Defense Centers for Public Health - Aberdeen

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Toxicology Report No. S.0089968-23, May 2024
Toxicology Directorate

Analysis of Kinetics and Potential Biotransformation of PFAS in *Peromyscus* with an Updated Dataset

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ACKNOWLEDGEMENTS

The authors would like to thank Caroline Procell, Mark Way, Jarod Hanson, and Rebecca Kilby for their contributions to these experiments.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
23-04-2024	Technical	January 2023 – February 2024

4. TITLE AND SUBTITLE
Toxicology Report No. S.0089968-23, February 2024
Toxicology Directorate
Analysis of kinetics and potential biotransformation of PFAS in *Peromyscus* with an updated dataset.

5a. CONTRACT NUMBER

5b. GRANT NUMBER
ER-2625

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
S.0089968-23

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Defense Centers for Public Health-Aberdeen
Toxicology Directorate
8300 Ricketts Point Road
Aberdeen Proving Ground, MD 21010

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Defense Program

10. SPONSOR/MONITOR'S ACRONYM(S)
SERDP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
[same as 5f]

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This Technical Report describes a toxicokinetic model-based analysis of an updated dataset from samples archived from a study where *Peromyscus leucopus* were exposed orally to several individual PFAS for 28 days (Toxicological Report No. S.0043781-18). The results of this Technical Report indicate that the observed decreases in PFHxS and 6:2 FTS serum concentrations is likely a result of integrated toxicokinetic processes (i.e., uptake rate, elimination rate, and volume of distribution). This is in contrast to the working hypotheses of Toxicological Report No. S.0043781-18 that either females reached peak serum concentration prior to males (PFHxS) or biotransformation drove serum reductions (6:2 FTS). Based on this toxicokinetic analysis, elimination rate appears to drive the sex-difference observed in maximal serum concentrations in *Peromyscus* exposed to PFHxS. In *Peromyscus* exposed to 6:2 FTS there is little indication that loss of 6:2 FTS in serum could be explained by a gain in other PFAS. Excretion, rather than biotransformation, appears to drive the decrease in serum concentrations over the course of exposure.

15. SUBJECT TERMS
PFAS, 6:2 FTS, PFHxS, *Peromyscus*, toxicokinetics, biotransformation

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Andrew East
U	U	U	U	32	**19b. TELEPHONE NUMBER** *(Include area code)* 410-417-1216

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ANALYSIS OF KINETICS AND POTENTIAL BIOTRANSFORMATION OF PFAS IN PEROMYSCUS WITH AN UPDATED DATASET
FEBRUARY 2024

1. PURPOSE

This report communicates analysis of an updated dataset to advance the findings in the Strategic Environmental Research and Development Program (SERDP) Project ER-2625 (APHC 2021b).

2. REFERENCES AND TERMS

See Appendix A for literature cited and glossary or common terms.

3. AUTHORITY

This work is authorized by SERDP ER-2625.

4. FINDINGS

4.1 Introduction

Narizzano et al. (2021a) presented a series of relationships (ratios) that related oral daily exposures and serum concentration of single per- and polyfluoroalkyl substances (PFAS) in *Peromyscus leucopus* (white-footed mouse) across timepoints that are relevant for standardized toxicology tests: day 0, 21, and 28. The original hypothesis evaluated was that PFAS concentrations in serum would be increased beyond day 0 and reach steady state by day 28. If the hypothesis was supported, the interpretation would be that uptake had occurred and that effects observed after 28 days of dosing were a function of consistent internal PFAS concentrations. The Narizzano et al. (2021a) data indicate clear support for uptake across the time window, but variable support for stability of serum concentrations between days 21 and 28. Specifically, male, but not female, animals exposed to perfluorohexane sulfonate (PFHxS) via oral gavage exhibited a drastic decrease in serum concentrations between day 21 and 28. The working hypothesis was that female animals reached a similar peak serum concentration earlier than males and that activation elimination pathways/processes also occurred earlier in females (Niu et al. 2023, Louisse et al. 2023, among others).

Additionally, animals exposed to 6:2 fluorotelomer sulfonate (6:2 FTS) via oral gavage showed decreased serum concentration between days 21 and 28 (Figure 1, Narizzano et al. (2021a)). Working hypotheses to explain this observation in 6:2 FTS-exposed animals include activated elimination pathways/processes or activated biotransformation/metabolic processes.

To explore these hypotheses, archived samples from the original time points (days 0, 21, and 28) and archived samples collected but not analyzed in the original work from days 7 and 14 were analyzed in an updated targeted PFAS quantification method and for a target suite of potential transformation products (in 6:2 FTS treatment samples only).

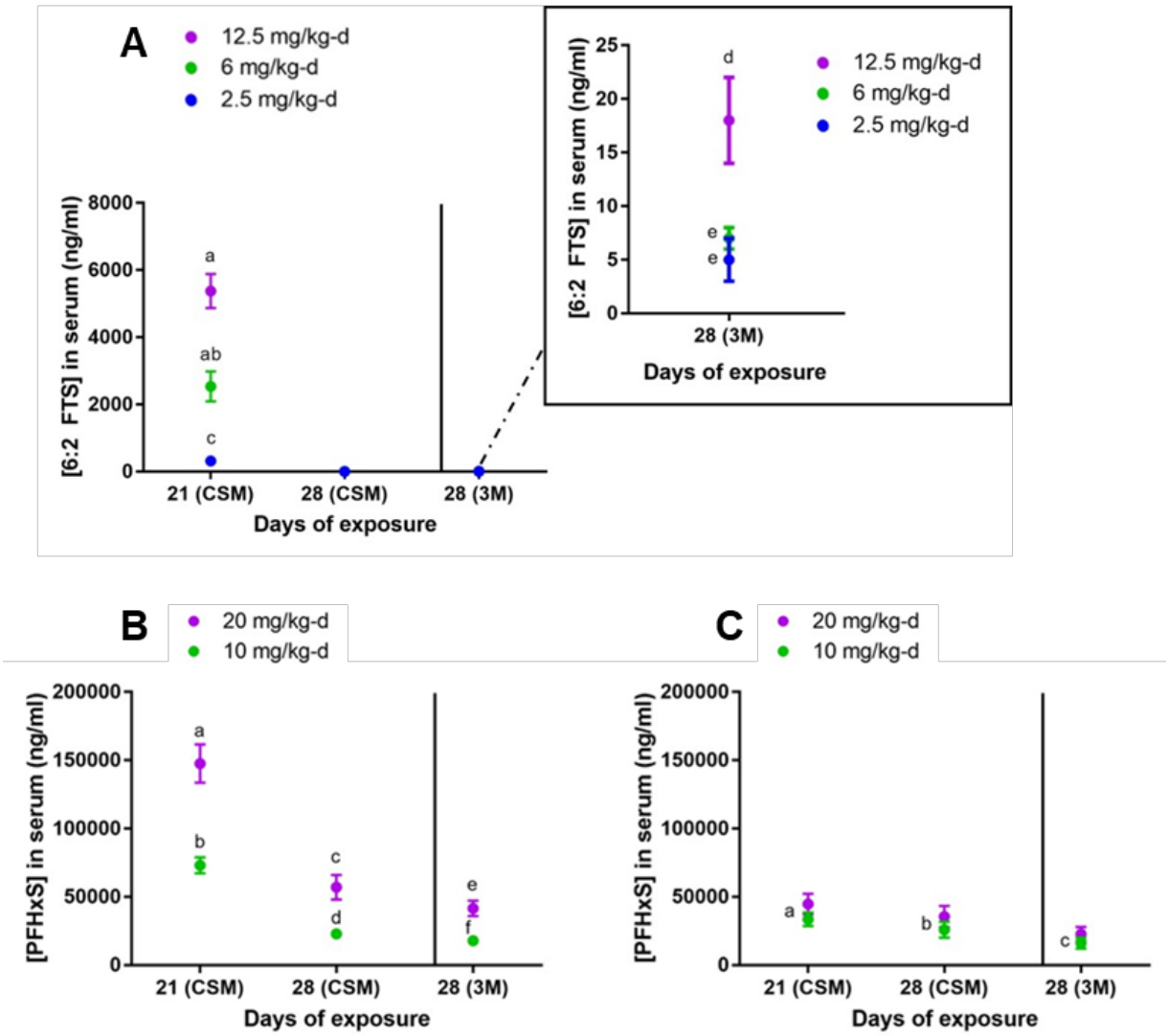

Figure 1. Serum Concentrations of 6:2 FTS and PFHxS in *Peromyscus*

Legend: A = 6:2 FTS males and females, B = PFHxS males, C = PFHxS females, mg/kg-d = milligrams per kilogram per day, CSM = Colorado School of Mines

Notes: Data for days 21 and 28 are shown, when dosed daily at various concentrations. Points are mean and error bars are mean±SEM. CSM=Colorado School of Mines data, 3M=3M data. Reproduced with permission from Narizzano et al. (2021a); see original for more details.

