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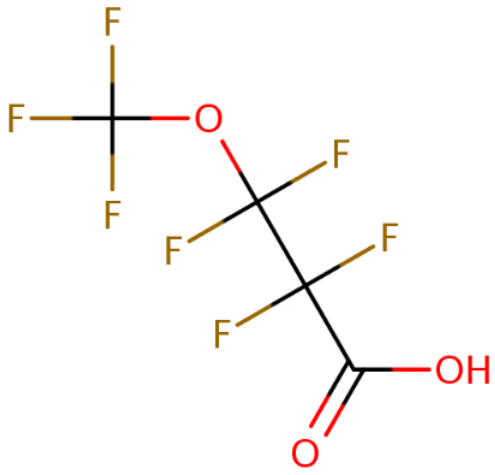
EPA Transcriptomic Assessment Product (ETAP) for Perfluoro-3-Methoxypropanoic Acid



March 2024

Center for Computational Toxicology and Exposure (CCTE) &
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Office of Research and Development
U.S. Environmental Protection Agency

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ABBREVIATIONS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ADQ	Audit of Data Quality
ANOVA	Analysis of Variance
BCTD	Biomolecular and Computational Toxicology Division
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower Bound
BW	Body Weight
CASRN	Chemical Abstracts Service Registry Number
CCCB	Computational Chemistry and Cheminformatics Branch
CCED	Chemical Characterization & Exposure Division
CCTE	Center for Computational Toxicology and Exposure
CPAD	Chemical & Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CPM	Counts Per Million
CTBB	Computational Toxicology and Bioinformatics Branch
DAF	Dosimetric Adjustment Factor
DNA	Deoxyribonucleic acid
DTT	Division of Translational Toxicology (formerly Division of the National Toxicology Program)
ECHA	European Chemicals Agency
EPA	U.S. Environmental Protection Agency
ETAP	EPA Transcriptomic Assessment Product
ETTB	Experimental Toxicokinetics and Toxicodynamics Branch
FDR	False Discovery Rate
GEO	Gene Expression Omnibus
GO	Gene Ontology
HED	Human Equivalent Dose
HERO	Health and Environmental Research Online
IDA	Information Dependent Acquisition
IUPAC	International Union of Pure and Applied Chemistry
LC/MS	Liquid Chromatograph Mass Spectrometry
LOAEL	Lowest Observed Adverse Effect Level
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NOAEL	No Observed Adverse Effect Level
OECD	Organization for Economic Cooperation and Development
ORD	Office of Research and Development
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
POD	Point of Departure
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QTOF	Quadrupole Time-of-Flight
RNA	Ribonucleic acid

SIDS	Screening Information Dataset
SMILES	Simplified Molecular-Input Line-Entry System
SPRI	Solid-Phase Reversible Immobilization
TEAB	Toxic Effects Assessment Branch
ToxValDB	US EPA Chemicals Dashboard ToxVal database
TRV	Transcriptomic-based Reference Value
UF	Uncertainty Factors
WOS	Web of Science

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1. BACKGROUND

EPA Transcriptomic Assessment Products (ETAPs) are developed by the Office of Research and Development (ORD), United States Environmental Protection Agency (EPA) to provide transcriptomic-based reference values (TRV). To the extent possible based on the currently available evidence, the objective of this human health assessment is to provide a TRV with the level of confidence and caveats outlined in the *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (EPA 2024). The TRV is defined as an estimate of a daily oral dose to the human population that is likely to be without appreciable risk of adverse non-cancer health effects over a lifetime. The TRV is derived from a transcriptomic point-of-departure (POD) with uncertainty factors applied to reflect limitations of the data used. The transcriptomic POD is defined as the dose at which there were no coordinated transcriptional changes that would indicate a potential toxicity of concern. The coordinated transcriptional changes used to identify the POD do not necessarily discriminate between specific hazards, adverse or adaptive effects, nor are they used to infer a mechanism or mode of action. While a TRV is expressly presented as a chronic value in an ETAP, it may also be applicable across other exposure durations of interest including short-term and subchronic. This approach has been previously used by EPA in certain risk assessment applications (e.g., Provisional Peer-Reviewed Toxicity Value [PPRTV] assessments) wherein a chronic non-cancer reference value has been adopted as a conservative estimate for a subchronic non-cancer reference value when data quality and/or lack of duration-relevant hazard and dose response data preclude direct derivation.

The ETAP is intended to be applied to substances with no existing or publicly accessible repeated dose toxicity studies or human evidence suitable for use as a POD and reference value derivation. The assessment is not intended to represent a comprehensive treatise on the chemical. The ETAP is not a risk assessment because it does not include an exposure assessment nor an overall risk characterization. Further, the human health assessment does not address the legal, political, social, economic, or technical considerations involved in risk management. The ETAP can be used by EPA, states, tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate statutes, if, and when, it is necessary to take action to address potential risk associated with human exposures to the ETAP chemical. ETAP assessments may be updated to incorporate new data or methodologies that might impact the reference values, or, retired if traditional toxicity studies and an associated human health assessment are published. The general methods associated with conducting the systematic literature survey and animal study are provided in *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (EPA 2024).

2. ASSESSMENT REVIEW

The methods for developing the ETAP outlined in this document have been internally reviewed by Office of Research and Development (ORD) scientists and management. The methods have been externally peer reviewed by the EPA Board of Scientific Counselors and subject to public comment (EPA 2024).

All activities and testing in this ETAP are covered under a standard EPA Category A Quality Assurance Project Plan (QAPP). The ETAP has undergone an Audit of Data Quality (ADQ) by an EPA Quality Assurance (QA) team and review by at least two ORD technical experts. This ETAP has followed the methods outlined in the *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (EPA 2024). Due to the extensive review of the standardized methods and to facilitate the rapid development, execution, and release, this ETAP did not receive independent peer review. The EPA BOSC has endorsed not adding external peer review for individual ETAPs that are the product of a peer reviewed and approved standardized process without assessment or judgments.

3. CHEMICAL IDENTITY AND PHYSICAL PROPERTIES

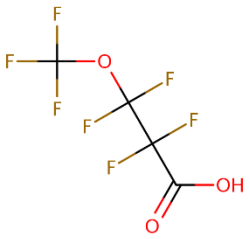
Table 3-1. Chemical identity and physical-chemical properties of perfluoro-3-methoxypropanoic acid	
Property	**Value**
Chemical structure	
DTXSID	DTXSID70191136
CASRN	377-73-1
IUPAC name	2,2,3,3-Tetrafluoro-3-(trifluoromethoxy)propanoic acid
Synonyms	Perfluoro-3-methoxypropanoic acid 2,2,3,3-Tetrafluoro-3-(trifluoromethoxy)propanoic acid Perfluoro-4-oxapentanoic acid Propanoic acid, 2,2,3,3-tetrafluoro-3-(trifluoromethoxy)- Perfluoromethoxypropionic acid BRN 1795024 PERFLUORO PFMPA PFMOPrA PFMPA PF4OPeA PF-4O-PeA PFPE-2
Color/Form	Liquid
Molecular formula	C4HF7O3
SMILES	OC(=O)C(F)(F)C(F)(F)OC(F)(F)F
Molecular weight (g/mol)	230.038
Density (g/cm^3 at 20°C)	1.65[a]
Boiling point (°C) (@ 0.01 mm Hg)	129[a]
Melting point (°C)	4.29[a]
LogP: octanol-water	3.15[a]

Table 3-1. Chemical identity and physical-chemical properties of perfluoro-3-methoxypropanoic acid	
Property	**Value**
Henry's law constant ($atmm^3$/mole at 25°C)	3.03e-10[a]
Water solubility (mg/L)	4.78e-2[a]
Vapor pressure (mm Hg)	3.51[a]

[a] Predicted from EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/chemical/details/DTXSID70191136). Average values presented where available.

4. LITERATURE SURVEY

4.1. DATABASE SEARCH

The databases listed below were searched on December 19, 2022, by an EPA information specialist and the results stored in the Health and Environmental Research Online (HERO) database[1]. The literature search focused only on the chemical name (and synonyms) with no language or date limitations. Full details of the search strategy for each database are presented in Appendix I.

- PubMed (National Library of Medicine)
- Web of Science (Clarivate)
- ProQuest (Clarivate)

Other searches were performed in European Chemicals Agency (ECHA) registration dossiers, EPA ChemView, National Toxicology Program (NTP) database, Organization for Economic Cooperation and Development (OECD) Screening Information Dataset (SIDS) database, and EPA ECOTOX database.

4.2. SEARCH RESULTS

No adequate studies were located regarding toxicity of perfluoro-3-methoxypropanoic acid to humans or animals via oral exposure. No human health relevant studies were identified from searches of journal databases (Appendix I). Similarly, no records were identified from searches of ECHA registration dossiers, EPA ChemView, OECD SIDS database, EPA ECOTOX database, or NTP database of finalized reports or in progress studies.

