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BIOCONCENTRATION AND TISSUE DISTRIBUTION OF PERFLUORINATED ACIDS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract—Rainbow trout (*Oncorhynchus mykiss*) were exposed simultaneously to a homologous series of perfluoroalkyl carboxylates and sulfonates in a flow-through system to determine compound-specific tissue distribution and bioconcentration parameters for perfluorinated acids (PFAs). In general, PFAs accumulated to the greatest extent in blood > kidney > liver > gall bladder. Carboxylates and sulfonates with perfluoroalkyl chain lengths shorter than seven and six carbons, respectively, could not be detected in most tissues and were considered to have insignificant bioconcentration factors (BCFs). For detectable PFAs, carcass BCFs increased with increasing length of the perfluoroalkyl chain, ranging from 4.0 to 23,000, based on wet weight concentrations. Carboxylate carcass BCFs increased by a factor of eight for each additional carbon in the perfluoroalkyl chain between 8 and 12 carbons, but this relationship deviated from linearity for the longest PFA tested, possibly because of decreased gill permeability. In general, half-lives (3.9–28 d) and uptake rates (0.053–1,700 L/kg/d) also increased with increasing length of the perfluoroalkyl chain in all tissues. Sulfonates had greater BCFs, half-lives, and rates of uptake than the corresponding carboxylate of equal perfluoroalkyl chain length, indicating that hydrophobicity, as predicted by the critical micelle concentration, is not the only determinant of PFA bioaccumulation potential and that the acid function must be considered.

Keywords—Bioaccumulation Fish Perfluorinated acid Perfluorooctane sulfonate Surfactants

INTRODUCTION

Perfluorinated acids (PFAs) are a class of anionic fluorinated surfactants characterized by a perfluoroalkyl chain and a sulfonate or carboxylate solubilizing group. Perfluorinated acids have been used increasingly over the past 20 years because of their temperature and chemical stability, lipophobicity, and effectiveness as surfactants at low concentrations [1]. The total production of fluorinated surfactants (anionics, cationics, and neutrals) was 200 t in 1979 [1], whereas in 2000, the total production of one PFA, perfluorooctane sulfonate (PFOS), was nearly 3,000 t [2]. Although this production volume represents only a small fraction of total surfactant manufacturing (i.e., $\ll$0.01%) [2,3], PFOS recently emerged as a global contaminant after its detection in humans and wildlife from various geographic locations.

By using liquid chromatography–tandem mass spectrometry (LC-MS-MS) [4,5], human serum was found to contain ng/ml concentrations of PFOS, perfluorohexane sulfonate (PFHxS), perfluorooctanoate (PFOA), and perfluorooctane sulfonylamide [5]. Soon thereafter, wildlife samples collected in various global locations also were observed to contain PFOS [6,7], and organisms consuming fish, such as predatory birds and mink, contained greater concentrations of PFOS than their food sources [6,8]. The wide distribution of PFAs in the environment, despite low production volumes, is largely a result of recalcitrance to biotic and abiotic degradation mechanisms [9]. Furthermore, atmospheric transport of PFAs may be accommodated through recently detected transient volatile derivatives [10], and direct atmospheric emission of perfluoroalkyl carboxylates (C_3–C_{14}) occurs through thermolysis of polytetrafluoroethylene [11]. Because of concerns regarding their ubiquitous distribution in humans and wildlife, as well as their environmental persistence, the main manufacturer of PFAs currently is phasing out the production of long-chain perfluorinated acids [2].

Dietary exposure of fish to PFAs does not result in biomagnification [12]; however, the major uptake route for waterborne xenobiotics in fish is directly across the gills [13], and direct uptake of chemicals from water (i.e., bioconcentration) is probably much more important than accumulation from food (i.e., biomagnification) [14]. Many hydrocarbon surfactants are known to bioconcentrate in fish [3,15–19], and the European Economic Community has even adopted surface activity as an indicator of a compound's bioconcentration potential [20]. The predictably low Henry's law constant for PFAs indicates that they will accumulate in the aquatic environment [21,22], making fish a relevant test organism for PFA bioaccumulation testing. Under field conditions, fish collected downstream of a fire-fighting foam spill contained higher concentrations of several PFAs than fish collected upstream [23].

We describe here the results of a tissue distribution and bioconcentration study with rainbow trout (*Oncorhynchus mykiss*) exposed simultaneously to a suite of perfluoroalkyl carboxylates and sulfonates of varying fluorinated chain lengths in a flow-through aqueous exposure. We report compound-specific bioaccumulation parameters determined by liquid chromatography–tandem mass spectrometry (LC-MS-MS), and discuss their relation to physicochemical properties and structure.

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MATERIALS AND METHODS

Standards and reagents

Standards of potassium perfluorobutane sulfonate (PFBS), potassium PFHxS (99.9%), and potassium PFOS (86.4%) were provided by the 3M Company (St. Paul, MN, USA). Standards of perfluoropentanoic acid (97%), perfluoroheptanoic acid (PFHpA, 99%), PFOA (98%), perfluorononanoic acid (PFNA, 97%), perfluorodecanoic acid (PFDA, 98%), perfluoroundecanoic acid (95%), perfluorododecanoic acid (PFDoA, 95%), and perfluorotetradecanoic acid (PFTA, 97%) were purchased from Sigma-Aldrich (Oakville, ON, Canada), and perfluorohexanoic acid (95%) was obtained from Oakwood Research Chemicals (West Columbia, SC, USA). Ammonium acetate (98%) and tetrabutylammonium hydrogensulfate were purchased from Sigma-Aldrich, anhydrous sodium carbonate (99.8%) was purchased from J.T. Baker (Phillipsburg, NJ, USA), and methyl-*tert*-butyl ether (99.5%) was purchased from EM Science (Gibbsburg, NJ, USA).

Fish rearing

Rainbow trout were purchased from Rainbow Springs (Thamesford, ON, Canada) and were allowed to acclimate to laboratory conditions for two weeks before exposure. Carbon-filtered and dechlorinated water (with Na_2SO_3) was maintained at 12°C, and a 12-h photoperiod was used. Fish were fed daily at a rate of 1.5% body weight per day, corrected for growth throughout the experiment. Trout feed was purchased from Martin Mills (Tavistock, ON, Canada).

