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Total body burden and tissue distribution of polyfluorinated compounds in harbor seals (*Phoca vitulina*) from the German Bight

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ABSTRACT

Total body burden and tissue distribution of polyfluorinated compounds (PFCs) were investigated in harbor seals (*Phoca vitulina*) from the German Bight in 2007. A total number of 18 individual PFCs from the following groups could be quantified in the different tissues: perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFSAs) and their precursors perfluorinated sulfinates (PFSiAs), perfluorinated sulfonamides, and sulfonamido ethanols. Perfluorooctanesulfonate (PFOS) was the predominant compound in all measured seal tissues (up to 1665 ng g^{-1} wet weight in liver tissue). The dominant PFCAs were perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA), but their concentrations were much lower compared to PFOS. The mean whole body burden in harbor seals of all detected PFCs was estimated to be 2665 ± 1207 μg absolute. The major amount of the total PFCs burden in the bodies was in blood (38%) and liver (36%), followed by muscle (13%), lung (8%), kidney (2%), blubber (2%), heart (1%), brain (1%), thymus (<0.01%) and thyroid (<0.01%). These data suggest large differences in body burden and accumulation pattern of PFCs in marine mammals.

1. Introduction

Recently polyfluorinated compounds (PFCs) were discovered as emerging persistent organic pollutants. PFCs are widely used as processing additives during fluoropolymer production and as surfactants in consumer applications, including surface coatings for carpets, furniture and paper products over the past 50 years (Kissa, 2001). From the production and use of these products, PFCs can be released into the environment. Scientific concern about PFCs increased due to their global distribution and ubiquitous detection in the environment, especially in marine mammals (Giesy and Kannan, 2001). PFCs in general bind to blood proteins (Jones et al., 2003) and the longer-chained PFCs are known to bioaccumulate (Martin et al., 2004). Toxic effects in biota like neuroendocrine effects (Austin et al., 2003) and peroxisome proliferation (Goecke-Flora and Reo, 1996) were demonstrated. In addition, positive correlation between infection diseases of river otters and diet of high concentration of PFCs was observed (Kannan et al., 2006).

Relatively little is known about the total body burden of PFCs in organisms. For the calculation of the total body burden the concentration in liver tissue and plasma are often used. These estimations are often potential sources of errors because little is known about the distribution of PFCs in the whole body. In addition, bioaccumulation evaluations may be overestimated when using liver and plasma concentrations.

The bioconcentration factors (BCF), half-lives and uptake rates increased with increasing perfluoroalkyl chain length in all tissues of rainbow trouts (*Oncorhynchus mykiss*) exposed with perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFSAs) in a flow-through system (Martin et al., 2003a). Longer-chained PFCAs, perfluorobutanesulfonate (PFBS) and perfluorooctanesulfonate (PFOS) were quantified in kidney, liver, blubber, muscle, tracheo-branchial muscle and spleen in harbor seals (*Phoca vitulina*) from the Dutch Wadden Sea (van de Vijver et al., 2005). Another study determined PFCs in different tissues from ringed seals (*Phoca hispida*), where the highest whole body distribution was observed in blood, muscle and liver with PFOS as the predominant compound (Sturman et al., 2007).

The object of this study was to determine concentrations and burden of PFCs in various tissues of harbor seals (*P. vitulina*) from the German Bight. To better understand the mechanisms and pathways of PFCs in marine wildlife, we examined the compound-specific distribution in liver, kidney, lung, heart, blood, brain, muscle, thyroid, thymus and blubber of harbor seals.

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2. Materials and methods

2.1. Sample collection

The harbor seals ($n = 4$) were collected at the German Bight in 2007. All harbor seals were stranded and shot by trained personal due to severe illness such as bronchopneumonia and septicemia, the carcass were then post-mortem at the Research and Technology Centre Westcoast (FTZ) according to the protocol described by Siebert et al. (2007) (Table 1). Between finding and dissection the carcass was frozen in a plastic bag at −20 °C to minimize any degradation of PFC precursors. The age was determined based on the length of the animal, filling of tooth, growth layers in the tooth, date of birth of harbor seals in the sampling area and date of finding (Siebert et al., 2007). Length and weight of the seals were measured and organs were examined macroscopically. Organs were weighed before post-mortem subsampling. All tissue samples for PFC analysis were taken with stainless steel instruments, placed into polypropylene (PP) bags and stored in a −20 °C freezer until analysis.

2.2. PFC analysis

A list of the native and mass-labelled standards including their acronyms, formula, supplier and purity are presented in Table 2.

Table 1
General informations of the four analysed harbor seals (*Phoca vitulina*) from the German Bight including the organ and tissue weights (g).

	Harbor seals (*Phoca vitulina*)			
Sex	♂	♀	♂	♂
Age (years)	<1	<2	<2	<1
Date of finding	26/11/2007	05/12/2007	05/12/2007	24/11/2007
Date of dissection	24/01/2008	24/01/2008	24/01/2008	24/01/2008
Place of finding	Büsum, Germany	Helgoland, Germany	Helgoland, Germany	Sylt, Germany
Standard length (cm)	85	88	97	84
Blubber thickness breast/sternum (mm)	15	18	22	20
Blubber thickness dorsal/neck (mm)	11	18	15	15
Blubber in %	18.3[a]	27.2[a]	24.6[a]	21.4[a]
Tissue and organ weight (g)				
Liver	725	856	974	1196
Kidney	160[b]	213[b]	181[b]	223[b]
Lung	435[c]	445[c]	435[c]	535[c]
Heart	171	199	157	230
Blood	2610[d]	2670[d]	2610[d]	3210[d]
Brain	283[e]	265[e]	283[e]	223
Muscle	5011[f]	5126[f]	5011[f]	6163[f]
Thyroid	0.60[b]	0.78[b]	1.15[b]	0.69[b]
Thymus	-[g]	1.40	0.52	0.57
Blubber	3181[h]	4846[h]	4281[h]	4571[h]
∑Tissue and organ weight	12,593	14,644	14,662	16,372
Whole body mass (g)	17,400	17,800	17,400	21,400

