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Tissue-Specific Concentrations and Patterns of Perfluoroalkyl Carboxylates and Sulfonates in East Greenland Polar Bears

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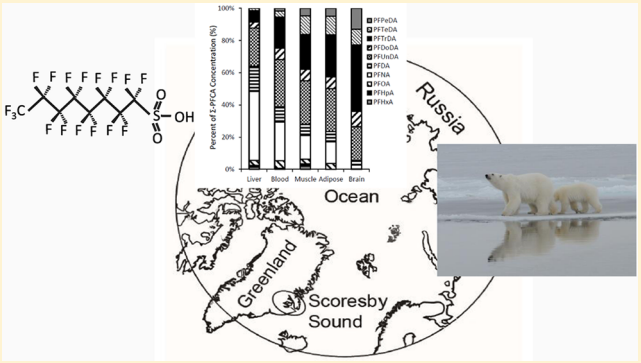
Supporting Information

ABSTRACT: Several perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs) of varying chain length are bioaccumulative in biota. However, wildlife reports have focused on liver and with very little examination of other tissues, and thus there is a limited understanding of their distribution and potential effects in the mammalian body. In the present study, the comparative accumulation of C_6 to C_{15} PFCAs, C_4, C_6, C_8 and C_{10} PFSAs, and select precursors were examined in the liver, blood, muscle, adipose, and brain of 20 polar bears (*Ursus maritimus*) from Scoresby Sound, Central East Greenland. Overall, PFSA and PFCA concentrations were highest in liver followed by blood > brain > muscle ≈ adipose. Liver and blood samples contained proportionally more of the shorter/medium chain length (C_6 to C_{11}) PFCAs, whereas adipose and brain samples were dominated by longer chain (C_{13} to C_{15}) PFCAs. PFCAs with lower lipophilicities accumulated more in the liver, whereas the brain accumulated PFCAs with higher lipophilicities. The concentration ratios (±SE) between perfluorooctane sulfonate and its precursor perfluorooctane sulfonamide varied among tissues from 9 (±1):1 (muscle) to 36 (±7):1 (liver). PFCA and PFSA patterns in polar bears indicate that the pharmacokinetics of these compounds are to some extent tissue-specific, and are the result of several factors that may include differing protein interactions throughout the body.



INTRODUCTION

Poly- and per-fluoroalkyl substances (PFASs) are some of the newer emerging contaminants that have been detected in Arctic biotic and abiotic compartments, and retrospective temporal reports have shown that in general their levels have been steadily increasing since the 1970s.[1−3] The two groups of PFASs that are of concern in Arctic ecosystems are perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs).[1,3] In general, reports have shown that PFSAs with chain lengths > C_6 and PFCAs > C_8 tend to bioaccumulate and biomagnify in aquatic food webs.[2] Transportation of PFCAs and PFSAs to the Arctic is thought to occur via two main pathways: atmospheric and oceanic transport. Due to their high water solubilities, PFCAs and PFSAs are transported directly to the Arctic via oceanic transport.[4] Alternately, atmospheric pathways transport volatile precursors like fluorotelomer alcohols (FTOHs), fluorotelomer unsaturated carboxylates (FTUCAs), and perfluoroalkyl sulfonamides (PFOSAs) to the Arctic, where they can degrade to PFCAs or PFSAs either via atmospheric oxidation, abiotic hydrolysis, abiotic photolysis, or biotic degradation (among other processes).[1,5−8]

Polar bears (*Ursus maritimus*) are apex predators and may serve as indicators of pollution effects at higher trophic levels in Arctic marine ecosystems. It has been reported that polar bears contain some of the highest PFAS concentrations in their liver relative to other wildlife worldwide, and in particular for the highly bioaccumulative perfluorooctane sulfonate (PFOS).[1−3] Among subpopulations, polar bears from East Greenland have among the highest PFOS concentrations as well as for PFCAs.[9] Recent exponential increases (from about 2000 to 2006) have been reported for perfluorodecanoic acid (PFDA), perfluorotridecanoic acid (PFTrDA) and PFOS (as high as 6340 ng/g ww) in the livers of East Greenland polar bears.[10] Finally, numerous contaminant and biomarker correlative studies suggest that East Greenland polar bears are at risk from

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persistent organic pollutant (POP)-mediated immune and reproductive effects.[3]

The majority of PFCA and PFSA (and precursor) studies have focused on liver concentrations due to high hepatic concentrations, whereas a few (including human studies) have examined concentrations in blood.[9,11,12] For mammalian wildlife, the distribution of PFCAs and PFSAs in tissues and body compartments within a whole animal has only been reported in liver, plasma, and select tissues in harbor seals (*Phoca vitulina*) and harbor porpoises (*Phocoaena phocoena*) from Europe, and bottlenose dolphins (*Tursiops truncates*) from the southeastern United States.[13−16] In Arctic mammalian wildlife, limited PFSA and PFCA data are available for the liver, blood, and blubber of ringed (*Pusa hispida*) and bearded seals (*Erignathus barbatus*) from the Canadian Arctic.[17]

While the transport and distribution mechanisms of PFCAs, PFSAs, and their precursors throughout the body have not been fully elucidated, laboratory exposure studies have shown that PFCAs (e.g., perfluorooctanoic acid; PFOA) and PFSAs (e.g., PFOS) tend to accumulate in protein-rich tissues, such as the liver, blood, and yolk sac.[13,18] The presence of PFOS in liver and plasma has been shown to be associated with proteins such as serum albumin and fatty acid binding proteins.[19,20] We recently reported that, specifically within regions of the brain of polar bears, longer-chain PFCAs (C_{10} − C_{15}) are strongly correlated with extractable lipid content.[21] In previously reported biotic studies, only nonsignificant correlative relationships have been reported between lipid content and, for example, PFOS concentrations in liver or plasma samples.[22−24] The present study is an expanded comparison of the distribution and patterns of PFCAs and PFSAs and selected precursors in the body of highly exposed East Greenland polar bears by examination in the liver, blood, muscle, adipose, and brain.

