Tissue uptake, distribution, and elimination of perfluoroalkyl substances in juvenile perch through perfluorooctane sulfonamidoethanol based phosphate diester dietary exposure

Juliette Gaillard, Bruno Veyrand, Marielle Thomas, Xavier Dauchy, Virginie Boiteux, Philippe Marchand, Bruno Le Bizec, Damien Banas, Cyril Feidt

Tissue Uptake, Distribution, and Elimination of Perfluoroalkyl Substances in Juvenile Perch through Perfluorooctane Sulfonamidoethanol Based Phosphate Diester Dietary Exposure

Juliette Gaillard*[1]; Bruno Veyrand[2]; Marielle Thomas[1]; Xavier Dauchy[3]; Virginie Boiteux[3]; Philippe Marchand[2]; Bruno Le Bizec[2]; Damien Banas*[1]; Cyril Feidt*[1]

*damien.banas@univ-lorraine.fr

[1]UR Animal et Fonctionnalités des Produits Animaux, Universitéde Lorraine, EA 3998, BP 20163, F-54 505 Vandoeuvre-lès-Nancy, France
[2]Atlanpôle−La Chantrerie, ONIRIS, USC 2013 LABERCA, BP 50707, F-44307 Nantes, France
[3]Nancy Laboratory for Hydrology, Water Chemistry Department, ANSES, 54000 Nancy, France

ABSTRACT:
Perfluorooctane sulfonamidoethanol based phosphate diester (SAmPAP) is a potential perfluorooctanesulfonate (PFOS) precursor. To examine whether SAmPAP exposure would result in fish contamination by perfluoroalkyl and polyfluoroalkyl substances (PFASs), juvenile Eurasian perch were dietarily exposed to this compound (dosed group) or exposed to the same tank water but fed control feed (control group). SAmPAP and metabolites were monitored in the muscle, liver, and serum during the 45-day exposure phase and 35-day depuration phase. SAmPAP was only detected in the dosed group and the absorption efficiency (0.04−2.25%) was very low, possibly related to its low bioavailability in the gastrointestinal tract, steric constraints in crossing biological membranes, and clearing by enterohepatic circulation. Although SAmPAP was biotransformed and eliminated at a slow rate (t1/2 > 18 days), its biomagnification factor was low. The observed metabolites in fish were N-ethyl perfluorooctane sulfonamidoacetic acid, perfluorooctane sulfonamidoacetic acid, perfluorooctane sulfonamide, and PFOS. Considering that SAmPAP was the only source of PFASs in the tanks, the occurrence of metabolites indicates that SAmPAP could be biotransformed in fish and contribute to PFOS bioaccumulation. However, levels of metabolites were not significantly different in the dosed and control groups, indicating that metabolite excretion followed by re-exposure to these metabolites from water was the main uptake route.
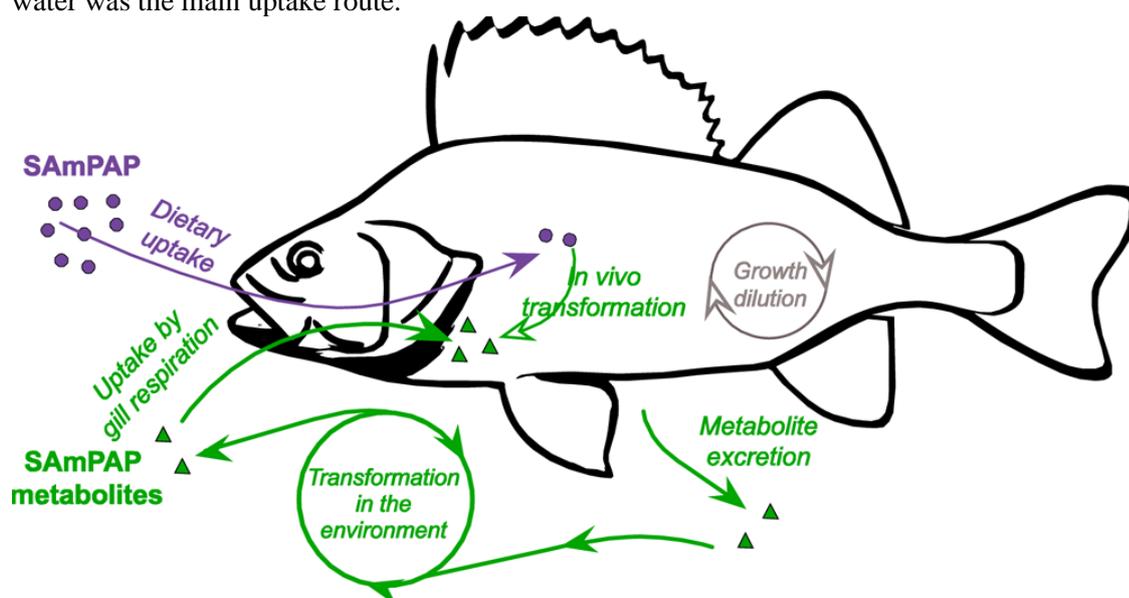
1. INTRODUCTION

Perfluorooctanesulfonate (PFOS) is one of the most abundant per- and polyfluoroalkyl substances (PFASs) detected in wildlife and humans worldwide[1-3] and has been associated with endocrine-disrupting and developmental effects in both wildlife and humans[4-6]. Dietary uptake of PFOS is suggested to be an important route to the total exposure for the general adult human population[7,8] and it has been shown that freshwater fish is a significant determinant of internal exposure to PFOS for specificpopulations, such as anglers[9-11]. PFOS concentrations are indeed particularly high in freshwater species, such as common carp or Eurasian perch[12,13]. A primary research interest is to determine to what extent the body burden of PFOS in aquatic organisms is due to the degradation of its higher molecular weight derivatives, referred to as PFOS precursors (PreFOS)[14-18]. It has been estimated that PreFOS may be among the largest potential historical reservoirs of PFOS, given production volumes [19]. Perfluorooctane sulfonamidoethanol based phosphate diester (SAmPAP) is a typical PreFOS that was introduced in 1974 for use in foodcontact paper and packaging[17,20] and constituted one of 3M's major fluorochemical output before it was phased out in the United States, although it is still produced in some places, including China[21]. SAmPAP and potential SAmPAP transformation products have been recently detected in aquatic sediments to which SAmPAP would be expected to partition preferentially[22-25]. While SAmPAP was predicted to be recalcitrant to both aqueous hydrolysis and microbial degradation[26,27], biotransformation of SAmPAP through aqueous exposure in fish has been demonstrated[18]. However, little is known about SAmPAP biotransformation by aquatic organisms through dietary exposure and its potential to contribute to the bioaccumulation of PFOS. In the present study, fish were dietarily exposed to SAmPAP and levels of metabolites, including PFOS, were monitored in fish tissues in order to ascertain PFOS accumulation from SAmPAP exposure.

2. MATERIALS AND METHODS

2.1. <u>Chemicals</u>:

A list of analytical standards used in this study is provided in the Supporting Information (SI). Technicalgrade N-ethyl perfluorooctane sulfonamidoethanol (NEtFOSE)- based phosphate (SAmPAP, CAS 2965-52-8) originated from Defu and was provided by Holly Lee (Department of Chemistry, University of Toronto). The product was purified by Atlanchim (Saint-Herblain, France) in order to achieve a purity close to 99%. Potential impurities in the preparation were monitored by LC−MS using the full scan acquisition mode (m/z 60−800). No impurity was detected.