[1] EPA's HERO database is available at: https://hero.epa.gov/hero/

5. ANIMAL STUDY

The 5-day in vivo transcriptomic study used in this ETAP was performed consistent with the standard methods outlined in the EPA report *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (EPA 2024). Animal study parameters are outlined in the Table 5- 1.

5.1. STUDY PARAMETERS, GROSS OBSERVATIONS, AND SURVIVAL

Table 5-1. Summary of animal study parameters for perfluoro-3-methoxy propanoic acid

Parameter	Value
Species	Rat
Strain	Sprague Dawley
Sex	Males and females
Age	10 – 12 weeks post acclimation
Sample Size	n = 8 vehicle control; n = 5 treatment group
Route of Exposure	Oral gavage
Vehicle	Deionized water
Doses	0, 0.01, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0, 300.0 mg/kg-day
Dosing Frequency	Once per day
Dosing Duration	5 days
Sacrifice Time After Last Dose	24 h
Organs Evaluated	Adrenal gland, brain, heart, kidneys, liver, lung, ovary, spleen, testis, thyroid, thymus, and uterus

One male in the 30 mg/kg-day treatment group did not survive to termination (Table 5-2). One female rat in the 30 mg/kg-day and one female rat in the 300 mg/kg-day treatment group did not survive to termination. One female rat in the 300 mg/kg-day treatment group exhibited abnormal breathing and rales. A second female rat in the 300 mg/kg-day treatment group exhibited rales. All gross observations were noted on Days 5 and 6 on study. Detailed results from the animal study are presented in Appendix II.

Table 5-2. Survival of animals across doses for male and female rats treated with perfluoro-3-methoxypropanoic acid	
Sex	**Treatment Doses in mg/kg-day (Number of Animals Surviving Through Termination)**
Males	0 (8), 0.01 (5), 0.1 (5), 0.3 (5), 1.0 (5), 3.0 (5), 10.0 (5), 30.0 (4), 100.0 (5), 300.0 (5)
Females	0 (8), 0.01 (5), 0.1 (5), 0.3 (5), 1.0 (5), 3.0 (5), 10.0 (5), 30.0 (4), 100.0 (5), 300.0 (4)

5.2. TRANSCRIPTIONAL CHANGES

Pre-modeling dataset evaluation was performed to determine where there was adequate signal. All tissues passed the analysis of variance (ANOVA) cut-off of at least 1 gene with false discovery rate (FDR) corrected p-value < 0.05 for benchmark dose (BMD) modeling. Based on the pre-modeling probe filtering, the number of differentially expressed probes from male and female rats varied across sex and tissues (Table 5-3).

Table 5-3. Number of differentially expressed probes following exposure to perfluoro-3-methoxypropanoic acid*

Tissue	**Male**	**Female**
Adrenal Gland	170	327
Brain	103	110
Heart	252	296
Kidney	540	153
Liver	347	158
Lung	340	86
Ovary	NA	168
Spleen	163	89
Testis	120	NA
Thymus	121	419
Thyroid	277	166
Uterus	NA	183

*Based on Williams Trend test p-value < 0.05 and |Fold-Change| > 1.5. NA, not available; PMC, did not pass pre-modeling cut-off using one-way ANOVA with FDR corrected p-value < 0.05.

The female uterus had the Gene Ontology (GO) biological process class with the lowest median BMD value across tissues and in both sexes (Fig. 5-1; Table 5-4). The GO biological process class was negative regulation of cell motility (GO:2000146) with a median BMD value of 0.872 mg/kg-day and an associated median BMDL value of 0.121 mg/kg-day (Table 5-4).

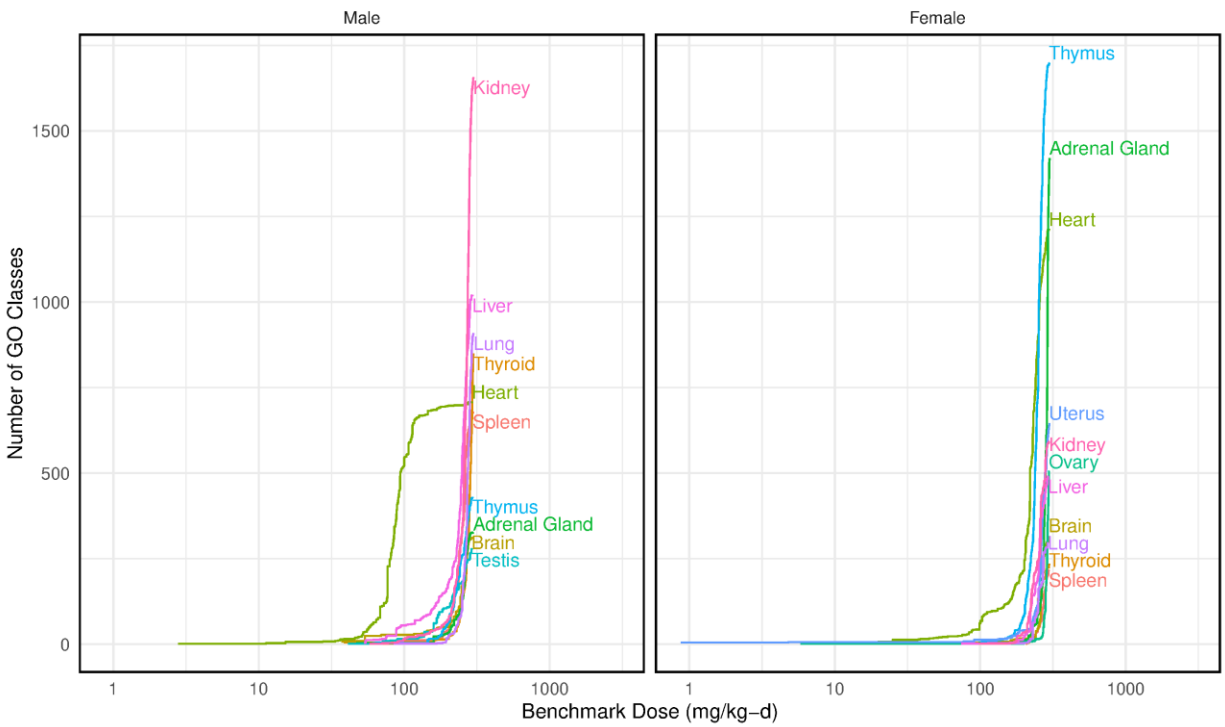


Figure 5-1. Accumulation plots of GO biological process classes by median benchmark dose value for each tissue in male (left) and female (right) rats exposed to perfluoro-3-methoxypropanoic acid.

Table 5-4. Lowest GO biological process class median benchmark dose values across tissues in male and female rats exposed to perfluoro-3-methoxypropanoic acid					
Tissue	**GO Accession**	**Gene Ontology Biological Process Class**	**# of Genes with BMD**	**BMD (mg/kg-day)**	**BMDL (mg/kg-day)**
Males					
Adrenal Gland	GO:0009896	positive regulation of catabolic process	3	4.68×10^{1}	1.08×10^{1}
Brain	GO:0033365	protein localization to organelle	3	3.70×10^{1}	8.35×10^{0}
Heart	GO:0048608	reproductive structure development	3	2.80×10^{0}	8.93×10^{-1}
Kidney	GO:1901568	fatty acid derivative metabolic process	3	5.76×10^{1}	3.77×10^{1}
Liver	GO:0006656	phosphatidylcholine biosynthetic process	3	5.30×10^{1}	3.53×10^{1}
Lung	GO:0070374	positive regulation of ERK1 and ERK2 cascade	3	8.45×10^{1}	1.13×10^{1}
Spleen	GO:0007519	skeletal muscle tissue development	3	6.30×10^{1}	3.87×10^{1}
Testis	GO:0090304	nucleic acid metabolic process	3	4.11×10^{1}	5.63×10^{0}
Thymus	GO:0010629	negative regulation of gene expression	3	6.64×10^{1}	4.09×10^{1}
Thyroid	GO:0071320	cellular response to cAMP	3	3.85×10^{1}	1.01×10^{1}
Females					
Adrenal Gland	GO:1901655	cellular response to ketone	5	9.78×10^{1}	5.15×10^{1}
Brain	GO:1901654	response to ketone	3	8.09×10^{1}	4.49×10^{1}
Heart	GO:1901216	positive regulation of neuron death	3	1.86×10^{1}	3.15×10^{0}
Kidney	GO:0042594	response to starvation	3	9.79×10^{1}	7.96×10^{1}
Liver	GO:0034641	cellular nitrogen compound metabolic process	5	7.44×10^{1}	4.50×10^{1}
Lung	GO:0032355	response to estradiol	3	1.20×10^{2}	5.39×10^{1}
Ovary	GO:0060612	adipose tissue development	3	5.80×10^{0}	6.38×10^{-1}
Spleen	GO:0045597	positive regulation of cell differentiation	3	1.53×10^{2}	1.10×10^{2}
Thymus	GO:0060070	canonical Wnt signaling pathway	3	9.00×10^{1}	4.97×10^{1}
Thyroid	GO:0045597	positive regulation of cell differentiation	3	2.06×10^{2}	1.41×10^{2}
Uterus	GO:2000146	negative regulation of cell motility	3	8.72×10^{-1}	1.21×10^{-1}

6. HUMAN EQUIVALENT DOSE AND TRANSCRIPTOMIC REFERENCE VALUE

6.1. POINT OF DEPARTURE

The transcriptomic point-of-departure for the study is 0.121 mg/kg-day. The point-of-departure is defined as the dose at which there were no coordinated transcriptional changes that would indicate a toxicity of concern. The coordinated transcriptional changes used to identify the POD do not necessarily discriminate between specific hazards, adverse or adaptive effects, nor are they used to infer a mechanism or mode of action.

