



Toxicokinetics of 8:2 fluorotelomer alcohol (8:2-FTOH) in male and female Hsd:Sprague Dawley SD rats after intravenous and gavage administration

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ABSTRACT

Fluorotelomer alcohols (FTOHs) are used in the production of persistent per- and polyfluorinated alkyl substances (PFAS). Rodents and humans metabolize FTOHs to perfluoroalkyl carboxylic acids which have several associated toxicities. Thus, understanding the toxicokinetics of these FTOHs and their metabolites will be useful for interpreting their toxicity for humans. Here, male and female Hsd:Sprague-Dawley SD rats were administered a single dose of 8:2-FTOH via gavage (males: 12, 24, 48 mg/kg; females: 40, 80, 160 mg/kg) or IV (males: 12 mg/kg; females: 40 mg/kg). Toxicokinetics of 8:2-FTOH and two primary metabolites, perfluorooctanoic acid (PFOA) and 7:3-fluorotelomer acid (7:3-FTA) were determined in plasma. Concentrations (total) of these chemicals were determined in the liver, kidney, and brain. There was rapid absorption and distribution of 8:2-FTOH after gavage administration in male rats. The plasma elimination half-life ranged from 1.1 to 1.7 hours. Kinetic parameters of 8:2-FTOH in females were similar to that in males. Bioavailability of 8:2-FTOH ranged from 22 to 41% for both sexes with no dose-dependent trends. 8:2-FTOH metabolites, PFOA and 7:3-FTA were detected in plasma following administration of the parent FTOH. Consistent with existing literature, the plasma half-life of PFOA was longer in males than in females (198–353 hours and 4.47–6.9 hours, respectively). The plasma half-life of 7:3-FTA was around 2–3 days in both sexes. 8:2-FTOH and 7:3-FTA were detected in all tissues; PFOA was found in the liver and kidney but not the brain. Detectable concentrations of metabolites persisted longer than the parent FTOH. These data demonstrate that in rats given a single gavage dose, 8:2-FTOH is rapidly absorbed, metabolized to form PFOA and 7:3-FTA, distributed to tissues, and eliminated faster than its metabolites. Sex differences were observed in the tissue distribution and elimination of PFOA, but not 8:2-FTOH and 7:3-FTA.

1. Introduction

Per- and polyfluorinated alkyl substances (PFAS) are used to repel water and oils for use on paper, food packaging products, furniture, and outdoor equipment. Due to their chemical properties, PFAS are resistant to degradation, bioaccumulate, and persist in the environment [1]. PFAS such as perfluorooctanoic sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been detected in human sera worldwide for many years [2–6]. In humans and laboratory animals, exposure to PFOS and PFOA has been associated with cancer, impairments in reproduction and development, hormonal changes, and liver toxicity [7–10], which led to regulatory actions and voluntary reductions of PFOA and PFOS production by major manufacturers in the US between 2000 and 2015.

During this time, concentrations of PFAS in sera of the US population decreased but, interestingly, there was a larger decrease in PFOS than PFOA concentrations [3,4]. Given that PFOS has a longer half-life than PFOA, the continued persistence of PFOA in sera suggests continued exposure to PFOA [11].

One explanation for the continued presence of PFOA is the continued use of fluorotelomer alcohols (FTOHs) in the manufacture of PFAS. Since the electrochemical fluorination process for PFAS synthesis was discontinued in 2003, telomerization, which synthesizes FTOHs that can be converted to other products, has become the primary industrial synthesis process [12]. FTOHs are a potential source of perfluorinated carboxylates (PFCAs) such as PFOA in the environment as they are converted into PFCAs by microbes [13] and abiotically [14].

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Furthermore, FTOH-derived PFCAs have been detected in rats [15] and are suspected to form in humans [16–18]. In 2006, worldwide production of FTOHs was estimated to be 20 million pounds per year [19].

Human exposure to environmental FTOHs comes from residual FTOHs in PFAS-containing products. Inhalation is a potential route of exposure due to the volatility of FTOHs. While manufacturing workers and ski wax technicians have high levels of exposure due to occupational use of PFAS-containing products [17], airborne FTOHs have also been detected in office environments, private homes, and furniture and outdoor equipment stores [20–23]. Ingestion may be another route of human exposure given the use of PFAS in food-contact coatings [24] and its presence in dust [25]. Exposure to FTOHs specifically has been associated with hepatotoxicity [26–28], increased breast cancer cell proliferation [29,30], and estrogenic activity [31,32]. Some effects on reproduction and development were observed, but these may be due to maternal toxicities [33,34]. It is possible that some of the toxicities of FTOHs are related to effects of their metabolites, either one specific metabolite or a combination of multiple metabolites [15]. Thus, increased understanding of the toxicokinetics of FTOHs and their metabolites may help delineate which toxicities are attributable to the parent compound versus the metabolites. Assessing the toxicokinetics of FTOHs will also be essential for contextualizing toxicological data and interpreting the relevance of animal data to humans.

8:2-FTOH is a fluorotelomer manufactured extensively around the world [35]. It is excreted primarily through the feces and bile [27] and has been shown to be metabolized to PFCAs, such as PFOA and fluorotelomer carboxylic acids, in rodents [15,36]. Although there is a shift towards the use of more short-chain PFAS (six carbons and less), long-chain PFAS and FTOHs are still in use and are present in the environment. Toxicokinetics of 8:2-FTOH have been reported following intraperitoneal injection [37], oral administration either once [27] or daily for multiple days [15], or inhalation [36] of the chemical. Many of these studies identify metabolites produced but not all evaluate their kinetics. This paper reports the toxicokinetic parameters of 8:2-FTOH and two primary 8:2-FTOH metabolites, PFOA and 7:3-fluorotelomer carboxylic acid (7:3-FTA), in adult male and female Harlan Sprague-Dawley rats following oral gavage exposure to 8:2-FTOH (Fig. 1).

