Non-targeted Analysis for Identification of PFAS Biotransformation Products in Rat Plasma

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Introduction

Per- and polyfluorinated substances (PFAS) are dispersed throughout the environment due to widespread commercial use, which leads to exposure and the potential for adverse health effects. Toxicological data are available for only a few of the nearly 10,000 currently known PFAS. The USEPA is using short term PFAS exposure studies to:

- generate interim risk assessment data for priority chemicals concern to states and EPA Regions,
- explore possible biotransformations in mammals,
- and further understanding of the biological responses to exposure.

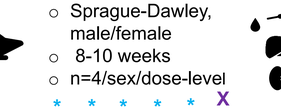
Biotransformations of PFAS telomers have been observed in microbes and fish, but few other PFAS or species have been studied. Here we present results of non-targeted analysis (NTA) to identify potential biotransformation products (PBTPs) in plasma from rats exposed over five days to perfluoro(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid (PF-TODoA), a perfluoroether carboxylic acid oligomer of GenX, and begin to investigate biological response.

5-Day *In Vivo* Repeat 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

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



* = Oral gavage. X = Rats euthanized.

Analytical Chemistry

- Plasma (25 µL) prepared by protein precipitation with acidified acetonitrile
  - Chromatography: Phenomenex Kinetex XB-C18 (100 x 2.1 mm)
  - 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
  - Negative ion electrospray ionization (-ESI)
  - Information Dependent Acquisition (IDA) and Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH) scanning

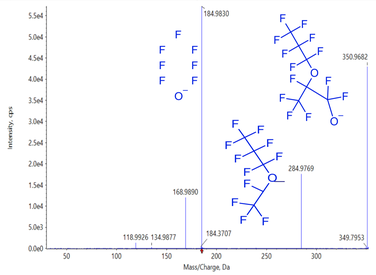
Data Handling

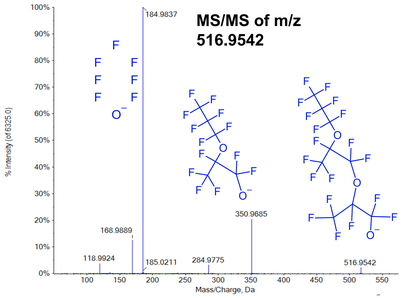
Data processing, library searching (NIST 17, Sciex Fluorochemical, and in-house libraries), formula finding, peak picking and alignment, normalization, and statistical analysis were performed using Sciex OS 2.1.0 and MarkerView 1.3.1. Features were filtered by log fold change, $p < 0.05$, negative mass defect, and abundance. A screening list of PBTPs was generated using Biotransformer 3.0.[1] MetFrag[2] was used to generate *in-silico* spectra of potential candidates.

Results

PF-TODoA
CASRN: 65294-16-8
DTXSID70191136

The MS/MS spectrum of the in-source fragment of **PF-TODoA, also known as HPFO-TeA,** shows characteristic fragment ions that are also expected to appear in PBTP spectra.



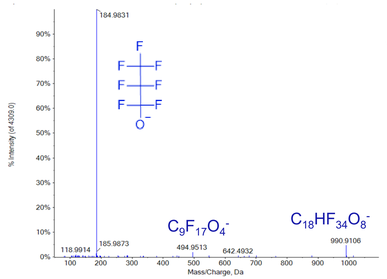


Predicted PBTPs

Data were screened for predicted PBTPs. Exact masses observed in MS scans suggested several predicted PBTPs were present, but only the ion of *m/z* 516.9542 gave an MS/MS spectrum. The fragment ions observed at *m/z* 350.9 and *m/z* 184.9 are characteristic of PF-TODoA and consistent with sequential cleavages of C_3F_6O. An *in-silico* spectrum generated for PubChem CID 13244841 (ion structure shown) with MetFrag matched all experimental fragment ions. The data suggest O-dealkylation of PF-TODoA to yield an alcohol with the formula $C_9HF_{19}O_3$.

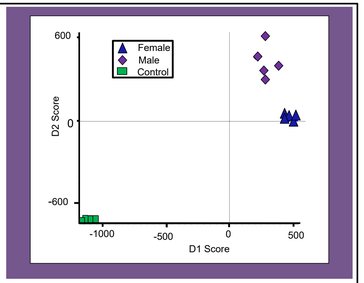
Additional PBTPs

Fold changes, negative mass defect, and characteristic fragment ions were also used to filter for PBTPs. Shown here 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 a $C_3F_7O^-$ ion. The neutral monomer mass fits to $C_9HF_{17}O_4$ within 5 ppm. The spectrum and retention time matched to those for a commercial standard of perfluoro(2,5-trimethyl-3,6-trioxanonanoic) acid (HFPO-TA), another perfluoroether carboxylic acid oligomer of GenX.

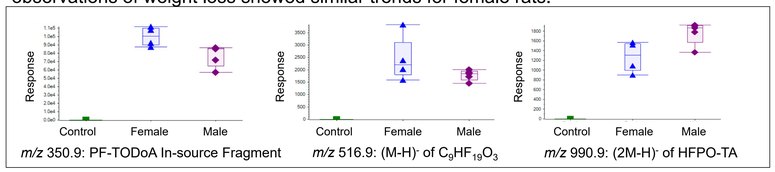


Statistical Analysis

NTA data from plasma of male and female rats exposed to 17 mg/kg/day PF-TODoA were normalized and analyzed by supervised principal component analysis (PCA-DA). The scores plot showed separation of the data for exposed samples from study controls. Data from male and female rats clustered apart from controls and distinct from each other.



Differences in normalized responses of selected significant ions between groups are shown in the plots below. Targeted determination of internal dose for PF-TODoA and clinical observations of weight loss showed similar trends for female rats.[3]



m/z 350.9: PF-TODoA In-source Fragment | *m/z* 516.9: $(M-H)^-$ of $C_9HF_{19}O_3$ | *m/z* 990.9: $(2M-H)^-$ of HFPO-TA

Conclusions

NTA of plasma from exposed rats provided evidence of PF-TODoA biotransformation and insights into biological response to PF-TODoA exposure.

- The ion of one predicted PBTP, *O*-dealkylation to yield an alcohol, was tentatively identified with level 3 confidence[4] as hexafluoro-2[hexafluoro-2-heptafluoropropoxy)propoxy]propan-1-ol (CID 13244841).
- The significance of the ion of *m/z* 990.9106 was highlighted through data filtering. Acquisition of a reference spectrum identified the compound as the GenX oligomer perfluoro(2,5-trimethyl-3,6-trioxanonanoic) acid (HFPO-TA) with level 1 confidence.
- Sex-based differences observed after PF-TODoA exposure were visualized through statistical analysis of the data set, which included features with endogenous and exogenous sources. Clinical observations of toxicity including weight loss also showed differences by sex. The greater weight loss observed for female rats may be related to the higher abundances of PF-TODoA in female plasma.

References

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