Presence of Anionic Perfluorinated Organic Compounds in Serum Collected from Northern Canadian Populations

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Introduction

Perfluorinated organic compounds are used in a wide variety of consumer and industrial products and applications, ranging from personal care products and cleaning solutions, to grease resistant coatings for fabric and paper and emulsifiers in the production of polymers.[1] Perfluorinated compounds such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are persistent and bioaccumulative. PFOS and PFOA have been detected in biota sampled from around the world[2], including the Canadian Arctic.[3,4] Evidence from various laboratory experiments suggest that these perfluorinated compounds can elicit negative effects, including peroxisome proliferation[5] and possibly hepatocarcinogenesis.[6] PFOA and PFOS also appear to biomagnify in marine food webs, in a similar fashion as traditional organohalogenated POPs like the recalcitrant PCB congeners.[4,7]

Indigenous northern Canadian populations such as the Inuit and Inuvialuit often hunt and consume marine mammals, including beluga, narwhal, and seal, as part of their traditional diet. Thus, segments of these populations are often exposed to higher levels of POPs than southern populations and other consumers of market foods. This higher exposure is reflected in plasma concentrations of traditional POPs such PCBs.[8] There is a question of whether a similar situation occurs for PFOS, PFOA, and similar perfluorinated compounds. This preliminary survey analyzed a suite of perfluorinated sulfonates and carboxylates in 23 pooled archived samples of human plasma collected from various northern Canadian populations.

Materials and Methods

Samples. Maternal and umbilical cord blood samples were collected from 1994 to 2001 from individuals from various communities in the Northwest and Nunavut Territories for measurement of a range of persistent organic pollutants and toxic metals.[9] Ethnicities and region of origin for each sample donor was recorded. Blood samples were centrifuged to obtain plasma fractions, which were stored at -20^{o}C in glass containers. Remnant plasma samples (560 in total) were pooled to form 10 maternal and 13 cord composite samples based on geographical region in which donors resided and donor ethnicities (Inuit, Dene/Métis, and Caucasian).

Chemicals and Materials. The following perfluorinated compounds (purity >95%) were used as standards for the analysis of perfluorinated carboxylates and sulfonates in archived human plasma: perfluorohexanoic acid (Fluka, Buchs, Switzerland), perfluoroheptanoic acid (Aldrich, Oakville,

ON), perfluoroctanoic acid (Aldrich, Oakville, ON), perfluorononanoic acid (Aldrich, Oakville, ON), perfluorodecanoic acid (Aldrich, Oakville, ON), perfluoroundecanoic acid (Aldrich, Oakville, ON), perfluorododecanoic acid (Aldrich, Oakville, ON), perfluorotetradecanoic acid (Aldrich, Oakville, ON), tetrabutylammonium perfluorobutane sulfonate (Fluka, Buchs, Switzerland), and tetraethylammonium perfluorooctane sulfonate (Aldrich, Oakville, ON). Perfluoro-3,7-dimethyloctanoic acid (SynQuest Labs, Alachua, FL) was used as an internal standard for all analytes. Standards were prepared in a 1:1 (v/v) methanol/water solution. Rabbit plasma (Biomeda, Foster City, CA) was used as the standard matrix during the analyses. The same rabbit plasma was also used as a standard blank. Purified water (see under "*Plasma Extraction*") was used as a sample blank. An archived pool of human serum prepared from Canadian Red Cross human serum samples collected in 1994 was used as a quality control (QC) sample.

Plasma Extraction. Pooled human plasma samples, standards prepared in rabbit plasma, human serum quality control samples, and water and rabbit plasma blanks (500 uL sample size for all) were extracted following the ion pair extraction procedure described in Hansen et al.[10] One rabbit plasma blank, one water sample blank, one human serum QC sample, and six standards were run with every set of human plasma samples. Perfluoro-3,7-dimethyloctanoic acid was used as a surrogate standard for all perfluorinated compounds, and was added to all samples and blanks prior to the initial addition of ion pair extraction reagents.

All water and methyl-*tert*-butyl ether used in the method were Milli-Q purified (Millipore, Billerica, MA) and ACS reagent grade, respectively. Both solvents were passed through a glass column containing Amberlite XAD-7 resin (Aldrich, Oakville, ON) to remove any possible perfluorinated contaminants. Methanol (OmniSolv® grade, EMD Chemicals, Darmstadt, Germany) was used without extra purification. All solvents were kept in glass containers.

