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Spatial and Temporal Trends of Perfluorinated Compounds in Beluga Whales (*Delphinapterus leucas*) from Alaska

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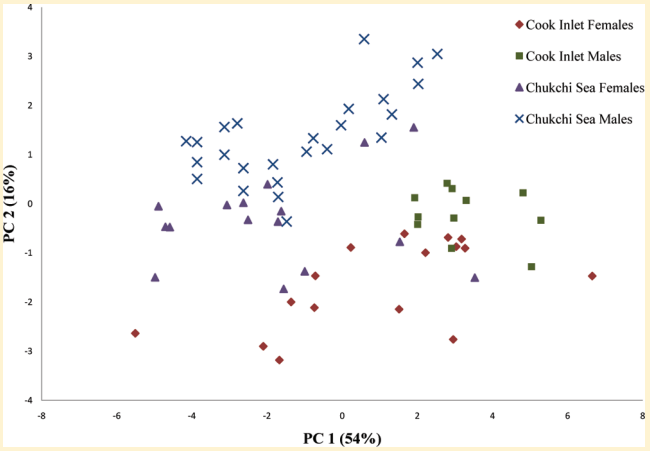
Supporting Information

ABSTRACT: Wildlife from remote locations have been shown to bioaccumulate perfluorinated compounds (PFCs) in their tissues. Twelve PFCs, consisting of perfluorinated carboxylic (PFCA) and sulfonic (PFSA) acids as well as the perfluorooctane sulfonate (PFOS) precursor perfluorooctane sulfonamide (PFOSA), were measured in livers of 68 beluga whales (*Delphinapterus leucas*) collected from two subpopulations, Cook Inlet and eastern Chukchi Sea, in Alaska between 1989 and 2006. PFOS and PFOSA were the dominant compounds measured in both beluga stock populations, with overall median concentrations of 10.8 ng/g and 22.8 ng/g, respectively. Long-chain perfluorocarboxylates, PFCAs (9 to 14 carbons), were detected in more than 80% of the samples. Perfluoroundecanoic acid (PFUnA) and perfluorotridecanoic acid (PFTriA) made up a large percentage of the PFCAs measured with median concentrations of 8.49 ng/g and 4.38 ng/g, respectively. To compare differences in location, year, sex, and length, backward stepwise multiple regression models of the individual and total PFC concentrations were used. Spatially, the Cook Inlet belugas had higher concentrations of most PFCAs and PFOS ($p < 0.05$); however, these belugas had a lower median concentration of PFOSA when compared to belugas from the eastern Chukchi Sea ($p < 0.05$). Temporal trends indicated most PFCAs, PFHxS, PFOS, and PFOSA concentrations increased from 1989 to 2006 ($p < 0.05$). Males had significantly higher concentrations of PFTriA, ΣPFCA, and PFOS ($p < 0.05$). Perfluorononanic acid (PFNA) and PFOS showed a significant decrease in concentration with increasing animal length ($p < 0.05$). These observations suggest the accumulation of PFCs in belugas is influenced by year, location, sex, and length.



INTRODUCTION

Perfluorinated compounds (PFCs), mostly perfluorinated carboxylic (PFCA) and sulfonic (PFSA) acids, have been identified in wildlife worldwide;[1−6] with some of the highest levels of PFCs being measured in marine mammals from the Arctic.[1,3−5,7,8] It is not exactly clear how PFCs are transported to Arctic wildlife, but it is thought to happen via two major routes: atmospheric transport of volatile precursors and oceanic transport from their release in lower latitudes.[2,9,10] A previous study indicates atmospheric transport having a greater influence on PFC contamination in the Arctic by examining the spatial trends of PFCs in ringed seal (*Phoca hispida*) populations from East and West Greenland.[3] Another study noted that circulation patterns in the Arctic Ocean may also have an effect of spatial differences of PFCs in the Arctic region.[11] Due to the large distribution of some Arctic species it is important to understand if there are similarities or differences in the concentrations and patterns among species from different locations. While there have been numerous studies focusing on PFCs in wildlife from the Canadian Arctic, few studies have looked at the wildlife in the Alaskan Arctic.[1,12] Recognizing possible spatial patterns will help highlight sources of PFCs to the Arctic and help examine atmospheric and oceanic transport.

The beluga whale, *Delphinapterus leucas*, is an Arctic species that feeds close to the top of the food chain, providing the potential to bioaccumulate persistent contaminants in their tissues. Belugas are expected to contain relatively high concentrations of PFCs because of their trophic level and their long life span (>30 yr).[13] Previous studies examining PFCs in beluga livers from the Canadian Arctic have shown individual PFC concentrations over 150 ng/g.[7,14] However, there is no information about the concentration of PFCs in belugas from Alaska.

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In Alaskan waters there are two genetically isolated populations of beluga whales: Cook Inlet and Bering Sea, the Bering Sea population is represented by four stocks; Bristol Bay, eastern Bering Sea, eastern Chukchi Sea, and Beaufort Sea.[15,16] The Alaskan Peninsula geographically isolates the Cook Inlet animals (living year round in Cook Inlet and in the Gulf of Alaska immediately outside Cook Inlet) from the Bering Sea animals.[17] Since 1992, liver samples have been collected as part of the Alaska Marine Mammal Tissue Archival Project (AMMTAP) from belugas taken during Alaska Native subsistence hunts in Cook Inlet.[18] In addition, liver samples have been collected from the eastern Chukchi Sea stock during subsistence hunts. As there are no overlapping areas of distribution between the Cook Inlet and eastern Chukchi Sea animals, having two distinct populations for comparison provides an opportunity to evaluate possible sources of contamination in the North American Arctic. The Cook Inlet belugas are thought to be affected more by local anthropogenic sources coming from the surrounding more urbanized area of Anchorage, while the eastern Chukchi Sea animals will be affected more by atmospheric and oceanic transport. The eastern Chukchi Sea animals forage between the Russian and United States Arctic. There is a lack of data on perfluorinated compounds in marine mammals from this area of the Arctic and having animals from the Chukchi Sea is a unique opportunity to assess the influences of Asian and Russian production of PFCs to this area of the Arctic.

In this study, aliquots of Cook Inlet and eastern Chukchi Sea beluga livers were analyzed for 12 PFCs. The primary objective was to determine if temporal trends exist between PFC concentrations from these two groups of belugas and speculate the different sources of PFCs. Other objectives include examining geographical differences, gender differences in the two locations, and possible bioaccumulation of PFCs with total length.

