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Identification of Long-Chain Perfluorinated Acids in Biota from the Canadian Arctic

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Recently it was discovered that humans and animals from various urban and remote global locations contained a novel class of persistent fluorinated contaminants, the most pervasive of which was perfluorooctane sulfonate (PFOS). Lower concentrations of perfluorooctanoate, perfluorohexane sulfonate, and heptadecafluorooctane sulfonamide have also been detected in various samples. Although longer perfluoroalkyl carboxylates (PFCAs) are used in industry and have been detected in fish following a spill of aqueous film forming foam, no studies have been conducted to examine the widespread occurrence of long-chain PFCAs (e.g., $CF_3(CF_2)_xCOO^-$, where $x > 6$). To provide a preliminary assessment of fluorinated contaminants, including PFCAs, in the Canadian Arctic, polar bears, ringed seals, arctic fox, mink, common loons, northern fulmars, black guillemots, and fish were collected at various locations in the circumpolar region. PFOS was the major contaminant detected in most samples and in polar bear liver was the most prominent organohalogen (mean PFOS = 3.1 μg/g wet weight) compared to individual polychlorinated biphenyl congeners, chlordane, or hexachlorocyclohexane-related chemicals in fat. Using two independent mass spectral techniques, it was confirmed that all samples also contained ng/g concentrations of a homologous series of PFCAs, ranging in length from 9 to 15 carbons. Sum concentrations of PFCAs (∑PFCAs) were lower than total PFOS equivalents (∑PFOS) in all samples except for mink. In mink, perfluorononanoate (PFNA) concentrations exceeded PFOS concentrations, indicating that PFNA and other PFCAs should be considered in future risk assessments. Mammals feeding at higher trophic levels had greater concentrations of PFOS and PFCAs than mammals feeding at lower trophic positions. In general, odd-length PFCAs exceeded the concentration of even-length PFCAs, and concentrations decreased with increasing chain length in mammals. PFOS and PFCA concentrations were much lower for animals living in the Canadian Arctic than for the same species living in mid-latitude regions of the United States. Future studies should continue to monitor all fluorinated contaminants and examine the absolute and relative toxicities for this novel suite of PFCAs.

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Introduction

The pervasive contamination of wildlife (*1*−*7*) and the general human population (*8*, *9*) with perfluorinated acids and heptadecafluorooctane sulfonamide (FOSA) has been described in many recent publications. Perfluorooctane sulfonate (PFOS) is the dominant perfluorinated acid in wildlife samples, while perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and FOSA are detected only occasionally and at lower concentrations. In human serum, PFOA (mean = 6.4 ng/mL) and PFHxS (mean = 6.6 ng/mL) (*8*) are generally present at higher concentrations than in wildlife, perhaps indicating additional exposure through contact with commercial products containing perfluorinated acids or their derivatives.

These widespread observations quickly led to a voluntary manufacturing phase-out by the main producer of PFOS (*10*, *11*) and garnered the attention of national and international environmental protection agencies. Perfluorinated acids are a concern because they have no known route of biotic or abiotic degradation in the environment and are bioaccumulative when the perfluorinated chain reaches a length of between 6 and 7 carbons (*12*, *13*). The health effects associated with long-term exposure to perfluorinated acids are uncertain but are the subject of a current risk assessment (*14*). In animal studies and in vitro tests, certain perfluorinated acids are well characterized as potent peroxisome proliferators (*15*), as inhibitors of gap-junction intercellular communication (*16*), and as tumor promoters (*17*).

Perfluoroalkyl carboxylates (PFCAs), such as perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA), are used as polymerization aids in the manufacture of fluorinated polymers (*18*), yet there are few reports of their environmental distribution, except for PFOA. Furthermore, PFCAs ranging in chain length from 2 to 13 carbons (some evidence for longer ones) were identified as minor thermolysis products of poly(tetrafluoroethylene) (*19*, *20*), and the environmental significance of this observation has yet to be established. The only report of long-chain (i.e., >C8) PFCAs in biota is for fish collected from a creek following a spill of aqueous film forming foam (AFFF) (*21*). PFNA, PFDA, perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA), and perfluorotetradecanoate (PFTA) were detected in the livers of these fish. However, given that these chemicals are not listed as active ingredients in AFFF, it is probable that the spill was not their source. The unknown environmental distribution of PFCAs represents a serious knowledge gap that must be examined given their persistence, bioaccumulation potential, and potential to cause adverse toxicological effects.

In this study, we examine for the presence of fluorinated organics in the tissue of biological specimens collected from the Canadian Arctic. Liquid chromatography coupled with tandem and high-resolution mass spectrometry

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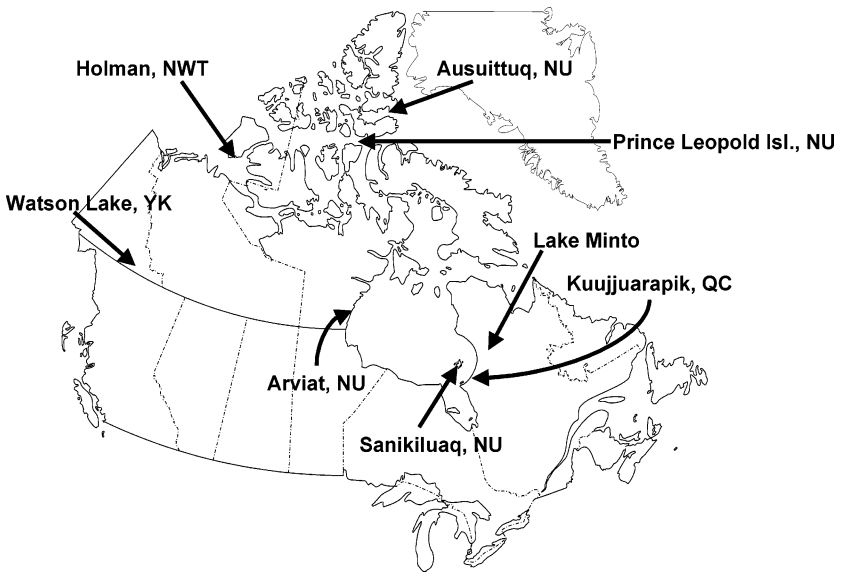

FIGURE 1. Map of Canada showing sampling locations in the circumpolar region.

