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Determination of Perfluorochemicals in Fish and Shellfish Using Liquid Chromatography−Tandem Mass Spectrometry

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Supporting Information

ABSTRACT: This paper reports the validation and application of a method for determination of 10 perfluorochemicals (PFCs) in retail fish and shellfish. The analytes of interest were 7 perfluorinated carboxylates and 3 perfluorinated sulfonates. Fish and shellfish samples were digested with a basic solution of 10 mM sodium hydroxide in methanol before sonication and solid phase extraction through weak anion exchange. Analysis was performed using liquid chromatography−tandem mass spectrometry. Recoveries from spiking five different types of fish and shellfish indicate that the method performs similarly with different fish types, and recoveries were over 90% for all analytes. Forty-six retail samples, collected between 2010 and 2012, including 13 different types of fish and shellfish were analyzed for PFCs. The 13 different types included the top 10 most-consumed fish and shellfish in the United States according to data collected by the National Fisheries Institute. Two Standard Reference Materials were also analyzed. Most fish and shellfish had no detected PFCs; only 11 samples of the 46 tested had detectable concentrations of PFCs.

KEYWORDS: *perfluorochemicals, method validation, fish, shellfish*

INTRODUCTION

Perfluorochemicals (PFCs) have been used in a wide variety of manufacturing processes and consumer goods (e.g., stain-resistant coatings for upholstery, nonstick cookware, and cosmetics).[1,2] Their widespread uses, broad range of applications, and environmental and biological persistence[3] have made PFCs a ubiquitous contaminant. PFCs have been detected in wildlife in remote locations and at various trophic levels[4] as well as in the blood, urine, and breast milk of humans who have not been occupationally exposed to the chemicals.[5−7] In addition to their persistence, PFCs have also been linked to developmental toxicity and immunotoxicity.[3] A majority of the US Environmental Protection Agency (EPA) Science Advisory Board has also noted PFOA is a "likely human carcinogen" according to hazard descriptors in the EPA cancer guidelines.[8] Given the ubiquitousness of the compounds, the long retention times in humans,[9] and the potential consequences of prolonged exposure it is important to better understand and quantify potential routes of human exposure.

Previous epidemiological studies report human PFC exposure occurs through the inhalation of house dust,[10] and air,[11,12] and the consumption of contaminated water[13] and food.[14] A number of researchers consider food to be the major exposure route for populations without occupational contact to PFCs.[5,6] Food can become contaminated by PFCs through endogenous exposure or during processing. For example, fish may incorporate PFCs from the water or sediments of their habitats,[15] or from their food sources.[16] Alternatively, PFCs may migrate into foods from food contact materials during processing, storage, and/or preparation.[17,18] While there have been broad PFC-related surveys of retail food in Canada,[14,19] and the U.K.,[20] no such survey exists for United States (US) retail foods other than milk.[21]

Fish is an important component of the US diet, with the 2010 per capita annual consumption of 15.8 pounds.[22] Based on dietary recommendations and an increased focus on healthier diets, it is likely that the amount of fish and shellfish in the US diet will increase. A number of studies by the US Environmental Protection Agency (EPA) have shown that wild caught fish can contain significant concentrations of PFCs.[23−25] For example, PFOS was detected in 73% of composite samples collected from the upper Mississippi River. Additionally, PFOS concentrations as high as 90 ng/g, which is above the Minnesota (MN) Department of Health's set action levels for wild caught fish,[26] have been reported from the same waterway. While these previous studies clearly establish that fish can be a source PFCs, they have tended to focus on fish from waterways with known PFC sources. This paper presents the first study of PFCs in retail fish and shellfish purchased in the continental US (Table 1) between 2010 and 2012. In an effort to gain an understanding of potential human exposure to PFCs from fish and shellfish consumption, even with a limited sample size, this study has sampled the ten most-consumed fish in the US according to data compiled by the National Fisheries Institute.[27]