Stock perfluorinated acid solution preparation

A stock solution containing all the test compounds was produced by first dissolving PFAs in a small amount of methanol, which subsequently was dissolved in 30 L of reverse-osmosis laboratory water. The resulting solution was contained in a polypropylene container and was stirred for 3 d before the beginning of the experiment. After allowing the solution to settle for several hours, some of the test compounds had not completely dissolved, based on the appearance of a white solid at the surface of the solution. This material was removed by filtering the entire solution through glass (GF/C) microfiber filters (Whatman, Kent, UK). The resulting solution was used for exposure and was constantly stirred while being delivered to exposure tanks.

Bioconcentration exposure

Juvenile rainbow trout (5–10 g) were exposed to a 1,000-fold dilution of the stock PFA solution in a flow-through exposure design for 12 d, followed by 33 d of depuration in clean water. Two days before the initial exposure, fish were transferred to glass aquaria lined with polypropylene bags. Perfluorinated acids have been reported to bind to glass surfaces [5], and the plastic bags were used as a precaution to minimize equilibration time after introduction of the test compounds. Initial biomass loading was 8 g/L in the exposure tank, and 2.5 g/L in the control tank. Fish growth was monitored by weighing the total biomass every 2 to 3 d throughout the course of the experiment.

Dilution water was gravity fed to each aquarium at 500 ml/min, and a peristaltic pump delivered the stirred stock solution into the dilution stream of the treatment tank at 0.5 ml/min. At time 0 of the exposure, an initial volume of the stock solution (45 ml) was added to the exposure aquarium (45 L) to immediately achieve the desired exposure concentration.

Three fish from the exposure tank and one from the control tank were sampled at each predetermined interval during the uptake phase of the experiment (4.5, 9, 18, 36, 72, 144, and 288 h). At 288 h, the remaining fish were transferred to new aquaria receiving clean water at 3 L/min. Extra care was taken to reduce contamination of the depuration phase tanks by allowing the fish to depurate for 5 min in a pail containing clean dilution water, and then transferring the fish by net to the depuration tanks. During the depuration phase, three fish from the treatment tank and one from the control tank were sampled at each time interval (4.5, 9, 18, 36, 72, 144, 288, 456, and 792 h). During the uptake phase, water samples (1 L) were collected below the surface in polypropylene bottles at 0.25, 4.5, 12, 18, 36, 72, 144, 197, 244, and 288 h. Water samples were also collected at 48 and 96 h of the depuration phase from both tanks to check for contamination.

Sampled fish were anesthetized with MS-222, a blood sample (50–200 μl) was drawn, and fish were subsequently killed by a blow to the head and cervical dislocation. An incision was made along the ventral surface from the anus to the gills, and the entire liver was removed for analysis. The gut, consisting of esophagus, stomach, pyloric ceca, spleen, and intestines, was removed but not analyzed. The blood, liver, and carcass samples were analyzed separately for PFAs at each sampling time to determine the kinetics of uptake and depuration.

Tissue distribution exposure

Four immature rainbow trout (30–48 g) were exposed in a separate tank under the same uptake conditions as bioconcentration fish. On day 12, three fish were randomly sampled and anesthetized with MS-222. Blood samples were immediately drawn and the fish were euthanized by a blow to the head and cervical dislocation. Fish were subsequently dissected to separate and remove the spleen, heart, liver, gall bladder, gonads, gills (bones removed), adipose tissue (separated from pyloric ceca), gut (including esophagus, stomach, intestine, and pyloric ceca), and kidney. A small sample of white muscle was also removed adjacent to the dorsal fin of each fish and separated from the skin.

Analysis of perfluorinated acids by liquid chromatography–tandem mass spectrometry

Liver and blood samples were homogenized in 15-ml plastic (polypropylene copolymer) centrifuge tubes containing 3 ml of Na_2CO_3 (0.25 M), 1 ml of water, 1 ml of the ion-pairing agent tetrabutylammonium hydrogensulfate (0.5 M adjusted to pH 10) [5], and 100 μl (25 ng) of the internal standard, PFNA. Carcass samples were first reduced to a fine powder by using a mortar and pestle with liquid nitrogen, and subsequently were homogenized in 50-ml plastic centrifuge tubes containing 10 to 20 ml of Na_2CO_3. An exact quantity (2–4 g) of the homogenate was then transferred to a separate centrifuge tube containing 1 ml of water, 1 ml of tetrabutylammonium hydrogensulfate, and 100 μl of PFNA.

The resulting homogenates were extracted with 5 ml of methyl-*tert*-butyl ether by shaking vigorously for 10 min, followed by centrifugation to isolate the organic phase. The methyl-*tert*-butyl ether supernatant was collected in a separate plastic tube, and this extraction process was repeated once more, combining the supernatants. The methyl-*tert*-butyl ether was blown to dryness under high-purity nitrogen gas, and the analytes were taken up in 1 to 2 ml of 50:50 (v/v) water:methanol

Table 1. Test compound acronym, structure, ion transition monitored, and mean aqueous exposure concentration ± 1 relative standard deviation (RSD)

Test compound	Acronym	Structure	Ion transition monitored by LC-MS-MS[a]	Waterborne concentration (μg/L) ± RSD (%)
Perfluorocarboxylates				
Perfluoropentanoic acid	PFPA	$CF_3(CF_2)_3CO_2H$	263 → 219	1.7 ± 11
Perfluorohexanoic acid	PFHxA	$CF_3(CF_2)_4CO_2H$	313 → 269	1.7 ± 10
Perfluoroheptanoic acid	PFHpA	$CF_3(CF_2)_5CO_2H$	363 → 319	1.6 ± 12
Perfluorooctanoic acid	PFOA	$CF_3(CF_2)_6CO_2H$	413 → 369	1.5 ± 13
Perfluorodecanoic acid	PFDA	$CF_3(CF_2)_8CO_2H$	513 → 469	0.71 ± 24
Perfluoroundecanoic acid	PFUnA	$CF_3(CF_2)_9CO_2H$	563 → 519	0.48 ± 26
Perfluorododecanoic acid	PFDoA	$CF_3(CF_2)_{10}CO_2H$	613 → 569	0.20 ± 30
Perfluorotetradecanoic acid	PFTA	$CF_3(CF_2)_{12}CO_2H$	713 → 669	0.014 ± 30
Perfluorosulfonates				
Perfluorobutane sulfonic acid	PFBS	$CF_3(CF_2)_3SO_3H$	299 → 99	1.4 ± 10
Perfluorohexane sulfonic acid	PFHxS	$CF_3(CF_2)_5SO_3H$	399 → 99	1.4 ± 11
Perfluorooctane sulfonic acid	PFOS	$CF_3(CF_2)_7SO_3H$	499 → 99	0.35 ± 26

[a] LC-MS-MS = liquid chromatography–tandem mass spectrometry.

by vortexing for 30 s. The solution was then filtered through 0.2-μm nylon filters into polypropylene vials for analysis.

