Final Draft
of the original manuscript:

Ahrens, L.; Siebert, U.; Ebinghaus, R.:
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In: Marine Pollution Bulletin (2009) Elsevier

DOI: 10.1016/j.marpolbul.2008.11.030

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.

Keywords: Total body burden; tissue distribution; harbor seal; PFCs; PFOS; PFOA

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 (*Phoca 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.

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. 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). 1 to 2 g 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, 50µL 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 to 50 ng mL^{-1} and 50

to 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 a few ng g^{-1} w.w. (PFOS), depending on the extracted tissue (Table S1 in the supporting information (SI)). Relative recoveries of the 36 analytes, which were corrected for IS recovery and background concentration, ranged between 56% (perfluorohexadecanoic acid (PFHxDA)) and 135% (n-ethylperfluorooctane sulfonamidoethanol (EtFOSE)) (Table S2 in the SI). The matrix effects of individual PFCs were determined in liver, kidney, lung, heart, blood, brain and muscle by analysis of a fortified extract (100 µL of a 0.1 µg/mL PFC standard solution), non-fortified extract and

solvent based standard solution (matrix effect = (response $_{\text{fortified extract}}$ − response $_{\text{non-fortified extract}}$) / response $_{\text{solvent based standard}}$) (Table S3 in the SI). Most PFCs showed similar matrix effects in different tissues and a low mean signal suppression of 0.88 to 0.98 except of 3,7-dimethylperfluorooctanoic acid (Me_2PFOA) with a low signal enhancement of 1.01. Only PFHxDA and perfluorooctadecanoic acid (PFOcDA) have a stronger signal suppression in some tissues with a maximum of 0.24 (kidney) and 0.11 (lung), respectively.

3. Results and discussion

3.1 Tissue distribution

Levels of PFCs in liver, kidney, lung, heart, blood, brain, muscle, thyroid, thymus and blubber in harbor seals are shown in Table 3. 18 of 40 target analytes were found in the tissues (i.e. C_4 to C_{10} PFSAs, perfluorooctanesulfinate (PFOSi), C_8 to C_{15} PFCAs, perfluorosulfonamide (FOSA) and n-methylperfluorobutane sulfonamidoethanol (MeFBSE)). To our knowledge, this is the first report of PFPS, PFNS, PFOSi and MeFBSE in detectable concentrations in biota samples from the German Bight. Among to all detected PFCs the predominated compound in all tissues was PFOS with an average of over 90%, followed by perfluorohexanesulfonate (PFHxS) (2.7%), perfluorononanoic acid (PFNA) (1.8%) and perfluorodecanoic acid (PFDA) (1.6%). The highest PFC sum concentrations were detected in liver (1071 ng g^{-1} w.w.), lung (462 ng g^{-1} w.w.) and blood (381 ng g^{-1} w.w.). The lowest PFC sum concentration was found in blubber with an average of 11.4 ng g^{-1} w.w. This confirms with the findings that PFCs bind to blood proteins instead of fatty tissue (Jones et al., 2003).

All PFSAs with a chain length of C_4 to C_{10} could be detected in the analysed tissues. Although 3M, the major producer of perfluorooctyl sulfonyl fluoride (POSF), which is a major precursor for several PFCs, voluntarily phased out the production in 2002, PFOS was still the dominated compound in biota samples. The former POSF-based products are now substituted by perfluorobutyl sulfonyl fluoride (PBSF)-based products (U. S. Environmental

Protection Agency, 2000). In the present study PFBS could be detected with a maximum concentration of 17 ng g^{-1} w.w. in blood, but usually the concentration was less than 0.5 ng g^{-1} w.w. The production shift to the shorter-chained PFBS could not be observed because of the lower accumulation potential of the C_4 in comparison to the C_8 PFSA (Martin et al., 2003b). In contrast to the PFSAs the PFCAs could only be detected from a chain length grater than C_8, this suggest a higher accumulation potential of PFSAs compared to PFCAs (Martin et al., 2003b). PFNA and PFDA were the dominated PFCAs, with increasing chain length up to C_{15} concentration levels decreased. Two sulfonamides, which are precursors of PFSAs and PFCAs (Martin et al., 2006), were also detected. FOSA was detected in all tissues with a maximum of 6.9 ng g^{-1} w.w. in blood and MeFBSE was only observed in thyroid tissue and blubber with up to 2.0 ng g^{-1} w.w.