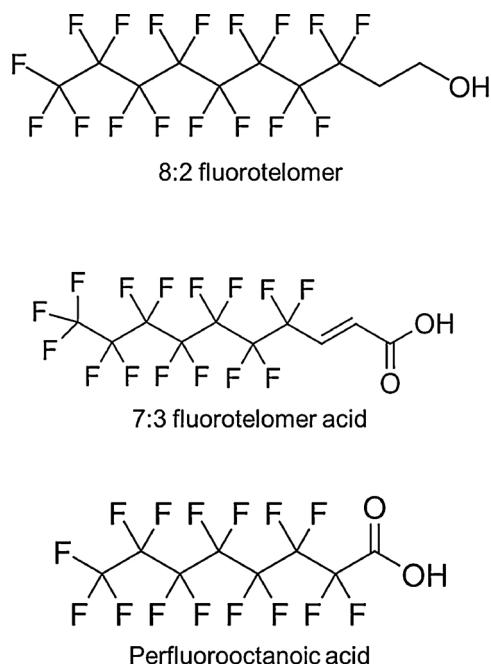


Fig. 1. Chemical structures of 8:2-fluorotelomer and its metabolites, perfluorooctanoic acid and 7:3 fluorotelomer acid.

2. Materials and methods

2.1. Chemicals and reagents

8:2-FTOH (CAS# 678-39-7; Lot# 09115HE; Sigma-Aldrich, St. Louis, MO) was purchased commercially (Fig. 1). Chemical identity was confirmed by infrared spectroscopy, ^{13}C and ^{19}F nuclear magnetic resonance spectroscopy and mass spectrometry. Purity (98.4%) was determined using gas chromatography (GC) with flame ionization. There were three reportable impurities: 1.3% was a perfluoroalkyl compound similar in structure to 8:2-FTOH, 0.19% was allylic 8:2-FTOH, and 0.11% was a perfluoroalkyl compound dissimilar to 8:2-FTOH. 2-perfluorooctyl-(1,1- $^2\text{H}_2$)-(1,2- $^{13}\text{C}_2$)-ethanol (MTHFPD) and perfluoro-n-(1,2,3,4- $^{13}\text{C}_4$)octanoic acid (MPFOA) to be used as internal standards were obtained from Wellington Laboratories (Guelph, Ontario). All other reagents were obtained from commercial sources.

2.2. Animals

These studies were conducted in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities and approved by Battelle's (Columbus, OH) Animal Care and Use Committee. Animal care was performed according to the *Guide for the Care and Use of Laboratory Animals* [38]. Studies were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulation [39].

Male and female Hsd:Sprague Dawley SD rats, approximately 7 weeks old, were purchased from Harlan Laboratories, Inc. (now Envigo, Inc., Indianapolis, IN). Prior to shipment, jugular catheters were implanted in rats for IV administration. Irradiated NTP-2000 feed (Zeigler Brothers, Inc., Gardners, PA) and municipal tap water was provided *ad libitum*. Animals were maintained on a 12:12 light cycle in a room at 64–79 °F with 30–70% relative humidity. Prior to dosing, rats were randomized using a partitioning algorithm program (Xybion PATH/TOX SYSTEM, Xybion Medical Systems Corporation, Version 4.2.2) to ensure mean body weights of rats in each group were similar. Additional animals were included as replacements for when dosing was believed to be incomplete or an insufficient volume of blood was collected. Animals were approximately 8 weeks old at the time of dosing.

2.3. Dose administration and sample collection

Dose formulations were prepared in 1:1:8 Cremophor:ethanol:water for gavage (2.4, 4.8, 8, 9.6, 16 and 32 mg/mL) and IV (3 or 10 mg/mL) and analyzed using a validated GC with electron capture detection method (range, 1.5–80 mg/mL; $r \geq 0.99$; precision $\leq 5\%$ relative standard deviation; accuracy, $\leq \pm 10\%$ relative error). All formulations were within 10% of target concentrations. Prior to study initiation, stability ($\leq \pm 10\%$ of day 0) of both oral and IV formulations was confirmed for up to 42 days when stored in sealed amber bottles with Teflon-lined lids at ambient or refrigerated conditions.

The study design is given in Table 1. A single bolus IV in 4 mL/kg (males: 12 mg/kg; females: 40 mg/kg) or a gavage dose in 5 mL/kg (males: 12, 24, 48 mg/kg; females: 40, 80, 160 mg/kg) was administered to non-fasted rats based on individual body weight on the day of dosing. Due to the anticipated differences in PFOA toxicokinetics from previous literature, females received higher doses than males. Following dose administration, retro-orbital blood (~ 0.7 mL) was collected from each rat after anesthesia with 70% CO_2/O_2 at 11–12 timepoints (Table 1). Time points were selected based on preliminary studies (data not shown) and literature. Each animal was bled for a maximum of two time points and three animals were bled at each time point. Samples were collected into a tube containing ethylenediaminetetraacetic acid, gently inverted, and placed on wet ice until separated into plasma. Plasma was separated by centrifugation at 1750 x g for 10 min at 4 °C and stored in at -20 °C until analysis. In specific gavage dose groups

Table 1

The route of administration, dose, and time points for a single dose of 8:2-FTOH administered to Hsd:Sprague Dawley SD rats.

Route of Administration	Sex	Dose (mg/kg)	Dose (mmol/kg)	Time Points (hours)
IV	Male	12	0.026	0.083, 0.25, 0.5, 0.75, 1, 3, 6, 9, 12, 24, 192
	Female	40	0.086	0.083, 0.25, 0.5, 0.75, 1, 3, 6, 9, 12, 24, 48, 192
Gavage	Male	12	0.026	0.083, 0.25, 0.5, 0.75, 1, 3, 6, 9, 12, 24, 48, 192
		24	0.052	
	48	0.103		
	Female	40	0.086	0.083, 0.25, 0.5, 0.75, 1, 3, 6, 9, 12, 24, 48, 192
		80	0.172	
160	0.345			

(24 mg/kg for males and 80 mg/kg for females), following blood collection, the liver, kidney, and brain were collected at 0.5, 1, 3, 6, and 12-h time points to measure 8:2-FTOH, PFOA, and 7:3-FTA tissue concentrations.

2.4. Sample preparation and analysis

An analytical method using protein precipitation followed by high performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) was used to quantitate 8:2-FTOH, PFOA (CAS# 335-67-1), and 7:3-FTA (CAS# 812-70-4) (Fig. 1) in HSD rat plasma, liver, kidney, and brain. The validation included an assessment of linearity (r), inter- and intraday accuracy (estimated as standard error, RE), and inter- and intraday precision (estimated as relative standard deviation, RSD), absolute recovery, and limit of detection (LOD). Analytical method validation parameters are given in Supplemental Table S1-S4.

