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Environmental Chemistry

Bioconcentration of per- and polyfluoroalkyl substances and precursors in fathead minnow tissues environmentally exposed to aqueous film-forming foam–contaminated waters

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Abstract: Exposure to per- and polyfluoroalkyl substances (PFAS) has been associated with toxicity in wildlife and negative health effects in humans. Decades of fire training activity at Joint Base Cape Cod (MA, USA) 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 bioconcentration potential of PFAS in complex environmental mixtures, a mobile laboratory was established to evaluate the bioconcentration of PFAS from AFFF-impacted 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. The ∑PFAS concentrations in groundwater increased from approximately 10,000 ng/L at day 1 to 36,000 ng/L at day 21. The relative abundance of PFAS in liver, kidney, and gonad shifted temporally from majority perfluoroalkyl sulfonamides (FASAs) to perfluoroalkyl sulfonates (PFSAs). By day 21, mean ∑PFAS concentrations in tissues displayed a predominance in the order of liver > kidney > gonad. Generally, bioconcentration factors (BCFs) for FASAs, perfluoroalkyl carboxylates (PFCAs), and fluorotelomer sulfonates (FTS) increased with degree of fluorinated carbon chain length, but this was not evident for PFSAs. Perfluorooctane sulfonamide (FOSA) displayed the highest mean BCF (8700 L/kg) in day 21 kidney. Suspect screening results revealed the presence of several perfluoroalkyl sulfinate and FASA compounds present in groundwater and in liver for which pseudo-bioconcentration factors are also reported. The bioconcentration observed for precursor compounds and PFSA derivatives detected suggests alternative pathways for terminal PFAS exposure in aquatic wildlife and humans. *Environ Toxicol Chem* 2024;00:1–12. © 2024 The Author(s). *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Bioaccumulation; Environmental partitioning; Per- and polyfluoroalkyl substances; Superfund site

INTRODUCTION

Fluorinated surfactants are part of an environmentally relevant class of contaminants known as per- and polyfluoroalkyl substances (PFAS). These substances exhibit a range of physicochemical properties such as high heat resistance, hydrophobicity, lipophilicity, and low surface tension. These properties have made PFAS effective in the development of aqueous film-forming foams (AFFFs) and water- and stain-resistant products since the 1950s (Buck et al., 2011; Gewurtz et al., 2014; Pabon & Corpart, 2002; Paul et al., 2009). Decades of manufacturing and AFFF use have rendered PFAS ubiquitous in the environment (Ahrens et al., 2015; Houtz et al., 2013; Lau et al., 2007; Schultz et al., 2004). Applications of AFFFs in the environment are associated with PFAS contamination of wastewater, drinking water, small finfish, fish harvested by local communities, and human serum (Gewurtz et al., 2014; Hansen et al., 2016; Houtz et al., 2016; Oakes et al., 2010; Sunderland et al., 2019). Contamination of natural

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resources links dietary exposure as a main pathway for PFAS exposure in wildlife and humans (De Silva et al., 2021; Sunderland et al., 2019). Exposure to PFAS has been linked to altered immune function, cancer, thyroid disease, adverse reproductive effects, and alterations in gene expression across a variety of mammals (including humans) and aquatic organisms (Ankley et al., 2005; Grandjean et al., 2012; Halldorsson et al., 2012; Lau et al., 2007; Lee et al., 2020; Melzer et al., 2010). In addition to understanding persistence and toxicity, investigation into PFAS biological partitioning is necessary to support the development of PFAS regulatory limits.

A key facet in determining PFAS environmental fate and biological interaction in aquatic organisms is understanding tissue–water partitioning. Bioconcentration and bioaccumulation studies provide opportunities to evaluate tissue–water partitioning in relation to PFAS physicochemical properties and may elucidate predictabilities in partitioning. Controlled bioconcentration studies provide aqueous exposures in which targeted chemicals are ideally fully dissolved. This is different from field-based bioaccumulation observations, which take into account all potential chemical exposure routes (i.e., water, diet, sediments; Burkhard, 2021).

Owing to their ubiquity and persistence, two commonly reported perfluoroalkyl acid (PFAA) subgroups in bioconcentration and bioaccumulation studies are the perfluoroalkyl sulfonates (PFSAs; C_nF_{2n+1}–SO_3^-) and perfluoroalkyl carboxylates (PFCAs; C_nF_{2n+1}–COO^-). Observations of PFAS in aquatic organisms, namely fish, show trends of increasing bioconcentration factors (BCFs) and bioaccumulation factors with each additional CF_2 moiety in legacy PFSAs and PFCAs, which is attributed to increased hydrophobicity (Conder et al., 2008). Additional evidence exists for the propensity of PFSAs to bioconcentrate or bioaccumulate at a higher degree than PFCAs (Conder et al., 2008; Houde et al., 2011; Martin et al., 2003; Shi et al., 2018). Similarly, uptake rates for PFSAs and PFCAs in fish have also been shown to increase with fluorinated carbon chain length, whereas elimination rates tend to decrease (Fang et al., 2016; Zhong et al., 2019). Differences in uptake and elimination, and therefore accumulation, are also evident for linear and branched PFAS isomers in fish (Chen et al., 2015; Zhong et al., 2019). Variations in physicochemical properties may suggest propensities for specific compounds to partition to tissue-specific targets with the potential to influence toxicological outcomes.

Highly perfuse tissues such as liver and kidney are often key sites of increased PFAS accumulation (Bangma et al., 2017; Goeritz et al., 2013; Martin et al., 2003), although gonads may contribute a significant fraction to whole body burden distribution (Ahrens et al., 2015). Per- and polyfluoroalkyl substance target organ toxicity is well documented for many aquatic species (Lee et al., 2020), and in some instances, reproductive toxicities may present a cascade of toxicological effects that impact generations of offspring (Du et al., 2009). Thus, obtaining empirical evidence on bioconcentration and bioaccumulation behavior based on associations with physicochemical properties is critical for understanding predictability of PFAS partitioning to tissue-specific compartments and toxicological outcomes.

To date, numerous studies have observed bioconcentration of PFAS in laboratory-controlled exposures involving teleost fish and invertebrates (Burkhard, 2021), many of which investigated the behavior of single compounds or mixtures of legacy PFSAs and PFCAs. Bioconcentration studies utilizing water from contaminated sites offer assessment of the bioconcentration of PFAS in chemical mixtures under realistic environmental conditions. Given the manufacturing shifts in AFFFs from legacy C6- and C8-based PFSAs as the predominant additives, fluorinated alternatives are becoming more environmentally relevant. Evidence of these shifts has been established by the growing presence of fluorotelomer-based compounds at fire training and disaster sites (Oakes et al., 2010; Schultz et al., 2004), precursor compounds involved in biotransformation (Harding-Marjanovic et al., 2015; Weber et al., 2017), and nontarget discovery of emerging PFAS in AFFFs produced by electrochemical fluorination (ECF) and telomerization (Barzen-Hanson et al., 2017). The discovery of novel and emerging PFAS using high-resolution mass spectrometry (HRMS) has evolved at a pace beyond which many analytical standards are made available, presenting challenges for risk assessments and regulatory decision-making. This results in the use of qualitative approaches to characterize the enrichment of identifiable structures in samples. For instance, the use of pseudo-bioaccumulation factors to describe enrichment of AFFF-derived PFAS precursors discovered in exposed mouse serum has been proposed (McDonough et al., 2020). Characterization of the structures of emerging PFAS and their precursors in environmental mixtures and biota is critical to understanding their biological interaction and effects.