(LC/MS/MS) revealed that PFOS, FOSA, PFHxS, and PFOA are not the only fluorinated organic contaminants of biological significance. Using standardized criteria for identification, we have confirmed and quantified several novel long-chain PFCAs in animals from remote areas. The toxicological implications for wildlife and people in the Canadian Arctic are unknown but are linked given that many of these animals serve as important traditional foods for indigenous peoples in the Canadian north.

Methods

Sample Collection. Sample collection was performed by subsistence hunters and trappers. The location and year of sample collection are shown in Figure 1 and Table 1. Ringed seals from Holman/Ulukhaqtuuq, NT, were collected in 2001, while seals from Ausuittuq/Grise Fjord, NU, were collected in spring of 1998 as part of the Northwater Polynya study (*22*). Common loons were collected around Kuujjuarapik, QC, in 1992, while northern fulmars and black guillemots were collected from Prince Leopold Island in 1993. Arctic fox were collected from Arviat in March, 2001 (*23*). Fish samples were collected from the mouth of the Great Whale River at Kuujjuarapik and in Lake Minto, Quebec, in July 2002 with the assistance of the local Cree Trappers Association. Samples of mink from the Yukon were collected by trappers in winter 2001/2002 from the southwestern part of the Territory east of Watson Lake, in the area of Blind Lake and Crow River. Polar bears were collected in February 2002 in eastern Hudson Bay, near Sanikiluaq, by Inuit hunters. Whole or partial liver samples were immediately removed from the animal at the time of collection and stored in clean glass containers lined with hexane-rinsed aluminum foil or directly in aluminum foil packaging. Samples were shipped frozen and remained frozen at −20 °C until the time of analysis.

Standards and Reagents. Potassium PFHxS (99.9%), FOSA (99.9%), and potassium PFOS (86.4%) were provided by the 3M Co. (St. Paul, MN). Standards of perfluoroheptanoic acid (PFHpA, 99%), PFOA (98%), PFNA (97%), PFDA (98%), PFUnA (95%), PFDoA (95%), and PFTA (97%) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Ammonium acetate (98%) and tetrabutylammonium hydrogensulfate (TBAS) were purchased from Sigma-Aldrich, anhydrous sodium carbonate (99.8%) from J. T. Baker (Phillipsburg, NJ), and methyl-*tert*-butyl ether (MTBE) from EM Science (99.5%, Gibbsburg, NJ).

Analysis of Perfluorinated Acids by LC/MS/MS. A small amount of tissue (0.5 g) was removed from the whole liver or partial liver for analysis. To minimize any potential contamination, the liver was cut open and an internal sample was removed for analysis. Liver tissue was then 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 TBAS (0.5 M adjusted to pH 10), and 100 μL (25 ng) of the internal standard, PFHpA. The resulting homogenates were extracted with 5 mL of MTBE by shaking vigorously for 10 min, followed by centrifugation to isolate the organic phase. The MTBE supernatant was collected in a separate plastic tube, and this extraction process was repeated once more, combining the supernatants. The MTBE was blown to dryness under high-purity nitrogen gas, and the analytes were taken up in 1 mL of 50:50 water/methanol by vortexing for 30 s. The concentrates were then filtered through 0.2 μm nylon filters into polypropylene vials for analysis.

Routine quantitative instrumental analysis and analyte confirmation by secondary mass transition analysis were performed by LC/MS/MS using previously described conditions (*12, 13*). Water and methanol solvents (0.01 M ammonium acetate) were delivered at a total flow rate of 250 μL min^{-1} by a Waters 600S controller, and samples were injected (10 μL) with a Waters 717 plus autosampler (Waters, Milford, MA). Chromatography was performed on a Genesis C8 column (2.1 × 50 mm, Jones Chromatography, Lakewood, CO). Initial mobile phase conditions were 80:20 water/methanol, followed immediately by an 8 min ramp to 0:100, a 2 min hold, and reverting to initial conditions at 10 min. The detector was a Micromass Ultima (Micromass, Manchester, U.K.) triple quadrupole mass spectrometer equipped with an electrospray source operating in negative ion mode. Data were acquired by tandem mass spectrometry using a multiple reaction monitoring (MRM) method that monitored one to three mass transitions (parent → daughter ion) for each compound (Table 2). The desolvation temperature was 210 °C, and the source block was maintained at 150 °C. Desolvation gas flow was between 600 and 700 L h^{-1}, and the capillary voltage was always 2.75 kV.

Accurate mass measurements were performed on a QSTAR quadrupole-time-of-flight (QTOF) mass spectrometer (MDS Sciex, Concord, ON, Canada) equipped with an electrospray ionization source and accurate mass capabilities.