Direct analysis of uptake phase water samples was possible for all PFAs except for PFDoA and PFTA, which were below the limit of detection (~0.4 μg/L). For direct analysis, 1-L samples were shaken vigorously and 1 ml was withdrawn from below the surface of each with a polypropylene syringe. These subsamples were then filtered through 0.2-μm nylon filters into polypropylene shell vials for analysis. These samples were analyzed without PFNA, and instead used external standards and quantitation by standard curve analysis. To determine PFDoA and PFTA uptake concentrations (36, 72, and 197 h), and to check for PFA contamination of depuration water, 25 ml of each water sample was removed and filtered (0.2-μm nylon) into a 50-ml plastic centrifuge tube containing 0.6 g of Na_2CO_3, 1 g of tetrabutylammonium hydrogensulfate, and 100 μl of PFNA. Perfluorinated acids were extracted two times by using 20-ml aliquots of methyl-*tert*-butyl ether, centrifuging, combining the supernatants, and analyzing further in the same manner as tissue samples.

Instrumental analysis was performed by LC-MS-MS by using previously described conditions [4,23]. Water and methanol solvents (0.01 M ammonium acetate) were delivered at a total flow rate of 300 μl/min by a Waters 600S controller (Milford, MA, USA), and samples (25 μl) were injected with a Waters 717 plus autosampler. Chromatography was performed on a Genesis C8 column (2.1 × 50 mm, Jones Chromatography, Lakewood, CO, USA). Initial mobile phase conditions were 90:10 (v/v) water:methanol for 30 s, followed by a 10-min ramp to 0:100, a 3.5-min hold, and reverting to initial conditions at 14 min. The detector was a Quattro LC (Micromass, Manchester, UK) equipped with an electrospray interface operating in negative ion mode. Data were acquired by MS-MS by using a multiple reaction monitoring method that monitored a single transition (parent → daughter ion) for each compound (Table 1). Desolvation temperature was 350°C, and the source block was maintained at 150°C. Desolvation gas flow was between 600 and 700 L/h, and the capillary voltage always was 2.75 kV.

Quantitation was performed relative to PFNA by using a standard curve constructed from known quantities of standards extracted from water in the same manner as tissue samples. All samples were blank subtracted before quantitation, and standard injections were made every six to nine samples to monitor sensitivity drift. Blank concentrations were absent for most PFAs; however, PFOA and PFOS were detected on occasion below 10% of sample concentrations.

Data analysis

Fish weight (FW) was best predicted by the exponential growth model, $FW = a \cdot \exp(g \cdot t)$, where a is a constant, g is the growth rate, and t is the time. All tissue concentrations were corrected for growth dilution by determining the percent increase in FW at each sampling interval, relative to $t = 0$, by using the predicted exponential growth rate equation. The depuration rate constants (k_d) were determined by linear regression after fitting the growth corrected depuration concentrations ($C_{fish(t)}$) to the first-order decay model $C_{fish(t)} = a \cdot \exp(-k_d \cdot t)$, where a is a constant. Depuration half-life was calculated by the formula $\ln(2)/k_d$.

Uptake rate constants (k_u) were determined by using iterative nonlinear regression (Systat®, Ver 9.0, Systat Software, Richmond, CA, USA), by fitting the growth-corrected tissue concentrations to the integrated form of the kinetic rate equation for constant aqueous exposure [14]

$$C_{fish(t)} = [(k_u)(C_w)/(k_d)] \cdot [1 - \exp(-k_d \cdot t)] \quad (1)$$

where C_w is the average exposure water concentration and k_d is a fixed parameter. Bioconcentration factors (BCFs) were calculated by the quotient (k_u/k_d).

RESULTS AND DISCUSSION

Fish mortality, growth, and liver somatic index

Only one fish (2%) died during the uptake phase, and no statistically significant difference was found in the rate of growth for exposed fish relative to controls ($p = 0.41$; Table 2). The mean initial fish mass (Table 2) was greater in this study relative to the dietary accumulation study [12], resulting in a lower rate of growth. No statistically significant difference was found between the liver somatic index of exposed and control fish ($p = 0.854$; Table 2). Perfluorinated acids cause hepatomegaly in rodents upon exposure via peroxisome proliferation [24], but this effect was not apparent in these fish under this exposure scenario based on liver mass.

Table 2 Uptake and depuration phase duration, growth rate constant, and the associated coefficient of determination, mortality, and liver somatic index (LSI) for exposed and control juvenile rainbow trout used in bioconcentration testing

	Uptake period (d)	Depuration period (d)	Mean initial fish mass (g)	Growth rate (10^{-3} g/d) (r^2)	Mortality (%)	LSI[a] (%)
Exposure tank	12	33	7.3	4.9 (0.50)	2	1.1 ± 0.03
Control	12	33	7.9	5.1 (0.43)	0	1.1 ± 0.07

[a] Average of all fish sampled throughout the uptake and depuration period in each tank, for each experiment. Linear regression revealed no increase or decrease in LSI throughout.