A basic digestion and weak anion exchange extraction method[23] adapted from the method used for the analysis of cow's milk[21] is applied to the determination of 10 PFCs (7 perfluorinated carboxylates and 3 perfluorinated sulfonates). Forty-six retail samples consisting of 13 different types of fish and shellfish, purchased from 11 different locations, were analyzed. Two Standard Reference Materials (SRMs) from the

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Table 1. Sample Summary: Fish and Shellfish Type, Quantity (Farm Raised, Wild Caught, Origin Unknown), and Purchase Location[a]

fish	no. of samples[b]	purchase location (no. of samples)
crab meat (s)	1 (1, 0, 0)	Washington, .DC.
shrimp (s)	9 (1, 1, 7)	Orlando, FL (5); Memphis, TN (3); Nashville, TN (2)
striped bass (e)	10 (3, 3, 4)	New York, NY (9); Cherry Hill, NJ (1)
catfish (f)	13 (8, 1, 4)	Indianola, MS (5); Dallas, TX (5); Tampa, FL (2); Orlando, FL (1)
clams (s)	1 (0, 1, 0)	Washington, D.C.
cod (s)	1 (0, 0, 1)	Washington, D.C.
flounder (s)	1 (0, 0, 1)	Washington, D.C.
pangasius (f)	1 (1, 0, 0)	Washington, D.C.
pollock (s)	1 (0, 0, 1)	Huntington Beach, CA
tuna (can and pouch) (s)	3 (0, 1, 1)	Chicago, IL
salmon (e)	2 (1, 1, 0)	Washington, D.C.
scallops (bay and sea) (s)	2 (0, 2, 0)	Washington, D.C.
tilapia (e)	1 (1, 0, 0)	Washington, D.C.

[a]All samples collected 2010−2012. (f) indicates freshwater fish. (s) indicates saltwater fish. (e) indicates euryhaline fish. [b]In parentheses: number of farm raised, wild caught, and origin unknown.

National Institute of Standards and Technology (NIST) were also analyzed.

MATERIALS AND METHODS

Standards, Reagents, and Materials. Eight stable isotope labeled PFCs, perfluoro-*n*-[1,2-$^{13}C_2$]hexanoic acid ($^{13}C_2$-PFHxA), perfluoro-*n*-[1,2,3,4-$^{13}C_4$]heptanoic acid ($^{13}C_4$-PFHpA), perfluoro-*n*-[1, 2, 3, 4-$^{13}C_4$]octanoic acid ($^{13}C_4$-PFOA), perfluoro-*n*-[1,2-$^{13}C_2$]-decanoic acid ($^{13}C_2$-PFDA), perfluoro-*n*-[1, 2-$^{13}C_2$]undecanoic acid ($^{13}C_2$-PFUnDA), perfluoro-*n*-[1, 2-$^{13}C_2$]dodecanoic acid ($^{13}C_2$-PFDoDA), sodium perfluoro-1-[1, 2, 3-$^{13}C_3$]hexanesulfonate ($^{13}C_3$-PFHxS), and sodium perfluoro-1-[1, 2, 3, 4-$^{13}C_4$]perfluorooctanesulfonate ($^{13}C_4$-PFOS), were used as internal standards (ISs), and were purchased from Wellington Laboratories (Guelph, Canada) along with sodium perfluoro-1-hexanesulfonate (PFHxS) 98%. All of the ISs and PFHxS were dissolved in methanol (50 μg/mL). Perfluoroheptanoic acid (PFHpA) 99%, perfluorooctanoic acid (PFOA) 96%, perfluorononanoic acid (PFNA) 97%, perfluorodecanoic acid (PFDA) 98%, perfluoroundecanoic acid (PFUnDA) 98%, perfluorododecanoic acid (PFDoDA) 95%, tetrabutylammonium perfluorobutanesulfonate (PFBS) 98%, and ammonium acetate 99.99% were obtained from Aldrich Chemicals (St. Louis, MO, USA). Perfluorooctanesulfonic acid (PFOS) potassium salt 98% standard was obtained from Alfa-Aesar (St. Louis, MO, USA). Perfluorohexanoic acid (PFHxA) was purchased from Oakwood Products Inc. (West Columbia, SC, USA). Fish standard reference materials (SRM 1946 and SRM 1947) were purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). LC−MS grade Optima water and methanol, ammonium hydroxide (29%), polypropylene (PP) centrifuge tubes (1.5, 15, and 50 mL), polystyrene (PS) 5 mL tubes, and colorpHast pH papers were purchased from Fisher Scientific (Pittsburgh, PA, USA). A Minimate Plus chopper/grinder was purchased from Cuisinart (East Windsor, NJ). Oasis weak anion exchange (WAX) solid phase extraction (SPE) cartridges containing 150 mg (6 cc) sorbent with a 30 μm particle size were purchased from Waters (Milford, MA, USA). PP high pressure liquid chromatography (HPLC) vials (0.3 mm) with polyethylene (PE) septa were purchased from Agilent Technologies (Palo Alto, CA, USA). Fish and shellfish homogenate digests were centrifuged using a Fisher 2100 R centrifuge, model 120 (Needham Heights, MA, USA). Fish and shellfish sample extracts were centrifuged as a final clean up step before LC−MS/MS analysis using a Thermo Scientific Sorvall Legend Micro 21 centrifuge from Thermo Scientific (Asheville, NC, USA). Fish and shellfish homogenates in PP centrifuge tubes were sonicated at room temperature using a Branson 2510R-DTH from Branson Ultrasonics Corporation (Danbury, CT, USA).