We investigated bioconcentration of legacy and emerging PFAS by exposing fathead minnows (*Pimephales promelas*) to AFFF-impacted groundwater at a historical fire-training area (FTA) by flow-through design (Barber et al., 2023). Prior work at the site has established that approximately 80% of the AFFF used at the site was ECF-based (Ruyle et al., 2023). Use of a mobile laboratory provided an opportunity to assess PFAS bioconcentration from environmental mixtures that incorporates the dynamic conditions intrinsic to natural systems. Using HRMS, we quantified targeted PFAS in fish tissues (liver, kidney, and gonads) and groundwater, and we incorporated suspect screening. Liver, kidney, and gonad were selected because of mounting evidence of preferential PFAS accumulation in highly perfuse tissues (Bangma et al., 2017; Goeritz et al., 2013; Martin et al., 2003). In addition to the potential for gonads to be sites of PFAS predisposition to ova, gonads may also comprise a significant fraction of total PFAS body burden (Ahrens et al., 2015), which prompted our investigation into bioconcentration behavior in reproductive tissue. Overall, our aims were to (1) identify the presence of precursor and novel PFAS in groundwater and fish liver, (2) derive tissue-specific BCFs for 21 targeted PFAS, (3) investigate the relationship between bioconcentration and chemical properties of PFAS with varying fluorinated carbon chain lengths and functional groups, and (4) derive pseudo-bioconcentration factors (BCF_{pseudo}) for 14 compounds identified by suspect screening.

MATERIALS AND METHODS

Exposure site characterization

Joint Base Cape Cod is a military base located on the western reaches of Cape Cod. Prior to 1985, Joint Base Cape Cod conducted fire-training exercises and utilized broadscale application of AFFFs at a site previously demarcated as fire training area-1 (FTA-1; Weber et al., 2017). The use of AFFF resulted in release of PFAS to the vadose zone, where leachate is expected to contribute to PFAS groundwater contamination over centuries without remediation efforts (Ruyle et al., 2023). The hydrological flow path of the groundwater plume presents broader biogeochemical dynamics that result in seasonal fluctuations in PFAS concentrations in connected surface waters (Tokranov et al., 2021) where PFAS exposure to aquatic organisms may pose an ecological risk.

Environmental mixture exposures

Environmental PFAS exposure of the fathead minnow, a model toxicology organism, was conducted over a 21-day period in July and August of 2019 at the US Geological Survey Cape Cod Toxic Substances Hydrology Research Site on Joint Base Cape Cod. Reproductively recrudescent male and female hatchery-reared fathead minnows ($n = 24$; Aquatic Biosystems) were exposed on site to the contaminated groundwater plume downgradient of FTA-1 within flow-through mobile laboratories (Barber et al., 2023; Vajda et al., 2011). Details of the mobile laboratory setup are in the Supporting Information. This specific study was designed to investigate bioconcentration and neither morphological nor behavioral assessments were included within the experimental procedures. Treatment groups consisted of four different sampling points for groundwater exposure—day 0 (control), and days 1, 7, and 21. Fathead minnows corresponding to the day 0 control group were never exposed to groundwater plumes at FTA-1, but rather were sampled directly from the laboratory water in which they were shipped. Tissues collected and extracted for PFAS analysis included liver, kidney, and gonad. Weekly water samples (1-L high-density polyethylene bottles, $n = 4$) were also collected and stored on ice until frozen.

Water and tissue preparation

Briefly, water samples were prepared for direct injection and tissue samples were extracted with 0.1 M formic acid in methanol prior to instrumental analysis. Additional details on water and tissue sample collection and preparation as well as standards and reagents used are in the Supporting Information.

Instrumental analysis

Quantification of PFAS in groundwater and fathead minnow tissue samples from days 0, 1, 7, and 21 was performed using a SCIEX ExionLC AC ultra-high-performance liquid chromatography system coupled to a SCIEX X500R quadrupole time-of-flight tandem mass spectrometer. Refer to the Supporting Information for details on instrument parameters for sample analysis.

Suspect screening analysis

For suspect screening analysis, groundwater and fathead minnow liver samples from day 0 (control) and day 21 treatment groups were measured to identify additional PFAS. Details on sample preparation for suspect screening are in the Supporting Information.

Quality assurance/quality control

All targeted PFAS results were recovery-corrected using the corresponding mass-labeled surrogate standards spiked into the sample prior to extraction (Supporting Information S2, Table S1). Recoveries of mass-labeled standards are reported for groundwater (Supporting Information S2, Table S2) and tissue (Supporting Information S2, Table S3) samples. Details on the construction of matrix-matched calibration curves and determination of limits of detection (LODs) for each target analyte are included in the Supporting Information S1, Table S4.

Deriving BCFs

We considered bioconcentration as the uptake of water and contaminants through the gill epithelia of the fathead minnow. The BCFs can be derived with Equation (1):

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

where C_{tissue} is the concentration in the specific tissue sample (μg/kg wet wt), and C_{water} is the concentration in the weekly water sample (μg/L). The BCFs for day 7 tissues were determined based on average water concentrations between day 1 and day 7. Similarly, BCFs for day 21 tissues were determined based on the days 1, 7, and 21 average water concentrations. A BCF value > 1 L/kg indicates that bioconcentration has occurred in the tissue. More information on the applicability of BCF determinations is included in the Supporting Information.

Deriving pseudo-bioconcentration factors

In the absence of analytical PFAS standards, peak areas of suspect and precursor compounds present in both day 21 water and fathead minnow livers were first normalized to the nearest mass-labeled internal standard peak areas in the suspect screening acquisition (Supporting Information S2, Table S5), and then normalized to sample mass. The normalized peak areas were used to calculate corresponding pseudo-bioconcentration factors ($\mathrm{BCF}_{\mathrm{pseudo}}$) following Equation (2):

$$\mathrm{BCF}_{\mathrm{pseudo}} = A_{i,\mathrm{tissue}}/A_{i,\mathrm{water}} \quad (2)$$

where $A_{i,\mathrm{tissue}}$ is the normalized peak area of the compound present in fathead minnow liver tissue, $A_{i,\mathrm{water}}$ is the normalized

peak area of the compound present in water, and units are in L/kg. The BCF_{pseudo} values calculated for compounds identified in water and liver are reported in the Supporting Information S2, Table S6. Similar to the BCF calculations performed as previously described in the *Deriving BCFs* section, a BCF_{pseudo} value >1 may indicate potential bioconcentration of the suspect compound of interest in the tissue.

