Time trend studies of perfluorinated compounds in beluga (Delphinapterus leucas) from SE Baffin Island

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Introduction

Perfluorinated compounds (PFCs) are a diverse group of compounds with unique chemical features. They have both hydro- philic and phobic properties and are used as surfactants in a wide range of commercial and residential applications. The perfluoroalkyl- carboxylic (PFCAs) and sulfonic (PFSAs) acids, two classes of PFCs, are both resistant to degradation or metabolism, and this contributes to their bioaccumulation and persistence.

Time trend studies on animals are useful in constructing a chronology of chemical contamination as changes in the inputs of chemicals into the environment are reflected in changes in chemical concentrations in wildlife over time[1]. Because of its unique physical and biological characteristics, the arctic environment has become a sentinel for studies on temporal trends of halogenated organic pollutants.

Our objective of this research was to determine if they are changes in concentrations of the PFCs in beluga whales from the Canadian arctic over time. In addition to the suite of PFCAs (C_8 to C_{12}) and PFOS, N-methyl- and perflurooctanesulfonamide (N-EtPFOSA and PFOSA), two metabolic precursors of PFOS in fish[2], and a suite of fluorotelomer saturated (FTCAs: $C_nF_{2n+1}CH_2COOH$, n = 6, 8 and 10) and unsaturated acids (FTUCAs: $C_nF_{2n}CHCOOH$, n = 6, 8 and 10) were analyzed in animals from our archives.

Material and Methods

Chemicals. Mass labelled ^{13}C-perfluorodecanoic acid (^{13}C-PFDA) and ^{13}C-PFOA, the suite of labelled and native saturated (6:2, 8:2 and 10:2 FTCAs) and unsaturated fluorotelomer acids (6:2, 8:2 and 10:2 FTUCAs), perfluorooctanesulfonamide (PFOSA), d_5-ethyl-PFOSA and d_3-methyl-PFOSA were obtained from Wellington Laboratories (Guelph, ON). The nomenclature of the FTCAs and FTUCAs was been described elsewhere[3]. Tetrahydro-PFOS (4H-PFOS), PFOS, PFOA and the analogous suite of carboxylic acids: perfluoro-nonanoic (PFNA), -decanoic (PFDA), -undecanoic (PFUA) and –dodecanoic (PFDoDA) acids were obtained from SynQuest Laboratories (Alachua, FL). Optima grade methanol and water were obtained from Caledon Laboratories (Georgetown, ON).

Samples. Male beluga from Pangnirtung, SE Baffin Island (1982, 1986, 1992, 1995, 2002), were selected for study. Eight animals were selected for study from each year. Archived liver were shipped in plastic bags and stored at -30^{o}C.

Extraction and Analysis. PFCs in liver samples were analyzed using an extraction method developed in our laboratory[4]. Approximately 5 g of liver was homogenized in a polypropylene tube. A sub-sample (~0.1 g) was removed and spiked with recovery internal standards [^{13}C-PFDA (for PFCAs), 4H-PFOS (for PFOS), and labelled 6:2, 8:2 and 10:2 FTCAs (for FTCAs) and FTUCAs (for FTUCAs) and d_5-ethyl-PFOSA (for PFOSA)]. Extractions were performed using 2 mL of optima grade methanol, vortexing for 1 minute and centrifuging at 3,500 *rpm* for 10 minutes. This was repeated 2 times and each time the methanol extract was removed and combined. The combined extract was then solvent reduced to ~ 1mL and transferred to a microcentrifuged vial and centrifuged at 13,500 *rpm* for 10 minutes. The supernatant was then carefully removed and transferred to a HPLC injection vial and spiked with instrument performance internal standard (^{13}C-PFOA and d_3-methyl-PFOSA). Injections were made on a C_{18} reverse phase HPLC column onto a Sciex 2000 triple quadrupole mass spectrometer.

