Internal Dose of Selected PFAS (Perfluoro-3-methoxypropanoic acid and) in Rat Plasma from Short Term Dosing Studies

Aero Renyer[1], Denise K. MacMillan[2], Michael DeVito[2], Michael F. Hughes[2], and Leah C. Wehmas[2]

1 – Oak Ridge Institute for Science and Education (ORISE)

2 - U.S. Environmental Protection Agency, Center for Computational Toxicology and Exposure

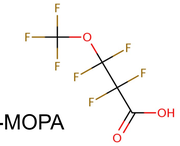
Aero Renyer | Renyer.Aero@epa.gov | 919-541-4438

Introduction

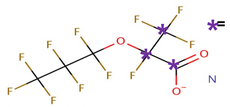
- Per- and polyfluoroalkyl substances (PFAS) are of growing concern due to their increasing environmental presence and persistence.
- Current estimates put the number of PFAS over 4700 yet only a select few have toxicological data available for public review and use.
- Several data-poor PFAS are also known to have high exposure potential to people.
- We are using a rat model and 5-day oral gavage exposures over multiple dose levels to provide a more rapid assessment of potential toxicity than the current 90-day and 2-year approaches.
- Quantitation of internal dose, along with toxicogenomics and thyroid hormone analysis, could yield primary points of departure based upon benchmark dose response modeling and enable timely risk prioritization for less-well studied PFAS.
- ***Plasma concentrations after exposures to perfluoro-3-methoxypropionic acid (PF-MOPA) are presented.***

Approach

1) Develop a single robust, reproducible, non-selective extraction method for multiple PFAS from rat plasma.
2) Develop a targeted LC/MS/MS method with sufficient sensitivity for detection of PFAS in plasma at all dose levels.
3) ***Apply method to plasma collected 24 hours after the 5-day exposure***



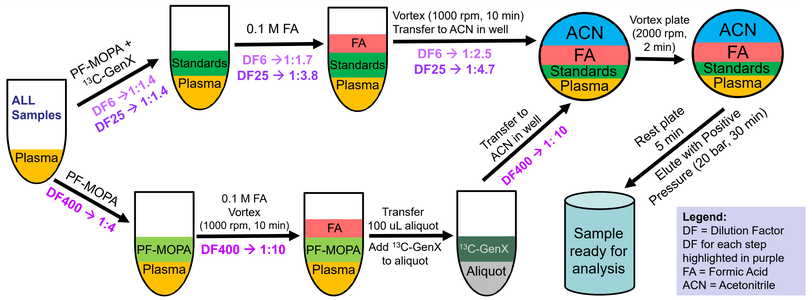
PF-MOPA



*= Carbon-13 isotope

Tetrafluoro-2-(Heptafluoropropoxy-propanoic acid) (^{13}C-GenX, internal standard)

Sample Preparation



Dilution Factor (DF) Volume Table

Plasma, µL	PF-MOPA stock OR MeOH, µL	^{13}C-GenX stock, µL	^{13}C-GenX stock conc. (ng/mL)	Formic acid, µL	Acetonitrile, µL	Total DF
25	5	5	500	25	100	1.4*1.7*2.5 = 6
25	5	5	1,000	100	500	1.4*1.38*4.7 = 25
25	100	20	1,000	875	900	4*10*10 = 400

Sample Prep: Samples are diluted as shown above. After dilution, protein precipitation (PPT) extraction was completed by applying the samples to a Biotage (Uppsala, Sweden) PPT+ 96-well plate to wells containing acetontrile. The plate is vortexed at 2000 rpm for 2 min then eluted using positive pressure (20 bar max for 30 min). The eluate is transferred to an autosampler vial and stored at -20 °C pending analysis.

Analytical Approach

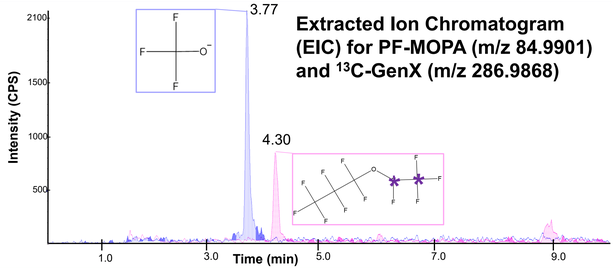
- LC/MS/MS was performed in negative ion, multiple reaction monitoring (MRM) with electrospray ionization (ESI) on a Sciex (Framingham, MA) X500R QTOF equipped with a Phenomenex (Torrance, CA) Kinetex XB-C18 (100 x 2.1 mm, 2.6 µm) column.
  - Flow rate: 0.200 mL/min
  - Injection volume: 5 µL
  - Mobile Phases:
    - A: 95:5 H_2O:MeOH with 4 mM NH_4formate
    - B: 95 MeOH:5 H_2O with 4 mM NH_4formate

Gradient

Time	%A	%B
0.00	98	2
1.00	35	65
5.00	0	100
7.00	0	100
7.10	98	2
10.0	98	2

MRM Fragments

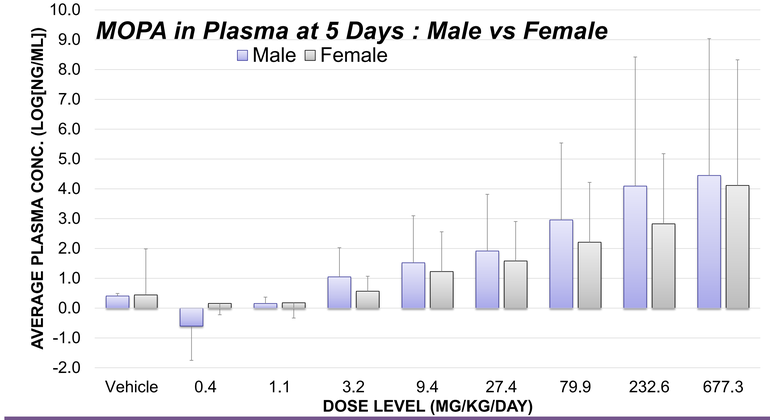
Compound	Molecular Ion	Fragment Ion
PF-MOPA	228.97	84.9901
^{13}C-GenX	331.98	286.9868



Extracted Ion Chromatogram (EIC) for PF-MOPA (m/z 84.9901) and ^{13}C-GenX (m/z 286.9868)

Results

Plasma Concentrations: PF-MOPA was detected above the limit of quantitation (1 ng PF-MOPA/mL plasma) in all dose levels and vehicles. PF-MOPA was not detected in method blanks. Concentrations in male rat plasma ranged from 56 µg/mL (677.3 mg/kg/day) to 1.38 ng/mL (0.4 mg/kg/day). Concentrations were between 2-18 times lower in female rat plasma in samples at and above the dose level of 3.2 mg/kg/day. A wide biological variation was also observed within all dose levels.



Conclusions

- **We developed a PPT-LC/MS/MS quantitative method for PF-MOPA in rat plasma.**
- Post 5-day exposure, male rat plasma had concentrations as high as 56 µg/mL, with female rat plasma having lower concentrations across the 6 highest dose levels.
- **The data suggests sex-related differences in processing of PF-MOPA.**
- Plasma concentrations paired with toxicokinetic data and body mass difference (BMD) analysis are compared to and potentially augment the transcriptomic point of departure (POD) results.
- *Our next step is to apply this non-selective method to 5-day oral gavage samples for Perfluoro-(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid.*