Data and statistical analysis

Data were analyzed using GraphPad Prism (Ver. 9.3.1; GraphPad Prism Software) and Microsoft Excel (Ver. 16.60). Mean values for PFAS concentrations were determined from values >LOD. Tissue-specific data on fathead minnows were corrected to respective sample mass and results from males and females combined to increase sample size within treatment groups ($n = 6$). For days 7 and 21 liver samples, one male and one female liver were used to develop the extraction and analytical method, and so composites for these treatment groups were constructed as $n = 4$. Logarithm-transformed BCF and BCF_{pseudo} values were compared with fluorinated carbon chain length (nCF_2), as well as PFAS molecular weight to explore relationships between PFAS bioconcentration and physicochemical properties using linear regression and Pearson correlation. See the Supporting Information for more details on the statistical approaches for comparing bioconcentration and PFAS physicochemical properties.

RESULTS

PFAS concentrations in water

In water samples, 21 of the 41 targeted compounds were detected, with perfluoroethylcyclohexane sulfonate (PFECHS) only detected in the day 21 water sample (Supporting Information S2, Table S7). Generally, concentrations of detected compounds increased over time, whereas the relative abundance of PFAS subgroups remained stable across weekly water samples (Supporting Information S3, Figure S1). Perfluoroalkyl sulfonates were the most abundant subgroup in weekly samples, comprising 73% of the sum of targeted PFAS (∑PFAS) at days 1 and 7 and 71% at day 21. Linear (L-) and branched (Br-) isomers of both perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) were predominant, with aqueous concentrations from day 21 quantified at 14,000 and 6200 ng/L for L-PFOS and Br-PFOS, and 3800 and 660 ng/L for L-PFHxS and Br-PFHxS, respectively (Supporting Information S2, Table S7). The perfluoroalkyl sulfonamides (FASAs) maintained a relative composition of 12% of ∑PFAS across samples. L-perfluorohexane sulfonamide (L-FHxSA) and Br-perfluorohexane sulfonamide (Br-FHxSA) displayed the highest concentrations of the FASAs, at 2200 ng/L L-FHxSA and 1000 ng/L Br-FHxSA in the day 21 water samples. For the PFCAs, L-perfluorooctanoate (L-PFOA) was predominant (2000 ng/L) over Br-PFOA throughout the duration of the experiment (Supporting Information S2, Table S7). The fluorotelomer sulfonates (FTS) comprised the lowest fraction (3%–4% of ∑PFAS) across sampling days; only 6:2 FTS and 8:2 FTS were detected in each weekly water sample.

Functional group distributions in fish tissue

Of the 41 targeted compounds, 23 PFAS had detection frequencies >60% in tissue samples. The relative abundance of PFAS subgroups in tissue compartments varied throughout the duration of groundwater exposure (Figure 1). At day 1, ∑FASAs comprised 83%, 87%, and 78% of ∑PFAS concentrations in

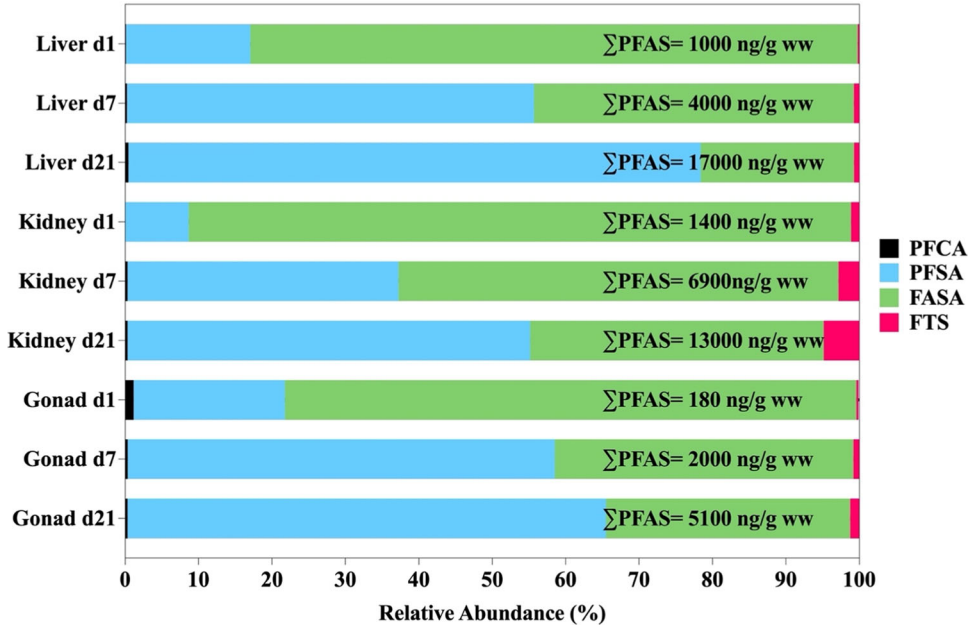

FIGURE 1: Relative abundance of per- and polyfluoroalkyl substances (PFAS) quantified in tissue-specific samples by compound subgroup and corresponding to the day of exposure to fire training area-1 (FTA-1) groundwater. FASA = perfluoroalkyl sulfonamides; FTS = fluorotelomer sulfonates; PFCA = perfluoroalkyl carboxylates; PFSA = perfluoroalkyl sulfonates.

liver, kidney, and gonad, respectively. Major shifts in relative compositions were evident by day 7 in liver, kidney, and gonad in, which ∑PFSAs comprised 53%, 38%, and 58% of ∑PFAS, respectively. By day 21 of groundwater exposure, the total mean concentrations of PFAS subgroups revealed ∑PFSA > ∑FASA > ∑FTS > ∑PFCA across tissue types. Overall, the PFSA and FASA groups comprised the bulk of tissue-specific PFAS burdens compared with the PFCA and FTS groups. Mean concentrations of ∑PFAS were highest in liver, followed by kidney and gonad.