TABLE 1. Mean, Minimum, and Maximum Concentration (ng/g) of All Fluorinated Contaminants in All Liver Samples Analyzed[a]

	site and year		PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrA	PFTA	PFPA	PFOS	FOSA
polar bear ($n = 7$)	Sanikiluaq, NU	mean	8.6	180	56	63	6.2	11	0.51	<0.5	3100	12
Ursus maritimus	2002	min	2.9	108	35	56	4.7	7.5	<0.5	<0.5	1700	<0.5
		max	13	230	76	78	8.2	14	1.1	<0.5	>4000	44
arctic fox ($n = 10$)	Arviat, NU	mean	<2.0	22	14	13	1.5	2.2	<0.5	n.d.	250	19
Alopex lagopus	2001	min	<2.0	2.2	1.9	0.78	<0.5	<0.5	<0.5	n.d.	6.1	<0.5
		max	<2.0	86	72	55	4.8	7.1	1.9	n.d.	1400	110
ringed seal ($n = 9$)	Holman, NT	mean	<2.0	5.9	2.1	3.3	0.44	0.57	<0.5	n.d.	16	0.36
Phoca hispida	(Ulukhaqtuuq)	min	<2.0	3.3	0.98	1.4	<0.5	<0.5	<0.5	n.d.	8.6	<0.5
	2001	max	<2.0	8.8	3.1	5.4	0.74	0.94	<0.5	n.d.	23	0.52
ringed seal ($n = 10$)	Grise Fjord, NU	mean	<2.0	4.9	2.9	3.8	0.76	0.95	n.d.	n.d.	19	2.0
Phoca hispida	(Ausuittuq)	min	<2.0	2.4	2.1	2.0	0.56	0.68	n.d.	n.d.	10	<0.5
	1998	max	<2.0	8.1	3.8	5.9	1.3	1.6	n.d.	n.d.	37	5.5
mink ($n = 10$)	Yukon	mean	<2.0	16	3.7	4.3	<0.5	<0.5	n.d.	n.d.	8.7	1.4
Mustela vison	Watson Lake Area	min	<2.0	2.0	0.69	<0.5	<0.5	<0.5	n.d.	n.d.	1.3	<0.5
	2001	max	<2.0	35	9.0	12	0.76	0.83	n.d.	n.d.	20	2.4
common loon ($n = 5$)	Kuujjuarapik, QC	mean	<2.0	<0.5	<0.5	1.3	<0.5	0.88	<0.5	<0.5	20	5.9
Gavia immer	1992	min	<2.0	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	11	2.0
		max	<2.0	<0.5	0.55	2.2	0.74	1.5	<0.5	<0.5	26	13
northern fulmar ($n = 5$)	Prince Leopold Isl.,	mean	<2.0	<0.5	n.d.	<0.5	<0.5	<0.5	n.d.	n.d.	1.3	n.d.
Fulmarus glacialis	NU	min	<2.0	<0.5	n.d.	<0.5	<0.5	<0.5	n.d.	n.d.	1.0	n.d.
	1993	max	<2.0	0.50	n.d.	<0.5	<0.5	<0.5	n.d.	n.d.	1.5	n.d.
black guillemot ($n = 5$)	Prince Leopold Isl.,	mean	<2.0	n.d.	n.d.	<0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cepphus grylle	NU	min	<2.0	n.d.	n.d.	<0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	1993	max	<2.0	n.d.	n.d.	<0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
white sucker ($n = 3$)	Kuujjuarapik, QC	mean	<2.0	1.0	2.6	6.2	1.3	2.7	0.76	n.d.	7.6	13
Catostomus commersoni	2002	min	<2.0	0.61	1.7	3.9	0.65	1.4	<0.5	n.d.	6.5	10
		max	<2.0	1.7	3.1	8.5	1.8	3.7	1.2	n.d.	8.6	18
brook trout ($n = 2$)	Kuujjuarapik, QC	mean	<2.0	6.2	2.5	5.7	1.5	1.4	0.27	n.d.	39	2.8
Salvelinus fontinalis	2002	min	<2.0	5.9	2.3	4.9	0.83	1.1	0.22	n.d.	29	2.0
		max	<2.0	6.5	2.8	6.5	2.2	1.7	0.32	n.d.	50	3.5
lake whitefish ($n = 2$)	Kuujjuarapik, QC	mean	<2.0	3.2	1.5	3.7	1.2	5.5	1.7	n.d.	12	14
Coregonus clupeaformis	2002	min	<2.0	2.4	1.2	2.7	0.69	2.7	1.1	n.d.	12	14
		max	<2.0	4.0	1.8	4.7	1.8	8.3	2.3	n.d.	12	15
lake trout ($n = 1$)	Lac Minto, QC		<2.0	3.4	2.0	6.1	2.30	4.8	0.63	n.d.	31	6.8
Salvelinus namaycush	2002											
northern pike ($n = 1$)	Kuujjuarapik, QC		<2.0	<0.5	2.0	2.9	0.83	1.3	0.35	n.d.	5.7	8.7
Esox lucius	2002											
Arctic sculpin ($n = 1$)	Kuujjuarapik, QC		<2.0	2.2	0.52	1.1	0.55	1.7	<0.5	n.d.	12	18
Myoxocephalus scorpioides	2002											

[a] The species, site and time of collection, and sample number (n) are also shown.

Samples were injected manually to the same column used for routine analysis, and an Agilent 1100 capillary LC system (Agilent, Palo Alto, CA) delivered a gradient elution of methanol and water. Mass calibration was performed externally.