EXPERIMENTAL SECTION

Standards and Chemicals. Perfluoroalkyl sulfonates (C_4, C_6, C_8, C_{10}), perfluoroalkyl carboxylates (C_6−C_{15}), and precursor compounds including perfluoroalkyl sulfonamides (FOSA, Me-FOSA), fluorotelomer alcohols (6:2, 8:2, 10:2 FTOHs), and perfluoroalkyl unsaturated carboxylates (6:2, 8:2, 10:2 FTUCAs) (all >98% purity) were purchased from Wellington Laboratories (Guelph, ON, Canada). Isotopically labeled standards were added to all samples for an internal standard reference. A complete list of all target compounds and internal standards can be found in SI Table S1. All solvents used were HPLC grade, including methanol obtained from Caledon Laboratories (Georgetown, ON, Canada) and Milli-Q water, treated on-site.

Sample Collection. All tissue samples were taken from 20 polar bears (14 males, 6 females) from the region of Scoresby Sound (69°-74°N, 20°-25°W), East Greenland between January and March 2006. Samples included liver tissue (n = 19), whole blood (n = 19), somatic muscle (n = 20) and adipose tissue (n = 20). Subcutaneous fat samples were collected from the base of the tail (rump fat), and muscle samples were collected from the sternocleidomastoid muscle in the neck. In addition, eight different regions of the brain were sampled as well: pons and medulla (n = 14), cerebellum (n = 15), frontal cortex (n = 16), occipital cortex (n = 17), temporal cortex (n = 15), striatum (n = 11), thalamus (n = 8), and hypothalamus (n = 4). The brain tissue remaining after dissection (n = 15) was also collected for PFAS analysis. Samples were collected on-site less than one hour post-mortem and kept frozen (−20 °C) until their arrival at the Institute of Bioscience (Aarhus University, Roskilde, Denmark). After the subsequent dissectioning, subsamples were shipped frozen to Environment Canada (Ottawa, Canada) and were kept at −40 °C until the time of analysis.

Ages of the 20 bears ranged from 3 to 19 years for males, and from 4 to 15 years for females. Ages were determined by counting the annual growth layer in the cementum of the I_3 tooth after decalcification, thin layer sectioning (14 μm), and staining with toluidine blue, as previously described.[25]

PFAS Sample Extraction and Lipid Determination. All samples were spiked with 10 ng of isotopically labeled internal standards. Internal standards were used to help quantify data, as well as calculate percent recoveries. Labeled standards were available for all target compounds with the exception of PFSAs (C_4, C_{10}) and PFCAs (C_7, C_{13}, C_{14}, C_{15}). For the compounds for which no labeled standard was available, a surrogate was used. Surrogates were chosen based on the closest retention times such that PFBS and PFHpA used ^{13}C-PFHxA as a surrogate; PFDS used ^{13}C-PFUnDA as a surrogate; and PFTrDA, PFTeDA, and PFPeDA used ^{13}C-PFDoDA as a surrogate.

Extraction and analysis of all PFAS target compounds followed Chu and Letcher and Greaves et al.[21,26] Briefly, either 0.1 g (liver) or 1.0 g (brain, adipose, muscle, blood) of tissue was homogenized with 10 mM potassium hydroxide in 80/20 (v/v) acetonitrile/water. The PFASs were then extracted from the solution using Oasis WAX cartridges to give two fractions: the first (neutral) fraction contained the FTOHs, and the FOSAs; the second (acidic) fraction contained the PFCAs, PFSAs, and FTUCAs. Full details on the cleanup process via solid phase extraction can be found in Greaves et al.[21]

Furthermore, the lipid content was determined for all samples. The method used to extract the lipid from the liver, brain, muscle, and adipose has previously been described,[21,27] and is detailed in the Supporting Information (SI).

PFAS Instrumental Analysis. The first fraction containing all neutral PFASs was analyzed using HPLC-APPI(−)-MS/MS. The second fraction containing all acidic PFASs was analyzed using HPLC-ESI(−)-MS/MS. Analysis was done using a Waters 2695 HPLC coupled with a Waters Quatro Ultima triple quadrupole, and has previously been described.[21] The column used was an ACE C18, 50 mm L × 2.11 mm i.d., 3 μm particle size, from Canadian Life Science (Peterborough, ON, Canada). Quantification of the target compounds by calibration curves and labeled internal standards permitted the correction of any ionization suppression or enhancement that may have occurred as a result of matrix-dependent effects such as coeluting ions. All PFAS quantification was done using MassLynx 4.0 (Waters, 2002).

