Historical Concentrations of Perfluorinated Compounds in Canadian Blood Donors

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Introduction

In a similar fashion as other populations, Canadians are exposed to perfluorinated compounds. Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) have been measured in the plasma of Canadians during a pilot study conducted in 2004[1]. The sources of exposure to these compounds likely includes consumption of contaminated food[2], dust ingestion[3], and use of treated consumer items [4].

Another potential source of perfluorocarboxylates and perfluorosulfonates for Canadians could be exposure to precursor compounds, such as perfluorooctanesulfonamides (PFOSAs) and N-ethylperfluorosulfonamidoethanols (FOSEs), which may be metabolised to persistent perfluorocarboxylates and perfluorosulfonates[5;6]. PFOSAs have been detected in foods[7], and FOSEs and PFOSAs have been detected in indoor air in Canadian homes[8].

In this work, archived plasma samples obtained from Canadian adult blood donors were analyzed for a suite of perfluorinated compounds including PFOSAs, FOSEs, perfluorocarboxylates (PFCAs), and perfluorosulfonates (PFSs).

Materials and methods

Plasma samples. Archived plasma samples from 148 individual blood donors were obtained from the Canadian Red Cross. Expressed consent was provided by the Canadian Red Cross for the analysis of the plasma samples for environmental chemical contaminants. Plasma was obtained through the Canadian Red Cross in the first half of 1994 from 13 different cities across Canada. General details on the samples are provided in Table 1. The 148 individual samples from adult donors were collected by apheresis where whole blood is drawn, cells are separated fromthe plasma *in situ*, and the cells returned to the donor. Collected plasma was stored in pre-cleaned glass or polycarbonate jars at -20^{o}C until to chemical analysis. Expressed consent was provided by the Canadian Red Cross for the analysis of the plasma samples for environmental chemical contaminants. Approval for the analyses was also obtained from the Health Canada Research Ethics Board

Analysis of PFOSAs and FOSEs in plasma. A new analytical method was developed and validated for the analysis of 4 perfluorooctylsulfonamides (N,N'-Me_2FOSA, N-MeFOSA, N-EtFOSA, PFOSA) and 2 perfluorooctylsulfonamidoethanols (NMeFOSE, NEtFOSE). The method used liquid-liquid extraction to isolate the analytes from plasma, followed by instrumental analysis by gas chromatography-positive chemical ionization-mass spectrometry. Briefly, approximately 1 mL of thawed plasma was fortified with recovery internal standards (D-N-EtPFOSA and D-N-EtFOSE; Wellington Laboratories, Guelph, ON, Canada). Formic acid was added to the plasma, and the mixture sonicated for 15 min. After sitting, 4 mL of water that had been passed through XAD2 resin was added and the mixture was extracted with 2 x 3 mL of hexane, followed by 1 x 2 mL of isooctane. The organic layers were combined, and reduced in volume using a warm water bath and N_2 (g). Instrument performance internal standards (D-N-MePFOSA, and D-N-MeFOSE; Wellington Laboratories) were added to extracts just prior to GC-MS analysis.

PFOSAs and FOSEs were analyzed using a modification of an existing gas chromatographic-mass spectrometric method[9]. Quasi-molecular ions formed using methane positive chemical ionization were monitored in the selected ion monitoring mode.

Analysis of PFCAs and PFSs in plasma. An existing extraction and liquid chromatography mass spectrometry analytical method[1] was used to analyze anionic perfluorinated compounds in the archived plasma samples. Four

perfluorocarboxylates [perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA)] and three perfluorosulfonates [perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), and perfluorooctanesulfonate (PFOS)] were covered by the method. Shorter carbon chain length perfluorocarboxylates were not chromatographically retained, and thus not included in the analytical method. In addition, two longer chain length perfluorocarboxylates [perfluorododecanoate (PFDoDA) and perfluorotetradecanoate (PFTeDA)] were not reproducibly recovered by the extraction method and were excluded from the data analysis.

Results and discussion

PFOSAs and FOSEs in plasma. Method recoveries, precision, and detection limits were characterized using fortified rabbit plasma. Average ± standard deviation recoveries for all analytes were 84 ± 14% (n=120). Method detection limits were in the range of 0.1 – 5 ng/mL.

Only one compound - perfluorooctanesulfonamide (PFOSA) – was observed in plasma samples at concentrations above the method detection limits. PFOSA was detected in 15 of the 148 samples, at a mean concentration of 0.10 ng/mL plasma wet weight, when non-detects were set equal to zero. The range of PFOSA concentrations detected above the method detection limits were 0.41 – 2.6 ng/mL. The PFOSA concentrations observed are similar to those reported in other studies that have detected PFOSA in serum from the United States[10], Spain[11], Poland[12], and Sweden[13].

There was no correlation between PFOSA and PFOS concentrations in the analyzed plasma samples. The lack of correlation may have been driven by the large difference in the frequency of detection of the two analytes – 10% and 99% for PFOSA and PFOS, respectively. Weak correlations have been reported by other groups[10;11], and are mechanistically logical considering that PFOSA has been shown to be metabolized to PFOS.

The apparent absence of other potential PFOS-precursors in plasma may be an indication that these compounds are too labile to be detected in plasma. The PFOSAs and FOSEs may be rapidly metabolized or otherwise transformed to more stable compounds, such as PFOS. It is also possible that direct exposure to these compounds is not great enough to result in detectable levels in plasma.

