Evaluations of *in vivo* and *in vitro* Dosimetry and Metabolic Biotransformation of the Hexafluoropropylene Oxide Homologue HFPO-TeA, an Emerging Per- and Polyfluoroalkyl Substance (PFAS)

Denise MacMillan, Aero Renyer, Marci Smeltz, Matt Phillips, Krishna Ravindra, Michael DeVito, Michael F. Hughes, Leah Wehmas, and Barbara A. Wetmore

US Environmental Protection Agency, Office of Research and Development,
Center for Computational Toxicology and Exposure

This presentation does not necessarily reflect USEPA policy.

Evaluation of *in vivo* and *in vitro* Dosimetry and Metabolic Biotransformation of HFPO-TeA

- United States Environmental Protection Agency's PFAS response
- HFPO-TeA background
- *In vivo* Assays
- Non-targeted analysis for discovery of *in vivo* and *in vitro* biotransformation products
- Next steps and future direction

PFAS Strategic Roadmap: EPA's Commitments to Action 2021–2024

"For far too long, communities across the United States have been suffering from exposure to PFAS pollution. As the science has continued to develop, we know more now than ever about how PFAS build up in our bodies over long periods of time, and how they can cause adverse health effects that can devastate families."

RESEARCH

Invest in research, development, and innovation to increase understanding of PFAS exposures and toxicities, human health and ecological effects, and effective interventions that incorporate the best available science.

RESTRICT

Pursue a comprehensive approach to proactively prevent PFAS from entering air, land, and water at levels that can adversely impact human health and the environment.

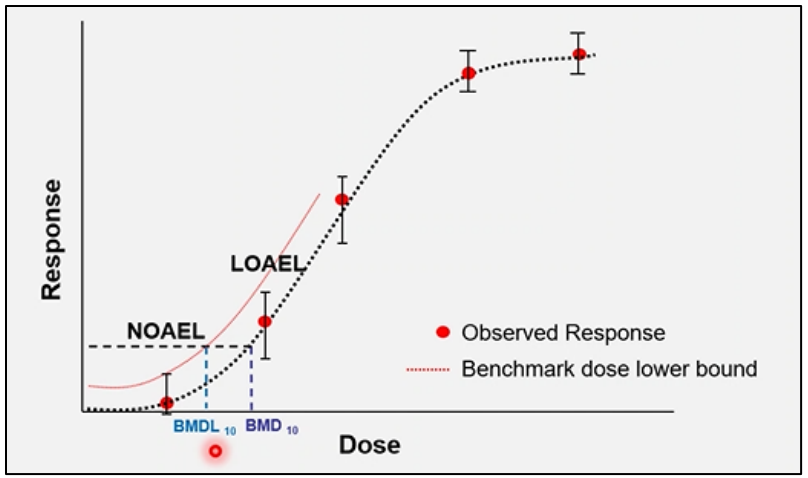
REMEDIATE

Broaden and accelerate the cleanup of PFAS contamination to protect human health and ecological systems.

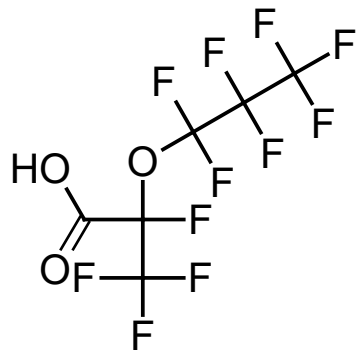
https://www.epa.gov/system/files/documents/2021-10/pfas-roadmap_final-508.pdf

Advancing the Science to Assess Human Health

- With toxicological data available for only a small fraction of the 1000s of known PFAS, the USEPA is developing New Approach Methods (NAMs) to evaluate potential toxicity.
- Interim Transcriptomic Assessment Product (ITAP) research
  - Short term exposure assessment using animal model
    - Propose interim benchmark dose levels,
    - Promote understanding of the biological responses to exposure,
    - Evaluate cross species and system-specific responses using *in vitro* assays,
    - Explore possible biotransformations to understand metabolism and response pathways.

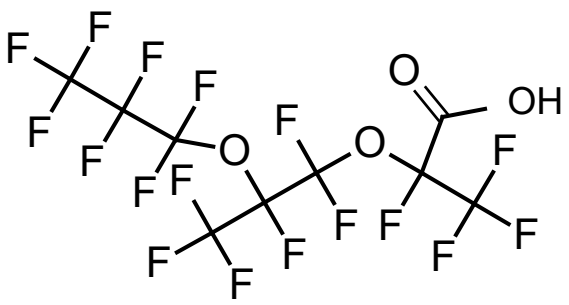


HFPO-DA and Oligomers



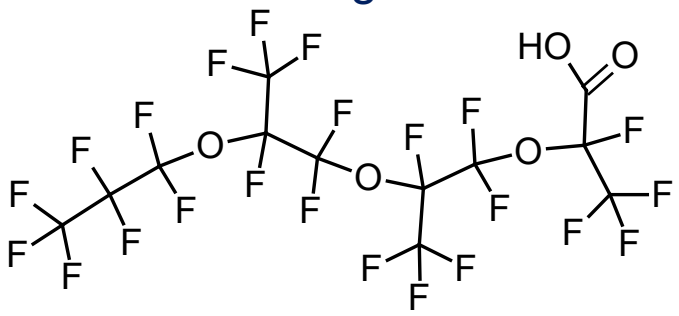
HFPO-DA
GenX
$C_6HF_{13}O_3$

- Replacements for PFOA after voluntary phase out in 2006.
- Ether linkages added to increase ease of degradation: perfluoroalkyl ether carboxylic acids (PFECAs).
- Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) identified in Cape Fear River of North Carolina in 2015.
- GenX and trimer acid HFPO-TA detected downstream of fluorochemical plants in Europe and Asia. Tetramer acid HFPO-TeA prevalent in China.
- Potential hazard of HFPO-TeA of interest to States and EPA regions.



HFPO-TA
$C_9HF_{18}O_4$

HFPO-TeA
$C_{12}HF_{23}O_5$



Environ. Sci. Technol., 2015, 49, 11622-11630. *Environ. Sci. Technol.*, 2017, 51, 17, 9553−9560. *Environ. Int.*, 2020, 137, 2020, 105583-105594.

ITAP Research Areas

In Vivo Assays

- In Life Observations – Response to Exposure
- Thyroid Hormones - Endocrine Disruption
- Liver Lipidomics – Signaling, Inflammation, energy, transport
- Transcriptomics – Benchmark Dose Prediction
- Dosimetry – Distribution and Biological Response
- Biotransformations – Xenobiotic Metabolism

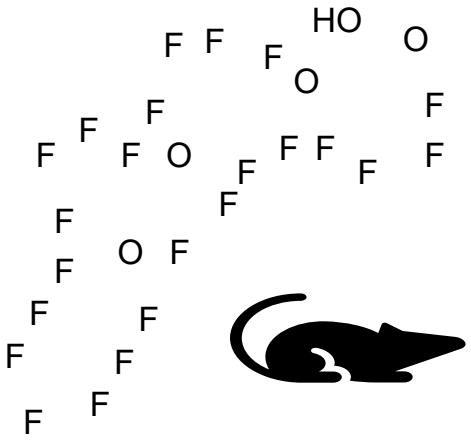
In Vitro Assays

- Plasma Protein Binding – Availability for Metabolism
- Hepatocyte Clearance- Concentration over Time
- Biotransformations - Cross Species and Cell Types