Stock solutions of chemical analyte were prepared in methanol and further diluted in the same solvent to generate concentrations of standards in the working range. Stock solutions of the internal standard (IS) was prepared in methanol and diluted in 4:1 ASTM Type 1 water:methanol to generate working IS solutions. Matrix calibration curves were prepared in duplicate by adding the standard solutions to blank HSD rat plasma/tissue. Quality control (QC) samples were prepared in blank HSD rat plasma/tissue using a procedure similar to that for the matrix standards, using an independent stock solution. Matrix blanks were prepared the same as matrix standards except the addition of the analyte.

Sample tissues (100–300 mg) were homogenized in a volume of ASTM Type 1 water that was 10x the weight of wet tissue prior to analysis. For analysis of 8:2-FTOH, 30 μ L of plasma was combined with 30 μ L of IS solution (10 ng/mL MTHPPD in methanol). In tissues, 100 μ L of homogenized tissue was combined with 50 μ L of 200 ng/mL MTHPPD in methanol and 200 μ L of 0.05 N potassium hydroxide in 1:1 water:methanol. For analysis of PFOA and 7:3-FTA, 100 μ L of plasma was combined with 100 μ L of the IS solution 20 ng/mL MPFOA. In tissues, 100 μ L of homogenized tissue was combined with 200 μ L of 50 ng/mL MPFOA. Samples (i.e. tissue samples) were neutralized with hydrochloric acid and vortexed, centrifuged, and an aliquot of 10–15 μ L of the supernatant was removed for analysis. Matrix calibration standards, QC samples and matrix blanks were prepared in the same manner as study samples and run with each batch of samples.

The HPLC-MS/MS system used was a Shimadzu (Kyoto, Japan) or Agilent 1200 (Santa Clara, CA) HPLC coupled to a Sciex API 5000 or Sciex Q Trap 4000 mass spectrometer (Toronto, Canada). Chromatography was performed using a Gemini-NX column (C18, 50 x 2.0 mm, 5 μ m for 8:2-FTOH or 3 μ m for metabolites; Phenomenex, Torrance, CA). For the analysis of 8:2-FTOH, mobile phases A (water) and B (methanol) were run with a linear gradient from 5% B (1.6 min) to 100% B over 0.9 min at a flow rate of 0.25 mL/min. For the analysis of PFOA and 7:3-FTA, mobile phases A (aqueous 10 mM ammonium

formate/0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) were run with a linear gradient from 10% B to 90% B in 0.6 min and held there for 2.4 min at a flow rate of 0.5 mL/min. The Turbo Ionspray™ (Sciex) ionization source was operated in negative ion mode with a source temperature of 350 °C (Sciex API 5000) or 500 °C for the (Sciex Q Trap 4000) and an ion spray voltage of –4500 V. Transitions monitored were m/z 463→403, 413→169, 441→337, 467→406 and 417→169 for 8:2-FTOH, PFOA, 7:3-FTA, (13 C)POE, and (13 C) PFOA, respectively.

A quadratic regression with 1/x weighting was used to relate LC-MS/MS analyte to IS peak area response ratio to the concentration of 8:2-FTOH. The concentration of 8:2-FTOH in samples was calculated using the response ratio, the regression equation, initial sample volume, and dilution when applicable. The concentration in plasma was expressed as ng/mL and in tissues as ng/g. All concentrations above the limit of quantitation (LOQ) were reported. Data from study samples were considered valid if: the matrix calibration curve was linear ($r \geq 0.99$), at least 75% of matrix standards were within 15% of nominal (20% at the LOQ), and at least 67% of the QC samples were within 15% of nominal values.

2.5. TK analysis

WinNonlin (Version 5.0.1, Pharsight Corporation, Mountain View, CA) was used for TK modeling of data. Concentrations below the LOQ were not used in the analysis. For 8:2-FTOH plasma data, individual animal concentration time data were used to find a best-fit model. One- and/or two-compartment models were tested based on the appearance of the plasma concentration time curve. The model and weighting factor that resulted in the best goodness-of-fit were used to calculate primary and secondary TK parameters based on conventional mathematical equations [40]. A two-compartmental model was used to calculate TK parameters following IV and gavage administration using the equation:

$$C_{(t)} = Ae^{-\alpha t} + Be^{-\beta t}$$

For 8:2-FTOH metabolites PFOA and 7:3-FTA, the plasma concentration-time profiles were assessed using noncompartmental analysis. Bioavailability was calculated using the area under the curve (AUC) with the equation:

$$F\% = [AUC_{\infty(\text{gavage})}/(\text{Dose}_{(\text{gavage})})] / [AUC_{\infty(\text{IV})}/(\text{Dose}_{(\text{IV})})] * 100$$

To facilitate comparisons, plasma and tissue concentrations and systemic exposure parameters were converted to a molar basis using the molecular weight of 8:2-FTOH (464 g/mol), PFOA (414 g/mol), and 7:3-FTA (442 g/mol).

3. Results

Data from this study are available at <https://doi.org/10.22427/NTP-DATA-002-02182-0003-0000-8>. There were no animals found dead or moribund due to toxicity and no treatment-related clinical signs observed following IV or gavage administration at any dosage level for 8:2-FTOH.

3.1. Plasma 8:2-FTOH Toxicokinetics

Plasma concentrations of 8:2-FTOH were below the LOQ by day 8. The plasma concentration-time profiles for IV administration were best described by a two-compartment model with first order elimination. For gavage administration, a two-compartment model with first order input and first order elimination was used. Plasma concentrations over time are shown in Fig. 2. Since males and females were given different doses of 8:2-FTOH, dose-adjusted plasma concentrations over time are shown in Figure S1.

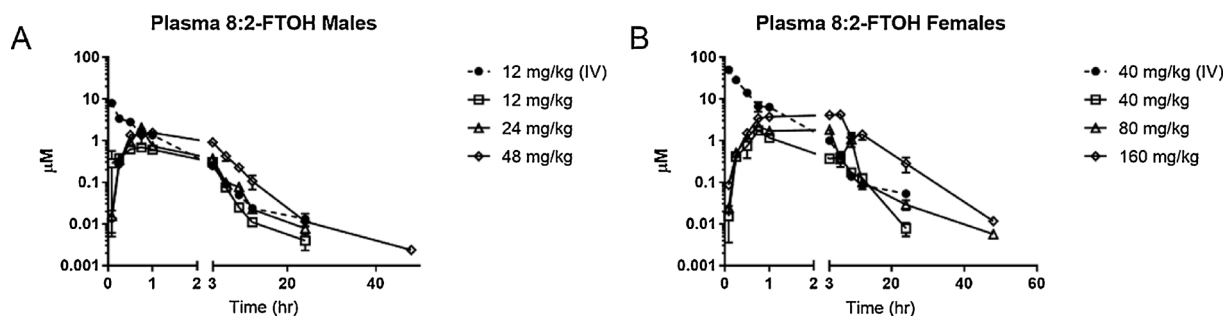


Fig. 2. Plasma concentrations (μM ; mean and SEM) of 8:2-FTOH in male (A) and female (B) Sprague Dawley rats ($N = 3$) after a single administration of 8:2-FTOH (one IV dose, three gavage doses).