PFHpA was spiked into each homogenate as an internal standard before extraction after determining that it was not detectable in unspiked tissue. Mean recovery of all analytes from all biota samples was greater than 80%, based on triplicate analysis of livers (0.5 g) spiked with each compound (~500−1000 ng), indicating accurate recovery and that significant electrospray ionization suppression was negligible. Quantification was performed based on the relative response of each analyte to PFHpA using a standard curve constructed from known quantities of standards extracted from water in the same manner as tissue samples. Concentrations were not adjusted for the purity of standards. Standard injections were made every six to nine samples to monitor sensitivity drift, and the coefficient of determination for standard curves always exceeded 0.98 between 2.5 and 1000 pg of analyte injected. This range was appropriate for quantification of all analyte concentrations in all animals except for PFOS in polar bears. Detectable responses that resulted in concentrations below our lowest standard were reported as <0.5 ng/g, while any response having a signal-to-noise ratio less than 3 was reported as nondetectable (n.d.). For calculation of mean concentrations, a concentration of 0.25 ng/g was arbitrarily assumed for concentrations reported as <0.5 ng/g. For analysis of PFOS in polar bears, it was necessary to add a higher standard (equivalent of 10 ng injected). Unfortunately, this resulted in nonlinearity at high concentrations, and thus any PFOS concentration exceeding the highest standard by 2-fold was reported as >4000 ng/g. This situation arose for 3 out of 7 polar bear samples analyzed in this study.

An instrumental blank response was always present for PFOA using the Waters 600 pump, which made quantification of PFOA difficult or impossible in most animals due to our resulting method detection limit of 2 ng/g. The background response could be minimized and stabilized, but not eliminated, by reducing the column equilibration time between sample injections. The precise source of the contamination is unknown but was localized to the liquid chromatograph and is presumably a result of PFCAs leaching out from internal fluorinated polymers. For example, PFCA salts are reportedly utilized as fluoropolymer polymerization aids (*18*). The Agilent 1100 capillary LC system did not have any measurable background contamination.

Results and Discussion

Confirmation of PFCAs in Biota. Tentative detection of a homologous series of PFCAs in biota was originally based on retention time and a decarboxylation mass transition corresponding to an authentic standard (Figure 2). Confirmation of analyte identity was then performed by comparing the relative response of two secondary mass transitions (i.e., parent→daughter) to the primary decarboxylation transition. For all analytes, the relative response of all secondary mass

TABLE 2. Confirmation Error and Criteria for Analyte Accurate Mass Determination and for the Ratio of Secondary to Primary Tandem Mass Spectrometry Transition for a Polar Bear Extract

	accurate mass determination		ratio of secondary to primary MS/MS transitions				
analyte	calculated mass	QTOF mass (ppm error)	mass transition[a]	polar bear ratio ($n = 5$)	authentic standard ratio	% error	% allowed[b]
PFOA	412.9663	412.9679 (3.9)	(413>369)[1]				
PFNA	462.9631	462.9650 (4.1)	(463>419)[1]				
			(463>0.219)[2]	0.130	0.133	5.1%	30%
			(463>169)[2]	0.047	0.050	6.2%	50%
PFDA	512.9599	512.9606 (1.4)	(513>469)[1]				
			(513>269)[2]	0.169	0.169	0.28%	30%
			(513>219)[2]	0.185	0.188	1.6%	30%
PFUnA	562.9567	562.9567 (0.0)	(563>519)[1]				
			(563>269)[2]	0.210	0.214	1.8%	25%
			(563>219)[2]	0.159	0.164	3.3%	30%
PFDoA	612.9536	612.9564 (4.6)	(613>569)[1]				
			(613>319)[2]	0.199	0.186	6.6%	30%
			(613>269)[2]	0.188	0.175	6.7%	30%
PFTrA	662.9505	662.9640 (20)	(663>619)[1]				
			(663>319)[2]	detected	*c*		
			(663>269)[2]	detected	*c*		
PFTA	712.9472	<LOD	(713>669)[1]				
			(713>219)[2]	0.035	0.029	16%	50%
			(713>169)[2]	0.014	0.014	1.5%	50%
PFPA	762.9440	not detected	(763>719)[1]				
			(763>169)[2]	nondetect	*c*		
			(763>319)[2]	nondetect	*c*		
PFOS	498.9303	498.9278 (5.0)	(499>99)[1]				
PFHxS	398.9365	398.9408 (11)	(399>99)[1]				
FOSA	497.9461	497.9477 (3.2)	(498>78)[1]				
			(498>169)[2]	0.028	0.035	26%	50%

[a] Primary transition is indicated by superscript 1; secondary transition is indicated by superscript 2. [b] Tolerance according to ref *24* based on percent abundance relative to primary transition. [c] No authentic standard was available.

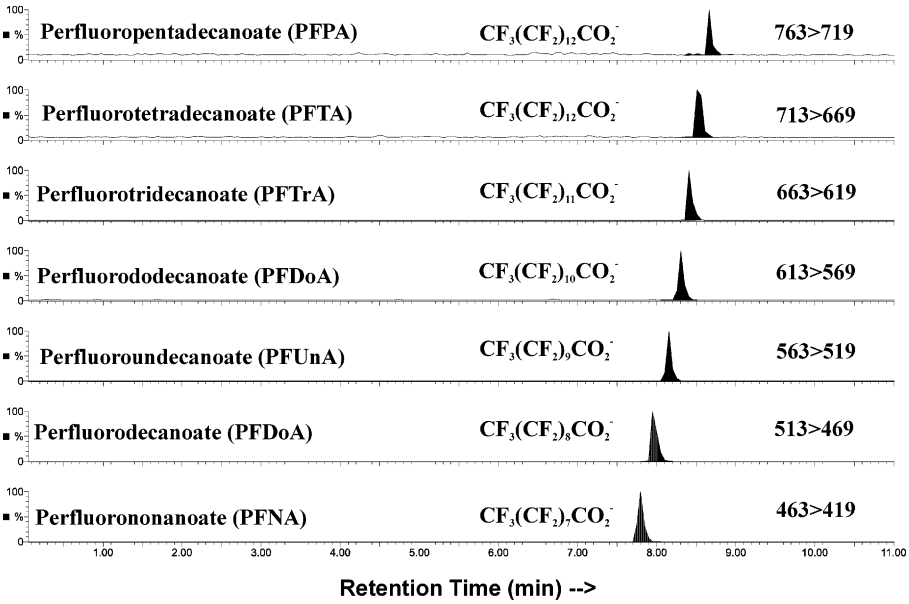

FIGURE 2. Tandem mass spectrometry chromatogram of polar bear extract showing the homologous series of PFCAs detected and later confirmed.

