# PERFLUOROOCTANE SULFONATE AND RELATED PERFLUORINATED HYDROCARBONS IN HARBOR SEALS (*PHOCA VITULINA CONCOLOR*) FROM THE NORTHWEST ATLANTIC

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## Introduction

Perfluorooctane sulfonate (PFOS) and related perfluorinated organic compounds (PFCs) are ubiquitous global contaminants of anthropogenic origin that are widely distributed in the environment, in humans, and in wildlife.[1-3] PFCs are extremely persistent and bioaccumulative, although unlike neutral organochlorines (PCBs), their accumulation is not driven by lipophilicity, as they bind to specific proteins in blood plasma and liver.[4] High concentrations of PFOS have been found in liver tissue of marine mammals,[3-5] with higher concentrations occurring in animals living near urbanized or industrialized areas. PFCs have also been detected in marine mammals from remote areas,[3,5-10] suggesting atmospheric transport of volatile precursor compounds and/or transport in ocean currents.

The mechanisms and pathways leading to the presence of perfluorinated compounds in wildlife and humans are not well characterized, but it is likely that there are multiple sources of the compounds[1]. For over 40 years, PFCs have been used in a variety of industrial and consumer products, including protective coatings for carpets and apparel, nonstick cookware, paper coatings, insecticide formulations, and surfactants in fire-fighting foams. Concerns about its environmental persistence and toxicity led to a phase-out of PFOS from the US market in 2000;[11] however, perfluoroocatanonic acid (PFOA) and longer chain perfluorinated carboxylic acids (PFCAs) continue to be manufactured as emulsifiers and additives in the polymerization process. These long-chain PFCAs are more bioaccumulative than PFOS and have similar toxic properties. Although relatively little is known about the long-term effects of exposure, five different pathways have been proposed for PFC-related carcinogenicity, reproductive, developmental, and other types of toxicity including elevated mitochondrial fraction catalase activity; cell membrane disruption; hepatic peroxisome proliferation; increased estrogen and decreased testosterone levels; and decreased thyroid hormone levels.[12]

Studies on the occurrence of PFCs in marine mammals have focused on Europe, the Arctic, and the US Pacific coast.[3-10] The occurrence of these compounds in marine mammals from the US northwestern Atlantic has not been previously reported. This is one of the most industrialized regions in the world, and environmental contamination has been a concern since at least the 1950s. At the top of the food chain, harbor seals (*Phoca vitulina concolor*) inhabit near-shore waters and are an important sentinel species for coastal contamination.[13] Central to their migratory range, the Gulf of Maine is a shallow, semi-enclosed sea receiving significant riverine, urban, agricultural, and industrial pollutant inputs from large urban centers in the Northeast as well as via long-range atmospheric transport. PCB burdens in these seals are relatively high on a global scale, similar to levels reported in seals from polluted regions of Europe and Asia.[13] Here we report, for the first time, the presence of PFOS and related perfluorinated sulfonic acids (PFSAs) as well as a suite of PFCAs ranging in carbon length from 7 to 12 in harbor seals from the northwestern Atlantic.

## Materials and Methods

**Samples.** Liver samples were collected over a six-year period (2000 - 2005) from 25 stranded harbor seals (6 adult females, 3 adult males, 7 female pups, 9 male pups) along the northwestern Atlantic coast at locations ranging from Maine to Massachussetts (Fig. 1). Seals were weighed, and standard length and axillary girth were measured. Age was estimated based on body size. Condition indices were calculated by dividing axillary girth/standard length and body weight/standard length. Liver samples were stored in a freezer at -40°C until analysis.

**Chemical Analysis.** Concentrations of perfluorinated acids in liver tissue were determined by the ion pairing liquid extraction method described elsewhere[5]. Briefly, liver samples (0.3 g) were homogenized in 3 mL of Milli-Q water. A 2-mL aliquot was spiked with 5 ng of PFBS, 5 ng of $^{13}$C PFOA, and 5 ng of $^{13}$C PFOS as internal standards. One mL of 0.5 M tetrabutyl-ammonium hydrogen sulfate solution, 2 mL of sodium carbonate buffer (0.25M, pH 10), and 5 mL methyl-*tert*-butyl ether (MTBE) were added to the sample. The organic layer was separated by centrifugation, and the extraction was repeated with 5 mL of MTBE. The extracts were combined and evaporated to dryness under a gentle flow of nitrogen, before being reconstituted in 1 mL of methanol, vortexed, and filtered into an autosampler vial with polypropylene cap.