PFCs are distributed on a global scale, with highest concentrations found close to urbanized/industrialized regions like Europe and USA (e.g. 970-3680 ng g^{-1} w.w. PFOS in Mink livers from Midwestern U.S.), while the Southern Hemisphere was less contaminated with PFCs (e.g. <0.08-3.52 ng g^{-1} w.w. PFOS in elephant seal livers from the Antarctic) (Giesy and Kannan, 2001; Tao et al., 2006). In most studies PFOS was the dominating PFC in marine animals (Houde et al., 2006). PFCAs were detected in seal liver in the Canadian Arctic in the same range than those in this study, except of PFOS, which was found one magnitude lower (Butt et al., 2007; Sturman et al., 2007). A similar trend was observed in pelicans (*Pelecanus occidentalis*) from Columbia where the concentration of PFOS in liver, kidney, lung, heart, brain and muscle from harbor seals were, on average, one to two magnitudes lower than in this study (Olivero-Verbel et al., 2006). This may be the result of the higher pollutant area around the North Sea in comparison to the Arctic and Columbia. The tendency of increasing PFOS concentration in the different tissues was comparable to this study in harbor seals (*Phoca vitulina*) (kidney > liver > blubber), ringed seals (*Phoca hispida*) (liver >

lung > heart and liver > spleen > kidney, respectively) (Sturman et al., 2007) and rainbow trouts (*Oncorhynchus mykiss*) (blood > kidney > liver) (Martin et al., 2003a).

3.2 Total body burden

The calculation of the whole body burden distribution based on the individual tissue masses of the whole organs (concentration in the sub-sample x tissue weight) (see Table 1). The organs from thymus, thyroid, liver, kidney, heart and one brain were tared directly. The content of blood, not directly tared brains, lung and muscle was calculated based on the mass information found in the literature (Stewardson et al., 1999; Bininda-Emonds, 2000; Burns et al., 2005). The blubber content was estimated with the dorsal blubber thickness, standard length and body mass as described by Ryg et al. (1990). The calculation included all examined tissues, which was around 75% of the whole body weight. Among others the skeleton and the pelt was unaccounted for the calculation because the extraction would be too difficult and the expected PFC concentrations very low. The mean whole body burden in harbor seals of all detected ∑PFCs was estimated to be 2665 ± 1207 µg absolute (Table 4, n = 4). The greatest proportion had PFOS (2477 ± 1122 µg absolute) which could be have potential developmental, reproductive, systemic and neuroendocrine effects to mammals (Austin et al., 2003). The high PFCs body burden which was found in this study could also have effects on the immune system and physiological functions (Kannan et al., 2006). The four seals investigated in the present study were all in moderate nutritional status. The main pathological findings were bronchopneumonia caused by parasitic and bacterial infection partly with final septicemia (personal communication with Ursula Siebert). Therefore an effect of PFCs on the health status can not be routed out.

Distribution of individual PFC in different tissues of harbor seals is presented in Figure 2. The whole body burden was related as follows: Blood ≈ liver > muscle > lung > kidney ≈ blubber > Heart ≈ brain >> thymus > thyroid (Figure 1). Blood and liver contributed three-fourths of the whole body burden for PFCs, but the composition differed from compound to

compound. For monitoring of PFCs in marine mammals in the North Sea it would be meaningful to collect liver or blood samples, because there were found an effectively accumulation of these compounds resulting in the highest concentrations of all examined tissues. Muscle and blubber tissue corresponded approximately to two-thirds of sum weight of all analysed tissues, but the PFC body burden of this both tissues was only 13 and 2%, respectively. However, the pattern of PFCs varied depending on the functional group and fluorinated chain length. It is noticeable that the proportion of the PFSAs in liver increased with increasing chain length, whereas the short-chained PFBS and PFPS were found with over 90% in blood. Otherwise, the PFCAs showed no obvious differences in the pattern between the different tissues. FOSA was mostly distributed in blood and MeFOSE in blubber. These different patterns indicated a compound-specific persistence of PFCs in different tissues of harbor seals.

4. Conclusion

In comparison to this study, PFC concentrations in ringed seals from the Canadian Arctic and elephant seals from the Antarctic were by a factor of ~100 and ~1000-10000 lower, respectively (Giesy and Kannan, 2001; Tao et al., 2006). The occurrence of high concentrations of PFCs in harbor seals in the German Bight suggests that these compounds should also be found at several levels in the marine food chain. This could possibly be problematic, because the North Sea is an important source for the fishery and shrimp industry of the countries bordering to the North Sea. A recent study has shown high concentrations of PFCs were found in plasma and whole blood of the Swedish population, which were usually much higher than other detected persistent organic pollutants (Kärrman et al., 2006). Little information is available on the exposure of PFCs in the marine environment, further investigations about the accumulation potential and whole body burden in marine wildlife are necessary to assess potential adverse effects of PFCs. This study provides advice on the

analysis of the whole body burden in harbor seals for individual PFCs, which is relevant for calculation of the bioaccumulation potential of these compounds in marine mammals.