Table 2

Summary of toxicokinetic parameters (mean \pm SEM, $N = 3$) of 8:2 fluorotelomer in plasma after a single IV (italicized) or gavage dose (three dose levels) in male and female Hsd:Sprague Dawley SD Rats.

Dose (mg/kg)	Male				Female			
	12 (IV)	12	24	48	40 (IV)	40	80	160
C_{\max}^a (μM)	6.51 \pm 0.84	0.754 \pm 0.127	1.01 \pm 0.20	1.49 \pm 0.29	37.9 \pm 5.8	1.25 \pm 0.33	2.039 \pm 0.438	4.40 \pm 0.91
C_{\max}/Dose ($\mu\text{M}/\text{mg}/\text{kg}$)	0.542 \pm 0.070	0.063 \pm 0.011	0.042 \pm 0.009	0.031 \pm 0.006	0.950 \pm 0.145	0.031 \pm 0.008	0.025 \pm 0.005	0.027 \pm 0.006
T_{\max}^a (hr)	–	0.545 \pm 0.167	0.853 \pm 0.226	1.37 \pm 0.300	–	0.921 \pm 0.303	2.40 \pm 0.510	2.76 \pm 0.410
$\alpha T_{1/2}$ (hr)	0.508 \pm 0.060	1.32 \pm 0.38	0.702 \pm 1.190	1.00 \pm 2.58	0.475 \pm 0.055	0.617 \pm 4.150	2.08 \pm 2.62	1.59 \pm 16.90
$\beta T_{1/2}$ (hr)	6.62 \pm 0.95	13.0 \pm 11.3	5.16 \pm 1.16	6.65 \pm 1.20	7.33 \pm 1.38	7.52 \pm 2.50	9.48 \pm 5.05	5.40 \pm 0.76
Elimination $T_{1/2}$ (hrs)	0.651 \pm 0.069	1.76 \pm 0.46	1.17 \pm 1.80	1.67 \pm 3.99	0.563 \pm 0.062	1.68 \pm 10.60	2.33 \pm 2.70	3.37 \pm 28.70
AUC ($\mu\text{M}\cdot\text{hr}$)	6.12 \pm 0.45	2.52 \pm 0.39	3.58 \pm 0.52	8.06 \pm 1.14	30.8 \pm 2.8	6.85 \pm 0.99	14.8 \pm 2.6	44.0 \pm 5.2
AUC/Dose ($\mu\text{M}\cdot\text{hr}/\text{mg}/\text{kg}$)	0.510 \pm 0.037	0.210 \pm 0.032	0.149 \pm 0.022	0.168 \pm 0.024	0.770 \pm 0.070	0.171 \pm 0.025	0.185 \pm 0.033	0.275 \pm 0.032
CL (mL/hr/kg)	4230 \pm 310	10,300 \pm 1600	14,500 \pm 2,100	12,800 \pm 1,800	2,800 \pm 250	12,600 \pm 1,800	11,600 \pm 2,000	7,860 \pm 900
V_1^b (mL/kg)	3970 \pm 520	26,000 \pm 6800	24,500 \pm 38,400	30,900 \pm 75,800	2,270 \pm 340	30,500 \pm 193,000	39,200 \pm 49,000	38,200 \pm 327,000
V_2^c (mL/kg)	7990 \pm 1570	41,500 \pm 38,000	33,400 \pm 21,200	37,000 \pm 35,500	4,270 \pm 1110	67,200 \pm 126,000	12,800 \pm 10,100	12,100 \pm 190,000
F^b (%)	–	41	29	33	–	22	24	36

C_{\max}/Dose = dose-adjusted C_{\max} .

CL = clearance.

F = bioavailability.

AUC/Dose = dose-adjusted area under the curve.

^a Predicted from two-compartment model with first order elimination for IV, two-compartment model with first order elimination and first order input for gavage.

^b Volume of distribution to central compartments.

^c Volume of distribution to peripheral compartments.

Kinetic and systemic exposure parameters are reported in Table 2. Male rats administered an IV dose (12 mg/kg) had a C_{\max} of 6.5 μM (Table 2). The α and β half-lives were 0.5 and 6.6 h, respectively. The plasma elimination half-life was around a half hour (0.65 h). AUC was 6.12 $\mu\text{M}\cdot\text{hr}$ and clearance was 4230 mL/hr/kg. Female rats administered 40 mg/kg had similar alpha, beta, and elimination half-lives compared to males (0.48, 7.33, and 0.56 h respectively). Dose-adjusted C_{\max} , dose-adjusted AUC, and clearance were also similar to that in males.

With gavage administration, the T_{\max} in males ranged from 0.55 to 1.37 h and increased with increasing doses. The elimination half-life was 1.17 to 1.76 h (Table 2). The volume of distribution greatly exceeded the estimated aqueous volume of total body water for rats (668 mL/kg) at all doses suggesting distribution of 8:2-FTOH into tissues. There were no major differences in the dose-adjusted C_{\max} , dose-adjusted AUC, and clearance with increasing dose. In females, the T_{\max} , elimination half-life, dose-adjusted C_{\max} , and clearance were similar to that in males. Unlike males, the dose-adjusted AUC increased with dose and clearance dropped by almost half in females with the highest dose, 160 mg/kg. The bioavailability of 8:2-FTOH ranged from 29 to 41% in males and 22 to 36% in females. There was a slight increase in bioavailability with increasing dose only in female rats.

3.2. Plasma 8:2-FTOH metabolite toxicokinetics

The plasma concentration-time profiles of PFOA and 7:3-FTA for male and female rats administered IV and gavage doses of 8:2-FTOH (Fig. 3, dose-adjusted in Figure S1) were assessed using noncompartmental analysis. When 8:2-FTOH was administered by gavage, PFOA was not detectable in plasma at the first timepoint (0.083 h) in either sex or at the last time point (192 h) in females. 7:3-FTA was not detectable in plasma at the first timepoint (0.083 h) in any males or females in the 12 mg/kg dose group.