Perfluoroalkyl sulfonates. For day 21 tissues, mean ∑PFSA concentrations were highest in liver (13,000 ng/g wet wt) and kidney (7300 ng/g wet wt), followed by gonad (3300 ng/g wet wt). Generally, mean concentrations of individual PFSAs increased in tissues over the duration of the experiment (Supporting Information S2, Table S8). Of the 12 targeted PFSAs, only L-PFHxS, L-PFOS, and Br-PFOS were detected in all tissue samples across the days 1, 7, and 21 treatment groups. The ∑PFSA burdens were dominated by L-PFOS, which comprised approximately 50% of ∑PFSAs in day 21 liver (6600 ng/g wet wt), 61% in kidney (4400 ng/g wet wt), and 50% in gonad (1700 ng/g wet wt), respectively (Supporting Information S2, Table S8). By day 21, approximately 21% of ∑PFSAs were comprised of L-PFHxS in liver (2700 ng/g wet wt) and kidney (1500 ng/g wet wt), and 28% of ∑PFSA in gonad (910 ng/g wet wt), respectively. Both Br-PFHxS and Br-PFOS were present at lower mean concentrations than their linear isomers, with highest mean tissue concentrations similarly observed at day 21 (Supporting Information S2, Table S8). Mean concentrations of Br-PFHxS and Br-PFOS were highest in the order of liver > kidney > gonad (Figure 2A). From day 7 to 21, the mean concentration of Br-PFHxS in kidney appeared stable but continued to increase in liver and gonad (Supporting Information S2, Table S8). Perfluoroethylcyclohexane sulfonate was detected in 100% of day 21 liver and gonad samples, with the highest reported concentration in liver (18 ng/g wet wt). Additional compounds with 100% detection frequency in tissue samples at day 21 included perfluoropentane sulfonate (PFPeS) and perfluoroheptane sulfonate (L-PFHpS). These mean concentrations were generally at least 1 order of magnitude lower than the PFHxS and PFOS isomers. The PFSAs detected in

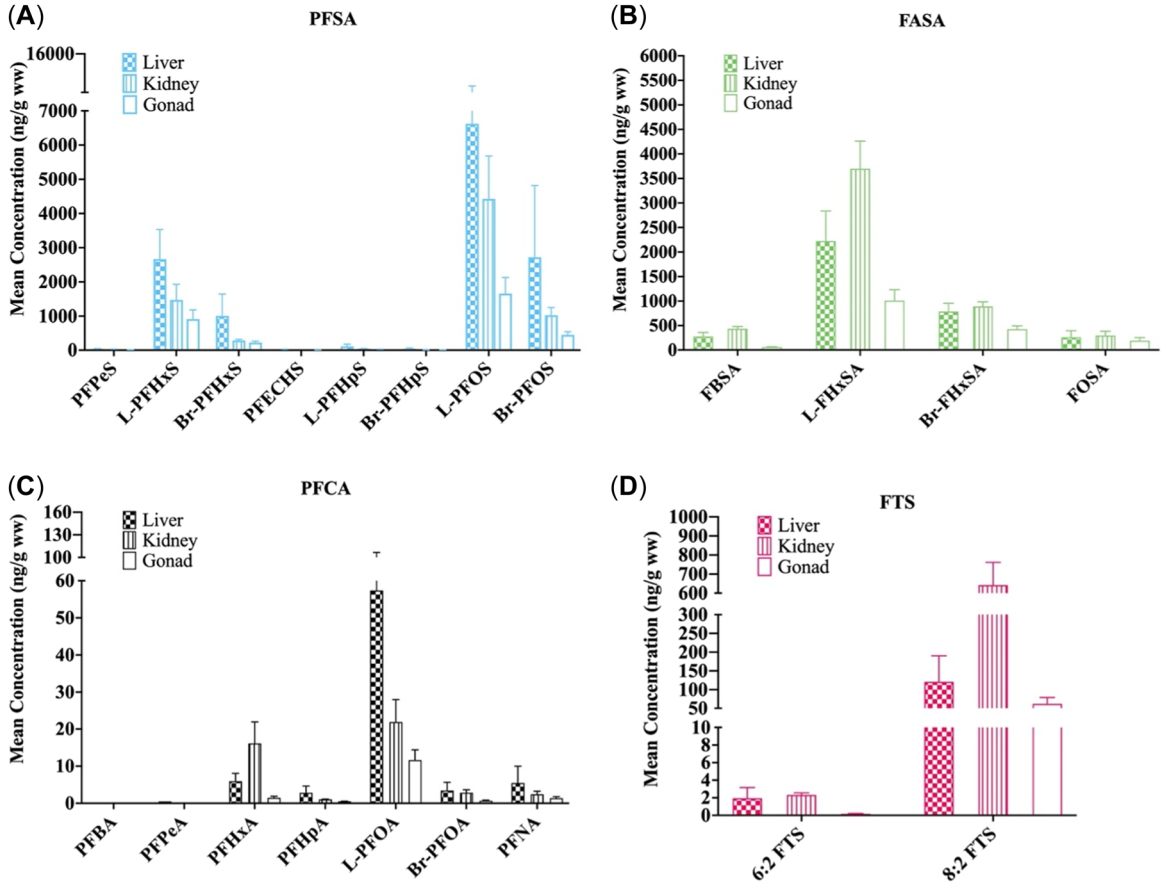

FIGURE 2: Mean tissue-specific concentrations (ng/g wet wt) of per- and polyfluoroalkyl substances (PFAS) after a 21-day exposure to fire training area-1 (FTA-1) groundwater for compounds detected in both tissue and water by PFAS subgroup: (**A**) perfluoroalkyl sulfonates (PFSA; C5–C8), (**B**) perfluoroalkyl sulfonamides (FASA; C4–C8), (**C**) perfluoroalkyl carboxylates (PFCA; C4–C8), (**D**) 6:2 and 8:2 fluorotelomer sulfonates (FTS). Error bars represent the standard error on $n = 6$ samples for kidney and gonad, and $n = 4$ samples for liver. Br- = branched; FBSA = perfluorobutane sulfonamide; FHxSA = perfluorohexane sulfonamide; FOSA = perfluorooctane sulfonamide; L = linear; PFBA = perfluorobutanoate; PFECHS = perfluoroethylcyclohexane sulfonate; PFHpA = perfluoroheptanoate; PFHpS = perfluoroheptane sulfonate; PFHxA = perfluorohexanoate; PFHxS = perfluorohexane sulfonate; PFPeA = perfluoropentanoate; PFPeS = perfluoropentane sulfonate; PFOA = perfluorooctanoate; PFOS = perfluorooctanesulfonate.

tissue but not in water samples included perfluorononane sulfonate (PFNS) and perfluorodecane sulfonate (PFDS).

Perfluoroalkyl sulfonamides. Perfluorobutane sulfonamide (FBSA), L-FHxSA, Br-FHxSA, and perfluorooctance sulfonamide (FOSA) were detected in 100% of samples across treatment groups. Generally, mean concentrations of ∑FASAs were highest in kidney (5300 ng/g wet wt), followed by liver (3500 ng/g wet wt) and gonad (1700 ng/g wet wt) by day 21. At day 21, L-FHxSA was the most prevalent sulfonamide moiety, comprising 63% of ∑FASAs in liver (2200 ng/g wet wt), 69% in kidney (3700 ng/g wet wt), and 60% in gonad (1000 ng/g wet wt; Figure 2B; Supporting Information S2, Table S8). Compositional profiles of Br-FHxSA ranged from approximately 23% of the ∑FASA liver burden (790 ng/g wet wt), to 17% of kidney (890 ng/g wet wt) and 25% of gonad (420 ng/g wet wt). Contributing the least to ∑FASAs were FBSA and FOSA, with mean concentrations being highest in day 21 kidney at 430 and 300 ng/g wet wt, respectively.