Acknowledgements

We kindly acknowledge the German Federal Environmental Foundation for sponsoring the project. The pathological investigations on the seals were funded by the Ministry of Agriculture, Environment and Rural Development of Schleswig-Holstein, Germany.

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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	12593	14644	14662	16372
Whole body mass (g)	17400	17800	17400	21400

[a] The percent blubber content of the body mass was calculated by the formula B% = 4.44 + 5693 * (√(standard length (m) / body mass (kg)) * 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 [a] (Ryg et al., 1990).

Table 2
Analytes, acronyms, formula, supplier, purity, precursor and product ions for the MS/MS detection.

Analyte	Acronym	Formula	Supplier (purity)	Precursor/ product ion [m/z]
Perfluorobutane sulfonate	PFBS	$C_4F_9SO_2O^-$	Fluka (97%)	298.877/ 79.8
Perfluoropentane sulfonate	PFPS	$C_5F_{11}SO_2O^-$	n.a.	348.939/ 79.8
Perfluorohexane sulfonate	PFHxS	$C_6F_{13}SO_2O^-$	Fluka (98%)	398.894/ 79.8
Perfluoroheptane sulfonate	PFHpS	$C_7F_{15}SO_2O^-$	Well. Lab.[a] (>98%)	449.034/ 79.3
Perfluorooctane sulfonate	PFOS	$C_8F_{17}SO_2O^-$	Well. Lab.[a] (>98%)	498.971/ 79.7
Perfluorononane sulfonate	PFNS	$C_9F_{19}SO_2O^-$	n.a.	548.926/ 79.8
Perfluorodecane sulfonate	PFDS	$C_{10}F_{21}SO_2O^-$	Well. Lab.[a] (>98%)	598.896/ 79.5
6:2 fluorotelomer sulfonate	6:2 FTS	$C_6F_{13}C_2H_4SO_3^-$	ABCR (98%)	426.925/ 406.7
Perfluoro-1-hexane sulfinate	PFHxSi	$C_6F_{13}SO_2^-$	Well. Lab.[a] (>98%)	382.865/ 319.0
Perfluoro-1-octane sulfinate	PFOSi	$C_8F_{17}SO_2^-$	Well. Lab.[a] (>98%)	482.824/ 418.9
Perfluoro-1-decane sulfinate	PFDSi	$C_{10}F_{21}SO_2^-$	Well. Lab.[a] (>98%)	582.934/ 518.9
Perfluorobutanoic acid	PFBA	C_3F_7COOH	ABCR (99%)	112.900/ 168.7
Perfluoropentanoic acid	PFPA	C_4F_9COOH	Alfa Aesar (98%)	262.825/ 218.9
Perfluorohexanoic acid	PFHxA	$C_5F_{11}COOH$	Fluka (97%)	312.934/ 268.8
Perfluoroheptanoic acid	PFHpA	$C_6F_{13}COOH$	Lanc. Syn.[b] (98%)	362.950/ 318.9
Perfluorooctanoic acid	PFOA	$C_7F_{15}COOH$	Lanc. Syn.[b] (95%)	412.987/ 368.9
Perfluorononanoic acid	PFNA	$C_8F_{17}COOH$	Lanc. Syn.[b] (97%)	462.908/ 418.9
Perfluorodecanoic acid	PFDA	$C_9F_{19}COOH$	Lanc. Syn.[b] (97%)	512.876/ 469.0
Perfluoroundecanoic acid	PFUnDA	$C_{10}F_{21}COOH$	ABCR (96%)	562.865/ 519.0