MATERIAL AND METHODS

Extraction and Analysis. Sixty-eight beluga liver samples were selected for analysis from the National Marine Mammal Tissue Bank (NMMTB). The National Institute of Standards and Technology (NIST) maintains the NMMTB to provide archived samples for retrospective analysis. Information regarding study area, sample collection, and sample information is provided in the Supporting Information.

Samples, calibrants, quality control materials, and blanks were extracted using a method similar to the potassium hydroxide (KOH) in methanol method as described previously,[19] and a solid phase extraction (SPE) cleanup step was also described previously.[20] NIST control material (Beluga whale liver homogenate, QC97LH02) was analyzed with each set of samples for quality control. Briefly, 0.5 g of sample was used for the extraction. Milli-Q water (0.5 mL) was added to each sample. The internal standard (IS) solution was gravimetrically added to each sample (Table S1). Three mL of 0.01 mol/L of KOH in methanol was added to the samples, and samples were vortexed, sonicated for 30 min, and centrifuged at 2500 rpm for 5 min. The supernatant was transferred to a glass culture tube. The original sample tube received an additional 3 mL of 0.01 mol/L of KOH in methanol, and the extraction procedure was repeated. Both extracts were combined and evaporated to approximately 3 mL and filtered using a Whatman UniPrep 0.2 μm filter (Stanford, ME). Samples were further evaporated to 1 mL. Ten mL of 50% (volume fraction) formic acid (98%, Fluka) in Milli-Q water was added to each sample. A Waters Oasis Weak Anion Exchange (WAX) SPE (3 cc, 60 mg, 30 μm; Milford, MA) cartridge was conditioned with methanol and water, and samples were loaded onto the SPE columns on the RapidTrace workstations (Caliper, Hopkinton, MA). Extracts were concentrated in volume, spiked with $^{13}C_2$−PFOA (the recovery standard), vortexed, and transferred to autosampler vials.

Samples were injected onto a liquid chromatograph (Agilent 1100 HPLC, Palo Alto, CA) interfaced to a negative electrospray ionization tandem mass spectrometer (LC-MS/MS) (API 4000, Applied Biosystems-MDS Sciex, Foster City, CA) using the NIST method described in Keller et al.[20] The MS/MS method included the optimization parameters for each analyte, and two to three of the most abundant transitions for each PFC were monitored.

PFOSA Extraction. The WAX SPE method described above performs well for the acidic compounds, perfluorocarboxylates (PFCAs) and perfluorosulfonates (PFSAs); however, the recovery of neutral PFOSA using this method is low (<10% for 14 samples after using the WAX method). Subsamples of these along with four samples that had good recovery and the quality control material were extracted again using the same KOH method described above but employed a different cleanup method using the Supelco, Supelclean ENVI-Carb SPE (3 cc, 250 mg 120−400 mesh; Bellefonte, PA) on the RapidTrace workstation. The ENVI-Carb cartridge was conditioned with 6 mL of methanol followed by 6 mL of water. Samples (evaporated to 3 mL) were loaded on the SPE cartridge and eluted immediately using 4.5 mL of methanol. PFOSA in this subset of beluga liver extracts was analyzed using a different LC column, the Phenomonex Luna PFP (2) column (50 mm × 3.0 mm × 5 μm). The solvent gradient started at 60% methanol and 40% 20 mmol/L ammonium acetate in water (flow rate of 0.3 mL/min) and then increased to 65% methanol by 5 min, held for 5 min, and then increased to 80% methanol by 15 min, held for 5 min, before reverting to original conditions at 20.5 min with a 10 min hold.

Samples that were extracted for PFOSA and cleaned with the ENVI-Carb SPE method showed recovery greater than 70%. Concentrations of PFOSA from the four samples that had satisfactory recovery using the WAX SPE method showed good agreement in measured PFOSA concentrations, and the percent difference between the two methods ranged from 3 to 10%. PFOSA concentrations in the quality control material QC97LH2 were similar to concentrations measured previously at the NIST laboratory. Quality assurance information is included in the Supporting Information.

Statistical Methods. All statistical analyses were performed using JMP 7.0.2 (SAS Institute, Cary, NC). Statistical tests (details provided in the Supporting Information) were performed for PFNA, PFDA, PFUnA, PFDoA, PFTriA, PFTA, PFHxS, PFOS, PFOS, total perfluorocarboxylate (ΣPFCA), and total PFC (ΣPFC) concentrations, which were the compounds detected in >70% of the samples. Compounds concentrations less than the reporting limit (RL) were set equal to half the RL prior to running the statistical tests. Backward stepwise multiple regressions were preformed on the concentrations with the beluga location, year, sex, and animal length used as the independent variables. When year was shown to be significant based on the regression, the log−linear regressions, based on the annual geometric means, were modeled using the Arctic Monitoring and Assessment Programme (AMAP) PIA program.[21]

Table 1. Concentrations of PFCs (ng/g) in Beluga Liver Samples[a]

	Cook Inlet						eastern Chukchi Sea					
	males			females			males			females		
	range	median	n > RL (n = 11)	range	median	n > RL (n = 16)	range	median	n > RL (n = 25)	range	median	n > RL (n = 16)
PFNA*	0.454−3.08	1.79	11	<0.502−5.67	1.66	15	0.170−2.55	0.670	25	<0.180−5.46	0.960	13
PFDA	0.894−4.11	3.15	11	0.309−6.98	1.95	16	0.553−5.38	2.15	25	0.514- 14.3	1.59	16
PFUnA*	7.90−27.2	20.0	11	<0.678−41.6	11.8	15	<0.497−20.8	6.76	24	1.67−49.0	4.40	16
PFDoA*	1.01−3.48	2.38	11	0.365−5.55	1.88	16	0.230−3.10	0.898	25	0.191−5.55	0.841	16
PFTriA*	6.17−48.0	20.0	11	<0.357−82.8	9.77	12	<0.262−14.3	2.53	21	<0.356−17.6	1.46	13
PFTA*	1.11−13.3	2.94	11	<0.552−21.3	1.46	12	<0.659−7.99	0.982	23	<0.323−4.36	1.55	11
PFHxS*	<0.0600−0.644	0.306	10	<0.0649−3.55	0.301	15	<0.0246−0.366	0.120	15	<0.0261−0.378	0.139	9
PFOS*	14.4−30.4	22.5	11	4.61−70.3	13.0	16	4.29−28.4	9.20	25	1.81 −38.1	4.76	16
PFOSA*	4.52−17.9	11.4	11	10.4−27.8	18.4	16	17.7−63.8	31.8	25	11.2−65.7	27.8	16

[a] n > RL indicates the number of samples above the reporting limit (RL). Values were calculated with half the RL substituted for nondetects as described in the methods section, but values shown as "<" a specified number describe the actual RL. Compounds marked with asterisks indicate there are significant differences between beluga stock of the log-transformed concentrations ($p < 0.05$).