transitions in a polar bear extract were very close to those produced by injection of an authentic standard, and the error was within the tolerable limits defined by the Commission of the European Communities (*24*). Perfluorotridecanoate (PFTrA) and perfluoropentadecanoic acid (PFPA) could not be confirmed by this method due to a lack of authentic standards; however, secondary mass transitions analogous to other PFCAs were detected for PFTrA, and retention times were appropriate for both PFTrA and PFPA relative to PFTA (i.e., PFTrA eluted before PFTA, while PFPA eluted after PFTA) (Figure 2). FOSA was also confirmed in biota based on retention time and the relative intensity of two mass transitions relative to an authentic standard (Figure 2); a third transition could not be identified in standards or biota.

Confirmation of all analytes was further attempted by accurate mass measurements using liquid chromatography coupled to the QTOF mass spectrometer. For most PFCAs, the resultant accurate mass measurements were within 5 parts per million (ppm) of the calculated mass (Table 2), resulting in confident confirmation. For PFTrA and PFHxS, instrument response was near to detection limits, thus resulting in only tentative confirmation due to poor ion

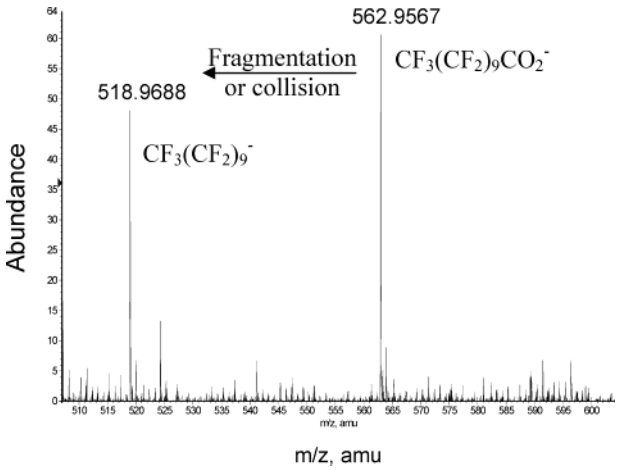

FIGURE 3. Full scan high-resolution mass spectrum for a polar bear extract showing the accurate mass associated with the negative molecular ion of PFUnA (*m/z* 562.9567) and of its characteristic fragment or daughter ion (*m/z* 518.9688).

statistics and a relatively large error (i.e., 20 and 11 ppm, respectively). The full scan mass spectra achieved by QTOF provided further evidence that the detected masses were indeed PFCAs due to the observation of a diagnostic fragment, or daughter ion. For PFNA, PFDA, PFUnA, and PFDoA, the decarboxylated negative ion was detected at the appropriate accurate mass. An example of this is shown in Figure 3 for PFUnA. Unfortunately, PFTA and PFPA could not be detected above background on the QTOF instrument, and thus no additional confirmation could be achieved for these acids. Overall, however, the weight of evidence suggested that long-chain PFCAs, ranging from PFOA to PFPA, are present in biota from the Canadian Arctic.

Concentrations in Biota. Although there is great spatial variation in the samples analyzed, it can be generalized from the data that mammals feeding at higher trophic levels had higher concentrations of PFOS and PFCAs than mammals feeding at lower trophic levels. The same general conclusions were made by Giesy and Kannan (*7*) for PFOS concentrations in a survey of global wildlife. Polar bear livers had the highest concentration of each perfluorinated acid among all animals analyzed. The concentration of PFOS always exceeded 1 μg/g (mean PFOS = 3.1 μg/g) in the polar bears livers, making PFOS the most prominent individual organohalogen contaminant detected in polar bears to date. There are very few reports of persistent organochlorine concentrations in polar bear liver with which to compare directly; however, PFOS liver concentrations exceed individual polychlorinated biphenyl (PCB) congeners, chlordane components, and hexachlorocyclohexane isomers quantified in polar bear subcutaneous fat (*25*). The concentrations of PFOS in polar bear liver reported here are also 10-fold higher than those reported by Giesy and Kannan (*7*) for Alaskan polar bears (mean = 350 ng/g). Sanikiluaq Island is at considerably lower latitude and perhaps closer to regional sources of PFOS. Due to the high method detection limit for PFOA, polar bears were the only animals to have quantifiable concentrations of PFOA, ranging from 2.9 to 8.6 ng/g. The homologous series of longer PFCAs, ranging in length from 9 to 15 carbons, were also detected in polar bears (Figure 2). The mean concentration of individual PFCA homologues in polar bear liver ranged from <0.5 to 180 ng/g and generally decreased with increasing perfluorinated chain length. The concentrations of these novel PFCAs in polar bear liver are at least an order of magnitude lower than that of PFOS, but PFNA, PFDA, and PFUnA concentrations exceed PFOA and FOSA concentrations (Table 1).

In general, PFOS was the major fluorinated contaminant in all animals, except for mink, followed by PFNA. The concentrations of PFOS exceeded PFNA concentrations by between 3- and 20-fold in most animals. However, for unknown reasons, average mink PFNA concentrations were approximately twice as high as PFOS ($n = 10$), although both concentrations were relatively low. PFOA could not be detected above the method detection limit (2 ng/g) in any animal except polar bear. Given the relative concentration of PFOA in polar bears, however, it may be reasonable to assume that PFOA concentrations are similar to PFDoA concentrations for most other animals. Overall, it may be generalized that PFOA is only a minor contributor to the overall burden of PFCAs in all biota samples. For example, in polar bear liver, PFOA concentrations were more than an order of magnitude lower than PFNA concentrations and contributed only 3% to total PFCAs.