Water concentrations

During the uptake period the average exposure water concentrations were between 0.014 and 1.7 μg/L for individual PFAs (Table 2 and Fig. 1). The low exposure concentrations for longer PFAs were a result of their low water solubility, whereas more soluble PFAs were close to the nominal concentration (~2 μg/L). Perfluorinated acid concentrations were relatively stable throughout the uptake phase after an initial decrease between 0.25 and 24 h (Fig. 1). This decrease was more pronounced for more hydrophobic compounds (i.e., PFOS > PFHxS; Fig. 1B), suggesting that rapid uptake by fish was influencing water concentrations to some extent despite flow-through conditions. As biomass was removed through sampling, and as uptake rates decreased, the water concentrations stabilized or increased slightly at later sampling intervals, and approached the initial concentrations (i.e., 0.25 h). The mean water concentration was used as a constant estimate of C_w in Equation 1 for determination of k_u. Perfluorinated acids could not be detected in depuration water above the limit of detection (~5 ng/L) in control or treated fish tanks.

Tissue distribution

In general, PFA concentrations were greatest in the blood > kidney > liver > gall bladder, and lowest in the gonads > adipose > muscle tissue (Fig. 2). Within the blood, the plasma contained between 94 and 99% of total PFAs, with only a minor fraction detectable in the cellular fraction. Perfluorinated acids also were detectable in the gills, suggesting that this was

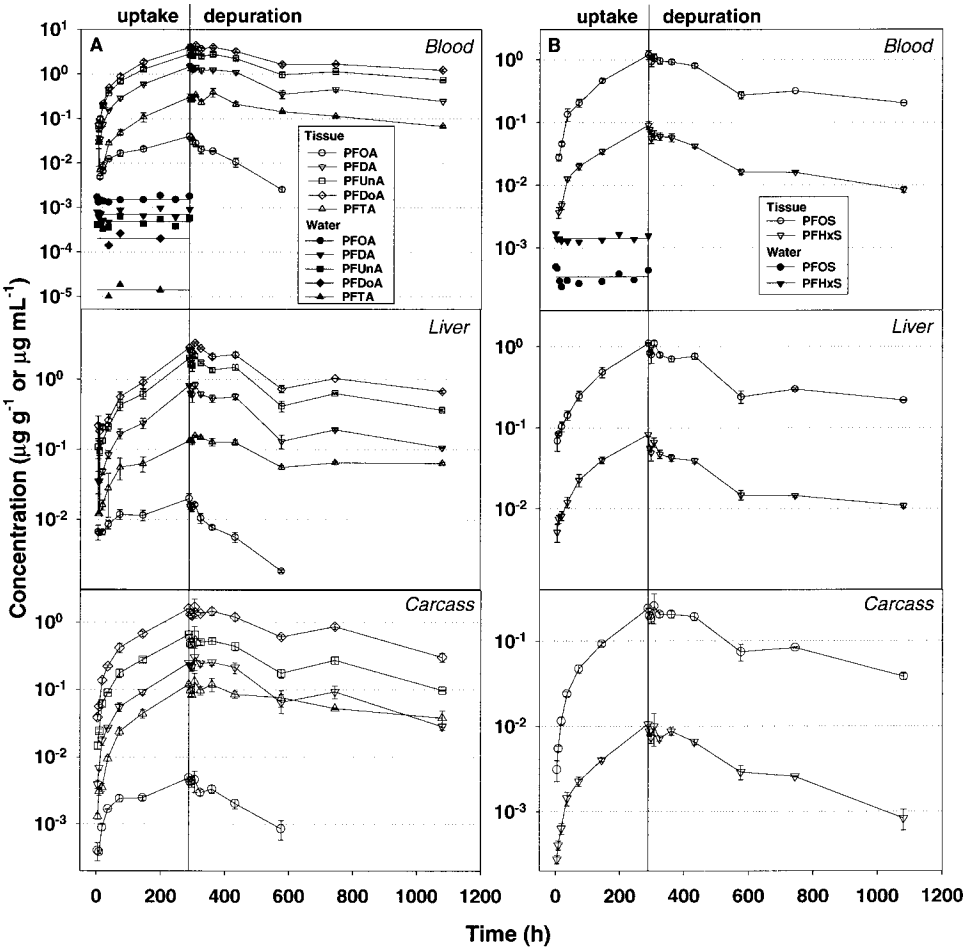

Fig. 1. Growth-corrected uptake and depuration phase concentrations for (**A**) perfluoroalkyl carboxylates and (**B**) perfluoroalkyl sulfonates in blood, liver, and carcass. Each tissue concentration represents the mean of three different fish (± 1 standard error). Aqueous exposure concentrations during the uptake phase are shown in **A** and **B** for carboxylates and sulfonates, respectively. See Table 1 for definitions of test compounds.

