids may have limited absorption

Tiered Testing

- Tier I consists of physical-chemical properties and *in vitro* testing to inform and guide whether additional short-term *in vivo* toxicity and/or toxicokinetic tests should be considered
- Tier II consists of testing to inform which species and doses to use in Tier III testing
- Tier III consists of testing to identify dose levels (i.e., NOAELs)

Physical/Chemical Properties

- Estimation procedures
  - We use OPERA version 2.9 for estimating p-chem properties
  - OPERA is considered one of the best available tools for predicting p-chem properties for PFAS (doi: 10.1002/etc.4681)
- Available data
  - We search Reaxys (Beilstein), our internal CBI databases, and the ECHA Registered Substances Database for available data
- Uncertainties
  - PFAS are difficult to test
  - Testing requirements are strongly coupled to p-chem properties so incorrect estimates can cause delays

In Vitro Approaches

- *In Vitro* methods are generally employed in Tier 1
- Gases (group #4) use more extensive *in vitro* testing in support of human-relevant methods
- Skin and eye effects will employ *in vitro* methods as they are well-accepted for these endpoints
- Genotoxicity/mutagenicity will employ *in vitro* methods in a battery
- *In vivo* data for skin, eye, and genotox endpoints will be considered if the data already exist and are considered of acceptable quality

Hazard Screening

- Portal of entry effects
  - Irritation/corrosion (eyes, skin, lung)
  - Lung overload
  - Disruption of lung surfactant
- Systemic effects
  - Liver toxicity
  - Neurotoxicity
  - Reproductive/Developmental toxicity

Hazard Screening, cont.

- Factors considered:
  - Structural features
  - *In silico* predictive tools
  - Available data
  - Test results (as they come in)
- EPA is required to consider all available toxicity data per TSCA §4(h)
- Available data will be posted to the docket
- All existing *in vivo* toxicity studies will be evaluated in accordance with the TSCA Systematic Review data quality evaluation metrics

Hazard Screening, cont.

- *In silico* predictive tools
  - OPERA (physical-chemical properties)
  - OncoLogic (carcinogenicity)
  - OECD QSAR Toolbox skin and respiratory sensitization profilers (sensitization)
  - Others?

Bioaccumulation

- PFAS chemicals are of high concern not only due to their intrinsic toxicity, but also because their long biological half-lives mean that very small exposures can lead to high body burdens over time
- However—many PFAS covered by the National Testing Strategy are likely to be reactive (e.g., HFPO and HFPO-DAF)
- Hydrolysis data, *in vitro* testing, and toxicokinetics studies will provide key information to further refine categories of PFAS based on bioaccumulation concerns

Toxicokinetics

- Toxicokinetic data are *critical*
- Toxicokinetics (TK) and Absorption, Distribution, Metabolism, and Elimination (ADME) are related concepts
  - ADME can be measured individually *in vitro* and/or *in vivo*
  - TK is generally an integrated picture that includes ADME but may not measure ADME as individual pieces
- Category Definition: The use of the # of carbon atoms/chain length metric to define terminal categories is based in part on the fact that shorter chains are eliminated more rapidly than longer chains and therefore are generally "less toxic"
- Testing Refinement: Identify species/sex sensitivities

Toxicokinetics, cont.

- Tracking: The tracks treat solids, liquids, and gases differently and this is partly due to their routes of elimination
  - Insoluble solids are difficult to eliminate from the lung following inhalation exposure because they don't dissolve in lung fluid
  - Insoluble solids are not well absorbed via the oral route and tend to be eliminated unchanged in feces
  - Gases and volatile liquids have an extra route of elimination through exhaled air; not solely dependent on urinary elimination
- Hazard Identification: While slow elimination does not in-and-of itself constitute a "hazard" it tends to increase the overall hazard (in a broader sense) of a chemical because even small exposures to the chemical can build up over time to toxic internal levels
- Dose-Response Assessment: Because TK studies do not measure health outcomes, you can't get dose-response data from them, but they can be used later on in dose-response assessment

Higher Tier Testing

- For most chemicals moving forward, the higher tier testing will consist of:
  - OECD 417 Toxicokinetics (Oral)
  - OECD 417 Toxicokinetics (Inhalation)
  - OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.
- Other higher tier tests may be required depending on available data (for example, HFPO test order)

Decision Points

- Decision points include on-ramps, off-ramps, and method refinements
- Examples:
  - On-Ramp: If surface tension test shows a reduction in surface tension to ≤45 mN/m at conc. 0.5% wt% in water at 20 °C, then a critical micelle concentration (CMC) test must be run
  - Off-Ramp: If biosolubility testing gives solubility >100 mg/L, then short-term repeated dose inhalation study not required
  - Refinements: Use TK testing in rats and mice to guide selection of species for repeated-dose studies (cuts animal use in half)

Ref: HERO IDs 10284414 and 11311210

Study Design/Conduct

- Generally an OECD test guideline will be specified
- Additional requirements are included in Appendix E of the orders
  - Example: Increase sample size; measure chemical concentration in serum
- Company/consortium sends draft study plans to EPA for review before initiating study
- Study reports will be evaluated for acceptability using the TSCA Systematic Review data quality evaluation metrics