6.2. HUMAN EQUIVALENT DOSE

The point-of-departure is scaled to a Human Equivalent Dose (HED) using the interspecies bodyweight dosimetric adjustment factor (DAF) and a reference human body weight of 80 kg (Table 6-1).

Table 6-1. Calculation of the $BMDL_{HED}$ for perfluoro-3-methoxypropanoic acid

Endpoint	Sex	Organ	BMDL (mg/kg-day)	Terminal Rat Body Weight (kg)	Dose Adjustment Factor (DAF)	$BMDL_{HED}$ (mg/kg-day)
Transcriptional changes	Female	Uterus	0.121	0.227	0.231	0.0279

$$BMDL_{HED} = BMDL \times \frac{BW_{Rat}^{1/4}}{BW_{Human}^{1/4}} = 0.121 \text{ mg/kg-day} \times \frac{0.227 \text{ kg}^{1/4}}{80 \text{ kg}^{1/4}} = 0.0279 \text{ mg/kg-day}$$

The $BMDL_{HED}$ for perfluoro-3-methoxypropanoic acid is 0.0279 mg/kg-day.

6.3. TRANSCRIPTOMIC REFERENCE VALUE

The application of uncertainty values follows the guidelines described in the *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (EPA 2024). The quantitative application of uncertainty factors for Intraspecies Variability (UF_H), Animal-to-Human Interspecies Variability (UF_A), Subchronic-to-Chronic Duration Extrapolation (UF_S), Extrapolation of a Lowest Observed Adverse Effect Level (LOAEL)-to-No Observed Adverse Effect Level (NOAEL) (UF_L), and Database (UF_D) are provided in Table 6-2.

Table 6-2. Uncertainty factors used in the calculation of the TRV for perfluoro-3-methoxypropanoic acid		
UF_H	10	A UF_H of 10 is applied to account for interindividual variability in the susceptibility of the human population due to both intrinsic and extrinsic factors that can influence the response to dose and the absence of chemical-specific information to assess toxicokinetic and toxicodynamic variability in humans.
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between animals and humans following oral exposure. Cross-species dosimetric adjustment (HED calculation) was performed using default allometric $BW^{3/4}$ scaling between rats and humans. A factor of 3 is applied to account for residual toxicokinetic uncertainty and potential toxicodynamic differences across species.
UF_S	1	A UF_S of 1 is applied due to the use of a transcriptomic POD from the GO biological process class with the lowest median BMD gene set following a 5-day *in vivo* study. The transcriptomic POD under these conditions has been shown to be concordant with apical/phenotypic PODs from chronic studies.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database.
	300	Composite UF = $UF_H \times UF_A \times UF_L \times UF_S \times UF_D$

Using the $BMDL_{HED}$ from the transcriptional changes in the female rat uterus of 0.0279 mg/kg-day (27.9 µg/kg-day), the TRV was calculated based on the following equation:

$$TRV = \frac{BMDL_{HED}}{\left(UF_H(10)\ X\ UF_A(3)\ X\ UF_L(1)\ X\ UF_S(1)\ X\ UF_D(10)\right)} = \frac{0.0279\ \text{mg/kg-day}}{300}$$
$$= 0.00009\ \text{mg/kg-day}$$

The TRV for perfluoro-3-methoxypropanoic acid is 0.00009 mg/kg-day (0. 09 µg/kg-day) and is an estimate of a daily oral dose to the human population that is likely to be without appreciable risk of adverse non-cancer health effects over a lifetime. The TRV is derived from a transcriptomic POD with UFs applied to reflect limitations of the data used.

7. APPENDIX I

7.1. POPULATIONS, EXPOSURES, COMPARATORS, AND OUTCOME (PECO) CRITERIA

PECO criteria were used to focus the research questions, search terms, and inclusion/exclusion parameters in the systematic evidence map process. The PECO criteria used for perfluoro-3-methoxypropanoic acid are provided in Table 7-1.

Table 7-1. Summary of PECO elements and associated evidence.

PECO element	Evidence
Populations	**Human**: Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Non-human mammalian animal species (whole organism) of any lifestage (including fetal, early postnatal, adolescents and adults).
Exposures	**Relevant forms:** [substance X] (CAS number) Other forms of [chemical X] that readily dissociate (*e.g.*, list any salts, etc.). Known metabolites of interest, including metabolites used to estimate exposures to [chemical X]. **Human:** Any exposure to [chemical X] via [oral or inhalation] route[s]. Studies will also be included if biomarkers of exposure are evaluated (*e.g.*, measured chemical or metabolite levels in tissues or bodily fluids), but the exposure route is unclear or likely from multiple routes. Other exposure routes, such as those that are clearly dermal, are tracked during title and abstract screening and tagged as "potentially relevant supplemental material." **Animal:** Any exposure to [chemical X] via [oral or inhalation] route[s] of >1 day duration, or any duration assessing exposure during reproduction or development. Studies involving exposures to mixtures will be included only if they include an experimental arm with exposure to [chemical X] alone. Other exposure routes, including [dermal or injection], are tracked during title and abstract as "potentially relevant supplemental material."

Table 7-1. Summary of PECO elements and associated evidence.	
PECO element	**Evidence**
Comparators	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits), or exposure for shorter periods of time, or cases versus controls, or a repeated measures design. However, worker surveillance studies are considered to meet PECO criteria even if no statistical analyses using a referent group is presented. Case reports or case series of > 3 people will be considered to meet PECO criteria, while case reports describing findings in 1–3 people will be tracked as "potentially relevant supplemental material." **Animal:** A concurrent control group exposed to vehicle-only and/or untreated control (control could be a baseline measurement, *e.g.*, acute toxicity studies of mortality, or a repeated measure design).
Outcomes	All health outcomes (cancer and non-cancer). In general, endpoints related to clinical diagnostic criteria, disease outcomes, biochemical, histopathological examination, or other apical/phenotypic outcomes are considered to meet PECO criteria.

7.2. LITERATURE SEARCH STRATEGY AND RESULTS

The literature search strategy and search results are summarized in Table 7-2.

Table 7-2. Literature search strategy and search results for perfluoro-3-methoxypropanoic acid		
Search	**Search Strategy**	**Date and Results**
WOS	TS="2,2,3,3-Tetrafluoro-3-(trifluoromethoxy)propanoic acid" OR TS="377-73-1" OR TS="O=C(O)C(F)(F)C(F)(F)OC(F)(F)F" OR TS="Perfluoro-3-methoxypropanoic acid" OR TS="Perfluoro-4-oxapentanoic acid" OR TS="Propanoic acid, 2, 2, 3, 3- tetrafluoro- 3-(trifluoromethoxy) -" OR TS="BRN 1795024" OR TS="Perfluoromethoxypropionic acid" OR TS="PERFLUORO PFMPA" OR TS="PF4OPeA" OR TS="PF-4O-PeA" OR TS="PFMOPrA" OR TS="PFMPA" OR TS="PFPE-2" OR TS="Propionic acid, 2,2,3,3-tetrafluoro-3-(trifluoromethoxy)-"	12/19/2022 5 results
PubMed	"2,2,3,3-Tetrafluoro-3-(trifluoromethoxy)propanoic acid"[tw] OR "377-73-1"[tw] OR "377-73-1"[rn] OR "O=C(O)C(F)(F)C(F)(F)OC(F)(F)F"[tw] OR "Perfluoro-3-methoxypropanoic acid"[tw] OR "Perfluoro-4-oxapentanoic acid"[tw] OR "Propanoic acid, 2, 2, 3, 3- tetrafluoro- 3-(trifluoromethoxy) -"[tw] OR "BRN 1795024"[tw] OR "Perfluoromethoxypropionic acid"[tw] OR "PERFLUORO PFMPA"[tw] OR "PF4OPeA"[tw] OR "PF-4O-PeA"[tw] OR "PFMOPrA"[tw] OR "PFMPA"[tw] OR "PFPE-2"[tw] OR "Propionic acid, 2,2,3,3-tetrafluoro-3-(trifluoromethoxy)-"[tw]	12/19/2022 4 results
ProQuest	ABSTRACT,TITLE("2,2,3,3-Tetrafluoro-3-(trifluoromethoxy)propanoic acid") OR ABSTRACT,TITLE("377-73-1") OR ABSTRACT,TITLE("O=C(O)C(F)(F)C(F)(F)OC(F)(F)F") OR ABSTRACT,TITLE("Perfluoro-3-methoxypropanoic acid") OR ABSTRACT,TITLE("Perfluoro-4-oxapentanoic acid") OR ABSTRACT,TITLE("Propanoic acid, 2, 2, 3, 3- tetrafluoro- 3-(trifluoromethoxy) -") OR ABSTRACT,TITLE("BRN 1795024") OR ABSTRACT,TITLE("Perfluoromethoxypropionic acid") OR ABSTRACT,TITLE("PERFLUORO PFMPA") OR ABSTRACT,TITLE("PF4OPeA") OR ABSTRACT,TITLE("PF-4O-PeA") OR ABSTRACT,TITLE("PFMOPrA") OR ABSTRACT,TITLE("PFMPA") OR ABSTRACT,TITLE("PFPE-2") OR ABSTRACT,TITLE("Propionic acid, 2,2,3,3-tetrafluoro-3-(trifluoromethoxy)-")	12/19/2022 3 results
Total unique references found		**5**