2.2. <u>Food Preparation</u>:

Two batches of commercial fish feed (Le Gouessant, Sturgeon Grower coul 5, 47% protein, 13% fat, 1.9% fiber, 11% ash) were prepared, one for the dosing of SAmPAP and one for the control feed. SAmPAP-spiked fish feed (target concentration 5 μg/g) was prepared using methanol as a vector followed by complete evaporation of the solvent (further details are presented in the SA). Spiked feed samples were taken every day of the exposure phase during preparation of meals for the dosed fish and pooled for analysis. The SAmPAP concentration in the dosed feed (n = 6) was 1631 ± 95 ng/g food. Large discrepancies between the target concentration (5000 ng/g food) and the actual concentration (1631 ng/g food) in the dosed feed may be due to solubility problems and sorption onto the labware walls. When preparing daily rations, subsamples (≈1 g) of each of the experimental feeds were collected. The control and dosed feed collected daily were pooled in separate jars. Both pooled samples were kept at −20 °C. These pooled samples of dosed and control feeds were analyzed, using the same method performed on fish, in order to measure feed contamination.

2.3. <u>Fish Care and Sampling</u>:

The present study was approved by the local Ethical Committee for Animal Experimentation (no. 02664.01). Juvenile perch (∼60 g) were purchased from a farm raising fish in a closed system (Asialor, Dieuze, France) and allowed to acclimate for a month prior to chemical exposure. Two weeks prior to chemical exposure, 90 fish were distributed in five glass tanks (280 L) in an aerated, recirculated system (circular pump and biological filter). During the experimentation, the light/dark period was 16 h/8 h. The water temperature was maintained at 22 °C, the pH at 8, and the dissolved oxygen at 7 mg/L[28]. Several steps were taken to control the fish environment, including a daily renewal of one-third of the water with a fresh water supply, daily draining of uneaten food and feces, and continuous filtration over an active carbon unit (Norit, 3−6 mm). Among the five tanks, three tanks were designated for the SAmPAP bioaccumulation test and two tanks were designated for the blank control (Figure 1).
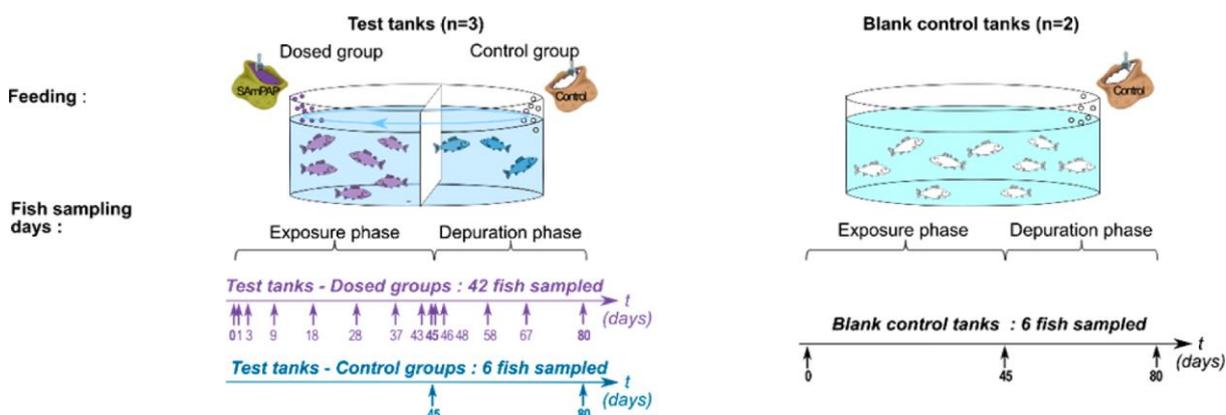
Figure 1. Experimental design.

Each of the three test tanks were divided into two connected compartments, separated by a screen through which water but no feed pellets could pass. During the exposure phase (45 days), fish in the first compartment of the test tanks (dosed group), received the SAmPAP dosed feed and fish in the second compartment (control group) received the control feed. The purpose of including fish that were not dietarily exposed to SAmPAP in the same tank as the SAmPAP dosed fish was to determine the extent of uptake from water that may occur. In addition, to evaluate water contamination, five water samples

(one from each tank) were collected at the start and at the end of the exposure phase. Water samples were stored at −20 °C until further analysis. During the depuration phase (35 days), all fish were fed control feed. The daily rate of feeding was equal to 1.5% of the mean fish weight. To maintain the same feeding rate, the amount of feed distributed was recalculated once a week using the weight of the fish sampled most recently. Different sampling schedules were applied to fish from (i) the dosed groups in the test tanks, (ii) the control group in the test tanks, and (iii) the blank control tanks (Figure 1). Fish sampling always occurred before feeding. For a given time point, three fish from the dosed group (one from the first compartment of each test tank); three fish from the control groups (one from the second compartment of each test tank), and two fish (one from each blank control tank) were collected. Each fish was anaesthetized by tricaine methanesulfonate. Whole blood was collected through puncture with syringes in the caudal vein. Fish were subsequently euthanized by a lethal overdose of tricaine methanesulfonate. Each fish was weighed and dissected to collect the liver and muscle tissue without skin. Livers and muscle of each fish were weighted. All samples were stored at −20 °C until further analysis.

2.4. Chemical Analysis:
2.4.1. Water Samples.
Water samples were extracted and analyzed using previously developed methods[29,30]. Three sample treatments were applied depending on the considered compound. Detailed extraction methods, chromatographic gradients, and instrumental conditions are provided in the SI. The analytical methods were validated according to SANCO 11945/2013 guidelines, except for analytes analyzed by direct injection [perfluorooctane sulfonamide (FOSA), perfluorooctane sulfonamidoacetic acid (FOSAA), Nethyl perfluorooctane sulfonamidoacetic acid (NEtFOSAA), and SAmPAP]. Mean recoveries for N-methyl perfluorooctane sulfonamide (NMeFOSA), N-ethyl perfluorooctane sulfonamide (NEtFOSA), and NEtFOSE in water samples ranged from 85 to 103% at 20 ng/L (quantification limit). Mean recoveries (n = 5) for PFHpS and PFOS in water samples ranged from 81% to 95% at 4 ng/L (quantification limit). The quantification of the four PFASs analyzed by direct injection was based on a standard addition calibration. Detection limits were 1 ng/L for FOSA and NEtFOSAA and 5 ng/L FOSAA and SAmPAP. The reliability of the results was ensured by within-run and intrasample controls systematically made for each sample batch. The within-run controls consist of calibration check standards inserted throughout the sample batch. Intrasample controls consist of spiking the analytes in the actual samples in order to verify the accuracy of the analytical method.

2.4.2. Food and Fish Samples. For food and fish sample analysis, 5 g (±500 mg) (for muscle or liver) or 1mL (for serum) was placed into a polypropylene centrifuger tube (50 mL), in which a mixture of labeled precursors was added (internal standards including 13C4-PFOS). For each sample as well as the blank, solid−liquid extraction was performed using sequentially 10 mL of ultrapure water, 200 μL of formic acid, and 15 mL of acetonitrile. The samples were then agitated via vortex for about 30 s. A mixture composed of MgSO4:NaCl (6:1.5, w/w) was added (7.5 g) and directly followed by vortex agitation in order to avoid agglomeration. After centrifugation at 2500g for 5 min, the supernatant layer was transferred into another 50 mL centrifuge tube, where 2.07 g of the mixture MgSO4:C18:EnviCarb (1.8:0.18:0.09, w/w/w) was added, followed by agitation via vortex for 30 s. After centrifugation at 2500g for 5 min, 8 mL of the purified extract was evaporated under nitrogen to 500 μL. The extract was then filtered on a 0.2 μm filter and transferred into 1.5 mL microtubes. The final volume was adjusted to 500 μL using acetonitrile. Before analysis, 50 μL of 13C8-PFOS (used as external standard) and 150 μL of the previous extract were transferred into a polypropylene vial. Seven standard solutions in methanol containing different amounts of target analytes (0, 0.5, 1, 2, 5, 10, 20 ng) and a fixed amount of internal standard (5 ng) were prepared to obtain calibration curves for each compound. Blank samples containing only extraction solvent and internal standard were included in all batches of analysis. Recoveries were in-between 30% and 50%, depending on the analytes and the matrices. The recovery was systematically corrected by the internal standard. Analyses were performed by liquid chromatography coupled to tandem mass spectrometry (LC−MS/MS) using an HPLC pump with a binary gradient system (Agilent Technologies, Santa Clara, CA) coupled to a 6410 triple quadrupole instrument (Agilent Technologies, Santa Clara, CA) or gas chromatography coupled to tandem mass spectrometry using positive chemical ionization (GC−PCI-MS/MS) using an Agilent 7000 instrument

(Agilent Technologies, Santa Clara, CA) for volatile compounds (NMeFOSE and NEtFOSE). Two diagnostic signals (qualifier and quantifier SRM transitions, respectively) were monitored for each target compound.