4.2 Methods

4.2.1 Samples

The samples described in this analysis were archived from studies described in Narizzano et al. (Narizzano et al 2021a, (U.S. Army Public Health Center (APHC) 2021b).

Briefly, *P. leucopus* (white-footed mouse) were exposed to PFHxS and 6:2 FTS via daily oral gavage for 28 days. Two analytically verified dose concentrations were used for PFHxS (10 and 20 milligrams per kilogram per day (mg/kg-d)) and three were used for 6:2 FTS (2.5, 6, and 12.5 mg/kg-d). Blood samples were collected via submandibular venipuncture on study days 0, 7, 14, and 21. After 28 doses, animals were euthanized, and trunk blood was collected. All blood samples were allowed to clot and centrifuged for 20 min at 600 x g (times gravity). Serum was decanted and frozen at -80°C.

The Defense Centers for Public Health-Aberdeen (DCPH-A, formerly Army Public Health Center (APHC)) Institutional Animal Care and Use Committee approved the animal care and use procedures (protocol no. 67-17-04-01). Animal care and use were conducted according to The Guide for the Care and Use of Laboratory Animals (National Research Council 2011) and all applicable federal and Department of Defense regulations (Army Regulation 40-33, DoD Instruction 3216.01). The DCPH-A Animal Care and Use Program is fully accredited by AAALAC® International.

In Narizzano et al. (2021a, APHC 2021b), only days 0, 21, and 28 were analyzed; in the present analysis, archived samples from other timepoints were quantified. For PFHxS, only the high treatment (20 mg/kg-d) was analyzed. Archived samples were pooled (to ensure sufficient sample mass) by sex on days 7 and 14 (see Table 1). For 6:2 FTS, high, medium, and low (12.5, 6, 2.5 mg/kg-d) treatments were analyzed. Archived samples were pooled by sex on days 7, 11, 14, 21, and 28, as available.

Based on sample volume and sample availability, it was not always possible to ensure number of pools, number of animals per pool, consistency of animals in pools, or, in some cases, the identity of the animals in each pool. Accordingly, data are generally presented via measure of center and dispersion as compared to individual values.

Table 1. Number of Archived Samples Analyzed According to Treatment and Exposure

	Dose	Days	Sex	Number of Pools	Animals in Pools
PFHxS	High, 20 mg/kg-d	7	M	2	2, 5
			F	1	7
		14	M	2	5, 2
			F	2	4, 3
6:2 FTS[a]	High, 12.5 mg/kg-d	7	M	2	NR, NR
			F	4	1, NR, NR, NR
		14	M	2	NR, NR
			F	1	NR
		21	M	1	7
			F	1	6
		28	M	1	NR
			F	2	4, 3
	Medium, 6 mg/kg-d	7	M	2	5, 4
			F	2	4, 4
		14	M	2	3, 5
			F	2	4, 3
		21	M	1	7
			F	1	5
		28	M	1	NR
			F	1	NR
	Low, 2.5 mg/kg-d	7	M	2	4, 5
			F	2	4, 4
		14	M	2	4, 5
			F	2	4, 3
		21	M	1	8
			F	1	6
		28	M	1	NR
			F	1	NR

Legend:
mg/kg-d = milligrams per kilogram per day
NR = not reported
M = male
F = female
Notes:
Note that prior analysis indicates no influence of sex on 6:2 FTS serum concentrations, so while the sexes are separated here for completeness, they are combined in following analyses.

4.2.2 Analytical Chemistry

Original samples were extracted and quantified at two laboratories (Colorado School of Mines, "CSM" and 3M®, "3M") to demonstrate interlaboratory verification and subsequent reduction in reporting limits, as described by Narizzano et al. (2021a, APHC 2021b). The method at CSM was published in Reiner et al. (2009). The method of 3M was published in Ehresman et al. (2007), Olsen et al. (2009), and Sundstrom et al. (2012). A critical observation is that analytical work in Narizzano et al. (2021a, APHC 2021b) was targeted to single PFAS based upon treatment group in all samples except for the high dose treatment, 6:2 FTS, day 28 samples. A

nontargeted analysis was performed by CSM to identify potential metabolic/transformation products of 6:2 FTS, as described in Narizzano et al. (2021a). In brief, chromatographic features of the 6:2 FTS samples were compared against those of control samples and cross-referenced against in-house and National Institute of Standards and Technology (NIST) libraries.

In the present effort, pooled samples were shipped to SGS Axys in January of 2023. Samples arrived in acceptable conditions and were maintained at -20°C until sample preparation and analysis. Two specific methods were utilized across these samples. All samples (i.e., from 6:2 FTS and PFHxS-dosed animal serum) were quantified using a quantitative UPLC-MS/MS procedure named "SGS AXYS Method MLA-110: Analytical Procedure for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples, Solids, Tissues by LC-MS/MS. Based on third draft EPA Method 1633. Additional Matrices Analyzed by MLA-110 Include AFFF Products, Blood, Serum and Solvent Extracts by LC-MS/MS." Table B-1 lists the 40-PFAS targeted analyte list from the 1633 methods (see also https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas). Samples from animals exposed to 6:2 FTS were also analyzed for two potential fluorotelomer transformation products: 6:2 FTCA (6:2 fluorotelomer carboxylic acid) and 6:2 FTUCA (6:2 fluorotelomer unsaturated carboxylic acid) via a semi-quantitative UPLC-MS/MS extended EPA Method 1633 (3rd draft) named "SGS Axys Method MLA-121 draft." Briefly, the MLA-121 extension is a unique instrument protocol run with sample extracts and chromatographic method of the 1633 sample prep and run. These two targeted molecules can occur from desulfonation, oxidation, and reduction reactions with fluorotelomer sulfonate molecules (as reviewed in Evich et al. 2023; among others). Full analysis reports (including lab flags, QAQC information, etc.) from SGS Axys are available upon request of the author. All data reported here are either 'no-flag' or as 'estimate' from above linear range of calibration curve.

4.2.3 Quantitative Methods

Data from the present analysis and from Narizzano et al. (2021a, APHC 2021b) were concatenated by animal number when possible (Table 1). For those samples where individual animal assignment to pools was not possible (i.e., those data were not available), pooled data were used to derive summary statistics. Accordingly, summary statistics that require sample size (i.e., standard error of mean (SEM)) use the sample size of number of unique values—these values represent either individual or pooled samples.

For day 28 data, where data from the present analysis and Narizzano et al. (2021a, APHC 2021b) were combined, only the 3M data were included. These methods had the most similar reporting limits.

All data were fit with toxicokinetic models of the form in Tarazona et al. (2015, 2016) to use uptake, elimination, and volume of distribution parameters to explore hypothesized explanations of decreases in serum concentrations between days 21 and 28. All analyses and figure generation occurred using the R language for statistical computing (version 4.3.1, R Core Project, 2024) and tidyverse packages (Wickham et al., 2019). Toxicokinetic model parameters were estimated in a stepwise fashion. Uptake parameters were estimated using the linear methods of Organisation for Economic Co-operation and Development (OECD) Test No. 305 (OECD 2012). The slope of a linear regression of the natural log serum concentration against

time when uptake is clearly the dominant process. In this case, data through day 21 was used to estimate uptake parameters in the 6:2 FTS samples and data through day 28 was used to estimate uptake parameters in the PFHxS samples. Elimination and volume of distribution (Vd) was estimated using non-linear least squares methods, a Port algorithm, with bounded estimates (0 to 1 for elimination, and 0 to 100 for volume of distribution), and the nlsLM function from the minpack.lm package (Elzhov et al., 2023). Parameter estimate uncertainty was estimated using non-parametric bootstrap methods from the nlsBoot function in the nlstools package (Baty et al., 2015).