RESULTS AND DISCUSSION

PFCs were detected in all beluga liver samples (Table 1); with PFDA, PFDoA, PFOS, and PFOSA detected in all samples. PFNA, PFUnA, PFTriA, PFTA, and PFHxS were detected less frequently (72% to 97% of the samples). Short-chain PFCs (PFOA, PFHpA, and PFHxA) were detected infrequently (<2%), so these short-chain PFCs were not included in the statistical analysis. ΣPFC measurements ranged from 17.5 ng/g to 240 ng/g (Supporting Information, Table S2). PFOS and PFOSA accounted for greater than 50% of the ΣPFCs measured (Figure 1). The concentrations of PFOS ranged from 1.81 ng/g to 70.3 ng/g (median: 10.8 ng/g). PFOSA was also found ranging from 4.52 ng/g to 65.7 ng/g (median: 22.8 ng/g). The long-chained, PFCAs with odd numbers of carbons, PFUnA and PFTriA, were detected at higher concentrations compared to even numbered, long-chained PFCAs. PFUnA comprised approximately 15% of the ΣPFCs measured (median: 8.49 ng/g) and PFTriA made up 7% of the ΣPFCs measured (median: 4.38 ng/g).

Similar ranges of concentrations have been measured in beluga whales from the northeastern and western Canadian Arctic (Table 2).[7,14,22] The relatively high body burden of PFOSA in beluga whales has also been seen in previous studies.[7,14] Higher measurements of PFTA were found in this study compared with beluga whales from northeastern Canada.[7] The prevalence of PFTA that was not seen in the earlier study suggests there are different transport pathways and/or sources of PFTA that exists in Alaska not found in the northeastern Canadian Arctic.[7] As seen before in Arctic biota, the odd-chain length PFCAs exceed the concentration of the even-chain length PFCAs.[23] It has been hypothesized that fluorotelomer alcohol (FTOH) degradation and the ensuing bioaccumulation explains this pattern observed in wildlife.[9] Specifically, the degradation of an FTOH will result in equal yields of odd and even chain-length PFCA, but the bioaccumulation potential of the odd-chain PFCA is greater than the even-chain PFCA. In this study there were statistically significant ($p < 0.05$) associations between all individual PFCAs, between PFCAs and PFOS, and between PFHxS and PFOSA, but not between PFOSA and other compounds (Table S3). These associations are similar to those seen previously in multiple Arctic species (Arctic fox, ringed seal, mink, and polar bears) suggesting exposure to PFCs is from similar transport pathways and/or sources,[23] except for PFOSA.

The effect of beluga location, year, sex, and length on the concentration of individual and total PFCs was examined using backward stepwise regressions (Table S4). ΣPFC concentrations were significantly higher in the Cook Inlet belugas compared to the eastern Chukchi Sea belugas ($p < 0.05$, see Table 2). Also, spatial trends in PFNA, PFUnA, PFDoA, PFTriA, PFTA, ΣPFCAs, PFHxS, PFOS, and PFOSA were observed between the Cook Inlet and the eastern Chukchi Sea animals, with significantly higher ($p < 0.05$) concentrations of PFNA, PFUnA, PFDoA, PFTriA, PFTA, PFHxS, and PFOS in belugas from Cook Inlet (Tables 2 and S4). Contrarily, the Chukchi Sea belugas had significantly higher concentrations of PFOSA ($p < 0.05$). The general patterns of PFCs in liver samples from the Cook Inlet population were different when compared with the patterns of PFCs from the eastern Chukchi Sea belugas (Figure 1), as PFOSA comprised greater than 50% of the ΣPFCs measured in the eastern Chukchi Sea animals and less than 35% of the ΣPFCs in the Cook Inlet belugas. PFOS and PFUnA were found at the second and third highest percentages of PFCs measured in the eastern Chukchi Sea samples. The most dominant PFC found in belugas from Cook Inlet was PFOS, followed by PFTriA, then PFOSA. The differences between the concentrations and patterns in the two beluga groups suggest there are either different transport processes and/or sources of PFCs for the two locations.

The source of PFCs to the Arctic has been explained by two main mechanisms; long-range atmospheric transport and oceanic transport. The atmospheric transport of persistent organic pollutants from low- and midlatitudes to the Arctic has previously been shown,[24] and it is thought the long-range atmospheric transport of volatile precursors (FTOHs and *N*-ethyl perfluorooctanesulfonamide) could explain the presence of long-chain PFCAs and PFOS in the Arctic.[23,25,26] At this time, measurements of PFCs and their precursors in the Pacific atmosphere do not exist; however, the presence of FTOHs and perfluoroalkyl sulfonamides in the Atlantic atmosphere gives further evidence that atmospheric transport may occur and contribute to PFC contamination seen in remote locations like the Arctic.[25] The biotransformation of *N*-ethyl perfluorooctanesulfonamide to