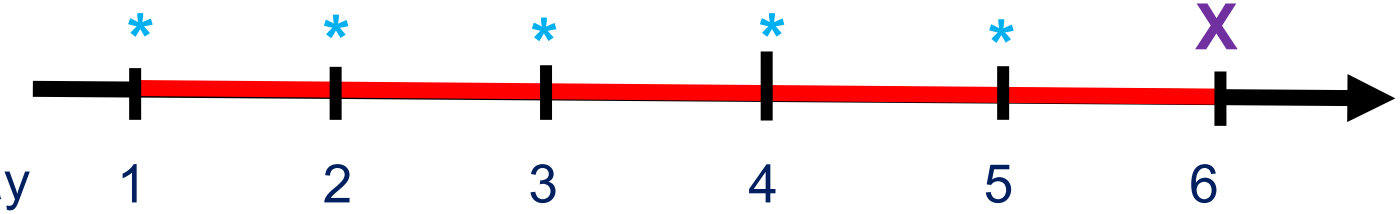
Five Day, Repeat Dose Exposure Study

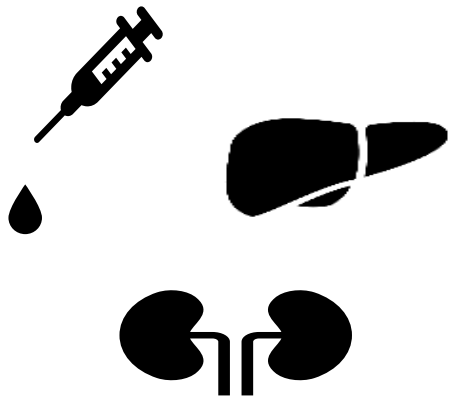
HFPO-TeA dose formulation

- Vehicle Control + 8 Dose-levels
- Vehicle = Water
- Range: 0.3 – 335.2 mg/kg/day
  ~1/2 $\log_{10}$ spacing



- Sprague-Dawley Rats
- 8-10 weeks
- n = 4/sex/dose-level





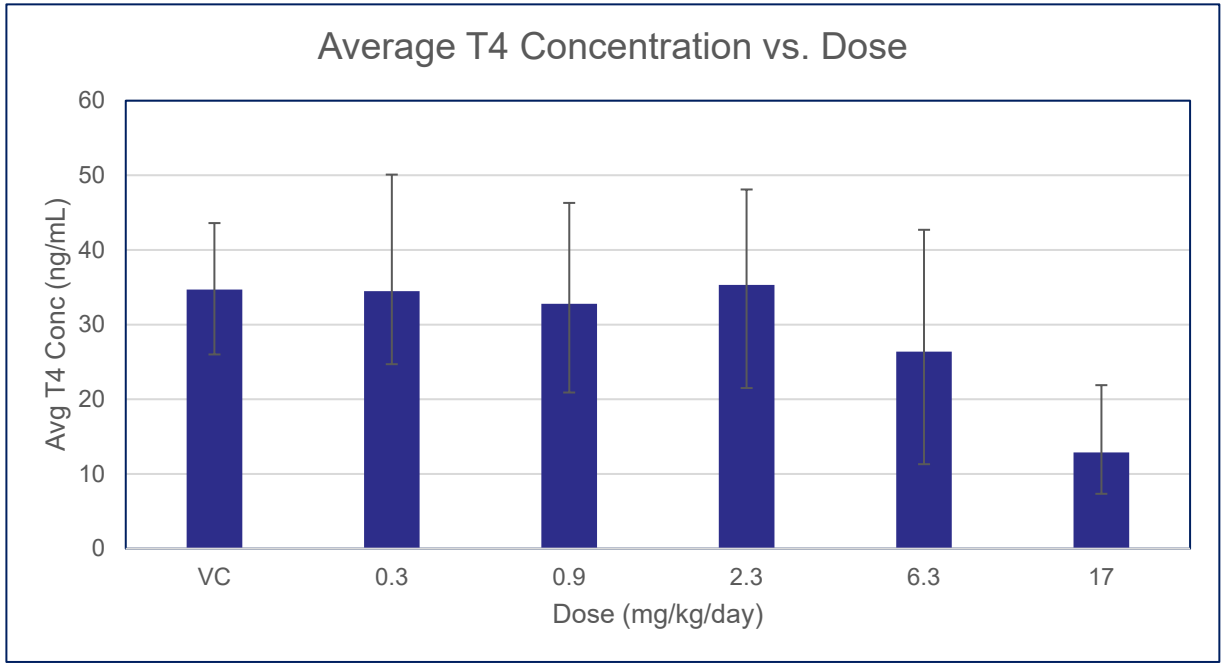
* = Oral gavage. X = Rats euthanized.

In Life Observations

- Evidence of Chronic Toxicity
  - Thinning hair
  - Hair standing on end
  - Hunching
  - Abnormal breathing
  - Weight loss
  - Lethargy
  - Lethality > 17 mg/kg/day

Dose Level (mg/kg/day)	Male Avg. Weight Change (g)	Female Avg. Weight Change (g)
Vehicle	31.33	3.73
0.3	37.20	6.48
0.9	39.60	12.18
2.3	39.30	12.30
6.3	31.78	-17.75
17	-51.48	-55.18

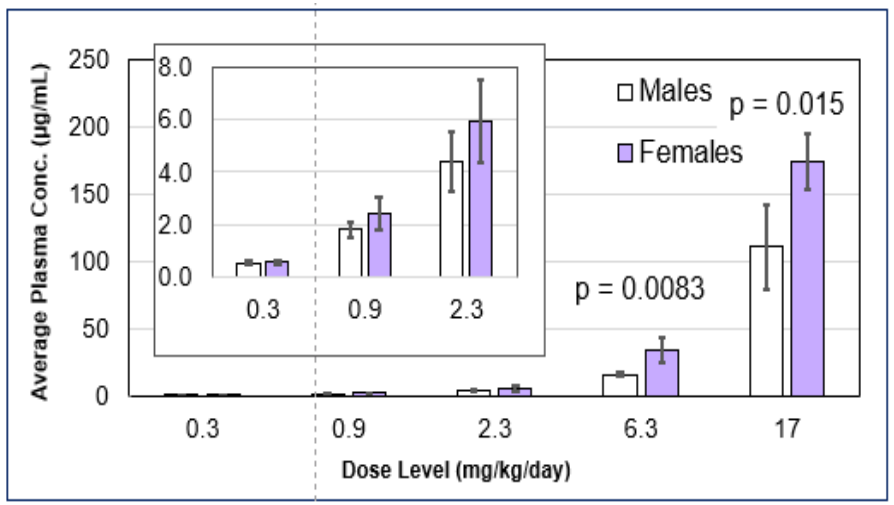
Thyroid Hormones

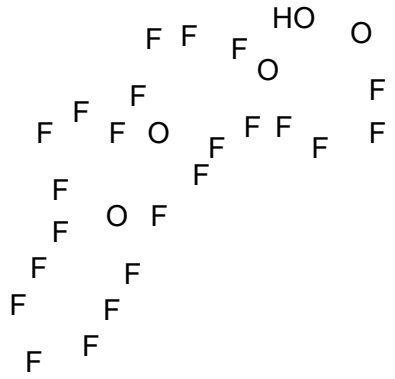


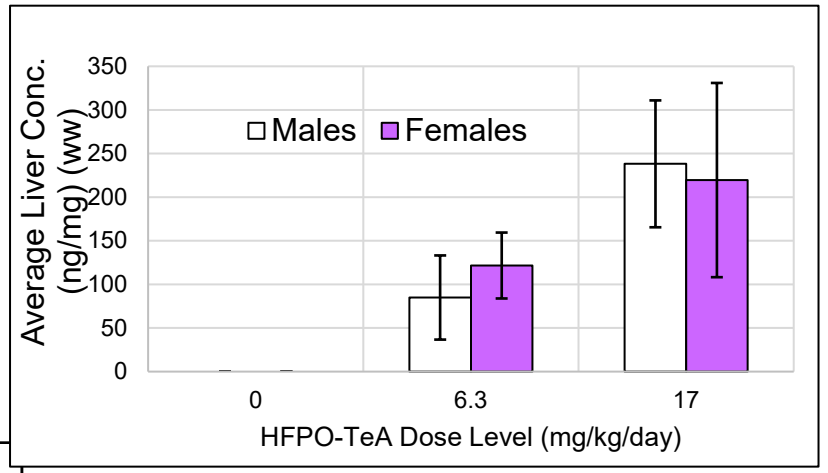
- For T4 at 17 mg/kg/day compared to Vehicle Control, p = 2.09E-06.
  - Decreased T4 observed by Conley *et al.* after exposure to GenX and PFOS.
- Results for T3 and rT3 decreased only slightly at 17 mg/kg/day.
- Similar concentrations for male and female rats.