Perfluorododecanoic acid	PFDoDA	$C_{11}F_{23}COOH$	Alfa Aesar (96%)	612.991/ 568.9
Perfluorotridecanoic acid	PFTriDA	$C_{12}F_{25}COOH$	Well. Lab.[a] (>98%)	663.094/ 618.9
Perfluorotetradecanoic acid	PFTeDA	$C_{13}F_{27}COOH$	Alfa Aesar (96%)	713.036/ 669.0
Perfluorotridecanoic acid	PFPDA	$C_{14}F_{29}COOH$	n.a.	762.980/ 718.9
Perfluorohexadecanoic acid	PFHxDA	$C_{15}F_{31}COOH$	Alfa Aesar (95%)	812.840/ 769.1
Perfluoroheptadecanoic acid	PFHpDA	$C_{16}F_{33}COOH$	n.a.	862.980/ 818.9
Perfluorooctadecanoic acid	PFOcDA	$C_{17}F_{35}COOH$	Alfa Aesar (97%)	912.870/ 869.0
3,7-dimethylperfluorooctanoic acid	Me_2PFOA	$C_9F_{19}COOH$	Alfa Aesar (97%)	512.885/ 468.9
N-methylperfluorobutane sulfonamide	MeFBSA	$C_4F_9SO_2NH(CH_3)$	3M (n.a.)	311.914/ 218.8
Perfluorooctane sulfonamide	FOSA	$C_8F_{17}SO_2NH_2$	ABCR (97%)	497.896/ 77.9
N-methyl perfluorooctane sulfonamide	MeFOSA	$C_8F_{17}SO_2NH(CH_3)$	3M (n.a.)	511.849/ 168.9
N-ethyl perfluorooctane sulfonamide	EtFOSA	$C_8F_{17}SO_2NH(C_2H_5)$	ABCR (95%)	526.008/ 169.0
N-methylperfluorobutane sulfonamidoethanol	MeFBSE	$C_4F_9SO_2N(CH_3)C_2H_4OH$	3M (n.a.)	416.047/ 59.0
N-methyl perfluorooctane sulfonamidoethanol	MeFOSE	$C_8F_{17}SO_2N(CH_3)C_2H_4OH$	3M (n.a.)	616.004/ 58.9
N-ethyl perfluroctane sulfonamidoethanol	EtFOSE	$C_8F_{17}SO_2N(C_2H_5)C_2H_4OH$	3M (n.a.)	630.109/ 58.8
2-Perfluorohexyl ethanoic acid	FHEA	$C_6F_{13}CH_2COOH$	Well. Lab.[a] (>98%)	376.945/ 292.8
2-Perfluorooctyl ethanoic acid	FOEA	$C_8F_{17}CH_2COOH$	Well. Lab.[a] (>98%)	476.909/ 392.9
2-Perfluorodecyl ethanoic acid	FDEA	$C_{10}F_{21}CH_2COOH$	Well. Lab.[a] (>98%)	577.011/ 493.0
2H-Perfluoro-2-octenoic acid	FHUEA	$C_6F_{12}CHCOOH$	Well. Lab.[a] (>98%)	356.885/ 293.0
2H-Perfluoro-2-decenoic acid	FOUEA	$C_8F_{16}CHCOOH$	Well. Lab.[a] (>98%)	456.803/ 292.8
2H-Perfluoro-2-dodecenoic acid	FDUEA	$C_{10}F_{20}CHCOOH$	Well. Lab.[a] (>98%)	556.937/ 493.1
Perfluoro-1-hexane[$^{18}O_2$]sulfonate	[$^{18}O_2$]-PFHxS	$C_6F_{13}S[^{18}O_2]O^-$	Well. Lab.[a] (>98%)	402.981/ 83.9
Perfluoro-1-[1,2,3,4-13C]octanesulfonate	[$^{13}C_4$]-PFOS	$C_4F_9[1,2,3,4\text{-}^{13}C_4]F_8SO_2O^-$	Well. Lab.[a] (>98%)	502.899/ 79.5
Perfluoro-1-[1,2,3,4-13C]octanesulfinate	[$^{13}C_4$]-PFOSi	$C_4F_9[1,2,3,4\text{-}^{13}C_4]F_8SO_2^-$	Well. Lab.[a] (>90%)	486.859/ 422.9
Perfluoro-n-(1,2,3,4-$^{13}C_4$)butanoic acid	[$^{13}C_4$]-PFBA	$2,3,4\text{-}^{13}C_3F_7{}^{13}COOH$	Well. Lab.[a] (>98%)	216.823/ 171.8
Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	[$^{13}C_2$]-PFHxA	$C_4F_9[2\text{-}^{13}C]F_2{}^{13}COOH$	Well. Lab.[a] (>98%)	314.891/ 269.9
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	[$^{13}C_4$]-PFOA	$C_4F_9[2,3,4\text{-}^{13}C_3]F_6{}^{13}COOH$	Well. Lab.[a] (>98%)	416.978/ 371.8
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	[$^{13}C_5$]-PFNA	$C_4F_9[2,3,4,5\text{-}^{13}C_4]F_8{}^{13}COOH$	Well. Lab.[a] (>98%)	467.907/ 423.0
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	[$^{13}C_2$]-PFDA	$C_8F_{17}{}^{13}CF_2{}^{13}COOH$	Well. Lab.[a] (>98%)	514.944/ 469.8
Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	[$^{13}C_2$]-PFUnDA	$C_9F_{19}{}^{13}CF_2{}^{13}COOH$	Well. Lab.[a] (>98%)	564.959/ 519.8
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	[$^{13}C_2$]-PFDoDA	$C_{10}F_{21}{}^{13}CF_2{}^{13}COOH$	Well. Lab.[a] (>98%)	614.913/ 569.9
N-methyl-d_3-perfluoro-1-octanesulfonamide	d_3-N-MeFOSA	$C_9D_3HF_{17}NO_2S$	Well. Lab.[a] (>98%)	514.920/ 168.8
N-ethyl-d_5-perfluoro-1-octanesulfonamide	d_5-N-EtFOSA	$C_{10}D_5HF_{17}NO_2S$	Well. Lab.[a] (>98%)	530.984/ 168.8
2-(N-deuteriomethylperfluoro-1-octanesulfoneamido)-1,1,2,2-tetradeuterioethanol	d_7-N-MeFOSE	$C_8F_{17}SO_2N(CD_3)C_2D_4OH$	Well. Lab.[a] (>98%)	623.058/ 58.9
2-(N-deuterioethylperfluoro-1-octanesulfoneamido)-1,1,2,2-tetradeuterioethanol	d_9-N-EtFOSE	$C_8F_{17}SO_2N(C_2D_5)C_2D_4OH$	Well. Lab.[a] (>98%)	639.054/ 58.9
2-Perfluorohexyl-[1,2-$^{13}C_2$]ethanoic acid	[$^{13}C_2$]-FHEA	$C_6F_{13}{}^{13}CH_2{}^{13}COOH$	Well. Lab.[a] (>98%)	378.912/ 294.0
2-Perfluorooctyl-[1,2-$^{13}C_2$]ethanoic acid	[$^{13}C_2$]-FOEA	$C_8F_{17}{}^{13}CH_2{}^{13}COOH$	Well. Lab.[a] (>98%)	478.911/ 393.8
2-Perfluorodecyl-[1,2-$^{13}C_2$]ethanoic acid	[$^{13}C_2$]-FDEA	$C_{10}F_{21}{}^{13}CH_2{}^{13}COOH$	Well. Lab.[a] (>98%)	579.017/ 494.1
2H-Perfluoro-[1,2-$^{13}C_2$]-2-octenoic acid	[$^{13}C_2$]-FHUEA	$C_6F_{12}{}^{13}CH{}^{13}COOH$	Well. Lab.[a] (>98%)	358.907/ 294.0
2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid	[$^{13}C_2$]-FOUEA	$C_8F_{16}{}^{13}CH{}^{13}COOH$	Well. Lab.[a] (>98%)	458.903/ 393.8
2H-Perfluoro-[1,2-$^{13}C_2$]-2-dodecenoic acid	[$^{13}C_2$]-FDUEA	$C_{10}F_{20}{}^{13}CH{}^{13}COOH$	Well. Lab.[a] (>98%)	558.955/ 494.0
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-EtFOSAA	$C_8F_{17}SO_2N(C_2D_2C_2D_3)C_2H_2CO_2H$	Well. Lab.[a] (>98%)	589.015/ 418.7