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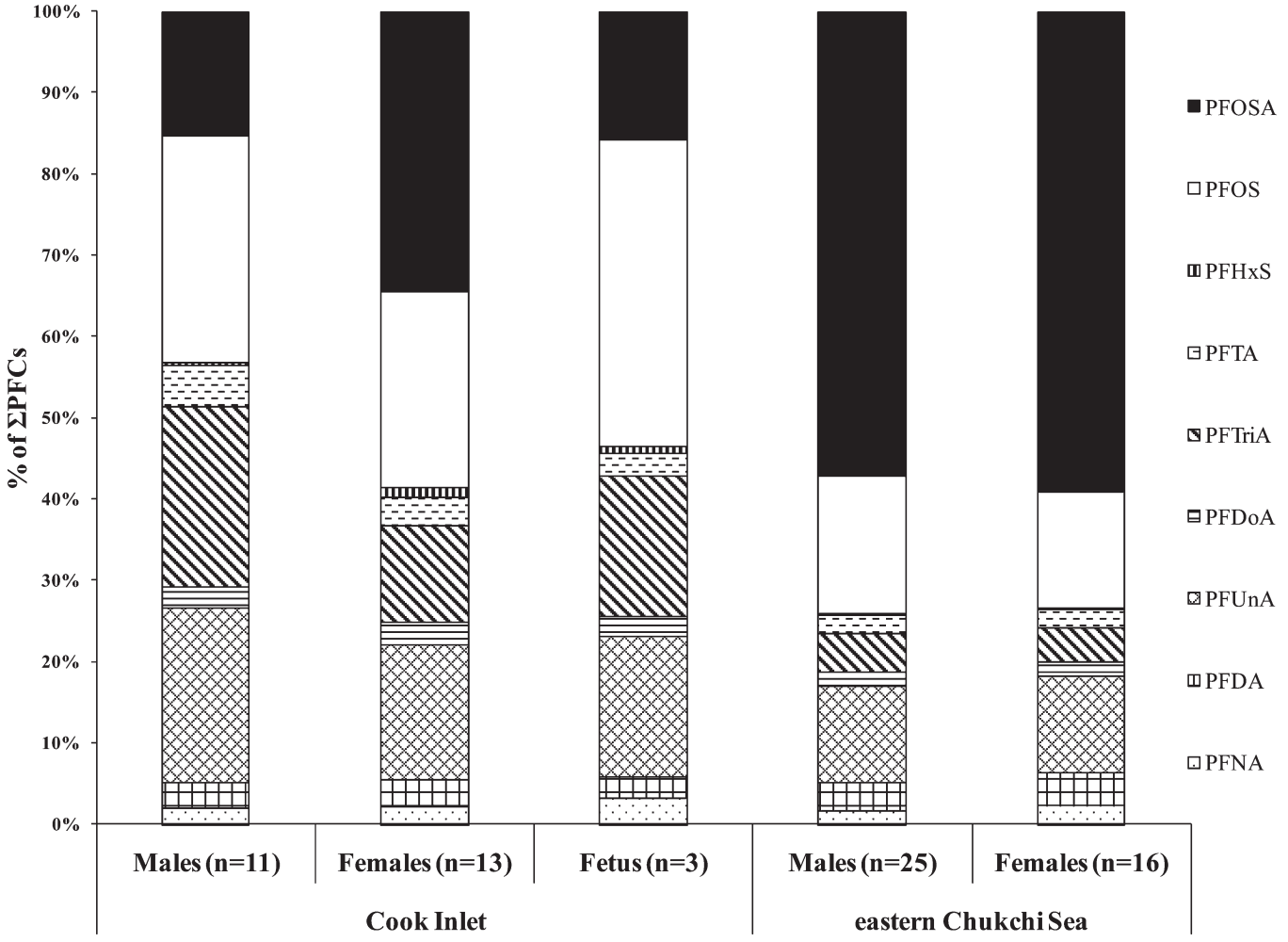

Figure 1. Patterns of individual PFCs as percent of ΣPFC in the livers of beluga whales.

Table 2. Concentration Ranges of PFCs (ng/g) in Beluga Livers from Arctic Locations[e]

location	southern Alaska/ Cook Inlet (n = 27, males and females)[a]	northeastern Alaska/ eastern Chukchi Sea (n = 41, males and females)[a]	northeastern Canada/ Hudson Bay (n = 22, males and females)[b]	Canada/ Newfoundland (n = 5, males only)[c]	western Canada/ Hedrickson Island (n = 10, males only)[d]
years	1992−2006	1989−2000	1999−2003	1996	2007
PFNA	<RL−5.67	<RL−5.46	0.69−6.58	n.m.	5.72−20.3
PFDA	0.309−6.98	0.514−14.3	2.0−18.9	n.m.	5.87−33.9
PFUnA	<RL−41.6	<RL−49.0	3.8−39.6	n.m.	6.47−30.7
PFDoA	0.365−5.55	0.191−5.55	0.80−9.89	n.m.	<RL−5.39
PFTriA	<RL−82.8	<RL−17.6	n.m.	n.m.	n.m.
PFTA	<RL−21.3	<RL−7.99	<RL−2.35	n.m.	n.m.
PFHxS	<RL−3.55	<RL−0.378	<RL−3.76	n.m.	n.m.
PFOS	4.61−70.3	1.81 −38.1	3.0−109	9.8−15.8	4.25−20.3
PFOSA	4.52−27.8	11.2−65.7	4.94−156	n.m.	7.76−24.5
ΣPFC	18.6−240	19.0−149	NA	NA	NA

[a] This study. [b] 5. [c] 20. [d] 12. [e] <RL = below the reporting limit; n.m. = not measured; NA = not applicable.

PFOSA and subsequently PFOS has been shown to occur in liver microsomes of rainbow trout.[26] If a volatile precursor such as *N*-ethyl perfluoroocatanesulfonamide is transported to the Arctic atmospherically and accumulated by an animal, then it may biologically transform it to PFOSA and subsequently PFOS. The higher concentration of PFOSA in the eastern Chukchi Sea belugas may indicate an increased presence of it in the eastern Chukchi Sea and/or the presence of atmospheric precursor. Measurements of the volatile precursors in the atmosphere of the eastern Chukchi Sea and Cook Inlet could confirm the hypothesis.

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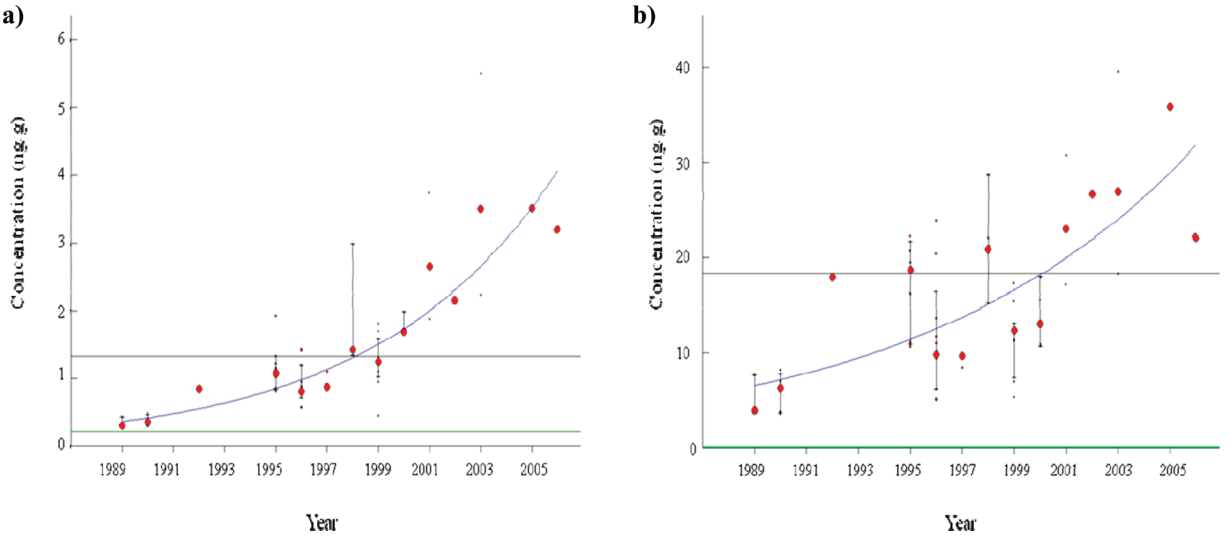