Perfluoroalkyl carboxylates. Mean concentrations of ∑PFCAs were highest in the order of liver > kidney > gonad. The day 21 mean concentrations of ∑PFCAs in liver, kidney, and gonad were 79, 53, and 18 ng/g wet wt, respectively. The ∑PFCAs 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. Both L-PFOA and Br-PFOA were more prominent in day 7 and 21 tissues than in day 1 tissues, with L-PFOA being the most abundant isomer (Supporting Information S2, Table S8). The compound 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 ($n < 7$ perfluorinated carbons) quantified in tissue replicates ranged from 17% to 100% and was variable over exposure days (Supporting Information S2, Table S8). Perfluorobutanoate (PFBA) was only detected in day 1 gonad. Perfluoropentanoate (PFPeA) was detected in day 7 and 21 gonad as well as day 21 liver, although detection frequencies only ranged from 17% to 33% of samples (Supporting Information S2, Table S8).

Fluorotelomer sulfonates. Only 6:2 FTS and 8:2 FTS were detected in tissue samples, not 4:2 FTS. In the day 1 treatment group, 6:2 FTS was not detected in tissue samples. Detection frequency of 6:2 FTS generally ranged from 25% to 83% of samples between day 7 and 21 tissues and was more prominent in day 21 kidneys (Supporting Information S2, Table S8). 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 21 kidney samples. The ∑FTS comprised approximately 5% of ∑PFAS in kidney, 1.2% in gonad, and 0.71% in liver, by day 21 of groundwater exposure. Mean concentrations of 6:2 FTS at day 21 were highest in the order of kidney > liver > gonad (Figure 2D). 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 wet weight in day 21 kidney, followed by 120 and 62 ng/g wet weight in day 21 liver and gonad, respectively (Supporting Information S2, Table S8).

Bioconcentration behavior and PFAS properties

Across tissue types, logarithm-transformed BCFs (log BCFs) increased with fluorinated carbon chain length (Figure 3): strong Pearson's correlations were observed for PFCAs ($r \geq 0.68$) and FASAs ($r = 1.0$), but not PFSAs ($r \leq 0.49$; Supporting Information S2, Table S9a). Log BCFs for 6:2 FTS and 8:2 FTS displayed an increase with fluorinated carbon chain length, although not enough data were available for assessment with Pearson correlation (Supporting Information S2, Table S9a). Similar results were evident in the analysis of log

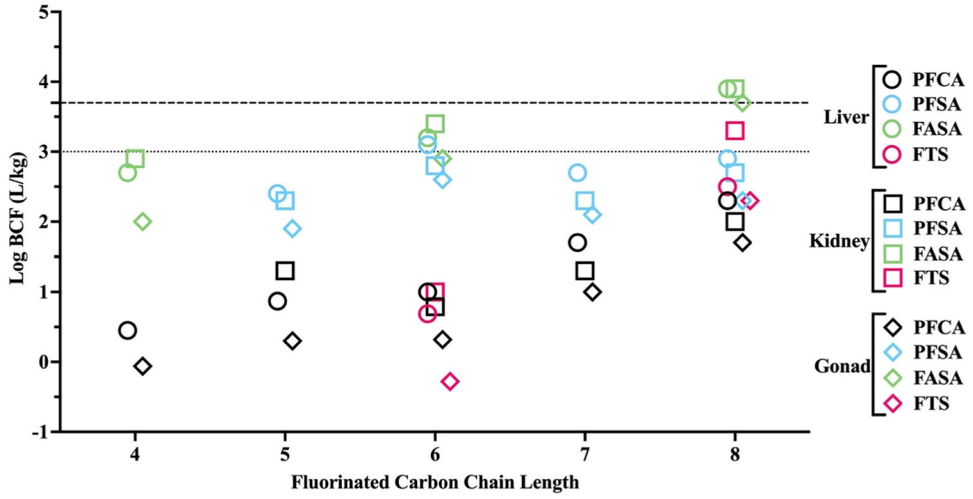

FIGURE 3: Logarithm-transformed bioconcentration factors (log BCF; L/kg) of per- and polyfluoroalkyl substances by compound subgroup in relation to compound fluorinated carbon chain length (nCF_2). Lower and upper bounds (dotted and dashed lines, respectively) demarcate thresholds in which compounds begin to be recognized as "bioaccumulative" according to regulatory criteria under the Toxic Substances Control Act (≥1000 and <5000 L/kg; USEPA, 1998). FASA = perfluoroalkyl sulfonamides; FTS = fluorotelomer sulfonates; PFCA = perfluoroalkyl carboxylates; PFSA = perfluoroalkyl sulfonates. For other abbreviations, see Figure 2 legend.

BCF relative to molecular weight (Supporting Information S2, Table S9b). Slopes representing the increase in log BCF with fluorinated carbon chain length varied between 0.26 and 0.45 for PFCAs and 0.25 and 0.43 for FASAs across liver, kidney, and gonad (Supporting Information S2, Table S9a). For PFCAs, mean day 21 BCFs ranged from 0.87 L/kg (PFPeA) to 180 L/kg for perfluorononanoate (PFNA), increased with degree of fluorination, and were generally higher for PFOA and PFNA in liver, compared with kidney and gonad (Supporting Information S2, Table S10). The BCFs for individual FASAs were generally higher for kidney, followed by liver and gonad. The day 21 tissue-specific BCFs for L-FHxSA revealed that kidney (2800 L/kg) was greater than liver (1700 L/kg) and gonad (750 L/kg). The highest BCFs were observed at day 21 for FOSA, at 8700 L/kg for kidney, 7600 L/kg for liver, and 5500 L/kg for gonad. Increases in log BCFs were also evident for FTS of increasing chain length, with slopes ranging from 0.91 to 1.3 (Supporting Information S2, Table S9a).

Log BCFs and fluorinated carbon chain length of PFSAs were not strongly correlated (Pearson's) in liver ($R^2 = 0.24$, $r = 0.49$), kidney ($R^2 = 0.12$, $r = 0.34$), or gonad ($R^2 = 0.13$, $r = 0.35$), indicating that fluorinated carbon chain length is not a strong indicator of bioconcentration for PFSAs compared with the other PFAS groups in our study (Supporting Information S2, Table S9a). General observations of day 21 PFSAs mean BCFs revealed that Br-PFHxS had the highest BCF values, followed by L-PFHxS, and then L-PFOS and Br-PFOS (Supporting Information S2, Table S10). The highest reported BCF for Br-PFHxS was 2600 L/kg in day 21 liver. The BCFs for Br-PFHxS in kidney decreased from 1200 to 740 L/kg from day 7 to 21, and a similar decrease was evident in gonad (Supporting Information S2, Table S10). The day 21 BCFs for L-PFHxS were highest in liver (1200 L/kg), followed by kidney (680 L/kg) and gonad (420 L/kg; Supporting Information S2, Table S10). Generally, L-PFOS BCFs were lower than those of L-PFHxS, and by day 21 were 730 L/kg (liver), 490 L/kg (kidney), and 180 L/kg (gonad). Mean BCFs were similar for L-PFOS (730 L/kg) and Br-PFOS (720 L/kg) in day 21 liver. In day 21 gonad, mean BCFs for Br-PFOS and L-PFHpS were found to be equivalent at 120 L/kg (Supporting Information S2, Table S10).