Future Directions

- Test order templates
- Improved instruction to companies regarding study design/test guideline requirements
- Analysis of species sensitivity
- Enhanced use of *in silico* and *in vitro* methods
- Development of predictive models

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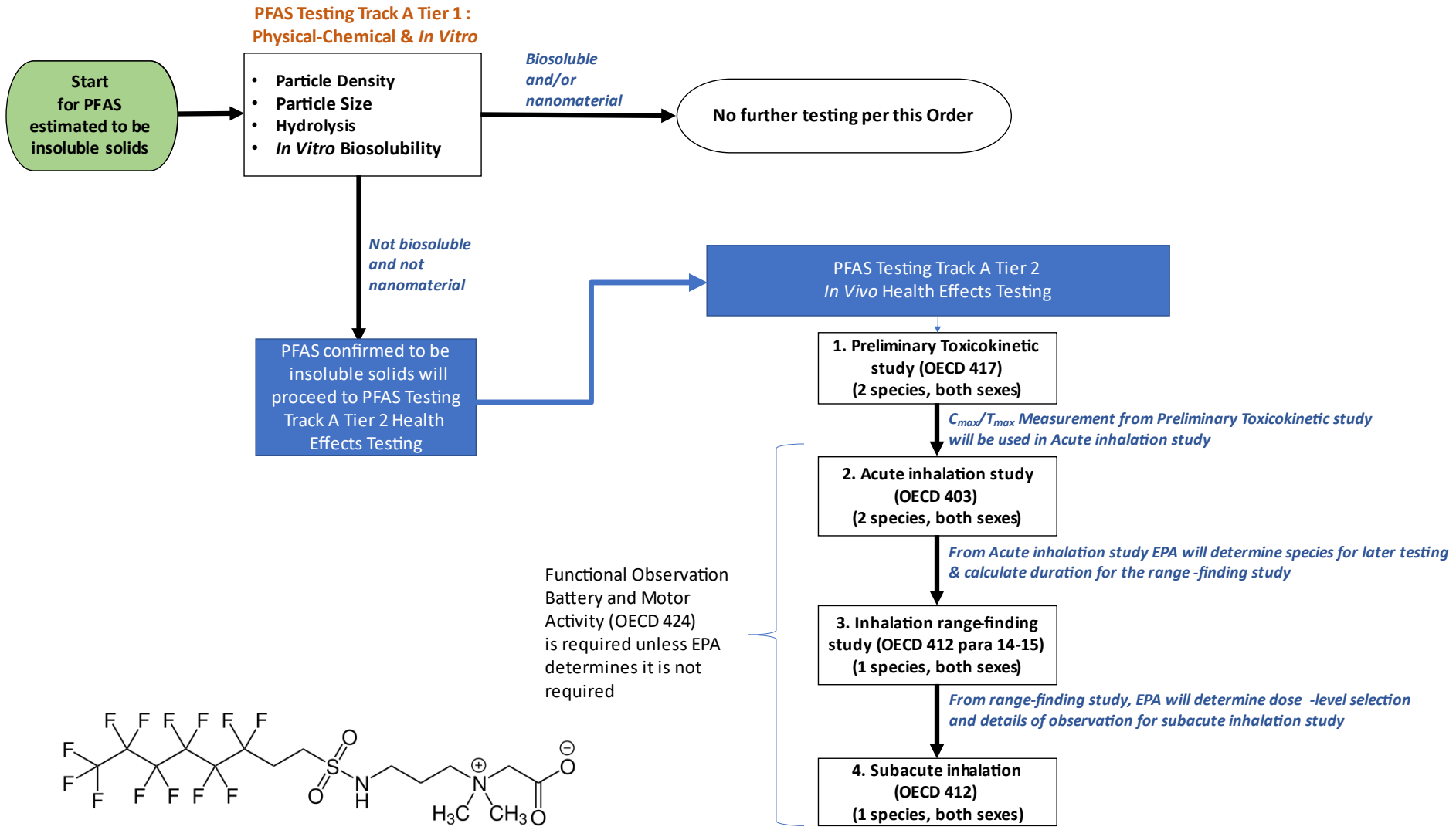
Pocket slides