The five unique references that did not meet PECO criteria are:

1. Miller, KE; Strynar, MJ. (2022). Improved Tandem Mass Spectrometry Detection and Resolution of Low Molecular Weight Perfluoroalkyl Ether Carboxylic Acid Isomers Environmental Science & Technology Letters 9:747-751. http://dx.doi.org/10.1021/acs.estlett.2c00509 HERO ID: 10584196
2. Wan, Y; Li, Z; Huang, Z; Hu, B; Lv, W; Zhang, C; San, H; Zhang, S. (2022). Wafer-Level Self-Packaging Design and Fabrication of MEMS Capacitive Pressure Sensors http://dx.doi.org/10.3390/mi13050738 HERO ID: 10603997

3. Woodlief, T; Vance, S; Hu, Q; Dewitt, J. (2021). Immunotoxicity of per- and polyfluoroalkyl substances: Insights into short-chain PFAS exposure Toxics 9:100. http://dx.doi.org/10.3390/toxics9050100 HERO ID: 9959537
4. Zhang, W; Cao, H; Liang, Y. (2021). Plant uptake and soil fractionation of five ether-PFAS in plant-soil systems Science of the Total Environment 771:144805. http://dx.doi.org/10.1016/j.scitotenv.2020.144805 HERO ID: 9952516
5. Kometani, N; Kaneko, M; Morita, T; Yonezawa, Y. (2008). The formation of photolytic silver clusters in water/supercritical CO2 microemulsions Colloids and Surfaces A: Physicochemical and Engineering Aspects 321:301-307. http://dx.doi.org/10.1016/j.colsurfa.2008.02.005 HERO ID: 5387167

There were no unique references that met PECO criteria and were suitable for use as a POD and reference value derivation.

8. APPENDIX II

8.1. DETAILED ANIMAL STUDY REPORT PERFLUORO-3-METHOXYPROPANOIC ACID

8.1.1. OVERVIEW

Male and female Sprague Dawley rats were exposed via oral gavage for 5 days with nine doses of perfluoro-3-methoxypropanoic acid and a vehicle control. At the end of the exposure period, gene expression changes were measured in the liver, kidney, spleen, thyroid, heart, adrenal gland, ovary (female), testes (male), thymus, uterus (female), brain, and lung. Dose response analyses of the gene expression changes were performed using BMD modeling and the results summarized by GO biological process classes. The GO biological process class with the lowest median BMD value was identified across all the tissues examined in either sex. The median BMDL associated with the identified GO biological process class was selected as the transcriptomic POD and used to derive the TRV.

8.1.2. DOSE FORMULATIONS AND PRE-ADMINISTRATION ANALYSIS

8.1.2.1. Chemical Procurement, Purity, Stability, and pH

Perfluoro-3-methoxypropanoic acid was purchased from Synquest Laboratories (Part # 2121-3-66; Lot # 00016952). Identity and purity (> 98%) of the chemical was confirmed at the US EPA using liquid chromatography/tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) in negative ion Information Dependent Acquisition (IDA) mode on a Sciex X500R quadrupole time-of-flight (QTOF) mass spectrometer. Purity of the standard (Figure 8-1) is ≥ 98% by this method and is consistent with the certificate-of-analysis. Perfluoro-3-methoxypropanoic acid eluted at 4.93 min. All other observed signal was observed in the solvent blank.

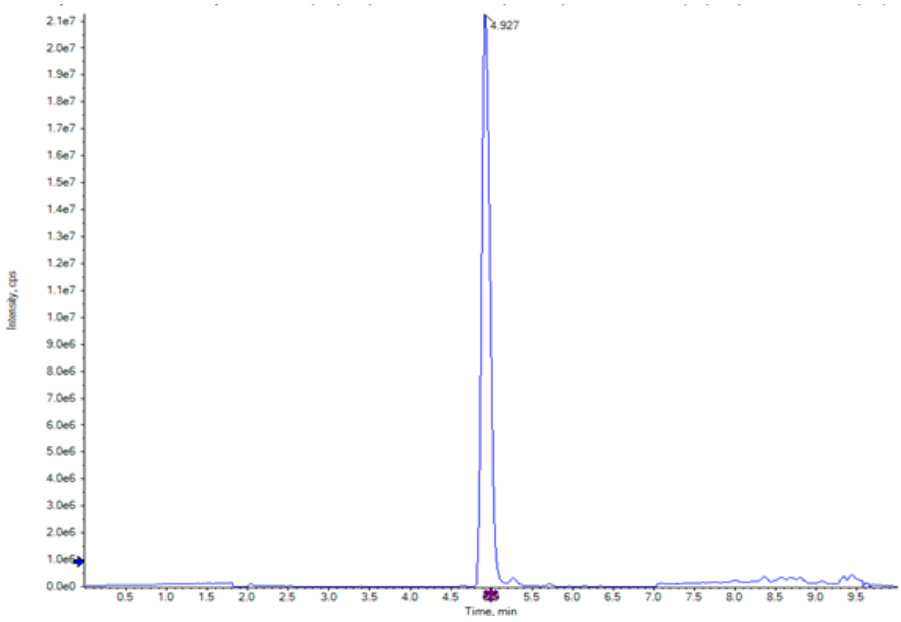


Figure 8-1. Total ion chromatogram of a 100 µg/mL perfluoro-3-methoxypropanoic acid standard used for purity confirmation.

The concentration and pH stability of perfluoro-3-methoxypropanoic acid was evaluated initially and after 5 days at 4 °C. Perfluoro-3-methoxypropanoic acid solutions were prepared in deionized water at 60.9 mg/mL and 1.89 µg/mL. Solutions were sonicated for 20 minutes to ensure dissolution of the standard. Stability measurements were made immediately after the initial preparation as well as on Day 5. Solutions were stored at 4 °C in the dark between measurements. The solutions were diluted with methanol for measurement of concentration stability by peak area. The stability was determined by LC/MS/MS on a Sciex X500R QTOF using negative ion ESI high resolution-multiple reaction monitoring (HR-MRM) and information dependent acquisition modes. The experiment was performed twice to confirm initial results. The average peak areas were used to calculate an average percent loss less than 10% observed over 5 days. Average percent recovery over 5 days was 98.8 ± 14.1% for the 60.9 mg/mL solution and 93.6 ± 12.5% for the 1.89 µg/mL solution.

Prior to measurement of pH, the 60.9 mg/mL solutions in water were brought to room temperature. A Corning Pinnacle 530 pH meter with FisherBrand Accumet pH probe was used to measure pH. The initial pH of the freshly prepared 60.9 mg/mL solution was 1.03 for Experiment #1 and 0.56 for Experiment #2. The pH was adjusted with 2 M NaOH to 4.40 for Experiment #1 and 4.65 for Experiment #2, both of which were within the targeted 4.5 ± 0.2 pH unit range. The pH on Day 5 was measured to be 6.46 for Experiment #1 and 7.65 for Experiment #2.

8.1.2.2. Dose Selection and Dosing Solution Preparation

At the time of study initiation, perfluoro-3-methoxypropanoic acid did not have any existing acute or repeat dose toxicity studies to assist in identifying the potential dose range in the study. An

initial dose-range finding study was performed in male and female rats with dose levels ranging from 1.0 - 1000 mg/kg-day. In both males, some lethality was observed at the highest two doses. In females, some lethality was observed at the high dose. Initial transcriptomic analysis of select tissues demonstrated gene set BMDL's were at or below the lowest dose. Based on these results, the present study was performed with nine dose levels plus a vehicle control. The high dose was set at 300 mg/kg-day range due to the observed lethality in both sexes. The next seven dose levels were set at half-log10 intervals and lowest dose at a full log10 lower than the eighth dose. The expected and observed concentrations of the dosing solutions are presented in Table 8-1.