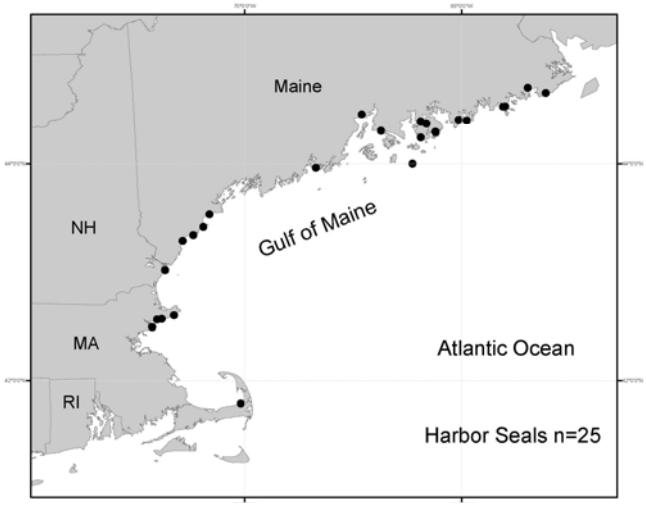


**Figure 1.** Map of the Gulf of Maine showing harbor seal sampling locations

Separation of perfluorinated acids was performed using an Agilent 1100 high performance liquid chromatograph (HPLC). Ten µL of the extracts were injected onto a 50 x 2mm (5 µm) Keystone Betasil $C_{18}$ column. A gradient mobile phase of methanol and 2mM ammonium acetate was used. At a flow rate of 300 µL/min, the mobile phase gradient was ramped from 10% to 25% methanol in 7 min and then to 100% methanol at 10 min, held at 100% methanol for 2 min, and then ramped down to 10% methanol. For quantitative analysis the HPLC was interfaced with an Applied Biosystems API 2000 tandem mass spectrometer (MS/MS) in negative electrospray ionization mode. Analyte ions were monitored using multiple reaction monitoring (MRM) mode. Parent and daughter ion transitions monitored for detection of PFOSA, PFDS, PFOS, PFHS, PFBS, $^{13}$C-PFOA, $^{13}$C-PFOS, PFHpA, PFOA, PFNA, PFDA, PFUnDA, and PFDoA. Quantitation was performed using a linear regression fit analysis weighted 1/x of a single unextracted calibration curve. Seven-point calibration curves were produced from concentrations of 0.1 to 100 ng/mL. The coefficient of determination ($r^2$) for each calibration was > 0.99. Quality-control standards were measured after every 10 samples. All procedural blank peak areas were less than half the determined limit of quantitation (LOQ) for each analyte. The LOQ was estimated as three times the lowest concentration point on the calibration curve, which is accurately measured within +/- 30 % of its theoretical value. Matrix spikes were performed for liver samples by spiking 5-10 ng of each target analyte, and passing through the whole analytical procedure. Recoveries of target analytes from the matrixes were between 63 and 99%. Mean recoveries of internal standards spiked to samples were between 68 and 81%. Concentrations are reported on a wet weight (ww) basis.

**Results and Discussion**

Four PFSAs (PFOS, PFHxS, PFDS, PFOSA) and six PFCAs (PFOA, PFNA, PFDA, PHFpA, PFUnDA, PFDoDA) were detected in harbor seal liver samples (Table 1). PFOS, detected in all samples, was the predominant compound, with concentrations ranging from 8.0 to 869 ng/g ww (mean: 162.3 ng/g ww). The highest PFOS concentration was found in a female harbor seal pup from the mid-Maine coast. PFDS, PFOSA, and PFHxS were found at relatively low concentrations (0.4 – 8.8 ng/g ww) and a detection frequency of 24, 20, and 16%, respectively. Long-chain PFCAs ($C_7$ - $C_{12}$) detected in harbor seals were an order of magnitude lower than PFSAs, with average concentrations ranging from 1.6 to 10.6 ng/g ww. Perfluoroundecanoic acid (PFUnDA) was detected in 96% of our samples (mean: 9.2 ng/g ww) and was the major contributor to the PFCA burden in harbor seal liver. Interestingly, a PFCA profile dominated by PFUnDA was reported in birds and fish from the Canadian arctic[6] and Lake Ontario[14] and in ringed seals from Greenland[9], whereas for most marine mammals, including harbor seals from the Dutch Wadden Sea[15], ringed seals from the Canadian Arctic[6] and polar bears from Greenland[7], the PFCA profile is dominated by perfluorononanoic acid (PFNA). Although PFNA was detected in only 40% of our samples, it should be noted that PFNA exceeded PFUnDA concentrations in some samples. PFOA, which has received attention for its toxic effects in humans[12] but is only sporadically detected in marine mammals[4] was found in 12% of our samples.