Environ. Int., 2022, 160, 107056. *Environ. Health Perspect.*, 2019, 127, 37008.

Plasma and Liver Dosimetry

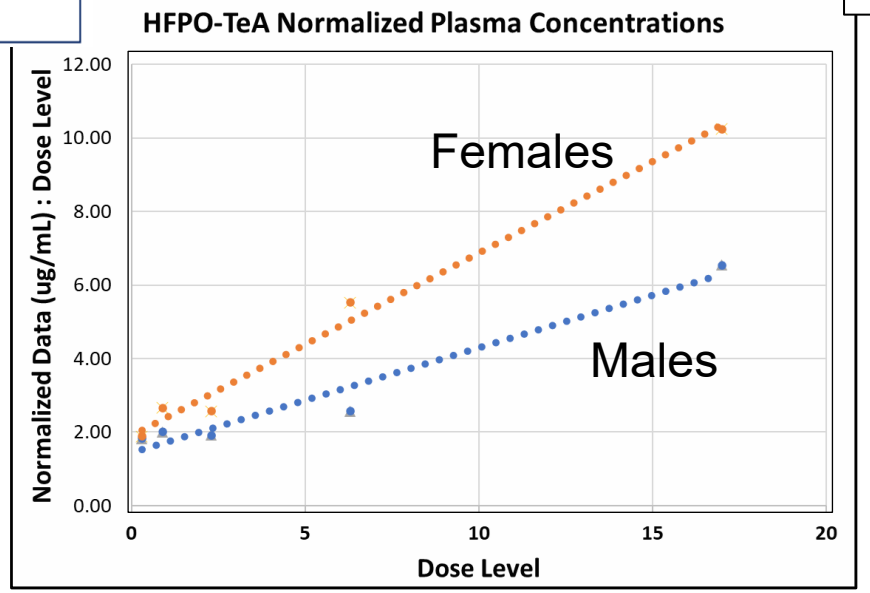






HFPO-TeA internal dose concentrations tended to be lower for males for plasma only.

Significant sex-associated differences observed at two highest plasma doses.



High biological variability in internal dose for high dose livers.

Plasma internal dose concentrations increase linearly, suggesting bioaccumulation.

Biotransformations Non-Targeted Analysis Workflow

Protein Precipitation or Solid Phase Extraction

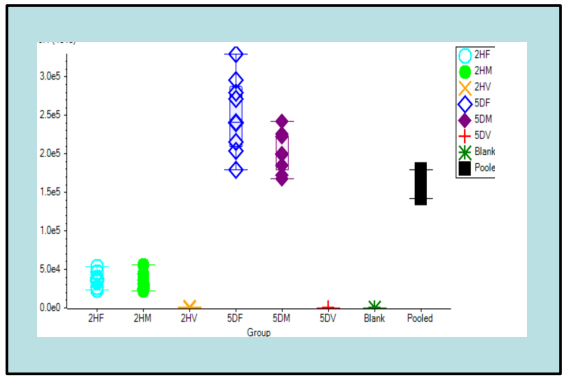
High Resolution, Accurate Mass, Data Dependent and Data Independent Scan Modes

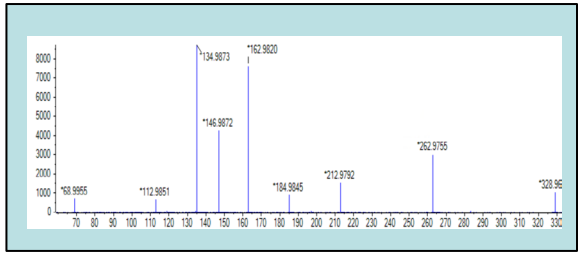
Peak Picking, Grouping, Alignment
Sciex OS 2.1

Data Filtering

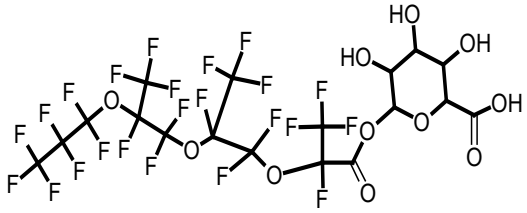
Mass Defect
Sample/Blank Ratio
Characteristic Fragments
Fold Change and p Value
Sciex MarkerView 1.3

Data Visualization





Spectral Library Searches - NIST 2017; Sciex Fluorochemical 2.0; In-House PFAS
In Silico Metabolite Predictions – Chemical Transformations Simulator; Biotransformer 3.0
In Silico Fragmentation Predictions - MetFrag

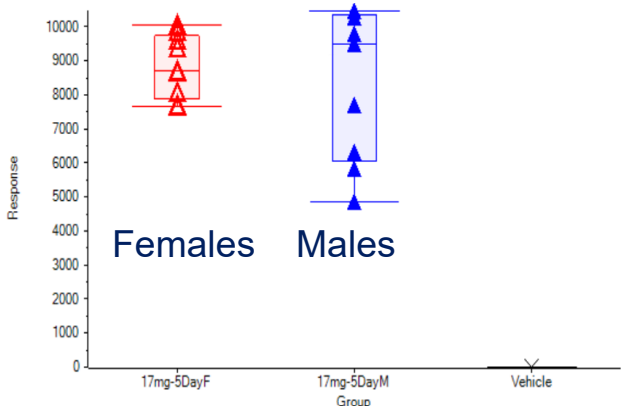


Identification

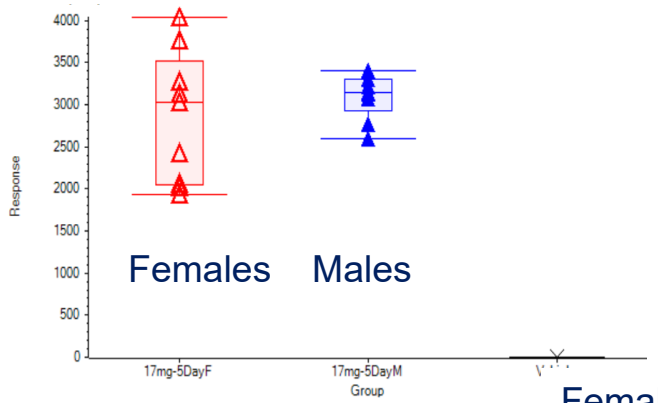
Ions of Interest

Several ions with high fold changes versus vehicle controls and negative mass defect were selected for further evaluation.