4.3 Results and Discussion

4.3.1 PFHxS Kinetic Analysis

In the combined datasets (this analysis, Narizzano et al. 2021a, and APHC 2021b), male and female PFHxS serum concentrations in *Peromyscus* dosed via oral gavage to 20 mg/kg-d increased through 14-21 days and then decreased by day 28, regardless of sex (Figure 2, Figure 3). However, male animals appear to have higher serum concentrations overall and tended to decrease later than females. In relation to the original day 21 and 28 analysis (Narizzano et al. 2021a), the new data for days 7 and 14 (plus repeated days 21 and 28) indicate that males reach higher serum concentrations than females. Based on the present analysis, we can reject the hypothesis that female animals reached similar maximal concentrations earlier than male animals (e.g., at day 7 or 14, per Narizzano et al. 2021a).

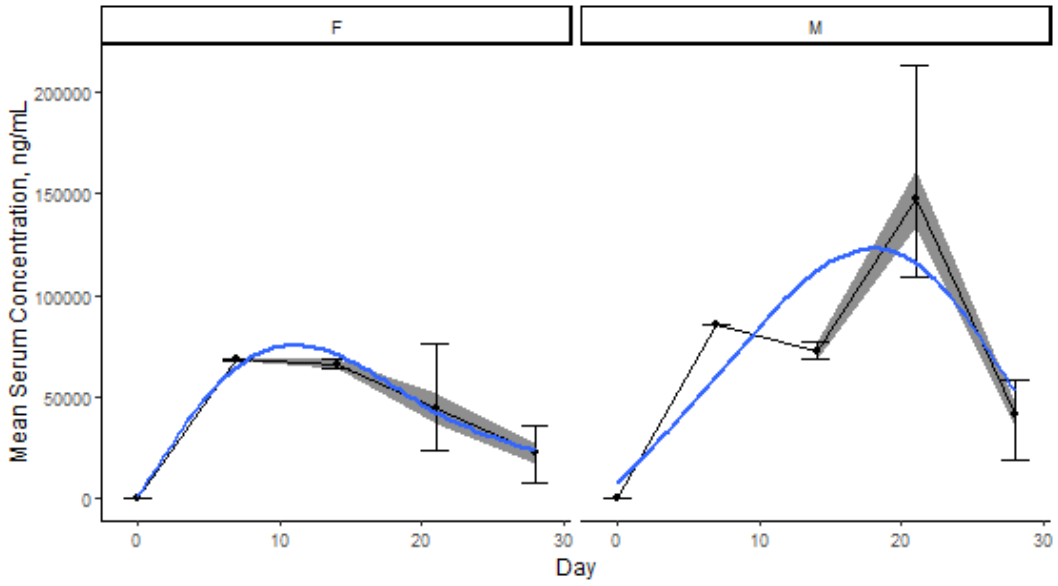

Figure 2. PFHxS Serum Concentrations (ng/mL) in Female and Male *Peromyscus*

Legend: F = female, M = male, ng/mL = nanograms per milliliter

Notes: Animals exposed orally to 20 mg/kg-d PFHxS relative to time. Points are mean of pooled or individual samples, ribbon is mean±SEM, and error bars 95% confidence intervals. Blue line is thin plate smoother with 4 knots in generalized additive model fit per sex individually to highlight sex-dependent change in peak concentrations and relative rates of uptake and elimination.

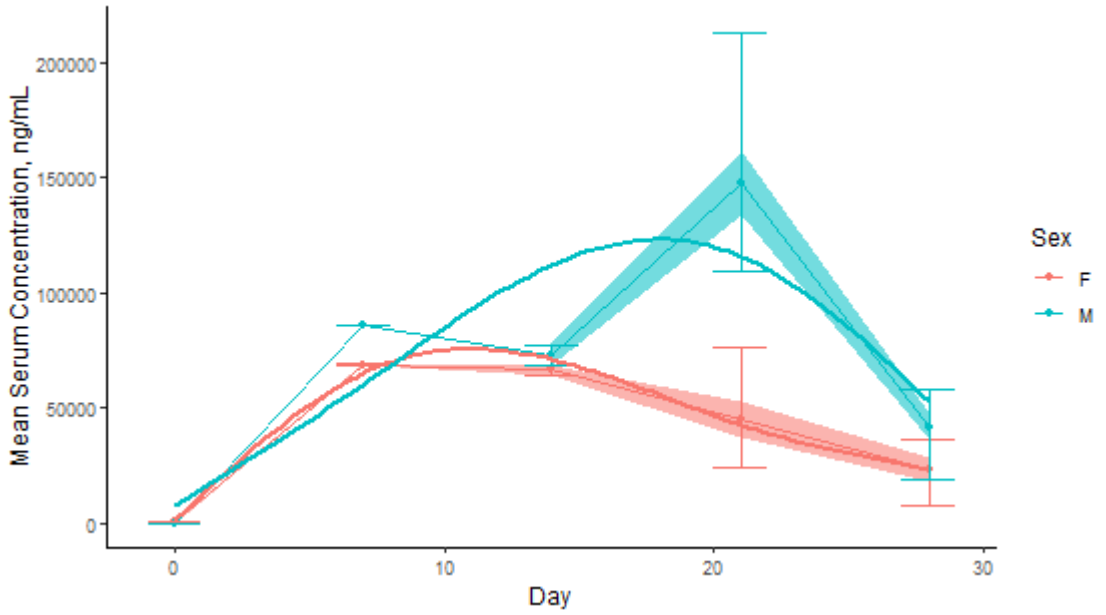

Figure 3. Serum Concentrations of PFHxS in *Peromyscus* Orally Exposed to 20 mg/kg-d PFHxS relative to Time and Sex

Legend: F = female, M = male, ng/mL = nanograms per milliliter

Notes: Points are mean of pooled or individual samples, ribbon is mean±SEM, and error bars 95% confidence intervals. Lines are thin plate smoother with 4 knots in generalized additive model fit per sex individually to highlight sex-dependent change in peak concentrations and relative rates of uptake and elimination.

Analysis via fitting a toxicokinetic model (Tarazona et al. 2015, 2016) indicates that differences in elimination rate may be explanatory as there are small differences in uptake rate and volume of distribution (Table 2, see Appendix C for more details on toxicokinetic analysis). However, given that the day 21 data nearly have an outlier-like effect (Figure 4) and elimination data are not available, further explanation with this dataset remain limited.

Table 2. Toxicokinetic Model (Tarazona et al. 2015 and 2016) Parameters for Male and Female *Peromyscus* Exposed to 20 mg/kg-d PFHxS via Oral Gavage

		Estimate	95% CI
Male	**Uptake**	0.309	0.425, 0.194[a]
	Elimination	0.014	0, 0.035[b]
	Vd	0.00018	0.00013, 0.00024[b]
Female	**Uptake**	0.258	0.374, 0.141[a]
	Elimination	0.056	0.044, 0.069[b]
	Vd	0.00018	0.00016, 0.00021[b]

Legend: mg/kg-d = milligrams per kilogram per day, CI = confidence interval
Vd = volume of distribution
Notes:
[a] via estimate±1.97*SE
[b] via non-parametric bootstrap (999 resamples)

