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Introduction

Per- and polyfluorinated substances (PFAS) are frequently used in industrial processes and commercial products, leading to environmental contamination, exposure, and the potential for adverse health effects. With toxicological data available for only a few of the 1000s of known PFAS, the USEPA is using short term PFAS exposure studies to:

- propose interim benchmark dose levels,
- promote understanding of the biological responses to exposure,
- and explore possible biotransformations in mammals.

Biotransformation of a few categories of PFAS such as telomers have been observed in microbes and fish, but few other species. Here we present results of non-targeted analysis (NTA) of plasma from rats exposed over five days to multiple concentrations of perfluoro(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid (PF-TODoA) to identify potential biotransformation products (PBTPs).
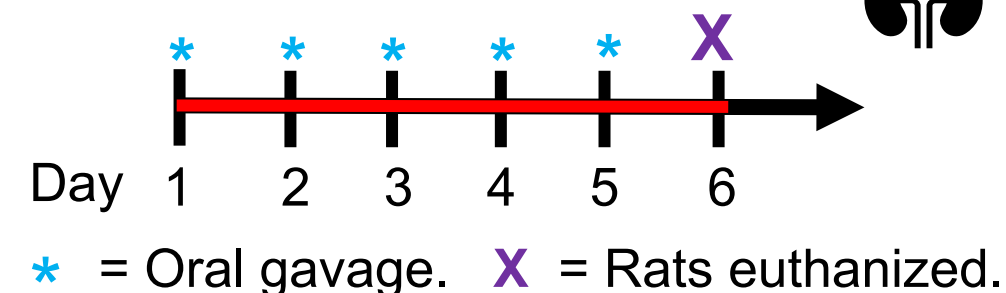
Methods

Exposure Study

PF-TODoA dose formulation

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

Five-day, repeat dose rat study and in-life analyses

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

Day 1 2 3 4 5 6

* = Oral gavage.   X = Rats euthanized.
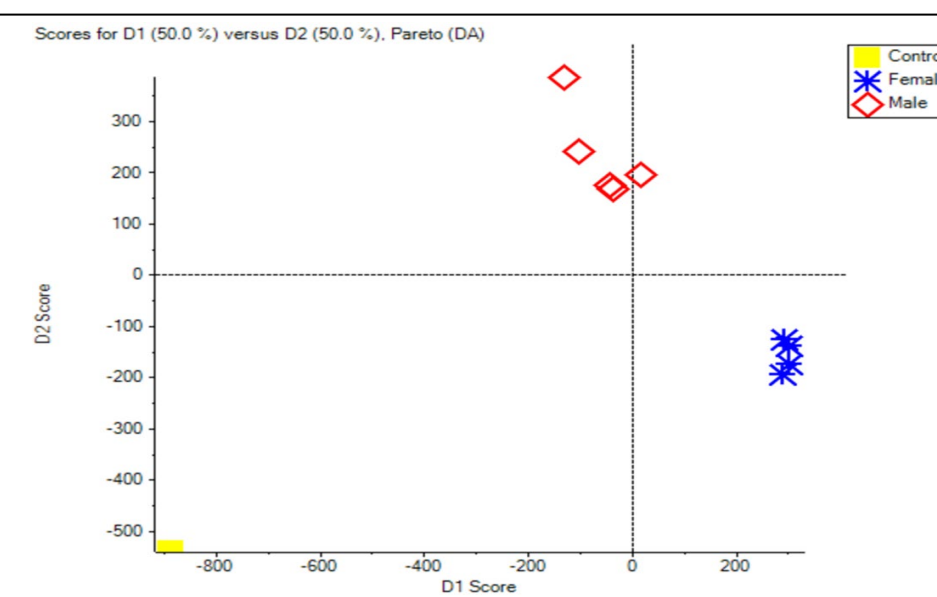
Analytical Chemistry

- Plasma (25 µL) was diluted and prepared by protein precipitation with acidified acetonitrile.
  - Chromatography: Phenomenex Kinetex XB-C18 (100 x 2.1 mm, 2.6 µm) with a 30-min linear gradient of A: 95:5 H_2O:MeOH and B: 95:5 MeOH:H_2O, both containing 4 mM ammonium formate.
- NTA acquired with Sciex X500R QTOF.
  - Electrospray ionization (ESI)
  - Negative ion mode
  - Independent Data Acquisition (IDA) and Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH) scanning.