Suspect screening identification of additional PFAS in groundwater and fathead minnow liver

A total of 14 additional compounds were identified by suspect screening analysis in both FTA-1 groundwater and fathead minnow liver from day 21 samples. Nine of the 14 compounds were variations of the perfluoroalkane sulfonates (keto-PFSA, oxa-PFHpS, perfluoroalkane sulfinates, perfluoropropane sulfonate, and 8-pentafluorosulfide-perfluorooctane sulfonate [8-F5S-PFOS]), two were perfluoroalkane sulfonamides, two possessed a perfluoroalkane sulfonamide base structure, and one compound belonged to a group of fluorotelomers (Supporting Information S2 Table S5).

Keto-perfluoroalkane sulfonate homologs (K-PFHxS, K-PFHpS, and K-PFOS) were detected in 100% of liver replicates, and the same detection frequency existed for perfluoropentane sulfinate (PFPeSi) and perfluorohexane sulfinate (PFHxSi), but not perfluorooctane sulfinate (PFOSi; 75%). Perfluoropropane sulfonamide (FPrSA), perfluoropentane sulfonamide (FPeSA), 8-F5S-PFOS, and N-sulfo propyl perfluorohexane sulfonamide (N-SP-FHxSA) were detected in 100% of liver samples (Supporting Information S2, Table S5).

We reported BCF_{pseudo} determinations utilizing peak area ratios of compounds detected in tissue and water via HRMS suspect screening. Individual peak areas of suspect compounds in tissue samples were used to calculate mean BCF_{pseudo} values for those respective compounds. It is important to note that BCF_{pseudo} values are an approximation that bears increased uncertainty given the lack of analytical standards needed for quantitation. Mean BCF_{pseudo} values of perfluoroalkane sulfinates were highest among PFSA variants, increasing from 1600 L/kg (PFPeSi) to 3400 L/kg (PFHxSi), and then decreasing with an increase in fluorinated carbon chain length to 1900 L/kg (PFOSi; Supporting Information S2, Table S6). For keto-PFSAs, mean BCF_{pseudo} values decreased consecutively from 530 L/kg (K-PFHxS) to 30 L/kg (K-PFOS). The mean BCF_{pseudo} value calculated for perfluoropropane sulfonate (PFPrS) was 1.2 L/kg, corresponding with detection in only 50% of liver samples. Low detection frequencies (25%) and low BCF_{pseudo} values (3.3 L/kg) were determined for N-sulfo propyl dimethyl ammonio propyl perfluoropentane sulfonamide (N-SPAmP-FPeSA) and 8:2 fluorotelomer sulfoxide amido sulfonate. Linear regression and Pearson correlation of targeted FASA homologs combined from quantitative and suspect screening analyses revealed a distinct positive correlation (Figure 4) with bioconcentration enrichment (log BCF) in liver with the degree of fluorinated carbon chain length of the compounds ($R^2 = 0.96$, $r = 0.98$, $p = 0.018$).

DISCUSSION

To our knowledge, and from peer review (Burkhard, 2021), these on-site, flow-through fish exposure experiments at Joint Base Cape Cod FTA-1 (Barber et al., 2023; Vajda et al., 2011) are among the first experimental exposure studies to incorporate teleost fish to determine the bioconcentration behavior of PFAS directly from an AFFF-impacted groundwater plume. The groundwater plume at FTA-1 contains a complex mixture of linear and branched isomers of PFSAs and FASAs, indicative of AFFF produced through ECF (Ruyle et al., 2021). The presence of fluorotelomers also provides evidence of AFFF derived from telomerization (Houtz et al., 2013; Moody & Field, 2000).

Correlations were evident between fluorinated carbon chain length and BCFs of FASAs, FTS, and PFCAs quantified via target analysis. These relationships, however, were more complex for PFSAs, with bioconcentration increasing from C5 to C6, but decreasing from C6 to C8. Similar relationships existed for log BCFs compared with the molecular weight of individual compounds (Supporting Information S3, Figure S2 and Table S9b). The observed bioconcentration for PFSAs was

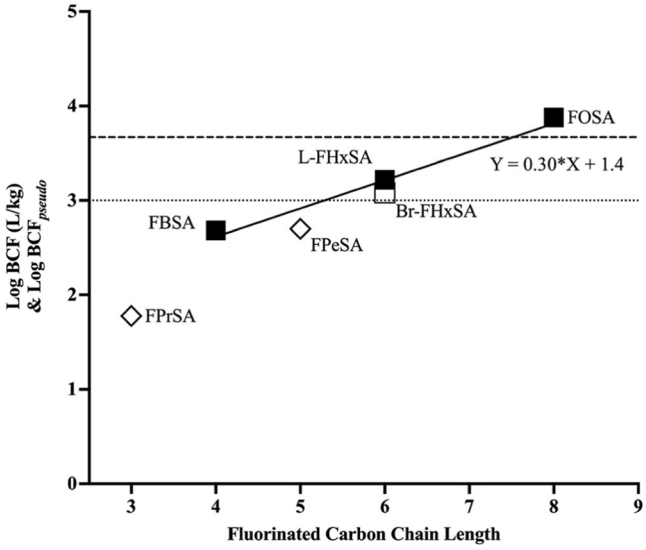

FIGURE 4: Plot of fluorinated carbon chain length versus mean log bioconcentration factor (BCF) and log BCF_{pseudo} for perfluoroalkyl sulfonamides (FASAs) included in the suspect screening analysis. The line of best fit was fitted according to targeted compounds and includes branched perfluorohexane sulfonate (Br-FHxSA); the equation of this line is also presented. Uncertainty of log BCF_{pseudo} values was determined based on the vertical distance of log BCF_{pseudo} from the line of best fit (Supporting Information S2, Table 12). Log BCF_{pseudo} values for C3 and C5 FASAs were calculated using only the day 21 normalized peak areas for water samples. Mean values represent $n = 4$ replicates/data point. Linear regression and Pearson correlation revealed strong positive correlations ($R^2 = 0.96$, $r = 0.98$, $p = 0.018$) with increasing fluorinated chain length and the degree of bioconcentration for the targeted FASA identified. Log BCF for targeted FASAs are identified by solid squares; Log BCF for branched perfluorohexane sulfonamide (Br-FHxSA) is represented by a hollow square, and log BCF_{pseudo} values for perfluoropropane sulfonamide (FPrSA) and perfluoropentane sulfonamide (FPeSA) are represented by hollow diamonds. Lower and upper bounds (dotted and dashed lines, respectively) demarcate thresholds in which compounds begin to be recognized as "bioaccumulative" according to regulatory criteria under the Toxic Substances Control Act (≥1000 and <5000 L/kg; USEPA, 1998). BCF = bioconcentration factors. For other abbreviations, see Figure 2 legend.

higher than that of PFCAs of equivalent fluorinated carbon chain lengths. With the exception of PFHxA, the mean BCFs for PFCAs in our study align with trends in a previous study that showed increases in bioconcentration and bioaccumulation of PFCAs in fish in accordance with fluorinated carbon chain length (Conder et al., 2008).