PFCAs and PFSs in plasma. Recoveries of the analytes ranged from 99 to 122%; method detection limits ranged from 0.07 to 0.54 ng/mL plasma. Eight samples were run in duplicate to access the mean percent difference between duplicate measurements which was 10%.

PFOA and PFOS were detected in all samples, at concentrations ranging from 0.16 to 14.3 ng/mL (geomean = 0.99) and 0.56 to 39.8 ng/mL (geomean = 7.16), respectively. Other analytes were detected less frequently, and at lower concentrations. Perfluorononanoate was detected in 43% of the samples analyzed; the range of concentrations detected was 0.16 to 0.68 ng/mL. Perfluoroheptanoate and perfluorohexanesulfonate were both detected in 39% of the samples analyzed, at concentrations ranging from 0.17 to 3.10 ng/mL and 0.36 to 4.45 ng/mL, respectively. Perfluorodecanoate was only detected in 8% of the samples analyzed, and perfluorobutanesulfonate was not detected in any samples (method detection limit = 0.54 ng/mL).

PFOA and PFOS concentrations observed were lower than those reported for in a pilot study conducted in 2002 [1]. Median PFOS and PFOA concentrations were 27.4 and 2.3 ng/mL respectively, for the samples collected from 56 donors (> 20 years old) in the Ottawa area. Concentrations of these two compounds in the plasma collected in 1994 were approximately one third to one half of the values reported for the 2002 study.

The factors that are contributing to the differences between the concentrations measured in samples collected in 1994 versus 2002 are not readily apparent. The differences may be potentially due to changes in the exposure of Canadians to PFOS, PFOA, and any precursors between the mid-nineties and early 2000s. Another possibility is changes in analytical method sensitivity. The method used to analyze the 2002 samples had higher method detection limits. This could inflate the calculated median concentrations of PFOA (15 out of the 56 samples analyzed did not contain PFOA above the method detection limit). However, the median concentration of PFOS would not be affected by the change in method sensitivity because all 2002 samples contained detectable levels of this analyte. It is also possible that the median age of donors differed during the 1994 and 2002 sampling periods; results from this study suggest that concentrations of PFCAs and PFSs decrease with increasing age (see below). Individual age data was not collected in the 2002 Canadian study; therefore this possibility cannot be tested.

There are currently no other data available for corroboration of the potential increasing trend of PFOS and PFOA in Canadians from 1994 to 2002. There are no other data on PFCA or PFS concentrations in serum or plasma collected from non-occupationally exposed North American donors in the mid-nineties. One study conducted in Maryland, United States has analyzed serum collected in 1974 and 1989[14]. Concentrations of PFOS in paired samples increased by 25%; PFOA concentrations increased two-fold over the two sampling time points. However, there was no indication of further increase when results were compared to plasma samples collected in 2001 and analyzed in a previous study[15]. A Japanese study has also observed an increase in PFOA serum concentrations from 1983 to 1999 of approximately 0.5 ng/mL per year; the trend for PFOS measured in the same samples was not statistically significant[16].

Statistical analyses on a model containing donor age, sex, and location (i.e. the city in which the blood collection centre was located) demonstrated that both age and location were significantly associated with the concentrations of total PFCAs and PFSs determined in plasma. Analyte concentrations decreased with an increase in donor age. The relationship between concentrations and age was not as strong in females as in the male sub-set. Other studies have examined the relationship between plasma and serum PFA concentrations and donor age, and only one up to this point has noted a significant association between the two variables[17]. In this study, Fromme et al. noted that plasma PFA concentrations increased with increasing age in females only.

The relevance of the statistical significance of location is not apparent, since detailed information on the donors' actual residence location and length of time at this residence prior to blood donation was not available.

No differences in total PFCA and total PFS concentrations were observed between males and females. A similar absence in sex-based concentration difference was noted in the 2002 Canadian study[1]. However, many other studies have noted for selected compounds, such as PFOS and PFOA, concentrations in males are often significantly greater than females.[11;15;18]

Overall, the results of this study have highlighted many avenues for future research. Research questions derived from this study touch upon factors governing the absence of PFOSAs and FOSEs in plasma, changes in Canadians' exposure to PFCAs and PFSs over time, and changes in Canadians' PFA body burdens with age.

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Table 1. Summary of results of total perfluorocarboxylates (SPFCAs) and perfluorosulfonates (SPFSs) measured in plasma from Canadian blood donors. Geometric mean (range) concentrations are provided.

City of blood collection	n samples	SPFCAs (ng/mL)	SPFSs (ng/mL)
Vancouver, BC	14	0.91 (0.19-4.8)	5.3 (0.56-43)
Calgary, AB	7	1.0 (0.15-1.7)	4.4 (0.63-27)
Edmonton, AB	8	1.6 (0.35-4.0)	10.2 (3.2-24)
Saskatoon, SK	7	0.82 (0.30-3.4)	5.5 (1.1-27)
Winnipeg, MB	11	1.3 (0.60-3.2)	9.1 (3.8-26)
London, ON	11	1.5 (0.83-3.0)	12.8 (5.0-31)
Hamilton, ON	12	1.8 (0.86-4.1)	12.1 (4.5-26)
Toronto, ON	12	2.6 (0.30-12)	15.2 (4.0-37)
Ottawa, ON	2	0.65 (0.60-0.71)	5.2 (4.2-6.3)
Montreal, QC	14	0.69 (0.24-1.9)	3.7 (0.80-14)
Quebec City, QC	14	0.71 (0.16-4.1)	4.6 (0.77-27)
Halifax, NS	7	1.5 (0.65-2.3)	11.7 (6.5-31)