Data Handling   Data processing, library searching, formula finding, diagnostic screening for characteristic fragment ions, peak picking and alignment, normalization, and statistical analysis were performed using Sciex OS 2.1.0 and MarkerView 1.3.1. A screening list of PBTPs of PF-TODoA was generated using Biotransformer 3.0 (www.biotransformer.ca).[1]

Results

Supervised Principal Component Analysis (PCA)

Plasma from male and female rats exposed to 17 mg/kg/day PF-TODoA were analyzed by NTA. Data were aligned and normalized prior to visualization by supervised PCA. The scores plot showed separation of the data for exposed samples from controls. Data from male and female rats clustered apart from controls and distinct from each other.


Predicted PBTPs

PF-TODoA

CASRN: 65294-16-8
DTXSID70191136

Data were screened for the presence of predicted PBTPs. Several were potentially observed, and some at such low abundances that MS/MS spectra could not be obtained.

| Predicted Biotransformation	Product	Formula	[M-H]⁻ Exact Mass	Observed (Yes/No) / Retention Time	MS/MS Spectrum?
Loss of CO_2	2H-Perfluoro(5,8-dimethyl-3,6,9-trioxadodecane)	$C_{11}HF_{23}O_3$	616.9480	Yes/ 18.6 min	No
Dealkylation	Loss of C_3F_8	$C_9HF_{15}O_5$	472.9506	Yes/ 17.1 min	No
Reduction to primary alcohol	Perfluoropropanol	C_3HF_7O	184.9837	Yes/16.3 min, 18.6 min, and 20.0 min	Yes (20.0 min)
O-Dealkylation	Loss of $C_6F_{14}O$	$C_6HF_9O_4$	306.9653	Yes/27.8 min	No
Dealkylation of carboxylate terminus	Perfluoro-2-methyl-3-oxahexanol	$C_6HF_{13}O_2$	350.969	Yes/18.6 min and 20.0 min:	No
Formation of keto carboxylic acid	3,3,3-trifluoro-2-oxopropanoic acid	$C_3HF_3O_3$	140.9799	No	No
O-dealkylation	Loss of $C_3F_4O_2$	$C_9HF_{19}O_3$	516.9544	Yes/20.0 min	Yes
O-glucuronidation	PF-TODoA O-Glucuronide	$C_{18}H_9F_{23}O_{11}$	836.9699	No	No

The MS/MS spectrum of the PF-TODoA in-source fragment ion shows characteristic fragment ions that are expected to also appear in PBTP spectra.
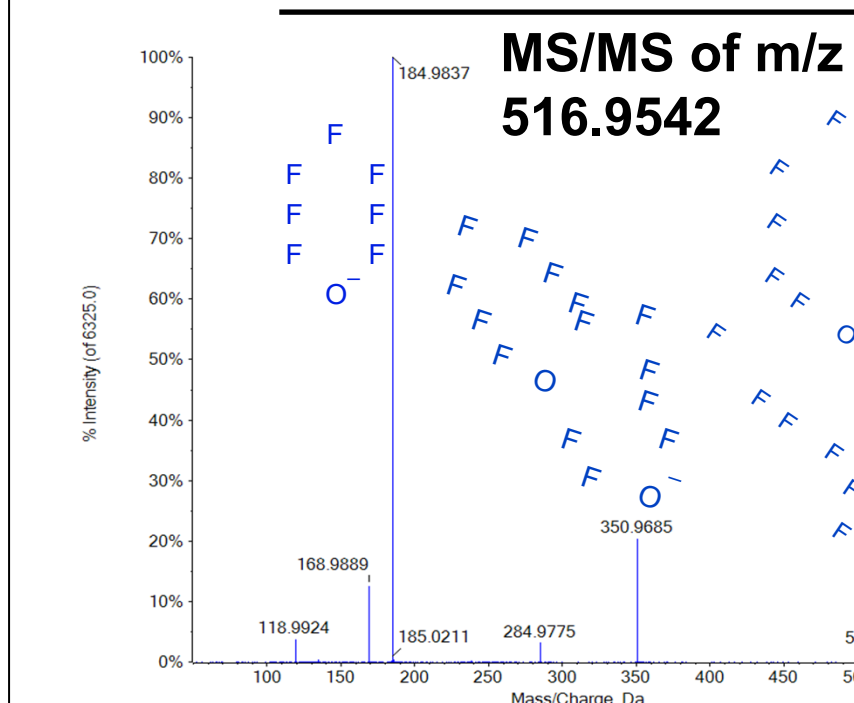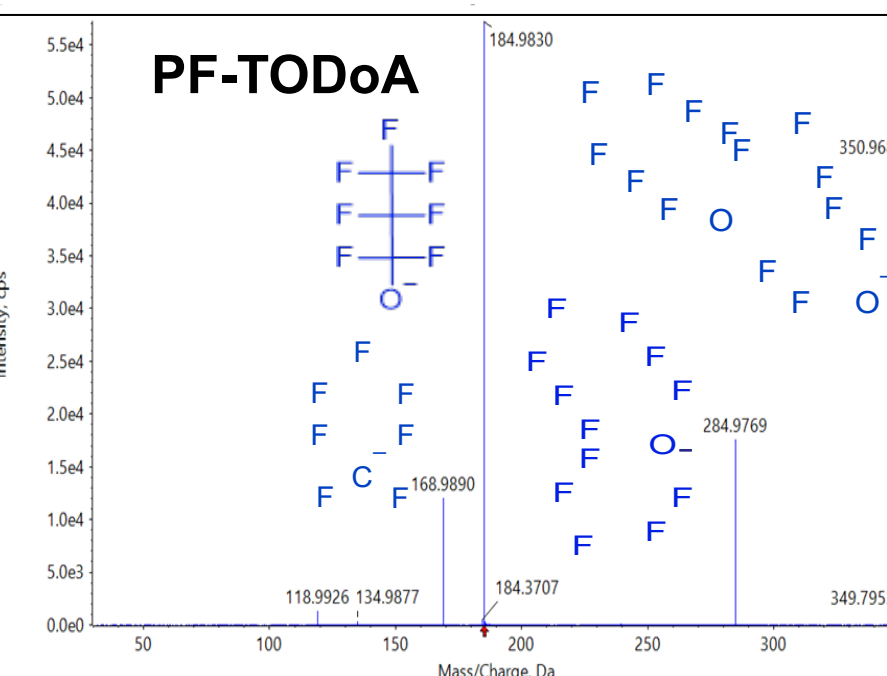