Toxicokinetic and systemic exposure parameters for PFOA and 7:3-FTA in plasma are reported in Table 3. Because there were few differences in metabolite parameters due to route of administration, only data for gavage administration will be discussed. In males, the T_{\max} of PFOA after gavage administration of 8:2-FTOH was observed at 24 h for most dose groups. The elimination half-life of PFOA ranged from 198 to 353 h. The AUC increased in a dose-dependent manner. In females, the T_{\max} was earlier than in males, occurring between 3 to 6 h. After adjustment for dose, there was no apparent difference in PFOA C_{\max} between sexes. However, the dose-adjusted AUC_{∞} was 36- to 48-fold lower in females compared to males, which corresponded to a faster elimination half-life (6.4–12.0 h) in females. For 7:3-FTA, the T_{\max} in males occurred between 3–6 h and the elimination half-life ranged from 52.5 to 105 h. The AUC increased in a dose-proportional manner. There

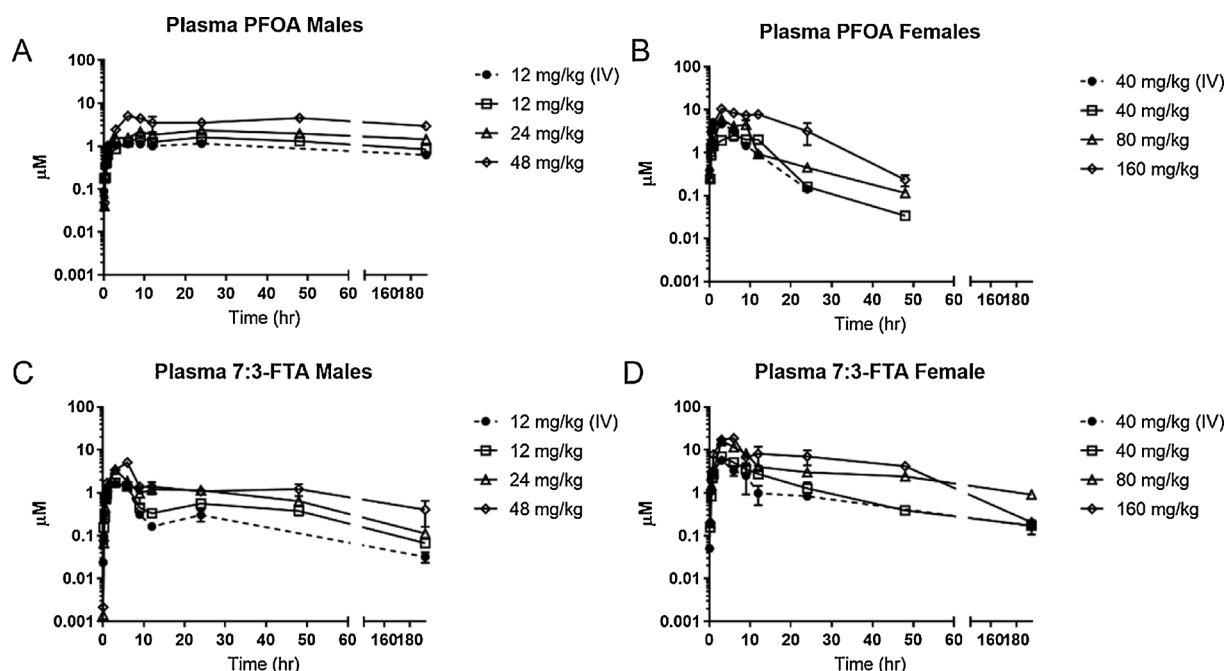


Fig. 3. Plasma concentrations (μM ; mean and SEM) of 8:2-FTOH metabolites, PFOA (A, B) and 7:3-FTA (C, D), in male and female Sprague Dawley rats ($N = 3$) after a single administration (one IV dose, three gavage doses) of 8:2-FTOH.

were no sex differences in toxicokinetic parameters for 7:3-FTA.

3.3. Tissue concentrations of 8:2-FTOH, PFOA, and 7:3-FTA

Concentrations of 8:2-FTOH, PFOA, and 7:3-FTA were measured over a period of 12 h in tissues from male and female rats following gavage administration of 24 mg/kg and 80 mg/kg, respectively. In both sexes, concentrations of 8:2-FTOH were highest in the liver, followed by brain and kidney (Fig. 4A, B). PFOA was detected in the liver and kidney but not in the brain (except one female brain measurement at the 6-h timepoint). At 12 h, concentrations of PFOA were similar in both sexes but appeared to plateau in males yet decrease in females starting at 3 h (Fig. 4C, D). 7:3-FTA concentrations in both sexes were highest in the liver, followed by kidney then brain. By the 12-h timepoint, tissue concentrations of 7:3-FTA plateaued or were decreasing

slightly in both males and females (Fig. 4E, F). Females had slightly higher concentrations of 7:3-FTA in all tissues.

To evaluate the amount of accumulation of these chemicals into tissues, tissue:plasma ratios were calculated, since concentrations in whole blood were not measured in this study. In male rats, tissue:plasma ratios of 8:2-FTOH exceeded one at all timepoints, showing distribution of the fluorotelomer into all measured tissues (Fig. 5A, B). Liver:plasma ratios were the highest compared to the kidney:plasma and brain:plasma ratios for all three analytes. In males, the tissue:plasma ratios in liver, kidney, and brain increased by 3- to 4-fold over the time period measured. Brain:plasma ratios of 8:2 FTOH were generally higher than kidney:plasma ratios. In females, the liver:plasma ratios of 8:2-FTOH were slightly lower than ratios in males, but still exceeded 1. Female kidney:plasma and brain:plasma ratios of 8:2 FTOH were similar to ratios in corresponding male tissues. However,

Table 3

Summary of toxicokinetic properties of 8:2 fluorotelomer (8:2-FTOH) metabolites, perfluorinated octanoic acid (PFOA) and 7:3 fluorotelomer acid (7:3-FTA), in plasma after a single IV or gavage dose (three dose levels) of 8:2-FTOH in male and female Hsd:Sprague Dawley SD Rats^{a,b}.