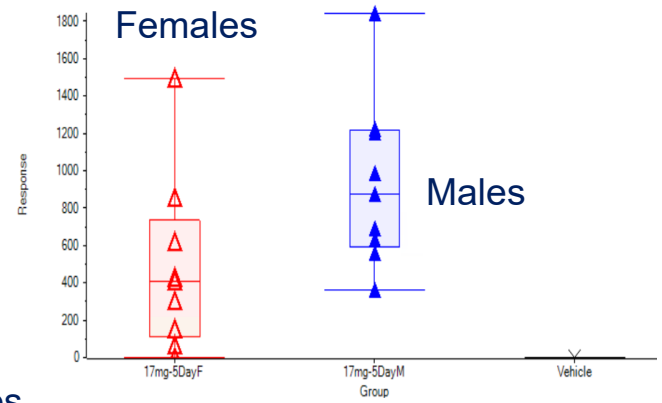
m/z 328.9670 Log (FC) = 9.5



m/z 516.9530 Log (FC) = 8.5

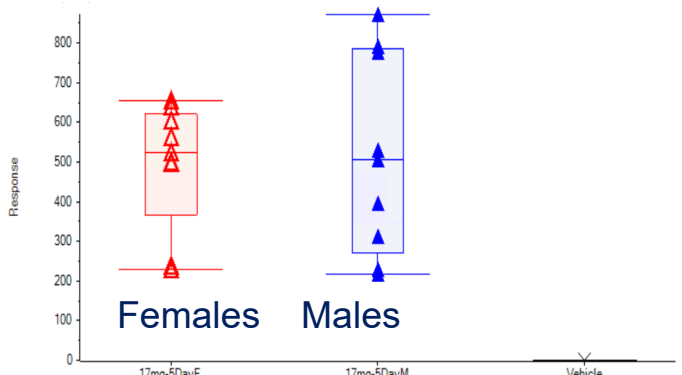


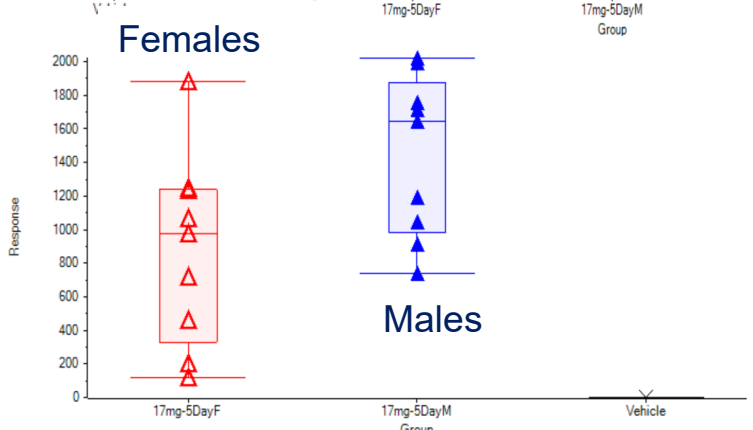
m/z 990.9120 Log (FC) = 7.7



m/z 723.9126
Log (FC) = 7.7

Co-elutes with m/z 328.9670





m/z 1012.8940
Log (FC) = 7.9

Co-elutes with m/z 990.9120
Δ 22 Da suggests potential Na adduct

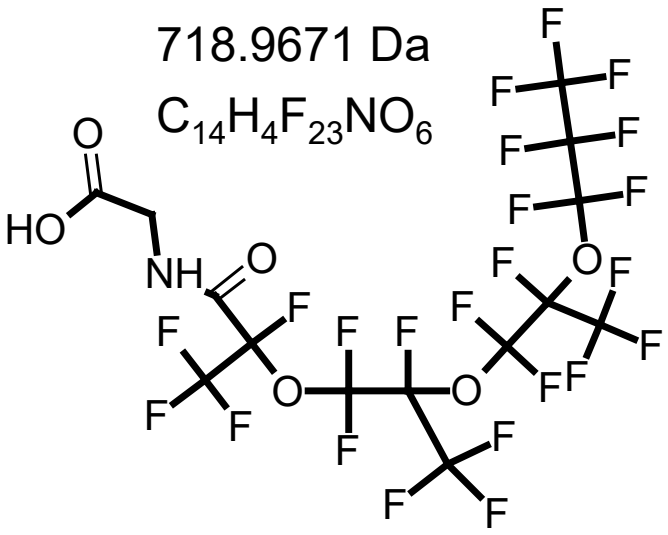
Predicted Biotransformation Products - Mammalian