Complex mixtures, such as those included in our study, present a variety of PFAS showing differences in bioconcentration behavior from what has previously been observed. For instance, previous work has demonstrated a propensity for PFOS to bioconcentrate by 1 order of magnitude higher than PFHxS in fish, but at environmentally relevant concentrations and compositions, we observed an opposite relationship (Martin et al., 2003; Yeung & Mabury, 2013; Supporting Information S2, Table S11). Discrepancies between these results may be due to different exposure methods and the reporting of specific isomers. In the present study, concentrations of PFOS isomers in exposure water (Supporting Information S2, Table S7) were generally much higher than those of PFHxS, as were mean concentrations in tissue samples (Supporting Information S2, Table S8). The mean BCFs for PFHxS were generally higher compared with those for PFOS (Supporting Information S2, Table S10), which may be attributed to the presence in exposure water of precursors, particularly FHxSA, which undergo biotransformation to PFHxS as the terminal degradation product. A similar result was observed by Barber et al. (2023). The presence of multiple isomers in exposure water should be considered because previous research emphasizes notable differences in uptake and elimination kinetics (Zhong et al., 2019), and biotransformation potential in fish (Chen et al., 2015).

Accumulation of branched isomers in tissue compartments was generally lower than the respective linear isomers, which supports previous observations of isomer-specific accumulation and elimination (Chen et al., 2015; Fang et al., 2014; Sharpe et al., 2010; Zhong et al., 2019). This partitioning behavior is evident when mean concentrations of PFOA, PFHxS, PFHpS, PFOS, and FHxSA isomers are compared in tissue samples (Supporting Information S2, Table S8). In addition, detection frequencies of L-PFOA and L-PFHpS were more prevalent across treatment groups in comparison to Br-PFOA and Br-PFHpS. Although subtle differences existed for mean tissue concentrations of L-PFHxS and Br-PFHxS in day 1 tissues, days 7 and 21 tissues revealed preferential accumulation of the linear isomer. The compound Br-PFHxS was generally detected in 100% of samples, though it was only detected in a single kidney sample from the day 1 treatment group. The lack of branched isomer detection in kidneys may be attributed to preferential elimination of branched isomers from them. A PFHxS precursor, Br-FHxSA, was detected in 100% of samples. Mean concentrations of Br-FHxSA were generally higher than those of Br-PFHxS, although an opposite relationship was reflected in day 7 liver and gonad, and day 21 liver (Supporting Information S2, Table S8), which may reflect the influence of biotransformation. Mean BCFs determined for Br-PFHxS were generally higher than those for L-PFHxS, and provided plausible evidence of preferential biotransformation of branched precursor FASAs into terminal PFSA degradation products in the branched form. Preferential biotransformation of branched FOSA isomers into Br-PFOS was previously demonstrated in vivo with crucian carp and in vitro with carp liver and kidney cytosol (Chen et al., 2015). Mean BCFs for Br-FHxSA were generally lower than for the linear isomer (Supporting Information S2, Table S10).

Differences in mean BCFs for PFCAs and PFSAs of equivalent fluorinated carbon chain length in our study highlight the importance of the terminal head group on PFAS. Similar differences in this trend were also reported for whole-body BCFs in fathead minnows exposed to FTA-1 groundwater in previous experiments (Barber et al., 2023). Plausible explanations may include biotransformation of sulfonamide moieties into PFSA terminal degradation products. Prior work also demonstrated that PFSAs have stronger interactions than PFCAs of the same fluorinated carbon chain length when bound to human liver fatty acid binding protein (L-FABP) amino acid residues (Zhang et al., 2013). The observed differences in BCFs for PFCAs and

PFSAs in fathead minnow liver and kidney may be partly explained by different compound affinities for L-FABP and other endogenous ligands such as organic anion transporter proteins in the kidney (Ng & Hungerbühler, 2013).

Generally, by day 21, the highest average ∑PFAS concentrations were detected in liver > kidney > gonad, which differs from previous laboratory observations in rainbow trout, in which concentrations were highest in blood > kidney > liver > gallbladder > gonads > adipose tissue > muscle (Martin et al., 2003). However, a similar relationship to our study was previously determined when average ∑PFAS concentrations were compared in liver and gonad from wild fish exposed to AFFFs in the natural setting (Ahrens et al., 2015). Differences in PFAS concentrations in tissues across studies could be driven by laboratory versus environmental settings, including dosing and exposure methods, and the likelihood of other co-contaminants in the environment that could influence PFAS biological partitioning. Consideration must also be given to increasing water concentrations over time. Such an increase may have delayed approaching chemical equilibrium during uptake, which adds complexity in quantitatively assessing biotransformation and bioconcentration dynamics.

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 samples (Supporting Information S2, Table S8). Variations in detection frequency and tissue concentrations may be due to the propensity of PFAS to interact with biological constituents and mechanisms that influence internal distribution (Allendorf et al., 2019; Bischel et al., 2010; Han et al., 2003; Khazaee et al., 2021; Luebker et al., 2002), although interindividual variability among test organisms must also be considered (Shi et al., 2018). Detection of FBSA, L-FHxSA, Br-FHxSA, and FOSA across all replicates in treatment groups exposed to AFFF-impacted groundwater has provided valuable insights into an emerging group of PFAS alternatives known for their prevalence at AFFF-impacted sites and widespread geographic distribution but are not yet widely represented in the biological literature (Ahrens et al., 2015; Chu et al., 2016; Houtz et al., 2013; Kaboré et al., 2022). Few studies had detected FBSA in biota until more recently (Chu et al., 2016; Kaboré et al., 2022; Pickard et al., 2022), and fewer have investigated effects-based biological responses to FBSA exposures. More recently, controlled aqueous exposures of embryonic zebrafish to FBSA, among other short-chain PFAS alternatives, revealed FBSA to be the most bioaccumulative, although the BCFs determined did not exceed regulatory thresholds. However, FBSA was the only compound to induce phenotypic responses that resulted in developmental malformations at 120 h post fertilization and also induced transcriptomic disruptions at 48 h post fertilization (Rericha et al., 2022).