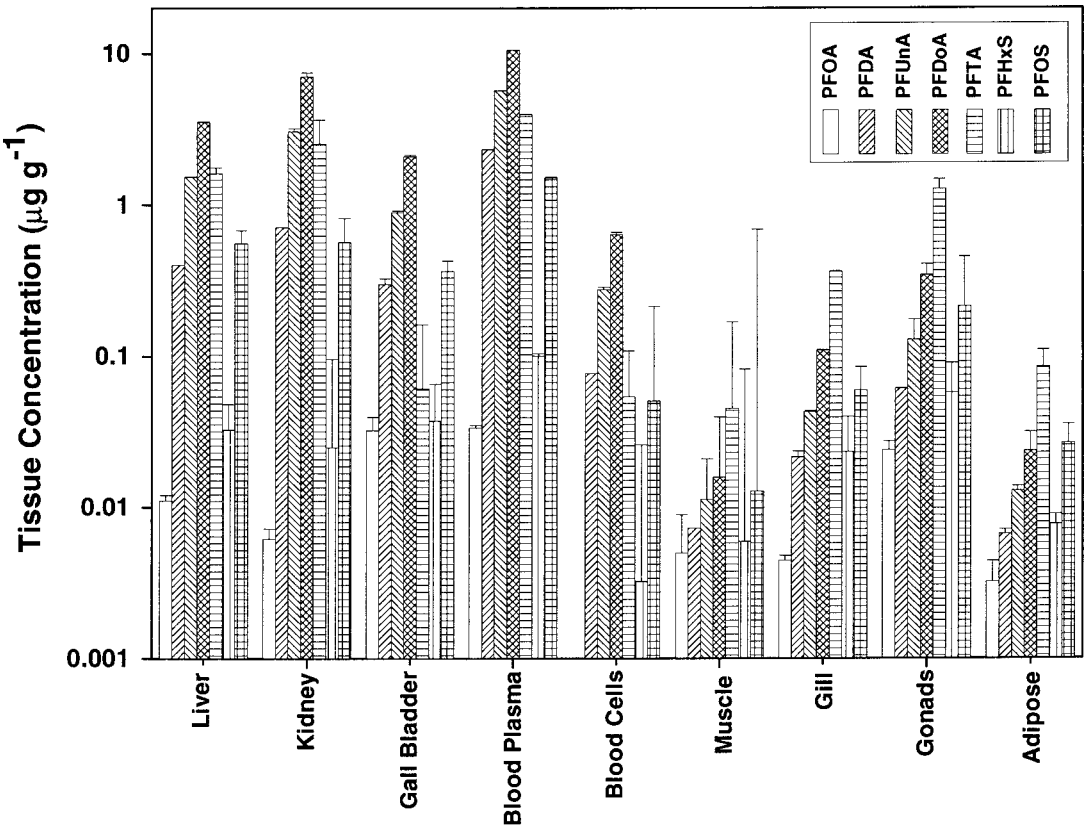

Fig. 2. Perfluorinated acid concentrations, ± 1 standard error, in various fish tissues after a 12-d aqueous exposure. See Table 1 for definitions of test compounds.

the site of uptake, depuration, or both, as has been determined for other xenobiotics [13], including surfactants [15]. Recovery of analytes from the heart and spleen was low (<10% based on internal standard response), thus preventing quantitation of PFAs in these tissues. In almost all surfactant bioconcentration tests for which tissue-specific data have been presented, the highest analyte (i.e., radiolabel) concentrations typically are found in the gall bladder, probably a result of metabolism in the liver [15]. The gall bladder concentrations determined herein may be lower than in previous surfactant studies [15] because the fish were fed daily, thus preventing concentration and accumulation of the bile [25].

Unlike lipophilic chlorinated organic pollutants, PFAs did not accumulate preferentially in adipose tissue. For example, dioxin congeners accumulated in the ceca and depot fat of rainbow trout to a greater extent than kidney, spleen, muscle, or skin tissue [26]. The tissue distribution of PFDA in male rats is similar to our results for rainbow trout, except that rat liver contained by far the greatest concentrations, followed by blood > kidney > heart > fat > testis > muscle [27]. This may be partially attributable to the lipophobic properties of PFAs; however, PFAs also have a high affinity for plasma albumin [28], and it has been hypothesized that PFAs may bind to hepatic proteins such as fatty acid–binding proteins [27], thus explaining the high PFA concentrations in blood and liver, respectively. The relatively high concentrations of PFAs in rainbow trout kidney may simply reflect the high perfusion of blood, and do not automatically indicate that urinary elimination is a significant depuration route. For example, despite high kidney concentrations of PFDA in exposed rats, the primary mode of depuration was feces, with only minor excretion in urine [27].

The route by which humans are exposed to PFAs is not well characterized, but consumption of fish meat may well be a significant source of exposure. Assuming that muscle comprises 67% of a rainbow trout by weight [29], the meat could therefore contain 22, 61, and 81% of the total body burden of PFOS, PFHxS, and PFOA, respectively, based on the tissue concentrations (Fig. 2).

Bioconcentration parameters

Similar to the results of a PFA dietary accumulation study [12], only carboxylates with more than six perfluoroalkyl carbons, and sulfonates with more than four perfluoroalkyl carbons were detected in blood, liver, and carcass at all sampling times (Fig. 1). Shorter PFAs are expected to have insignificant bioconcentration potential, but their uptake or depuration kinetics could not be determined here, although the half-life of PFBS in liver of rainbow trout was 3.3 d in a dietary accumulation study [12]. Recovery of most PFAs from liver and carcass is quantitative [12], and recovery from blood has already been demonstrated by Hansen et al. [5] for PFOS, PFHxS, and PFOA. We made no attempt to monitor for metabolites of PFAs because the existing literature suggests that biotransformation will be insignificant or absent [27,30,31], and we assumed that depuration was entirely a function of elimination. Visual observation of depuration data indicated possible biphasic depuration for most PFAs in blood, liver, and carcass (Fig. 1); however, this could not be demonstrated statistically because of the small sample size. Linear regression

Table 3. Rate of uptake (k_u), depuration (k_d), half-life (d), steady-state bioconcentration factor (BCF; estimated by k_u/k_d), and the 12-d accumulation ratio (AR), calculated at the end of the uptake period. Error represents ± 1 standard error, and a number in parentheses represents the coefficient of determination (r^2) for the corresponding regression analysis

Test compound[a]	k_u (L/kg/d)	$k_d \times 10^{-3}$ (l/d)	Half-life (d)	BCF (L/kg)	12-d AR
Carcass					
PFOA	0.53 ± 0.041 (0.80)	130 ± 17 (0.76)	5.2 ± 0.67	4.0 ± 0.60	3.2
PFDA	29 ± 1.0 (0.95)	62 ± 8.2 (0.71)	11 ± 1.5	450 ± 62	350
PFUnA	120 ± 4.8 (0.95)	46 ± 6.5 (0.68)	15 ± 2.2	2,700 ± 400	1,400
PFDoA	700 ± 29 (0.94)	38 ± 5.5 (0.68)	18 ± 2.6	18,000 ± 2,700	6,600
PFTA	580 ± 26 (0.93)	24 ± 5.5 (0.45)	28 ± 6.4	23,000 ± 5,300	8.500
PFOS	53 ± 1.3 (0.98)	48 ± 6.5 (0.70)	15 ± 2.0	1,100 ± 150	690
PFHxS	0.62 ± 0.021 (0.96)	65 ± 6.2 (0.82)	11 ± 1.0	9.6 ± 0.99	7.6
Blood					
PFOA	4.1 ± 0.31 (0.83)	150 ± 53 (0.83)	4.5 ± 1.6	27 ± 9.7	25
PFDA	160 ± 4.6 (0.97)	58 ± 7.2 (0.97)	12 ± 1.5	2,700 ± 350	1,900
PFUnA	500 ± 18 (0.96)	46 ± 5.3 (0.96)	15 ± 1.8	11,000 ± 1,400	5,500
PFDoA	1,700 ± 65 (0.96)	41 ± 4.3 (0.96)	17 ± 1.7	40,000 ± 4,500	18,000
PFTA	1,400 ± 120 (0.82)	48 ± 5.28 (0.82)	15 ± 1.6	30,000 ± 4,200	20,000
PFOS	240 ± 8.4 (0.90)	57 ± 6.7 (0.90)	12 ± 1.4	4,300 ± 570	3,100
PFHxS	5.3 ± 0.48 (0.76)	70 ± 6.5 (0.76)	10 ± 0.94	76 ± 9.7	59
Liver					
PFOA	1.4 ± 0.13 (0.65)	180 ± 13 (0.91)	3.9 ± 0.28	8.0 ± 0.59	12
PFDA	74 ± 5.3 (0.84)	65 ± 8.9 (0.69)	11 ± 1.5	1,100 ± 180	1,100
PFUnA	260 ± 20 (0.84)	55 ± 7.7 (0.68)	13 ± 1.7	4,900 ± 770	3,800
PFDoA	910 ± 79 (0.78)	50 ± 6.7 (0.70)	14 ± 1.9	18,000 ± 2,900	11,000
PFTA	960 ± 98 (0.69)	31 ± 5.5 (0.60)	22 ± 3.7	30,000 ± 6,000	8,700
PFOS	260 ± 17 (0.88)	50 ± 7.4 (0.66)	14 ± 2.0	5,400 ± 860	2,900
PFHxS	5.8 ± 0.31 (0.91)	58 ± 7.0 (0.75)	12 ± 1.5	100 ± 13	54