Figure 2. Temporal trend of a) PFNA ($R^2 = 0.92$) and b) PFOS ($R^2 = 0.64$) concentrations (ng/g) in beluga livers from Alaska drawn with AMAP PIA program. Data presented as the predicted concentrations of PFNA and PFOS based on the regression model taking into account significant factors such as beluga population, sex, and length. Individual data points are black, medians are red, and error bars are the 95% confidence intervals. The blue lines are the log linear regression, black horizontal lines indicate the overall mean, and green lines are the reporting limit. Similar trends were seen for PFDA, PFUnA, PFDoA, and PFHxS (Figure S1) as well as ΣPFCAs, PFOSA, and ΣPFCs (data not shown).

Localized releases of PFCs are other potential sources of PFC exposure for the belugas. The Cook Inlet beluga population is considered to be a geographically isolated population and individuals are found in Cook Inlet year round.[15] The range of Cook Inlet belugas puts them in close proximity to Anchorage, Alaska, an urban area where human abundance and anthropogenic sources are higher compared to the eastern Chukchi Sea. Another factor that could contribute to the differences seen between Cook Inlet and eastern Chukchi Sea is the feeding habits of the two beluga groups. The diets of the two groups can differ depending on location of their summering areas and prey that are available.[18] Hence, the differences in PFC patterns in Cook Inlet and eastern Chukchi Sea belugas are likely caused by atmospheric transport, oceanic transport, local inputs and/or prey preferences, or a combination of all these. Future studies could focus on tracking sources of PFCs to the Arctic.

Among the PFCs analyzed, concentrations of PFNA, PFDA, PFUnA, PFDoA, ΣPFCAs, PFHxS, PFOS, PFOSA, and ΣPFCs showed significant increases from 1989 to 2006 in Alaskan beluga whales ($p < 0.05$) (Figures 2 and S1). In fact exponential increases were observed over this time period without any noticeable recent stabilization. Other studies have shown temporal increases of PFC concentrations in marine mammals. For example, one study has shown an increase in PFDA, PFUnA, and PFOS concentrations from 1982 to 2003 in ringed seal livers from Greenland.[3] In another study, the concentrations of PFNA, PFDA, and PFOS measured in Baikal seal livers from 2005 showed an increase compared to samples collected in 1992.[27] A recent study measuring PFCs in peregrine falcon eggs (*Falco peregrines*) shows a similar exponential increase for PFNA, PFDA, PFUnA, PFDoA, PFHxS, and PFOS in eggs collected from 1974−2007.[28] In contrast, recent measurements of PFOS concentrations in sea turtle plasma and serum have shown a decrease from 2000 to 2008.[29] Measurements from human plasma and milk from the United States and Europe have shown a decrease in PFOS concentrations after 2000.[30,31] These decreases are attributed to the phase out of PFOS-based chemistry from the former main manufacturer. Differences in temporal trend data have been attributed to differences in collection locations and localized sources of direct PFC input.[29] It is thought wildlife samples from remote locations may show a reduction in PFC concentrations in years to come due to the delay from long-range transport of PFCs and their precursors.[29]

The two beluga populations are geographically separated; therefore, the annual trends of PFCs were also examined in the two stocks separately (Table S5). The Cook Inlet belugas tended to have smaller slopes of temporal increases compared to the eastern Chukchi Sea belugas, especially for PFOS, PFNA, PFDA, and PFUnA. The smaller annual increase seen in the Cook Inlet belugas is suggestive of a decrease of PFC inputs from local sources in and around Cook Inlet. The larger annual increase in the eastern Chukchi Sea belugas suggests there are significantly higher inputs of PFCs likely caused by atmospheric transport, oceanic transport, and/or inputs coming from Asia and Russia.

Since PFOSA is a precursor of PFOS, the ratio PFOSA/PFOS was also examined (Table S2). This ratio ranged from 0.3 to 17 depending on the beluga location, year, sex, and length. The belugas from Cook Inlet had a significantly lower PFOSA/PFOS ($p < 0.05$) compared to the belugas from the eastern Chukchi Sea. While there is no significant annual trend in PFOSA/PFOS in the Cook Inlet belugas, there is a significant ($p < 0.05$) yearly decrease of 5.6% of PFOSA/PFOS in the Chukchi Sea belugas (Table S5). The annual decrease of PFOSA/PFOS in the Chukchi Sea belugas could suggest the increase of PFOS and/or PFOS precursors (not including PFOSA) being transported to the Chukchi Sea. Little is known about the production statistics of PFCs from Asia and Russia. It has been estimated that the production of PFOS based chemistry from China has increased since 2003,[32] but little production information is available for Russia. The Chukchi Sea belugas forage in Arctic water between the United States and Russia and based on prevailing wind and ocean currents, these animals may be more reflective of PFCs emissions coming from Asia and Russia.

Table 3. Concentrations and Ratios of PFCs in Two Matched Mother and Fetus Pairs[a]

	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS	PFOSA	ΣPFC
mother-1 (ng/g)	0.690	0.727	4.32	0.756	<RL	<RL	3.55	10.6	11.1	31.7
fetus-1 (ng/g)	2.57	1.75	12.8	1.93	15.4	4.69	1.15	39.6	14.7	94.6
ratio-1 (mother to fetus)	0.27	0.42	0.34	0.39			3.08	0.27	0.76	0.34
mother-2 (ng/g)	1.61	2.30	10.8	1.25	5.86	1.48	1.35	17.1	14.4	56.1
fetus-2 (ng/g)	3.56	4.86	28.8	3.75	28.4	3.16	1.32	70.3	19.6	164
ratio-2 (mother to fetus)	0.45	0.47	0.37	0.33	0.21	0.47	1.02	0.24	0.73	0.34

[a] <RL = below the reporting limit.