	Dose of 8:2-FTOH (mg/kg)	C_{\max}^c (μM)	C_{\max}/Dose ($\mu\text{M}/\text{mg}/\text{kg}$)	T_{\max}^c (hr)	Elimination $T_{1/2}$ (hr)	AUC ($\mu\text{M}\cdot\text{hr}$)	AUC / Dose ($\mu\text{M}\cdot\text{hr}/\text{mg}/\text{kg}$)
PFOA (M)	12 (IV)	1.29	0.108	24	225	425	35.4
	12 (Gavage)	1.79	0.149	24	198	515	42.9
	24 (Gavage)	2.61	0.109	24	269	1010	42.1
	48 (Gavage)	5.65	0.118	6	353	2490	51.8
PFOA (F)	40 (IV)	5.77	0.144	1	4.47	47.6	1.18
	40 (Gavage)	2.85	0.071	6	6.35	44.2	1.11
	80 (Gavage)	6.69	0.084	3	12.0	69.8	0.872
	160 (Gavage)	11.8	0.074	3	6.97	226	1.41
7:3-FTA (M)	12 (IV)	1.69	0.141	3	60.6	46.8	3.90
	12 (Gavage)	1.80	0.150	3	56.1	69.0	5.75
	24 (Gavage)	3.64	0.152	3	52.5	124	5.18
	48 (Gavage)	5.36	0.112	6	105	267	5.56
7:3-FTA (F)	40 (IV)	5.95	0.149	3	71.2	158	3.93
	40 (Gavage)	7.35	0.184	3	40.0	150	3.74
	80 (Gavage)	17.6	0.220	3	99.0	615	7.69
	160 (Gavage)	19.4	0.121	6	33.0	722	4.51

^a Predicted from non-compartmental analysis (NCA).

^b NCA does not calculate error.

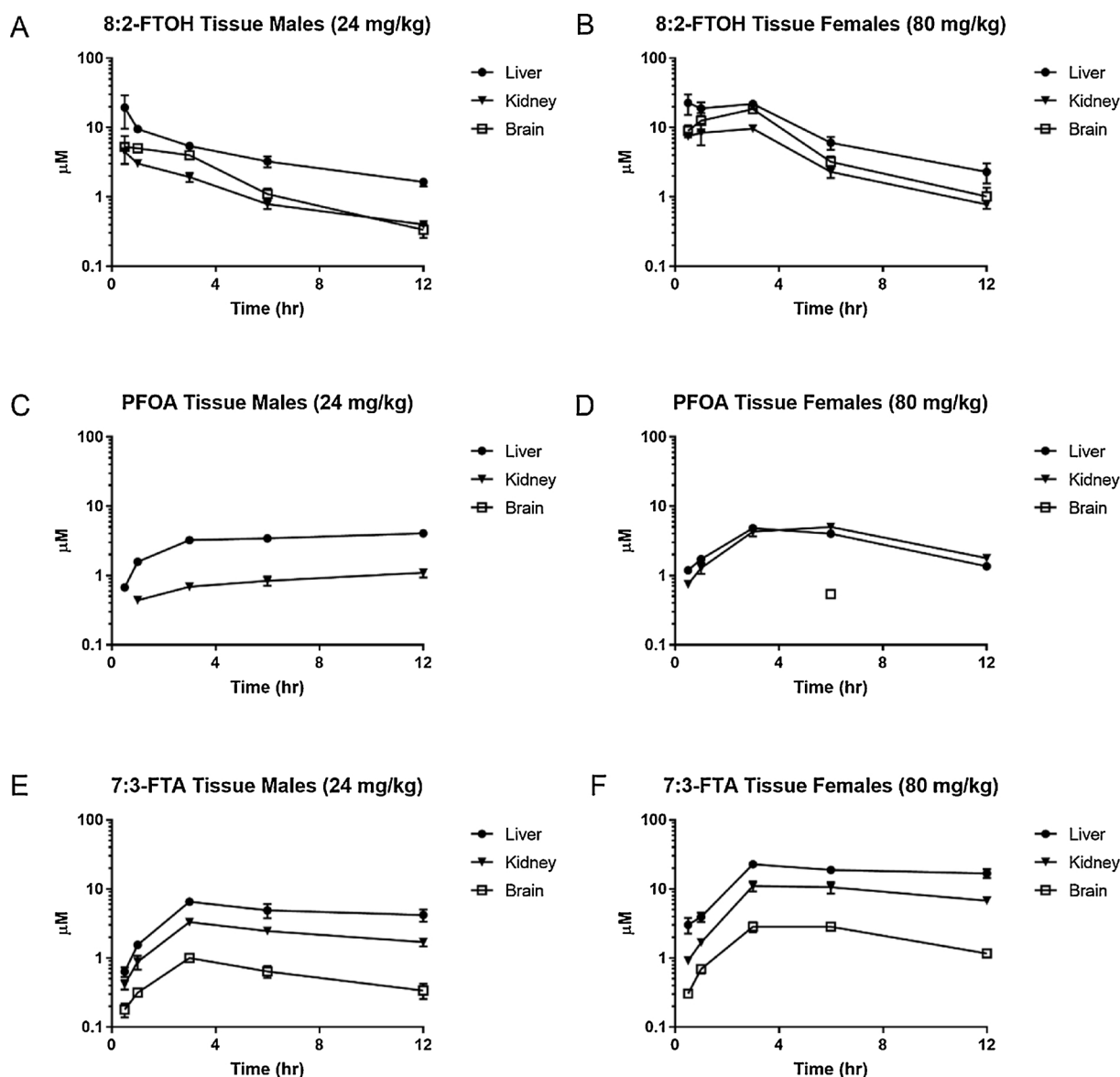


Fig. 4. Tissue concentrations (μM ; mean and SEM) of 8:2-FTOH (A,B) and its metabolites PFOA (C,D) and 7:3-FTA (E, F) in liver, kidney, and brain of male and female Sprague Dawley rats ($N = 3$) administered via gavage 24 mg/kg and 80 mg/kg 8:2-FTOH, respectively.

tissue:plasma ratios in females had a smaller increase over time than those in males.

For PFOA, the tissue:plasma ratios in males were > 1.0 in the liver but < 1.0 in the kidney (Fig. 5C, D) and stayed consistent over time. Liver:plasma ratios of PFOA in females were lower than ratios in males. Unlike males, the kidney:plasma and liver:plasma ratios tended to increase over time in females, suggesting elimination from the plasma. For 7:3-FTA, the liver:plasma ratio in male rats increased 2.5-fold over time while the 7:3 FTA kidney:plasma ratios increased by 1.6-fold (Fig. 5E, F). Brain:plasma ratios of 7:3-FTA were consistent over time and were lower than kidney:plasma 7:3-FTA ratios. Tissue:plasma ratios of 7:3-FTA in females followed similar trends to those observed in males.