Calibration Standards and Quantification. A 1000 ng/mL working stock solution of each of the 7 carboxylic acids and 3 sulfonate salts was prepared in a mixture of water/methanol (30/70 v/v) and stored in a 50 mL PP centrifuge tube with PP screw cap. From this stock solution two more working stock solutions of 100 and 10 ng/mL were volumetrically prepared in water/methanol (30/70). A 300 ng/mL IS solution, which contained all of the ISs, was prepared in a mixture of water/methanol (30/70). Using the 3 working stock solutions, calibration standards were produced by adding 37.5 ng of IS (125 μL of 300 ng/mL IS mixture) and an aliquot of the appropriate working stock and diluting to 5 mL with water/methanol (50/50). Calibration solutions were stored in PP centrifuge tubes with PP screw caps at 4 °C, until analysis.

Calibration standards (0.2−100 ng/mL) were analyzed daily, prior to and throughout the analysis of the sample sets. Each standard was run 2 or 3 times, depending on the size of the sample set, and replicates were averaged to generate the calibration curve each day. Calibration curves were prepared by determining the nonweighted simple linear regression for the area ratio (analyte:internal standard) versus concentration ratio (analyte:internal standard) for all standards in the run. The coefficients of determination (R^2) for all analytes were ≥0.99. All of the peak integration and mass spectrometry data processing was performed with MassHunter Quantitative Analysis (Version B.03.01, Agilent Technologies). Microsoft 2010 Excel (Microsoft Corp., Redmond, WA) was used for all additional data processing. All concentrations reported were determined using the mass of the free acetate or sulfonate ions and not the corresponding salts.

Sample Collection and Treatment. Retail fish and shellfish samples (46) were collected from 11 areas across the continental US (Table 1). Processed tuna in cans and pouches of different brands came from a single location. Six units of the same lot were purchased for each product. A single sample number was assigned to each product. All other samples were received frozen or chilled, placed at −20 °C upon receipt, and thawed as needed for sampling and homogenization. The types of fish sampled included the ten most-consumed fish in the US.[27] Fish were assigned a sample number upon receipt, with each fish species from the same location being assigned a single sample number. To evaluate within sample differences, a number of samples were divided into subsamples for processing and analysis. If a sample (location and type) contained multiple fillets, each fillet was assigned a subsample number. For shellfish such as shrimp, clams, and scallops, composites of 8−15 g were created and assigned a subsample number. Generally, 3 to 10 shrimp, clams or bay scallops were used to create a subsample; however, in one instance a single, large sea scallop weighing approximately 25 g was identified as a subsample. For tuna and crab samples, because it was impossible to determine which meat came from separate fish, homogenates were