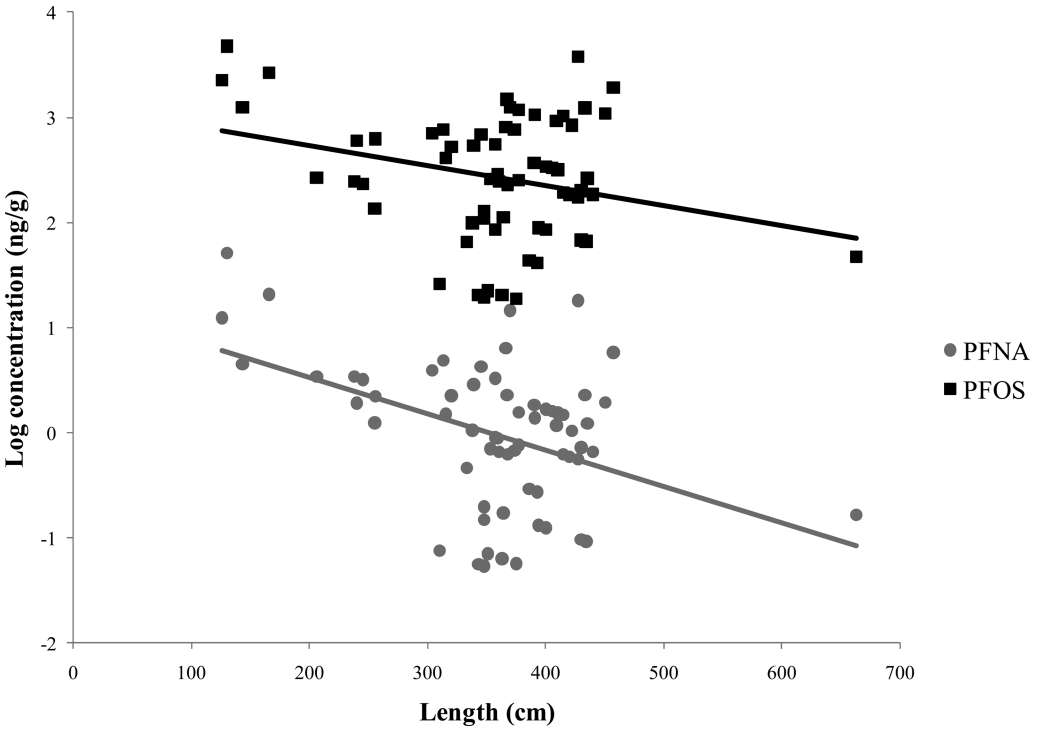

Figure 3. Linear regression between length and log concentration of PFCs (PFNA and PFOS) in livers of belugas from Alaska: Coefficient of determination (R^2) for PFNA = 0.18 and PFOS = 0.074. Data presented as the predicted concentrations of PFNA and PFOS based on the regression model taking into account significant factors such as beluga population, year, and sex. For both regressions $p < 0.05$.

For most compounds male belugas had higher concentrations of PFCs when compared with females. Males had significantly higher concentration of PFTriA, ΣPFCAs, and PFOS compared to the females ($p < 0.05$) (Supporting Information, Table S4). In contrast, female belugas had significantly higher concentrations of PFNA ($p < 0.05$) (Supporting Information, Table S4). Gender related differences may be a result of higher dietary intake and/or gender differences in elimination (urinary and fecal excretion). Although pharmacokinetic studies do not exist for cetaceans and therefore not comparable, other mammal pharmacokinetic studies have shown gender differences in the elimination half-life of some PFCs.[33,34] Other important elimination pathways in female belugas are placental and lactational transfer from mother to calves.

This study included three fetus samples; all were females from Cook Inlet with a total length not exceeding 150 cm. These fetus samples had a PFC pattern more similar to the males than females from Cook Inlet (Figure 1). Paired mother samples were available for two of the fetus liver samples. By examining the ratios of PFC concentrations in the mother to the calf, these samples were used to look at offloading of PFCs in belugas in utero (Table 3). A ratio equal to one indicates equilibrium between the mother and the calf, a ratio greater than one represents retention in the mother, and a ratio less than one represents offloading from mother to calf. Ratios between mother and fetus samples were less than one for all PFCs except PFHxS, suggesting offloading from mother to fetus in utero for most PFCs. Similar to previous human and rodent studies, maternal transfer of PFCs has been shown in utero and through lactation.[35−37] In wild populations, data from mother-calf pairs of bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay showed higher concentrations of ΣPFCs in calves when compared to their mothers, suggesting in utero and/or lactational transfer of PFCs occurs in marine mammals.[38]

There was a significant decrease ($p < 0.05$) of PFNA and PFOS with increasing beluga length in the backward stepwise regression (Figure 3). However, the relationship was weak with coefficients of determination (R^2) for PFNA and PFOS equal to 0.18 and 0.074, respectively. One beluga was quite large (663 m), potentially applying a leverage effect on the regression line. When this sample was removed from the regression there was still a significant decrease ($p < 0.05$) for PFNA and PFOS; however, the relationship was again weak (R^2 of 0.17 and 0.0526,

respectively). Beluga length did not significantly influence concentrations of any other PFCs in this study. A previous study has suggested that some PFCs decrease with length in bottlenose dolphins,[38] while another study has reported an increase of PFCs with length in sea turtles.[39] Furthermore, there are numerous studies that have observed no significant trends between PFC concentrations and length.[38,40] These conflicting results call to the importance of studying species-specific accumulation of PFCs, and this emphasizes that length-analyte relationships are complicated. Potentially there could be an influence of age on the bioaccumulation rate in the belugas. However, age information is not available, and length cannot be used as a proxy for age of belugas; therefore, we cannot infer any age relationships in this study.

Since beluga meat and blubber is consumed by Alaskan Natives, it is important to consider their exposure from these sources as well as other dietary routes of exposure. It has been suggested that food consumption is a primary source of PFOS exposure to the Nunavik Inuit adults from northern Quebec, with daily fish intake and marine mammal consumption being strong predictors of PFOS concentrations.[41,42] While the liver of belugas is not consumed as part of subsistence food, blubber and mammal meat is consumed. Recently, it has been shown that PFCs partition significantly into the blood and liver of belugas, but some PFCs are also found in the blubber and muscle tissue.[7] It is reasonable to assume the consumption of beluga meat by Alaskan Natives is a potential source of PFC exposure.

In summary, this study showed that the Cook Inlet animals, compared with eastern Chukchi Sea belugas, have different concentrations and patterns of PFCs. The Cook Inlet belugas have significantly higher concentrations of long-chain PFCAs, PFHxS, and PFOS compared to the eastern Chukchi Sea belugas. Differences suggest different sources or transport pathways of these compounds, which can be related to the geographic differences in long-range atmospheric transport of PFCs, oceanic transport of PFCs, local releases, and/or feeding habits. Besides the geographical differences, concentration differences were related to year, sex, and/or length. The toxicological implications of PFC concentrations in the hepatic tissue of beluga whales are unknown, but their potential impact on beluga whale health and human health should be considered.

