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Bioconcentration of PFAS from AFFF Environmental Exposure: Evidence of Precursor Bioaccumulation in Fathead Minnows

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BIOCONCENTRATION OF PFAS FROM AFFF ENVIRONMENTAL EXPOSURE: EVIDENCE OF PRECURSOR BIOACCUMULATION IN FATHEAD MINNOWS

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ABSTRACT

Exposure to per- and polyfluoroalkyl substances (PFAS) has been associated with toxicities in wildlife and negative health effects in humans. Decades of fire training activity at Joint Base, Cape Cod, Massachusetts incorporated the use of aqueous film forming foam (AFFF) which resulted in long-term PFAS contamination of sediments, groundwater, and hydrologically connected surface waters. To explore the bioaccumulative potential of PFAS residing in complex environmental mixtures, a mesocosm was established to evaluate bioconcentration of PFAS from AFFF-contaminated groundwater by flow-through design. Fathead minnows (n = 24) were exposed to PFAS in groundwater over a 21-day period and tissue specific PFAS burdens in liver, kidney, and gonad were derived at three different time points. ∑PFAS concentrations in groundwater increased temporally, ranging from approximately 10,000 ng/L at day 1 to 36,000 ng/L at day 21. Relative compositions of PFAS functional groups in liver, kidney, and gonad shifted temporally from majority FASA to PFSA. By day 21, mean ∑PFAS concentrations in tissues displayed predominance in order of: liver > kidney > gonad. Generally, bioconcentration factors (BCFs) for perfluoroalkyl sulfonamides (FASA), perfluoroalkyl carboxylic acids (PFCA), and fluorotelomer sulfonates (FTS) increased with degree of fluorinated chain length, but this was not evident for perfluoroalkyl sulfonic acids (PFSA). Perfluorooctane sulfonamide displayed the highest mean BCF (7,700 L/kg) in day 21 kidney. Generally, mean BCFs for FASA were within or above bioaccumulative thresholds outlined by the U.S. Environmental Protection Agency's (US EPA) Toxic Substances Control Act (TSCA) criteria, "B".

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PREFACE

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INTRODUCTION

Fluorinated surfactants constitute an environmentally relevant class of contaminants known as per- and polyfluoroalkyl substances (PFAS). Unique to PFAS chemistry is the strength of the C-F bond, which provides resilience to withstand biotic and abiotic degradation in the environment. PFAS exhibit a suite of physicochemical properties such as high heat resistance, hydrophobicity, lipophobicity, as well as low surface tension that has made them suitable for a broad spectrum of manufactured water and stain-repellent products, herbicides, and aqueous film forming foams (AFFF) since the 1950s (Pabon and Corpart 2002; Paul et al., 2009; Buck et al., 2011; Gewurtz et al., 2014). Decades of manufacturing and use have rendered PFAS ubiquitous and persistent in nature with recalcitrance linked to several environmental reservoirs, including aquatic ecosystems, groundwater, sediment, soil, and biota (Shultz et al., 2004; Houtz et al., 2013; Ahrens et al., 2015; Lau et al., 2007). Globally, PFAS have been detected in biota in ecosystems adjacent to industrial point sources (Houde et al., 2014; Gewurtz et al., 2014; Shi et al., 2015; Munoz et al., 2019; Guillette et al., 2020) and in remote polar regions (Haukas et al., 2007). Dietary exposure is a main pathway for PFAS exposure in wildlife and humans (Sunderland et al., 2019), resulting in altered immune function, cancer, thyroid disease, adverse reproductive effects, and alterations in gene expression across a variety of mammals (to include humans) and aquatic organisms (Lau et al., 2007; Grandjean et al., 2012; Melzer et al., 2010; Halldorsson et al., 2012; Ankley et al., 2005). Thus, understanding origin, transport and fate, and biological stressors of these persistent organic pollutants

is critical for establishing human health advisories and evaluating ecotoxicological impacts in biota (Lau et al., 2007).

Where AFFF have been applied at fire-training areas (FTA) and locations of fuel fire incidences, elevated concentrations of perfluoroalkyl acids (PFAA) and their precursors have been detected in soils and sediments (Houtz et al., 2013), surface water (Nakayama et al., 2007; Oakes et al., 2010; Abercron et al., 2019), groundwater (Houtz et al., 2013; Schultz et al., 2004; Backe et al., 2013; Filipovic et al., 2015; Weber et al., 2017), and biota (Oakes et al., 2010; Kärrman et al., 2011; Ahrens et al., 2015; Taylor et al., 2018). Prior literature has a continued focus on PFAA, which comprise two major functional groups: perfluoroalkyl carboxylic acids ($C_nF_{2n+1}COOH$, PFCA) and perfluoroalkyl sulfonic acids ($C_nF_{2n+1}SO_3H$, PFSA). Long chain PFCA ($n \geq 7$ carbons), PFSA ($n \geq 6$ carbons), and PFAA precursors exhibit bioaccumulative behaviors and toxicological impacts in humans and other biota to include birds, mammals, fish, and crustaceans (Haukas et al., 2007; Miyake et al., 2007; Death et al., 2021; Oakes et al., 2010; Munoz et al., 2017; Shi et al., 2015; Taylor et al., 2018; Fair et al., 2019). Their environmental ubiquity, bioaccumulative behavior, and toxicological impacts have resulted in agency and government regulatory action to curb further release and exposure (Buck et al., 2011; Armitage et al., 2006; Armitage et al., 2009a, 2009b).

Two key production pathways of PFAS are electrochemical fluorination, which yields even and odd number of carbon atoms in poly- and perfluorinated chains, and telomerization, which results in telomers containing only an even number of carbons within the poly- and perfluorinated chains (Moody and Field, 2000).

Electrochemical fluorination and telomerization have been pathways for the development of AFFF since the 1960s by the United States Navy and 3M Company (Schultz et al., 2004). AFFF were originally developed to extinguish hydrocarbon-based fuel fires at airports, military bases, and municipal firefighter training facilities. Thus, understanding the production pathway of AFFF – i.e., manufacturer, production process, and year of production - can allude to the specific PFAS moieties that can be anticipated within an area at which AFFF were applied (Moody and Field, 2000).

The major contributor to the efficiency of AFFF are the PFAA that effectively serve as vapor sealants that prevent reignition of fuel and solvents by lowering surface tension between aqueous surfactant solution and the hydrocarbon phase (Moody and Field, 2000; Kishi and Arai, 2008; Oakes et al., 2010). During the 1960s and early 1970s, it was understood that the 3M Company manufactured AFFF containing PFCA, whereas through the 1970s to 2001, incorporation of PFSA, namely perfluorooctane sulfonic acid (PFOS), was included until production was phased out in 2002 (Houtz et al., 2013; Lau et al., 2007). Aside from PFAA, the 3M Company also exploited the similar chemical properties that polyfluorinated surfactants offer by incorporating four to six carbon chain length homologs containing sulfonamide moieties (Houtz et al., 2013). Polyfluoroalkyl substances, now recognized as PFAA precursors, are another major constituent in AFFF and can transform into PFAA through biotic and abiotic degradation processes (Weber et al., 2017; Houtz et al., 2013). For instance, the use of perfluorooctane sulfonamide (FOSA), a precursor of PFOS and common AFFF constituent, has exhibited recalcitrance in sediment, water, and internal tissue compartments of native fish (Ahrens et al., 2015). Consequently,

use of AFFF in the environment has led to major concerns for groundwater quality with various sites reporting groundwater concentrations of perfluorooctanoic acid (PFOA) and PFOS above the U.S. Environmental Protection Agency lifetime drinking water health advisory level (70 ng/L) for the combined concentration of PFOA and PFOS (Weber et al., 2017; Sunderland et al., 2019).

Unlike legacy, lipophilic persistent organic pollutants (POP), understanding toxicokinetics and partitioning of PFAS is unreliably predicted via traditional octanol-water partitioning coefficient (K_{ow}), given their proteinophilic nature. Instead, PFAS hazard assessment is met with empirical derivations for BCF or BAF to reflect the magnitude by which these compounds accumulate in various tissue compartments (Shi et al., 2018; Arnot and Gobas, 2006). Previous bioaccumulation studies have alluded to preferential accumulation of PFAS in blood and liver, a likely result of PFAS affinity for serum albumin or liver fatty acid binding protein (Ng and Hungerbühler, 2014; Chen et al., 2008; Bischel et al., 2010; Hebert et al., 2010). Consequently, the highest bioaccumulation is typically reported in livers of fish-eating animals living near industrialized areas (Lau et al., 2007). More recently, two hypotheses have been proposed to offer explanations of the pathways in which PFAS and other ionogenic compounds mechanistically bioaccumulate: i) phospholipid-bilayer partitioning and ii) noncovalent interactions with proteins (i.e., serum albumin, fatty acid binding proteins (FABP), organic anion transport proteins (OATP)) (Shi et al., 2018; Armitage et al., 2012; Armitage et al., 2013; Ng and Hungerbühler, 2014). In vitro pharmacokinetic (PK) and pharmacodynamic (PD) studies have previously confirmed high protein affinity of PFAS specifically in blood plasma and liver, as well as isolated proteins

and serum (Ng and Hungerbühler, 2014; Luebker et al., 2002; Han et al., 2003; Zhang et al., 2013; Khazaee et al., 2021). A common PK/PD approach to quantitatively investigate the exposure-effect relationships of pharmaceutical drugs is to determine the free drug concentration at specific biological sites. The free drug concentrations, recognized as the fraction unbound, provides a quantitative index with which to relate drug efficacy and biological interaction (Riccardi et al., 2018). Physicochemical properties of targeted compounds have previously been shown to influence tissue fraction unbound (Ryu et al., 2019), yet, to our knowledge, this specific approach has not been conducted in PFAS research with fish.

Environmental and toxicological studies have attempted to allude to the behavior of PFAS bioaccumulation with respect to tissue compartments, sex-specific bioaccumulation or elimination, and source of environmental exposures (Shi et al., 2018; Ng and Hungerbühler, 2014; Armitage et al., 2012; Ahrens et al., 2015; Liu et al., 2019). For instance, organic anion transporters and analogous renal transporters are expressed differently in sex-specific ratios, which can lead to differences in PK observations of PFAA accumulation and elimination (Han et al. 2012). While toxicology and PK studies are often designed to illicit a dose-response based on ex situ concentrations of contaminants, ecotoxicology approaches target anthropogenic point-sources associated with environmental pollution of targeted contaminants. One such commonality across recent research studies is FTA where AFFF has been applied to evaluate bioaccumulation occurrence and tissue concentrations (Oakes et al., 2010; Gewurtz et al., 2014; Houtz et al., 2016; Kärrman et al., 2011). The pronounced concentrations of PFAS in contaminated groundwater plumes associated with FTA

can serve as a valuable point source of AFFF leachate and impacts to groundwater recharge zones and downstream reservoirs (Weber et al., 2017). Exploring the dynamics of bioconcentration based on environmental mixtures related to AFFF contamination as a form of dose-dependence is not well represented in literature and may lead to more conclusive experimental evidence towards bioaccumulation potential of PFAS moieties and the biological response induced at environmental concentrations.