[a] Refer to Table 1 for definitions of test compounds.

analysis of all growth-corrected depuration data resulted in acceptable coefficients of determination (Table 3).

For detected PFAs, depuration rate constants generally decreased with increasing length of the perfluoroalkyl chain for carboxylates and sulfonates in all tissues, ranging from 0.024 to 0.180/d, representing biological half-lives in the range of 3.9 to 28 d. For carboxylates, positive linear relationships were observed between half-life and perfluoroalkyl chain length for the carcass and liver, but not the blood (Fig. 3). In blood, the relationship deviated from linearity for the compound with the longest chain length, PFTA, which was eliminated more quickly than expected from extrapolation of the observations from PFAs with shorter chain lengths. Perfluorotetradecanoic acid probably partitioned out of the blood into the carcass, rather than being eliminated, as has been shown for other anionic surfactants [18]. Based on the linear regression equations (Fig. 3), half-life increased by 2.8 to 3.8 d for each additional carbon in the perfluoroalkyl chain. Similar analysis could not be ap-

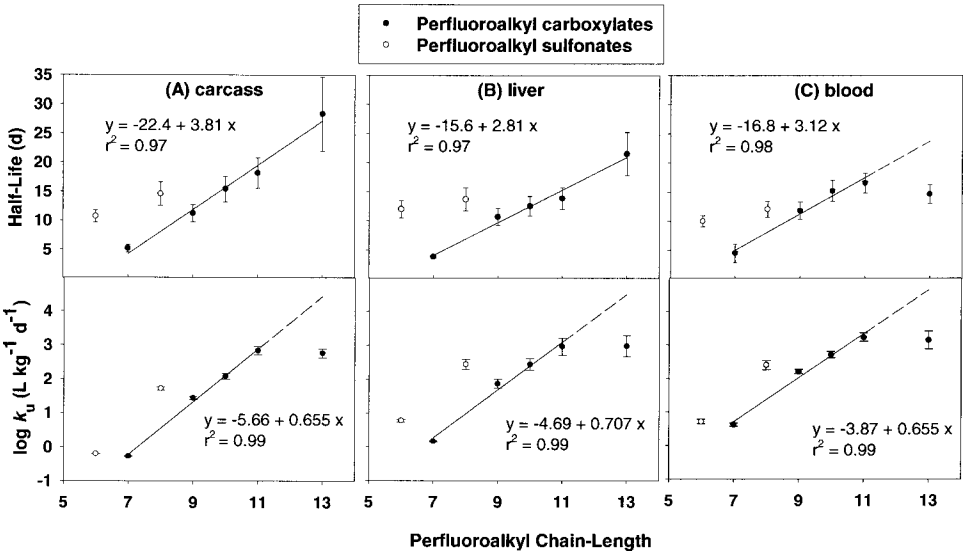

Fig. 3. Half-life (ln $2/k_d$) and uptake rate (log k_u) relationships with perfluoroalkyl chain length of carboxylates and sulfonates for (**A**) carcass, (**B**) liver, and (**C**) blood. Linear regression was applied to perfluoroalkyl carboxylate data, and the resulting equation and coefficient of determination (r^2) are shown. The dashed portion of the regression line is an extrapolation and indicates that perfluorotetradecanoic acid was not included in the regression analysis.

plied to the sulfonates because only two were detectable; however, in all tissues, the half-lives of PFHxS and PFOS were greater than those of carboxylates of equivalent perfluoroalkyl chain length (Fig. 3).

The depuration half-lives determined in this study are slightly greater than were determined in a dietary PFA exposure study [12]. Assuming that the mode of exposure is not important, we suggest that some of this variability may be attributable to the difference in fish size. For example, at the beginning of the depuration period in this study, the average trout mass was 8.4 g, compared with only 4.0 g in the dietary exposure study [12]. Hydrophobic organochlorine compounds previously have been demonstrated to have longer half-lives in larger fish [32,33], even when controlling for growth dilution. Body size also may help to explain the variability among half-lives reported for humans (1,428 d [2]), monkeys (180 d [34]), and male rats (89 d [34]). In comparison, the half-life in fish carcass was approximately 15 d for juvenile trout, but the possibility of elimination through the gills cannot be ruled out as an additional depuration mechanism for fish.