■ ASSOCIATED CONTENT

Supporting Information. Material and methods, supplemental tables (Tables S1−S5), and a figure (Figure S1) are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Kannan, K.; Yun, S. H.; Evans, T. J. Chlorinated, Brominated, and Perfluorinated Contaminants in Livers of Polar Bears from Alaska. *Environ. Sci. Technol.* **2005**, *39* (23), 9057–9063.

(2) Smithwick, M.; Norstrom, R. J.; Mabury, S. A.; Solomon, K.; Evans, T. J.; Stirling, I.; Taylor, M. K.; Muir, D. C. Temporal trends of perfluoroalkyl contaminants in polar bears (*Ursus maritimus*) from two locations in the North American Arctic, 1972−2002. *Environ. Sci. Technol.* **2006**, *40* (4), 1139–43.

(3) Bossi, R.; Riget, F. F.; Dietz, R. Temporal and Spatial Trends of Perfluorinated Compounds in Ringed Seal (*Phoca hispida*) from Greenland. *Environ. Sci. Technol.* **2005**, *39* (19), 7416–7422.

(4) Butt, C. M.; Mabury, S. A.; Muir, D. C.; Braune, B. M. Prevalence of long-chained perfluorinated carboxylates in seabirds from the Canadian Arctic between 1975 and 2004. *Environ. Sci. Technol.* **2007**, *41* (10), 3521–8.

(5) Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G. Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006**, *40* (11), 3463–3473.

(6) Butt, C. M.; Berger, U.; Bossi, R.; Tomy, G. T. Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci. Total Environ.* **2010**, *408* (15), 2936–2965.

(7) Kelly, B. C.; Ikonomou, M. G.; Blair, J. D.; Surridge, B.; Hoover, D.; Grace, R.; Gobas, F. A. Perfluoroalkyl contaminants in an Arctic marine food web: trophic magnification and wildlife exposure. *Environ. Sci. Technol.* **2009**, *43* (11), 4037–43.

(8) Dietz, R.; Bossi, R.; Riget, F. F.; Sonne, C.; Born, E. W. Increasing perfluoroalkyl contaminants in east Greenland polar bears (*Ursus maritimus*): A new toxic threat to the Arctic bears. *Environ. Sci. Technol.* **2008**, *42* (7), 2701–2707.

(9) Ellis, D. A.; Martin, J. W.; DeSilva, A. O.; Mabury, S. A.; Hurley, M. D.; SulbaekAndersen, M. P.; Wallington, T. J. Degradation of Fluorotelomer Alcohols: A Likely Atmospheric Source of Perfluorinated Carboxylic Acids. *Environ. Sci. Technol.* **2004**, *38* (12), 3316–3321.

(10) Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H. Sources, Fate and Transport of Perfluorocarboxylates. *Environ. Sci. Technol.* **2006**, *40* (1), 32–44.

(11) Armitage, J. M.; MacLeod, M.; Cousins, I. T. Modeling the global fate and transport of perfluorooctanoic acid (PFOA) and perfluorooctanoate (PFO) emitted from direct sources using a multispecies mass balance model. *Environ. Sci. Technol.* **2009**, *43* (4), 1134–40.

(12) Hart, K; Gill, V. A.; Kannan, K. Temporal Trends (1992−2007) of Perfluorinated Chemicals in Northern Sea Otters (*Enhydra*

lutris kenyoni) from South-Central Alaska. *Archiv. Environ. Contam. Toxicol.* **2009**, *56* (3), 607–614.

(13) Burns, J.; Seaman, G. Investigations of belukha whales in coastal waters of western and northern Alaska. II Biology and ecology. *U.S. Dep. Com. NOAA/OSCEAP Final Rep. 56* **1986**, 221–357.

(14) Tomy, G. T.; Pleskach, K.; Ferguson, S. H.; Hare, J.; Stern, G.; Macinnis, G.; Marvin, C. H.; Loseto, L. Trophodynamics of some PFCs and BFRs in a western Canadian Arctic marine food web. *Environ. Sci. Technol.* **2009**, *43* (11), 4076–81.

(15) Frost, K.; Lowry, L. Distribution, abundance and movements of beluga whales, *Delphinapterus leucas*, in the coastal waters of western Alaska. *Can. Bull. Fish Aquat. Sci.* **1990**, *224*, 39–57.

(16) O'Corry-Crowe, G. M.; Suydam, R. S.; Rosenberg, A.; Frost, K. J.; Dizon, A. E. Phylogeography, population structure and dispersal patterns of the beluga whale, *Delphinapterus leucas*, in the wesern Nearctic by mitochondrial DNA. *Mol. Ecol.* **1997**, *6*, 955–970.

(17) Laidre, K. L.; Shelden, K. E. W.; Rugh, D. J.; Mahoney, B. A. Beluga, *Delphinapterus leucas*, distribution and survey effort in the Gulf of Alaska. *Mar. Fish. Rev.* **2000**, *63* (3), 27–36.

(18) Becker, P. R.; Krahn, M. M.; Mackey, E. A.; Demiralp, R.; Schantz, M. M.; Epstein, M. S.; Donais, M. K.; Porter, B. J.; Muir, D. C. G.; Wise, S. A. Concentrations of polychlorinated biphenyls (PCB's), chlorinated pesticides, and heavy metals and other elements in tissues of belugas, *Delphinapterus leucas*, from Cook Inlet, Alaska. *Mar. Fish. Rev.* **2000**, *62* (3), 81–98.

(19) Taniyasu, S.; Kannan, K.; So, M. K.; Gulkowska, A.; Sinclair, E.; Okazawa, T.; Yamashita, N. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *J. Chromatogr., A* **2005**, *1093* (1−2), 89–97.

(20) Keller, J. M.; Calafat, A. M.; Kato, K.; Ellefson, M. E.; Reagen, W. K.; Strynar, M.; O'Connell, S.; Butt, C. M.; Mabury, S. A.; Small, J.; Muir, D. C.; Leigh, S. D.; Schantz, M. M. Determination of perfluorinated alkyl acid concentrations in human serum and milk standard reference materials. *Anal. Bioanal. Chem.* **2010**, *397* (2), 439–51.

(21) Bignert, A. PIA statistical application developed for use by the Arctic Monitoring and Assessment Programme. Available at http://www.amap.no (accessed month day, year).

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

(23) Martin, J. W.; Smithwick, M. M.; Braune, B. M.; Hoekstra, P. F.; Muir, D. C. G.; Mabury, S. A. Identification of Long-Chain Perfluorinated Acids in Biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004**, *38* (2), 373–380.