Table 2. MS/MS Quantitative Transitions, Collision Energies, and Calculated Method Detection Limits (MDLs) for PFCs

compound	MDL (n = 9)[a] (ng/g)	percursor ion	product ion: quantifier/qualifier	collision energy (eV)	IS used
perfluorobutanesulfonate (PFBS)	0.44	299	80/99	41	$^{13}C_3$-PFHxS
perfluorohexanesulfonate (PFHxS)	0.55	399	80/99	50	$^{13}C_3$-PFHxS
perfluorooctanesulfonate (PFOS)	0.88	499	99/80	60	$^{13}C_4$-PFOS
perfluorohexanoic acid (PFHxA)	1.14	313	269/119	2	$^{13}C_2$-PFHxA
perfluoroheptanoic acid (PFHpA)	1.28	363	319/169	3	$^{13}C_4$-PFHpA
perfluorooctanoic acid (PFOA)	0.31	413	369/169	4	$^{13}C_4$-PFOA
perfluorononanoic acid (PFNA)	0.60	463	419/219	4	$^{13}C_4$-PFOA
perfluorodecanoic acid (PFDA)	0.47	513	469/219	3	$^{13}C_4$-PFDA
perfluoroundecanoic acid (PFUnDA)	0.46	563	519/269	2	$^{13}C_2$-PFUnDA
perfluorododecanoic acid (PFDoDA)	0.57	613	569/169	8	$^{13}C_2$-PFDoDA
$^{13}C_3$-PFHxS		402	80/99	50	
$^{13}C_4$-PFOS		503	99/80	60	
$^{13}C_2$-PFHxA		315	270/120	2	
$^{13}C_4$-PFHpA		367	322/172	3	
$^{13}C_4$-PFOA		417	372/172	4	
$^{13}C_2$-PFDA		515	470	3	
$^{13}C_2$-PFUnDA		565	520/320	8	
$^{13}C_2$-PFDoDA		615	570/319	8	

[a] n = 9 indicates the number of replicate aliquots processed to determine the MDL.

Table 3. Percent Recovery for Spikes of Different Fish/Shellfish Tissues

fish type	n	PFBS	PFHxS	PFOS	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA
catfish	6	105 (3)[a]	99 (4)	100 (4)	98 (6)	98 (6)	99 (4)	101 (5)	97 (3)	99 (2)
pollack	6	104 (8)[b]	96 (3)[c]	101 (4)	96 (8)	100 (6)	101 (5)	92 (10)	95 (2)	97 (4)
shrimp	3	101 (11)	103 (2)	105 (1)	103 (4)	100 (6)	98 (3)	104 (3)	97 (2)	102 (2)
striped bass	3	98 (5)	92 (18)	98 (9)	94 (15)	91 (10)[d]	94 (3)	98 (9)	94 (3)	95 (4)
processed tuna	3	92 (6)	99 (4)	96 (4)	96 (8)	97 (4)	95 (2)	90 (1)	97 (1)	95 (3)

[a] % RSD is shown in parentheses. [b] n = 5. [c] n = 4. [d] n = 2.

created in place of subsamples. For tuna, each package (can or pouch) was identified as a single homogenate. For crab, 2 aliquots (8.79 and 12.82 g) were sampled from the 1 pound container, designated as homogenate 1 and 2 and processed and analyzed separately. Two reference materials of fish tissue from Lake Superior (SRM 1946) and Lake Michigan (SRM 1947) were obtained from NIST and stored at −80 °C.

