Challenges with testing volatile PFAS by *in vitro* and *in vivo* inhalation studies

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Test orders to fill data gaps for inhalation exposure

First two test orders:

June 16, 2022

- 6:2 fluorotelomer sulfonamide betaine

January 4, 2023

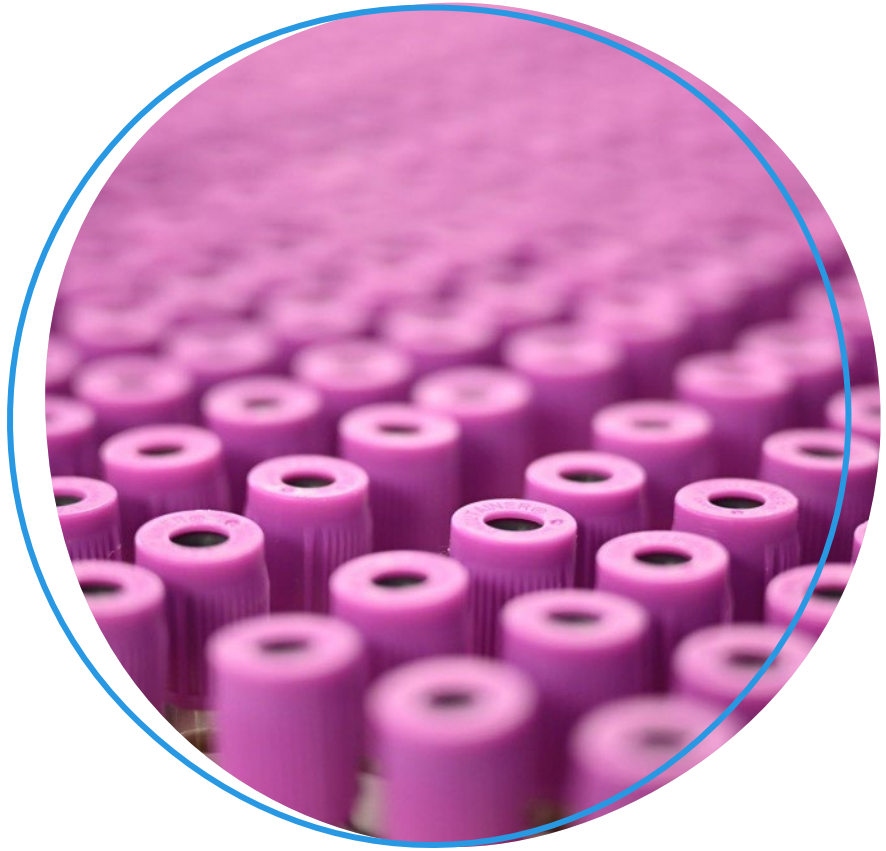
- Trifluoro(trifluoromethyl)oxirane (hexafluoropropylene oxide; HFPO)

Tier 1

- Physical-chemical properties
- *In vitro* testing (biosolubility, primary cell culture)
- *In vivo* testing (ADME)

Tier 2

- *In vivo* testing (ADME, acute, subacute, reproductive, neurotoxicity and carcinogenicity)



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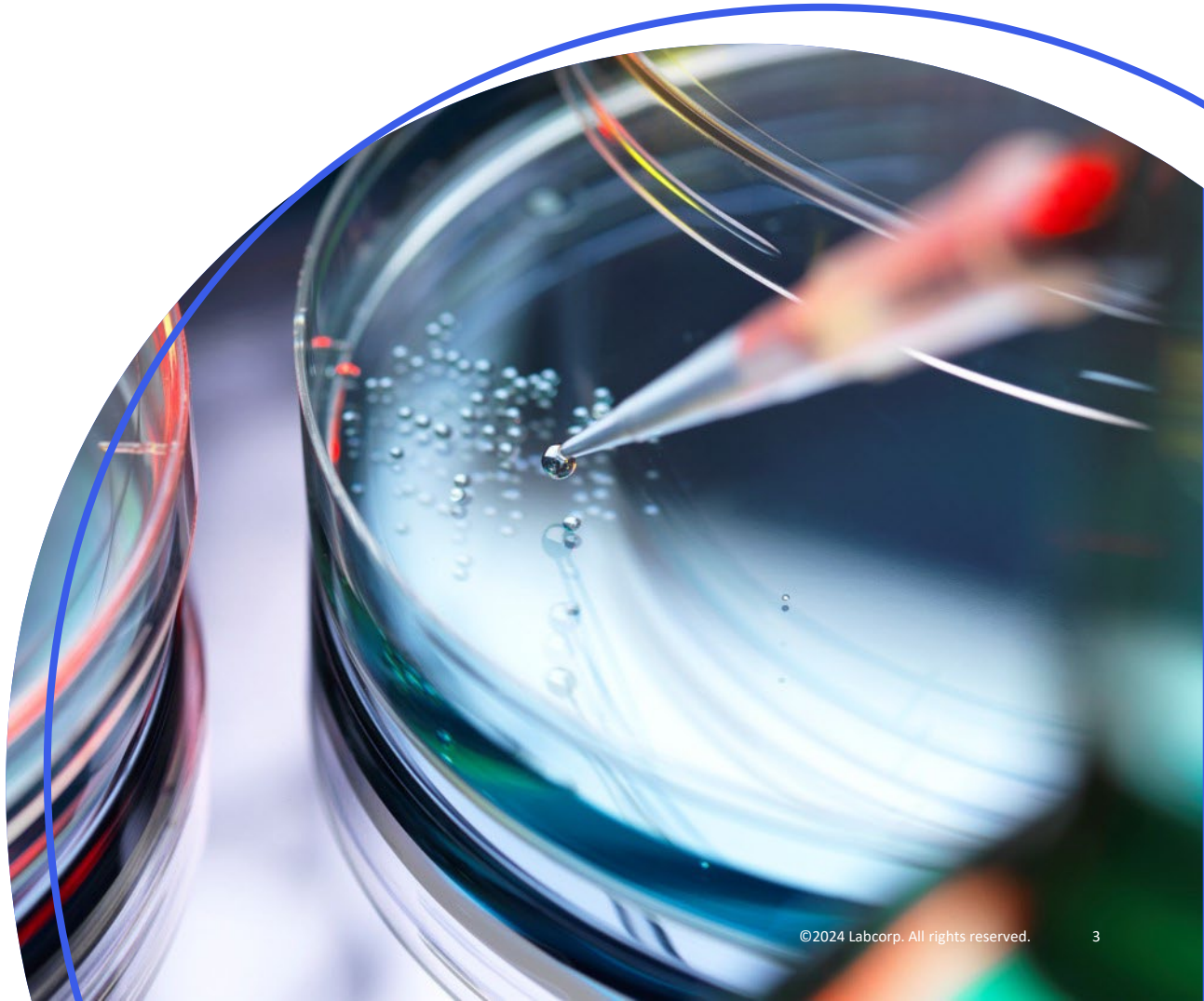
In vitro and primary cell culture testing