QA/QC. Two types of blanks were employed in this study. Instrument blanks were injections of methanol run after every five samples and were used to contamination from the LC/MS/MS instrument. Extraction (or method) blanks consisted of Optima grade methanol, and were extracted along with every 8 samples (total 5 blanks). Extraction blanks were used to monitor the potential for contamination to occur during extraction and work-up of the sample. PFC concentrations in samples were blank corrected by subtracting the signal from extraction blanks from the sample signals. Average recoveries of ^{13}C-PFDA, 4H-PFOS and d_5-ethyl-PFOSA were 83 ± 7, 112 ± 24 and 71 ± 11%, respectively. Samples were corrected for recoveries only when recoveries were less than 100%. Method detection limits were estimated to be 0.3 ng/g for PFOA and PFNA, 0.1 ng/g for PFDA, 0.03 ng/g for PFUA, 0.04 ng/g for PFDoDA, 0.02 ng/g for PFOS and 2 pg/g for PFOSA. For calculation of mean concentrations, a concentration of ½ of the MDLs was assumed in those instances that PFC concentrations were below MDLs. Matrix effects were assessed by comparing the ion signals of the ^{13}C-PFOA and d_3-methyl-PFOSA that were intentionally fortified into the extracts prior to injection to that of their respective ion signals from an injection made in methanol. Ion suppression was only observed for the d_3-methyl-PFOSA and a correction was applied to the PFOSA ion signal.

Results and Discussion

Plots of the time-trend log concentration of PFCAs (C_8-C_{12}), PFOS and PFOSA are shown in Figure 1. PFOSA concentrations were consistently higher than any other PFC examined in this study and were higher than the sum of all PFCAs. PFOS concentrations were generally similar to the ΣPFCA concentrations. FTCAs, FTUCAs and N-EtPFOSA were below detection limits in all samples.

PFCAs. There were exponential increases in both PFOA and PFDA concentrations from 1982 to 2002 with respective doubling times of 6 and 11 years. PFUA, the dominant PFCAs in these animals, showed a linear increase throughout the time period (r^2=0.25, p=0.001). No clear trends were observed in PFNA and PFDoDA concentrations except that both peaked in 1995.

PFOS and PFOSA. The concentration profile for both PFOSA and PFOS were similar. This is perhaps not too surprising considering that PFOSA is a metabolic precursor to PFOS and contributing to the overall burden of PFOS in these animals[2]. Consistent with our earlier findings, N-EtPFOSA was not detected in beluga from the eastern Canadian Arctic and suggests that either these animals were not exposed to the compound or that it is quickly metabolized to PFOSA[5].

PFOSA and PFOS concentrations were found to be increasing up until 2002 although there was a small decrease in concentration in 1995 for both compounds.