The dominant PFCA detected in all mammals was PFNA, and concentrations generally decreased for all other PFCA homologues with increasing perfluoroalkyl chain length. This trend is contrary to the bioaccumulation potential of PFCAs, whereby bioaccumulation increases with increasing perfluoroalkyl chain length (*12*, *13*), thus suggesting that the abiotic environmental burden of PFCAs is probably skewed toward the shorter PFCAs, such as PFNA. Although not always statistically significant, a trend was observed in all animals whereby mean odd-chain-length PFCA concentrations exceeded the mean concentrations of the corresponding shorter, even-chain-length PFCAs. For example in mammals, PFUnA (C11) concentrations were statistically greater than PFDA (C10) concentrations in both ringed seal populations ($\alpha = 0.05$), but not in polar bears ($p = 0.13$) or fox ($p = 0.59$). Furthermore, PFTrA (C13) concentrations were statistically greater than PFDoA (C12) concentrations in arctic fox and polar bears ($\alpha = 0.05$), but not in Holman ($p = 0.063$) or Grise Fjord ($p = 0.067$) ringed seals. These trends can be visualized by examining the contamination profile for polar bears and ringed seals (Figure 4). The contamination profile for birds and fish was different from that observed in mammals (Table 1). The dominant PFCA in northern fulmars, common loons, and all fish was PFUnA, not PFNA, and there were lower concentrations of longer and shorter homologues alike (Table 1). However, birds and fish were similar to mammals in that the same general odd/even pattern was observed (i.e., PFUnA > PFDA, and PFTrA > PFDoA) and was statistically significant among common loons and fish ($\alpha = 0.05$).

The reason for the difference in contamination profiles of mammals and birds or fish is unknown, but the consistent odd−even pattern may be indicative of the source(s). For example, fluorotelomer alcohols (FTOHs) are only manufactured in even chain lengths but have been reported to yield even- and odd-chain-length PFCAs upon degradation. For instance, 8:2 FTOH (i.e., $CF_3(CF_2)_7CH_2CH_2OH$) degraded to PFOA and PFNA, and 10:2 FTOH (i.e., $CF_3(CF_2)_9CH_2CH_2OH$) degraded to PFDA and PFUnA in wastewater treatment sludge (*26*). Metabolism of FTOHs is an unlikely source of PFCAs to the Arctic because, although FTOHs are detectable in the atmosphere (*27*) and degrade slow enough to allow for long-range transport, FTOHs are not expected to be deposited to the biosphere (*28*). A more feasible source of PFCAs to the Arctic involves tropospheric oxidation of FTOHs; however, the atmospheric degradation products of FTOHs have not yet been determined, and this suggestion remains tentative. An alternative potential source is the thermolysis of poly(tetrafluoroethylene), which produces gas-phase PFCAs ranging from trifluoroacetic acid to PFTA (*19*, *20*); however, further study would be required to determine if such a source could lead to long-range transport and the observed odd−even contamination profile.

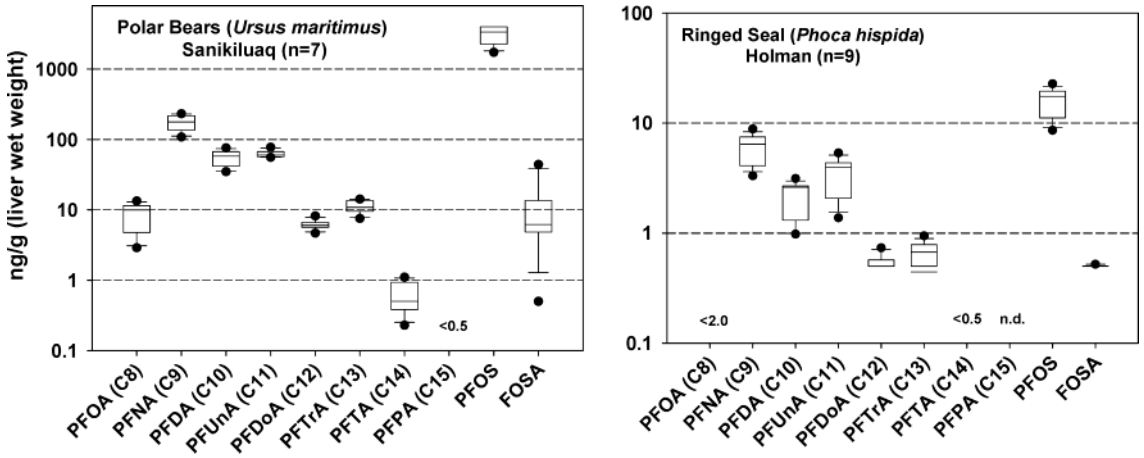

FIGURE 4. Box-plot profile for fluorinated contaminants in polar bears (Sanikiluaq) and ringed seal (Holman), showing the 5th, 10th, 25th, 75th, 90th, and 95th centile of concentrations and the median (middle bar).