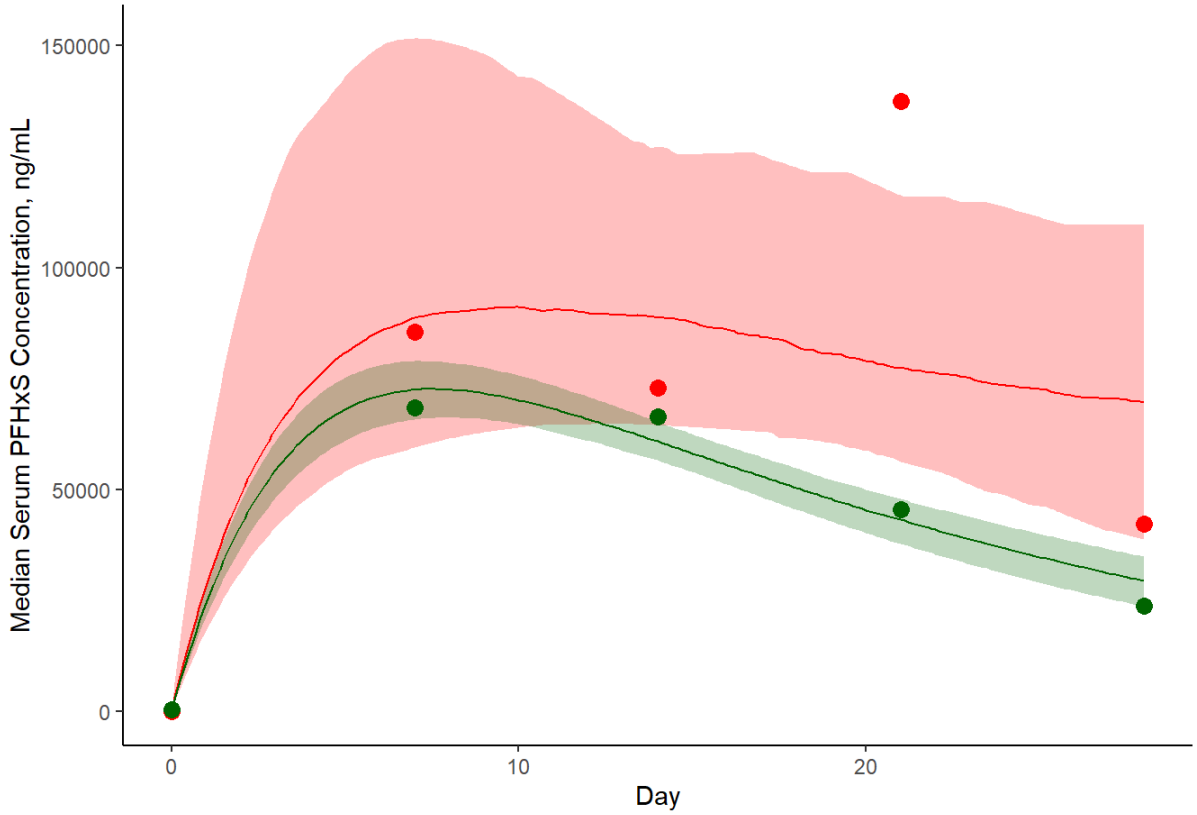

Figure 4. Median Serum Concentrations of PFHxS in *Peromyscus* Orally Exposed to 20 mg/kg-d PFHxS Relative to Time

Legend: Red = male, Green = female, mg/kg-d = milligrams per kilogram per day, ng/mL = nanograms per milliliter

Notes: Estimated (solid line) and confidence intervals (ribbon) for median (points) serum PFHxS concentrations in male (red) and female (green). Model fits here are based on median concentrations.

4.3.2 PFHxS Treatment Background PFAS

In *Peromyscus* exposed to 20 mg/kg-d PFHxS for 28 days, samples from days 7 and 14 were available to assess for concurrent PFAS in serum. There were five other PFAS observed in serum— perfluorooctane sulfonate (PFOS), perfluoropentane sulfonate (PFPeS), perfluorobutane sulfonate (PFBS), perfluorononanoate (PFNA), and 6:2 FTS (Figure 5). They were, in all cases, more than 2 orders of magnitude less in mean concentration and do not have substantially different patterns longitudinally compared to mean PFHxS. Accordingly, they are likely background signals from purity limitations of the PFHxS salt used for dosing solutions, feed/bedding/water for the animals, or some unknown source.

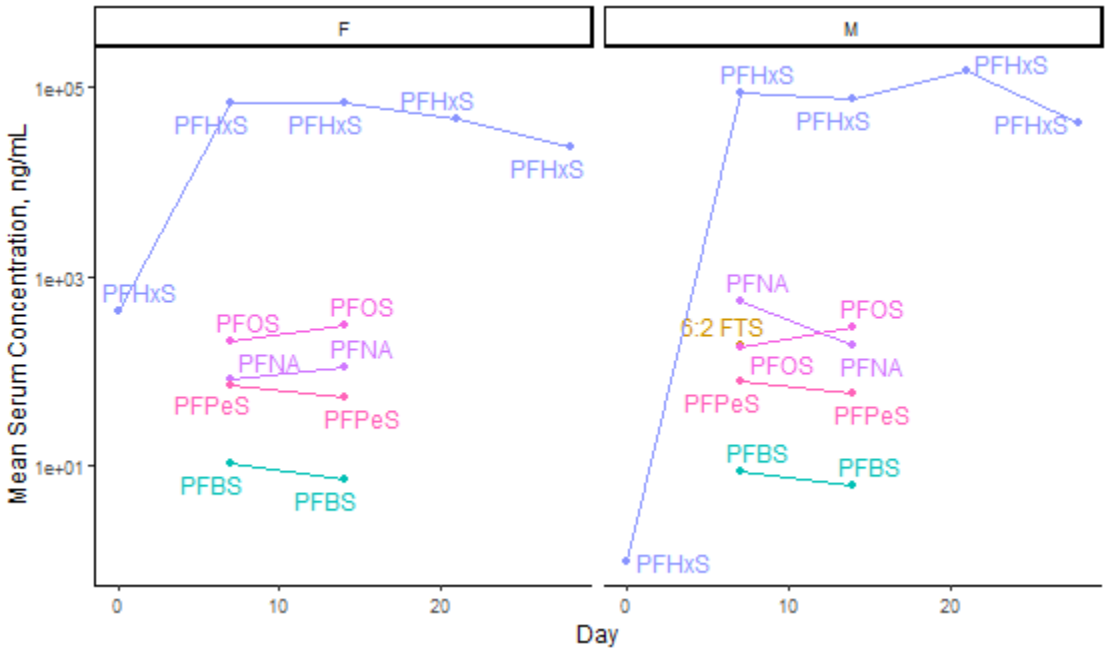

Figure 5. Mean Serum PFAS Concentrations of Female and Male *Peromyscus* Orally Exposed to 20 mg/kg-d PFHxS Relative to Time

Legend: F = female, M = male, mg/kg-d = milligrams per kilogram per day, ng/mL = nanograms per milliliter

Notes: Points are means, and only days 7 and 14 were available for EPA method 1633 PFAS analytes per sample. There is only a single observation of 6:2 FTS in males, hence no line and a single label. Note the $\log_{10}$ scaled y-axis.

4.3.3 6:2 FTS Kinetic Analysis

In the combined datasets (this analysis, Narizzano et al. 2021a, and APHC 2021b), 6:2 FTS exposed *Peromyscus* serum samples were combined by sex (see Narizzano et al. 2021a and APHC 2021b) across all three doses. In all treatments (2.5, 6, or 12.5 mg/kg-d), serum concentrations were stable or increasing on days 7 through 21 and then decreased drastically to background levels between days 21 and 28 (Figure 6).

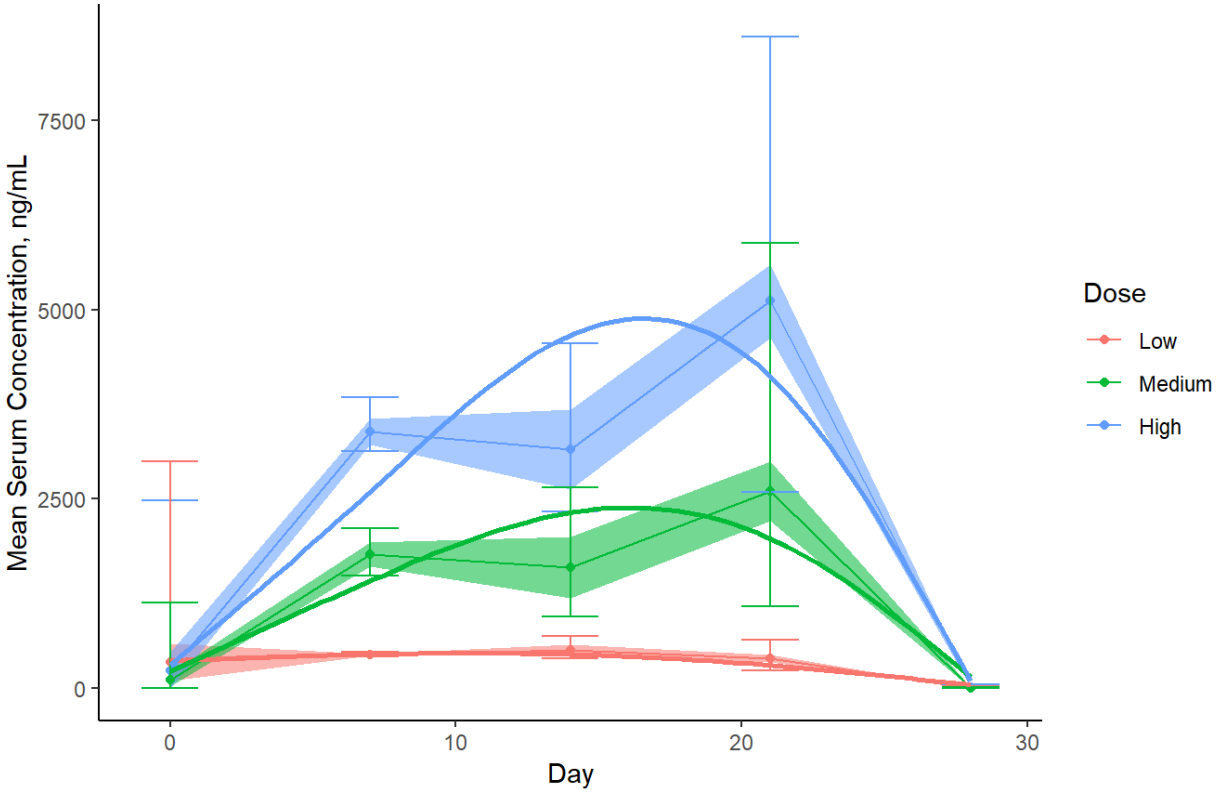

Figure 6. 6:2 FTS Serum Concentrations (ng/mL) in Combined Sex Peromyscus Relative to Oral Exposure (2.5, 6, or 12.5 mg/kg-d 6:2 FTS) and Time