Dosing solutions of perfluoro-3-methoxypropanoic acid were prepared by the contracted laboratory (Inotiv, Morrisville, NC) in deionized water and the pH was adjusted with 2 M NaOH to 4.5. Dosing solution concentrations were confirmed at the US EPA. Dosing solutions were diluted with methanol, spiked with 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropyl)-$^{13}C_3$-propanoic acid ($^{13}C_3$-GenX) as an internal standard, and vortexed to mix. The dilutions were analyzed by LC/MS/MS in ESI negative ion HR-MRM mode on a Sciex X500R QTOF mass spectrometer. The final perfluoro-3-methoxypropanoic acid concentrations for the dosing solutions are presented in Table 8-2.

The perfluoro-3-methoxypropanoic acid concentrations observed in dosing solution dilutions were quantitated using internal standard calibration. The calibration curve included 5 or more points. Calibration for the analyte was acceptable; the Pearson correlation coefficient (r) was ≥ 0.99 and calculated concentrations of calibration standards were within ± 20% of actual, except for the low standard which was acceptable with a readback concentration within ± 30% of actual. The calibration was verified with a second source standard that gave a calculated concentration within ± 20% of actual. Continuing calibration acceptability was verified before and after analysis of approximately 30 or fewer samples and daily by analysis of continuing calibration standards that gave acceptable calculated concentrations within ± 20% of actual.

The samples were prepared and analyzed together in a batch with a matrix blank, laboratory control sample (LCS), and laboratory control sample duplicate (LCSD) spiked at a concentration below the mid-point of the calibration curve. Perfluoro-3-methoxypropanoic acid was not detected above the limit of quantitation (LOQ) in the matrix blank. The LOQ was set at the lowest calibration standard of 30 ng/mL. A laboratory duplicate (LD) of project sample 0.002 mg/mL was also included. The relative percent difference for the LD compared to the project sample was 2.0%. Recoveries for the LCS and LCSD were within the acceptance range of ± 30% of actual.

Table 8-1. Dosing solution concentrations (mg/ml) for perfluoro-3-methoxypropanoic acid									
Expected Concentration (mg/ml)	0.002	0.02	0.06	0.2	0.6	2	6	20	60.0
Observed Concentration (mg/ml)	0.00292	0.0342	0.0864	0.274	0.737	2.90	8.85	22.8	83.7
% Difference[a]	37.4	52.4	36.1	31.2	20.5	36.7	38.4	13.1	33.0
[a]% Difference = [abs(a - b)/(a+b)/2] × 100%; where a is the expected concentration and b is the observed concentration.									

8.1.3. ANIMAL HUSBANDRY AND TREATMENT

All procedures in the study were in compliance with the Animal Welfare Act Regulations, 9 CRF 1-4. All animals were treated according to the Guide for the Care and Use of Laboratory Animals. Animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and maintained at 20–22°C, 30-70% humidity, and a 12:12 h photoperiod (lights off at 1800 hours). Male and female Sprague Dawley rats (8-10 weeks old; Crl:CD(SD)) were received at Inotiv (Morrisville, NC) from Charles River Laboratories (Raleigh NC), housed two per polycarbonate cage (23 cm x 44 cm) with micro-isolator tops and heat-treated hardwood bedding (Northeastern Products Crop., Warrensburg, NY). Rats received food (Certified Purina Pico Chow No. 5002; Ralston Purina Co., St. Louis MO) and reverse osmosis treated tap water (City of Durham, NC) *ad libitum*. Animals were acclimated for 2 weeks and were uniquely identified by ear punch prior to study start. The animals were assigned to a dose group using a procedure that stratifies animals across groups by body weight, such that the mean body weight of each group is not statistically different from any other group using analysis of variance (ANOVA) (Statistical Analysis System version 9.2, SAS Institute, Cary, NC). Only clinically healthy animals were used for allocation. Five male and female rats per dose group received the test article (0.01, 0.1, 0.3, 1.0, 3.0, 10, 30, 100, and 300 mg/kg-day) solubilized in deionized water and eight males and females received deionized water vehicle alone via gavage at a dose volume of 5 ml/kg for five consecutive days. Dose volumes were adjusted on a daily basis based on individual animal body weights. Dosing solutions were placed on a stir plate at least 30 minutes prior to dosing and continuously stirred. Dosing occurred at the same time each day (±1 hour) and animals were euthanized 24 hours (±1 hour) after the 5th dose. Animals were observed twice daily on weekdays and once daily on weekends. Clinical observations were performed within two days of arrival, when allocating animals to dose groups, prior to first gavage administration, and at termination. Body weights were collected within two days of arrival, when allocating animals to dose groups, prior to each administration, and at termination. Animal studies were performed by Inotiv (Morrisville, NC).

8.1.4. GROSS OBSERVATIONS, SACRIFICE, AND TISSUE COLLECTION

Male rats from the lowest six doses survived until scheduled termination (Table 8-2). One male rat in the 30 mg/kg-day treatment group died prior to the scheduled termination. One female rat in the 30.0 and 300 mg/kg-day treatment groups died prior to the scheduled termination. One female rat in the 300 mg/kg-day treatment group exhibited abnormal breathing and rales. A second female rat in the 300 mg/kg-day treatment group had rales. All gross observations were noted on Days 5 and 6 of the study.

Table 8-2. Exposure doses and survival of animals for male and female rats treated with perfluoro-3-methoxypropanoic acid

Sex	Exposure Doses in mg/kg-day (Number of Animals Surviving Through Termination)
Males	0 (8), 0.01 (5), 0.1 (5), 0.3 (5), 1.0 (5), 3.0 (5), 10.0 (5), 30.0 (4), 100.0 (5), 300.0 (5)
Females	0 (8), 0.01 (5), 0.1 (5), 0.3 (5), 1.0 (5), 3.0 (5), 10.0 (5), 30.0 (4), 100.0 (5), 300.0 (4)

Surviving animals were sacrificed 24 hours (±1 hour) after administration of the final dose. Rats were euthanized by carbon dioxide asphyxiation and death confirmed by exsanguination. At the time of sacrifice, blood (~2.0 mL) was collected from each animal at termination using cardiac puncture and placed into a four K3EDTA tubes, centrifuged at 1500 × *g* for 15 min at 4°C, and the plasma removed. Plasma was stored in six aliquots of 100 µl and frozen at or below -70°C in cryotubes after collection for potential analysis. The time of blood collection and necropsy were recorded for all animals. The left lobe of the liver, kidneys, spleen, thyroid, heart, adrenal glands, ovaries (female), testes (male), thymus, uterus (female), brain, and left lung were removed and preserved for RNA isolation. Necropsies were completed by noon. Animal sacrifice and tissue collection was performed by Inotiv (Morrisville, NC).

8.1.5. RNA ISOLATION AND TRANSCRIPTOMIC MEASUREMENTS

The left lobe of the liver, right kidney, spleen, heart, testes (male), thymus, uterus (female), brain, and left lung were removed. Samples from these larger tissues were cubed into ~5 mm^3 pieces and divided amongst three cryotubes (except adrenal glands, thyroid, and ovaries, which were split amongst two cryotubes due to small tissue size). Two of the cryotubes contained RNAlater™ (ThermoWaltham, MA) while the third cryotube containing from the larger tissue samples was frozen in liquid nitrogen and stored at approximately -80°C. The samples in RNAlater™ were stored at room temperature for same-day processing or 4°C until ready for processing to total RNA. Remaining tissues were then stored at approximately -20°C for up to 3 weeks before being transferring to approximately -80°C. Approximately 10 mg of liver and spleen or 20 mg of all other tissues were transferred by a clean single-edged razor blade to a microplate preloaded with 50 µL of

RNAlater™. Samples were processed for RNA purification using the RNAdvance purification kit (Beckman Coulter). For each plate of tissues in RNAlater™, the tissue fragment was transferred from RNAlater™ to a dry Kimwipe to remove excess liquid, and then placed into a deep well homogenization microplate preloaded with 400 µL of Lysis Buffer with Proteinase K and containing two stainless steel balls. The homogenization plate was shaken on a plate shaker at an optimized homogenization speed for 10 minutes. After shaking, homogenates were transferred to an RNA purification deep-well microplate and transferred to a 37°C water bath for 25 minutes of incubation. After incubation, homogenates were frozen at approximately -80°C until processing to purified RNA.

RNAs were purified in a semi-automated process using the RNAdvance protocol and a BioMek FX liquid handler. Bead binding buffer containing paramagnetic solid-phase reversible immobilization (SPRI) beads was added under rapid mixing. Following the addition of beads, plates were incubated at room temperature before magnetic bead capture and washing. Nucleic acids were bound to the SPRI beads and cellular material was washed off. The residual DNA was removed enzymatically with DNase, washed multiple times, and the purified RNA was eluted in 40 µL of nuclease-free water. RNA purity and quantity were evaluated using OD 260/230 and OD 260/280 measurements.