To better understand the bioaccumulative behavior of PFAS in environmental mixtures associated with FTA and prolonged AFFF application, this study constructed a mesocosm which utilized AFFF-contaminated groundwater to dose fathead minnows. The objectives of this study were to: (i) derive empirical results on bioconcentration potential of legacy and emerging PFAS that contribute to tissue-specific burdens; (ii) evaluate PFAS affinity for liver-binding through quantification of the fraction unbound; and (iii) delineate relationships of bioconcentration with PFAS molecular weight and chain-length within specific tissue compartments.

MATERIALS AND METHODS

Exposure Site Characterization

Joint Base Cape Cod (JBCC) is located on the western reaches of Cape Cod, Massachusetts, USA and serves as a joint military base for the United States Army, Air Force, and Coast Guard (Figure 1) (Weber et al., 2017). Throughout 1958 to 1997, JBCC conducted fire-training exercises that required broadscale application of AFFF at an FTA, which resulted in release of PFAS to the surrounding terrestrial and aquatic

environments. This specific FTA on JBCC is now demarcated as the U.S. Geological Survey (USGS) Cape Cod Toxic Substances Hydrology Research Site. Weber et al. (2017) previously characterized the PFAS composition of groundwater samples and sediment cores originating from the FTA. Their results indicated that sediments below the FTA and the former wastewater treatment effluent infiltration beds act as continuous PFAS sources that contribute to the contamination of the groundwater plume. Notably, groundwater well S488 located downgradient of FTA-1 (Figure 1), contained PFOS concentrations ranging between 42 and 59% of the molar sum of PFAS quantified across groundwater wells and reaching concentrations up to 63 µg/L (Weber et al., 2017).

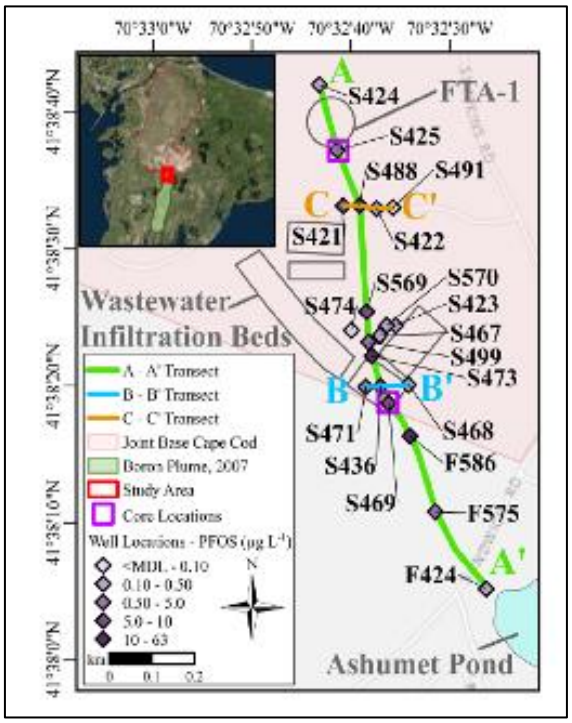


Figure 1: Map of Joint Base Cape Cod (JBCC) fire-training area (FTA) sampling sites and associated groundwater access wells (Weber et al., 2017).

Environmental Mixture Exposures

Environmental PFAS exposure of a model toxicology organism, *Pimephales promelas*, commonly known as the fathead minnow, was conducted over a 21-day period during the months of July and August of 2019 at the U.S Geological Survey (USGS) Cape Cod Toxic Substances Hydrology Research Site on JBCC. Here, sexually recrudescent male and female hatchery-reared fathead minnows (n = 24) were exposed to the contaminated groundwater plume downgradient of FTA-1 from groundwater well S488 (Figure 1) via continuous flow-through systems. To meet the requirements for continuous flow-through design, the USGS Hydrological-Ecological Interactions Branch established an enclosed mobile laboratory to which the continuous flow-through system was affixed. The tissue samples collected from each fathead minnow respective of the exposure treatment included the following: carcass (including swim bladder), brain, heart, kidney, liver, gonad, gills, gut, plasma, and remaining blood cell pellet after separation of plasma. Weekly water samples (1L HDPE bottles, n = 3) were also collected and stored on ice until frozen. All water and tissue samples were transported frozen on ice to the University of Rhode Island Graduate School of Oceanography to be analyzed for PFAS tissue burden.

Table 1: Mobile fish lab fathead minnow dosing exposure treatment groups and simultaneous water sample quantity.

Day #	Treatment	Sex (M/F)	Water Samples (1 L)
1	FTA 100%	n = 3 M n = 3 F	n = 1
7	FTA 100%	n = 3 M n = 3 F	n = 1
21	FTA 100%	n = 3 M n = 3 F	n = 1

Standards and Reagents

Formic acid (≥ 99%), liquid chromatography-mass spectrometry (LC-MS) grade acetic acid (≥ 99%), and LC-MS grade methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). Analytical PFAS standards were purchased from Wellington Laboratories (Ontario, Canada). Dispersive ENVI-Carb (Supelco) was purchased from Sigma-Aldrich.

Preparation of Water

Weekly water samples (n = 3) (Table 1) were prepared for direct injection on LC-MS/MS. Water samples (1 L) were allowed to equilibrate to room temperature and were then placed in an ultrasonic bath for ~ 20 min. Following sonication, approximately 5 mL of water sample was transferred into a 15 mL Corning® Falcon conical centrifuge tube with 5 mL of LC/MS-grade methanol. Mass-labeled internal standard (4 ng/sample) and native PFAS mix (4 ng/sample) were added to respective

samples and were then vortexed for 2 min. 10 μL of LC/MS-grade acetic acid was added to each sample and the samples were vortexed for ~ 30 s. Approximately 1 mL of sample was transferred to a 1.5 mL Eppendorf® microcentrifuge tube and the sample was centrifuged at 10,000 rpm for 5 min. 980 μL of the supernatant was transferred to a gas chromatograph vial and 20 μL of a mass-labeled injection standard (2 ng/sample) was added to quantify instrumental recoveries.

Laboratory PFAS Tissue Extraction

Tissue samples were individually homogenized in an OMNI® Bead Ruptor Elite™ until a wet tissue slurry remained. Solvent extraction of PFAS from homogenized tissues was conducted using LC/MS-grade methanol and 0.1 M formic acid in LC/MS-grade methanol. First, 150 μL of LC/MS-grade methanol was added to each sample followed by the addition of mass-labeled surrogate PFAS standard mixture (4 ng/sample). Two process blanks per sample batch were included in the procedure (n = 8), each spiked with the same mass-labeled standard mixture (4 ng/sample). Fortified samples were then vortexed for ~ 20 s and then were allowed to equilibrate at room temperature for ~ 30 min. Following equilibration at room temperature, 200 μL of 0.1 M formic acid in LC/MS-grade methanol was added to each sample to precipitate biological matrix. Samples were then vortexed for ~ 30 s and then centrifuged at 10,000 rpm for 5 min on a Thermo Scientific mySPIN™ 12 mini centrifuge. The supernatant was then transferred to a new 1.5 mL Eppendorf® microcentrifuge tube. Next, 400 μL of LC/MS-grade methanol was added to each tissue pellet and the vortex and centrifugation steps were repeated. Supernatants were

combined. The methanolic extracts were then evaporated to ~ 500 μL under a gentle stream of nitrogen gas and then stored at -15 ºC overnight.

For sample clean-up, cold, methanol extracts were centrifuged at 10,000 rpm for 5 min to further remove precipitated tissue matrix and the supernatant was transferred into tubes with ~10mg of dispersive ENVI-carb (Supelco®). Samples were then vortexed for ~ 30 s and then centrifuged at 10,000 rpm for 5 min and the supernatant was transferred and stored at -15 ºC until instrumental analysis.

LC-MS Analysis of Water and Tissue

Quantification of PFAS in water and tissue was performed using a SCIEX ExionLC AC UHPLC system coupled to a SCIEX X500R quadrupole time-of-flight tandem mass spectrometer (QTOF MSMS). A Phenomenex Gemini 3μm C18 110Å 50x2mm LC analytical column preceded with a Phenomenex SecurityGuard cartridge was used for the analyte separation. Another Phenomenex Gemini 5um C18 110 Å 50x4.6mm LC analytical column was used to delay the PFASs instrumental contribution. For the quantification of the target analytes, a HRMSMS (MRM HR) method was used. The negative ESI with the following parameters was used: curtain Gas at 30 psi, ion source gas 1 at 40 psi, ion source gas 2 at 60 psi, temperature 450°C.

QA/QC

All results were recovery corrected using the corresponding mass-labeled surrogate standards spiked into the sample prior to extraction as described above. Recoveries of mass-labeled standards ranged 45 ± 3.0% to 150 ± 20 % in water and 37

± 9.4% to 180 ± 93% in tissue specific samples (SI Table 3, SI Table 4). Unexposed fathead minnow liver, kidney, and gonad were extracted following the same solvent extraction to construct a seven-point matrix matched calibration curve. A set of procedural blanks (n = 8) was processed the same way as the samples to calculate limits of detection (LODs). The LODs were calculated as an average blank value plus 3 times it's standard deviation. Consecutively, the average of the procedural blanks was subtracted from the samples above LOD. When a compound was not detected in the blanks, instrumental detection limits were used (SI Table 5).

Deriving Bioconcentration Factors

Bioconcentration is considered to occur mainly across gill epithelia of the fathead minnow. Bioconcentration factors can be derived with Eq. (1):

$$BCF = C_{tissue}/C_{water} \quad (1)$$

where C_{tissue} is the concentration in the specific tissue sample (μg/kg ww) and C_{water} is the concentration in the weekly water sample (μg/L). Calculations for BCFs were applied to each tissue-specific sample respective of treatment group and reported as a mean BCF where concentrations for tissue-specific replicates. Inter-individual variability of reported concentrations and bioconcentration factors are present throughout the dataset, which led to several instances in which a mean BCF was only calculated based on two quantifiable tissue concentrations above the LOD. In the instance in which only one reportable concentration was above the LOD, the BCF was also calculated and reported. A BCF value > 1 indicates that bioconcentration has occurred in the tissue. BCFs were compared with fluorinated chain length (nCF_2), as

well as PFAS molecular weight to explore relationships between PFAS bioconcentration and physicochemical properties.