PFAS	Uses	Issuance Date	Companies	Testing
6:2 FTSB	- surface-active agent - fire-fighting foam agent	6-Jun-22	Chemours, Dupont de Nemours Inc, E.I. du Pont de Nemours and Company Tyco Fire Products LP, National Foam Inc	Tier 1: particle density OECD 109, Aerodynamic Particle Size Distribution, Hydrolysis as a function of pH OECD 111, Biosolubility Tier 2: Prelim TK for ADME OECD 417, Acute inhalation OECD 403, Inhalation range-finding OECD 412, Subacute inhalation 28-day OECD 412
HFPO	- reactant for plastics material and resin manufacturing - in other basic organic chemical manufacturing	4-Jan-23	3M, Chemours, Dupont de Nemours Inc, E.I. du Pont de Nemours and Company	Tier 1: Hydrolysis as a function of pH OECD 111, In vitro Respiratory Tract Epithelial Toxicity, Partition coefficient and ADME inhalation; Tier 2: Two-gen OECD 416, Dev/neurotox OECD 426, Subchronic neurotox OECD 424, Combined chronic/carcinogenicity OECD 453
HFPO-DAF	- reactant in other basic chemical manufacturing	14-Aug-23	3M, Chemours, E.I. du Pont de Nemours and Company	Tier 1.1: Melting Point/Melting Range OECD 102, Boiling Point OECD 103, Vapor Pressure OECD 104, Water Solubility OECD 105, Determination of pH, Acidity and Alkalinity OECD 122, Hydrolysis as a Function of pH OECD 111. Tier 1.2: specific protocol may depend on results of the Tier 1.1. Vapor Pressure test In Vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method OECD 431, In Vitro Membrane Barrier Test Method for Skin Corrosion OECD 435. Tier 1.3: As required based on results of Tier 1.1. Hydrolysis as a Function of pH and Tier 1.2 Skin Corrosion tests Skin Absorption: In Vitro Method OECD 428, Defined Approaches on Skin Sensitization OECD 497, In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method OECD 439



Category: unclassified, greater than or equal to 8 carbon atoms

Physical-Chemical & *In Vitro*

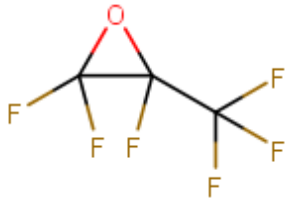
Start for PFAS estimated to be insoluble and/or highly volatile gases

- Water Solubility (*WS*)
- Boiling Point
- Hydrolysis
- Vapor Pressure
- Henry's Law Constant (*HLC*)
- *In Vitro* Epithelial Cell Assay

Not a gas
OR
WS ≥ 100 mg/L
AND
HLC < 0.1 atm·m³/mol

No further testing per this Order

WS < 100 mg/L
OR
HLC ≥ 0.1 atm·m³/mol



Category: others, cyclic, volatile, sub-cluster 1

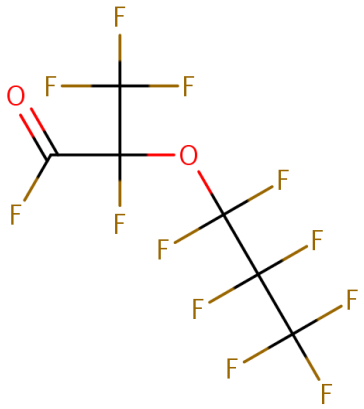
1. **Partition Coefficient and ADME Inhalation Study (2 species, both sexes)**

Review Tier 2 Results
Select most sensitive species (rats or mice)

2. **2-Generation Reproduction Toxicity (OECD 416) (1 species, both sexes)**
3. **Developmental Neurotoxicity Study (OECD 426) (1 species, both sexes)**
4. **Subchronic Neurotoxicity Study in Rodents (OECD 424) (1 species, both sexes)**
5. **Cancer Inhalation Bioassay (OECD 453) (1 species, both sexes)**

Note: Higher tier testing on HFPO was triggered based on available data, including OECD 403 and 422 studies via the inhalation route

HFPO-DAF



Category: “Others, lt8, sub-cluster 1, sub-sub-cluster 2

Tiering of Tests for HFPO-DAF

Tier 1.1

- Bovine Corneal Opacity Test (OECD 437)
- Hydrolysis as a function of pH (OECD 111)
- Vapor pressure (OECD 104)
- Melting point/melting range (OECD 102)
- Water solubility (OECD 105)
- pH, acidity and alkalinity determination (OECD 122)
- Boiling point (OECD 103)
- Corrosive to eyes? — Yes → Stop
- Neutral hydrolysis half-life ($t_{1/2}$) — <3 hrs → Stop
- If VP ≥75 mmHg, use closed system

Tier 1.2

- RHCE for eye hazard ID (OECD 492B) — ≥3 hrs AND Not corrosive to eyes
- One genotoxicity test:
  - *In vitro* mammalian chromosomal aberration test (OECD 473)
  - *In vitro* mammalian cell mutation test with thymidine kinase gene (OECD 490)

  — ≥3 hrs AND <48 hrs
- Bacterial reverse mutation test (OECD 471)
- And one more genotoxicity test:
  - *In vitro* mammalian chromosomal aberration test (OECD 473)
  - *In vitro* mammalian cell mutation test with thymidine kinase gene (OECD 490)
  - *In vitro* mammalian cell micronucleus test (OECD 487)

  — ≥48 hrs
- *In vitro* skin corrosion: RhE (OECD 431)
- *In vitro* membrane barrier for skin corrosion (OECD 435)
- Yes
- Corrosive to skin? — Stop
- No
- ≥3 hrs AND Not corrosive to skin

Tier 1.3

- *In vitro* skin absorption (OECD 428)
- Skin sensitization defined approaches (OECD 497)
- *In vitro* skin irritation: RhE (OECD 439)

Key

Checkpoint; Decision; Stop; Tier 1.1 Test; Tier 1.2 Test; Tier 1.3 Test