Quality Assurance and Quality Control. Method limits of quantification (MLOQs) were based on signal-to-noise ratios of 10, or approximately 3 times the coefficient of variation of the noise. MLOQs ranged from 0.1 to 0.2 ng/g ww in all tissues for PFCAs and C_4, C_6, and C_{10} PFSAs, and 3.1 ng/g ww for PFOS. Analytes were identified based on their relative chromatographic retention times to authentic reference standards, and their characteristic multiple reaction monitoring transitions (SI Table S1).

Prior to extraction, all samples were spiked with 10 ng of each isotopically labeled internal standard. Relative response factors were generated based on identically spiked calibration

Table 1. Arithmetic Mean Concentrations (ng/g ww ± SE) and Tissue Lipid Contents for All Detected Compounds[a]

Chemical Abbreviation	Chemical Trivial Name	Tissue	Liver (n = 19)	Blood (n = 19)	Brain (n = 115)	Muscle (n = 20)	Adipose (n = 20)
	Lipid Content (% ± SE):		6.04 ± 0.61	1.36 ± 0.13	11.2 ± 0.4	1.61 ± 0.46	63.8 ± 1.4
		Perfluoroalkyl Carboxylates (PFCAs)					
PFHxA	perfluorohexanoic acid		18.5 ± 0.6	nd	nd	0.27 ± 0.05	nd
PFHpA	perfluoroheptanoic acid		8.39 ± 1.36	0.50 ± 0.06	nd	0.19 ± 0.02	nd
PFOA	perfluorooctanoic acid		39.2 ± 3.4	3.53 ± 0.31	0.27 ± 0.06	0.42 ± 0.05	0.42 ± 0.10
PFNA	perfluorononanoic acid		497 ± 44	17.8 ± 1.6	1.87 ± 0.14	1.99 ± 0.18	1.57 ± 0.23
PFDA	perfluorodecanoic acid		184 ± 17	7.05 ± 0.79	2.12 ± 0.13	1.00 ± 0.10	0.74 ± 0.14
PFUnDA	perfluoroundecanoic acid		276 ± 24	21.8 ± 2.6	16.0 ± 0.7	3.67 ± 0.37	3.07 ± 0.45
PFDoDA	perfluorododecanoic acid		41.5 ± 3.3	5.16 ± 0.57	7.21 ± 0.33	0.98 ± 0.08	0.84 ± 0.13
PFTrDA	perfluorotridecanoic acid		82.1 ± 7.2	14.3 ± 1.5	31.4 ± 1.5	2.96 ± 0.23	3.05 ± 0.34
PFTeDA	perfluorotetradecanoic acid		18.0 ± 0.7	2.66 ± 0.14	7.45 ± 0.34	1.56 ± 0.22	1.32 ± 0.15
PFPeDA	perfluoropentadecanoic acid		0.81 ± 0.08	1.35 ± 0.13	10.4 ± 0.5	0.65 ± 0.07	0.57 ± 0.07
Σ-PFCA			1160 ± 90	74.3 ± 6.9	77.0 ± 3.3	13.7 ± 0.9	11.7 ± 1.4
		Perfluoroalkyl Sulfonates (PFSAs)					
PFHxS	perfluorohexane sulfonate		30.9 ± 2.1	18.0 ± 1.1	1.37 ± 0.10	1.87 ± 0.11	1.55 ± 0.20
PFOS	perfluorooctane sulfonate		3270 ± 290	128 ± 17	35.2 ± 2.0	15.9 ± 1.7	15.4 ± 1.9
PFDS	perfluorodecane sulfonate		nd	1.28 ± 0.22	1.30 ± 0.15	1.40 ± 0.04	nd
Σ-PFSA			3310 ± 300	147 ± 18	38.0 ± 2.0	19.4 ± 1.8	18.0 ± 2.1
		Perfluoroalkyl Sulfonamides (PFOSAs)					
FOSA	perfluorooctane sulfonamide		151 ± 32	3.50 ± 0.99	1.33 ± 0.10	1.56 ± 0.13	0.69 ± 0.10
		Fluorotelomer Unsaturated Carboxylates (FTUCAs)					
6:2FTUCA	2H-perfluoro-2-octenoic acid		0.98 ± 0.22	nd	nd	0.02 ± 0.01	nd
8:2FTUCA	2H-perfluoro-2-decenoic acid		1.13 ± 0.12	nd	nd	nd	nd
10:2FTUCA	2H-perfluoro-2-dodecenoic acid		nd	nd	nd	0.08 ± 0.02	nd
Σ-PFAS			4630 ± 390	225 ± 24	116 ± 5	34.5 ± 2.7	30.1 ± 3.5

[a]All compounds reported were found above the method limit of quantification (MLOQ) in at least 50% of the samples for a given tissue. nd, Not detected.

curve solutions, allowing percent recoveries to be calculated. As a result, PFAS concentrations were inherently recovery corrected. Percent recoveries for all detected compounds ranged from 51.9% ± 1.0 (PFHxA) to 84.9% ± 1.8 (PFUnDA) (SI Table S1).