Legend: Low = 2.5 mg/kg-d, Medium = 6 mg/kg-d, High = 12.5 mg/kg-d, ng/mL = nanograms per milliliter, mg/kg-d = milligrams per kilogram per day

Notes: Points are mean of pooled or individual samples, ribbon is mean±SEM, and error bars 95% confidence intervals. Lines are thin plate smoother with 4 knots in generalized additive model fit per treatment individually to highlight treatment-dependent change in peak concentrations and relative rates of uptake and elimination.

When evaluated by eye in the higher treatments (medium and high, 6, and 12.5 mg/kg-d), the general trajectories appear similar, suggesting potential for similar toxicokinetic parameters (Figure 7).

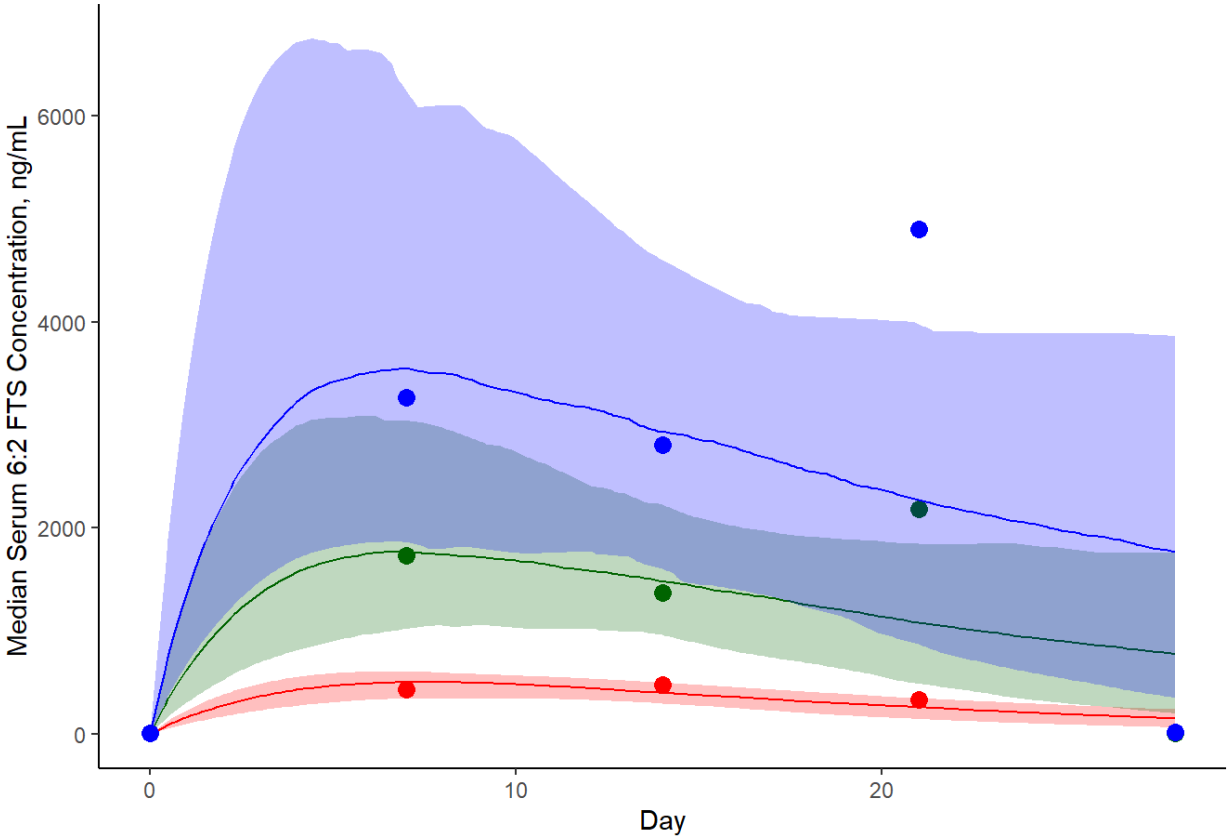

Figure 7. Estimated and Confidence Intervals for Median Serum PFHxS Concentrations in Combined Sex *Peromyscus*

Legend: Solid lines = estimated, Ribbon = confidence intervals, Points = median serum concentrations, ng/mL = nanograms per milliliter, mg/kg-d = milligrams per kilogram per day
red = 2.5 mg/kg-d, green = 6 mg/kg-d, blue = 12.5 mg/kg-d,
Notes: Mice exposed to 2.5, 6, or 12.5 mg/kg-d 6:2 FTS via oral gavage. Model fits here are based on median concentrations, not all data.

A toxicokinetic analysis by fitting models from Tarazona et al. (2015 and 2016) suggests that uptake rates may be increasing as doses increase and elimination and Vd are likely stable based on large overlap in 95% confidence intervals (CIs). Accordingly, the increase in serum concentrations observed in higher doses are most likely attributed to the dose level and that there is not a strong dose effect on toxicokinetics. We note also the similar limitation as observed in the PFHxS analysis that sans an elimination period and elevated day 21 serum concentrations, there remains uncertainty in model estimates.

Table 3. Toxicokinetic Model (Tarazona et al. 2015 and 2016) Parameters for Combined Sex *Peromyscus* Exposed to 2.5, 6, or 12.5 mg/kg-d 6:2 FTS via Oral Gavage

		Estimate	95% CI
Low, 2.5 mg/kg-d	**Uptake**	0.179	0.122, 0.236[a]
	Elimination	0.091	0.021, 0.186[b]
	Vd	0.0026	0.0011, 0.0044[b]
Med, 6 mg/kg-d	**Uptake**	0.277	0.219, 0.336[a]
	Elimination	0.042	0.020, 0.065[b]
	Vd	0.0021	0.0015, 0.0027[b]
High, 12.5 mg/kg-d	**Uptake**	0.348	0.295, 0.400[a]
	Elimination	0.052	0.026, 0.081[b]
	Vd	0.0018	0.0012, 0.0028[b]

Legend: mg/kg-d = milligrams per kilogram per day, CI = confidence interval
Vd = volume of distribution
Notes:
[a] via estimate±1.97*SE
[b] via non-parametric bootstrap (999 resamples)

4.3.4 6:2 FTS Treatment Background PFAS and Transformation Potential

In all samples analyzed for 6:2 FTCA and 6:2 FTUCA, no signals above limits of detection were reported. As stated previously, these molecules were targeted because they can occur from desulfonation, oxidation, and reduction reactions and fluorotelomer sulfonate molecules (as reviewed in Evich et al. 2023; among others).

One route to infer transformation products would be a clear negative correlation of other PFAS with 6:2 FTS. This is not observed clearly, for instance, PFNA (Figure 8, low treatment facet) appears to be increasing through exposure period, but decreases drastically between days 21 and 28, so it is unlikely that the increases emerge from a decrease in 6:2 FTS (i.e., transformation). In another instance, PFHxS is seen to have a high peak in the high treatment on day 21 (Figure 8, high facet), but in all other treatments, is decreasing throughout the exposure period. This signal may suggest, when compared against the stability of the other PFAS (e.g., 8:2 FTS, PFOS) that an elimination process that captures multiple PFAS may be activated. Subsequently, the decrease in 6:2 FTS may be as part of that process rather than biotransformation. Note also that dosing solutions of 6:2 FTS had high proportions of 8:2 FTS and would be explanatory of high 8:2 FTS observations.