Fraction Unbound Quantification with Equilibrium Dialysis

Equilibrium dialysis provides a quantitative method to determine free drug concentrations in PK/PD practices and is comparable to *in vivo* measurements of free drug concentrations (Liu et al., 2009; Ryu et al., 2019). Since insufficient amounts of fathead minnow liver were available from the mobile fish lab experiment, monkfish liver was acquired from local commercial fishermen (Boston, Massachusetts) to use as surrogate for fish liver matrix. Whole monkfish liver was rinsed thoroughly with 1X phosphate-buffered saline (pH 7.4) (PR1MA™, Midwest Scientific, Valley Park, Missouri) to remove residual blood. Approximately 10 g of liver was homogenized with a Tekmar Tissumizer (Teledyne Tekmar©, Mason, OH) in a 1:1 (w/v) dilution of tissue and 1X PBS (~10mL, pH 7.4) and stored at -80 ºC until analysis at Pfizer Worldwide Research & Development (Groton, Connecticut).

PFAS standards purchased from AccuStandard (New Haven, Connecticut) were prepared in DMSO stock solutions at 200 μm and were added in 1:100 ratio to tissue homogenates. The final concentrations of test compounds were 2 μm with 1% DMSO. Tissue homogenates with spiked test compounds were dialyzed against PBS at 37 ºC on an orbital shaker (VWR, Radnor, PA) at 200 rpm in a humidified CO_2 incubator (75% relative humidity, 5% CO_2/air) for 18 h in quadruplicate. Following incubation, samples were quenched with cold acetonitrile containing internal standards to precipitate proteins and tissues. Plates were sealed and mixed with a vortex mixer

for 1 min, then centrifuge at 3000 rpm at room temperature for 5 min. The supernatants were transferred to a new deep 96-well plate, dried, reconstituted in 1:1 water/acetonitrile, and analyzed using LC-MS/MS (Ryu et al., 2019).

LC-MS/MS Quantification

A LC-MS/MS system with a CTC PAL autosampler (LEAP Technologies, Carrboro, NC), a 1290 binary pump (Agilent, Santa Clara, CA) with HALO C18 UPLC column, 2.1 x 30 mm, 2.7 μm (Mac-Mod, Chadds Ford, PA), and a triple quadrupole 5500 mass spectrometer (Sciex, Foster City, CA) was used for sample analysis. Multiple reaction monitoring (MRM) was used to detect ion transitions of targeted compounds. A linear UPLC gradient was performed from 90% mobile phase A (0.1% formic acid in water) to 95% mobile phase B (0.1% formic acid in acetonitrile) over 1.2 min with a flow rate of 0.5 mL/min to elute compounds. Data acquisition and quantitation were performed using Analyst version 1.6.2 and MultiQuant version 3.0.2 (Applied Biosystems, Foster City, CA).

Calculation of Fraction Unbound and QAQC

The fraction unbound (f_u) was calculated based on Eq. (2) from which area ratios of targeted compounds to internal standard present in receiver and donor wells were quantified by LC-MS/MS.

$$f_u = \frac{\text{Receiver Area Ratio}}{\text{Donor Area Ratio}} \quad (2)$$

where liver homogenates were diluted, concentrations were corrected using Eqs. (3) and (4), where DF represents the dilution factor (Riccardi et al., 2015; Ryu et al., 2019).

$$\text{Diluted } f_{u,d} = \frac{\text{Receiver Area Ratio}}{\text{Donor Area Ratio}} \quad (3)$$

$$\text{Undiluted } f_u = \frac{1/DF}{(((1/f_{u,d}) - 1) + 1/DF)} \quad (4)$$

Assay recovery and stability calculation were also performed using Eqs. (5) and (6), respectively.

$$\%\ \text{Recovery} = \frac{\text{Donor Area Ratio} + \text{Receiver Area Ratio}}{\text{Donor Area Ratio at Time Zero}} \times 100\% \quad (5)$$

$$\text{Stability as \% Remaining} = \frac{\text{Area Ratio at 18 Hour}}{\text{Area Ratio at Zero Hour}} \times 100\% \quad (6)$$

Data and Statistical Analysis

Data were analyzed using GraphPad Prism (Version 9.3.1, GraphPad Prism Software, San Diego, CA) and Microsoft Excel (Version 16.60, Microsoft, Redmond, WA). Mean values for PFAS concentrations were determined from values above the LOD. Tissue specific data on fathead minnows were mass normalized and pooled as composites, combining male and female samples to increase sample size (n = 6). For day 7 and day 21 liver samples, one male and female liver were used to develop the

extraction and analytical method and so composites for these treatment groups were constructed as n = 4. Linear regressions were conducted to explore relationships of PFAS fluorinated chain length and tissue specific logarithm-transformed bioconcentration factors of the day 21 treatment groups (SI Table 9). Fluorinated chain length (nCF_2) is defined as the number of carbons with two or more chemically bound fluorine atoms. Additionally, since specific structures could not be determined for branched isomers, only linear forms of the isomeric structures were inputs for analysis with linear regression, except for PFECHS which was included in the analysis. The same linear regressions were also applied using PFAS molecular weight and similar results were present (SI Table 10). The same statistical tests were utilized to determine relatedness of the fraction unbound (f_u) with fluorinated chain length and molecular weight of PFAS included in the equilibrium dialysis experiment with monkfish liver. Logarithm transformations were conducted on f_u from equilibrium dialysis and Pearson correlation was applied to test for significance between logBCF of exposed fathead minnows and f_u of analogous compounds.

RESULTS

PFAS Concentrations in Water

20 of the 41 targeted compounds were detected across day 1, day 7, and day 21 water and perfluorocyclohexane sulfonate (PFECHS) was only detected in the day 21 water sample. Generally, concentrations of detected compounds increased across sampling days, while the composition of PFAS functional groups remained stable across weekly water samples (Figure 2). PFSA were the most abundant functional

group in weekly samples, comprising 73% of the total molar sum of PFAS at days 1 and 7 and 71% at Day 21. Linear (L-) and branched (Br-) isomers of both PFOS and perfluorohexane sulfonic acid (PFHxS) were predominant with exposure concentrations from day 21 quantified at 14,000 ng/L and 6,200 ng/L for L-PFOS and Br-PFOS, and 3,800 ng/L and 660 ng/L for L-PFHxS and Br-PFHxS, respectively. The perfluoroalkyl sulfonamides (FASA) maintained a relative composition at 12% of the total molar sum of PFAS across samples. Linear-perfluorohexane sulfonamide (L-FHxSA) and branched-perfluorohexane sulfonamide (Br-FHxSA) displayed the highest concentrations of the FASA, measured at 2,200 ng/L L-FHxSA and 1,000 ng/L Br-FHxSA in the day 21. For PFCA, perfluorohexanoic acid (PFHxA) was the most prominent compound at day 1 (380 ng/L) but was surpassed by PFOA at day 7 and day 21 in which PFOA was present at 860 ng/L and 2,000 ng/L, respectively. Fluorotelomer sulfonates (FTS) comprised the lowest molar fraction (3-4% of the total molar sum of PFAS) across sampling days and only 6:2 FTS and 8:2 FTS were detected in each weekly water sample.

Table 2: Water concentrations (ng/L) of PFAS detected in groundwater samples across exposure days. Included are the day 7 standard deviations based on analysis of duplicate day 7 samples.

	Exposure Day		
Compound	Day 1	Day 7 (ng/L ± SD)	Day 21
PFBA	66	91 ± 15	270
PFPeA	120	220 ± 5.5	410
PFHxA	380	760 ± 82	1500
PFHpA	120	230 ± 5.0	480
L-PFOA	390	860 ± 15	2000
Br-PFOA	29	68 ± 2.6	160
PFNA	21	26 ± 2.5	46
PFPeS	49	120 ± 2.8	220
L-PFHxS	840	1800 ± 31	3800
Br-PFHxS	160	330 ± 66	660
L-PFHpS	85	180 ± 0.20	380
Br-PFHpS	26	61 ± 14	150
L-PFOS	4400	8400 ± 440	14000
Br-PFOS	1800	3300 ± 100	6200
FBSA	250	560 ± 3.2	910
L-FHxSA	620	1200 ± 100	2200
Br-FHxSA	340	600 ± 11	1000
FOSA	30	34 ± 5.0	39
PFECHS	N.D.	N.D.	32
6:2 FTS	130	350 ± 8.5	930
8:2 FTS	230	280 ± 23	520

a: N.D. denotes the compound was not detected.
b: L- denotes linear form of the isomer and Br- denotes the branched forms

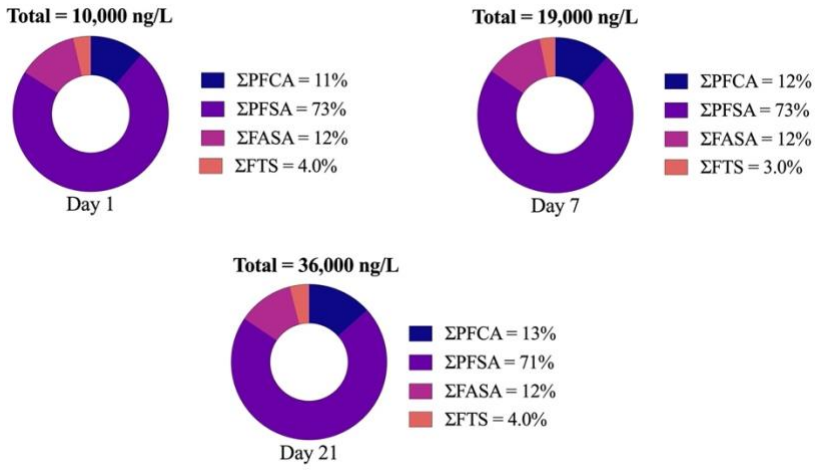


Figure 2: Relative composition of PFAS functional groups present in groundwater samples collected at the specific day of groundwater exposure. Total PFAS (∑PFAS) is presented in ng/g ww.

Functional Group Distributions in Fish Tissue

Of the 41 targeted compounds, 21 compounds were detected in both water and tissue. Relative compositions of PFAS functional groups in tissue compartments varied throughout duration of groundwater exposure (Figure 3). At day 1, ∑FASA comprised 83%, 87%, and 78% of the ∑PFAS concentrations in liver, kidney, and gonad, respectively. Major shifts in relative compositions were evident by day 7 in liver and gonad in which ∑PFSA comprised 55% of the ∑PFAS. By day 21 of groundwater exposure, total mean concentrations of functional groups revealed ∑PFSA > ∑FASA > ∑FTS > ∑PFCA across tissue types. Overall, PFSA and FASA groups comprised the bulk of tissue specific PFAS burdens when compared to PFCA and FTS. Mean concentrations of ∑PFAS were highest in liver, followed by kidney and gonad.