Glycine Conjugate –

- Human Phase II metabolism
- Endothelial cell (EC) metabolism

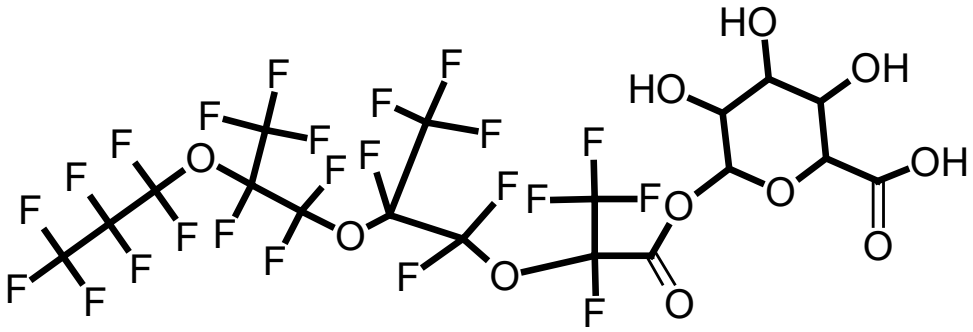
718.9671 Da

$C_{14}H_4F_{23}NO_6$



Glucuronidation –

- Human gut microbiota
- Phase II metabolism
- EC metabolism



837.9777 Da

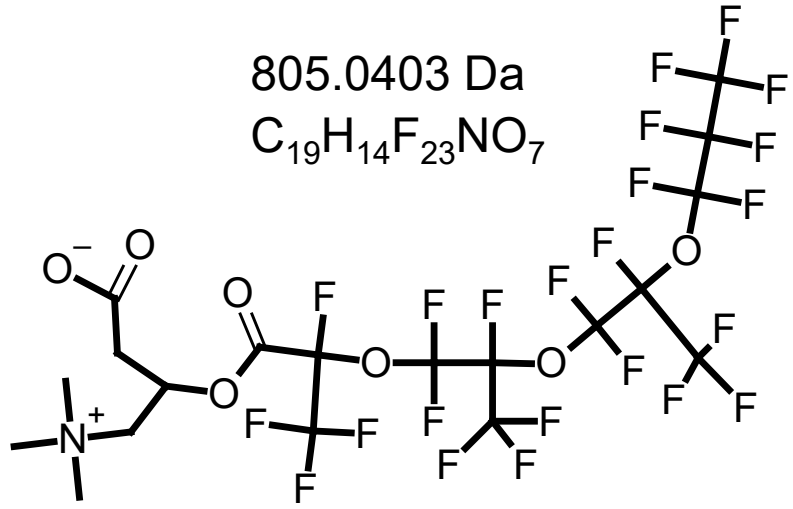
$C_{18}H_9F_{23}O_{11}$

Acyl carnitine–

- EC metabolism

805.0403 Da

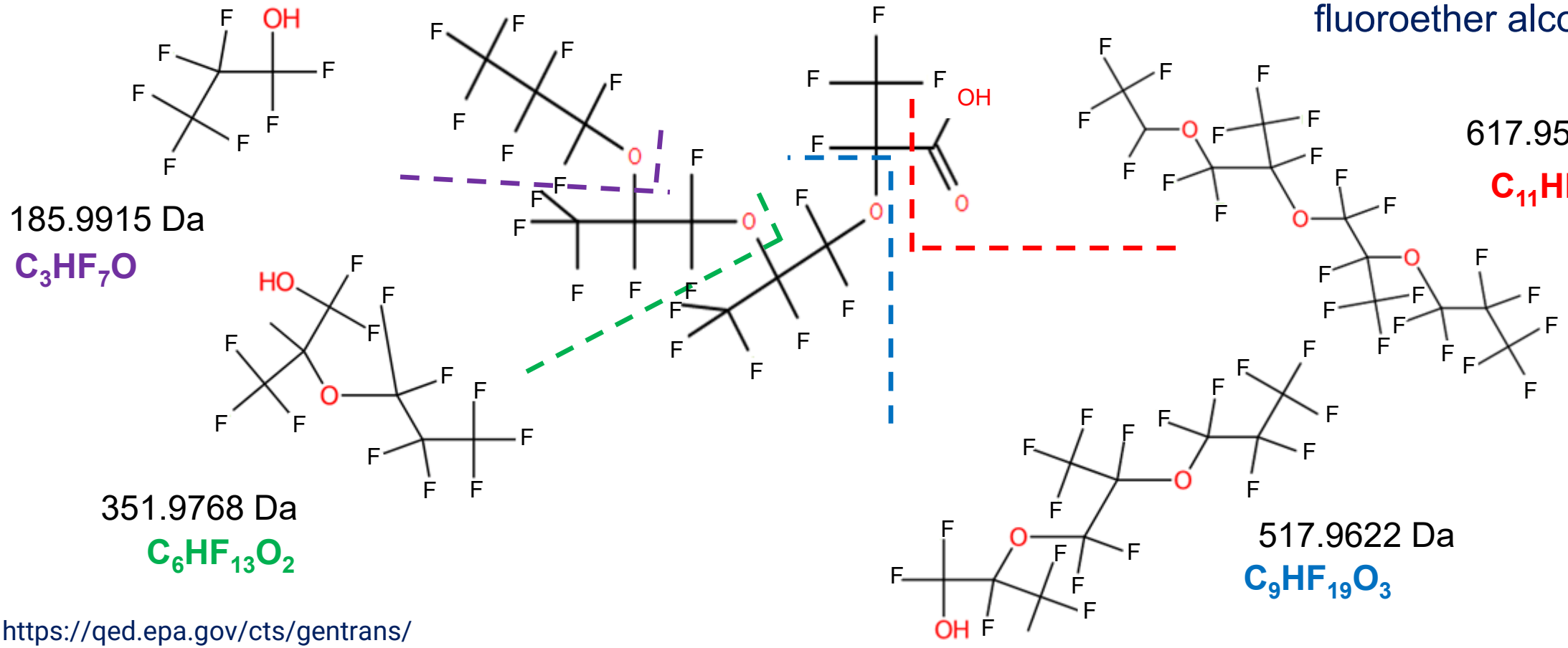
$C_{19}H_{14}F_{23}NO_7$



https://qed.epa.gov/cts/gentrans/

Predicted Biotransformation Products - Environmental Biocatalysis/Biodegradation

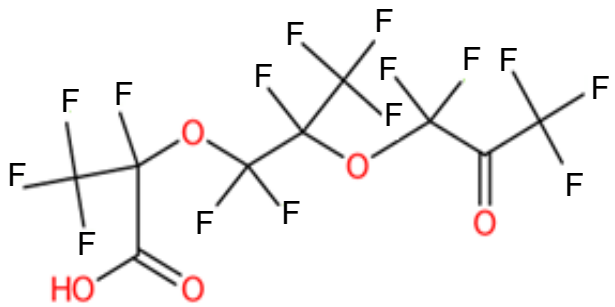
Dealkylation at carboxylate and reduction reactions to give fluoroether alcohols



https://qed.epa.gov/cts/gentrans/

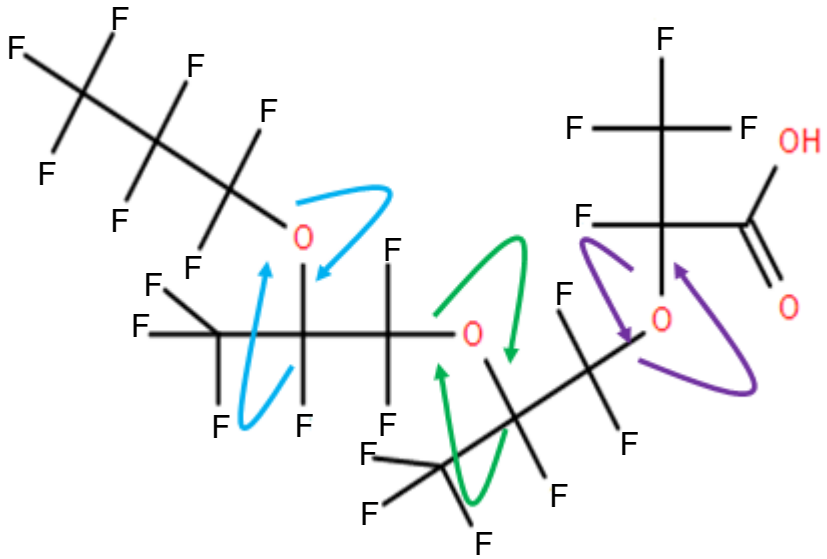
Predicted Biotransformation Products -
Environmental Biocatalysis/Biodegradation

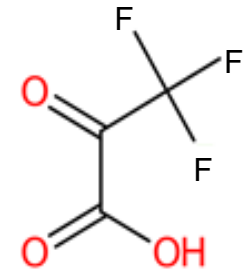
Dealkylation and fluorine rearrangement reactions to yield keto acids