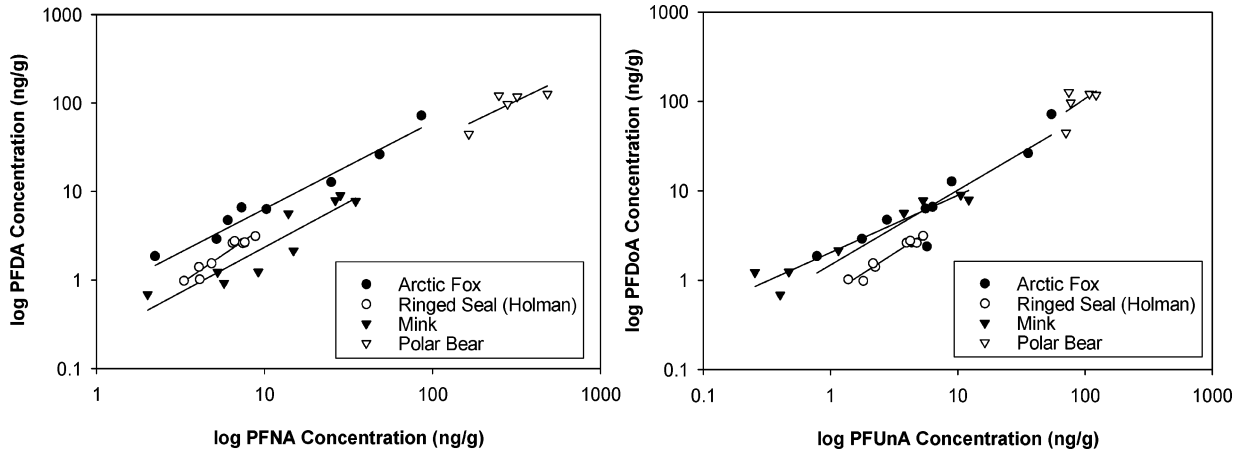

FIGURE 5. Linear regression of individual PFCA homologue concentrations in arctic fox, ringed seal, mink, and polar bear. A log transformation was performed to normalize the data.

There was very little variation of all analyte concentrations in both ringed seal and polar bear liver within each population. For the most part, the difference between the maximum and minimum concentration was less than a factor of 3. The same was not true for either arctic fox or mink liver. In these animals, there was generally more than an order of magnitude separating the minimum from the maximum concentration (Table 1). This variation is presumably a function of feeding habits for these species. For example, arctic fox are opportunistic feeders that may be influenced by terrestrial and/or marine food webs, depending on food availability, and this can influence chlorinated contaminant concentrations (*23*). Mink are also exposed to contaminants through both the terrestrial and the aquatic food web (*29*). Sex did not influence contamination levels in any organism, as determined by simple *t*-tests ($\alpha = 0.1$).

The only neutral fluorinated contaminant that was analyzed for was FOSA, which was detected in all animals except fulmars and guillemots and ranged in concentration from <0.5 to 110 ng/g. Surprisingly, arctic fox liver had a greater mean FOSA concentration than polar bears, despite having 5-fold lower concentrations of all perfluorinated acids. This may be a function of diet but could also indicate that polar bears have a greater metabolic capacity for degrading FOSA.

It was interesting that FOSA concentrations were lower than PFOS in all mammals and birds, but not in fish. With the exception of brook trout and lake trout, all fish had higher concentrations of FOSA than PFOS. It has been reported that the ultimate metabolic product of most PFOS derivatives is expected to be PFOS (*30*, *31*), thus indicating that FOSA in fish could be an important route of exposure to PFOS for piscivores. Although sample size was small, there was anecdotal evidence that trout have a higher capacity to metabolize FOSA to PFOS than other fish. For example, brook trout and lake trout had the lowest FOSA concentrations and the highest PFOS concentrations of all fish.

Other reports have shown statistical associations between PFOS and FOSA (*6*) as a demonstration that FOSA is a metabolic precursor to PFOS. Our results also revealed some statistical associations between PFOS and FOSA; however, they are not consistent for all animals and, therefore, it is not clear if these associations represent functions of metabolism or simple exposure. For example, there was a statistically significant positive association between PFOS and FOSA concentrations in mink and fox, but not in polar bears, loons, or Holman ringed seals. Furthermore, among all fish and Grise Fjord ringed seals, there was a statistically significant negative association between PFOS and FOSA. For PFCAs, metabolism is expected to be absent, and a comparison of any two PFCA homologue concentrations in any animal almost always produced a statistically significant positive linear association. For example, the linear regressions between PFNA and PFDA and between PFDA and PFUnA are shown in Figure 5. These associations suggest that exposure of each animal to each PFCA homologue occurs in tandem with exposure to all other PFCAs and, thus, that the source(s) of PFCAs is probably similar among all homologues.

Polar bears appear to be the most contaminated organisms with respect to fluorinated contaminants. Although geo-

TABLE 3. Sum of PFCAs (∑PFCAs) and Total PFOS Equivalents (∑PFOS) for Animals Analyzed in This Study Compared to ∑PCB Data from Previous Reports in Similar Canadian Samples

	∑PFCA[a] (ng/g)	∑PFOS[b] (ng/g)	∑PCB[c]	citation
polar bear	325	3112	4080 ng/g fat (Barrow)	35
			5000−11000 ng/g fat (Eastern Hudson Bay)	25
arctic fox	53	269	124 ng/g liver (Barrow and Holman)	23
			156 ng/g muscle (Arviat)	23
ringed seal, Holman	12	16	348 ng/g fat (Holman)	36[e]
ringed seal, Ausuittuq	13	21	male = 963, female = 483 ng/g fat (Ausuittuq)	22[e]
mink	24	10	4.3−79 ng/g liver (Northwest Territories)	29
common loon	2	26	430 ng/g muscle (Kuujjuarapik, QC)	37[d]
northern fulmar	<1	1	200 ng/g liver (Prince Leopold Isl., NU)	38[d]
black guillemot	<1	<1	80 ng/g liver (Prince Leopold Isl., NU)	38[d]
white sucker	15	21	13 ng/g (Northern Quebec Rivers)	39
northern pike	7	14	<15 ng/g muscle (Lac Bienville, NU)	29
Arctic sculpin	6	30		
brook trout	18	42	19 ng/g (Northern Quebec Rivers)	39
lake whitefish	17	27	<15 ng/g muscle (Lac Bienville, NU)	29
lake trout	19	38	898 ng/g liver (Lac Bienville, NU)	29