[a] Well. Lab. = Wellington Laboratories; [b] Lanc. Syn. = Lancaster Synthesis; n.a. = not available.

Table 3
Average concentration (ng g^{-1} wet weight), standard deviation (SD) and ranges of PFCs in different organs and tissues of harbor seals (*Phoca vitulina*) from the German Bight (n = 4).

		Liver	Kidney	Lung	Heart	Blood	Brain	Muscle	Thyroid	Thymus	Blubber
PFBS	mean ± SD	0.32 ± 0.34	0.10 ± 0.15	0.17 ± 0.16	0.13 ± 0.18	4.32 ± 8.45	n.d.	0.02 ± 0.05	0.11 ± 0.22	0.07 ± 0.12	n.d.
	range	0-0.78	0-0.32	0.06-0.41	0-0.39	0.03-17.0	n.d.	0-0.10	0-0.43	0-0.21	n.d.
PFPS [a]	mean ± SD	1.75 ± 2.46	0.09 ± 0.11	0.09 ± 0.05	0.04 ± 0.07	5.90 ± 11.66	n.d.	n.d.	n.d.	0.07 ± 0.06	n.d.
	range	0.13-5.38	0-0.24	0.04-0.15	0-0.15	0-23.4	n.d.	n.d.	n.d.	0-0.11	n.d.
PFHxS	mean ± SD	6.90 ± 4.03	5.68 ± 3.83	8.14 ± 4.82	4.32 ± 3.33	3.16 ± 1.08	1.58 ± 1.00	1.94 ± 1.41	4.11 ± 2.64	10.49 ± 6.19	0.66 ± 0.48
	range	1.11-10.4	1.05-9.84	1.91-13.3	0.60-7.64	1.67-4.13	0.27-2.48	0.34-3.74	0.68-6.87	3.38-14.6	0.14-1.30
PFHpS	mean ± SD	2.27 ± 2.29	1.24 ± 0.87	3.67 ± 2.67	1.32 ± 1.15	0.66 ± 0.66	0.66 ± 0.45	0.41 ± 0.32	1.22 ± 1.17	3.43 ± 2.51	0.10 ± 0.15
	range	0-5.43	0.15-2.09	0.83-7.12	0.08-2.82	0-1.58	0.23-1.15	0.02-0.71	0-2.78	0.84-5.86	0-0.32
PFOS	mean ± SD	1017 ± 536	288 ± 117	433 ± 227	143 ± 40	349 ± 370	99 ± 49	59 ± 52	62 ± 58	312 ± 136	8.91 ± 9.93
	range	559-1665	118-383	228-755	87-181	48-887	38-153	7.65-132	0-121	159-416	0-23
PFNS [a]	mean ± SD	0.74 ± 0.74	0.06 ± 0.09	0.11 ± 0.12	n.d.	0.10 ± 0.11	n.d.	n.d.	0.08 ± 0.15	0.11 ± 0.04	n.d.
	range	0.12-1.80	0-0.19	0-0.27	n.d.	0-0.25	n.d.	n.d.	0-0.30	0.06-0.14	n.d.
PFDS	mean ± SD	0.53 ± 0.38	0.14 ± 0.16	0.18 ± 0.16	0.06 ± 0.08	0.12 ± 0.13	0.04 ± 0.08	n.d.	0.12 ± 0.15	0.20 ± 0.08	0.03 ± 0.03
	range	0.11-1.02	0-0.37	0-0.38	0-0.16	0-0.31	0-0.16	n.d.	0-0.33	0.11-0.27	0-0.06
PFOSi	mean ± SD	0.05 ± 0.08	n.d.	0.02 ± 0.04	n.d.	0.03 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.
	range	0-0.16	n.d.	0-0.07	n.d.	0-0.09	n.d.	n.d.	n.d.	n.d.	n.d.
∑PFSAs/∑PFSiAs		1030	295	445	149	364	101	61	67	327	9.7
PFOA	mean ± SD	0.70 ± 0.59	0.40 ± 0.41	0.75 ± 0.46	0.42 ± 0.42	0.62 ± 0.58	0.06 ± 0.10	0.07 ± 0.11	0.09 ± 0.11	0.70 ± 0.25	0.03 ± 0.04