Extraction Methods. The extraction method used was based on a method used by Delinsky et al.[23] and is similar to the method previously validated for the determination of PFCs in cow's milk.[21] Fish tissue (no bones, shell, or skin) was combined with 3 g of water for every 1 g of fish, and homogenized using a food processor. The food processor was washed, rinsed with deionized water, and dried before the next use. A 2 g aliquot of fish homogenate and 15 ng of internal standard (50 μL of 300 ng/mL IS mixture) was added to a 15 mL centrifuge tube and vortexed for approximately 5 s. Sodium hydroxide (8 mL of 10 mM) in methanol (0.1% water by volume) was added to the fish homogenate, and the samples were vortexed and then sonicated for 30 min in a water bath at room temperature. After sonication, the samples were centrifuged (4000 rcf) at room temperature for 5 min. 3 mL of the supernatant was transferred into a new 50 mL centrifuge tube (for extraction), and the remaining solid pellet and liquid were discarded. The supernatant was diluted to 30 mL with water, vortexed, and loaded onto a WAX SPE cartridge, which had been preconditioned with 5 mL each of methanol (0.1% ammonium hydroxide, neat) and water. The sample was eluted to waste under vacuum, at a rate of about 2 drops/s, and the cartridge was washed with sodium acetate buffer (25 mM, pH = 4, 6 mL), followed by methanol (6 mL). The PFCs were eluted, without vacuum, using 0.1% ammonium hydroxide in methanol (6 mL) and concentrated to 0.3 mL under a steady stream of nitrogen at 60 °C. The concentrates were diluted (1:1) with water (0.3 mL) and briefly vortexed. The sample extracts were then transferred to a 1.5 mL PP centrifuge tube and centrifuged at 14000 rcf for 20 min. A 0.3 mL aliquot was placed in a PP autosampler vial with PE septa and stored at 4 °C until analysis. Negative controls (LC−MS grade water blanks) were processed with each sample set. Additionally positive controls (PFC spiked LC−MS grade water, 5 ng/g) were also processed, 1 for every 40.

LC−MS/MS. The LC−MS/MS conditions were similar to those previously reported for the determination of PFCs in cow's milk. Fish extracts were analyzed using an Agilent 1100 HLPC interfaced with an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA). A 10 μL aliquot of the extract was injected into a Pursuit XRs C_{18} column (150 × 2.0 mm, 3.0 μm; Agilent Technologies). PFCs were separated using methanol and water (2 mM ammonium acetate, 5% methanol) with a gradient from 60 to 95% methanol during the first 12 min. Methanol (95%) was maintained for another 1.5 min before re-equilibrating the column for 8 min prior to the next injection. All separations were performed at a flow rate of 0.3 mL/min and a column temperature of 35 °C, resulting in PFCs eluting between 2 and 13 min.

The mass spectrometer was equipped with an electrospray ionization (ESI) source, operating in the negative ion mode with a capillary voltage of 1000 V, 20 psi N_2 nebulizer gas at a flow of 6 L/min, N_2 collision gas, and a temperature of 300 °C. Two mass transitions were monitored for each of the 10 analytes (Table 2) using time segmented multiple reaction monitoring (MRM). Analytes were quantified using the first transition listed in Table 2. Analyte confirmation required the presence of both transitions and that the relative intensity ratios were within ±20% of the calibration standard.

Additionally, for confirmation the second transition required a S/N peak intensity of >3.

RESULTS AND DISCUSSION

Determination of Method Detection Limits. When analyzing a variety of food matrices with a single method, it is often necessary to evaluate and characterize the method performance for each matrix. Therefore, to determine if the extraction method was applicable to all fish and shellfish or if each type should be considered as a different matrix, an abbreviated spike recovery study was performed. Homogenates (2 g) of 5 types of fish/shellfish (shrimp, striped bass, catfish, pollock, canned tuna) were fortified with 50 μL of the 100 ng/mL working stock solution (water/methanol 30/70). The homogenates were vortexed for ~5 s and the fortified tissue samples were then processed according to the extraction method described above. The recoveries are listed in Table 3 with % RSDs. The range of recoveries for all the fortified fish and shellfish samples was 90−105%, indicating that the method described above is suitable for different types of fish.

Based on the comparable recoveries detected for all the fish tested, a single fish type, catfish, was used for the determination of the method detection limits (MDL). The MDL for each PFC was determined using the procedure described in 40.CFR part 136 appendix B.[28] Briefly, two aliquots of ~25 g of catfish homogenate were fortified with PFC mixture to create one homogenate with ~1.5 ng of PFC/g of fish and one homogenate with ~4 ng of PFC/g of fish. For each spiked homogenate, ten 2 g aliquots were processed through the fish extraction method and analyzed to determine PFC concentration in each aliquot. The variance and standard deviation of the 10 replicates were then used to determine the MDL. Eight of the PFCs had MDLs under 0.9 ng/g, and the remaining two compounds, PFHxA and PFHpA, had MDLs of 1.14 and 1.28 ng/g respectively (Table 2). The limit of quantitation (LOQ) is estimated as 3 times the MDL values.