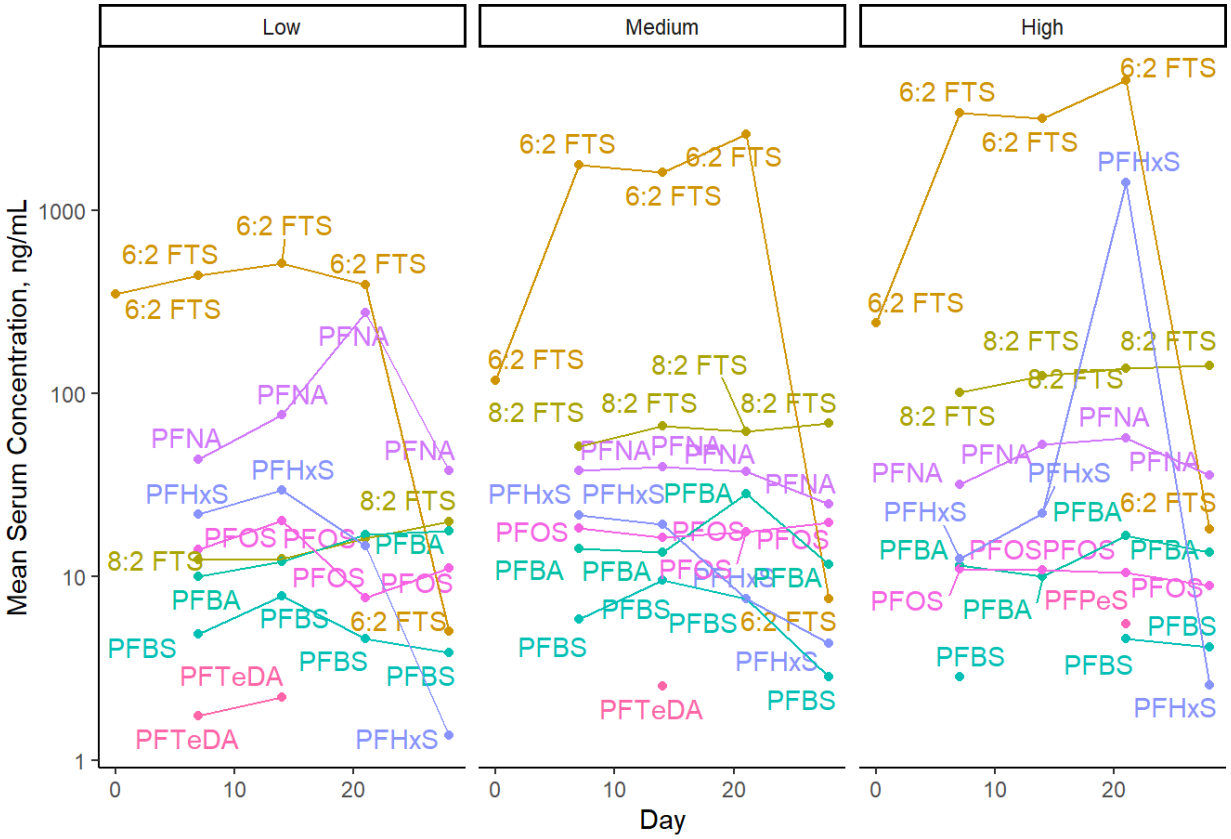

Figure 8. Mean Serum PFAS Concentrations of Combined Sex *Peromyscus* Exposed to Low, Medium, or High PFHxS Via Oral Gavage

Legend: Low = 2.5 mg/kg-d, Medium = 6 mg/kg-d, High = 12.5 mg/kg-d, mg/kg-d = milligrams per kilogram per day, ng/mL = nanograms per milliliter

Notes: Points are means from days where samples were available for EPA method 1633 PFAS analytes per sample. Single points and labels indicate single observations above reporting limits. Note the $\log_{10}$ scaled y-axis.

5. CONCLUSIONS

In conclusion, the observed decreases in PFHxS and 6:2 FTS towards the end of 28 days consecutive dosing is likely a result of the integrated toxicokinetic processes (i.e., uptake rate, elimination rate, volume of distribution). This is in contrast to the working hypotheses of Narizzano et al. (2021a) that either females reached peak serum concentration prior to males (PFHxS) or biotransformation drove serum reductions (6:2 FTS).

Based on this toxicokinetic analysis, elimination rate appears to drive the sex-difference observed in maximal serum concentrations in *Peromyscus* exposed to PFHxS. In *Peromyscus* exposed to 6:2 FTS there is little indication that loss of 6:2 FTS in serum could be explained by a gain in other PFAS. Excretion, rather than biotransformation, appears to drive the decrease in serum concentrations over the course of exposure. This observation aligns with suggestions that some of the observed variability in serum PFAS concentrations may be associated with organic anion transporters and their sex- and taxa-specific characteristics (Niu et al. 2023) in conjunction with concentration- and transporter-specific affinity for PFAS (Louisse et al. 2023).

Importantly, serum represents a carrier of PFAS throughout an organism, and PFAS are likely moving to other tissues with high PFAS affinity or being eliminated. The high serum concentrations observed early in the exposure period are signals of a ‘pulse’ of PFAS entering the serum from the gastrointestinal system prior to deposition in other tissues or elimination. Significantly, environmental scenarios relevant to risk assessment include high concentration sites and migratory animals. It is reasonable to expect some animals to have high, short exposures to PFAS that could drive dynamic serum concentrations.

6. POINTS OF CONTACT

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APPENDIX A

REFERENCES

APHC. 2021b. Public Health Report No. S.0043781-18. *Comparative serum pharmacokinetics of per- and polyfluoroalkyl substances (PFAS) in white-footed mice (Peromyscus leucopus)"* by AM Narizzano, ME Bohannon, and MJ Quinn. Aberdeen Proving Ground, Maryland.
https://apps.dtic.mil/sti/pdfs/AD1154445.pdf

Baty F, C Ritz, S Charles, M Brutsche, J-P Flandrois, and M-L Delignette-Muller. 2015. "A Toolbox for Nonlinear Regression in R: The Package nlstools." *Journal of Statistical Software* 66(5), 1-21.
doi: 10.18637/jss.v066.i05.

Ehresman DJ, JW Froehlich, GW Olsen, SC Chang, and JL Butenhoff. 2007. "Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals." *Environ Res* 103:176-184.
doi: 10.1016/j.envres.2006.06.008.

Elzhov TV, KM Mullen, A Spiess, and B Bolker. 2023. minpack.lm. *R Interface to the Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in MINPACK, Plus Support for Bounds*. R package version 1.2-4.
https://CRAN.R-project.org/package=minpack.lm.

Evich MG, MJB Davis, JP McCord, B Acrey, JA Awkerman, DRU Knappe, AB Lindstrom, et al. 2022. "Per- and polyfluoroalkyl substances in the environment." *Science* 375(6580).
doi: 10.1126/science.abg9065.

Louisse, J, L Dellafiora, JJ van den Heuvel, D Rijkers, L Leenders, J-L CM Dorne, A Punt, FG Russel, and JB Koenderink. 2023. "Perfluoroalkyl substances (PFASs) are substrates of the renal human organic anion transporter 4 (OAT4)." *Arch Toxicol* 97(3):685-696.
doi: 10.1007/s00204-022-03428-6.

Narizzano AM, ME Bohannon, AG East, C McDonough, S Choyke, CP Higgins, and MJ Quinn. 2021a. "Patterns in Serum Toxicokinetics in *Peromyscus* Exposed to Per- and Polyfluoroalkyl Substances." *Environ Toxicol and Chem* 40(10):2886–2898. doi: 10.1002/etc.5151

National Research Council. 2011. Guide for the Care and Use of Laboratory Animals. 8th ed. National Academies, Washington, DC. https://www.ncbi.nlm.nih.gov/books/NBK54050/

Niu, S, Y Cao, R Chen, M Bedi, AP Sanders, A Ducatman, and C Ng. 2023. "A State-of-the-Science Review of Interactions of Per-and Polyfluoroalkyl Substances (PFAS) with Renal Transporters in Health and Disease: Implications for Population Variability in

PFAS Toxicokinetics." *Environ Health Perspectives* 131(7):076002. doi:10.1289/EHP11885.

OECD. 2012. Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris. doi: 10.1787/9789264185296-en.

Olsen G, SC Chang, PE Noker, GS Gorman, DJ Ehresman, P Lieder, and J Butenhoff. 2009. "A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans." *Toxicology* 256:65-74. doi: 10.1016/j.tox.2008.11.008.