A sample blank was run every 10 samples to minimize background interference. Sample blanks were treated identically to all other samples, except no tissue was added to the vial. Traces of PFHxA (0.90 ng/g ww), PFHpA (0.80 ng/g ww), PFOA (0.92 ng/g ww), PFNA (1.04 ng/g ww), PFDA (0.93 ng/g ww), and PFUnDA (1.03 ng/g ww) were consistently (>70%) found in sample blanks. Therefore, background correction was done for all PFHxA, PFHpA, PFOA, PFNA, PFDA, and PFUnDA concentrations using the background levels established by sample blanks. All other PFAS concentrations were not background corrected, due to their absence from sample blanks. A control sample was also run every 10 samples to minimize day to day fluctuations in the lab, as well as to ensure reproducibility. Control samples were prepared using a National Wildlife Research Center (NWRC) in-house standard reference material consisting of pooled bird egg homogenate from double-crested cormorants (*Phalacrocorax auritus*) collected from the Great Lakes in northeastern North America in 2003. Control samples showed good reproducibility with an RSD of 13% for Σ-PFCA concentrations, and 18% for Σ-PFSA concentrations.

At NWRC, the Organic Contaminants Research Laboratory (OCRL) regularly participates in interlaboratory QA/QC exercises for PFAS analysis. Regular QA/QC exercises include participation in the Northern Contaminants Program interlab exercises, including in 2009, 2010, 2011, and 2012. For a more detailed account of QA/QC exercises, please see the SI.

Data Analysis. All statistical analyses were done using *Statistica 8.0* (StatSoft, 2008; Tulsa, OK). The normality of data was ensured by $\log_{10}$-transformation, followed by a Shapiro-Wilks test, with $p > 0.05$ indicating normality. Compounds for which more than half of the samples in a given tissue were below the MLOQ were not statistically analyzed. For all other compounds where the majority of samples were above the MLOQ, those samples below the MLOQ were assigned a concentration between 0 and the MLOQ, proportionally based on the sample's PFOS concentration so that the replacement values were proportional to the sample's overall contamination level. .

Correlations between PFAS concentrations and lipid content were done using correlation matrices, with a significance level $p \leq 0.05$ indicating statistical significance, and Pearson coefficient $r \geq 0.5$ indicating a strong correlation. Sex and age trends were examined using multivariate linear regression, with $\alpha = 0.05$. Statistical concentration differences between tissues were examined using one-way ANOVA, followed by a Tukey HSD test ($\alpha = 0.05$).

Brain samples consisted of eight different compartments. However, for the purposes of this paper, all brain samples were averaged per bear for all statistical analyses to give a representation of the entire brain. Information on PFAS concentration and pattern differences between brain compartments can be found in Greaves et al.[21]

RESULTS AND DISCUSSION

Relationship between PFSA and PFCA Concentrations and Extractable Lipid Content. A complete list of concentrations for all detected compounds can be found in Table 1. Significant correlations between lipid content and

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long-chain PFCAs (C_{10}–C_{15}) were found across the whole brain ($p \leq 0.047$), and are discussed in more detail in Greaves et al.[21] The liver also showed statistically significant correlations between extractable lipid content and PFOS, PFNA, and PFDA concentrations ($p \leq 0.032$) (Figure 1, SI Table S3). However, blood, muscle, and adipose tissue only showed a weak and nonsignificant ($p > 0.05$) correlations with lipid content.

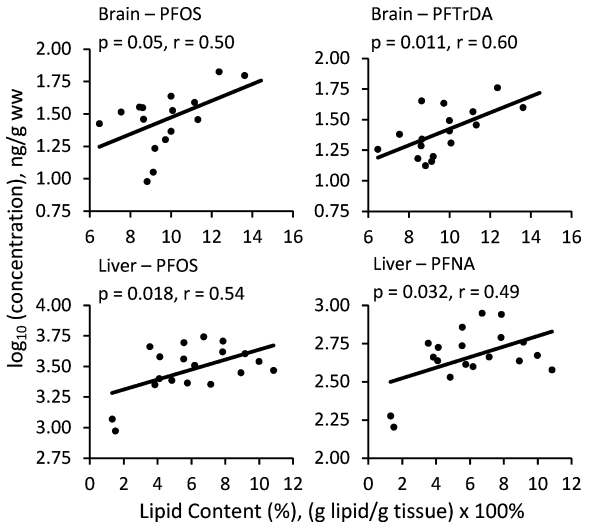

Figure 1. Correlative relationships between extractable lipid content and perfluoroalkyl carboxylate and sulfonate concentrations in both the brain (top), and liver (bottom). See Table 1 for the full chemical names of the abbreviations PFTrDA, PFOS, and PFNA.

Correlations between PFAS concentrations and extractable lipid content were not previously observed in studies on polar bear and mink (*Mustela vison*) liver or bottlenose dolphin (*Tursiops truncatus*) plasma[22−24] Longer-chain PFCAs greatly resemble saturated fatty acids. We hypothesize that the lipid correlations seen in the brain and liver are a result of PFCAs mimicking fatty acids. Within the brain, we had previously hypothesized that long-chain PFCAs associate with lipid-binding proteins that interact with the blood-brain barrier (BBB); when the lipid-binding proteins arrive at the BBB to replenish fatty acids in the brain, the long-chain PFCAs are transported into the brain.[21] Additionally, the liver has many lipid-related functions, including the synthesis of triglycerides and lipoproteins.[28] It is possible that due to their resemblance to fatty acids, the liver provides a suitable chemical environment for PFSAs and PFCAs, thus potentially explaining the correlative relationships.