Method Validation. Method robustness was evaluated by determining the recovery of all the PFCs in 3 different fish and shellfish species, each fortified at three different concentrations based on the procedure described in the FDA Foods Program Guidelines for Chemical Methods.[29] The three fish/shellfish (shrimp, catfish, tuna) were chosen to represent the largest differences in the fat, moisture, and protein compositions of the 13 types of fish/shellfish evaluated. The FDA Foods Program Guidelines recommend fortification at three different concentrations based on the LOQ of each analyte. Due to the differences in the LOQ for the 10 PFCs, the 10 compounds were divided into two sets based on LOQ and then fortification concentrations were determined. Set A contained PFOS, PFHxA, and PFHpA, which were added to the fish at 3.5, 7, and 14 ng of PFC/g of fish. Set B contained the remaining PFCs, which were added to the fish at 1.5, 3, and 6 ng of PFC/g of fish. Recoveries for all spike concentrations were over 70% (shrimp data shown in Figure 1, tuna and catfish data shown in Supporting Information Figures SI.1 and SI.2 respectively) with the exception of PFHpA in the lowest concentration shrimp and catfish fortifications. These shrimp and catfish had average recoveries of 63% and 68% respectively. Although the PFHpA recoveries were low, the values had a high repeatability, with RSDs of less than 2% for shrimp and catfish. The exact cause of the low PFHpA recovery was not evident, but it is likely contributing the high MDL (1.28 ng/g) established for this analyte.

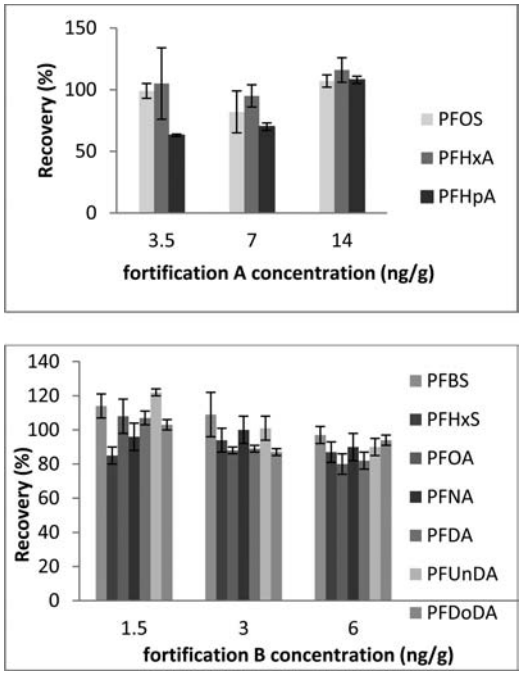

Figure 1. Fortification sets A and B, percent recoveries in shrimp.

Two NIST SRMs, 1946 (Lake Superior fish tissue) and 1947 (Lake Michigan fish tissue), were also analyzed using the method described in this paper. The SRMs are both adult lake trout (*Salvelinus namaycush*) collected in 1997, and reference values for only PFOS were established in 2012 (Table 4).[30,31]

Table 4. PFC Concentrations in SRMs 1946 and 1947 and NIST Reference PFOS Values[a]

SRM	NIST PFOS (ng/g)	PFOS (ng/g)	PFDA (ng/g)	PFUnDA (ng/g)
1946	2.19 ± 0.08	2.0* (16)	0.75* (4)	1.0* (24)
1947	5.90 ± 0.39	4.89 (13)	1.3* (70)	n/d

[a]An asterisk (*) designates values above MDL but below LOQ. % RSD is shown in parentheses.