	range	0-1.42	0-0.93	0.28-1.21	0-0.99	0-1.14	0-0.20	0-0.24	0-0.22	0.43-0.93	0-0.08
PFNA	mean ± SD	15.3 ± 5.75	3.64 ± 0.62	4.84 ± 2.06	2.07 ± 0.84	3.93 ± 2.08	1.20 ± 0.50	0.96 ± 0.35	1.90 ± 1.19	4.99 ± 1.22	0.61 ± 0.27
	range	8.27-22.3	2.97-4.22	2.06-6.55	1.13-2.86	0.88-5.54	0.77-1.91	0.45-1.25	0.80-3.58	3.60-5.91	0.30-0.89
PFDA	mean ± SD	15.2 ± 4.49	4.11 ± 2.09	5.18 ± 1.63	2.44 ± 1.06	4.38 ± 2.35	1.55 ± 0.47	1.09 ± 0.46	1.23 ± 0.59	5.65 ± 2.04	0.29 ± 0.22
	range	8.83-19.0	1.99-6.97	2.90-6.70	1.37-3.88	0.86-5.68	0.95-2.00	0.56-1.67	0.52-1.74	3.31-7.05	0.01-0.51
PFUnDA	mean ± SD	5.26 ± 1.59	1.92 ± 0.69	2.80 ± 0.88	1.36 ± 0.50	1.71 ± 0.84	1.06 ± 0.16	0.26 ± 0.16	0.31 ± 0.34	2.30 ± 0.57	0.088 ± 0.10
	range	3.29-6.62	0.95-2.54	1.71-3.74	0.75-1.92	0.46-2.26	0.90-1.20	0.10-0.42	0-0.61	1.65-2.70	0-0.19
PFDoDA	mean ± SD	1.47 ± 0.49	0.75 ± 0.37	1.10 ± 0.43	0.54 ± 0.25	0.47 ± 0.24	0.51 ± 0.36	0.06 ± 0.11	0.18 ± 0.35	0.42 ± 0.13	0.042 ± 0.067
	range	1.04-2.17	0.27-1.16	0.74-1.63	0.24-0.84	0.25-0.74	0-0.84	0-0.22	0-0.70	0.31-0.57	0-0.14
PFTriDA	mean ± SD	1.53 ± 0.55	1.01 ± 0.54	1.27 ± 0.63	0.77 ± 0.47	0.76 ± 0.34	0.73 ± 0.55	0.12 ± 0.83	0.51 ± 0.43	1.00 ± 0.16	0.12 ± 0.090
	range	0.74-1.96	0.58-1.74	0.54-2.02	0.33-1.31	0.29-1.03	0-1.31	0-0.19	0.04-1.07	0.86-1.18	0-0.20
PFTeDA	mean ± SD	0.22 ± 0.16	0.05 ± 0.06	0.16 ± 0.22	0.06 ± 0.12	0.08 ± 0.06	0.10 ± 0.12	n.d.	0.05 ± 0.07	0.21 ± 0.17	n.d.
	range	0.08-0.44	0-0.11	0-0.46	0-0.23	0-0.15	0-0.23	n.d.	0-0.15	0.07-0.40	n.d.
PFPeDA [a]	mean ± SD	n.d.	n.d.	0.13 ± 0.26	n.d.	0.04 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.
	range	n.d.	n.d.	0-0.53	n.d.	0-0.11	n.d.	n.d.	n.d.	n.d.	n.d.
∑PFCAs		39.7	11.9	16.2	7.65	12.0	5.20	2.55	4.27	15.3	1.17
FOSA	mean ± SD	1.55 ± 0.69	0.62 ± 0.44	0.40 ± 0.027	0.27 ± 0.18	5.06 ± 1.23	0.14 ± 0.14	0.07 ± 0.08	0.02 ± 0.02	0.46 ± 0.38	0.03 ± 0.04
	range	0.78-2.32	0-1.02	0.37-0.44	0.06-0.48	4.08-6.85	0-0.33	0-0.15	0-0.05	0.03-0.74	0-0.08
MeFBSE	mean ± SD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14 ± 0.27	n.d.	0.50 ± 1.00
	range	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0-0.55	n.d.	0-2.01
∑FOSA/FOSE		1.55	0.62	0.40	0.27	5.06	0.14	0.07	0.16	0.46	0.53
∑PFCs		1071	308	462	157	381	106	64	72	343	11