473.9584 Da

$C_9HF_{15}O_5$



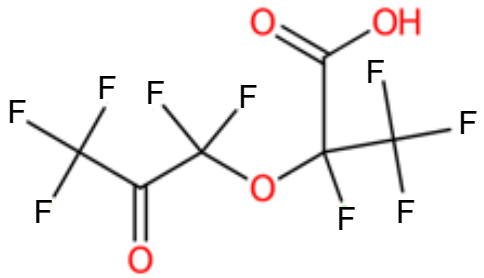


141.9877 Da

$C_3HF_3O_3$

307.9731 Da

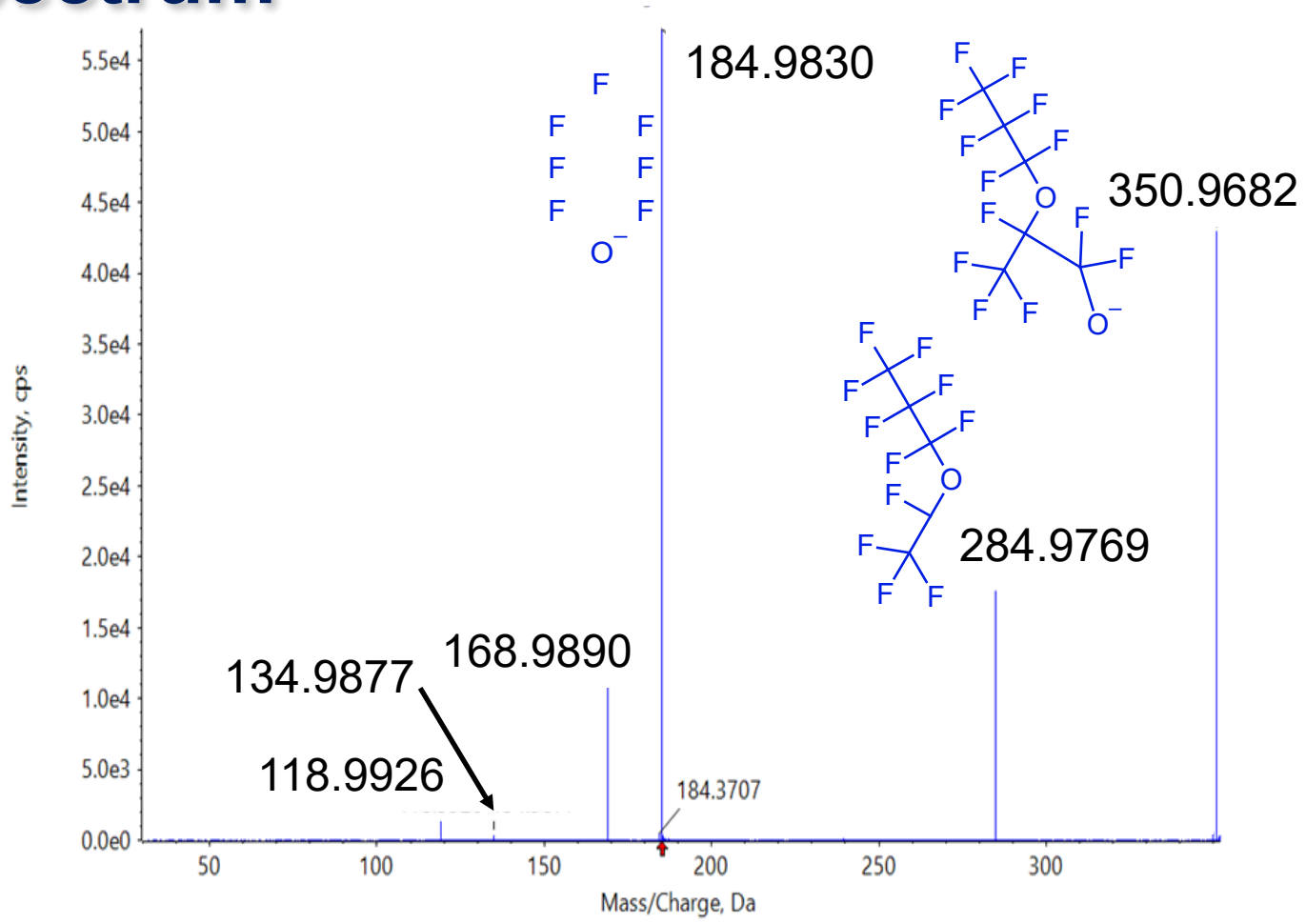
$C_6HF_9O_4$



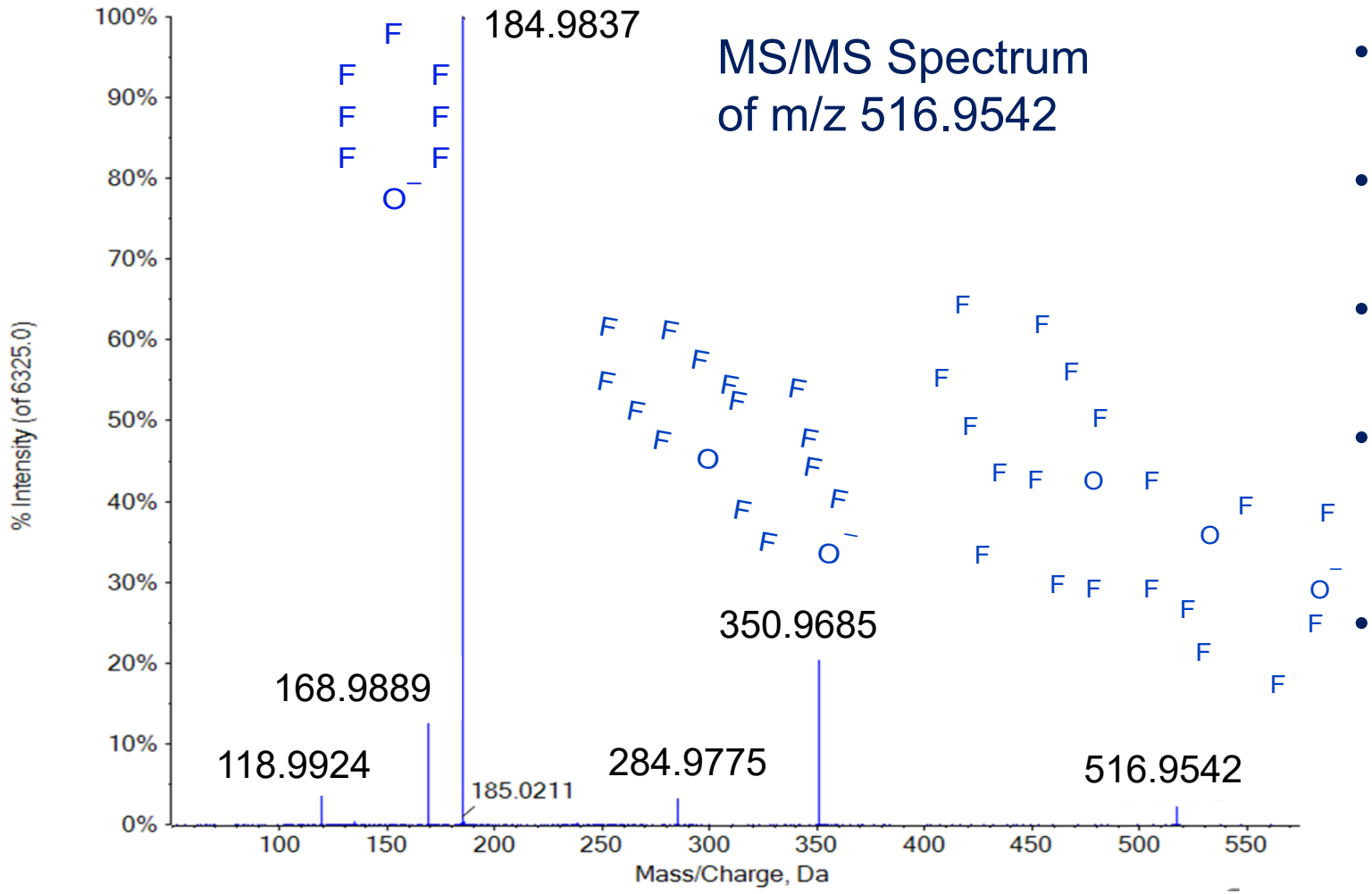
https://qed.epa.gov/cts/gentrans/

Characteristic Ions From HFPO-TeA MS/MS Spectrum

Fragment Ion, *m/z*	Fragment Ion Formula
350.9682	$C_6F_{13}O_2^-$
284.9769	$C_5F_{11}O^-$
184.9830	$C_3F_7O^-$
168.9890	$C_3F_7^-$
134.9877	$C_2F_5O^-$
118.9926	$C_2F_5^-$