An aliquot of each RNA sample from each tissue, was hybridized with the Biospyder TempO-Seq Rat S1500+ Surrogate v1.2 detector oligo pool mix consisting of 2,654 probes. The full probe set details are available under the National Center for Biotechnology Information Gene Expression Omnibus (GEO) accession: GSE260875. Hybridization was followed by nuclease digestion, ligation, and then heat denaturation. An aliquot of each ligation product was then transferred to a multi-well polymerase chain reaction (PCR) amplification microplate and amplified using sample-specific, "barcoded" primer pairs. The amplification products from each sample were then pooled into a single sequencing library and processed with a PCR clean-up kit prior to sequencing. Each sample was sequenced on an Illumina HiSeq 2500 at a target read depth of ~1 million mapped reads per sample.

8.1.6. SEQUENCE ALIGNMENT, SAMPLE NORMALIZATION, AND QUALITY CONTROL

The sequence alignment and sample normalization followed the process outlined in the *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (EPA 2024). Briefly, each FASTQ file was aligned to the TempO-Seq probe manifest using HISAT2 and imported directly into SAMtools to compute probe-level counts for each individual FASTQ file. Samples were evaluated for quality based on:

- Sequencing depth (i.e., total number of mapped reads). Samples with < 10% of target depth (100,000 mapped reads) were removed from further analysis.
- Fraction of uniquely mapped reads. Samples with < 50% of reads uniquely mapped to known probes were removed from further analysis.
- Probe coverage (i.e., total probes with at least 5 reads). Samples with < 1,200 covered probes were removed from further analysis.

The list of samples removed based on the alignment QC metrics are listed in Table 8-3.

Table 8-3. Samples removed based on QC metrics

Tissue	Animal ID	Sex	Dose Group	QC Issue
Liver	108	Male	vehicle	< 50% reads aligned
Heart	123	Male	0.1 mg/kg-day	< 50% reads aligned
Brain	143	Male	10 mg/kg-day	< 50% reads aligned
Adrenal	149	Male	30 mg/kg-day	< 50% reads aligned
Spleen	150	Male	100 mg/kg/day	< 50% reads aligned

Prior to performing downstream gene expression across samples, probe counts for each sample were normalized to adjust for differences in sequencing depth. For each treatment condition in each sex and tissue, raw probe counts for all samples were normalized within each sample as follows:

- All probes with a mean read count < 5 were removed, as these probes lack sufficient signal for reliable analysis.
- Each remaining probe count was normalized to Counts Per Million (CPM) = probe count * 1,000,000 / sum of all remaining probe counts in sample
- CPM values were transformed to $\log_2$ scale with added pseudo-count of 1 to prevent taking log of zero counts and ensuring a positive value for dose response modeling.

To identify potential outlier samples, a principal component analysis (PCA) was performed on subsets of samples corresponding to either: 1) All samples corresponding to same tissue, and sex, including matched vehicle controls ("treatment PCA"); and 2) All available vehicle controls corresponding to the same tissue and sex ("vehicle PCA"). Samples not meeting the sequencing quality metrics (e.g., < 50% of uniquely aligned reads) were excluded prior to PCA analysis. Outlier samples were identified based on the following considerations:

- Individual samples separated from all remaining samples on either principal component #1 (PC1) or principal component #2 (PC2) by >2x the span of all other samples on the corresponding PC were considered strong outliers and removed from further analysis.
- Individual samples separated by <2x the range of all other samples were considered moderate outliers, and additional exclusion criteria were considered:
    - Vehicle samples that appeared as moderate outliers on both a treatment PCA and vehicle PCA were excluded unless multiple controls from the same group appeared as outliers.
    - Moderate outlier samples with lower quality than corresponding tissue samples by one or more sequencing quality metrics (e.g., percentage of uniquely mapped reads) were excluded.
    - Samples that appeared as moderate outliers in both PC1 and PC2 with a relatively large Euclidean distance from all other remaining samples were excluded.

- Moderate outlier samples that were especially distant from corresponding replicates or similar doses were excluded.

When multiple outlier samples were present on the same PCA, they were only removed if each outlier sample corresponded to a different dose group, as these were unlikely to represent any reproducible dose-dependent effect. The outlier samples that were removed in the perfluoro-3-methoxypropanoic acid study are listed in Table 8-4. The National Center for Biotechnology Information GEO accession number for the dataset is: GSE260875.

Table 8-4. Samples removed based on PCA grouped by tissue and sex

Tissue	Animal ID	Sex	Dose Group
Spleen	210	Female	300 mg/kg-day
Thyroid	162	Female	Vehicle
Adrenal Gland	128	Male	0.3 mg/kg-day
Adrenal Gland	193	Female	10 mg/kg-day
Thymus	115	Male	0.01 mg/kg-day
Thymus	167	Female	Vehicle
Brain	113	Male	Vehicle
Testis	131	Male	1 mg/kg-day
Ovary	191	Female	3 mg/kg-day
Uterus	165	Female	Vehicle

The quality statistics for the remaining sequencing samples are provided in Table 8-5 and Figures 8-2, 8-3, and 8-4.

Table 8-5. Minimum and median TempO-Seq sequencing depth, mapping rate, and probe coverage statistics by tissue and sex

Sample Group		Sequencing Depth		Mapping Rate		Probe Coverage	
Tissue	**Sex**	**Minimum**	**Median**	**Minimum**	**Median**	**Minimum**	**Median**
Liver	Male	1319890	1567787	0.904299	0.910607	1336	1566
Liver	Female	1221565	1630937	0.883735	0.910119	1512	1627
Kidney	Male	1214393	1373827	0.883364	0.903278	1817	1951.5
Kidney	Female	830736	1307532	0.872645	0.884394	1876	2029
Spleen	Male	1170103	1525792	0.865306	0.875215	1714	1890
Spleen	Female	973288	1224717	0.861576	0.875101	1791	1913
Thyroid	Male	798876	1073374	0.810685	0.851559	1906	2029
Thyroid	Female	940246	1148191	0.785922	0.843692	1969	2044
Heart	Male	1091511	1392526	0.851941	0.867176	1796	1948
Heart	Female	1220552	1470762	0.824538	0.856716	1836	1977
Adrenal Gland	Male	1269412	1504351	0.865419	0.881572	1808	1926
Adrenal Gland	Female	1023761	1550673	0.94555	0.963216	1747	1950
Lung	Male	1074105	1348705	0.939957	0.962015	1920	2123.5
Lung	Female	1087649	1361548	0.935237	0.956288	2027	2144.5
Thymus	Male	522606	1393221	0.957296	0.965328	1664	1996
Thymus	Female	1201274	1470065	0.958982	0.966635	1863	2011.5
Brain	Male	883151	1281097	0.905357	0.969901	1835	2002
Brain	Female	1106019	1336732	0.953297	0.967818	1812	2011.5
Testis	Male	1058842	1407276	0.962576	0.97415	1929	2030
Ovary	Female	1029995	1681707	0.920002	0.956569	2027	2125
Uterus	Female	1258077	1668352	0.941067	0.956677	2006	2116

