Internal dose and in-life results of perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoic acid exposure from short term dosing studies in rats

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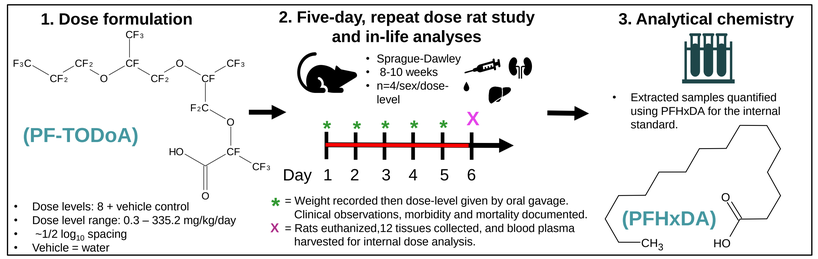
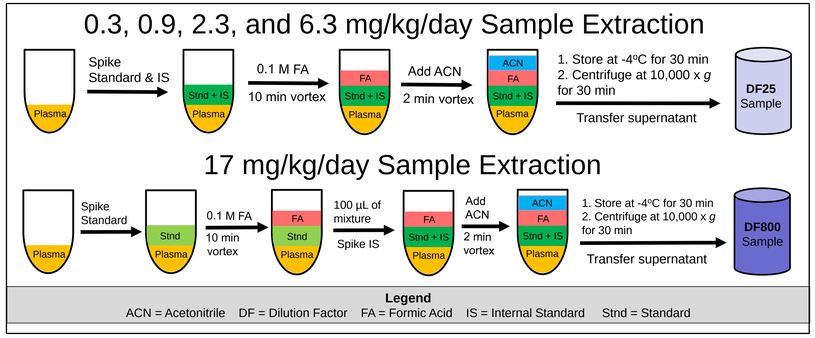
Introduction and Approach

- Per- and polyfluoroalkyl substances **(PFAS)** are widely-used industrial compounds.
- Current estimates of this growing class number over 4700 compounds.
- The majority of PFAS are lacking publicly available toxicological data.
- Our overall goal is to develop a more rapid assessment of potential toxicity than occurs with current 90-day and 2-year guideline bioassays.
- **Plasma concentrations and in-life observations for perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoic acid (PF-TODoA) are presented for dosimetry analysis, clinical response observation analysis, and potential correlation of the results.**



Sample Preparation



Dilution Factor Volume Guide

DF	Plasma (µL)	PF-TODoA stock (µL)	PFHxDA stock (µL)	PFHxDA stock conc. (ng/mL)	Formic acid (µL)	Acetonitrile (µL)
25	25	5	5	1000	100	500
800	25	100	10	1000	875	950

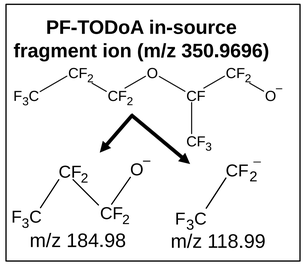
Analytical Methodology

- LC/MS/MS = Shimazdu LC20 (Kyoto, Japan) coupled to a Sciex (Framingham, MA) X500R QTOF
  - **Negative ion polarity, with electrospray ionization (ESI)**
  - **Multiple reaction monitoring (MRM)**
- Separation = Phenomenex (Torrance, CA) Kinetex XB-C18 (100 x 2.1 mm, 2.6 µm)
- Flow rate = 0.2 mL/min, injection volume = 5 µm
  - Mobile Phase A: 95:5 H_2O:MeOH, B: 95:5 MeOH:H_2O, both containing 4 mM ammonium formate

MRM Fragments

Compound	Molecular/In-Source Fragment Ion	Fragment Ion
PF-TODoA	350.9696	184.9837
PF-TODoA	350.9696	118.9919
PFHxDA	812.9482	768.9514

PF-TODoA in-source fragment ion (m/z 350.9696)



LC 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

In-Life Observations

- Rats from the 3 highest dose groups (45.9, 124, and 335.2 mg/kg/day) did not survive to scheduled termination.
- **Weight losses were observed for females at 6.3 mg/kg/day and for both sexes at 17 mg/kg/day.**

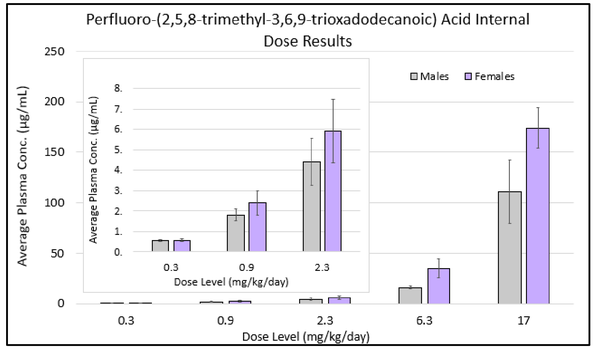
Weight Changes in Male and Female Sprague-Dawley Rats over 5-Day PF-TODoA Exposure

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

- Signs of toxicity observed with dose levels from 6.3 – 45.9 mg/kg/day (males) and 6.3 – 124 mg/kg/day (females) include: thinning of hair, piloerection, cold to touch, hunched, abnormal breathing, and lethargy.

Plasma Concentration Results

- All five measured dose levels contained PF-TODoA above the limit of quantitation (9 ng PF-TODoA/mL plasma).
- **Male rat plasma had lower PF-TODoA plasma concentrations across four of the five measured dose levels.**



Discussion and Conclusions

- **We observed greater female rat plasma concentration in this PF-TODoA dosing study. This is the *opposite trend* of what we observed in a perfluoro-3-methoxypropanoic acid (PF-MODA) 5-day oral dosing study where we observed greater male rat plasma concentrations.**
  - Both studied PFAS compounds are perfluorinated carboxylic acid-ethers.
  - The difference in accumulation trends is unexpected due to their similar structures and warrants additional investigation.
- **The data from our two studies suggests further interrogation of the sex-related effects of PFAS. We are not aware of any other published PFAS dosimetry studies that include both sexes.**
- Investigations of metabolomic, lipidomic, and transcriptomic data for potential pathway changes could indicate the onset of toxicity at lower dose levels than the observed clinical effects.
- **Further investigation is needed to determine if the greater weight loss observed in females during the study is linked to the corresponding higher PF-TODoA plasma concentrations in females versus males.**

References

1. Renyer A., MacMillan, D.K., et.al. Presented at the 61st Annual Society of Toxicology Meeting and ToxExpo, San Diego, CA March 27-31, 2022. Poster 3087