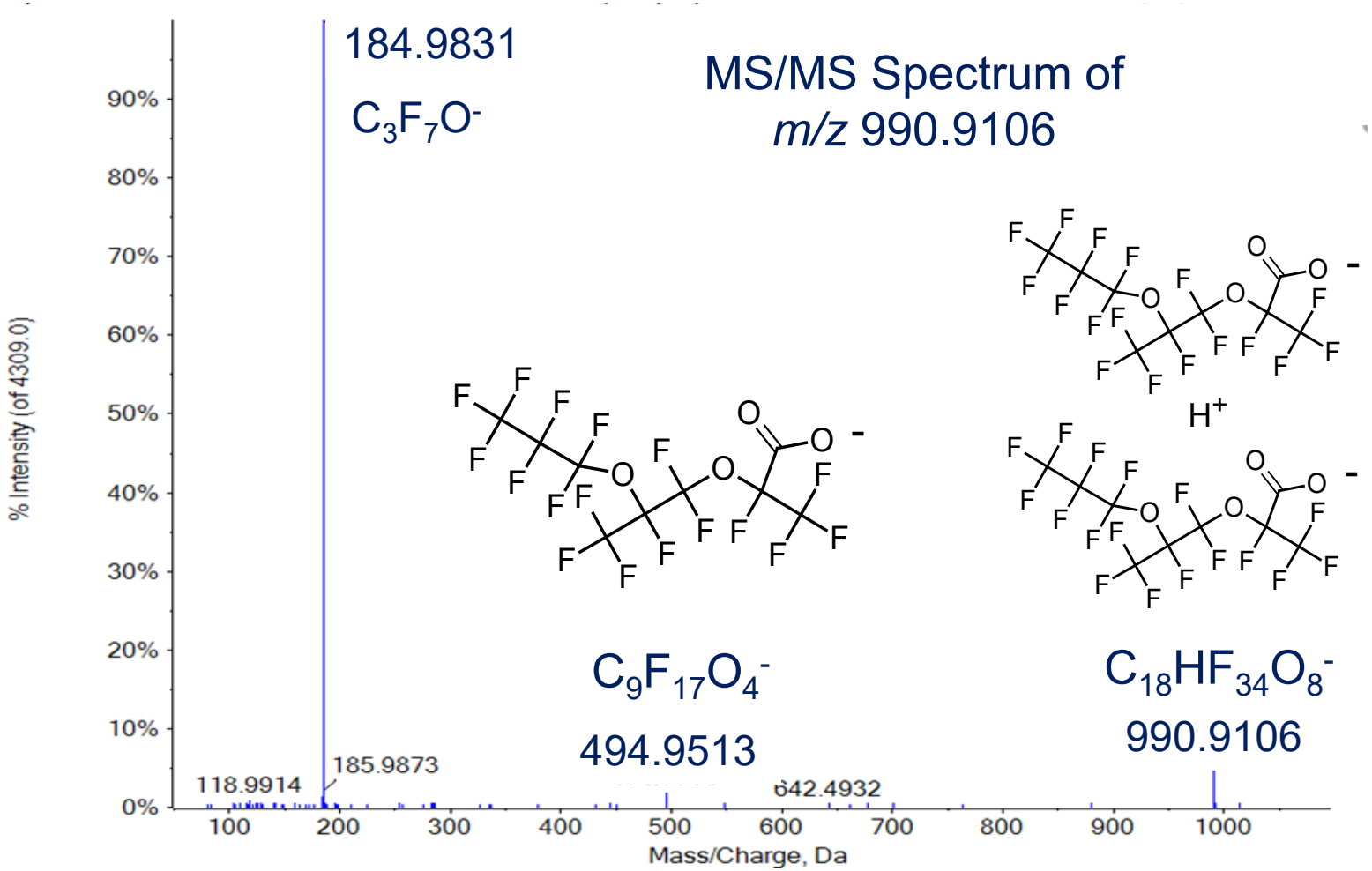
Biotransformation Products: Identified Compound 1



- Characteristic HFPO-TeA backbone fragments observed.
- Mass match with predicted metabolite.
- Spectral match to *in silico* MetFrag spectrum.
- Data suggests *O*-dealkylation of HFPO-TeA yielding $C_9HF_{19}O_3$, a perfluoro alcohol.
- ID with Schymanski level 3 Confidence – *Exact mass, formula, tentative candidate.*

Environ. Sci. Technol., 2014, 48, 2097−2098. https://ipb-halle.github.io/MetFrag/

Biotransformation Products: Identified Compound 2

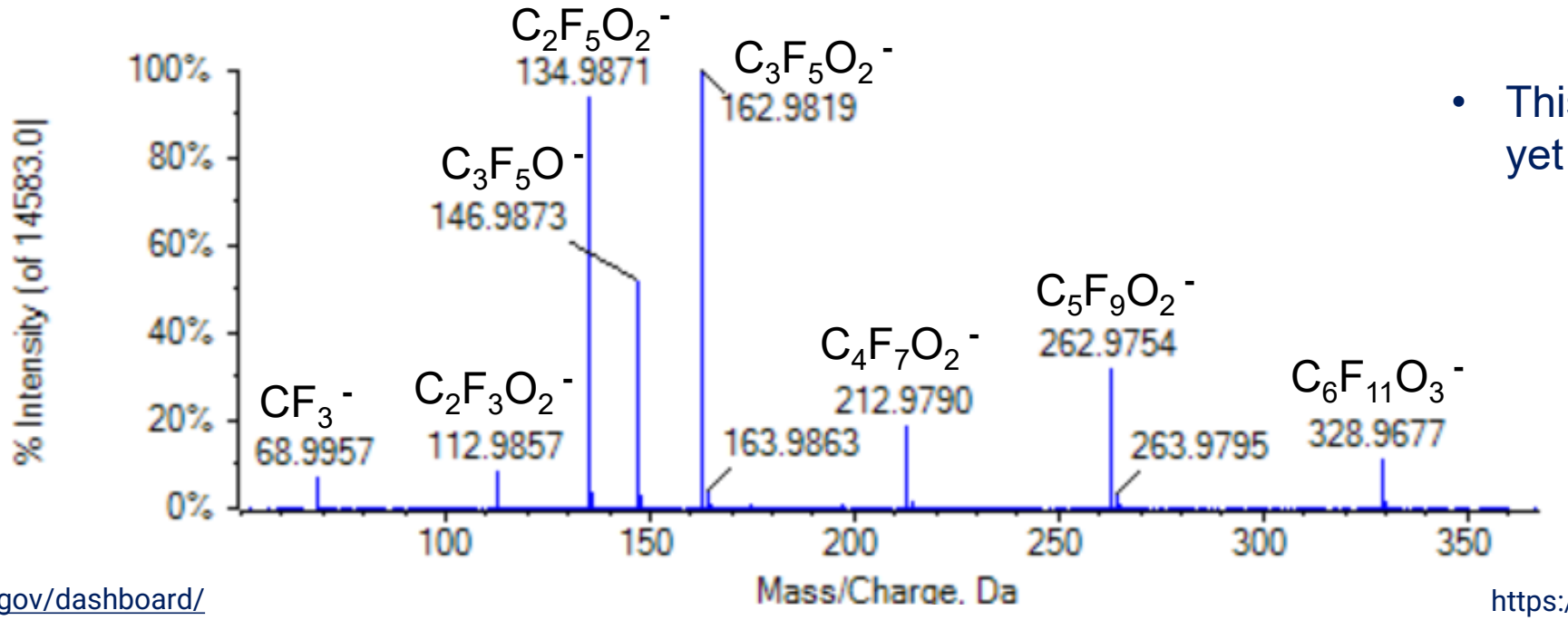


- *m/z* 494.9513 observed in *m/z* 990.9106 MS/MS spectrum.
  - Suggests higher mass ion is a dimeric species.
  - Monmer fits $C_9F_{17}O_4^-$ within 5 ppm.
  - Matches spectrum and retention time obtained with HFPO-TA standard.

  Co-eluting dimer Na adduct suggests carboxylic acid moiety.
- *m/z* 990.9106 is proton bound dimer of HFPO-TeA.
- ID with Schymanski Level 1 Confidence – *Confirmed structure with authentic standard.*

Biotransformation Products: Compound 3

- *m/z* 328.9677 matches to $C_6F_{11}O_3^-$ - same formula as the (M-H)- ion of GenX.
- Spectrum includes characteristic PFAS fragment ions; NOT consistent with GenX spectrum.
- Ion elutes at a retention time for a C10 ion, however, suggesting this species is an in-source fragment of a higher molecular weight PFAS.
- Ion of *m/z* 723.9126 co-elutes; MS/MS spectrum includes an *m/z* 328.9677 fragment ion. No formulas consistent with typical PFAS matched this mass in the CompTox Dashboard. A search in MetFrag did not yield a matching spectrum.