2.5. Data Analysis

All values reported are means (±standard errors) unless stated otherwise. All statistical analyses were performed using R version 3.0.2[31].

2.5.1. Physical Indices. Fish weight (FW in g) was best predicted by the exponential growth model, FW = a exp(kGt), where a is a constant, kG is the growth rate [in g/(g day)], and t is the time (in days). Differences between groups or experimental phases (i.e., exposure and depuration phases) were compared using the parametric method of grouped linear regression with covariance analysis. Liver somatic index (LSI in %) was calculated as LSI = 100 × (LW/FW), where LW is the liver weight (in g). The parametric one-way analysis of variance (ANOVA) was used to assess the differences in LSI among the different groups.

2.5.2. Dietary Uptake and Depuration of SAmPAP in Fish. Depuration rate constants (kT) were calculated by fitting tissue concentrations during the depuration phase to the first-order elimination equation, ln(CF) = a + kTt, where CF is the chemical concentration in fish (ng/g wet weight fish), a is a constant, t is time (days), and kT is the overall depuration rate constant (i.e., not growth corrected)[32,33]. To correct kT for growth dilution, the growth corrected elimination rate constant kTg was calculated as kTg = kT − kG, where kT is the overall depuration constant, and kG is the growth rate. Depuration half-lives (t1/2 or t1/2G) were calculated as ln(2/kT) or ln(2/kTg). Absorption efficiencies (α or αG) were determined for SAmPAP by using iterative nonlinear regression to fit data in the exposure phase to the integrated form of the kinetic rate equation for constant dietary exposure: CF = (αFCD/kT)[1 − exp(−kTt)] or CF = (αgFCD/kTg)[1 − exp(−kTgt)], where F is the feeding rate (1.5% FW) and CD is the chemical concentration in diet (ng/g food). Biomagnification factors (BMF and BMFg) were calculated for SAmPAP using the kinetic equation method (i.e., BMF = αF/kT or BMFg = αGF/ kTg).

2.5.3. Uptake and Depuration of SAmPAP Metabolites in Fish. The uptake and depuration of SAmPAP metabolites in fish can be expressed using a typical bioaccumulation mass balance model[32,33]. In addition to the distinction between uptake from the water and from the diet, a distinction was made between metabolite uptake resulting from in vivo transformation of precursor compounds, denoted "PrePF-based uptake", and metabolite uptake resulting from direct exposure, denoted "PFbased uptake"

$$dC_{F,PF}/dt = \left(k_{R,PF}C_{W,\ PF} + \sum_{PrePF} k_{R,PrePF}F_{biotransf}C_{W,PrePF}\right) + \left(k_{D,SAmPAP}F_{biotransf,SAmPAP}C_{D,SAmPAP}\right) - k_TC_{F,PF}$$

(equation 1)

where CF,PF is the concentration of the considered metabolite in fish (ng/g wet weight fish), CW,PF is the concentration of the metabolite in water (ng/mL water), CW,PrePF is the concentration of a precursor of the metabolite in water (ng/mL water), CD,SAmPAP is the concentration of SAmPAP in diet (ng/g food), kR,PF and kR,PrePF [mL water/(g ww fish d)] are the rate constants for uptake from the water of the metabolite or one of its precursors, kD,SAmPAP [g food/(g ww fish d)] is the rate constant for dietary uptake of SAmPAP, and Fbiotransf (unitless), the biotransformation factor of precursor compounds to metabolites.

2.5.4. Tissue Distribution. Liver-to-serum (LSRs), liver-to-muscle (LMRs), and serum-to-muscle (SMRs) ratios were calculated on each sampling day to evaluate partitioning of SAmPAP and metabolites in these compartments[34-36]. The burden of PFASs in the muscle, liver, and serum was calculated by multiplying tissue concentration by the mass or volume of the considered tissue. Muscle weight was calculated as 0.42 × FW, liver weight was measured at the time of sampling, and serum volume was calculated as 0.018 × FW[37,38].

3. RESULTS AND DISCUSSION

3.1. Fish Health.

The fish growth rates (kG) were 0.62 g/(g d), with no significant differences observed between the test and blank control tanks or between the uptake and depuration phases (Table S1, SI). Variability in size distributions among groups was reflected by a relatively low coefficient of determination ($r2 = 0.4$) value for growth rate. Mortality only occurred in the test tanks, with lower mortality in the dosed groups (4% mortality rate) compared to the control groups (33% mortality rate). The fact that mortality rates were higher in the test tanks compared to the blank control tanks contrasts a number of studies recording no mortality while having higher levels of PFSAs[14,18,39,40]. Mortality may have been induced from stress resulting from isolation of the control group from the larger dosed group, considering that juvenile perch is a gregarious fish. The mean LSI was 2.16%, which is consistent with previous studies[41]. No significant differences between the test and blank control tanks and no increase over time were observed, both of which suggest the absence of liver enlargement.

3.2. SAmPAP Dietary Bioaccumulation.

3.2.1. Background Concentrations. SAmPAP was not detected in either the control feed, samples collected from the test tank 1 h before dosing (day 0), or samples collected from the blank control tanks during the whole course of the experiment. SAmPAP background concentrations were therefore very low (below limits of detection).

3.2.2. Relative Importance of Uptake from Diet and Water in the Bioaccumulation of SAmPAP. In the dosed group, SAmPAP was detected in the muscle, liver, and serum samples. In the control group, SAmPAP was detected in the liver and serum samples at low levels and was not detected in the muscle samples (Table 1).

Table 1. Detection Frequencies and Concentrations (mean ± standard error) of SAmPAP and Metabolites in Muscle (ng/g wet weight), Liver (ng/g wet weight), and Serum (ng/mL) of Eurasian Perch at the End of the Exposure Phase and at the End of the Depuration Phase[a]

	Muscle	Muscle	Liver	Liver	Serum	Serum
	Dosed group	Control group	Dosed group	Control group	Dosed group	Control group
Detection Frequencies (%) during the Whole Course of the Experiment						
SAmPAP	71	Nd	93	83	86	17
NEtFOSAA	79	100	88	100	83	100
FOSAA	Nd	Nd	19	Nd	Nd	Nd
FOSA	79	100	76	100	71	100
PFOS	100	100	100	100	100	100
Concentrations (ng/g ww or ng/mL) at the End of the Exposure Phase						
SAmPAP	0.16 ± 0.08	0	5.99 ± 1.84*	0.44 ± 0.29*	9.37 ± 2.25*	0.67 ± 0.67*
NEtFOSAA	1.16 ± 0.40	0.93 ± 0.15	2.23 ± 0.33	1.82 ± 0.24	13.77 ± 3.02	11.61 ± 1.68
FOSAA	0	0	0	0	0	0
FOSA	1.31 ± 0.29	0.84 ± 0.30	1.94 ± 0.38	2.06 ± 0.50	2.29 ± 0.49	1.6 ± 0.31
PFOS	0.47 ± 0.12	0.53 ± 0.13	2.32 ± 0.73	3.11 ± 0.81	16.12 ± 3.71	16.13 ± 3.27
Σ SAmPAP metabolites	2.94 ± 0.19	2.30 ± 0.15	6.50 ± 0.94	7.00 ± 0.88	32.18 ± 4.21	29.34 ± 3.39
Concentrations (ng/g ww or ng/mL) at the End of the Depuration Phase						
SAmPAP	0.16 ± 0.03*	0	6.28 ± 1.85*	0.12 ± 0.06*	4.31 ± 1.25*	0*
NEtFOSAA	0.74 ± 0.25	0.85 ± 0.28	2.29 ± 0.67	1.63 ± 0.49	6.22 ± 2.15	9.79 ± 3.31
FOSAA	0	0	0.04 ± 0.04	0	0	0
FOSA	1.92 ± 0.86	1.36 ± 0.30	5.37 ± 1.73	4.06 ± 0.81	2.49 ± 0.81	3.26 ± 1.08
PFOS	1.34 ± 0.09	1.57 ± 0.22	11.60 ± 2.08	9.79 ± 0.83	39.23 ± 8.27	50.85 ± 8.48
Σ SAmPAP metabolites	4.01 ± 0.99	3.77 ± 0.57	19.30 ± 2.21	15.48 ± 1.15	47.94 ± 9.67	63.90 ± 10.87
[a]Differences between the dosed and control groups from the test tanks were evaluated by the unpaired Student's t-test (*p < 0.1).						