Effects from Sex and Age. We did not find any statistically significant differences between males and females for any PFAS in adipose, muscle, or brain samples. However, in blood, significantly higher concentrations of PFHpA were found in males, whereas PFNA was significantly higher in females. In the liver, PFHxS was found to be significantly higher in females (SI Table S4). All other compounds showed no sex differences for any tissue studied. These sex differences may be partially attributable to the uneven sex distribution (14 males, 6 females), and the limited number of females under study. The majority of samples showed no differences between accumulation profiles in males and females, similar to what was found by Smithwick et al.[11] As a result, further analysis of the data considered the present sample of bears as one group.

Other groups of POPs found in high concentrations in polar bears from East Greenland include the lipophilic polychlorinated biphenyls (PCBs) and chlordanes (CHLs).[29,30] These lipophilic compounds accumulate in lipid-rich tissue such as adipose, and have been found to leave the body through gestation and lactation in females.[3] Furthermore, lipophilic POP concentrations are often correlated with the lipid content in tissues.[31] As a result, sex differences are often seen for PCB and CHL concentrations. Maternal transfer of PFASs has been observed in mammals,[24,32] however the relative lack of gender differences observed for PFASs in these polar bears suggests that gestation and lactation are not major depuration pathways for females.

Both the brain and muscle showed significant correlations between Σ-PFCA concentrations and age (SI Figure S1). Significant correlations between Σ-PFSA concentrations and age were observed for the brain, muscle, and blood (SI Figure S1). These findings are similar to other polar bear studies in the past decade where significant PFOS age effects were shown in polar bear liver samples,[9,11] strengthening the argument that PFOS has a long half-life in the body and is retained in the body through enterohepatic recirculation.[33]

The exact half-lives of PFCAs and PFSAs in polar bears are not known, although half-lives in other animals have been reported to vary significantly. For example, PFOS has been shown to have half-lives ranging from 30 to 103 days in male Sprague–Dawley rats (*Rattus norvegicus*), 21 weeks in bottlenose dolphins, 5.4 years in humans, and 5.6 years in harbor seals.[5,34]

Polar bears undergo large fluctuations in mass and dietary consumption every year. During the hibernating winter months, polar bears undergo extreme fasting and adipose tissue deposits may be reduced as low as 10% of body mass.[35] Following hibernation, polar bears quickly feed on prey to regain body mass. Therefore, modeling the half-life and bioenergetics associated with accumulation and depuration of POPs is not straightforward. Regardless, the fact that PFCA and PFSA tissue concentrations increases with the age of the bear indicates that polar bears accumulate PFCAs and PFSAs faster than the clearance rate of excretion.

Distribution of PFCAs. Large tissue differences were seen in the contamination patterns of the PFCA concentrations. For instance, PFNA accounted for 42.6% of the overall PFCA burden in liver samples, whereas it only accounted for 2.4% of the overall PFCA burden in brain samples (Figure 2). Similarly, although in reverse, PFPeDA accounted for 13.1% of the overall PFCA burden in brain samples, whereas it only accounted for 0.07% of the overall PFCA burden in liver samples. As a result, the dominant PFCA varied from tissue to tissue, such that short/medium chain lengths (i.e., C_9–C_{10}) were proportionally higher in the liver and medium/longer-chain lengths (i.e., C_{13}–C_{15}) were proportionally higher in the brain. The dominant PFCA was PFNA (43%) in the liver, PFUnDA (29%) and PFNA (24%) in the blood, PFUnDA (27% and 26%) and PFTrDA (22% and 26%) in muscle and adipose, respectively, and PFTrDA (41%) in the brain. This pattern indicates that PFCAs are regionally distributed in the body partially based on their chain length.

Jing et al.[36] recently reported the experimentally determined octanol–water partitioning coefficients (log K_{OW} values) of PFCAs. There was a clear relationship between the chain length of the PFCA and its lipophilic behavior: longer chain lengths possessed higher log K_{OW} values, indicating that they have an

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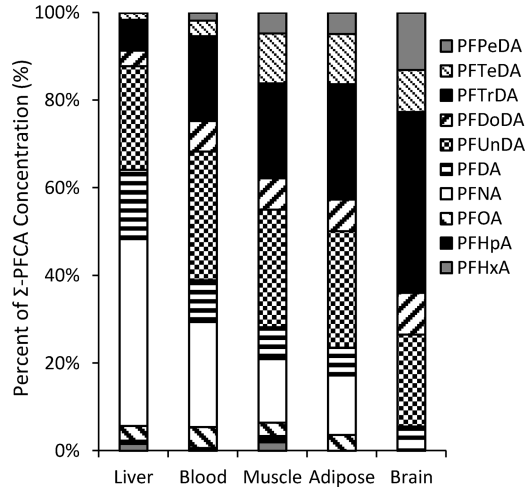

Figure 2. Percent compositions of Σ-perfluoroalkyl carboxylate (Σ-PFCA) concentrations from 20 East Greenland polar bears sampled in 2006. PFCA: $CF_3(CF_2)_nCOOH$. See Table 1 for the full chemical names of the abbreviations of the C_6–C_{15} chain length PFCAs.

increased solubility in nonpolar, lipid-soluble environments. PFCAs with lower lipophilicities accumulate in the liver, whereas PFCAs with higher lipophilicities accumulate in the brain. This relationship is not related to the lipid content found in the tissues, since muscle and adipose have roughly similar PFCA distribution patterns, although extremely different lipid contents (1.61% vs 63.8%, respectively). This favorably supports the hypothesis that tissue differences may be attributed to localized proteins that preferably bind to one specific chain length, and therefore direct the PFCA in question to a specific body region and tissue.