[a] The percent blubber content of the body mass was calculated by the formula $B\% = 4.44 + 5693 \times (\surd\ (\text{standard length (m)}/\text{body mass (kg)}) \times \text{dorsal blubber thickness}$ (Ryg et al., 1990).
[b] Sum of the right and left organ.
[c] Calculation based on a relative lung weight of 2.5% of the of the body mass (Stewardson et al., 1999).
[d] Calculation by 150 mL per kg body mass (Burns et al., 2005).
[e] It were uses the brain size of 283 g for males and 265 g for females (Bininda–Emonds, 1999).
[f] Calculation based on a relative muscle weight of 28.8% of the body mass (Burns et al., 2005).
[g] Thymus macroscopically not detectable.
[h] Calculation based on the percent blubber content of the body mass (Ryg et al., 1990).

Methanol (SupraSolv), acetonitrile (LiChrosolv) and acetic acid (glacial, >99%) were purchased from Merck.

PFCs in liver, kidney, lung, heart, blood, brain, muscle, thyroid, thymus and blubber of harbor seals were analysed described by Powley et al. (2005) with some modifications. Shortly, tissue subsample were homogenised in a ice bath using an Ultraturrax® disperser (T 25 basic Ultraturrax, IKA, Germany) with plastic dispersing (made of polycarbonate and polysulfone). One to two grams of tissue and 2 mL blood sample respectively were weighed in a PP tube and spiked with 10 ng of an internal standard (IS) mix (i.e. [$^{13}C_4$]-PFBA, [$^{13}C_2$]-PFHxA, [$^{13}C_4$]-PFOA, [$^{13}C_4$]-PFNA, [$^{13}C_4$]-PFDA, [$^{13}C_2$]-PFUnDA, [$^{13}C_2$]-PFDoA, [$^{18}O_2$]-PFHxS, [$^{13}C^4$]-PFOS, [$^{13}C^4$]-PFOSi, [$^{13}C_2$]-FHEA, [$^{13}C_2$]-FOEA, [$^{13}C_2$]-FDEA, >98%, [$^{13}C_2$]-FHUEA, [$^{13}C_2$]-FOUEA, [$^{13}C_2$]-FDUEA, d_3-MeFOSA, d_5-EtFOSA, d_7-MeFOSE, d_9-EtFOSE, 100 μL of a 0.1 μg mL^{-1} solution, see Table 2) to correct matrix effects as well as for losses sample extraction, concentration, and analysis. Tissues were extracted with 5 mL acetonitrile three times for 30 min in an ultrasonic bath at 30 °C. The combined extract was reduced to 2 mL unsing rotary evaporation and acidulated with 50 μL acetic acid. For clean-up Supelclean ENVI-Carb® cartridges (100 mg, 1 mL, 100–400 mesh, Supelco, USA) were used. The conditioning of the cartridge was carried out with 2 mL acetonitrile and 1 mL 20% acetic acid in acetonitrile. Afterwards, the sample extract and three times 1 mL methanol was given onto the cartridge and directly collected into another vial. The extracts were reduced to 150 μL under a nitrogen stream and 20 ng of an injection standard (InjS, d_5-EtFOSAA, 50 μL of a 0.4 μg mL^{-1} solution, see Table 2) was spiked to the final extract for corrections of instrumental drift and differences of the injection volume for instrumental analysis.

Concentrations of PFCs in samples were determined by high performance liquid chromatography with tandem mass spectrometer interfaced with an electrospray ionisation source in a negative-ion mode (HPLC-(−)ESI-MS/MS) as previously described (Yamashita et al., 2005). A detailed list of the precursor and product ions for the MS/MS can be found in Table 2. Quantification was done using response factors calculated by a ten-point calibration curve from 0.1 to 300 ng mL^{-1}. For quantification the linear range of 0.1–50 ng mL^{-1} and 50–300 ng mL^{-1} was used. Some PFSAs and sulfonamides showed more than one peak in the chromatogram, which is due to the presence of branched isomers resulting from the production process (Giesy and Kannan, 2002). These branched isomers could not be quantified precisely because of the lack of calibration standards. As the analytical standards are not available for perfluorinated pentane- and nonanesulfonate (PFPS, PFNS) and perfluorinated pentadecanoic and heptadecanoic acid (PFPDA, PFHpDA), they were integrated into the method taking the MS/MS parameters of the compound having one carbon atom less in the carbon chain and their calibration was used for the quantification. Hence, the results given for PFPS, PFNS, PFPDA and PFHpDA should be considered only as an estimation.

2.3. Quality control

Data quality assurance and quality control included method blanks, method detection limits (MDLs), method quantification limits (MQLs), matrix spike recovery rates, matrix effect and continuing calibration verification. For the method blank one mL of acetonitril was extracted in the same way as the natural samples. The MDLs and MQLs were calculated for substances which were found in real samples at a signal to noise (S/N) of 3 and 10, respectively. PFC recoveries were tested for liver tissues based on triplicate analysis of matrix spiked and extracted with the same analytical procedure.

All method blanks were under the MQL. The MQLs ranged from a few pg g^{-1} wet weight (w.w.) (e.g. perfluorooctanoic acid (PFOA)) to