At the end of the exposure phase, serum and liver concentrations were significantly different between the dosed and control groups, and concentrations measured in the control group were always <8% of corresponding concentrations in the dosed group. SAmPAP uptake from water was therefore considered a negligible uptake route in comparison to dietary uptake. Assimilation efficiency, depuration rate

constant, and biomagnification factors for SAmPAP were therefore calculated using the concentration data from the dosed group.

3.2.3. SAmPAP Uptake. During the exposure phase, fish from the dosed group received ≈24 ng of SAmPAP/g of fish, although some fish may have been feeding above or below the feeding rate (1.5%), as evidenced by the low coefficient of determination ($r2 = 0.4$) for the exponential growth model. SAmPAP was detected in the serum and liver samples of the dosed group within 1 day of exposure and in the muscle within 3 days of exposure (Figure 2).
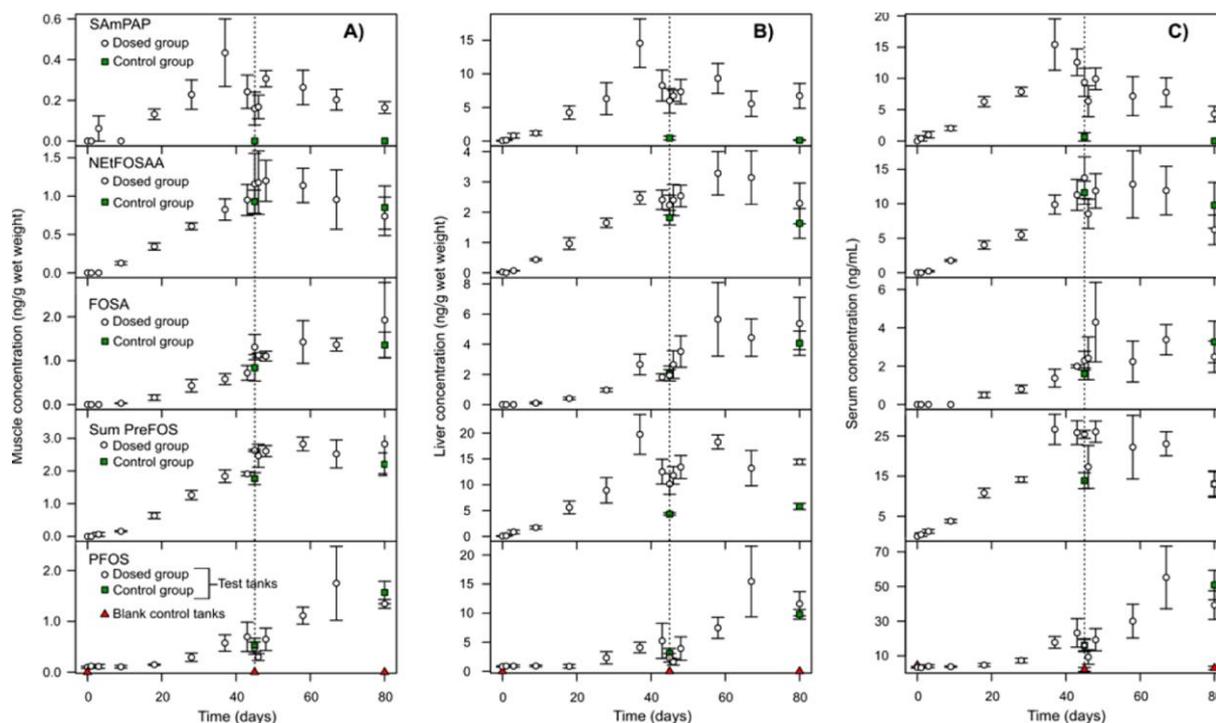

Figure 2. Evolution of concentrations of SAmPAP and potential metabolites in the muscle (A), liver (B), and serum (C) of Eurasian perch in the test tanks (dosed and control groups) and in the blank control tanks.

SAmPAP concentrations increased in the muscle, liver, and serum ($p < 0.05$) throughout the exposure phase. The absorption efficiencies observed for SAmPAP varied depending on the considered tissue ($\alpha = 0.04-2.25\%$, Table 2). SAmPAP absorption efficiencies were low when compared to other PFASs, such as FOSA (18%) or PFOS (120%), in rainbow trout[14,40] The reduced uptake of SAmPAP (molecular weight = 1204 amu, Table S1, SI) is consistent with the typical 500 amu molecular weight cutoff for oral absorption[42]. This low absorption efficiency can indeed be the result of the reduced bioavailability in the gastrointestinal tract and/or steric or electrostatic effects limiting diffusive mass transfer through the epithelium of the gastrointestinal tract[33]. The first pass effect is unlikely given the absence of SAmPAP metabolites in liver on the first day of exposure. Higher concentrations in serum and liver compared to muscle support the importance of SAmPAP enterohepatic circulation in perch, as suggested for PFOS, together with intrinsic partition of these chemicals to the liver[40,43]. However, it is unlikely that SAmPAP is reabsorbed across the intestinal mucosa following excretion via the bile, given the low absorption efficiency of SAmPAP.

3.2.4. SAmPAP Depuration. During the depuration phase, the concentration of SAmPAP decreased in the dosed group, but slopes of ln(concentrations or growth corrected concentrations) vs time (i.e., kT or kTg) were not statistically different from zero. Growth-corrected elimination rate constants ($kTg = kT − kG$)
were indeed negative values (i.e., increase in SAmPAP growthcorrection concentrations during the depuration phase) except in the serum, as previously observed for other extremely high KOW chemicals (log $KOW > 8$).[44] Loss by fecal egestion, biotranformation, or respiratory ventilation of SAmPAP were

therefore very slow compared to that of other PFASs[14,18,40]. In the absence of significant SAmPAP depuration, SAmPAP depuration rate constants and half-lives were estimated using the lower bounds 95% confidence interval of the slopes obtained (Table 2)[26].

Table 2. Depuration Rate Constant (kT or kTg), Depuration Half-Life (t1/2 or t1/2g), Assimilation Efficiency (α or αg), Biomagnification Factor (BMF or BMFg), and Estimated Time To Achieve Steady-State (tss) for SAmPAP Using Gross Concentration Data or Growth-Corrected Concentration Data

	α (%)	kT (day−1)[a]	t1/2 (day)[a]	BMF	Log(BMF)	(day)[a]
Gross Concentration						
muscle	0.04	>0.03	>27.5	0.0003	−3.6	>91.3
liver	1.33	>0.02	>32.7	0.0094	−2.0	>108.6
serum	2.25	>0.04	>18.2	0.0089	−2.1	>60.6
Growth-Corrected Concentration						
	α (%)	kT (day−1)[a]	t1/2 (day)[a]	BMF	Log(BMF)	(day)[a]
muscle	0.05	>0.02	>36.4	0.0003	−3.4	>120.8
liver	1.51	>0.02	>46.1	0.0151	−1.8	>153.1
serum	2.59	>0.03	>21.8	0.0122	−1.9	>72.3

[a]Estimated from the lower bounds 95% confidence interval of the slope.