Using the method described above, PFOS concentrations in SRM 1946 and 1947 were determined to be 2.0 and 4.89 ng/g, respectively. These concentrations are comparable to the NIST reference values, even though the PFOS concentration in SRM 1946 is below the method LOQ. PFDA and PFUnDA were the only other 2 PFCs detected in SRM 1946; PFDA was also detected in SRM 1947 (Table 4). The concentrations determined were below the LOQ of the method; however, the values are comparable to previously published results.[32] None of the remaining PFCs were detected, which is consistent with the reported results for both SRM 1946 and 1947. Although the nondetected analytes do not support the accuracy of the method, the lack of signal peaks for the undetected PFCs is further evidence, along with blank analyses, that there is not significant background signal from reagents or laboratory contamination.

Sample Analysis. Forty-six retail fish and shellfish samples were analyzed with the method detailed above; thirty-five (76%) of the samples had no detectable concentrations of PFCs. None of the catfish and salmon labeled as farm raised contained PFCs. However, given the number of samples with unknown origin and the small sample set, the data does not

Table 5. Fish/Shellfish Samples with Detected Concentrations of PFCs[a]

sample	PFHxS (ng/g)	PFOS (ng/g)	PFNA (ng/g)	PFDA (ng/g)	PFUnDA (ng/g)	PFDoDA (ng/g)
Crab						
Lanham						
homogenate 1	n/d	6.29 (30)	n/d	3.40 (16)	6.54 (10)	n/d
homogenate 2	n/d	n/d	n/d	0.73*	0.95*	n/d
Shrimp						
Orlando-1 (u)						
sub 1	n/d	0.97*	n/d	n/d	n/d	n/d
sub 2	n/d	n/d	n/d	n/d	n/d	n/d
Orlando-2 (w)						
sub 1	n/d	n/d	n/d	1.2*	2.47	n/d
sub 2	n/d	n/d	n/d	n/d	n/d	n/d
Memphis-1 (u)						
sub 1	n/d	3.26 (17)	1.2* (14)	0.93* (11)	n/d	n/d
sub 2	n/d	3.53	n/d	n/d	n/d	n/d
Memphis-2 (u)						
sub 1	n/d	2.6*	n/d	n/d	n/d	n/d
sub 2	n/d	2.3* (21)	n/d	n/d	n/d	n/d
Nashville (u)						
sub 1	n/d	4.86 (7)	n/d	0.76* (60)	0.99* (67)	n/d
sub 2	n/d	2.81	n/d	n/d	n/d	n/d
Striped Bass						
New York-1 (w)						
sub 1	n/d	1.5*	n/d	0.73*	1.4*	n/d
sub 2	n/d	1.1*	n/d	n/d	n/d	n/d
New York-2 (w)						
sub 1	n/d	2.91	1.4*	0.97*	2.17	1.1*
sub 2	n/d	2.0*	n/d	n/d	n/d	n/d
New York-3 (w)	n/d	3.60 (30)	n/d	n/d	n/d	n/d
New York-4 (f)	n/d	1.8* (65)	n/d	n/d	n/d	n/d
New York-5 (f)	0.66*	n/d	n/d	n/d	n/d	n/d

[a]An asterisk (*) designates value above MDL but below LOQ. % RSD is shown in parentheses. All concentrations are for fish wet weight. (f) indicates a farm raised sample. (w) indicates a wild caught sample. (u) indicates sample origin unknown.

represent a good comparison between wild and farm raised fish and shellfish. Table 5 summarizes the results of the 11 fish and shellfish samples that did contain detectable concentrations of PFCs. The only fish/shellfish with detectable concentrations of PFCs were shrimp, striped bass, and crab, with a crab homogenate having the highest PFC concentration (16.2 ng of total PFC/g of fish). PFOS was the most widely detected analyte, being measured in 9 of the 11 samples with detectable PFC concentrations. Additionally, PFOS was generally detected at higher concentrations than the other PFCs, except in crab homogenate #1, where PFOS and PFUnDA were measured at comparable concentrations (6.29 and 6.54 ng/g, respectively).