The MS/MS spectrum of the PBTP ion of m/z 516.9542 gives fragments that suggest O-dealkylation to yield an alcohol with the formula $C_9HF_{19}O_3$.

Fold changes, negative mass defect, and PFAS-characteristic fragment ions were also used to filter for PBTPs. Shown below is the MS/MS spectrum of a significant feature not found in controls. The dimer ion m/z 990.9106 ([2M-H]⁻) fragments to give a monomer ion of m/z 494.9 and $C_3F_7O^-$. The monomer mass fits to $C_9HF_{17}O_4$ within 5 ppm.
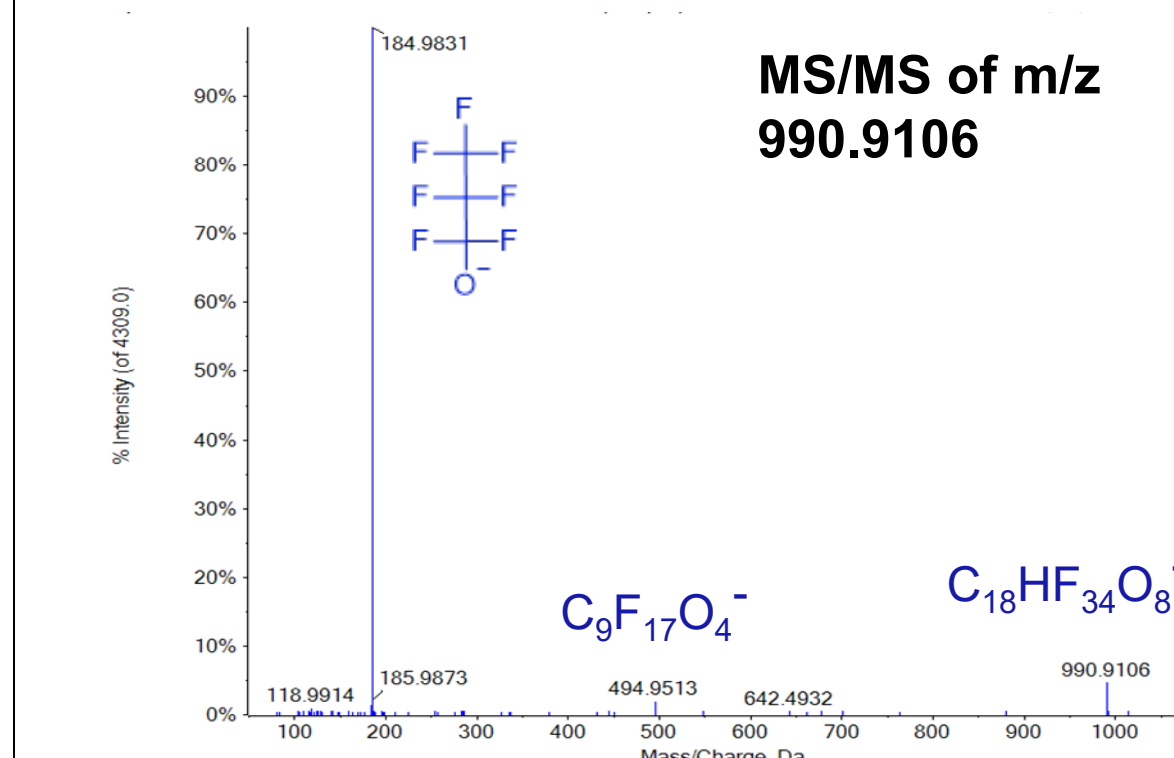
Discussion