The half-lives reported in the present study are much higher than those observed for SAmPAP in a bioconcentration study (i.e., 2.4−2.7 days in medaka)[18]. Differences between half-lives obtained from bioconcentration (i.e., chemicals enter the systemic circulation directly via the gills) and the present dietary accumulation study, together with higher concentrations in serum and liver compared to muscle, support the importance of SAmPAP enterohepatic circulation in perch, which is indeed suggestive of a prolonged retention of SAmPAP and longer half-life[40].

3.2.5. SAmPAP Biomagnification. The biomagnification factor (BMF) was calculated using the kinetic approach. The BMF was not significantly greater than 1, indicating that SAmPAP dietary exposure will not result in biomagnification in perch. If we examine the equation for biomagnification (BMF = $\alpha F/kT$) and set the absorption efficiency to 1%, the depuration rate kT must be 0.000 15 (i.e., half-life of 4621 days) to obtain a biomagnification factor of 1. The low BMF of SAmPAP was therefore strongly associated with its low absorption efficiency.

3.3. Intermediate and Terminal Metabolites Bioaccumulation.
3.3.1. Background Concentrations. PFOS was detected in fish serum from the blank control tanks, but there was no significant increase of PFOS concentrations over time, and serum concentrations were <13% and <7% of corresponding concentrations in the fish from the test tanks on day 45 and 80, respectively. Similarly, PFOS was detected in the predose fish (day 0) collected from the test tanks. PFOS background concentrations were low (0.1 ng/g muscle, 0.8 ng/g liver, and 1 ng/mL serum) but above the limits of detection in fish. These PFOS levels could be attributed to the presence of PFOS in the fish from the hatchery or to ambient levels in water and feed during the experiment, although PFOS was not detected in either of the two matrices. Assuming a bioconcentration factor of 4300, PFOS concentrations in the serum of the predose and control fish (1 ng/mL) could be the result of a water concentration below the quantification limit (i.e., water concentration of 0.25 ng/L when the quantification limit in water is 4 ng/L). Other potential SAmPAP metabolites were not detected in the predose fish collected from each test tanks or in the blank control tanks during the course of the experiment. These results suggest that the trends regarding PFOS and other PFASs observed in the test tanks were the result of SAmPAP transformation in the tanks and not attributable to background concentrations.

3.3.2. Relative Importance of Uptake from Diet vs Water in the Bioaccumulation of Metabolites.
Detection frequencies and concentrations of PFASs in the dosed and control fish from the test tanks can be found in Table 1. Four potential SAmPAP metabolites, including NEtFOSAA, FOSAA, FOSA, and PFOS, were detected. Occurrence of these compounds in the dosed and control fish from the test tanks provides evidence that SAmPAP is degraded in fish. No significant differences among NEtFOSAA,

FOSA, PFOS, or ΣSAmPAP metabolite concentrations in either muscle, liver, or serum were observed between the dosed and control groups at the end of the exposure phase or at the end of the depuration phase while FOSAA was only detected in the dosed group (Table 1). Considering that SAmPAP was the only source of PFASs in the tanks, the occurrence of metabolites indicates that SAmPAP could be biotransformed in fish including fish gut. However, the similarity of concentrations between the dosed and control groups indicates that fish in the test tanks were exposed to NEtFOSAA, FOSA, and PFOS through water, which was not the expected pathway. The fact that $uptake_{water} \gg uptake_{diet}$ indicates that SAmPAP ingestion and in vivo biotransformation did not directly result in the accumulation of metabolites but resulted in the excretion of metabolites that were subsequently absorbed from water. This could be the result of (i) low uptake rate of SAmPAP from diet (i.e., $k_{D,SAmPAP}$ in eq 1) compared to uptake rates from water (i.e., $k_{R,PF}$ or $k_{R,PrePF}$) and/or (ii) minor biotransformation contributing to tissue accumulation (i.e., $F_{biotransf,SAmPAP}$ in eq 1) of SAmPAP to metabolites in fish. An illustrative calculation was made with PFOS to evaluate the plausibility of these assumptions. In the present study, $k_{D,SAmPAP} \approx \alpha F \approx 0.01 \times 0.015 \approx 1.5 \times 10^{-4}$/day, while it has been reported that $k_{R,PFOS} \approx 100$/day.[39] The ratio $k_D/k_R \approx 1.5 \times 10^{-4}/100 \approx 1.5 \times 10^{-6}$ of the rate constants for dietary uptake and respiration includes differences in chemical uptake efficiencies from the water and diet. Assuming the production of 2 mol of PFOS from 1 mol of SAmPAP and using this k_D/k_R ratio of approximately 1.5×10^{-6}, the uptake rates by the two routes become equal when $C_{D,SAmPAP}/(2 \times C_{W,PFOS})$ is approximately $1/(2 \times 1.5 \times 10^{-6}) \approx 3 \times 10^{5}$, e.g., if $C_{W,PFOS} \approx 1$ ng/L and $C_{D,SAmPAP} \approx 1500$ ng/g, which was close to the actual SAmPAP concentration in the dosed feed (i.e., 1631 ng/g). This illustrative calculation emphasizes that, in addition to the low uptake rate of SAmPAP from diet, minor biotransformation of SAmPAP to PFOS could explain that $uptake_{water} \gg uptake_{diet}$. These results are consistent with the minor biotransformation of SAmPAP to PFOS in the rat after a single oral dose and confirm the slow rate of SAmPAP metabolism is fish tissues, as previously mentioned[45]. Surprisingly, SAmPAP metabolites were not detected in the water, except for NEtFOSAA, which was quantified in a sample collected at the end of the exposure in a single test tank at a concentration of 43 ng/L. Applying bioconcentration factors from previous studies and measured tissue concentrations to estimate water concentrations in the experimental tanks, as previously undertaken with PFOS background concentrations in predose fish, it appears that water concentrations were likely below the method detection limits of PFASs in water[15.39]. It should be noted that the increase in metabolite concentrations in the water from the test tanks during the exposure phase was limited by several processes, including water renewal and irreversible sorption on the tank filtration system and walls. Further observations on the transformation pathway, bioaccumulation, and elimination of SAmPAP metabolites were carried out from data obtained from the dosed group, as fish from the control group were only sampled at the end of the exposure phase and at the end of the depuration phase (Figure 1).

3.3.3. Metabolites Uptake from Water and Transformation Pathways of SAmPAP. Significant trends of greater concentrations of all metabolites except FOSAA as a function of exposure duration were observed in the muscle, liver and serum (Figure 2). This is consistent with a gradual transformation of SAmPAP to the more stable terminal product PFOS in the test tanks. The last compound to appear was PFOS at day 28, indicating that multiple transformations steps are necessary for its production. Proposed biotransformation pathways for SAmPAP to PFOS in fish are presented in the SI (Figure S1). In the present study, NEtFOSE was not detected in the test tanks, which could be the result of the partial volatility of this compound during sampling or of a relatively high detection limit. The lack of detection of NEtFOSE in the dosed fish may also be the result of a relatively short half-life in comparison to other PFASs[18.46]. Considering the metabolites detected in the test tanks and that FOSE was not analyzed, the following transformation pathways were suggested in accordance with previous studies:
(i) SAmPAP → NEtFOSE →NEtFOSAA,
(ii) SAmPAP→NEtFOSE→FOSE→FOSAA →FOSA→PFOS, and
(iii) SAmPAP→NEtFOSE→FOSE→ FOSA → PFOS[18.47.48].
The formation of NEtFOSA from
SAmPAP was however not observed, although this metabolite
was detected in Japanese medaka[18].