Subsamples, homogenates (crab and tuna), and replicate aliquots were analyzed throughout the study. Out of the 11 samples with detectable concentrations of PFCs, 8 had multiple subsamples or homogenates analyzed for all 10 PFCs, yielding a total of 80 paired results. A large percentage (74%) of the results showed no detectable PFCs in either subsample/homogenate; however, 21 of the paired results had detectable PFCs in one or both subsamples/homogenates (Table 5). Of these 21 detected pairs, 8 (10% of total) had concentrations that did not agree between subsamples/homogenates. These data indicate only a small variability between fillets of the same species purchased at the same location.

The results determined in this current work are similar to other published results for retail fish studies performed in Canada and Spain.[14,33−35] Tittlemier et al. have published multiple studies on Canadian salt and freshwater fish samples.[14,34,35] The Canadian total diet study analyzed composite samples of salt (haddock, cod, and sole) and freshwater (trout, pickerel) fish samples collected in 2004. Both fresh and saltwater composites had detectable concentrations of only PFOS, 2.0 and 2.6 ng/g respectively.[14] While none of the same fish species were tested in this study, PFOS concentrations ranged from >0.88 to 3.60 for both saltwater and fresh water finfish and are in agreement with values reported in the Canadian TDS. A second study with Canadian retail samples compared PFC concentrations in fish before and after cooking.[34] A raw catfish composite was analyzed and only PFOS was detected, with a concentration of 1.57 ng/g. None of the 13 catfish samples analyzed in this work had detectable concentrations of any PFC. A third study by Ostertag et al. analyzed clams collected in northern Canada and found only PFNA (0.5 ng/g),[35] which is comparable to the clam sample analyzed in this work (PFNA < 0.60 ng/g).

Ericson et al.,[33] using retail samples from the Catalan market in Spain, analyzed 4 groups of fish composites for the presence of PFOS, PFOA, and PFHpA. The 4 composites were white

fish (hake, whiting blue, sea bass, and monkfish), seafood (mussel and shrimp), canned fish (tuna, sardine, and mussel) and blue fish (salmon, sardine, and tuna). In agreement with the present study, Ericson and co-workers did not report detectable concentrations of PFOA or PFHpA in any of the composite samples. However, the PFOS concentrations determined by Ericson et al. were slightly lower than those found in the US retail samples. For example, PFOS in the Spanish shrimp/mussel composite was reported to average 0.148 ng/g, while the US retail samples ranged from <0.88 to 4.86 ng/g. Without further sample source and handling information it is difficult to determine what impact compositing and/or shrimp harvest location had on the determined PFOS concentrations.

Although there are no previous studies of PFC concentrations in US retail fish, an earlier publication of wild caught fish from the upper Mississippi River, where there are known sources of PFC emissions, did analyze carp fillets.[24] Of the 30 fish tested by Ye and co-workers, all contained PFOS at concentrations from 4.3 to 90 ng/g and over 73% contained PFDA, PFUnDA, and PFDoDA above the LOQ. However, a retail sample of tilapia fillets tested by Ye et al. did not contain detectable concentrations of PFCs and was used as their blank matrix. A comparison of the Ye et al. data to the current retail PFC concentrations indicates that there is a significant difference in PFC concentrations between retail and wild caught fish from locations with known sources of PFC emissions.

The current US study agrees with other retail studies from different countries, all finding PFCs to be either nondetectable in fish and shellfish or detectable in concentrations of parts per billion or less. The data presented, although only a limited sample size, does not indicate that consumption of US retail fish and shellfish represents a major pathway of consumer exposure to PFCs. Indeed 76% of the samples had no detectable concentrations of PFCs and the highest detected concentration of 6.29 ng/g of PFOS is significantly below the consumption advisory set forth by the Minnesota Department of Health (40 ng/g) for wild caught fish in Minnesota waters.[26] Clearly, in order to more accurately quantify the exposure, larger studies, focusing on retail samples of highly consumed fish, would be necessary.

■ ASSOCIATED CONTENT

Supporting Information

Figures illustrating % recoveries from the tuna and catfish fortification analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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