The rate of depuration for PFAs is generally more rapid than for persistent organochlorine contaminants, including polychlorinated biphenyls, toxaphene, hexachlorobenzene, mirex, and chlorinated alkanes [33,35]. However, the rate of depuration for PFAs, with the exception of PFOA, is slower than for any previously investigated surfactant in fish [15], and this may be partially attributable to the lack of metabolism or biotransformation [27,30,31]. The relatively slow PFA depuration half-lives, combined with the observations of high blood, liver, and gall bladder concentrations, also support the theory that PFAs enter into enterohepatic recirculation in fish, the process whereby compounds are continuously recycled between the blood, liver, gall bladder, and intestines, where resorption occurs via the portal vein. This is further supported by the observation of high PFA assimilation efficiencies [12], which indicate that intestinal resorption efficiency is similar to that of endogenous bile acids. Use of the relatively low assimilation efficiency of PFOA (59% [12]) as a measure of resorption from the gut during enterohepatic recirculation could explain why its half-life is shorter than for longer PFAs, which have a higher resorption capacity [12]. The divergence of sulfonate and carboxylate rates of depuration (Fig. 3) also could be attributable to the different efficiency of resorption from the gut during enterohepatic recirculation. For example, the assimilation efficiency of PFHxS, which has only six perfluoroalkyl carbons, is greater than that for PFOA, which has seven perfluoroalkyl carbons [12]. Also, short PFAs possibly are eliminated in the urine to a greater extent than longer PFAs, as has been shown for PFHpA in rats [24].

Uptake rate constants varied by a factor of 3,000 for different PFAs, and increased with increasing perfluoroalkyl chain length (Fig. 3), ranging from 0.53 to 1,700 L/kg/d for all tissues (Table 3). A similar trend, showing increased uptake rates with increasing alkyl chain length, has been reported for a series of cationic monoalkyltrimethyl ammonium surfactants [36]. In general, these PFA uptake rates may be considered very rapid and, to our knowledge, the carcass k_u reported here for PFDoA exceeds any previously reported rate of uptake for anionic surfactants (i.e., 642 L/kg/d reported by Tolls et al. [3]). Given equal perfluoroalkyl chain lengths, sulfonates were taken up at a greater rate than carboxylates, always lying above the regression line for carboxylates (Fig. 3). For carboxylates,

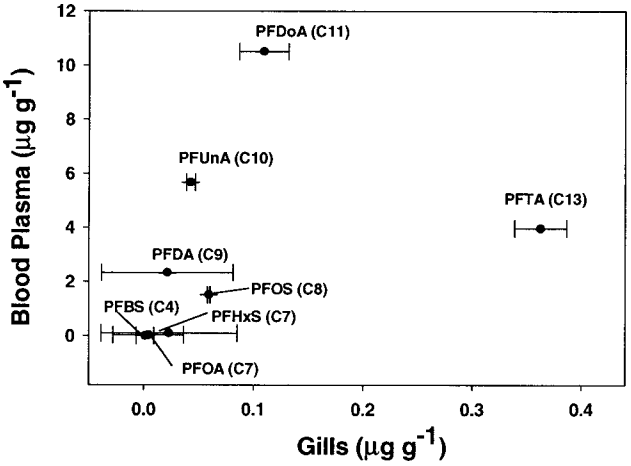

Fig. 4. Relationship between gill and blood concentrations, ± 1 standard error, in tissue distribution in fish. Each data point represents the mean of three fish, and is labeled with the perfluorinated acid and the length of the respective perfluoroalkyl chain in parentheses (e.g., C8 = eight carbons). See Table 1 for definitions of test compounds.

uptake increased in a linear manner with perfluoroalkyl chain length until the length reached 13 carbons (i.e., PFTA).

The rate of uptake for PFTA, presumably the most hydrophobic PFA tested, was less than expected based on extrapolation from shorter PFAs in liver, blood, and carcass (Fig. 3). Such a phenomenon is well characterized for hydrophobic chlorinated contaminants, and can result from exclusion based on limited diffusive mass transfer [37], molecular size [38], or from reduced bioavailability because of binding on dissolved or colloidal organic matter in the water column [39,40]. Reduced bioavailability because of association with dissolved and colloidal organic matter has been demonstrated for cationic surfactants [15], and anionic surfactants [41]. The octanol–water partition coefficient (K_{OW}) for PFTA is unknown; however, it is feasible that the long perfluoroalkyl chain of PFTA causes the molecule to act similar to superhydrophobic neutral organochlorines (i.e., log $K_{OW} > 6$), which interact with organic matter, resulting in an apparent reduction of the uptake rate [39]. Alternatively, based on the ratio of concentrations in the gills and blood plasma at day 12 of the tissue distribution study, the gills appear to be less permeable to PFTA than shorter PFAs (Fig. 4). Perfluorotetradecanoic acid was enriched at the gills relative to shorter perfluoroalkyl carboxylates and sulfonates, suggesting that gill permeation rates may limit uptake of PFTA, as has been shown for the high molecular weight cationic surfactants C_{16}-alkylpyridinium and $(C_{10})_2$-dialkyldimethylammonium [15]. A time course of the tissue distribution would allow for more conclusive arguments; however, gill permeation is known as the rate-limiting step for uptake of other anionic surfactants [18].

Steady-state carcass BCFs ranged from 4.0 to 23,000, and generally increased with the perfluoroalkyl chain length (Table 3 and Fig. 5). Similar trends, showing increased BCFs with increased alkyl chain length, have been reported for anionic linear alkylbenzene sulfonate surfactants [18] and some cationic surfactants [36]. For PFAs, blood and liver BCFs were 1 to 10 times greater than in the carcass (Table 3). Carboxylate BCFs were greatest in the blood, whereas sulfonate BCFs were greatest in the liver. Carcass BCFs closely approximate the whole-body BCF; however, removal of the liver and a portion of the blood from the intact fish resulted in a minor loss of

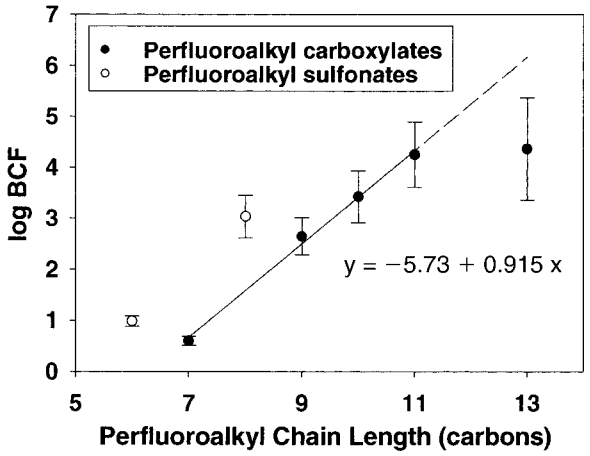

Fig. 5. Carcass bioconcentration factor (BCF) relationship with perfluoroalkyl chain length. Linear regression was applied to perfluoroalkyl carboxylate data, and the resulting equation and coefficient of determination (r^2) are shown. Perfluorotetradecanoic acid was not included in the regression analysis and the dashed portion of the regression line is an extrapolation of the regression equation.