3.3.4. Metabolites Depuration in Fish. During the depuration phase, there was no significant decrease in the concentration of SAmPAP metabolites, although fish were fed the control feed as previously reported[18]. The low depuration of PFOS in the present study is in contradiction with previous studies and most likely resulted from continuous biotransformation of precursors during the depuration phase rather than slow elimination of PFOS[40]. For NEtFOSAA only, slopes of ln(tissue concentrations) vs time were negative, although not statistically different from 0 (Figure 2). Differences in depuration kinetics among metabolites indicate that NEtFOSAA was generally depurated faster from fish than FOSA or PFOS. The reason for the lower elimination of FOSA and PFOS could be due to (i) continuing uptake from the water, (ii) in vivo biotransformation of precursors, and/or (iii) low elimination rates. Molar concentrations ratios of ΣPFOS precursors (i.e., sum of SAmPAP, NEtFOSAA, FOSAA, and FOSA) to PFOS were investigated as an indicator of degradation of precursors (Figure S2, SI). In the muscle and liver tissues, ΣPreFOS/PFOS were generally above 1, indicating higher concentrations of PreFOS compared to PFOS. In serum, the ratio was generally around 1, indicating similar concentrations between PFOS and precursors. For the three tissues, there was a significant increase in the ratio values during the exposure phase, while a significant decrease was observed during the depuration phase. These results reflect the shift from SAmPAP dosed feed to control feed and highlight the progressive transformation from a PreFOS-dominated bioaccumulation to a PFOS-dominated bioaccumulation.

3.4. Tissue Distribution of SAmPAP and Metabolites.
The mean concentrations of SAmPAP, NEtFOSAA, FOSAA, FOSA, and PFOS were higher in the liver and serum compared to muscle tissue (Table 3).

These results are consistent with (i) the demonstrated affinity of PFOS for fish serum albumin during extracellular transport[49] and (ii) the affinity of NEtFOSE, NEtFOSA, and PFOS for rat liver intracellular fatty acid-binding proteins during intracellular transport50. This pattern is indeed consistent with the resemblance of PFOS and PreFOS to fatty acids50-52.
Besides these general considerations, ratio values differed among PFASs, indicating that tissue distribution is also compound specific, suggesting differences in partitioning and toxicokinetics within the fish. The highest LMR and SMR were measured for SAmPAP, indicating very low muscle concentrations. SAmPAP was equally distributed to serum and liver with LSRs close to 1. NEtFOSAA was primarily distributed to serum compared to liver, which might be explained by its strong affinity to albumin. FOSA was the only PFASs primarily distributed to liver, which might be explained by its strong affinity for lipids, unlike other acidic analytes53. PFOS concentrations were higher in the liver and serum, and reported LMRs are consistent with other studies in freshwater fish[54,55]. PFOS was primarily distributed to serum compared to liver, which is consistent with previous studies36.39. When considering the weight of the tissue sampled, muscle tissue (42% of fish body weight) comprised the majority of the burden for FOSA (Table 3).

Table 3. Tissue Distribution of SAmPAP and Metabolites[a]

	distribution ratios[b]			proportional distribution in the three tissues (%)[c]		
	LMR	LSR	SMR	muscle	liver	serum
SAmPAP	35.66 ± 3.21	0.88 ± 0.09	39.37 ± 2.73	19.72 ± 2.19	40.84 ± 3.13	39.44 ± 2.75
NEtFOSAA	2.92 ± 0.16	0.27 ± 0.02	11.50 ± 0.58	55.25 ± 3.16	13.96 ± 3.27	30.79 ± 2.40
FOSA	3.25 ± 0.28	1.71 ± 0.27	2.34 ± 0.26	80.55 ± 1.05	11.68 ± 0.84	7.77 ± 0.78
PFOS	7.22 ± 0.40	0.23 ± 0.01	32.44 ± 1.27	37.47 ± 0.88	12.35 ± 0.46	50.18 ± 0.97

[a]Liver-to-muscle (LMR), liver-to-serum (LSR), and serum-to-muscle (SMR) ratios (mean ± standard error) were calculated using concentration data from the dosed group. FOSAA was only detected in the liver and ratios could therefore not be calculated. bDistribution ratios were calculated by dividing tissue concentrations. cThe burden of PFASs in the muscle, liver, and serum were calculated by multiplying tissue concentration by the mass or volume of the considered tissue. Proportional distributions in fish body were calculated as the relative importance of tissue burden relative to the sum of the three tissue burdens.

NEtFOSAA and PFOS were equally distributed between muscle and serum. SAmPAP was mainly distributed to liver and serum. Linear regression was used to test for temporal trends in the evolution of LSRs, LMRs, or SMRs during the exposure and depuration phases. When considering the evolution of distribution ratios during the experiment, SAmPAP ratios remained constant (see Figure S1 in the SI). LMRs and LSRs of NEtFOSAA and PFOS significantly decreased during the exposure phase and increased during the elimination phase. FOSA LSRs significantly decreased during the exposure phase

as well. An increase of LMRs and LSRs of PFOS during the depuration phase has already been reported in rainbow trout and could be attributable to longer elimination half-lives in the liver[56]. There was no significant difference of distribution ratios of NEtFOSAA, FOSA, and PFOS between the dosed and control groups from the test tanks at the end of the exposure and depuration periods.

3.5. Environmental Implications.

Examination of wildlife monitoring data demonstrates the signification of bioaccumulation potential for PFOS, with high trophic level predators often reported with higher PFOS concentrations[57,58]. However, laboratory-derived BMF values obtained by exposing juvenile rainbow trout to dietary PFOS were less than 1. Similarly, there appear to be some foodweb-specific differences in the biomagnification of PFOS in the environment. Martin et al.[59] and Asher et al.[60] measured the highest mean concentrations of PFOSA and PFOS in a benthic macroinvertebrate and slimy sculpin, occupying low trophic levels of Lake Ontario. Similarly, Gebbink et al.[24] found the highest concentrations of precursor compounds, mainly NEtFOSAA and FOSA, in zooplankton from the Baltic Sea. The occurrence of PreFOS in the environment (water or sediment) may indeed lead to the contamination of low trophic levels, while it appears higher trophic levels are mostly contaminated by PFOS. In the present study, perch were dietarily exposed to SAmPAP in a recirculated system. SAmPAP ingestion and in vivo biotransformation did not directly contribute to the body burden of PFOS in Eurasian perch. In vivo biotransformation of SAmPAP rather contributed to the metabolite (including PFOS) burden measured in water, which could be subsequently bioaccumulated in fish through reexposure to the dissolved metabolites.

Supporting Information

The Supporting Information is available free of charge at
https://pubs.acs.org/doi/abs/10.1021/acs.est.6b05598

REFERENCES

(1) Calafat, A. M.; Wong, L.-Y.; Kuklenyik, Z.; Reidy, J. A.; Needham, L. L. Polyfluoroalkyl Chemicals in the U.S. Population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003− 2004 and Comparisons with NHANES 1999−2000. Environ. Health Perspect. 2007, 115 (11), 1596−1602.

(2) Giesy, J. P.; Kannan, K. Global Distribution of Perfluorooctane Sulfonate in Wildlife. Environ. Sci. Technol. 2001, 35 (7), 1339−1342.

(3) Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Wouwe, N. V.; Yang, J. H.; et al. Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. Environ. Sci. Technol. 2004, 38 (17), 4489−4495.

(4) Du, Y.; Shi, X.; Liu, C.; Yu, K.; Zhou, B. Chronic effects of waterborne PFOS exposure on growth, survival and hepatotoxicity in zebrafish: A partial life-cycle test. Chemosphere 2009, 74 (5), 723−729.

(5) Lopez-Espinosa, M.-J.; Mondal, D.; Armstrong, B.; Bloom, M. S.; Fletcher, T. Thyroid Function and Perfluoroalkyl Acids in Children Living Near a Chemical Plant. Environ. Health Perspect. 2012, 120 (7), 1036−1041.