Perfluoroalkyl sulfonates – PFSA

Mean ∑PFSA concentrations were highest in liver (13,000 ng/g ww) and kidney (7,300 ng/g ww), followed by gonad (3,300 ng/g ww). Of the 12 targeted PFSA, only L-PFHxS, L-PFOS, and Br-PFOS were detected in all tissue samples across treatment groups. Relative compositions of L-PFOS contributed to the bulk of ∑PFSA burdens, comprising approximately 50% of ∑PFSA in day 21 liver and gonad and 61% in kidney. Day 21 mean concentrations of L-PFOS were 6,600 ng/g ww, 4,400 ng/g ww, and 1,700 ng/g ww in liver, kidney, and gonad, respectively (SI Table 7). By day 21, L-PFHxS comprised approximately 20% of ∑PFSA in liver and kidney and 28% of ∑PFSA in gonad. Mean concentrations of L-PFHxS in day 21 liver,

kidney, and gonad, reached 2,700 ng/g ww, 1,500 ng/g ww, and 910 ng/g ww, respectively. Br-PFHxS and Br-PFOS were present at lower mean concentrations than their linear isomers with highest mean tissue concentrations of branched isomers similarly observed at day 21 (SI Table 7). Mean concentrations of Br-PFHxS and Br-PFOS were highest in the order of liver > kidney > gonad (Fig. 4B). From day 7 to day 21, the mean concentration of Br-PFHxS in kidney appeared stable but continued to increase in liver and gonad (SI Table 7). PFECHS was detected in 100% of day 21 liver and gonad samples with the highest reported concentration in liver (18 ng/g ww). Additional compounds with 100% detection frequency in tissue samples at day 21 included perfluoropentane sulfonic acid (PFPeS) and perfluoroheptane sulfonic acid (L-PFHpS). Although, these mean concentrations were generally at least one order of magnitude lower than C6 or C8 PFSA. PFSA compounds detected in tissue but not in water samples included perfluorononane sulfonic acid (PFNS) and perfluorodecane sulfonic acid (PFDS).

Perfluoroalkyl sulfonamides - FASA

Relative compositions of ∑FASA were highest in day 1 samples across tissue types but decreased in importance over time (Figure 3). For example, in day 1 liver, ∑FASA comprised 830 ng/g ww, compared to 170 ng/g ww ∑PFSA, 2.4 ng/g ww ∑FTS, and 2.2 ng/g ww ∑PFCA. Perfluorobutane sulfonamide (FBSA), L-FHxSA, Br-FHxSA, and FOSA were detected in 100% of samples across treatment groups. Generally, mean concentrations of ∑FASA were highest in kidney, followed by liver and gonad across treatment days with concentrations reaching 5,300 ng/g ww, 3,500

ng/g ww, and 1,700 ng/g ww by day 21, respectively. At day 21, L-FHxSA was the most prevalent sulfonamide moiety, comprising 69% of ∑FASA in kidney, 63% in liver, and 60% in gonad. Mean concentrations of L-FHxSA quantified in kidney, liver, and gonad were 3,700 ng/g ww, 2,200 ng/g ww, and 1,000 ng/g ww, respectively (Fig. 4C). Compositional profiles of Br-FHxSA ranged from approximately 22% of ∑FASA liver burden, to 17% of kidney and 25% of gonad, reaching mean concentrations at day 21 of 790 ng/g ww, 890 ng/g ww, and 420 ng/g ww, respectively. FBSA and FOSA comprised the smallest fraction of liver, kidney, and gonad burdens. Mean concentrations of FBSA and FOSA were highest in day 21 kidney at 430 ng/g ww 300 ng/g ww, respectively.

Perfluoroalkyl carboxylates – PFCA

Mean concentrations of ∑PFCA were highest in order of liver > kidney > gonad. Day 21 mean concentrations of ∑PFCA in liver, kidney, and gonad were 79 ng/g ww, 53 ng/g ww, and 18 ng/g ww, respectively. ∑PFCA comprised the smallest fraction of ∑PFAS by day 21 across tissue types, ranging from 0.35% in gonad to 0.41% in kidney, and 0.46% in liver. The only PFCA detected in all sample replicates across treatments was L-PFOA. Br-PFOA was also detected in 100% of day 21 liver and gonad samples, but only in 67% of day 21 kidney samples. The detection frequency of short chain PFCA (n < 7 carbons) quantified in tissue replicates ranged from 17% to 100% and was variable over exposure days (SI Table 7).

Fluorotelomer sulfonates – FTS

4:2 FTS, 6:2 FTS, and 8:2 FTS were included in the targeted method but only 6:2 FTS and 8:2 FTS were detected in tissue samples. Detection frequency of 6:2 FTS generally ranged from 25% to 83% of samples and was more prominent in day 21 kidneys (SI Table 7). In contrast, the longer chain fluorotelomer, 8:2 FTS, was detected in 100% of liver and gonad samples across treatments and in 100% of day 7 and day 21 kidney samples. ∑FTS comprised approximately 5% of ∑PFAS in kidney, 1.2 % in gonad, and 0.71 % in liver, by day 21 of groundwater exposure. Day 21 mean concentrations of 6:2 FTS were highest in order of kidney > liver > gonad (Figure 4D). This similar relationship was conserved with 8:2 FTS, although mean concentrations of 8:2 FTS were higher. Mean concentrations of 8:2 FTS peaked at 640 ng/g ww in day 21 liver, followed by 120 ng/g ww and 62 ng/g ww in day 21 liver and gonad, respectively (Figure 4D).

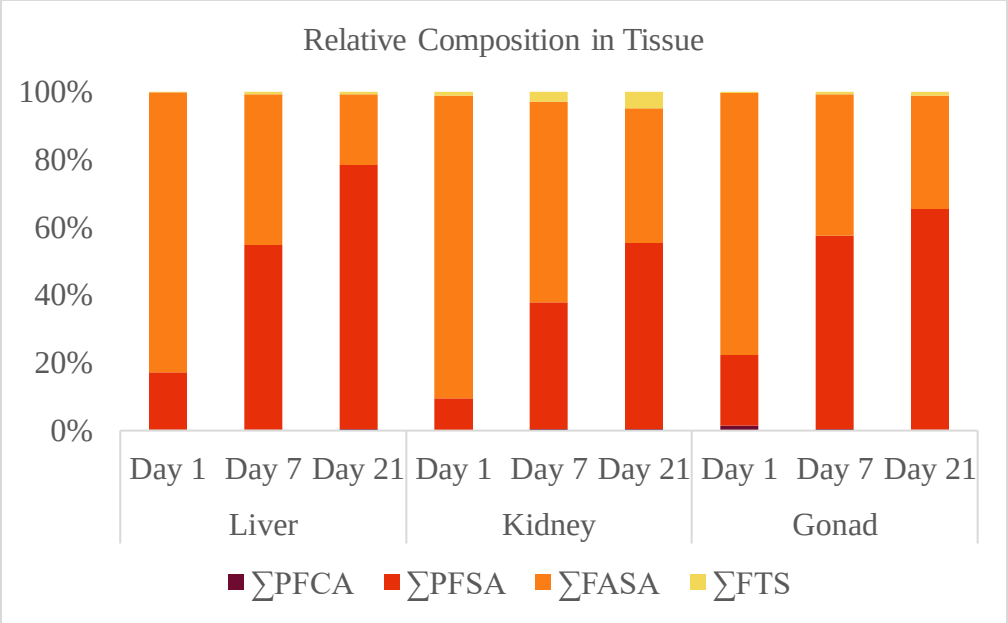
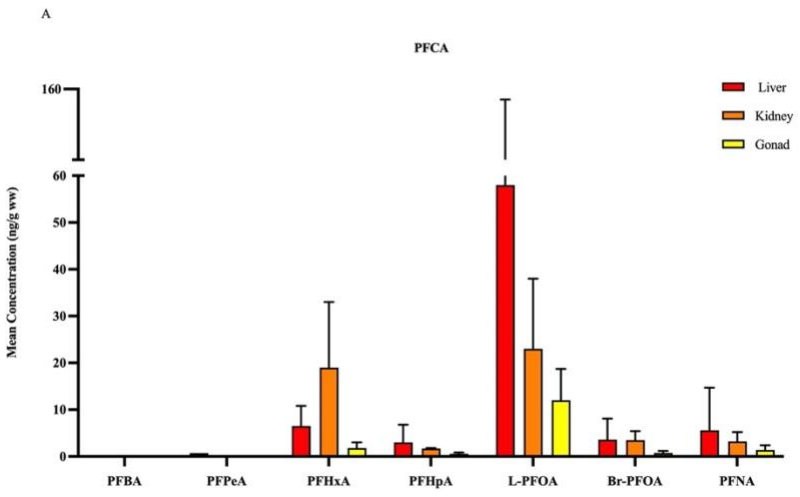
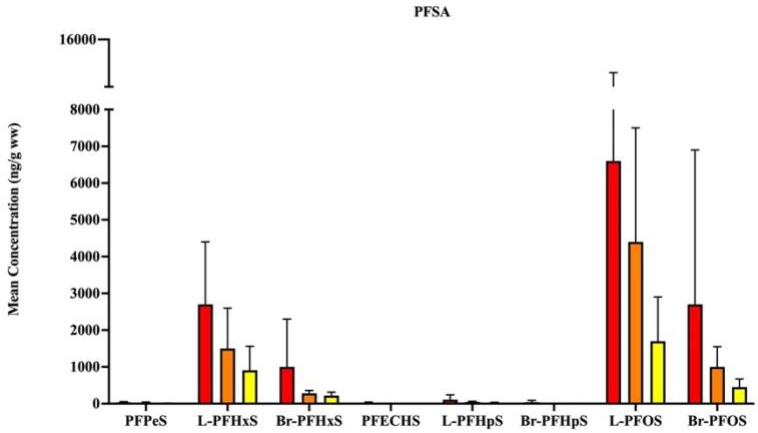


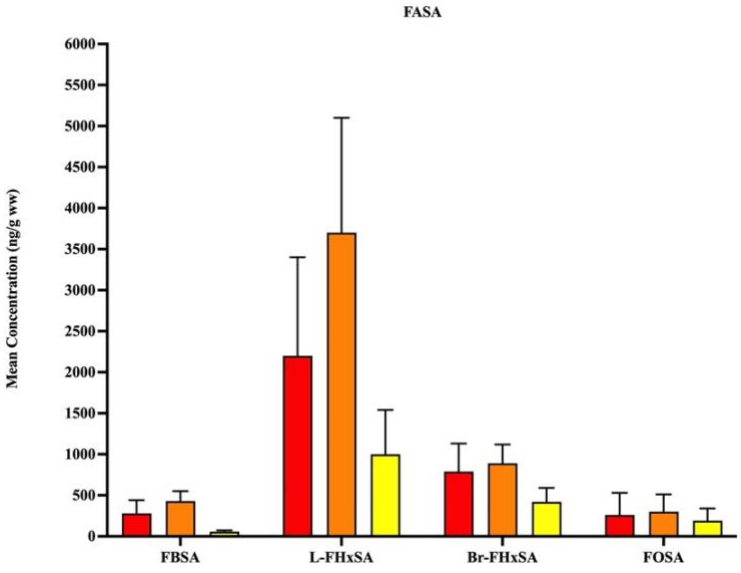
Figure 3: Relative compositions of PFAS functional groups quantified in tissue-specific samples from each dosing-exposure time point.