R Core Team (2023). _R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reiner JL, SF Nakayama, AD Delinsky, JP Stanko, SE Fenton, AB Lindstrom, and MJ Strynar. 2009. "Analysis of PFOA in dosed CD1 mice. Part 1. Methods development for the analysis of tissues and fluids from pregnant and lactating mice and their pups." *Reprod Toxicol* 27:360-364. doi: 10.1016/j.reprotox.2008.10.006.

Sundstrom M, SC Chang, PE Noker, GS Gorman, JA Hart, DJ Ehresman, A Bergman, and JL Butenhoff. 2012. "Comparative pharmacokinetics of perfluorohexanesulfonate (PFHxS) in rats, mice, and monkeys." *Reprod Toxicol* 33:441-451. doi: 10.1016/j.reprotox.2011.07.004.

Tarazona JV, C Rodríguez, E Alonso, M Sáez, F González, MD San Andrés, B Jiménez, and MI San Andrés. 2015. "Toxicokinetics of perfluorooctane sulfonate in birds under environmentally realistic exposure conditions and development of a kinetic predictive model." *Toxicology Letters* 232(2):363-368. doi: 10.1016/j.toxlet.2014.11.022.

Tarazona JV, C Rodríguez, E Alonso, M Sáez, F González, MD San Andrés, B Jiménez, and MI San Andrés. 2016. "Toxicokinetics of perfluorooctane sulfonate in rabbits under environmentally realistic exposure conditions and comparative assessment between mammals and birds." *Toxicology Letters* 241:200-206. doi: 10.1016/j.toxlet.2015.11.002.

Wickham H, M Averick, J Bryan, W Chang, LD McGowan, R François, G Grolemund, et at. 2019. "Welcome to the tidyverse." *Journal of Open Source Software* 4(43): 1686. doi:10.21105/joss.01686.

APPENDIX B

ANALYTICAL RESULTS

Table B-1. Target Analytes in EPA Method 1633 (3rd draft) Utilized in this Analysis by SGS Axys

Target Analyte Name	Acronym	Group
Perfluorobutanoic acid	PFBA	Perfluoroalkyl carboxylates
Perfluoropentanoic	PFPeA	
Perfluorohexanoic acid	PFHxA	
Perfluoroheptanoic	PFHpA	
Perfluorooctanoic acid	PFOA	
Perfluorononanoic acid	PFNA	
Perfluorodecanoic acid	PFDA	
Perfluoroundecanoic acid	PFUnA	
Perfluorododecanoic acid	PFDoA	
Perfluorotridecanoic acid	PFTrDA	
Perfluorotetradecanoic acid	PFTeDA	
Perfluorobutanesulfonic acid	PFBS	Perfluoroalkyl sulfonates
Perfluoropentanesulfonic acid	PFPeS	
Perfluorohexanesulfonic acid	PFHxS	
Perfluoroheptanesulfonic acid	PFHpS	
Perfluorooctanesulfonic acid	PFOS	
Perfluorononanesulfonic acid	PFNS	
Perfluorodecanesulfonic acid	PFDS	
Perfluorododecanesulfonic acid	PFDoS	
1H,1H,2H,2H-Perfluorohexanesulfonic acid	4:2 FTS	Fluorotelomer sulfonates
1H,1H,2H,2H-Perfluorooctanesulfonic acid	6:2 FTS	
1H,1H,2H,2H-Perfluorodecanesulfonic acid	8:2 FTS	
3-Perfluoropropyl propanoic acid	3:3 FTCA	Fluorotelomer carboxylates
2H,2H,3H,3H-Perfluorooctanoic acid	5:3 FTCA	
3-Perfluoroheptyl propanoic acid	7:3 FTCA	
Perfluorooctanesulfonamide	PFOSA	Perfluorooctane sulfonamides
N-methyl perfluorooctanesulfonamide	NMeFOSA	
N-ethyl perfluorooctanesulfonamide	NEtFOSA	
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	Perfluorooctane sulfonamidoacetic acids
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	Perfluorooctane sulfonamide ethanols
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSA	
Hexafluoropropylene oxide dimer acid	HFPO-DA	Per- and polyfluoroether carboxylates
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	
Perfluoro-3-methoxypropanoic acid	NFDHA	
Perfluoro-4-methoxybutanoic acid	PFMBA	
Nonafluoro-3,6-dioxaheptanoic acid	PFMPA	
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	Ether sulfonates
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	

Table B-2. Summary Statistics of PFHxS Serum Concentrations in *Peromyscus* Exposed via Oral Gavage to 20 mg/kg-d ("high") for 28 Consecutive Days

Day	Sex	#	Mean	Median	SD	SEM	97.5th	2.5th	Maximum	Minimum
0	F	7	429.2	429.2	605.6	428.2	836.1	22.4	857.5	1
	M	7	1	1	-	-	1	1	1	1
7	F	2	68450	68450	636.4	450	68877.5	68022.5	68900	68000
	M	1	85600	85600	-	-	85600	85600	85600	85600
14	F	2	66400	66400	3252.7	2300	68585	64215	68700	64100
	M	2	72950	72950	6434.7	4550	77272.5	68627.5	77500	68400
21	F	7	44793.4	45609.4	19771.5	7472.9	76376.7	23777.4	79465.5	22731.6
	M	7	147558.9	137451.4	37076.6	14013.7	213163.1	108860.7	223550.8	104936.5
28	F	6	22798.3	23775	12855.9	5248.4	35950	7822.5	36100	7390
	M	7	41628.6	42200	14965.6	5656.5	58155	18895	58500	16600

Legend:
F = female, M = male, mg/kg-d = milligrams per kilogram per day, # = number of samples,
SD = standard deviation, SEM = standard error of mean, ng/mL = nanograms per milliliter
Notes: Sorted by time and sex. Inclusive of acceptable values (all 'H' flag for estimated based on outside linear range). Units are ng/mL. The number of samples includes those that are pooled and those that are individual animals.

Table B-3. Summary Statistics of 6:2 FTS Serum Concentrations in *Peromyscus Leucopous* Exposed via Oral Gavage to 2.5, 10, or 20 mg/kg-d for 28 Consecutive Days

Dose	Day	#	Mean	Median	SD	SEM	97.5th	2.5th	Maximum	Minimum
Low	0	17	347.5	1	1015.6	246.3	2995.0	1	4043.6	1
	7	4	441.3	428.5	31.0	15.5	483.0	421.2	487	421
	14	4	511	473	134.7	67.4	687.6	399.0	703	395
	21	10	391.8	328.2936	148.3	46.9	637.0	235.6	659.6	228
	28	13	5.0	2.26	9.5	2.6	27.3	1.0	36.5	1
Medium	0	18	117.9	1	377.9	89.1	1125.2	1	1532.3	1
	7	4	1772.5	1735	317.7	158.9	2116.5	1492.3	2130	1490
	14	4	1598	1365	813.4	406.7	2648.3	943.9	2730	932
	21	15	2598.9	2180	1523.8	393.5	5878.3	1081.6	6599.1	1053.9
	28	16	7.6	7.125	4.5	1.1	16.4	2.2	17	2.04
High	0	18	241.1	1	1018.6	240.1	2485.9	1	4322.5	1
	7	4	3390	3270	343.3	171.7	3848.0	3136	3890	3130
	14	4	3155	2810	1057.2	528.6	4560.3	2336.3	4690	2310
	21	18	5113.4	4904.7	2050.5	483.3	8602.3	2587.2	9202.6	2457.7
	28	19	18.2	14.1	13.0	3.0	47.6	4.4	52.9	3.2

Legend:
Low = 2.5 mg/kg-d, Medium = 10 mg/kg-d, High = 20 mg/kg-d, SD = standard deviation,
SEM = standard error of mean, # = number of samples, mg/kg-d = milligrams per kilogram per day,
ng/mL = nanograms per milliliter
Notes: Sexes are combined, sorted by dose and time. Units are ng/mL. The number of samples includes those that are pooled and those that are individual animals where appropriate.

APPENDIX C

TOXICOKINETIC MODELING

PFHxS Uptake Rate Estimation

PFHxS uptake rates were based on the methods of OECD TG#305, which rely on a linear regression of the ln-transformed serum concentration against time. The slope of that line represents an unitless uptake rate that represents a multiplicative increase rate ($conc_{t+1}=conc_t+conc_t*rate$) (the use in the TG#305 is for elimination rate, which is more easily defined as a single process). Pilot regressions indicate that strictly linear-phase data (days 0 and 7) over predict uptake rates, and inclusion of 6:2 FTS day 28 data strongly underpredicted uptake rates. Accordingly, data used to estimate uptake in PFHxS was from days 0 through 28 and 6:2 FTS was days 0 through 21.