4. Discussion

A shift towards telomerization in the production method of PFAS has increased the production of FTOHs. Since metabolism of FTOHs produces PFCAs [13], understanding the toxicokinetics of FTOHs and their metabolites is essential for understanding animal toxicity data and

its relevance to humans. In the current investigation, we report the toxicokinetic parameters of 8:2-FTOH and its metabolites, PFOA and 7:3-FTA, after a single administration (IV or gavage) of 8:2-FTOH in male and female Sprague-Dawley rats. Due to the anticipated differences in PFOA toxicokinetics, females received higher doses of 8:2-FTOH than males.

After gavage administration, 8:2-FTOH was rapidly distributed to tissues, evidenced by a T_{max} of 0.5–3 h and high tissue:plasma ratios even at the earliest time point (0.5 h post-administration). The tissue:plasma ratios increased over time. The high volume of distribution that greatly exceeded the total body water volume in rats also indicates distribution into the peripheral compartment. Determined concentrations of 8:2-FTOH in the kidney and brain were both about a quarter of the liver concentration, which was similar to the ratio seen by Fasano et al. [27]. Fasano and collaborators found that in Crl:CD SD rats 168 h post-administration of 125 mg/kg [$3\text{-}^{14}\text{C}$] 8:2-FTOH, the liver had one of the highest amounts of radioactivity (9–17 $\mu\text{g/g}$), the kidney contained $\sim 5 \mu\text{g/g}$, and the brain $\sim 0.3 \mu\text{g/g}$ [27]. Out of these tissues, only the liver had significant sex differences in tissue concentration [27]. We found higher concentrations of 8:2-FTOH in the brain, but this

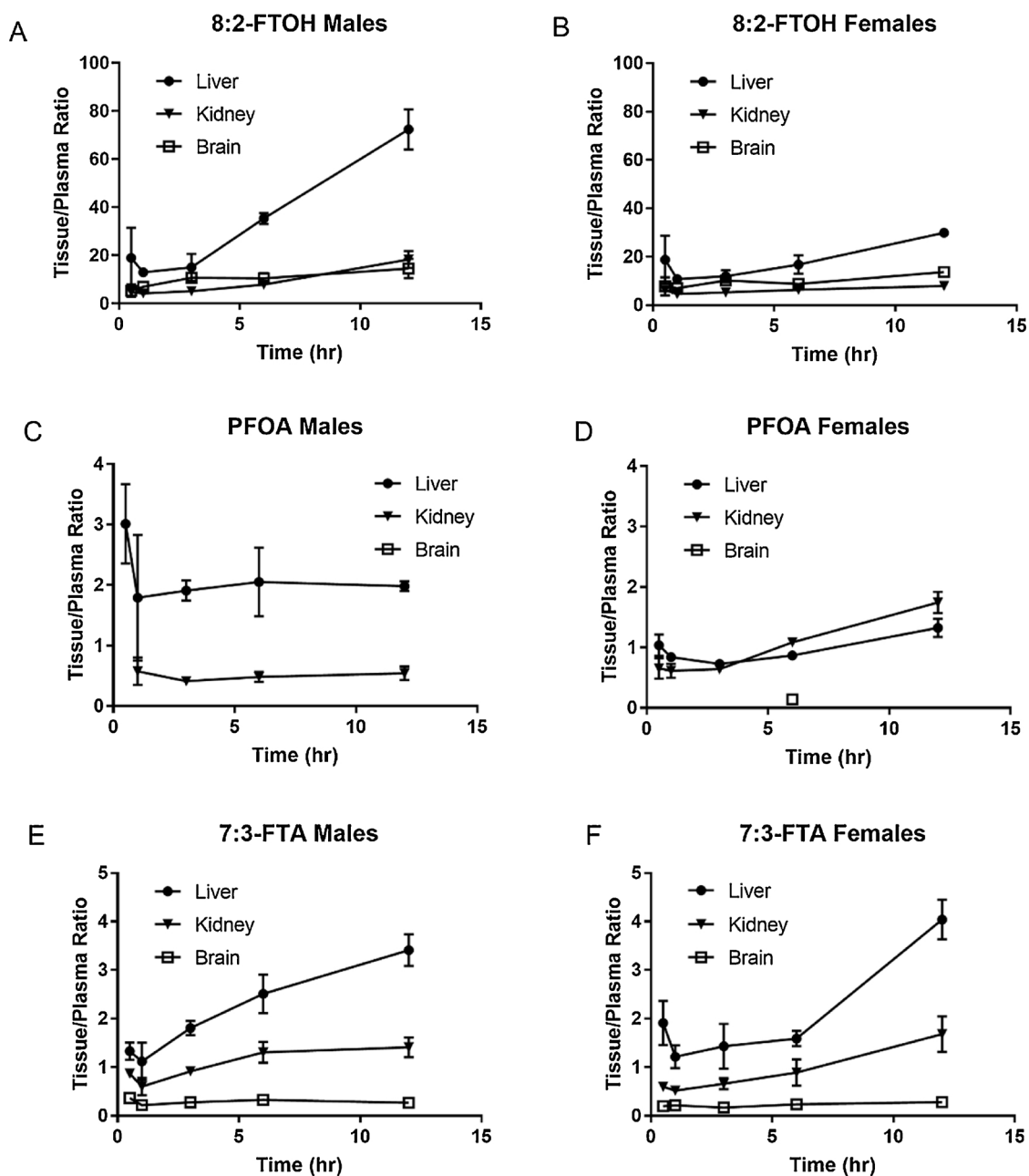


Fig. 5. Tissue:plasma ratios over time of 8:2-FTOH, PFOA, and 7:3-FTA in liver, kidney, and brain of male and female Sprague Dawley rats (N = 3) administered via gavage 24 mg/kg and 80 mg/kg 8:2-FTOH, respectively.

discrepancy may be due to time of tissue collection and the fact that Fasano et al. measured total radioactivity and not FTOH specifically.