B


PFSA
Mean Concentration (ng/g ww)
16000
8000
7000
6000
5000
4000
3000
2000
1000
0
PFPeS
L-PFHxS
Br-PFHxS
PFECHS
L-PFHpS
Br-PFHpS
L-PFOS
Br-PFOS

C


FASA
Mean Concentration (ng/g ww)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
FBSA
L-FHxSA
Br-FHxSA
FOSA

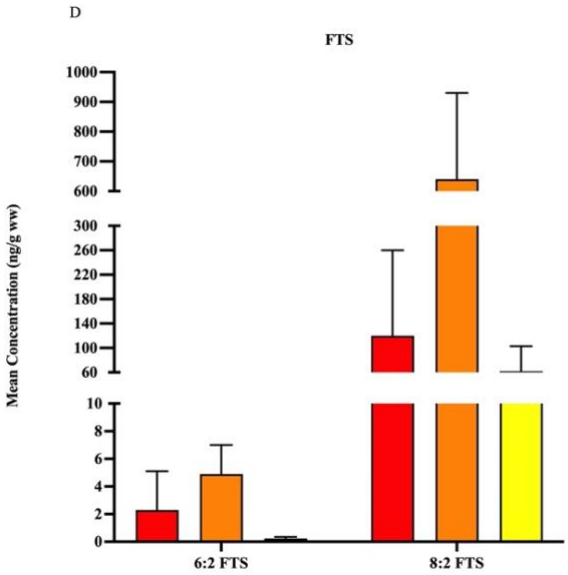


Figure 4: Day 21 tissue-specific concentrations (ng/g ww) of PFAS functional groups in which: (A) Perfluoroalkyl carboxylic acids (C4-C8), (B) Perfluoroalkyl sulfonic acids (C5-C8), (C) Perfluoroalkyl sulfonamides (C4-C8) (D) 6:2 and 8:2 fluorotelomer sulfonic acids. Error bars represent the standard deviation.

Bioconcentration Behavior and PFAS Properties

Across tissue types, a common trend was evident with BCF and fluorinated chain length in which strong correlations (Pearson's) existed for PFCA and FASA, but not PFSA (SI Table 9). Linear regression applied to BCF for 6:2 FTS and 8:2 FTS displayed perfect linearity (R^2 =1) with fluorinated chain length, although not enough data were available for analysis with Pearson correlation. Slopes representing the increase in BCF with fluorinated chain length varied between 0.27 and 0.45 for PFCA and 0.30 and 0.47 for FASA across liver, kidney, and gonad (SI Table 9). For PFCA, BCFs ranged from 1.7 L/kg (PFPeA) to 120 L/kg (PFNA), increased with degree of fluorination, and were generally higher for C7 or C8 PFCA in liver, compared to kidney and gonad (Figure 5). BCFs for individual FASA were generally higher for kidney, followed by liver and gonad. Day 21 tissue specific BCFs for L-FHxSA

revealed kidney (1,600 L/kg) was greater than liver (990 L/kg) and gonad (450 L/kg). BCFs at day 21 for FOSA are the highest reported in this study and were found to be 7,700 L/kg for kidney, 6,700 L/kg for liver and 4,900 L/kg for gonad. Strong increases in BCFs (Figure 5) were also evident in FTS of increasing chain length with slopes ranging from 0.97 to 1.3 (SI Table 9).

BCF and fluorinated chain length of PFSA were not strongly correlated (Pearson's) in liver ($R^2 = 0.12$, $r = 0.35$), kidney ($R^2 = 0.13$, $r = 0.36$), or gonad ($R^2 = 0.085$, $r = 0.29$), indicating fluorinated chain length is not a strong indicator of bioconcentration for this functional group in comparison to others in this study. Day 21 BCFs for L-PFHxS were highest in liver (690 L/kg), followed by kidney (380 L/kg) and gonad (240 L/kg) (SI Table 8). Temporally, L-PFOS BCFs were lower than L-PFHxS, and by day 21 were calculated to 460 L/kg (liver), 310 L/kg (kidney) and 120 L/kg (gonad). BCFs calculated for linear and branched isomers revealed Br-PFHxS BCFs were greater than L-PFHxS. The highest reported BCF for Br-PFHxS was 1,500 L/kg in day 21 liver. BCFs for Br-PFHxS in kidney decreased from 850 L/kg to 430 L/kg from day 7 to day 21, and a similar decrease was evident in gonad (SI Table 8). FTS displayed strong increases in BCF with increasing fluorinated chain length (Figure 5). BCFs for 8:2 FTS were highest in day 21 kidney (1200 L/kg).

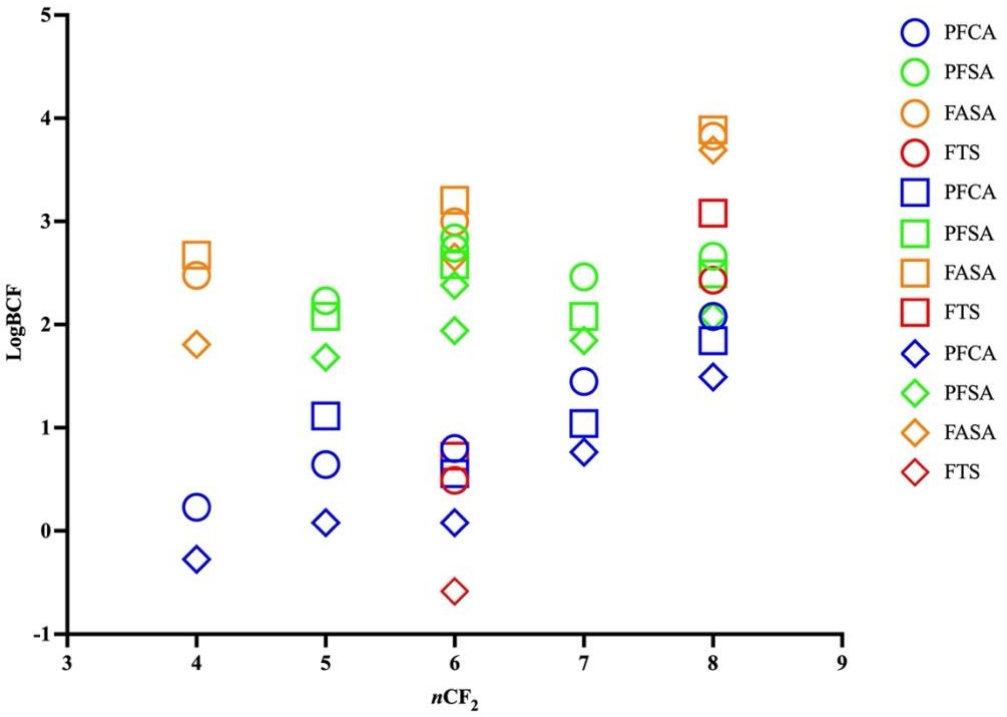


Figure 5: Day 21 logarithm transformed bioconcentration factors (BCF, L/kg) of PFAS functional groups in relation to fluorinated chain length (nCF$_2$). BCF corresponding to liver, kidney, and gonad are indicated by circles, squares, and diamonds, respectively.

PFAS Properties and Fraction Unbound

Results from the equilibrium dialysis experiment with test compounds are included in Table 3. While fraction unbound is often employed in PK/PD practices to predict drug interaction with biological targets, we exemplify the applicability in PFAS fate and transport through quantifying PFAS fraction unbound for monkfish liver matrix. Here, the fraction unbound represents the proportion of free PFAS that are not associated with liver matrix. Generally, f_u values displayed strong correlations and decreased with increasing fluorinated chain length (Pearson's $r(8) = -0.92$, $p \leq 0.0001$) and molecular weight (Pearson's $r(8) = -0.95$, $p \leq 0.0001$) (Figure 7 A, B). Short-chain PFCAs and PFSAs included in the experiment showed order of magnitude

differences in their amount of free concentration. This association, in part, may explain the reduced tissue burdens and bioconcentration behavior of these shorter chain compounds in comparison to their long-chain counterparts in the exposed fathead minnows (Figure 8).

Table 3: Monkfish liver fraction unbound (f_u) and Coefficient of Variation (CV%) determinations for targeted test compounds included in the equilibrium dialysis experiment. Assay recovery (%) and stability (%) are also reported.

Compound	f_u	Recovery	Stability
PFBA	0.51 (21%)	110 (5.0%)	100
PFPA	0.46 (27%)	110 (5.0%)	110
PFHxA	0.27 (24%)	120 (11%)	110
PFHpA	0.080 (17%)	97 (9.0%)	100
PFOA	0.085 (6.0%)	99 (2.0%)	100
PFNA	0.0095 (45%)	93 (9.0%)	100
PFBS	0.35 (11%)	120 (1.0%)	100
PFHxS	0.031 (25%)	120 (14%)	100
PFOS	0.0033 (11%)	77 (6.0%)	120
6:2 FTS	0.056 (28%)	99 (13%)	110

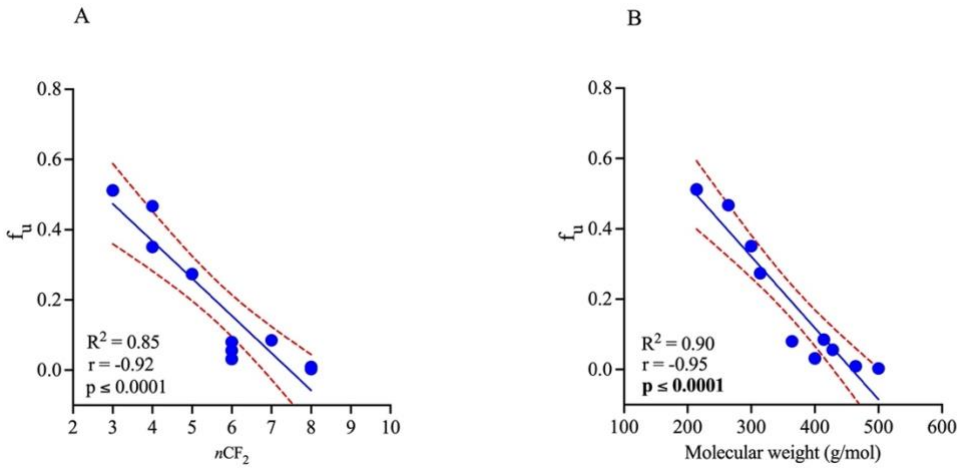


Figure 7: Fraction unbound (f_u) of targeted PFAS from equilibrium dialysis experiments in relation to (A) fluorinated chain length (nCF$_2$) and (B) molecular weight (g/mol). Linear regression and Pearson correlation (r) indicate relatedness of f_u and molecular weight (g/mol). Significant correlations are indicating in bold, $P < 0.05$. Red-dashed lines indicates the 95% confidence interval of the line of best fit.