**Table 1**. Mean concentrations of perfluorinated compounds (ng/g ww) in liver of stranded harbor seals from the NW Atlantic (N = 25).

| Compound | | Mean[a] | Min[b]-Max | % Detected |
|---|---|---|---|---|
| Perfluorohexane sulfonate | **PFHS** | 1.3 | (*0.4* - 1.7) | 16 |
| Perfluorooctane sulfonate | **PFOS** | 162.3 | (8.0 - 868.8) | 100 |
| Perfluorodecane sulfonate | **PFDS** | 2.6 | (*0.8* - 4.8) | 24 |
| Perfluorooctane sulfonamide | **PFOSA** | 5.0 | (*0.8* - 8.8) | 20 |
| **PFSAs** | **∑PFSAs** | 286.1 | (10.0-876.3) | 100 |
| Perfluoroheptanoic acid | **PFHpA** | 1.6 | (*0.8* - 1.6) | 8 |
| Perfluorooctanoic acid | **PFOA** | 6.7 | (*1.6* - 6.7) | 4 |
| Perfluorononanoic acid | **PFNA** | 10.6 | (*1.9* - 27.2) | 40 |
| Perfluorodecanoic acid | **PFDA** | 5.2 | (*1.9* - 7.1) | 28 |
| Perfluoroundecanoic acid | **PFUnDA** | 9.2 | (*0.8* - 18.2) | 96 |
| Perfluorododecanoic acid | **PFDoDA** | 4.6 | (*1.9* - 5.3) | 12 |
| **PFCAs** | **∑PFCAs** | 22.5 | (*8.9* - 54.2) | 96 |

**Correlations among PFCs.** With the exception of PFOSA, concentrations of PFSA homologues were intercorrelated in harbor seal liver samples ($p<.05$). Although PFOSA is a metabolic precursor of PFOS[10], a similar lack of correlation between PFOS and PFOSA was reported in ringed seals from Greenland[9] and in various Arctic biota,[6] which probably reflects species differences in metabolic capability as well as differences in direct exposure to PFOSA. Concentrations of PFCA homologues were also intercorrelated in our samples ($p\leq 0.001$), but they were not correlated with PFSAs. This is in contrast with recent findings of a positive PFOS:PFCA correlation in ringed seals and polar bears from Greenland[7,9] and bottlenose dolphins from the southeastern US.[16] Overall, our results suggest multiple sources of these chemicals.

**Influence of Age, Gender, and Condition.** Using a two-way ANOVA, gender and age were not significant influences on concentrations of PFOS in harbor seal liver, although concentrations tended to be marginally higher in pups than adults (Figure 2). These results suggest a different accumulation pattern in these seals from that observed for lipophilic organochlorine compounds (e.g., PCBs, DDTs) in which adult females carry significantly lower concentrations than males or pups, reflecting excretion of organochlorines through gestation and lactation.[13] As PFOS does not accumulate in lipid-rich tissues, maternal transfer may be a relatively minor means of elimination. Reports of age and gender effects of PFCs in marine mammals are inconsistent in the literature. Kannan et al.[4,5] found that PFOS concentrations in ringed seals from the Baltic Sea were not significantly correlated with gender and did not increase with age. A trend of decreasing PFOS concentrations with age was reported in some studies. Van de Vijver et al.[17] reported higher PFOS concentrations in juvenile harbor porpoises in the North Sea than in the adults. Smithwick et al.[7] found an increase of PFCs in Greenland polar bears up to age six, suggesting these compounds may be effectively eliminated in adult animals. In harbor seals from the Dutch Wadden Sea, an increasing trend in PFOS levels was found with age in liver but a different pattern was observed in kidney.[15] Several studies report higher PFOS concentrations in males than

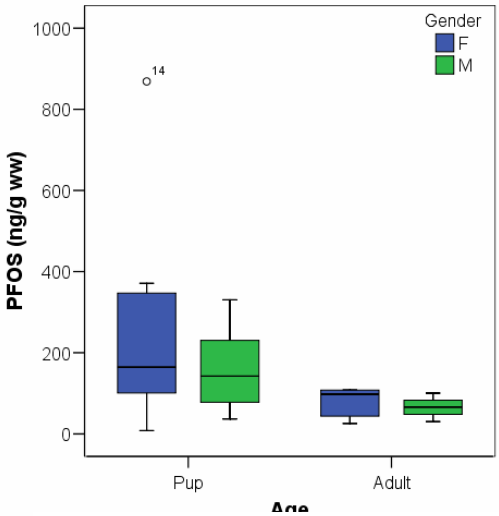


**Figure 2.** Concentration of PFOS (ng/g ww) in harbor seal livers by gender and age class. One outlier (>1.5 interquartile lengths from box edge) is indicated by a circle.

females,[4,5,18] while others have reported higher concentrations in females.[17] The lack of consistent age and gender effects in PFOS concentrations may reflect other factors such as developmental/reproductive parameters, trophic position, feeding ecology, and metabolic/excretion capacities in these species.