In our study, females and males had similar tissue concentrations and tissue:plasma ratios of 8:2-FTOH. Since whole blood concentrations of the chemicals were not measured, tissue:plasma ratios were used to compare tissue accumulation. The plasma:blood partitioning for FTOH has not been determined, though it has been shown that FTOHs bind to serum proteins during its metabolism [41]. In humans, PFOA and other anionic PFAS show preferential binding in plasma with plasma:blood ratios of around 2 [42,43]. Thus, using the assumption that FTOH, PFOA, and 7:3-FTA have plasma:blood ratios of ~2:1 in both male and female rats, tissue:plasma ratios reported in this study would underestimate tissue:blood ratios. However, comparisons across chemicals and sexes can still be made. Concentrations of 8:2-FTOH in tissues slowly decreased over time (Fig. 4), suggesting that there was minimal accumulation of the compound in tissues and that the rise in

tissue:plasma ratios observed was likely due to elimination from plasma.

The plasma elimination half-life of 8:2-FTOH ranged from 1 to 4 h in both sexes which is similar to what was found in Fasano et al. [27]. In Fasano et al. [27], plasma elimination half-life ranged from 1 to 5 h, depending on the dose, and was not significantly different between males and females. Although there were few differences between sexes in our single administration study, prolonged exposure may elicit sex differences, as was observed in a 45-day exposure study in CrI:CD SD rats which showed there were greater sex differences in plasma and tissue 8:2-FTOH concentrations on Day 45 than on Day 1 of dosing [15].

Metabolism of 8:2-FTOH has been shown to occur in humans, rodents, and trout [15,17,44]. In rats, 8:2-FTOH is metabolized in the liver to produce glucuronidated and sulfonated 8:2-FTOH compounds as well as oxidized fluorotelomer acids that are metabolized to terminal

perfluorinated acids (summarized in [15]). PFOA and 7:3-FTA are stable metabolites found in rats and humans after exposure to 8:2-FTOH [15,17,44]. Here, we substantiate these reports, showing increases in tissue and plasma concentrations of PFOA and 7:3-FTA after administration of 8:2-FTOH in rats. At the doses administered in this study, we did not observe evidence of metabolic saturation in males because dose-adjusted AUC and C_{\max} of 8:2-FTOH and its metabolites were similar across the gavage doses. However, in females, which were administered a higher dose, there may have been metabolic saturation at the highest dose of 160 mg/kg as the dose-adjusted AUC of 8:2-FTOH was higher compared to the lower dose females and all male groups. The 22–41% bioavailability across the doses in males and females is likely due, in part, to metabolism of 8:2-FTOH.

PFOA was detected in the liver and kidney but not in the brain, consistent with previous studies [45,46]. Tissue:plasma ratios of 7:3-FTA in the liver and kidney were similar to those of PFOA in the same organs except in the brain where it was present in both males and females. Concentrations of 7:3-FTA in the brain remained constant during the measured time. Females had slightly higher concentrations of 7:3-FTA in tissues than males, which was also observed in Fasano et al. [15].

The plasma C_{\max} of PFOA and 7:3-FTA were 2- to 8-fold higher than the parent chemical 8:2-FTOH and reached T_{\max} much later than 8:2-FTOH. Females had a substantially shorter elimination half-life of PFOA than males, which has also been previously reported [27,46–48], resulting in lower C_{\max} and AUC after normalizing for dose (Table 3). The sex difference in PFOA half-life has been attributed to differences in renal resorption due to differences in expression of organic ion transporters [48,49]. The half-life of 7:3-FTA has not been reported in gavage studies of 8:2-FTOH [15,27] but in an inhalation exposure study, the half-life was 4–7 hours for both males and females [36]. This half-life is much shorter than our findings of ~2–3 days, implying differences in kinetics depending on the route of FTOH exposure. In our study, there were no major differences in internal exposure (dose-adjusted C_{\max} or AUC) or kinetic parameters of 7:3-FTA between males and females.

In females, the AUCs of 7:3-FTA are ~3-fold higher than that of PFOA but this trend is reversed in males whereas the AUCs of 7:3-FTA are 7 to 9-fold lower than AUCs of PFOA (Table 3). Furthermore, in females, dose-adjusted AUC of 7:3-FTA were higher than that of PFOA while the opposite was true in males (Table 3), indicating that following exposure to 8:2-FTOH, the persistent metabolite in females is 7:3-FTA while in males it is PFOA. Findings from other studies also show this trend, where females generally had higher concentrations of upstream metabolites (8:2-FTOH, 8:2-fluorotelomer acid, 7:3-FTA, and unsaturated 7:3-FTA) while males had more downstream metabolites (PFOA, perfluorononanoic acid, perfluoroheptanoic acid) [15,36]. Since some studies have found precursor FTAs to be more toxic than PFCAs [50–52], higher concentrations of FTAs in female rodents may be of concern. These different metabolite profiles in males and females are likely primarily due to the major sex difference in the half-life of PFOA. Additionally, in our study, females were administered higher doses of 8:2-FTOH than males, potentially altering metabolism kinetics which could explain these sex differences in metabolite concentrations.

There are few data on the concentrations or kinetics of FTOHs and their metabolites in humans. At the time of writing, there are no kinetic studies in humans that can be compared to the parameters presented in this study. However, it is possible to compare concentrations of FTOH metabolites. In a study of 11 male ski wax technicians with high inhalation exposure to 8:2-FTOH, 1.9 to 630 ng/mL of PFOA and 0.05 to 3.9 ng/mL 7:3-FTA were found in their blood; concentrations of 8:2-FTOH were not measured [17]. In the present study, the lowest oral dose of 8:2-FTOH in males (12 mg/kg) produced a plasma PFOA C_{\max} of 1.98 μM (743 ng/mL) and plasma 7:3-FTA C_{\max} of 1.79 μM (795 ng/mL). Though it is difficult to determine if concentrations of these metabolites in humans is from direct exposure or due to metabolism of 8:2-

FTOH, it is conceivable that occupationally-exposed individuals may experience internal doses of the metabolites, particularly PFOA, similar to the animals in this study. For the general population, the contribution of 8:2-FTOH exposure to PFOA levels is estimated to be minimal [16,18,44]. However, the combined effects of low concentrations of 8:2-FTOH metabolites and other PFAS remain to be elucidated.

Overall, our study corroborates previously published kinetic studies of 8:2-FTOH and its metabolite profile. This study confirmed that 8:2-FTOH has a rapid plasma elimination half-life and is readily absorbed into tissues. Sex differences in kinetics and tissue distribution of 8:2-FTOH were not observed. PFOA and 7:3-FTA were stable metabolites detected in plasma and tissues of both male and female rats after exposure to 8:2-FTOH. At these doses, there was little evidence of 8:2-FTOH metabolic saturation. Consistent with previous studies, there were sex differences in elimination and distribution of PFOA but not of 7:3-FTA.

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