LC-MS/MS Analysis. LC-MS/MS analysis was performed following the method described by Lau.[11] Samples (10 uL injection) were chromatographed on a 2.1 x 100 mm Genesis C_{18} analytical column and C_{18} guard column (Jones Chromatography, Columbia, MD) installed on an HP 1100 binary pump high performance liquid chromatograph (Agilent, Palo Alto, CA). A 5 mM solution of ammonium formate in Milli-Q purified water and a 2:1 (v/v) solution of acetonitrile/methanol (OmniSolv® grade, EMD Chemicals, Darmstadt, Germany) were mobile phase solutions A and B, respectively. The perfluorinated analytes were chromatographically resolved using the following gradient program: 45% B at 0.150 mL/min for 1 min, increasing to 70% B over 4 minutes, 75% B over 8 minutes, and 90% B over 3 minutes, and then held at 90% B for 7 minutes. The column was then flushed with 90% B at 0.200 mL/min for 1 minute, and 45% B for 6 minutes. The liquid chromatograph was connected to a Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK). Samples were analyzed for perfluorocarboxylates and sulfonates using negative electrospray tandem mass spectrometry in the multiple reaction monitoring mode. Table 1 lists the monitored transitions for all analytes.

Table 1. MS/MS Instrumental Parameters.

Analyte	Transition	Cone Voltage (kV)	Collision Energy (eV)
perfluorobutane sulfonate (PFBS)	299 → 99	50	35
perfluorohexanoate (PFHxA)	313 → 268.8	30	10
perfluoroheptanoate (PFHpA)	363 → 318.8	30	10
perfluorooctanoate (PFOA)	413 → 368.8	30	10
perfluorononanoate (PFNA)	463 → 418.8	30	10
perfluorooctane sulfonate (PFOS)	499 → 99	50	40
	499 → 80*	50	40
perfluoro-3,7-dimethyloctanoate ($PFMe_2OA$)**	513 → 468.8	50	40
perfluorodecanoate (PFDA)	513 → 468.8	30	10
perfluoroundecanoate (PFUA)	563 → 518.8	30	11
perfluorododecanoate (PFDoA)	613 → 568.8	30	12
perfluorotetradecanoate (PFTeDA)	713 → 668.8	30	12

*used for quantitation
**surrogate standard

Data Analysis. Peak areas were integrated using MassLynx software provided as part of the LC-MS/MS system. Relative response factors were calculated as the ratio of analyte peak area to $PFMe_2OA$ area. Relative response factors of standards were corrected for the presence of PFHpA, PFOA, PFNA, and PFOS observed in the rabbit plasma blanks. Relative response factors of samples were corrected for the presence of PFHpA, PFOA, PFNA, and PFOS observed in the water blanks.

Method Detection Limits. MDLs were determined according to Winefordner and Long[12] using 9 replicates of extracted water. Only PFHpA, PFOA, PFNA, and PFOS were detected in the blanks. MDLs of the other analytes were assigned to be the lowest concentration in the standards that produced a peak with area at least 3 times greater than adjacent baseline noise. MDLs are listed in Table 2.

Table 2. Method detection limits (sample volume = 500 uL).

Analyte	MDL (ng/mL)
PFBS	4.00
PFHxA	0.6
PFHpA	0.36
PFOA	1.4
PFNA	0.38
PFOS	0.39
PFDA	0.6
PFUA	0.6
PFDoA	0.6
PFTeDA	0.6

Table 3. Mean analyte recoveries and relative standard deviations.

Analyte	% Recovery	RSD
PFBS	79	8
PFHxA	81	21
PFHpA	87	12
PFOA	80	25
PFNA	82	6
PFOS	79	11
PFDA	92	11
PFUA	84	8
PFDoA	68	8
PFTeDA	49	6

Method Recoveries. Method recoveries were evaluated by comparing relative response factors obtained from extracted rabbit plasma that had been fortified with perfluorocarboxylates and sulfonates prior to extraction and after completion of the work up (ie. after bringing the methyl-*tert*-butyl ether phases to dryness). Rabbit plasma (n=5) was fortified at two levels (1.2 and 2.4 ng perfluorocarboxylates/mL plasma, and 0.40 and 0.80 ng perfluorosulfonates/mL plasma). Recoveries did not significantly differ between the two fortification levels. Overall mean recoveries (n=10) are listed in Table 3.

Quality Control. PFHpA, PFOA, PFNA, and PFOS were observed above MDLs (means: 0.62, 3.10, 0.62, 36.9 ng/mL, respectively) in the three human serum QC samples analyzed. One pooled human plasma sample was also run in duplicate. Concentrations of PFOA and PFOS were within 20% of their respective mean

Results and Discussion

PFOS, PFOA, and PFNA were detected in all plasma samples analyzed. In addition, 70% of the samples contained detectable levels of PFHpA. PFHxA was detected in only one sample. PFOS was always the most abundant analyte, observed at blank corrected concentrations ranging from 2.8 to 57.9 ng/mL. PFOA was generally the next most abundant (0.48 to 5.46 ng/mL), followed by PFNA (0.11 to 1.98 ng/mL). The measured concentrations of PFOS and PFOA are similar to those reported in the literature for non-occupationally exposed southern populations (Table 4).