(6) Washino, N.; Saijo, Y.; Sasaki, S.; Kato, S.; Ban, S.; Konishi, K.; Ito, R.; Nakata, A.; Iwasaki, Y.; Saito, K.; et al. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ. Health Perspect. 2009, 117 (4), 660.

(7) Tittlemier, S. A.; Pepper, K.; Seymour, C.; Moisey, J.; Bronson, R.; Cao, X.-L.; Dabeka, R. W. Dietary Exposure of Canadians to Perfluorinated Carboxylates and Perfluorooctane Sulfonate via Consumption of Meat, Fish, Fast Foods, and Food Items Prepared in Their Packaging. J. Agric. Food Chem. 2007, 55 (8), 3203−3210.

(8) Vestergren, R.; Cousins, I. T. Tracking the Pathways of Human Exposure to Perfluorocarboxylates. Environ. Sci. Technol. 2009, 43 (15), 5565−5575.

(9) Denys, S.; Fraize-Frontier, S.; Moussa, O.; Bizec, B. L.; Veyrand, B.; Volatier, J.-L. Is the fresh water fish consumption a significant determinant of the internal exposure to perfluoroalkylated substances (PFAS)? Toxicol. Lett. 2014, 231 (2), 233−238.

(10) Yamada, A.; Bemrah, N.; Veyrand, B.; Pollono, C.; Merlo, M.; Desvignes, V.; Sirot, V.; Marchand, P.; Berrebi, A.; Cariou, R.; et al. Dietary exposure to perfluoroalkyl acids of specific French adult subpopulations: High seafood consumers, high freshwater fish consumers and pregnant women. Sci. Total Environ. 2014, 491−492, 170−175.

(11) Zhou, Z.; Shi, Y.; Vestergren, R.; Wang, T.; Liang, Y.; Cai, Y. Highly Elevated Serum Concentrations of Perfluoroalkyl Substances in Fishery Employees from Tangxun Lake, China. Environ. Sci. Technol. 2014, 48 (7), 3864−3874.

(12) Berger, U.; Glynn, A.; Holmström, K. E.; Berglund, M.; Ankarberg, E. H.; Törnkvist, A. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden − Analysis of edible fish from Lake Vättern and the Baltic Sea. Chemosphere 2009, 76 (6), 799−804.

(13) Yamada, A.; Bemrah, N.; Veyrand, B.; Pollono, C.; Merlo, M.; Desvignes, V.; Sirot, V.; Oseredczuk, M.; Marchand, P.; Cariou, R.; et al. Perfluoroalkyl Acid Contamination and Polyunsaturated Fatty Acid Composition of French Freshwater and Marine Fishes. J. Agric. Food Chem. 2014, 62 (30), 7593−7603.

(14) Brandsma, S. H.; Smithwick, M.; Solomon, K.; Small, J.; de Boer, J.; Muir, D. C. G. Dietary exposure of rainbow trout to 8:2 and 10:2 fluorotelomer alcohols and perfluorooctanesulfonamide: Uptake, transformation and elimination. Chemosphere 2011, 82 (2), 253−258.

(15) Chen, M.; Qiang, L.; Pan, X.; Fang, S.; Han, Y.; Zhu, L. In Vivo and in Vitro Isomer-Specific Biotransformation of Perfluorooctane Sulfonamide in Common Carp (Cyprinus carpio). Environ. Sci. Technol. 2015, 49 (23), 13817−13824.

(16) Lee, H.; De Silva, A. O.; Mabury, S. A. Dietary Bioaccumulation of Perfluorophosphonates and Perfluorophosphinates in Juvenile Rainbow Trout: Evidence of Metabolism of Perfluorophosphinates. Environ. Sci. Technol. 2012, 46 (6), 3489−3497.

(17) Martin, J. W.; Asher, B. J.; Beesoon, S.; Benskin, J. P.; Ross, M. S. PFOS or PreFOS? Are perfluorooctane sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane sulfonate (PFOS) exposure? J. Environ. Monit. 2010, 12 (11), 1979− 2004.

(18) Peng, H.; Zhang, S.; Sun, J.; Zhang, Z.; Giesy, J. P.; Hu, J. Isomer- Specific Accumulation of Perfluorooctanesulfonate from (N-Ethyl perfluorooctanesulfonamido)ethanol-based Phosphate Diester in Japanese Medaka (Oryzias latipes). Environ. Sci. Technol. 2014, 48 (2), 1058−1066.

(19) Paul, A. G.; Jones, K. C.; Sweetman, A. J. A First Global Production, Emission, And Environmental Inventory For Perfluorooctane Sulfonate. Environ. Sci. Technol. 2009, 43 (2), 386−392.

(20) Begley, T. H.; Hsu, W.; Noonan, G.; Diachenko, G. Migration of fluorochemical paper additives from food-contact paper into foods and food simulants. Food Addit. Contam., Part A 2008, 25 (3), 384−390.

(21) US Environmental Protection Agency. Fluorochemical Use, Ditribution and Release Overview; Report AR226-0550; USEPA, 1999. (22) Ahrens, L.; Yamashita, N.; Yeung, L. W. Y.; Taniyasu, S.; Horii, Y.; Lam, P. K. S.; Ebinghaus, R. Partitioning Behavior of Per- and Polyfluoroalkyl Compounds between Pore Water and Sediment in Two Sediment Cores from Tokyo Bay, Japan. Environ. Sci. Technol. 2009, 43 (18), 6969−6975.

(23) Benskin, J. P.; Ikonomou, M. G.; Gobas, F. A. P. C.; Woudneh, M. B.; Cosgrove, J. R. Observation of a Novel PFOS-Precursor, the Perfluorooctane Sulfonamido Ethanol-Based Phosphate (SAmPAP) Diester, in Marine Sediments. Environ. Sci. Technol. 2012, 46 (12), 6505−6514.

(24) Gebbink, W. A.; Bignert, A.; Berger, U. Perfluoroalkyl Acids (PFAAs) and Selected Precursors in the Baltic Sea Environment: Do Precursors Play a Role in Food Web Accumulation of PFAAs? Environ. Sci. Technol. 2016, 50 (12), 6354−6362.

(25) Higgins, C. P.; Field, J. A.; Criddle, C. S.; Luthy, R. G. Quantitative Determination of Perfluorochemicals in Sediments and Domestic Sludge. Environ. Sci. Technol. 2005, 39 (11), 3946−3956.

(26) Benskin, J. P.; Ikonomou, M. G.; Gobas, F. A. P. C.; Begley, T. H.; Woudneh, M. B.; Cosgrove, J. R. Biodegradation of N-Ethyl Perfluorooctane Sulfonamido Ethanol (EtFOSE) and EtFOSE-Based Phosphate Diester (SAmPAP Diester) in Marine Sediments. Environ. Sci. Technol. 2013, 47 (3), 1381−1389.

(27) Rayne, S.; Forest, K. Modeling the hydrolysis of perfluorinatedcompounds containing carboxylic and phosphoric acid ester functions and sulfonamide groups. J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng. 2010, 45 (4), 432−446.

(28) Lazartigues, A.; Thomas, M.; Banas, D.; Brun-Bellut, J.; Cren- Olivé, C.; Feidt, C. Accumulation and half-lives of 13 pesticides in muscle tissue of freshwater fishes through food exposure. Chemosphere 2013, 91 (4), 530−535.

(29) Bach, C.; Boiteux, V.; Hemard, J.; Colin, A.; Rosin, C.; Munoz, J.- F.; Dauchy, X. Simultaneous determination of perfluoroalkyl iodides, perfluoroalkane sulfonamides, fluorotelomer alcohols, fluorotelomer iodides and fluorotelomer acrylates and methacrylates in water and sediments using solid-phase microextraction-gas chromatography/mass spectrometry. J. Chromatogr. A 2016, 1448, 98−106.