[a] Sum of PFCAs, including PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTA, and PFPA. [b] Sum of PFOS and FOSA. [c] Concentrations are wet weight in tissue indicated, from the location in parentheses. [d] The same five samples that were analyzed for fluorinated contaminants in this study. [e] The same sample set of ringed seals analyzed in this study, but not necessarily the same animals.

graphic location may play some role, we suggest that the high contaminant concentrations in polar bears are mainly a function of their high trophic position and that the abiotic Arctic environment is less contaminated than source regions of North America, at least with respect to PFOS. When the mean concentration of PFOS is compared between the same species living in the Arctic and mid-latitude environments, it is evident that animals living in mid-latitudes are more heavily contaminated. For example, Giesy and Kannan (*7*) reported a mean liver PFOS concentration of 2630 ng/g for mink in the Midwest United Sates, compared to the mean value of 8.7 ng/g reported here for the same species in the southwest Yukon. Lake whitefish from Michigan waters had mean PFOS concentrations of 67 ng/g, compared to 12 ng/g reported here for the same species from the Great Whale River, Kuujjuarapik. Common loons from North Carolina averaged 290 ng/g of PFOS in the liver (*7*), compared to the same species in Kuujjuarapik (mean, 20 ng/g). Ringed seals from the Baltic Sea had liver PFOS concentrations exceeding 400 ng/g (*2*), compared to 16 and 19 ng/g for ringed seals from Holman and Grise Fjord, respectively. Further monitoring of animals is necessary to ascertain if these trends are also evident for PFCAs.

The two bird species collected from Prince Leopold Island provided a unique opportunity to examine the influence of diet and annual migration pattern on fluorinated contaminant concentrations. Guillemots had nondetectable concentrations of all analytes, whereas fulmars had quantifiable concentrations of PFOS and detectable, but nonquantifiable, concentrations of PFNA, PFUnA, PFDoA, and PFTrA. Guillemots stay close to their breeding grounds all year, migrating only until reaching ice-free coastal areas, feeding primarily on marine fish and amphipods at the ice edge (*32*). Fulmars also feed on marine amphipods and fish but are different from guillemots because they will occasionally scavenge marine mammal carcasses and because they migrate greater distances south toward the eastern coast of Canada (North Atlantic Ocean) in winter (*33*). Loons are different from both fulmars and guillemots because they feed primarily on freshwater fish and migrate great distances annually, some as far south as the Gulf of Mexico in winter (*32*), both factors which presumably result in increased dietary exposure.

Sum of PFCAs and PFOS Equivalents. The fluorinated contaminants reported here can be categorized as two distinct chemical classes: PFCAs (i.e., PFOA, PFNA, PFDA, etc.), and PFOS equivalents (i.e., PFOS and FOSA); it is assumed that FOSA is ultimately metabolized to PFOS (*30*). As a means of comparing the relative importance of these two chemical classes, we have calculated the sum of PFCAs (∑PFCAs) and the sum of PFOS equivalents (∑PFOS) and compared these to the sum of polychlorinated biphenyls (∑PCBs) for the same species from similar or identical Canadian locations in Table 3. It is not our intention to introduce the concept of ∑PFOS or ∑PFCAs as a risk assessment tool, as has often been attempted with chlorinated contaminants. When further toxicological information becomes available, the use of these terms should be considered, but until then it is questionable whether the mode of toxic action is similar among all PFCAs. For example, both PFOA and PFDA are potent peroxisome proliferators, but only PFOA induces tumors in rats (*34*).

∑PFOS exceeded ∑PFCAs in all samples except for mink (Table 3), due to the high concentration of PFNA in these samples. For ringed seal, mink, and most fish, ∑PFOS and ∑PFCA concentrations were not disparate, differing only by a factor of approximately 2. The greatest difference between ∑PFOS and ∑PFCA was in polar bears and arctic fox, wherein ∑PFOS exceeded ∑PFCA concentrations by at least 5-fold. The reason for the divergence of ∑PFOS and ∑PFCA at high trophic levels deserves attention in future studies because, as a group, PFCAs should biomagnify to a greater extent than PFOS based on their bioaccumulation factors (*12, 13*). One possibility is that metabolism of higher molecular weight PFOS precursors occurs in these mammals and this is contributing to ∑PFOS. However, this suggestion remains speculative because no efforts were made in this study to examine for the presence of PFOS derivatives other than FOSA.

∑PCBs for similar samples was comparable to ∑PFOS for polar bears, arctic fox, mink, and most fish, whereas ∑PCBs was much higher than either PFOS or ∑PFCAs in ringed seals, lake trout, and birds. In birds, although ∑PCBs was much higher than fluorinated contaminants, the relative trend of PCB contamination matches what is reported here for fluorinated contaminants (i.e., loons > fulmars > guillemots).

Overall, these two classes of fluorinated contaminants are present in biota on a similar scale as prominent classes of persistent organohalogen contaminants, such as PCBs. Future studies should continue to monitor PFCAs and PFOS-related contaminants and to explore the absolute and relative toxicity for these chemicals in wildlife. Concern has been raised about the possible human health risks associated with current PFOA exposure (*14*), and this is alarming because

longer PFCAs may act by similar toxicological mechanisms. Also, given that the animals investigated here serve as important dietary items for many communities in northern Canada and because human blood serum is already known to be contaminated with PFOS, FOSA, PFOA, and PFHxS (*8*), it seems prudent to examine for the presence of longer PFCAs in human tissue and to assess the toxicological risk associated with current exposure profiles.

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Note Added after ASAP

This paper was released ASAP on 11/25/2003 with the wrong year in refs 12 and 13. The correct version was posted 11/26/2003.

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