Since many proteins are to some extent tissue-specific, it is possible that different length PFCAs are distributed protein-specifically and thus tissue-specifically. For example, chylomicrons and very low density lipoproteins (VLDLs) are formed in the liver to transport fatty acids from the intestines to the liver, and the liver to adipose, respectively. Newsted et al.[37] showed that PFOS associated with VLDLs in the yolk of northern bobwhite quails (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*). It is therefore possible that long-chain PFCAs, greatly resembling saturated fatty acids, are transported from the liver to adipose tissue thus explaining their low liver levels and high adipose levels.

It is interesting to note that of the few tissue distribution studies reported to date, most showed inconsistent trends relating to the dominant PFCA by tissue. Other large marine mammals show very little tissue-to-tissue fluctuation in PFCA patterns. Ahrens et al.[13] reported on the whole body burden of PFASs in harbor seals from the German Bight and found that the dominant PFCA for all tissues studied was exclusively either PFNA or PFDA. In contrast, herring gulls (*Larus argentatus*) from the Great Lakes in northeastern America were recently shown to have varying chain-length dominance between tissues, similar to this study.[38] The reason for these large interspecies differences remains unclear, but may in part be due to differences in tissue-specific proteins, diet, excretion capabilities, and pathways, as well as exposure as a consequence of different geographical locations.

Distribution of PFSAs. For all tissues, PFOS was by far the most dominant PFSA, accounting for between 87% (blood) and 99% (liver) of all PFSAs detected. The average PFOS concentration in the liver was 3270 ng/g ww, whereas the next largest PFOS concentration was in the blood at 128 ng/g ww, an almost 26-fold difference (Table 1). This extreme dominance of PFOS has been seen in other arctic wildlife studies, and can partially be explained by its large bioconcentration factor, estimated between 1100 and 5400.[39] In the present polar bears, the clear dominance of PFOS in the liver compared to other tissues seems to indicate that either (a) the liver is more exposed to PFOS than other tissues, (b) the liver has a larger PFOS bioconcentration factor than other tissues, and/or (c) the metabolism of PFOS precursor compounds (e.g., FOSA) is much higher in the liver than other tissues. Although all three options are perhaps contributing factors, PFOS has been found to mimic bile acids and as a result undergo enherohepatic recirculation,[33] thus making it difficult to leave the liver.

Most toxicity studies have focused on the effects of PFOS, particularly in the liver and the brain. In the liver, PFOS and PFOA have been shown to be both peroxisome proliferators, and tumor promoters.[34,40] Yu et al.[41] also recently showed that hepatic exposure to PFOS increased the expression of both the organic anionic transporter protein (OATP2), as well as the multidrug resistance-associated protein (MRP2) in rats. Increased expression of OATP2 and MRP2 may lead to increased thyroid hormone metabolism, ultimately leading to structural brain damage, developmental delay and various neurological effects.[41] In the brain, prenatal PFOS exposure in rats lead to the up-regulation of glial fibrillary acidic protein (GFAP) and S100 calcium binding protein B (S-100β).[42] At low concentrations, these proteins act to promote brain development and maintain a homeostatic environment. However, at high concentrations, S-100β can lead to neuro-inflammation and neuronal dysfunction.[42] These studies suggest that exposure to PFOS will result in various toxicity effects, depending on the tissue exposed. Dietz et al.[10] showed that levels of, for example, PFOS in polar bears have been quickly increasing from 1996–2006, despite the recent voluntary phase-out of C_8–PFOSF chemistry by the 3M Company in 2002. These recent increases in PFAS concentrations stress the importance of understanding the pharmacokinetics of these compounds in the body, and their toxicity effects.

Distribution of Precursor Compounds. Of all the precursor compounds studied, the only compound to be consistently found above the MLOQ in all tissues was FOSA. The liver by far contained the highest concentrations of FOSA (151 ng/g ww), with a concentration more than 40 times higher than in blood, which was the second most contaminated tissue (3.50 ng/g ww).

The fluorotelomer unsaturated carboxylates (6:2, 8:2, and 10:2 FTUCAs) were found in the majority of liver and muscle samples, although at very low levels (Table 1). Very few studies have been able to quantify FTUCAs in biota, and are often only detected in lower trophic position species.[43,44] FTUCAs have been shown to biologically degrade to PFCAs,[6] and as a result of degradation processes, are often not detected in high trophic position species. The detection of FTUCAs in polar bear liver and muscle raises questions concerning their apparent biotransformation potential. Direct comparisons to polar bear prey (i.e., ringed seal, *Phoca hispida*, blubber) would be highly beneficial in understanding food web transfer and any potential biotransformation. Correlations between FTUCA and PFCA concentrations were examined with no significant correlations

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found. This may suggest that oceanic transport plays a larger role in PFCA localization in the arctic than atmospheric transport.