(24) Bard, S. M. Global transport of anthropogenic contaminants and the consequences for the Arctic marine ecosystem. *Mar. Pollut. Bull.* **1999**, *38* (5), 356–379.

(25) Dreyer, A.; Weinberg, I.; Temme, C.; Ebinghaus, R. Polyfluorinated Compounds in the Atmosphere of the Atlantic and Southern Oceans: Evidence for a Global Distribution. *Environ. Sci. Technol.* **2009**, *43* (17), 6507–6514.

(26) Tomy, G. T.; Tittlemier, S. A.; Palace, V. P.; Budakowski, W. R.; Braekevelt, E.; Brinkworth, L.; Friesen, K. Biotransformation of N-Ethyl Perfluorooctanesulfonamide by Rainbow Trout (*Onchorhynchus mykiss*) Liver Microsomes. *Environ. Sci. Technol.* **2004**, *38* (3), 758–762.

(27) Ishibashi, H.; Iwata, H.; Kim, E. Y.; Tao, L.; Kannan, K.; Amano, M.; Miyazaki, N.; Tanabe, S.; Batoev, V. B.; Petrov, E. A. Contamination and effects of perfluorochemicals in Baikal Seal (*Pusa sibirica*). 1. Residue level, tissue distribution, and temporal trend. *Environ. Sci. Technol.* **2008**, *42* (7), 2295–2301.

(28) Holmstrom, K. E.; Johansson, A. K.; Bignert, A.; Lindberg, P.; Berger, U. Temporal Trends of Perfluorinated Surfactants in Swedish Peregrine Falcon Eggs (*Falco peregrinus*), 1974−2007. *Environ. Sci. Technol.* **2010**, *44* (11), 4083–8.

(29) O'Connell, S. G.; Arendt, M.; Segars, A.; Kimmel, T.; Braun-McNeill, J.; Avens, L.; Schroeder, B.; Ngai, L.; Kucklick, J. R.; Keller, J. M. Temporal and Spatial Trends of Perfluorinated Compounds in Juvenile Loggerhead Sea Turtles (*Caretta caretta*) along the East Coast of the United States. *Environ. Sci. Technol.* **2010**, *44* (13), 5202–5209.

(30) Karrman, A.; Ericson, I.; van Bavel, B.; Darnerud, P. O.; Aune, M.; Glynn, A.; Lignell, S.; Lindstrom, G. Exposure of perfluorinated chemicals through lactation: Levels of matched human milk and serum and a temporal trend, 1996−2004, in Sweden. *Environ. Health Perspect.* **2007**, *115* (2), 226–230.

(31) Olsen, G. W.; Mair, D. C.; Church, T. R.; Ellefson, M. E.; Reagen, W. K.; Boyd, T. M.; Herron, R. M.; Medhdizadehkashi, Z.; Nobilett, J. B.; Rios, J. A.; Butenhoff, J. L.; Zobel, L. R. Decline in perfluorooctanesulfonate and other polyfluoroalkyl chemicals in American Red Cross adult blood donors, 2000−2006. *Environ. Sci. Technol.* **2008**, *42* (13), 4989–4995.

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

(33) Butenhoff, J. L.; Kennedy, G. L., Jr.; Hinderliter, P. M.; Lieder, P. H.; Jung, R.; Hansen, K. J.; Gorman, G. S.; Noker, P. E.; Thomford, P. J. Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. *Toxicol. Sci.* **2004**, *82* (2), 394–406.

(34) Kemper, R. A.; Jepson, G. W. Pharmacokinetics of perfluorooctanoic acid in male and female rats. *Toxicologist* **2003**, *72* (1-S), 148.

(35) Apelberg, B. J.; Witter, F. R.; Herbstman, J. B.; Calafat, A. M.; Halden, R. U.; Needham, L. L.; Goldman, L. R. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ. Health Perspect.* **2007**, *115* (11), 1670–6.

(36) Fenton, S. E.; Reiner, J. L.; Nakayama, S. F.; Delinsky, A. D.; Stanko, J. P.; Hines, E. P.; White, S. S.; Lindstrom, A. B.; Strynar, M. J.; Petropoulou, S. S. Analysis of PFOA in dosed CD-1 mice. Part 2. Disposition of PFOA in tissues and fluids from pregnant and lactating mice and their pups. *Reprod. Toxicol.* **2009**, *27* (3−4), 365–72.

(37) Lau, C.; Thibodeaux, J. R.; Hanson, R. G.; Rogers, J. M.; Grey, B. E.; Stanton, M. E.; Butenhoff, J. L.; Stevenson, L. A. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicol. Sci.* **2003**, *74* (2), 382–92.

(38) Houde, M.; Wells, R. S.; Fair, P. A.; Bossart, G. D.; Hohn, A. A.; Rowles, T. K.; Sweeney, J. C.; Solomon, K. R.; Muir, D. C. G. Polyfluoroalkyl Compounds in Free-Ranging Bottlenose Dolphins (*Tursiops truncatus*) from the Gulf of Mexico and the Atlantic Ocean. *Environ. Sci. Technol.* **2005**, *39* (17), 6591–6598.

(39) Keller, J. M.; Kannan, K.; Taniyasu, S.; Yamashita, N.; Day, R. D.; Arendt, M. D.; Segars, A. L.; Kucklick, J. R. Perfluorinated Compounds in the Plasma of Loggerhead and Kemp's Ridley Sea Turtles from the Southeastern Coast of the United States. *Environ. Sci. Technol.* **2005**, *39* (23), 9101–9108.

(40) Kannan, K.; Koistinen, J.; Beckmen, K.; Evans, T.; Gorzelany, J. F.; Hansen, K. J.; Jones, P. D.; Helle, E.; Nyman, M.; Giesy, J. P. Accumulation of Perfluorooctane Sulfonate in Marine Mammals. *Environ. Sci. Technol.* **2001**, *35* (8), 1593–1598.

(41) Dallaire, R.; Ayotte, P.; Pereg, D.; Dery, S.; Dumas, P.; Langlois, E.; Dewailly, E. Determinants of plasma concentrations of perfluorooctanesulfonate and brominated organic compounds in Nunavik Inuit adults (Canada). *Environ. Sci. Technol.* **2009**, *43* (13), 5130–5136.

(42) Weihe, P.; Kato, K.; Calafat, A. M.; Nielsen, F.; Wanigatunga, A. A.; Needham, L. L.; Grandjean, P. Serum Concentrations of Polyfluoroalkyl Compounds in Faroese Whale Meat Consumers. *Environ. Sci. Technol.* **2008**, *42* (16), 6291–6295.