Advancing utilization of New Approach Methodologies (NAMs)

- **Is the particle soluble in biologically relevant fluid?**
  - Gamble's solution, simulated epithelial lung lining fluid
- **Is a local exposure response elicited?**
  - Primary human respiratory tract epithelial cell culture
  - Mechanistic and future potential *in vitro* alternative



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Challenges with *in vitro* respiratory tract epithelial toxicity in primary human cell culture

Advantages
- Provides data for modeling and extrapolation to humans

Disadvantages
- Commercial availability is limited
  - Upper airway versus lower airway
- Availability of individual donors versus pooled
- Feasibility of repeated exposure
- Cost

Alternatives
- Pooled human donors
- Immortalized cell cultures, animal-derived primary cells (lung slices)

Acute
- Single exposure (4hr)
- 6 test concentrations
- 2 controls [mock-treatment (air-only) control and incubator control]

Short-term
- Repeated exposures (6hrs/day for 14 days)
- 2 test concentrations
- 2 controls [mock-treatment (air-only) control and incubator control]

Measurements
- Barrier integrity (TEER)
- H&E
- Cell viability (WST-8 viability assay and LDH release)
- Pro-inflammation (cytokines/chemokine levels)
- Morphology (light microscope observations)
- IHC for p63, MUC5AC, and FOXJ1 and of expected cell types to evaluate treatment-related de-differentiation/airway remodeling

Note: TEER, LDH, release, cytokine levels and light microscopy observations are non-destructive and must be performed on all wells.

Considerations for *in vivo* study conduct

Overall test order notes

- Previous rat inhalation via whole body exposure[1]
- ADME required in mouse and rat
  - May reveal species specific differences in metabolism
  - Mouse exposure limited by duration and smaller RMV
- OECD 416 generally conducted in rat
  - Mouse would require 2nd control group for lack of historical control data, and may not have all parameters available for evaluation
- OECD 426 and OECD 424 are done in rat
- OECD 453 can be done in either species

1 https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/5721/7/6/3

2. Health Effects: Inhalation Route

Tier 1

a. *In vitro* Respiratory Tract Epithelial Toxicity in Primary Human Cell Culture (**Appendix E**)

b. Partition Coefficient and ADME Inhalation Study (**Gargas, et al. (1986)**)

Tier 2

c. Two-Generation Reproduction Toxicity (**OECD 416 (2001)**)

d. Developmental Neurotoxicity Study (**OECD 426 (2007)**)

e. Subchronic Neurotoxicity Study in Rodents (**OECD 424 (1997)**)

f. Combined Chronic Toxicity/Carcinogenicity Studies (**OECD 453 (2018)**)

Considerations for *in vivo* study conduct

- **Understanding the objective of the customized study design**
  - Metabolism + acute toxicity
- **Implementing 3Rs while achieving study objectives**
  - Tissue sampling and lavage are terminal procedures
  - Pulmonary function baseline testing requires restraint
- **Logistical scheduling of evaluations**
  - Urine collection in metabolism caging
  - Clinical observations that capture potential neurobehavioral effects
- **Analytical measurement of atmosphere and biological samples (and interpretation of results)**
  - Evaporation, metabolism and hydrolysis

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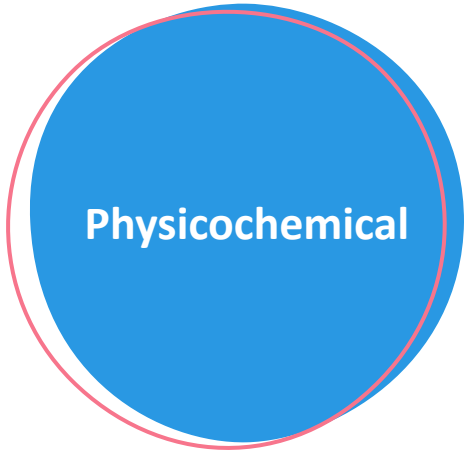
Summary



Is it respirable? Biosoluble? Volatile?

How is it metabolized?

Relevancy and availability of cell type

Robustness balanced with feasibility

Scientific limitations for analyses

Prioritize test parameters

Thank you