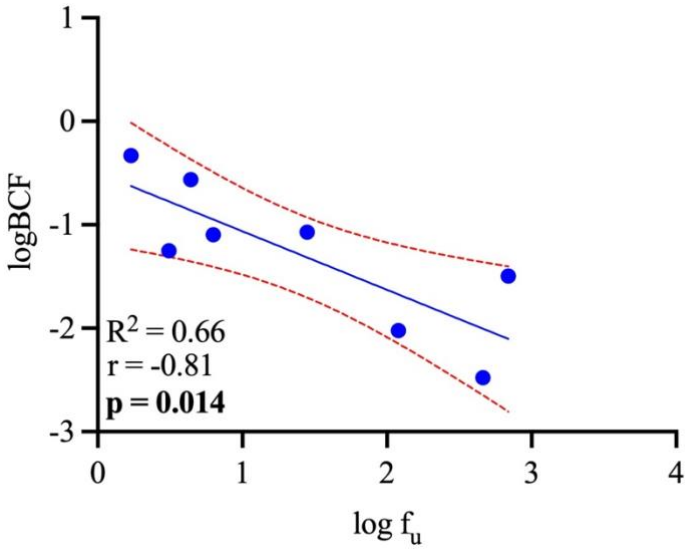


Figure 8: Logarithm transformed fraction unbound ($logf_u$) of targeted PFAS from equilibrium dialysis plotted against logBCF of fathead minnows for analogous compounds included in the groundwater exposure study. Linear regression and Pearson correlation (*r*) results indicated a significant negative correlation between the two variables, in bold, $P < 0.05$. Red-dashed lines indicates the 95% confidence interval of the line of best fit.

Discussion

To our knowledge, and from peer review (Burkhard, 2021), this is one of the first mesocosm studies to incorporate teleost fish to determine the bioaccumulation behavior of PFAS from an AFFF-contaminated groundwater plume. The groundwater plume at FTA-1 contained a complex mixture of linear and branched isomers as well as fluorotelomer-based compounds indicative of AFFF produced from electrochemical fluorination and telomerization manufacturing (Moody and Field, 2000; Houtz et al., 2013).

In this study, correlations were evident between fluorinated chain length and BCFs of FASA, FTS, and PFCA. These relationships, however, were not as evident

when analyzing PFSA where bioconcentration fluctuated with increases from C5 to C6 PFSA and decreased from C6 to C8. Similar relationships existed for BCFs compared with molecular weight of individual compounds. Bioaccumulation potential for PFSA was higher than PFCA of equivalent fluorinated chain length based on BCFs determined. BCFs for PFCA in this study support generalized distinctions regarding increases in bioconcentration with increases in fluorinated chain length (Conder et al., 2007).

Differences in bioconcentration for PFCA and PFSA of equivalent fluorinated chain length in this study highlight an importance of the terminal head group on PFAS compounds. It was previously demonstrated that PFSA have stronger interactions than PFCA of the same fluorinated chain length when bound to human L-FABP amino acid residues (Zhang et al., 2013). These differences in BCFs for PFSA and PFCA observed in fathead minnow liver and kidney in this study may, in part, be explained by different compound affinities for L-FABP and other transporters (Ng and Hungerbühler, 2014).

Generally, by Day 21, the highest average ∑PFAS concentrations were detected in liver > kidney > gonad, which differs from previous laboratory observations where concentrations were highest in blood > kidney > liver > gall bladder > gonads > adipose tissue > muscle in rainbow trout (Martin et al., 2003). However, a similar relationship to this study was previously determined when comparing average ∑PFAS concentrations in liver and gonad from wild fish exposed to AFFF in the natural setting (Ahrens et al., 2015). These differences in literature could be driven by laboratory versus environmental settings and the likelihood of

other co-contaminants in the environment that could influence PFAS biological partitioning.

Detection of PFAS varied greatly across tissue types with only L-PFHxS, L-PFOS, Br-PFOS, FBSA, L-FHxSA, Br-FHxSA, and FOSA being detected in 100% of tissue-specific replicates (SI Table 6). Variations in detects and tissue concentrations may be due to the propensity of PFAS to interact with biological constituents that influence internal distribution (Luebker et al., 2002; Han et al., 2003; Bischel et al., 2010; Allendorf et al., 2019; Khazaee et al., 2021).

Detection of FBSA, L-FHxSA, Br-FHxSA, and FOSA provided valuable insight on an emerging functional group of PFAS known for their prevalence at AFFF impacted sites and widespread geographic distribution but are not yet widely represented in biological literature (Houtz et al., 2013; Ahrens et al., 2015; Chu et al., 2016; Kaboré et al., 2021). The prevalence of FASA in fish tissue during early exposure to contaminated groundwater emphasizes their mobility in environmental fate and transport.

Temporally, mean BCFs for FOSA calculated for liver, kidney, and gonad were within or above the TSCA's regulatory range of 1,000-5,000 L/kg, in which compounds within or above criteria, "B", thresholds are recognized as "bioaccumulative" or "very bioaccumulative", respectively (USEPA, 1998). By day 21, L-FHxSA (1,600 L/kg kidney) and Br-PFHxS (1,500 L/kg liver) also fell within TSCA criteria, "B", thresholds. Generally, BCFs for FASA were much greater than PFSA of equivalent fluorinated chain length. This further supports plausible evidence that chemistry of the PFAS functional head group is a key determinant in partitioning

to biological matrices as described here by the magnitude of bioconcentration displayed by sulfonamide and sulfonate moieties in fathead minnow tissues.

Complex mixtures, such as the one included in this study, present a variety of PFAS moieties that showed differences in bioconcentration behavior from what has previously been observed. For instance, previous work demonstrated a propensity for PFOS to bioconcentrate an order of magnitude higher than PFHxS in fish, but at environmental concentrations, we establish an opposite relationship (Martin et al., 2003; Yeung and Mabury, 2013) (SI Table 5). A plausible explanation for this difference may be competition amongst compounds for binding sites associated with tissue compartments as well as biochemical variations in fathead minnows themselves. Furthermore, the presence of multiple isomers in exposure water should be considered since previous research emphasizes notable differences in uptake and elimination kinetics in fish. Accumulation of branched isomers in tissue compartments was generally lower than linear forms which supports earlier studies with teleost fish in laboratory and environmental settings (Sharpe et al., 2010; Chen et al., 2015). However, when examining fathead minnow liver, mean BCFs determined for Br-PFHxS were generally higher than L-PFHxS which may reflect higher uptake kinetics of the branched isomer or possibly metabolism, but does not necessarily indicate specific interaction with biological transporters. Little difference in mean BCFs were observed between L-PFOS and Br-PFOS as well as L-PFOA and Br-PFOA in liver.

The incorporation of a PK/PD approach to quantify the fraction unbound provides an alternative route to understand the affinity of PFAS for specific tissue matrices. As reported here, it appears that the amount of free, unbound PFAS not

associated with liver matrix is negatively correlated with chain length and molecular weight (Figure 7A, 7B). Although this relationship may explain the lower tissue burdens and bioconcentration of short chain compounds in fathead minnows exposed to AFFF-contaminated groundwater, it is also difficult to determine how increasing PFAS concentrations in groundwater over exposure duration also plays a role in competitive binding to sites associated with the biological matrices measured in this study. Future investigation with equilibrium dialysis to determine the fraction unbound within fish plasma may provide a greater understanding for PFAS available to partition to biological tissues once compounds diffuse across gill epithelia and are associated with plasma.

The controlled environmental exposure design presented an integrative way to investigate PFAS partitioning behavior within a model organism, though it was not without confounding elements. Due to temporal increases in tissue concentration for precursor compounds such as 8:2 FTS, it was difficult to determine whether biotransformation played a critical role in tissue specific burdens of probable PFCA degradation end products such as PFPeA, PFHxA, PFHpA, or PFOA (Wang et al., 2011; Houtz et al., 2012, 2013). Yet given the low prevalence of PFCA in this study, and their low BCFs, any metabolism will be of minor importance overall.

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SUPPORTING INFORMATION

Tables

SI Table 1. Common nomenclature in this study

Abbreviation	Common Name
AFFF	aqueous film forming foam
BAF	bioaccumulation factor
BCF	bioconcentration factor
FABP	fatty acid binding protein
FASA	perfluoroalkyl sulfonamides
FTA	fire-training area
f_u	fraction unbound
L-FABP	liver-fatty acid binding protein
OATP	organic anion transporter polypeptide
PD	pharmacodynamic
PFAA	perfluoroalkyl acids
PFAS	per- and polyfluoroalkyl substances
PFCA	perfluoroalkyl carboxylic acids
PFSA	perfluoroalkyl sulfonic acids
PK	pharmacokinetic

SI Table 2. Nomenclature for per- and polyfluoroalkyl substances (PFAS) in this study.

	Native abbreviation	Common name
Carboxylic Acids	PFBA	perfluorobutanoic acid
	PFPeA	perfluoropentanoic acid
	PFHxA	perfluorohexanoic acid
	PFHpA	perfluoroheptanoic acid
	PFOA	perfluorooctanoic acid
	PFNA	perfluorononanoic acid
	PFDA	perfluorodecanoic acid
	PFUnDA	perfluoroundecanoic acid
	PFDoDA	perfluorododecanoic acid
	PFTriDA	perfluorotridecanoic acid
	PFTeDA	perfluorotetradecanoic acid
Sulfonic Acids	PFBS	perfluorobutane sulfonic acid
	PFPeS	perfluoropentane sulfonic acid
	PFHxS	perfluorohexane sulfonic acid
	PFHpS	perfluorheptane sulfonic acid
	PFOS	perfluorooctane sulfonic acid
	PFNS	perfluorononane sulfonic acid
	PFDS	perfluorodecane sulfonic acid
Perfluoroalkyl sulfonamides	FBSA	perfluoro-1-butanesulfonamide
	FHxSA	perfluoro-1-hexanesulfonamide
	FOSA	perfluorooctane sulfonamide
Fluorotelomer sulfonates	4:2 FTS	4:2 fluorotelomer sulfonate
	6:2 FTS	6:2 fluorotelomer sulfonate
	8:2 FTS	8:2 fluorotelomer sulfonate
Perfluoroalkane Sulfonamidoacetic Acids	N-MeFOSAA	N-Methyl perfluorooctane sulfonamidoacetic acid
	br-N-MeFOSAA	
	N-EtFOSAA	N-Ethyl perfluorooctane sulfonamidoacetic acid
	br-N-EtFOSAA	
PFOA Replacement	ADONA	Dodecafluoro-3H-4,8-dioxanonanoate

SI Table 3: Extraction recovery of mass-labeled internal standard for direct injection analysis of groundwater samples.