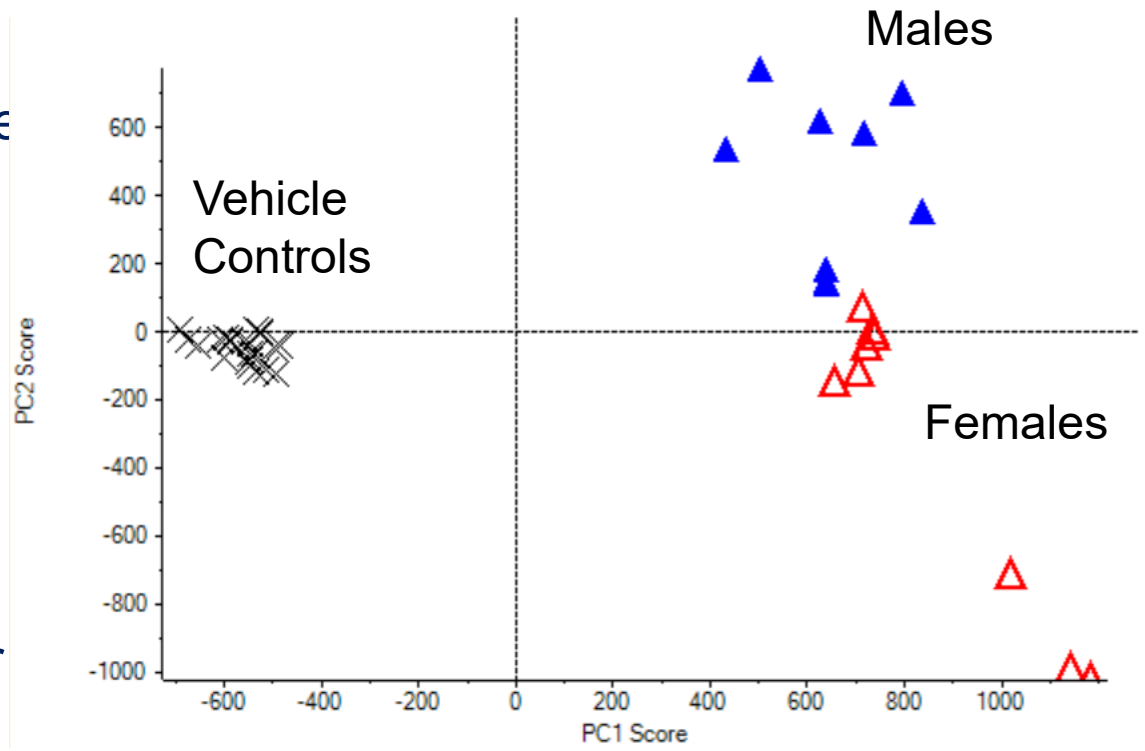
- This species is not yet identified.

https://comptox.epa.gov/dashboard/

https://ipb-halle.github.io/MetFrag/

Principle Component Analysis

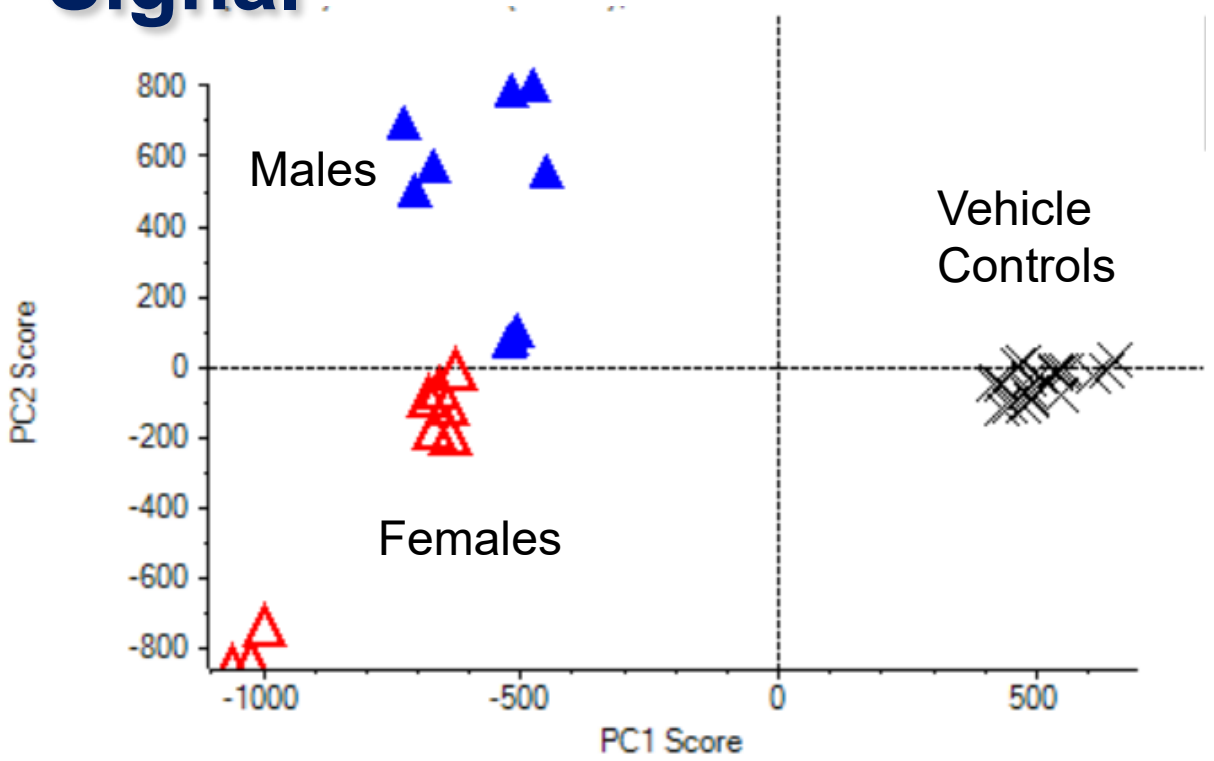
- Scores plot for PCA of all detecte ions (SWATH mode) – 17 mg/kg/day plasma and vehicle controls
- Exposed samples are clearly differentiated from vehicle controls.
- Loadings plot (*not shown*) indicates HFPO-TeA is driver for differentiation from vehicles.



- Exposed samples show general clustering according to sex.

Principle Component Analysis without HFPO-TeA Signal

- Signal for HFPO-TeA excluded from data analysis.
- Exposed samples are clearly differentiated from vehicle controls.
- Loadings plot (*not shown*) indicates non-PFAS drive differentiation from vehicles.



- Sex-associated clustering maintained independent of presence of HFPO-TeA signal.

Biotransformation and NTA Observations

- Potential biotransformation products (PBTPs) after HFPO-TeA exposure were observed in rat plasma.
- The presence of HFPO-TA after HFPO-TeA exposure was confirmed in plasma with standards. HFPO-DA was not observed.
- Preliminary vitro assays with rat hepatocytes suggest the presence of the ion of *m/z* 516.9 (perfluoro alcohol) but not HFPO-TA. The human hepatocyte assay with HFPO-TeA is under development.
- No PBTPs were observed in preliminary NTA of rat liver.
- Presence of characteristic fragment ions, negative mass defect, high fold change compared to vehicles, comparative retention times, and knowledge of PFAS ion behavior presented more opportunities for identification of PBTPs than library searching and use of Kendrick mass defect.
- Further investigation of ions already detected by NTA may provide insights into metabolic response to HFPO-TeA exposure.

Next steps and future direction

- Resolve identities for additional potential PFAS and other features in NTA data.
- Re-evaluate liver preparation to reduce potential interferences which may have prevented detection of low levels of PBTPs.
- Investigate sex-associated differences in exposure response.
- Optimize *in vitro* assays for rat and human hepatocytes and explore other cell models such as for gut and/or intestinal.
- Evaluate liver lipidome:
  - liver damage often has been observed after exposure to other PFAS,
  - preliminary transcriptomics results suggest disruption of genes associated with lipid regulation.
- Short term exposure studies planned for additional individual PFAS and mixtures.

Acknowledgements

Bioanalytical Chemistry

Aero Renyer*

Jermaine Ford

Amanda Brennan

Bob Payne*

Jackson Bounds*

In Vitro Assays

Barbara Wetmore

Marci Smeltz

Krishna Ravindra*

Matt Phillips*

ITAP Team

Leah Wehmas

Mike DeVito

Mike Hughes

Susan Hester

Adam Swank

Logan Everett

Rubia Martin*

*: Oak Ridge Institute for Science and Education

https://www.maxpixel.net/Sky-Maryland-Reflection-Chesapeake-Bay-Water-1310538