In this study, PFOS liver concentrations were generally higher in adult harbor seals with better body condition (based on biometric indices), although the relationship was not statistically significant. Similarly, Van de Vijver et al.[15] reported higher PFOS concentrations in the liver of harbor seals from the Wadden Sea with a good body condition (defined as absence of gross lesions at necropsy), although no link was established for harbor porpoises.

**Temporal and Spatial Trends.** An increasing trend in PFC concentrations over the past 20 to 30 years has been reported for marine mammals and seabirds[8, 9, 19]. For the period 2000 to 2005, we found no evidence of a time trend in PFOS or PFUnDA concentrations in these harbor seals (Figure 3), nor did we find significant spatial variations in concentrations ($p>0.5$). These results are interesting because harbor seals from the northwestern Atlantic are believed to be from one population and undergo seasonal migrations throughout their range. Their coastal distribution and trophic position, along with the influence of the semi-enclosed character of the waters may result in exposure to multiple pollution sources from large population and industrial centers in the region. Although the sample sizes in this study preclude any definite conclusions, a similar lack of a time trend was found in organochlorine concentrations (PCBs and DDTs) in these harbor seals over the ten-year period 1991 to 2001, and regional variations in concentrations were not found,[19] suggesting that persistent organohalogens are at equilibrium in this system.

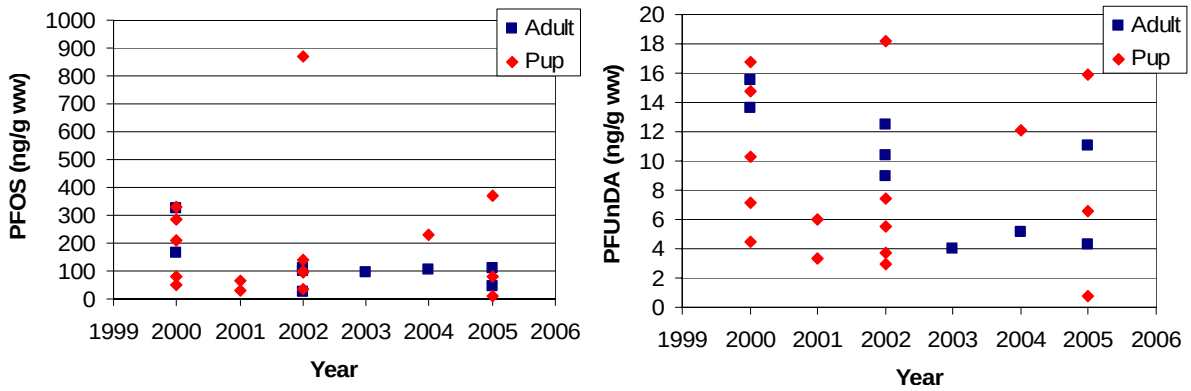


**Figure 3**. PFOS and PFUnDA concentrations (ng/g, ww) in liver of harbor seals from 2000 to 2005

**Global Comparisons**. On a global scale, mean PFOS concentrations in harbor seals from the northwestern Atlantic (162.3 ng/g, ww) are similar to those found in harbor seals from industrialized areas of Europe including the Dutch Wadden Sea (160 ng/g)[15] and the southern North Sea (range: <10 - 532 ng/g)[17] and gray seals from the Baltic Sea (214 ng/g).[4] PFOS concentrations in our samples were lower than those found in ringed seals from the Baltic (454 ng/g)[4] and bottlenose dolphins from Florida (489 ng/g)[2] and South Carolina (1,315 ng/g)[16] but an order of magnitude higher than those reported in harbor seals from the US Pacific coast (27 ng/g,ww).[3,5] Much higher concentrations of PFOS have been reported in polar bears from Greenland (2,470 ng/g)[7] and the Canadian Arctic (3,100 ng/g, ww)[6] and inland wildlife such as mink (2,630 ng/g) from the midwestern US.[18] Generally, marine mammals living in mid-latitudes near source regions appear to be more heavily contaminated, while the high concentrations in polar bears may be mainly a function of their high trophic position.[6]

To our knowledge, this is the first report of perfluorochemical contaminants in marine mammals from the US northwestern Atlantic region. An interesting observation is the presence of PFUnDA as the dominant PFCA compound in harbor seal liver, which is distinct from the profile found in most marine mammals. Compared with PCB concentrations recently reported in blubber of these seals (55,000 ng/g, lipid weight in adult males),[13] PFOS is a relatively minor contaminant, although the potential toxic effects of PFCs at these concentrations in harbor seals are unknown.

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