Functional Group	Fluorination	*n* (CF2)	Compound	Recovery (% ± SD)
1	Per-	3	PFBA	75 ± 3.4
		4	PFPeA	73 ± 3.7
		5	PFHxA	49 ± 2.0
		6	PFHpA	57 ± 2.6
		7	PFOA	69 ± 4.3
		8	PFNA	65 ± 2.7
		9	PFDA	87 ± 7.2
		10	PFUnDA	110 ± 4.4
		11	PFDoDA	110 ± 6.8
		13	PFTeDA	150 ± 20
2	Per-	4	PFBS	60 ± 5.6
		6	PFHxS	67 ± 2.3
		8	PFOS	67 ± 4.5
3	Per-	8	FOSA	77 ± 4.3
		8	MeFOSAA	71 ± 4.8
		8	EtFOSAA	83 ± 8.0
		8	MeFOSA	120 ± 5.9
		8	EtFOSA	45 ± 3.0
4	Poly-	5	HFPO-DA	100 ± 4.8
5	Poly-	4	4:2 FTS	72 ± 3.0
		6	6:2 FTS	82 ± 5.6
		8	8:2 FTS	120 ± 5.4

SI Table 4: Extraction recovery of mass-labeled internal standard from tissue-specific samples.

Functional Group	Fluorination	n (CF2)	Compound	Recovery (% ± SD)		
				Liver	Kidney	Gonad
1	Per-	3	PFBA	68 ± 5.9	77 ± 8.3	68 ± 16
		4	PFPeA	92 ± 19	81 ± 8.5	73 ± 11
		5	PFHxA	64 ±10	69 ± 7.2	63 ± 12
		6	PFHpA	60 ± 8.1	71 ± 7.5	58 ± 14
		7	PFOA	64 ± 5.6	73 ± 6.9	62 ± 12
		8	PFNA	53 ± 16	72 ± 10	51 ± 18
		9	PFDA	74 ± 6.6	81 ± 9.5	68 ± 12
		10	PFUnDA	73 ± 9.3	82 ± 8.6	70 ± 23
		11	PFDoDA	71 ± 12	79 ± 10	59 ± 23
		13	PFTeDA	72 ± 25	72 ± 23	51 ± 34
2	Per-	4	PFBS	83 ± 40	69 ± 11	81 ± 37
		6	PFHxS	69 ± 14	76 ± 7.1	64 ± 20
		8	PFOS	68 ± 4.2	72 ± 5.6	62 ± 12
3	Per-	8	FOSA	67 ± 26	83 ± 11	56 ± 19
		8	MeFOSAA	95 ± 47	106 ± 25	70 ± 24
		8	EtFOSAA	85 ± 44	100 ± 22	69 ± 27
		8	MeFOSA	59 ± 29	45 ± 14	61 ± 25
		8	EtFOSA	64 ± 32	43 ± 20	55 ± 25
4	Poly-	5	HFPO-DA	51 ± 24	52 ± 11	37 ± 9.4
5	Poly-	4	4:2 FTS	180 ± 93	130 ± 36	170 ± 99
		6	6:2 FTS	160 ± 90	140 ± 30	160 ± 60
		8	8:2 FTS	120 ± 50	110 ± 34	110 ± 31

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SI Table 5: Limits of detection (LOD) for groundwater and tissue-specific samples.

	Tissue	Water
Compound	LOD (ng/g)	LOD (ng/L)
PFBA	0.085	0.016
PFPeA	0.037	0.030
PFHxA	0.046	0.0082
PFHpA	0.013	0.0062
L-PFOA	0.015	0.014
Br-PFOA	0.0072	0.014
PFNA	0.0083	0.013
PFDA	0.014	0.012
PFUdA	0.0080	0.016
PFDoA	0.018	0.012
PFTrDA	0.012	0.0051
PFTeDA	0.059	0.015
PFPeS	0.011	0.011
L-PFHxS	0.024	0.020
Br-PFHxS	0.076	0.076
PFECHS	0.014	0.014
L-PFHpS	0.026	0.018
Br-PFHpS	0.035	0.018
L-PFOS	0.040	0.034
Br-PFOS	0.013	0.16
PFNS	0.018	0.018
PFDS	0.023	0.023
MeFBSA	0.018	0.018
FBSA	0.026	0.026
L-FHxSA	0.012	0.012
Br-FHxSA	0.012	0.012
FOSA	0.022	0.019
L-N-MeFOSAA	0.025	0.025
Br-N-MeFOSAA	0.13	0.13
L-N-EtFOSAA	0.042	0.042
Br-N-EtFOSAA	0.25	0.25
MeFOSA	0.034	0.034
EtFOSA	0.017	0.017
HFPO-DA	0.047	0.047
DONA	0.0055	0.0055
9ClPF3ONS	0.014	0.014
11ClPF3OUdS	0.014	0.014
4:2 FTS	0.011	0.011
6:2 FTS	0.025	0.013
8:2 FTS	0.0084	0.011

a: L- denotes the linear isomer and Br- denotes the branched.

SI Table 6: Comparison of liver bioconcentration factors (L/kg) derived from previous laboratory PFAS exposures.

	Martin et al. (2003)	Yeung and Mabury (2013)	**This study**
Exposure Type	Laboratory, standard PFAS mixture	Laboratory, AFFF (3M)	Environmental, AFFF contaminated groundwater
Species Tested	*O. mykiss*	*O. mykiss*	*P. promelas*
Compound	BCF (L/kg ± SE)	BCF (L/kg ± SE)	BCF (L/kg ± SD)
PFHxA	-	-	4.4 ± 2.9
PFHpA	-	-	6.3 ± 8.0
L-PFOA	8.0 ± 0.59	-	28 ± 49
Br-PFOA	-	-	22 ± 28
PFNA	-	-	120 ± 200
PFDA	1100 ± 180	-	-
PFUnDA	4900 ± 770	-	-
PFDoDA	18000 ± 2900	-	-
PFTeDA	30000 ± 6000	-	-
PFPeS	-	-	170 ± 87
L-PFHxS	100 ± 13	133 ± 46	690 ± 450
Br-PFHxS	-	-	1500 ± 1900
L-PFHpS	-	-	290 ± 340
Br-PFHpS	-	-	270 ± 320
L-PFOS	5400 ± 860	1650 ± 144	460 ± 610
Br-PFOS	-	-	440 ± 670
PFDS	-	1620 ± 343	-
FBSA	-	-	300 ± 180
L-FHxSA	-	-	990 ± 550
Br-FHxSA	-	-	760 ± 330
FOSA	-	-	6700 ± 6800
8:2 FTS	-	-	230 ± 270

SI Table 7: Summary statistics of PFAS concentrations (ng/g ww) reported from the analysis of Day 1, Day 7, and Day 21 treatment groups.

			Compound																												
Exposure Day	**Tissue Type**		**PFBA**	**PFPeA**	**PFHxA**	**PFHpA**	**L-PFOA**	**Br-PFOA**	**PFNA**	**PFDA**	**PFUdA**	**PFDoA**	**PFTrDA**	**PFPeS**	**L-PFHxS**	**Br-PFHxS**	**PFECHS**	**L-PFHpS**	**Br-PFHpS**	**L-PFOS**	**Br-PFOS**	**PFNS**	**PFDS**	**FBSA**	**L-FHxSA**	**Br-FHxSA**	**FOSA**	**6:2 FTS**	**8:2 FTS**
Day 1	Liver	Det %	<LOD	<LOD	<LOD	50%	<LOD	<LOD	50%	33%	67%	<LOD	33%	67%	100%	100%	<LOD	67%	<LOD	100%	100%	ND	ND	100%	100%	100%	100%	<LOD	100%
		Mean	-	-	-	0.99	-	-	0.26	0.27	0.44	-	0.24	0.65	24	31	-	1.2	-	94	23	-	-	44	600	150	38	-	2.4
		SD	-	-	-	0.67	-	-	0.1	0.067	0.21	-	0.0054	0.28	19	25	-	1.1	-	75	18	-	-	22	500	130	29	-	1.8
	Kidney	Det %	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	100%	17%	<LOD	<LOD	<LOD	100%	100%	ND	ND	100%	100%	100%	100%	<LOD	33%
		Mean	-	-	-	-	-	-	-	-	-	-	-	-	20	22	-	-	-	75	25	-	-	65	970	230	31	-	18
		SD	-	-	-	-	-	-	-	-	-	-	-	-	18	-	-	-	-	66	12	-	-	42	660	140	20	-	16
	Gonad	Det %	17%	<LOD	<LOD	33%	83%	<LOD	67%	67%	67%	50%	67%	67%	100%	100%	50%	33%	<LOD	100%	100%	ND	ND	100%	100%	100%	100%	<LOD	100%
		Mean	0.85	-	-	0.52	0.12	-	0.14	0.17	0.4	0.15	0.26	0.043	4.8	6.9	0.071	0.095	-	19	6.7	-	-	3.7	89	32	13	-	0.65
		SD	0	-	-	0.19	0.05	-	0.04	0.039	0.15	0.053	0.1	0.042	2.7	3.2	0.062	0.0039	-	12	5.3	-	-	1.3	58	22	12	-	0.33
Day 7	Liver	Det %	<LOD	<LOD	100%	100%	100%	100%	100%	75%	75%	<LOD	25%	100%	100%	100%	75%	100%	100%	100%	100%	75%	ND	100%	100%	100%	100%	25%	100%
		Mean	-	-	1.6	0.85	6.9	0.43	0.59	0.15	0.47	-	0.12	19	650	350	2.2	15	4.3	900	310	2.4	-	220	1100	340	97	1.9	28
		SD	-	-	1.1	0.83	9.2	0.46	0.57	0.026	0.23	-	-	5.9	380	120	1.2	12	1.6	1100	380	3.1	-	130	330	77	14	-	34
	Kidney	Det %	<LOD	<LOD	50%	33%	100%	33%	50%	<LOD	<LOD	<LOD	<LOD	83%	100%	100%	<LOD	83%	17%	100%	100%	ND	ND	100%	100%	100%	100%	50%	100%
		Mean	-	-	13	2.3	9.3	2.8	1.5	-	-	-	-	23	860	280	-	22	9.3	1100	310	-	-	410	2900	670	200	10	190
		SD	-	-	15	0.78	9.6	1.7	1	-	-	-	-	12	1100	200	-	19	-	980	290	-	-	200	1300	470	90	9.6	150
	Gonad	Det %	<LOD	33%	83%	100%	100%	100%	100%	83%	100%	50%	50%	100%	100%	100%	50%	100%	100%	100%	100%	50%	33%	100%	100%	100%	100%	50%	100%
		Mean	-	0.16	1.7	0.43	4.1	0.28	0.48	0.22	0.36	0.14	0.26	9.8	390	190	1.7	11	3.1	430	110	2.7	0.16	32	530	150	90	0.42	15
		SD	-	0.089	1.8	0.34	3.9	0.19	0.37	0.041	0.15	0.066	0.13	5	250	85	1.2	11	2.6	420	82	3.8	0.11	11	130	56	30	0.4	11
Day 21	Liver	Det %	<LOD	25%	100%	100%	100%	100%	100%	100%	75%	<LOD	25%	100%	100%	100%	100%	100%	100%	100%	100%	100%	ND	100%	100%	100%	100%	50%	100%
		Mean	-	0.7	6.5	3	58	3.6	5.6	0.7	1	-	0.14	36	2700	1000	18	110	41	6600	2700	26	-	280	2200	790	260	2.3	120
		SD	-	-	4.3	3.8	99	4.5	9.1	0.68	0.99	-	-	19	1700	1300	26	130	49	8700	4200	41	-	160	1200	340	270	2.8	140
	Kidney	Det %	<LOD	<LOD	67%	33%	100%	67%	83%	17%	67%	<LOD	<LOD	100%	100%	100%	<LOD	100%	67%	100%	100%	ND	ND	100%	100%	100%	100%	83%	100%
		Mean	-	-	19	1.7	23	3.5	3.2	0.88	1.4	-	-	27	1500	280	-	47	9.4	4400	1000	-	-	430	3700	890	300	4.9	640
		SD	-	-	14	0.11	15	1.9	2	-	0.97	-	-	17	1100	80	-	16	2.5	3100	550	-	-	120	1400	230	210	2.1	290
	Gonad	Det %	<LOD	17%	100%	100%	100%	100%	100%	83%	83%	17%	50%	100%	100%	100%	100%	100%	100%	100%	100%	50%	50%	100%	100%	100%	100%	50%	100%
		Mean	-	0.22	1.8	0.58	12	0.75	1.4	0.44	0.45	0.28	0.26	10	910	220	2.8	27	8.7	1700	450	9.2	0.054	58	1000	420	190	0.24	62
		SD	-	-	1.2	0.27	6.7	0.42	1	0.19	0.21	-	0.12	4.8	650	94	1.9	9.1	4	1200	220	8.1	0.043	15	540	170	150	0.11	41