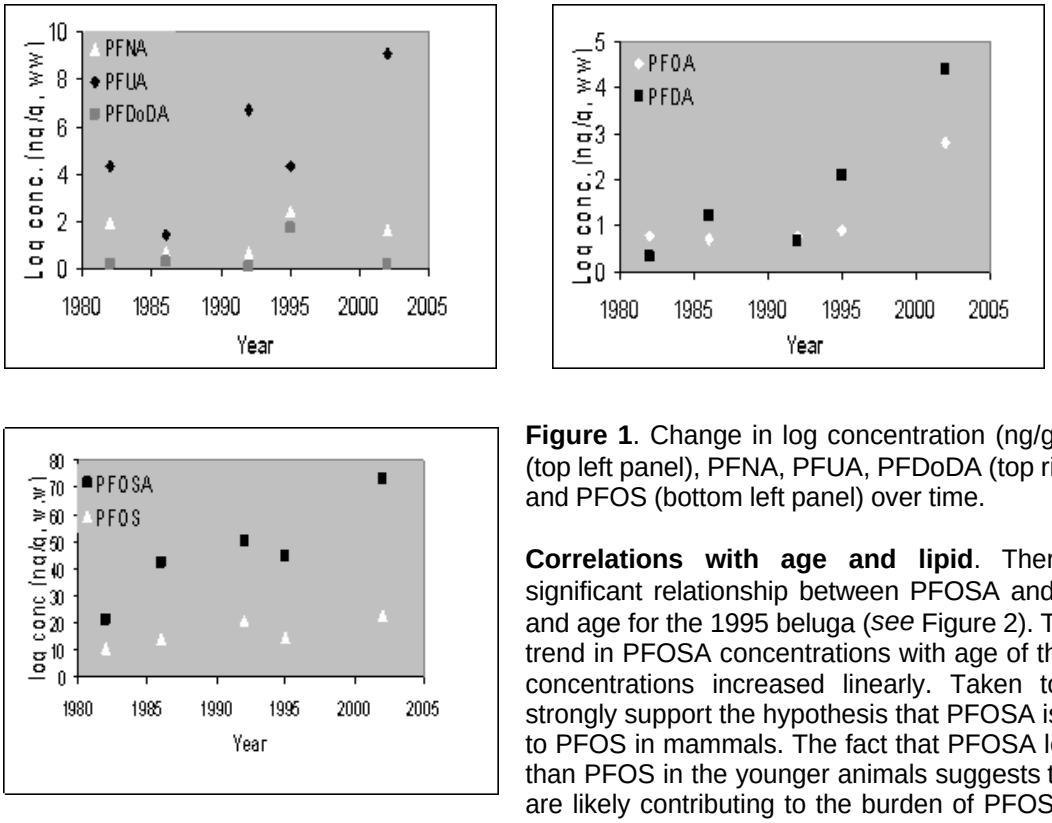

Figure 1. Change in log concentration (ng/g, ww) of PFOA, PFDA (top left panel), PFNA, PFUA, PFDoDA (top right panel) and PFOSA and PFOS (bottom left panel) over time.

Correlations with age and lipid. There was a statistically significant relationship between PFOSA and PFOS concentrations and age for the 1995 beluga (*see* Figure 2). There was a decreasing trend in PFOSA concentrations with age of the animals while PFOS concentrations increased linearly. Taken together, these results strongly support the hypothesis that PFOSA is a metabolic precursor to PFOS in mammals. The fact that PFOSA levels are much greater than PFOS in the younger animals suggests that PFOSA-precursors are likely contributing to the burden of PFOSA at a very young age and that older animals have a greater capacity to biotransform PFOSA to PFOS. No correlation was observed for any of the PFCs and lipid content.

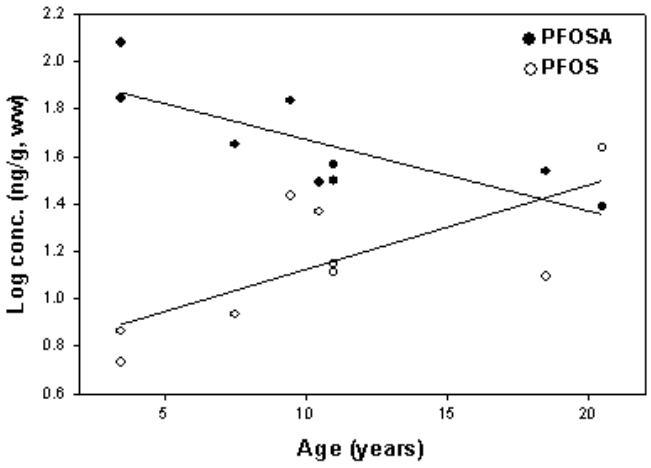

Figure 2. Correlation between PFOSA and PFOS log concentration in 1995 beluga and age. Regression analysis: log [PFOSA] = 1.9750 – 0.0302*(age), (r^2=0.63, p=0.01); log [PFOS] = 0.7688 + 0.0356*(age), (r^2=0.51, p=0.03).

Correlation amongst concentrations of PFAs. Correlations between PFCA chain length (and PFOS) and concentrations were observed for beluga from 1982. Statistically significant positive correlations were found

between PFOS and PFUA concentrations (r^2=0.57, p=0.05) and PFDA and PFUA concentration (r^2=0.64, p=0.05).

Acknowledgements

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References

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