Relationship between PFOS and FOSA Concentrations. PFOS is the major PFAS in the environment. It has been found worldwide, in mammals, birds, and fish and is most often the PFAS found at highest concentrations. It can be emitted into the environment directly from sources, as well as from less stable compounds, such as FOSA, that can degrade to PFOS.[5]

To determine the relationship between PFOS and FOSA, only samples that had both PFOS and FOSA concentrations above the MLOQ were analyzed, accounting for 81% of all samples. The brain, blood, adipose, liver, and muscle all showed significant positive correlations ($p \leq 0.038$) between PFOS and FOSA concentrations. Ratios of PFOS to FOSA ranged from 9 (±1):1 (muscle) to 36 (±7):1 (liver) (Table 2). Although the reason for the ratio differences between tissues remains unclear, these findings indicate perhaps one or more of the following: (a) tissues such as the liver and blood preferentially accumulate PFOS more strongly than other tissues; (b) the liver and blood may have more difficulty eliminating PFOS compared to other tissues; and/or (c) the liver may proportionally convert more FOSA to PFOS compared to other tissues. The exact mechanism by which FOSA is converted to PFOS has yet to be confirmed,[5] although Benskin et al.[8] showed that two enzymes from the cytochrome P450 family, CYP2C9 and CYP2C19, were majorly responsible for the metabolism of *N*-ethyl perfluorosulfonamide (*N*-EtFOSA) to PFOS in human liver microsomes. These CYPs are mostly present in the liver, for the purpose of oxidizing xenobiotic compounds. Although CYPs have been detected in tissues other than the liver, CYP2C9 and CYP2C19 have not been detected in the blood, and have been detected in very small quantities in the brain.[45,46] Consequently, the majority of their activity (and thus N-EtFOSA metabolism) occurs in the liver.

Table 2. Arithmetic Mean Ratio (± SE) between Perfluorooctane Sulfonate (PFOS) and Perfluorooctane Sulfonamide (FOSA) Concentrations in East Greenland Polar Bears[a]

Tissue	PFOS to FOSA Ratio (±SE)	Significance Level, p	Pearson Coefficient, r
Muscle (n = 18)	9 (±1): 1	0.029	0.51
Adipose (n = 15)	18 (±2): 1	0.033	0.55
Brain (n = 100)	25 (±1): 1	0.0001	0.79
Blood (n = 12)	29 (±4): 1	0.038	0.60
Liver (n = 19)	36 (±7): 1	0.023	0.53

[a]A significance level, $p < 0.05$ indicated statistical significance, and a Pearson coefficient, $r > 0.50$ indicated a strong correlation between PFOS and FOSA concentrations.

The PFOS to FOSA ratio in the liver (36.4 ± 6.7) is much lower than in previous studies of polar bears from East Greenland. Smithwick et al.[9] and Dietz et al.[10] both reported PFOS to FOSA ratios on the order of 200:1 to 250:1. However, these studies quantified the concentration of FOSA by using a surrogate internal standard (e.g., ^{13}C-Me-FOSA). The FOSA concentrations determined in this study were confirmed by HPLC-Q-TOF (quadrupole time-of-flight, SI Table S2). For further information regarding FOSA quantification, please refer to the SI. This data indicates that previous FOSA quantification using ^{13}C-Me-FOSA as an internal standard is more than likely underestimated, and that comparisons between FOSA data generated prior to 2010 should perhaps not be directly compared to FOSA data generated post 2010.

Estimated PFAS Tissue Burdens. The total PFAS tissue burdens were estimated for all tissues analyzed, with the exception of muscle (Table 3). To our knowledge, there has not been a study to correlate total skeletal muscle mass with overall polar bear body length or body mass. As a result, no reasonable skeletal muscle mass could be estimated. Estimates were based on tissue mass correlations from numerous sources.[35,47−49] The total body mass of the polar bears in this study was not recorded. However, the total brain mass was recorded for 16 of the 20 bears. Using eq 1 (below), a total body mass estimate was generated for each bear. Equations

Table 3. Total Tissue Burden (μg ± SE) Estimates of Per- and Polyfluoroalkyl Substances in the Liver, Blood, Adipose, And Brain of East Greenland Polar Bears[a]