(30) Boiteux, V.; Bach, C.; Sagres, V.; Hemard, J.; Colin, A.; Rosin, C.; Munoz, J.-F.; Dauchy, X. Analysis of 29 per- and polyfluorinated compounds in water, sediment, soil and sludge by liquid chromatography− tandem mass spectrometry. Int. J. Environ. Anal. Chem. 2016, 96 (8), 705−728.

(31) R Development Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing, 2013.

(32) Arnot, J. A.; Gobas, F. A. P. C. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environ. Toxicol. Chem. 2004, 23 (10), 2343−2355.

(33) Arnot, J. A.; Quinn, C. L. Development and Evaluation of a Database of Dietary Bioaccumulation Test Data for Organic Chemicals in Fish. Environ. Sci. Technol. 2015, 49 (8), 4783−4796.

(34) Nania, V.; Pellegrini, G. E.; Fabrizi, L.; Sesta, G.; Sanctis, P. D.; Lucchetti, D.; Pasquale, M. D.; Coni, E. Monitoring of perfluorinated compounds in edible fish from the Mediterranean Sea. Food Chem. 2009, 115 (3), 951−957.

(35) Peng, H.; Wei, Q.; Wan, Y.; Giesy, J. P.; Li, L.; Hu, J. Tissue Distribution and Maternal Transfer of Poly- and Perfluorinated Compounds in Chinese Sturgeon (Acipenser sinensis): Implications for Reproductive Risk. Environ. Sci. Technol. 2010, 44 (5), 1868−1874.

(36) Shi, Y.; Wang, J.; Pan, Y.; Cai, Y. Tissue distribution of perfluorinated compounds in farmed freshwater fish and human exposure by consumption. Environ. Toxicol. Chem. 2012, 31 (4), 717− 723.

(37) Holmes, W. N.; Donaldson, E. M. The Body Compartments and the Distribution of Electrolytes. In Fish Physiology. Excretion, Ionic Regulation, and Metabolism; Hoar, W. S., Randall, D. J., Eds.; Academic Press, 1969; Vol. 1, pp 1−89.

(38) Mathis, N.; Feidt, C.; Brun-Bellut, J. Influence of protein/energy ratio on carcass quality during the growing period of Eurasian perch (Perca fluviatilis). Aquaculture 2003, 217 (1−4), 453−464.

(39) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 2003, 22 (1), 196−204.

(40) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 2003, 22 (1), 189− 195.

(41) Mairesse, G.; Thomas, M.; Gardeur, J.-N.; Brun-Bellut, J. Appearance and technological characteristics in wild and reared Eurasian perch, Perca fluviatilis (L.). Aquaculture 2005, 246 (1−4), 295−311.

(42) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings1. Adv. Drug Delivery Rev. 2001, 46 (1−3), 3−26.

(43) Goeritz, I.; Falk, S.; Stahl, T.; Schäfers, C.; Schlechtriem, C. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 2013, 32 (9), 2078−2088.

(44) Gobas, F. A. P. C.; Muir, D. C. G.; Mackay, D. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. Chemosphere 1988, 17 (5), 943−962.

(45) Seacat, A. M.; Luebker, D. J. Diester- Pharmacokinetic Study in Rats; Study T-7043.1, DT-26; U.S. Environmental Protection Agency, 2000.

(46) Rhoads, K. R.; Janssen, E. M.-L.; Luthy, R. G.; Criddle, C. S. Aerobic Biotransformation and Fate of N -Ethyl Perfluorooctane Sulfonamidoethanol (N -EtFOSE) in Activated Sludge. Environ. Sci. Technol. 2008, 42 (8), 2873−2878.

(47) Xie, W.; Wu, Q.; Kania-Korwel, I.; Tharappel, J. C.; Telu, S.; Coleman, M. C.; Glauert, H. P.; Kannan, K.; Mariappan, S. V. S.; Spitz, D. R.; et al. Subacute exposure to N-ethyl perfluorooctanesulfonamidoethanol results in the formation of perfluorooctanesulfonate and alters superoxide dismutase activity in female rats. Arch. Toxicol. 2009, 83 (10), 909−924.

(48) Xu, L.; Krenitsky, D. M.; Seacat, A. M.; Butenhoff, J. L.; Anders, M. W. Biotransformation of N-Ethyl-N-(2-hydroxyethyl)-perfluorooctanesulfonamide by Rat Liver Microsomes, Cytosol, and Slices and by Expressed Rat and Human Cytochromes P450. Chem. Res. Toxicol. 2004, 17 (6), 767−775.

(49) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of perfluorinated fatty acids to serum proteins. Environ. Toxicol. Chem. 2003, 22 (11), 2639−2649.

(50) Luebker, D. J.; Hansen, K. J.; Bass, N. M.; Butenhoff, J. L.; Seacat, A. M. Interactions of flurochemicals with rat liver fatty acid-binding protein. Toxicology 2002, 176 (3), 175−185.

(51) Bischel, H. N.; MacManus-Spencer, L. A.; Luthy, R. G. Noncovalent Interactions of Long-Chain Perfluoroalkyl Acids with Serum Albumin. Environ. Sci. Technol. 2010, 44 (13), 5263−5269.

(52) Luo, Z.; Shi, X.; Hu, Q.; Zhao, B.; Huang, M. Structural Evidence of Perfluorooctane Sulfonate Transport by Human Serum Albumin. Chem. Res. Toxicol. 2012, 25 (5), 990−992.

(53) Ullah, S.; Huber, S.; Bignert, A.; Berger, U. Temporal trends of perfluoroalkane sulfonic acids and their sulfonamide-based precursors in herring from the Swedish west coast 1991−2011 including isomerspecific considerations. Environ. Int. 2014, 65, 63−72.

(54) Becker, A. M.; Gerstmann, S.; Frank, H. Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Two Fish Species Collected from the Roter Main River, Bayreuth, Germany. Bull. Environ. Contam. Toxicol. 2010, 84 (1), 132−135.

(55) Labadie, P.; Chevreuil, M. Partitioning behaviour of perfluorinated alkyl contaminants between water, sediment and fish in the Orge River (nearby Paris, France). Environ. Pollut. 2011, 159 (2), 391−397.

(56) Falk, S.; Failing, K.; Georgii, S.; Brunn, H.; Stahl, T. Tissue specific uptake and elimination of perfluoroalkyl acids (PFAAs) in adult rainbow trout (Oncorhynchus mykiss) after dietary exposure. Chemosphere 2015, 129, 150−156.

(57) Kannan, K.; Tao, L.; Sinclair, E.; Pastva, S. D.; Jude, D. J.; Giesy, J. P. Perfluorinated Compounds in Aquatic Organisms at Various Trophic Levels in a Great Lakes Food Chain. Arch. Environ. Contam. Toxicol. 2005, 48 (4), 559−566.

(58) Tomy, G. T.; Budakowski, W.; Halldorson, T.; Helm, P. A.; Stern, G. A.; Friesen, K.; Pepper, K.; Tittlemier, S. A.; Fisk, A. T. Fluorinated Organic Compounds in an Eastern Arctic Marine Food Web. Environ. Sci. Technol. 2004, 38 (24), 6475−6481.

(59) Martin, J. W.; Whittle, D. M.; Muir, D. C. G.; Mabury, S. A. Perfluoroalkyl Contaminants in a Food Web from Lake Ontario. Environ. Sci. Technol. 2004, 38 (20), 5379−5385.

(60) Asher, B. J.; Wang, Y.; De Silva, A. O.; Backus, S.; Muir, D. C. G.; Wong, C. S.; Martin, J. W. Enantiospecific Perfluorooctane Sulfonate (PFOS) Analysis Reveals Evidence for the Source Contribution of PFOS-Precursors to the Lake Ontario Foodweb. Environ. Sci. Technol. 2012, 46 (14), 7653−7660.