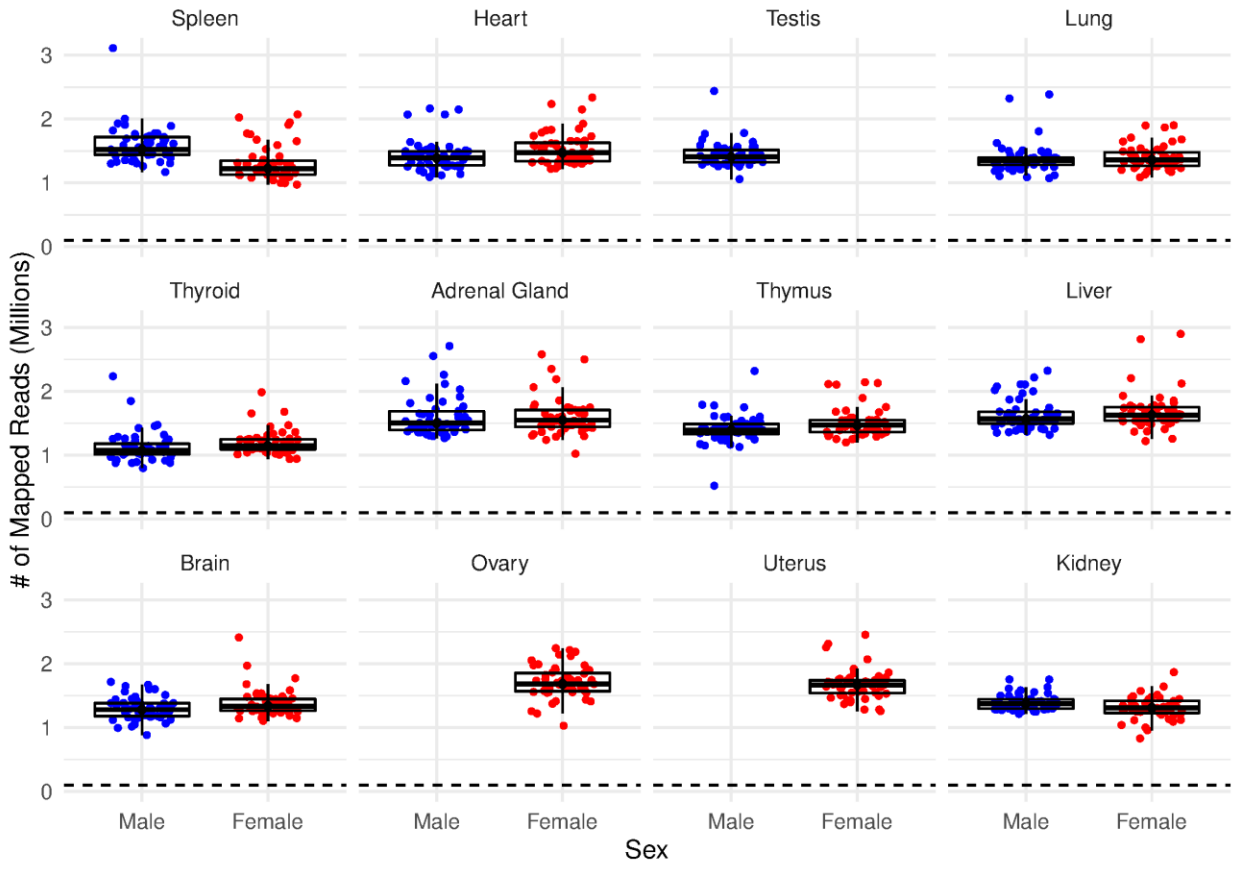


Figure 8-2. Distribution of sequencing depth (number of uniquely mapped reads) for each sample, grouped by tissue and sex.

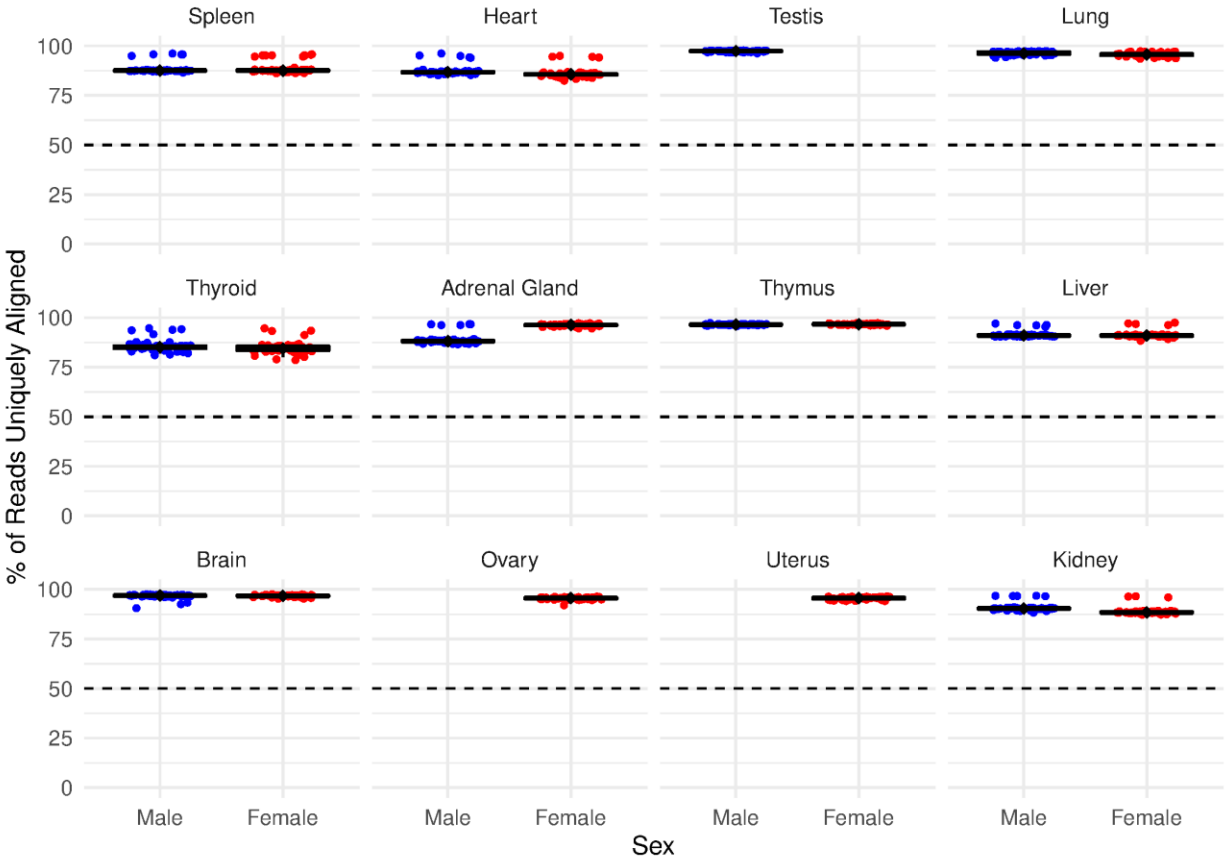


Figure 8-3. Distribution of mapping rate (% of reads uniquely aligned to probes) for each sample, grouped by tissue and sex.

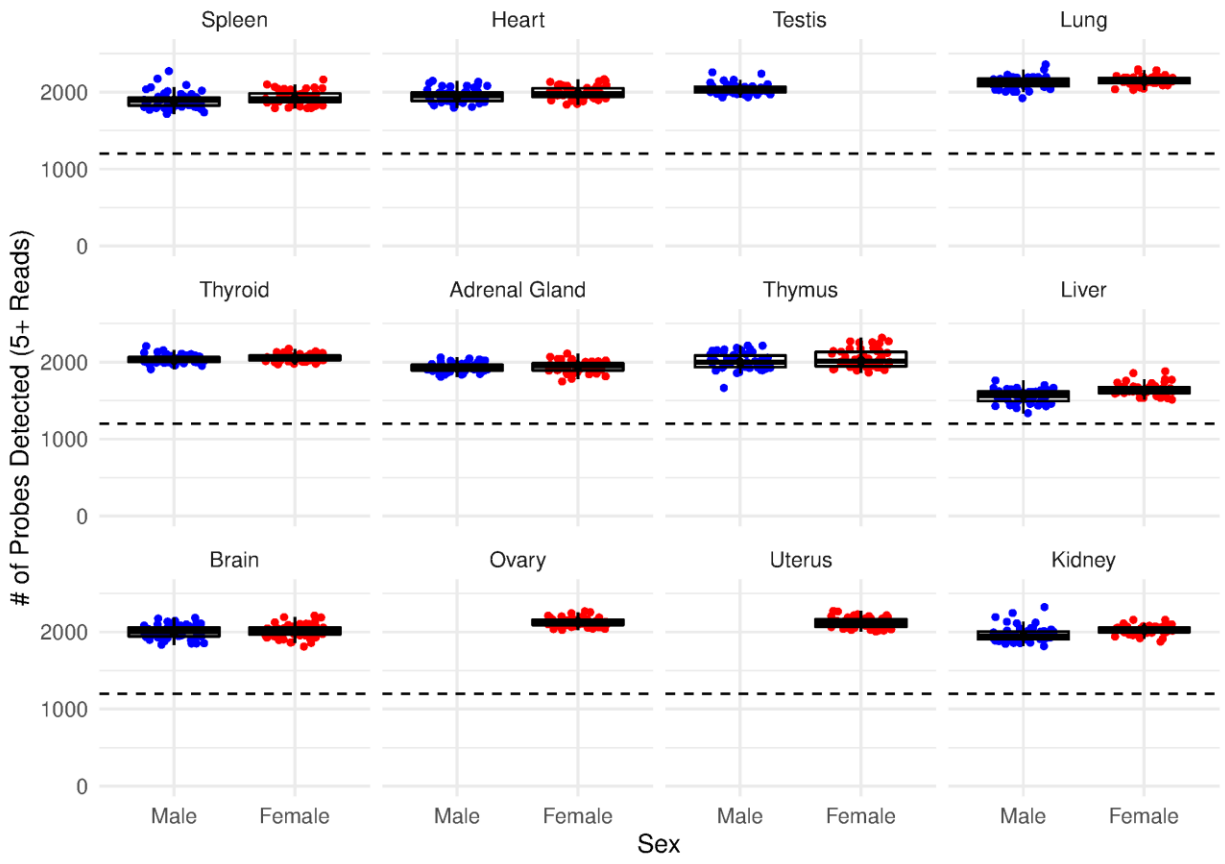


Figure 8-4. Distribution of probe coverage (number of probes detected with at least 5 reads) per sample, grouped by tissue and sex.