	Liver	Blood	Adipose	Brain
Mass	8.87 kg	16.6 kg	51.0 kg	0.39 kg
PFHxS	285 ± 23	302 ± 29	82.3 ± 13.9	0.54 ± 0.04
PFOS	30,110 ± 3540	2,180 ± 360	856 ± 155	15.1 ± 1.0
PFDS	70.8 ± 25.0	22.8 ± 4.5	64.4 ± 21.2	0.52 ± 0.07
Σ-PFSA	30,460 ± 3,570	2,510 ± 390	1,010 ± 170	16.2 ± 1.1
PFHxA	166 ± 12	nd	nd	nd
PFHpA	89.3 ± 13.2	8.51 ± 1.28	nd	nd
PFOA	355 ± 44	60.2 ± 6.5	23.6 ± 6.7	0.11 ± 0.03
PFNA	4,540 ± 530	294 ± 28	90.2 ± 17.5	0.77 ± 0.06
PFDA	1,710 ± 213	115 ± 14	44.4 ± 10.8	0.89 ± 0.06
PFUnDA	2,250 ± 320	351 ± 45	174 ± 36	6.81 ± 0.37
PFDoDA	382 ± 47	84.5 ± 11.1	47.7 ± 9.9	3.04 ± 0.16
PFTrDA	752 ± 101	240 ± 34	164 ± 27	13.4 ± 0.8
PFTeDA	162 ± 13	44.3 ± 3.8	68.6 ± 10.0	3.13 ± 0.17
PFPeDA	7.29 ± 0.90	23.0 ± 3.0	29.5 ± 5.1	4.39 ± 0.24
Σ-PFCA	10,720 ± 1210	1,220 ± 130	649 ± 111	32.7 ± 1.7
PFOSA	1,390 ± 400	58.9 ± 22.6	39.8 ± 6.5	0.57 ± 0.05
Σ-PFAS	42,400 ± 4,910	3,780 ± 510	1,680 ± 290	49.3 ± 2.6

[a]Average tissue masses were estimated based on eqs 1−4.

1−4, shown below, were used to approximate the tissue masses of all bears.

$$\text{mass brain(kg)} = \text{total body mass(kg)} \times 0.192\%(47) \quad (1)$$

$$\text{mass liver(kg)} = 0.087(\text{total body mass, kg})^{0.087}(48) \quad (2)$$

$$\text{mass blood(kg)} = 0.086(\text{total body mass, kg})^{0.99}(49) \quad (3)$$

$$\text{mass adipose(kg)} = \text{total body mass(kg)} \times 25\%(35) \quad (4)$$

As can be seen in Table 3, the liver contained the highest burden of PFASs (42 400 μg ± 4900 μg), followed by blood (3780 μg ± 510 μg), adipose (1680 μg ± 290 μg), and finally brain (49.3 μg ± 2.6 μg).

Gebbink et al.[27] estimated tissue burdens of various lipophilic organohalogen compounds such as polychlorinated biphenyls (PCBs), chlordanes (CHLs), and polybrominated diphenyl ethers (PBDEs), in polar bears from East Greenland. These OHCs have been shown to be endocrine disruptors causing thyroid hormone disruption (PCBs), serum vitamin A depletion (PCBs), decreased size of male sexual organs (PCBs, CHLs, and PBDEs), as well as immune suppression (PCBs), among others.[3] Most OHCs are lipophilic, and as such accumulate in fatty tissue such as adipose, over less fatty tissues. As a result, they found the total burdens of Σ-PCBs and Σ-CHLs in polar bear adipose tissue to be 840 mg and 134 mg, respectively.[27] These adipose burdens by far surpass the Σ-PFAS burden in adipose tissue found in this study (1.7 mg). However, the liver, blood and brain burdens of Σ-PCBs (25 mg, 1.5 mg, 18 μg, respectively) and Σ-CHLs (30 mg, 0.2 mg, 1.5 μg, respectively) are somewhat comparable to the Σ-PFAS burdens found in this study (42 mg, 3.8 mg, 49 μg, respectively), indicating that overall the liver, blood and brain are exposed to similar levels of PFASs, PCBs, and CHLs.

Implications. PFAS distribution in the body is largely unknown for wildlife including those from the Arctic. The results of the present study showed that large differences in tissue contaminant patterns exist in East Greenland polar bears. Extractable lipid content was examined as a possible causal factor for these differences. In the case of brain, and somewhat in liver, PFCA concentrations were correlated with extractable lipid. However, this relationship was not seen in blood, muscle, or adipose. Research is warranted in assessing what other factors, such as associations with particular proteins, degradation, and excretion are driving the observed PFAS accumulation differences in tissues. These differences in PFAS levels and patterns among the different tissues are of potential toxicological concern. PFAS-induced toxicity has been observed, often with different end points, depending on the exposed tissue. These tissue-specific PFAS patterns indicate possible tissue-specific absorption, metabolism, and excretion rates, perhaps due to different protein interactions from tissue to tissue. Many proteins in the body are to some extent tissue-specific, and therefore, by binding more favorably to one chain-length over another, or to one functional group over another, PFASs may distribute themselves in the body based on these protein affinities.

Select studies have examined the interaction of certain PFASs (e.g., PFOS, PFOA, FOSA) and select proteins, such as serum albumin, serum steroid binding proteins, lipoproteins, and CYP proteins, although these studies have focused on only a few compounds. Future work should consider examining the binding affinities of these proteins with an extensive suite of PFASs, so that it may help explain the tissue-specific pharmacokinetics observed in this study. Other future work should include analyzing PFAS concentrations in East Greenland ringed seals, in order to directly compare predator and prey. By understanding the PFAS profile in their prey, we can gain knowledge regarding the biotransformation, bioaccumulation, and metabolism of PFASs in polar bears.

■ ASSOCIATED CONTENT

Supporting Information

Additional information is available concerning tissue lipid determination, the quantification of FOSA, and past QA/QC exercises performed by the OCRL. Details regarding percent recoveries, method limits of quantification, and multiple reaction monitoring transitions are also provided. Finally, all significant lipid correlations, sex differences, and age trends are also provided. 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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