Table 4. Human sera and plasma PFOS and perfluorooctanoate PFOA concentrations.

Sampled population	mean PFOS (ng/mL)	mean PFOA (ng/mL)	Reference
female adults, northern Canada	36.9	2.2	this study
cord blood, northern Canada	16.7	3.4	this study
non-occupationally exposed adults, Ottawa, Ontario	28.8	3.4	13
commercially available human serum	28.4	6.4	10
senior citizens, Seattle, Washington	31.0 (geomean)	4.2 (geomean)	14
fluorochemical plant workers	1320	1780	15
non-occupationally exposed Americans	17.7	3.1	16
American Red Cross blood donors	34.9 (geomean)	4.6 (geomean)	17

Differences in ΣFOC concentrations between cord and maternal plasma sample types, and amongst geographical regions and ethnic groups, were examined using a three-way ANOVA (generalized linear model: ΣFOC = geographical region x sample type x ethnic group). ΣFOC concentrations were significantly higher in maternal plasma samples, as compared to cord plasma (Figure 1, p=0.01). This difference, not usually observed for lipid-normalized data on hydrophobic contaminants, may be due to differences in the amount or type of proteins available to bind FOCs in maternal and cord blood respectively, since protein binding has been implicated in the bioaccumulation of FOCs.[18] Ethnic groups (Table 5) were not significantly associated with ΣFOC concentrations (p=0.2, respectively); neither was the region in which the donors resided (p=0.09). The number of samples was too low to examine interactions amongst region, ethnicity and concentration of FOCs. A more powerful analysis could be done on a larger data set.

Figure 1. Mean ± std deviation ΣFOC concentrations in cord and maternal plasma.

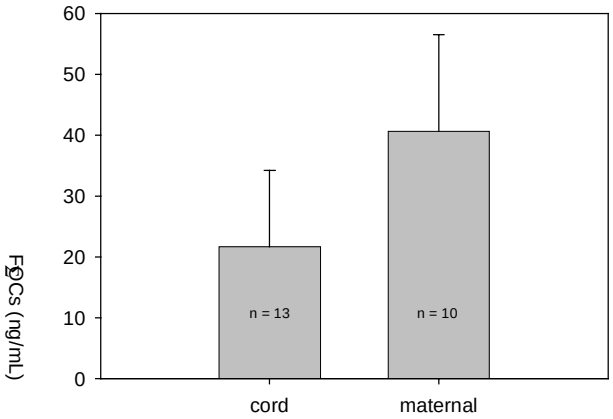

Table 5. Arithmetic mean plasma ΣFOC concentrations in ethnic groups.

Ethnic Group	n	mean ΣFOC (ng/mL)	range ΣFOC (ng/mL)
		Cord plasma	
Inuit	5	22.9	7.3-42.8
Dene/Métis	4	16.1	7.9-28.2
Caucasian	4	25.8	15.4-39.1
		Maternal plasma	
Inuit	3	42.8	29.4-57.7
Dene/Métis	3	36.0	21.7-61.3
Caucasian	4	42.3	21.7-58.7

The results of this preliminary survey demonstrate that populations residing in the Northwest and Nunavut Territories in northern Canada are exposed to FOCs. The presence of FOCs in cord blood plasma also indicates that exposure occurs in utero. This limited data set shows that FOC levels in these northern samples are very similar to FOC concentrations in southern population, and that there are no marked differences between the various ethnic groups (Inuit, Dene/Metis, Caucasian). The low samples numbers are insufficient to confidently examine whether or not there are differences in FOC exposure and body burdens amongst various ethnic groups within regions that consume different diets, but these preliminary data do not support such a conclusion.

There are likely a number of routes of exposure to FOCs which contribute to the observed plasma concentrations, including via environmental contamination and consumption of some traditional and country foods. However, exposure has also likely occurred via consumption of market foods

contained in packaging that has been treated with perfluoroalkyl-containing coatings.[19] This exposure route appears to have been decreasing in significance since the cease in production of compounds based on the perfluorooctanesulfonyl fluoride building block by 3M. The results of this small survey suggest that a diet based on traditional foods does not result in higher body burdens of FOCs as has been observed with other legacy POPs such as PCBs.

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