8.1.7. TRANSCRIPTOMIC DATA ANALYSIS AND GO BIOLOGICAL PROCESS SUMMARIZATION

Transcriptomic data analyses and GO biological process summarization were performed as described in the *Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs)* (EPA 2024). The dose response modeling was performed independently on each probe, sex, and tissue using BMDExpress 2.30 BUILD 0488. The digital object identifier (DOI) of the benchmark dose analysis files from BMDExpress and the OECD Omics Reporting Template are: https://doi.org/10.23645/epacomptox.25335844.

8.1.8. POINT-OF-DEPARTURE (POD) SELECTION

The GO biological process class with the lowest median BMD value in all tissues and both sexes was the negative regulation of cell motility in the female uterus (0.872 mg/kg-day). The median BMDL of 0.121 mg/kg-day associated with the identified GO biological process class was selected as

the POD. The human equivalent median BMDL was used to derive the TRV. The dose response changes of the probes for all 3 genes populating the negative regulation of cell motility GO biological process class are included in Figures 8-5 through 8-7.

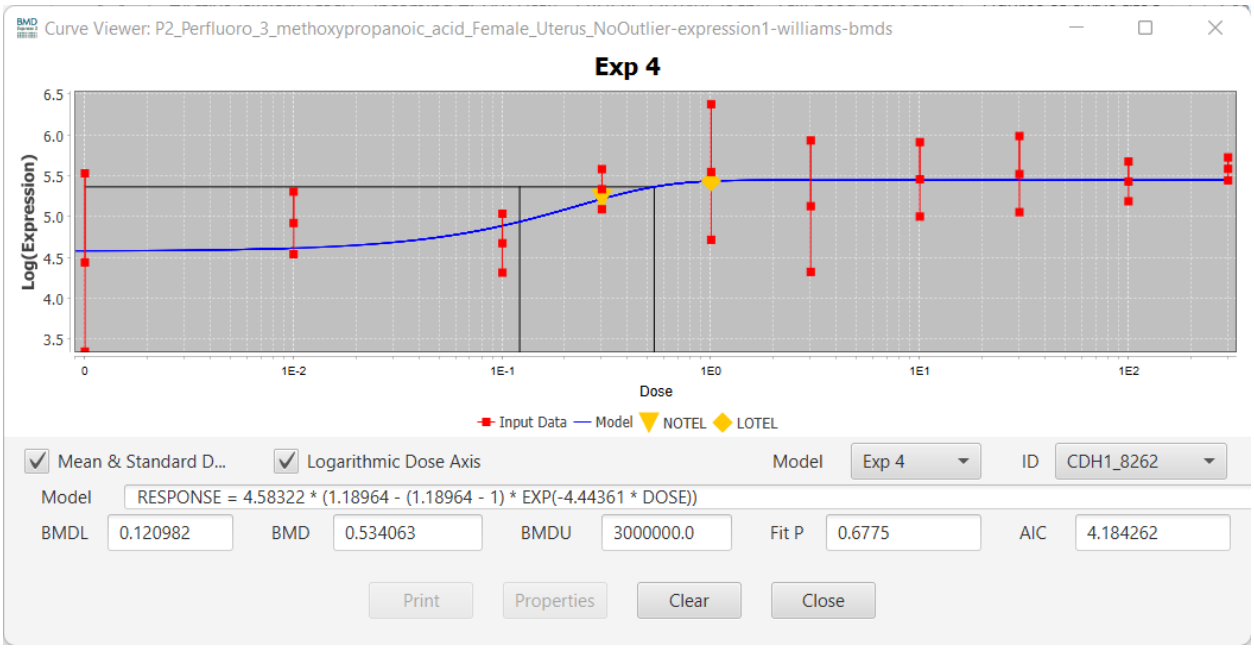


Figure 8-5. Dose response model for *Cdh1* expression (best model = Exp 4, BMD = 0.534, BMDL = 0.121 in mg/kg-day).

Figure 8-6. Dose response model for *Crk* expression (best model = Exp 2, BMD = 229.94, BMDL = 148.45 in mg/kg-day).

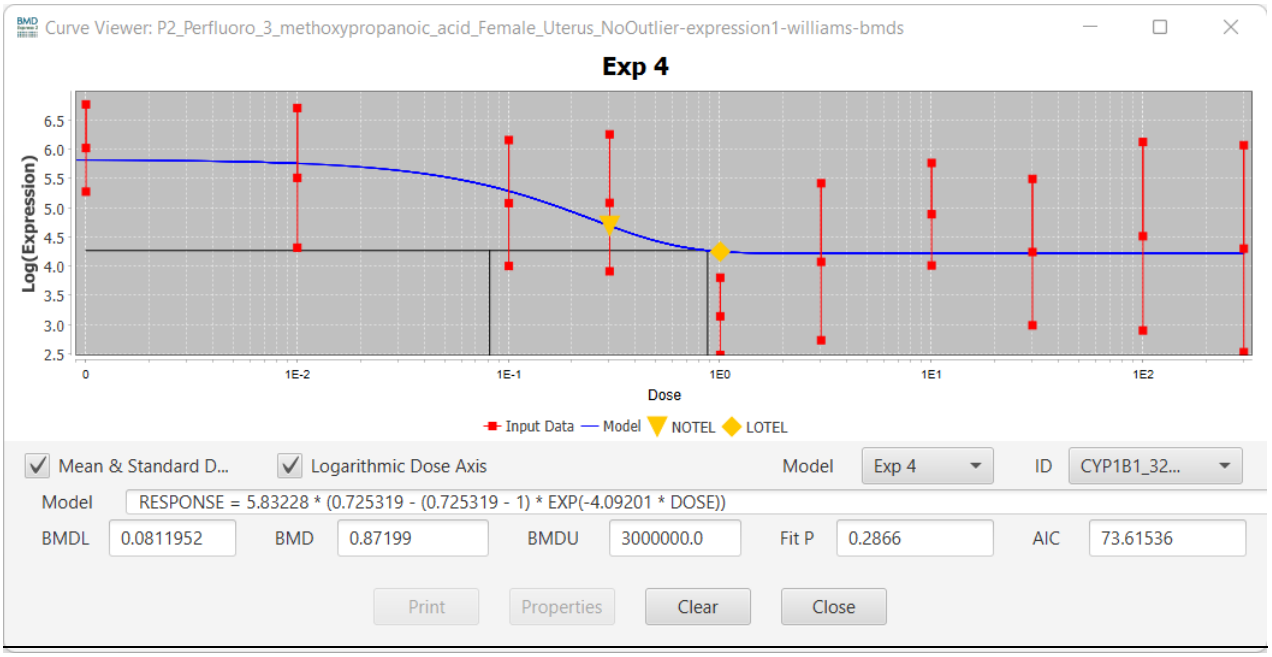


Figure 8-7. Dose response model for *Cyp1b1* (best model = Exp 4, BMD = 0.872, BMDL = 0.0812 in mg/kg-day).

8.1.9. TERMINAL BODY WEIGHTS

Body weight changes from Day 0 to sacrifice and body weight at sacrifice are provided in Tables 8-5 and 8-6.

Table 8-5. Body weight changes by perfluoro-3-methoxypropanoic acid in male rats		
Dose (mg/kg-day)	**Body Weight Mean (Std Dev)**	
	Terminal Body Weight (g)	**Body Weight Change (g)**
0	332.85 ± 34.41	30.54 ± 7.02
0.01	319.80 ± 23.52	28.70 ± 4.41
0.1	328.96 ± 16.59	34.62 ± 2.92
0.3	322.92 ± 18.25	30.28 ± 7.21
1.0	317.86 ± 26.76	28.72 ± 5.61
3.0	317.98 ± 20.51	30.02 ± 8.11
10	320.98 ± 24.85	19.62 ± 11.79
30	328.78 ± 28.63	28.30 ± 3.03
100	318.64 ± 19.10	20.48 ± 5.64
300	327.80 ± 51.00	17.08 ± 10.43
Table 8-6. Body weight changes by perfluoro-3-methoxypropanoic acid in female rats		
Dose (mg/kg-day)	**Body Weight Changes Mean (Std Dev)**	
	Terminal Body Weight (g)	**Body Weight Change (g)**
0	236.65 ± 27.20	9.89 ± 6.46
0.01	222.80 ± 16.90	2.76 ± 4.41

0.1	229.26 ± 15.18	5.38 ± 4.88
0.3	224.02 ± 9.41	4.02 ± 10.74
1.0	225.08 ± 24.03	5.28 ± 1.72
3.0	218.32 ± 25.57	-2.86 ± 7.50
10	236.52 ± 12.88	12.28 ± 6.74
30	234.20 ± 16.10	11.02 ± 4.96
100	231.84 ± 12.82	11.30 ± 7.23
300	212.22 ± 10.18	-9.60 ± 16.96

9. REFERENCES

EPA. 2024. Standard Methods for Development of EPA Transcriptomic Assessment Products (ETAPs). EPA/600/X-23/083. Research Triangle Park, NC:U.S. Environmental Protection Agency.