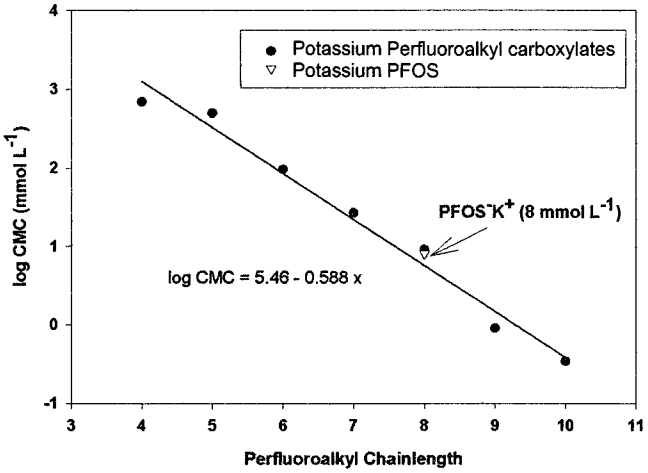

Fig. 6. Relationship determined by linear regression between the log critical micelle concentration (log CMC) and perfluoroalkyl chain length of potassium perfluoroalkyl carboxylate salts. The CMC for the potassium salt of perfluorooctane sulfonate ($PFOS^-K^+$) fits the relationship. All data have been taken from Kissa [42].

total PFAs. The resulting carcass contained between 85% (i.e., PFHxS) and 96% (i.e., PFTA) of total PFAs, depending on the test compound. Bioconcentration factors increased largely as a result of increasing uptake rate, whereas depuration rate varied only by a factor of eight for all tissues examined and had less relative influence on the observed BCF trends.

Because steady state was not achieved for any PFA, a 12-d accumulation ratio was calculated (Table 3) and compared to the estimated steady-state BCF. The 12-d accumulation ratio always was smaller than the BCF; however, for the PFAs with the shortest chain lengths, the 12-d accumulation ratio approached the estimated BCF to within 80% (i.e., PFOA). The tissue concentrations of PFAs with longer chain lengths were far from steady state at the end of the uptake period, reaching only 37% of the predicted steady-state value by day 12 (i.e., PFDoA and PFTA). The time to reach steady state is determined exclusively by the rate of depuration, and was predicted to exceed 10, 43, and 120 d for PFOA, PFOS, and PFTA, respectively [12]. The fact that steady state was not approached should not influence our estimates of the BCF calculated by the ratio of uptake and elimination rates. A previous comparison of the kinetic and steady-state approaches to the study of surfactant bioaccumulation indicated that both methods yield similar results, and that the one-compartment, first-order model was suitable [15].

Perfluoroalkyl carboxylate carcass BCFs (i.e., log BCF) increased linearly with perfluoroalkyl chain length from PFOA to PFDoA by a factor of eight for each additional fluorinated carbon in the chain (Fig. 5). The trend deviated from linearity for PFTA as a result of the decreased rate of uptake (Fig. 3A). Given equal perfluoroalkyl chain length, sulfonates bioconcentrated to a greater extent than carboxylates, largely as a result of higher uptake rates, but also because of lower depuration rates, and did not fit the same linear relationship for carboxylates, always lying above the linear regression line (Fig. 5). Furthermore, PFHxS, which has six perfluoroalkyl carbons, had a carcass BCF of 9.6, whereas PFHpA, which also has six perfluoroalkyl carbons, could not be detected in most tissues despite a higher exposure concentration (Table 1). In a dietary exposure of fish to PFAs, perfluoroalkyl sulfonates also bioaccumulated to a greater extent than carboxylates of equivalent perfluoroalkyl chain length, and a similar linear trend was observed for carboxylate bioaccumulation factors [12].

Bioconcentration potential historically has been well predicted by the K_{OW}, a measure of a substance's hydrophobicity. However, the K_{OW} is a problematic parameter for surfactants because of their tendency to aggregate at the interface of a liquid–liquid system. Here we have used the perfluoroalkyl chain length as a measure of hydrophobicity; however, Tolls and Sijm [16] suggested that a more meaningful measure of the relative hydrophobicity for surfactants is the critical micelle concentration (CMC), the concentration at which half of the molecules in solution are associated as micelles. Critical micelle concentration data are limited or nonexistent for many of the test compounds; however, the CMC of PFOS fits the relationship determined for several carboxylates of variable perfluoroalkyl lengths (Fig. 6) [42], indicating that the acid function has little influence on CMC. Analysis of our data indicates that bioaccumulation potential increases with decreasing CMC for carboxylates; however, the CMC alone does not explain the disproportionate accumulation of sulfonates relative to carboxylates. For PFAs, the acid function must be considered for prediction of the bioconcentration and bioaccumulation potential [12]. For perfluoroalkyl carboxylates, the slope of the log CMC data (Fig. 6) is remarkably similar, but opposite in sign, to the plot of log uptake rate in carcass (Fig. 3A), indicating that increased uptake for longer PFAs within a class may be a function of increasing hydrophobicity.

The linear CMC–BCF relationships, described by Tolls and Sijm [16], had relatively low coefficients of determination, and it was noted that the CMC might not be the most suitable parameter for describing hydrophobicity of surfactants. The suggestion was made that interfacial tension or reversed-phase chromatographic retention time might be more suitable parameters. When using our chromatographic methods, PFOS and PFNA (i.e., both of which have eight perfluoroalkyl carbons and similar CMCs) could not be time-resolved on a reversed-phase column, with PFNA eluting only 0.02 min after PFOS. Additional physical and chemical properties for this series of PFAs would be beneficial for future modeling efforts, particularly for the longer perfluoroalkyl carboxylates.

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