a. Compounds below limits of detection are indicated by, <LOD.

b. Compounds not detected are indicated by, ND.

SI Table 8: Summary of bioconcentration factors (L/kg) for PFAS reported from the analysis of Day 1, Day 7, and Day 21 treatment groups.

	Liver						Kidney						Gonad					
	Day 1		Day 7		Day 21		Day 1		Day 7		Day 21		Day 1		Day 7		Day 21	
Compound	BCF	*SD*	BCF	*SD*	BCF	*SD*	BCF	*SD*	BCF	*SD*	BCF	*SD*	BCF	*SD*	BCF	*SD*	BCF	*SD*
PFBA	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	13	-	NA	-	NA	-
PFPeA	NA	-	NA	-	1.7	-	NA	-	NA	-	NA	-	NA	-	0.75	0.41	0.53	-
PFHxA	NA	-	2.1	1.5	4.4	2.9	NA	-	17	20	13	9.1	NA	-	2.2	2.3	1.2	0.79
PFHpA	8.0	5.4	3.8	3.7	6.3	8.0	NA	-	9.9	3.4	3.6	0.2	4.3	1.5	1.9	1.5	1.2	0.58
L-PFOA	NA	-	8.0	11.0	28.0	49.0	NA	-	11	11	11	7.5	0.31	0.13	4.7	4.6	5.8	3.3
Br-PFOA	NA	-	6.3	6.7	22	28	NA	-	41	25	22	12	NA	-	4.1	2.8	4.7	2.6
PFNA	13	4.8	23	22	120	200	NA	-	56	39	70	43	6.6	1.9	19	14	31	22
PFPeS	13	5.7	160	50	170	87	NA	-	190	100	120	81	0.87	0.86	82	42	48	22
L-PFHxS	28	22	350	210	690	450	24	21	470	600	380	290	5.7	3.2	210	140	240	170
Br-PFHxS	200	160	1000	370	1500	1900	140	-	850	600	430	120	44	20	570	260	340	140
PFECHS	NA	-	750	430	550	830	NA	-	ND	-	NA	-	27	23	600	430	87	60
L-PFHpS	14	13	83	67	290	340	NA	-	120	110	120	41	1.1	0.047	64	64	70	24
Br-PFHpS	NA	-	71	26	270	320	NA	-	150	-	61	16	NA	-	51	42	57	26
L-PFOS	21	17	110	130	460	610	17	15	130	120	310	210	4.4	2.7	51	50	120	81
Br-PFOS	13	10	93	120	440	670	14	7.0	94	88	160	88	3.8	3.0	35	25	72	36
FBSA	170	87	400	230	300	180	260	160	740	360	470	130	15	4.9	58	19	64	16
L-FHxSA	970	810	940	280	990	550	1600	1100	2500	1100	1600	620	140	94	450	110	450	240
Br-FHxSA	450	390	570	130	760	330	670	430	1100	790	860	220	94	64	250	93	410	170
FOSA	1300	960	2800	410	6700	6800	1000	670	5700	2600	7700	5400	450	400	2600	890	4900	3900
6:2 FTS	NA	-	5.4	-	2.5	3.1	NA	-	30	28	5.3	2.3	NA	-	1.2	1.1	0.26	0.12
8:2 FTS	10	7.9	99	120	230	270	77	68	670	540	1200	560	2.8	1.4	54	41	120	79

a. NA denotes “not applicable” due to compound not being detected or below limits of detection.

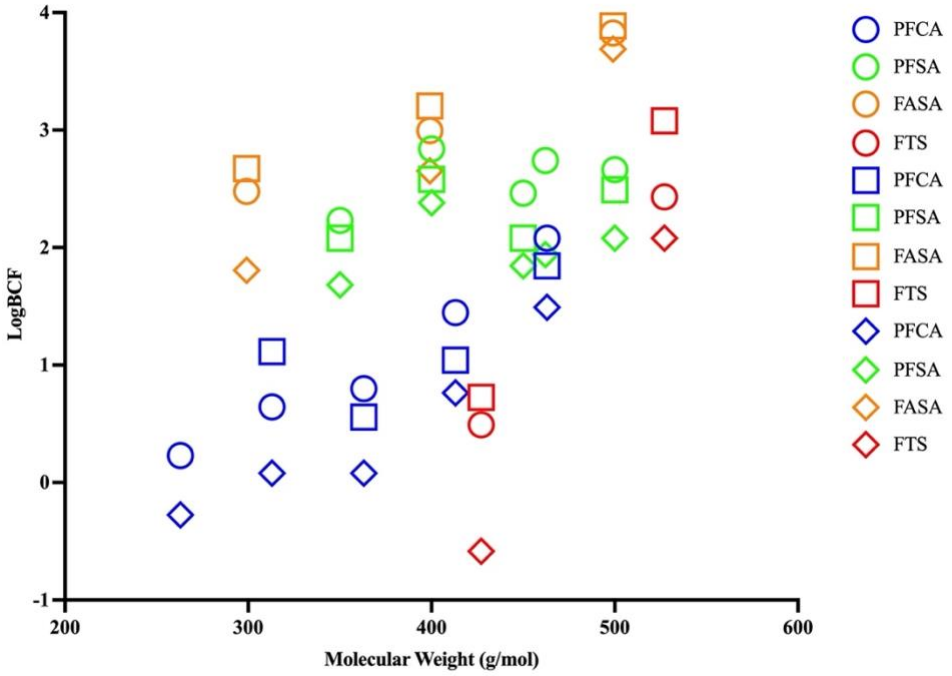
SI Table 9: Results from linear regression analysis delineating relatedness of PFAS fluorinated chain length with bioconcentration factors derived for compounds detected in each tissue compartment.

Tissue	Functional Group	Pearson *r*	R-squared	Slope ± SE
Liver	PFCA	0.98	0.96	0.45 ± 0.055
	PFSA	0.35	0.12	0.074 ± 0.12
	FASA	0.99	0.98	0.34 ± 0.045
	FTS	Too few pairs	1.0	0.97
Kidney	PFCA	0.65	0.42	0.27 ± 0.22
	PFSA	0.36	0.13	0.074 ± 0.14
	FASA	1.0	0.99	0.30 ± 0.022
	FTS	Too few pairs	1.0	1.2
Gonad	PFCA	0.95	0.90	0.42 ± 0.082
	PFSA	0.29	0.085	0.068 ± 0.13
	FASA	1.0	1.0	0.47 ± 0.027
	FTS	Too few pairs	1.0	1.3

SI Table 10: Results from linear regression analysis delineating relatedness of PFAS molecular weight (g/mol) with bioconcentration factors derived for compounds detected in each tissue compartment.

Tissue	Functional Group	Pearson *r*	R-squared	Slope ± SE
Liver	PFCA	0.98	0.96	0.0090 ± 0.0011
	PFSA	0.51	0.26	0.0021 ± 0.0021
	FASA	0.99	0.98	0.0067 ± 0.00090
	FTS	Too few pairs	1.0	0.019
Kidney	PFCA	0.65	0.42	0.0054 ± 0.0044
	PFSA	0.36	0.13	0.0015 ± 0.0027
	FASA	1.0	0.99	0.0061 ± 0.00043
	FTS	Too few pairs	1.0	0.024
Gonad	PFCA	0.95	0.90	0.0084 ± 0.0016
	PFSA	0.24	0.058	0.0011 ± 0.0025
	FASA	1.0	1.0	0.0094 ± 0.00055
	FTS	Too few pairs	1.0	0.027

Figures



SI Figure 1: Logarithm transformed bioconcentration factors (LogBCF, L/kg) plotted against PFAS molecular weight (g/mol). Strong linear correlations were present for PFCA, FASA, and FTS, however LogBCF showed weak correlation with PFSA molecular weight. BCFs corresponding to liver, kidney, and gonad are indicated by circles, squares, and diamonds, respectively.