Mean BCFs for FOSA calculated for liver, kidney, and gonad were generally within or above the US Environmental Protection Agency (USEPA) Toxic Substances Control Act (TSCA) thresholds (≥1000 and <5000 L/kg) in which chemicals are considered to display tendencies to bioaccumulate (USEPA, 1998). By day 21, L-FHxSA (1700 L/kg liver and 2800 L/kg kidney), Br-FHxSA (1200 L/kg liver and 1400 L/kg kidney), L-PFHxS (1200 L/kg liver), and Br-PFHxS (2600 L/kg liver) also fell within the TSCA bioaccumulation thresholds. Generally, BCFs for linear FASAs were greater than other PFAAs of equivalent fluorinated carbon chain length. This further demonstrated that the amine head group of the FASA molecule strongly influences tissue–water partitioning. Our results on FASA detection and bioconcentration suggests that FASAs have the potential to accumulate similarly to, or greater than other quantifiable PFAAs of similar chain length in our study. The prevalence of FASAs in fish tissue during early exposure to contaminated groundwater demonstrates their effective partitioning from aqueous media to biological endpoints. Observations of relative abundances among PFAS subgroups (Figure 1) generally revealed a predominance of FASAs in day 1 and 7 tissues. Relative abundances in day 7 liver and gonad revealed shifts from FASA to PFSA predominance. These shifts in relative abundance from FASAs to PFSAs may reflect biotransformation processes after uptake, higher elimination kinetics, or both. This piques our curiosity as to the mechanisms of biotransformation and whether biotransformation of certain FASAs is more likely to occur in liver and gonad, rather than kidney. Contemporary evidence of the bioaccumulative behavior and toxicological effects of sulfonamide moieties should prompt further investigation into the toxicological implications of exposure across taxa.

Suspect screening conducted on FTA-1 groundwater and exposed fathead minnow livers revealed the presence of PFSA and FASA derivatives previously reported in a variety of matrices including AFFF and commercial products, AFFF-impacted groundwater and drinking water, and human serum (Barzen-Hanson et al., 2017; McDonough et al., 2021; Rotander et al., 2015; Supporting Information S2, Table S5). Compounds such as N-SP-FHxSA and N-SPAmP-FPeSA belong to subgroups comprised of sulfonamide base structures that were previously discovered in AFFF and commercial products, but not in AFFF-impacted groundwater (Barzen-Hanson et al., 2017). In the present study we discovered the presence of these compounds in AFFF-impacted groundwater and in fathead minnow liver, although N-SPAmP-FPeSA was detected in day 21 FTA-1 groundwater and in only one liver sample with a $BCF_{pseudo} < 1$ L/kg. Low detection of this compound in liver samples may suggest low absorption, high clearance rates, and/or activity in biotransformation pathways. Additionally, the detection of FPrSA and FPeSA in FTA-1 groundwater and 100% of fathead minnow livers further emphasizes the richness of sulfonamide precursors in AFFF formulations and the potential for FASAs of varying chain lengths to accumulate in aquatic organisms. The same FASA homologs were previously detected in recreational fish samples collected from AFFF-impacted surface waters, which provides further evidence for the environmental ubiquity and bioaccumulative potential of FASA homologs (Pickard et al., 2022).

Keto-perfluorooctane sulfonate was previously detected in serum samples of firefighters and in human serum samples of a population exposed to AFFF-impacted drinking water (McDonough et al., 2021; Rotander et al., 2015). Like human

serum samples included in previous nontargeted approaches (McDonough et al., 2021), the same homologous series of keto-substituted PFSAs (K-PFHxS, K-PFHpS, and K-PFOS) was also found in the fathead minnow livers analyzed by HRMS suspect screening. Mean BCF_{pseudo} values decreased consecutively for keto-substitute PFSA homologs with increasing fluorinated carbon chain length (Supporting Information S2, Table S6). Order of magnitude differences were also observed when the mean BCF_{pseudo} values of K-PFHxS and K-PFOS were compared with sulfinate- and sulfonate-derived PFSAs of equivalent chain lengths. Additional perfluoroalkane sulfonate derivatives in fathead minnow livers included a homologous series of perfluoroalkane sulfinates (PFPeSi, PFHxSi, and PFOSi), O-PFHpS, and 8-F5S-PFOS, all of which were detected in 100% of liver samples except for PFOSi (75%). These compounds were also detected in FTA-1 groundwater.

The presence of PFHxSi provided further evidence linked to biotransformation of sulfonamide moieties. Ruyle et al. (2023) previously identified PFHxSi as an intermediate of perfluoroalkyl sulfonamido precursor biotransformation via nitrifying bacteria. The presence of PFHxSi and its homologs in groundwater and liver may suggest that nitrifying bacteria in soils and groundwater play a role in producing PFHxSi as a byproduct of nitrification. If such is the case, PFHxSi may have partitioned into surrounding porewaters after microbial turnover events at our study site, or plausibly, biotransformation of FASAs within fathead minnows themselves. The biotransformation of FHxSA into PFHxSi provides plausible evidence for the established presence of PFHxS and elevated accumulation of PFHxS in liver, kidney, and gonad at day 21. Degradation of FHxSA to PFHxS by this pathway may also imply biotransformation of FOSA to the degradation intermediate, PFOSi, before final degradation to PFOS, as previously proposed by Rhoads et al. (2008).

These results highlight the limitations of relying on targeted analytical methods when dealing with AFFF exposure, and the potential for biota from AFFF-impacted sites to contain PFAS precursors as bioaccumulative as PFOS. There is greater uncertainty in deriving the BCF_{pseudo} values based on peak area rather than recovery-corrected concentration, given the potential for different matrix effects in water versus tissues. At least for the FASAs, results from target and suspect screening approaches agree within a factor of 2 to 3 (Supporting Information S2, Table S12). It seems prudent to treat BCF_{pseudo} values as approximations, but they clearly demonstrate the potential for several FASAs to bioaccumulate.

These observations further emphasize the necessity for analytical standards required to quantify a broader scope of novel and emerging compounds. Future toxicokinetic studies utilizing environmental PFAS exposures and employing steady-state and depuration periods may provide further insights into the mechanistic relationships of PFAS partitioning required for bioaccumulation modeling and ecological risk assessments. The statistical approaches used in the present study to determine the relationships of PFAS physicochemistry and bioconcentration behavior support growing evidence for the possibility of predicting tissue enrichment based on fluorinated chain length and molecular weight for PFCAs, FASAs, and FTS, but our results do not yield statistical correlations to support these distinctions for PFSAs.

Utilization of field-derived point sources and mobile laboratories provided a blueprint for which future environmental dosing studies can be modified to characterize PFAS bioconcentrations in aquatic organisms. The PFAS analyzed in the present environmental exposure study emulate real-world compositions in terms of PFAS richness. Overall, our study emphasizes the importance of precursor compounds and emerging PFSA derivatives present in AFFF formulations that exhibit bioaccumulative potential in aquatic wildlife and humans and may enhance exposures to terminal PFAS of concern.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5926.

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