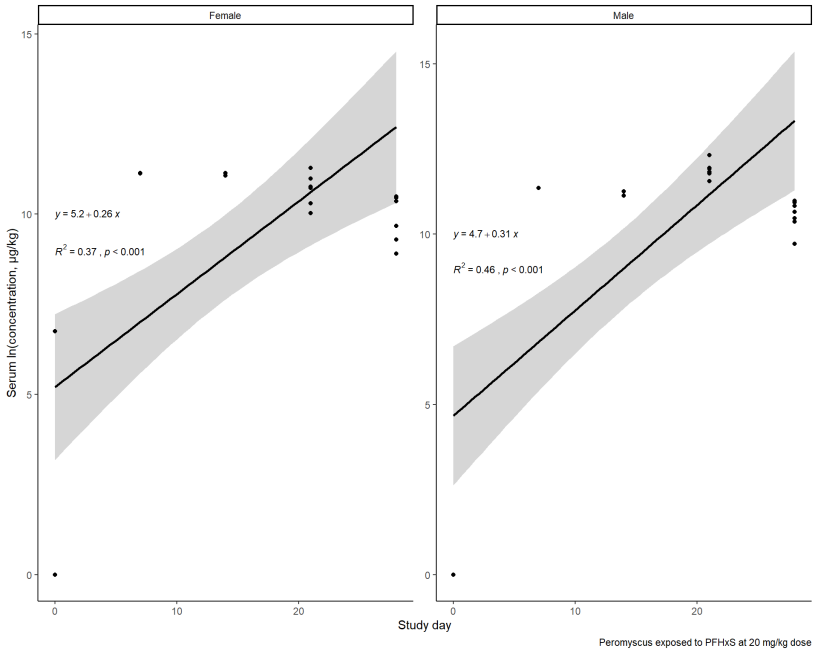

Figure C-1. Linear Regressions of ln(serum concentration, ng/mL) Through Day 28 in Male and Female *Peromyscus* Exposed to 20 mg/kg-d PFHxS

Legend: ln = natural log, mg/kg-d = milligrams per kilogram per day, ng/mL = nanograms per milliliter

Table C-1. Linear Regression Statistics for ln(serum concentration, ng/mL) Through Day 28 in Male and female *Peromyscus* Exposed to 20 mg/kg-d PFHxS

	Slope Estimate	Slope SE	Test Statistics	p-value
Female	0.2575416	0.05933411	4.340532	1.333581e-04
Male	0.3092966	0.05857960	5.279937	8.076789e-06

Legend: ln = natural log, mg/kg-d = milligrams per kilogram per day, ng/mL = nanograms per milliliter

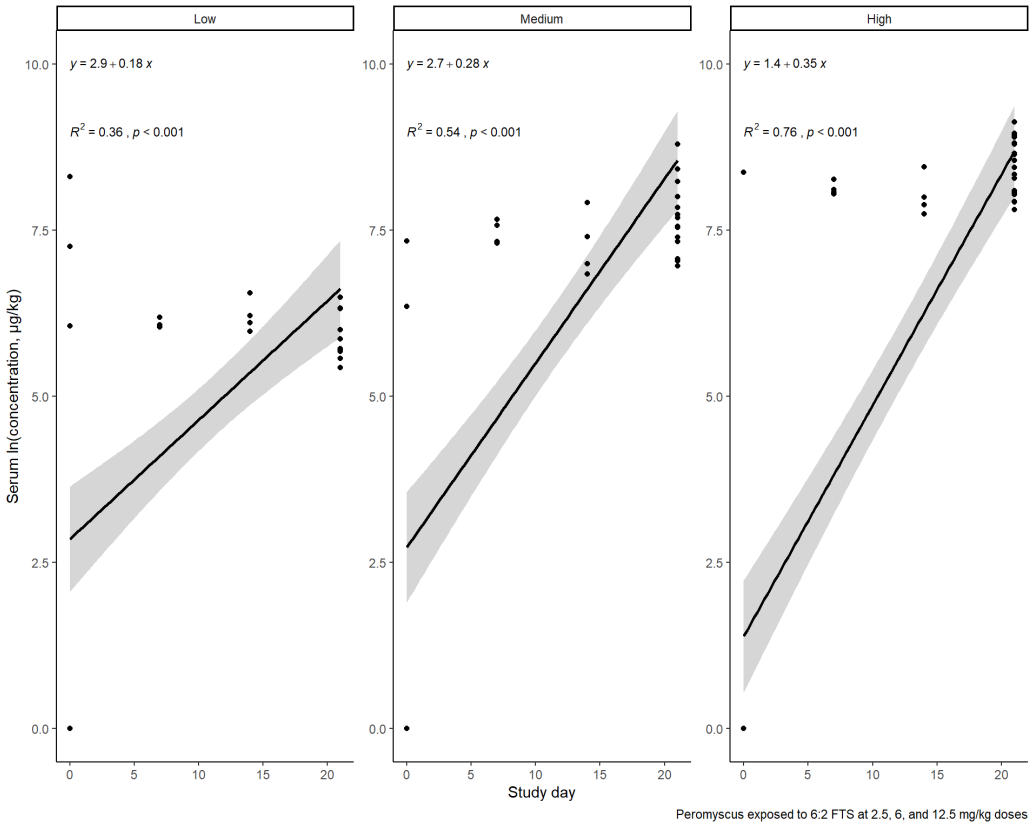

Figure C-2. Linear Regressions of ln(serum concentration, ng/mL) Through Day 28 in *Peromyscus* Exposed to 2.5, 6, or 12.5 mg/kg-d 6:2 FTS

Legend: ln = natural log, mg/kg-d = milligrams per kilogram per day, ng/mL = nanograms per milliliter

Table C-2. Linear Regression Statistics for ln(serum concentration, ng/mL) Through Day 28 in *Peromyscus* Exposed to 2.5, 6, or 12.5 mg/kg-d

	Slope Estimate	Slope SE	Test Statistics	p-value
Low, 2.5 mg/kg-d	0.1793322	0.02886224	6.213386	3.278126e-08
Med, 6 mg/kg-d	0.2771540	0.02964014	9.350630	4.109848e-14
High, 12.5 mg/kg-d	0.3477061	0.02675811	12.994422	2.948269e-18

Legend: ln = natural log
mg/kg-d = milligrams per kilogram per day
ng/mL = nanograms per milliliter

Example R code to fit model and make predictions out through 28 days. Values here are for high treatment of 6:2 FTS group. Data frame named `high62nlspredboot` would be used to generate a plot, `summary(high62nlsboot)` is used to extract model parameter estimates.

```
high62nlsboot <- nlsBoot(
  nlsLM(
    CONC ~1+((12.5*0.348)/(V*(0.348-k10)))*(exp(-k10*day)-exp(-0.348*day)),
    data=ftsonly|> filter(doselevel=="High")|>filter(!is.na(CONC)),
    start=list(V=0.01, k10=0.1),
    lower=c(0,0), upper=c(100,1), algorithm="port"
    ))
summary(high62nlsboot)
high62nlspredboot <- data.frame(
  nlsBootPredict(high62nlsboot,
                 newdata=data.frame(day=seq(from=0, to=28, by=0.1)),
                 interval="confidence"))
high62nlspredboot$day <- c(seq(from=0,to=28,by=0.1))
```

GLOSSARY

Acronyms/Abbreviations

APHC
Army Public Health Center

°C
degrees Celsius

CI
confidence interval

CSM
Colorado School of Mines

DCPH-A
Defense Centers for Public Health-Aberdeen

DOD
Department of Defense

EPA
U.S. Environmental Protection Agency

Ln
Natural log

mg/kg-d
milligrams per kilogram per day

MS/MS
tandem mass spectroscopy

N/A, NA
not applicable

ND
no data

ng/mL
nanogram per milliliter

OECD
Organisation for Economic Co-operation and Development

PFAS
per- and polyfluoroalkyl substances

QAQC
quality assurance and quality control

SD
standard deviation

SE, SEM
standard error, standard error of the mean

TG
Technical Guide

Unk
Unknown

UPLC
ultra-high performance liquid chromatography

Vd
Volume of distribution

x g
times gravity