Visualization of the NTA data showed a distinction between results for male and female rats. Targeted analysis for determination of internal dose showed similar results for PF-TODoA. [2] The NTA data expands that observation and provides a broad overview of response to exposure. Shown below are example box-and-whisker plots of SWATH peaks areas for PF-TODoA and two PBTPs.

PF-TODoA   m/z 516.9: (M-H)⁻ of $C_9HF_{19}O_3$ m/z 990.9: (2M-H)⁻ of $C_9HF_{17}O_4$
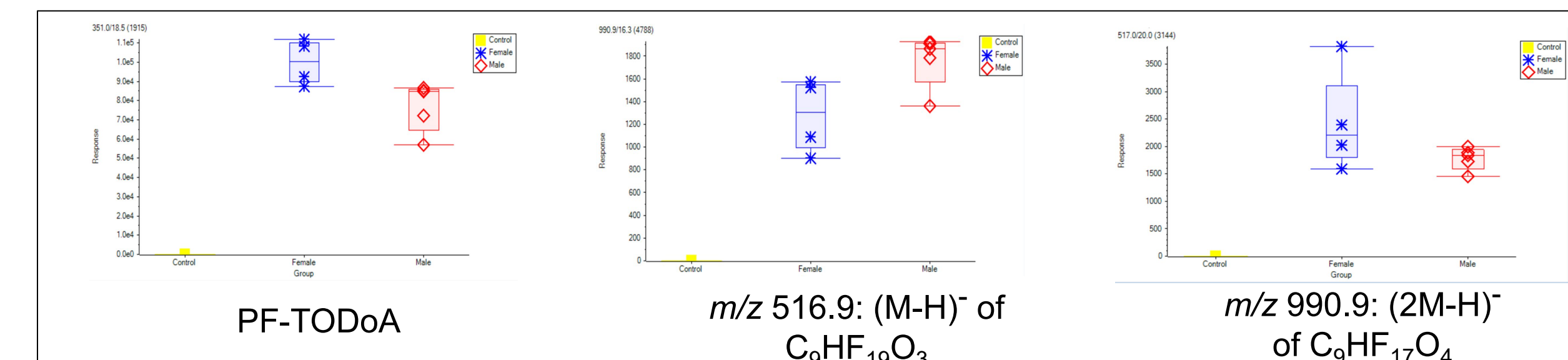
NTA enabled tentative identification of one predicted PBTP, a primary alcohol formed by cleavage of an ether bond and loss of the C_2F_4COOH headgroup from PF-TODoA. The observed mass agrees with the formula $C_9F_{19}O_3^-$ to within 5 ppm. The fragment ions observed at m/z 350.9685 and m/z 184.9837 are consistent with sequential cleavages of C_3F_6O. These data suggest the identity of the precursor may be a perfluoro-2,5-dimethyl-3,6-dioxanonanol, formula $C_9HF_{19}O_3$, with 2b level confidence[3]. A reference spectrum or standard could not be obtained to provide further verification.

A second species of interest, the ion of m/z 990.9106, was found by filtering NTA data for fold changes compared to controls, negative mass defect, and presence of diagnostic fragments such as $C_3F_7O^-$. The MS/MS spectrum suggests the ion is the (2M-H)⁻ dimer of m/z 494.9513 which gives a formula fit of $C_9F_{17}O_4^-$. Further structural clues could not be derived from the spectrum, and the monomer did not generate a separate MS/MS spectrum. These data suggest the precursor is $C_9HF_{17}O_4$ with level 4 confidence.

Conclusions

NTA of plasma from in vivo exposure to PF-TODoA was used to gain insights into biological response and potential biotransformation products. These data suggest gender-based differences upon PF-TODoA exposure. A more complete overview of metabolomic changes and transcriptomics could identify biological pathway changes and support benchmark dose level estimates.

The NTA results also suggest PF-TODoA is biotransformed to novel species in rats and provide clues to persistence of emerging PFAS after mammalian exposure.

References

1: Djoumbou Feunang et al., J Cheminformatics, 2019. doi: 10.1186/s13321-018-0324-5.
2. Renyer et al., ASMS 2022, Minneapolis, MN, Abstract 310541.
3. Schymanski et al., Env. Sci. Technol., 2014, 48, 2097-2098. doi: 10/1021/es5002105.