[a] have to be considered as estimates, because no standards were available for this compound; n.d. = not detected.

Table 4
Mean whole body burden and standard deviation for individual PFC in harbor seals (*Phoca vitulina*) from the German Bight in µg absolute (n = 4).

Analyte	Whole body burden [µg]
PFBS	12 ± 22
PFPS	17 ± 35
PFHxS	33 ± 9.1
PFHpS	8.6 ± 3.2
PFOS	2477 ± 1122
PFNS	1.0 ± 0.71
PFDS	1.0 ± 0.49
PFOSi	0.12 ± 0.17
PFOA	3.3 ± 1.6
PFNA	35 ± 7.8
PFDA	36 ± 7.8
PFUnDA	13 ± 2.8
PFDoDA	3.9 ± 1.1
PFTriDA	5.6 ± 1.1
PFTeDA	0.52 ± 0.21
PFPeDA	0.15 ± 0.14
FOSA	16 ± 3.3
MeFBSE	1.9 ± 0.01
∑PFCs	2665 ± 1207

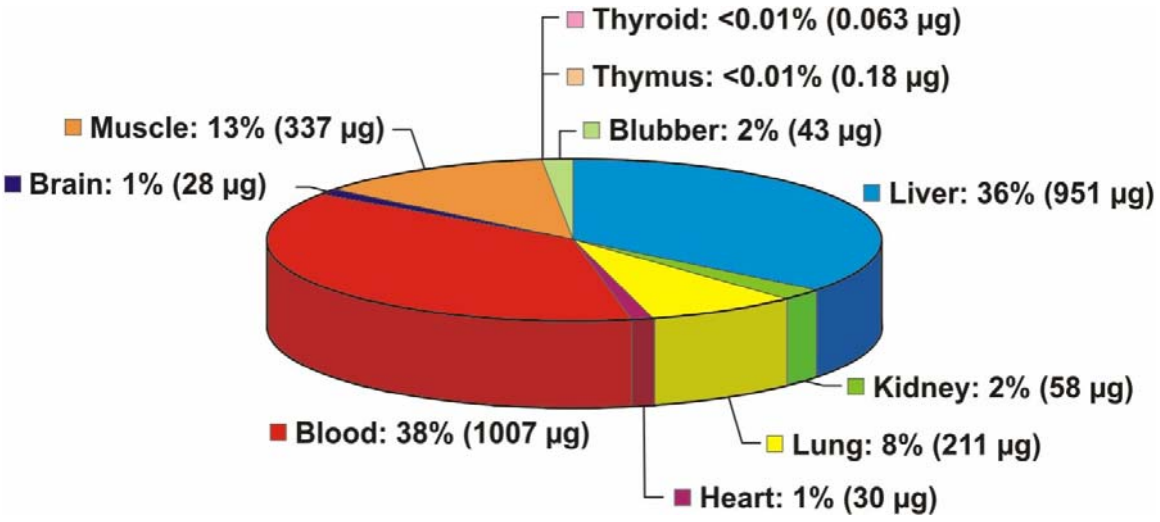

Fig. 1. PFC whole body burden distribution in percent and µg per tissue in brackets for harbor seals (*Phoca vitulina*) from the German Bight.

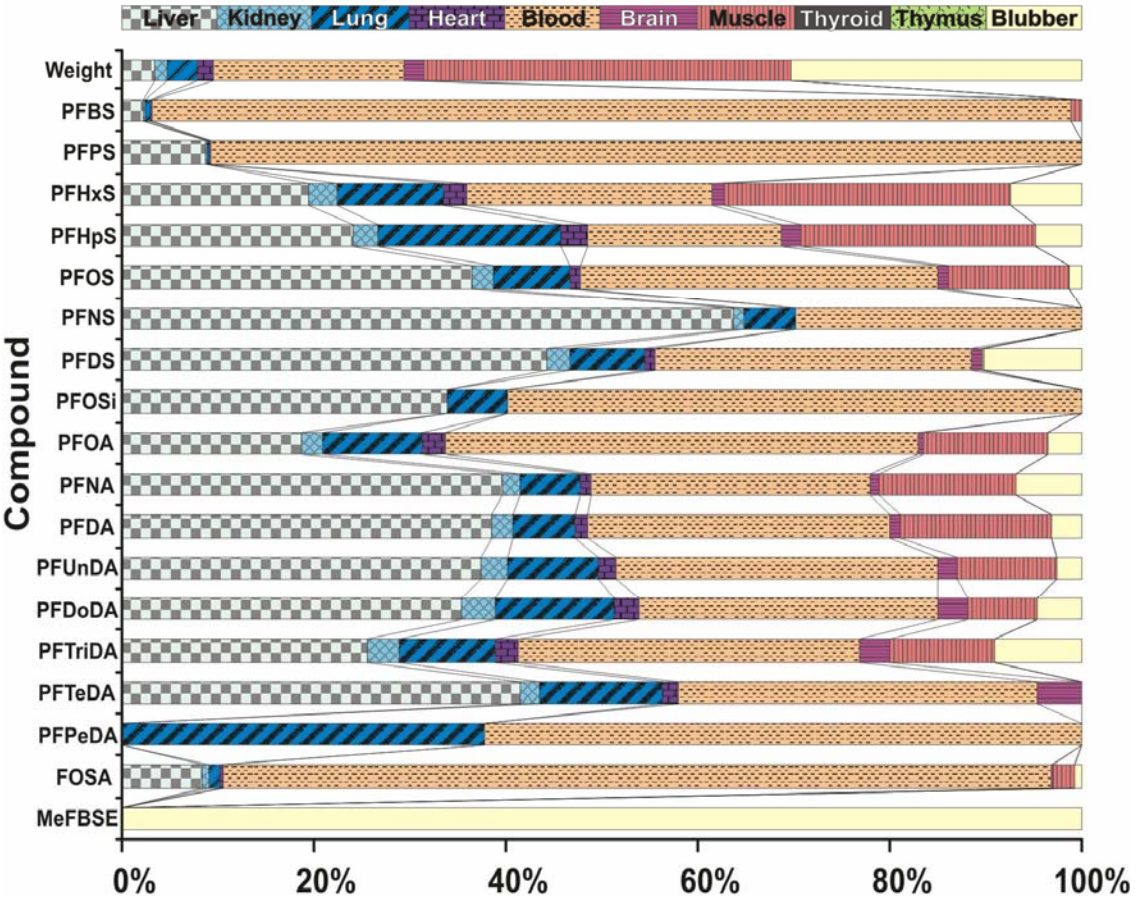

Fig. 2. Tissue distribution of PFC burdens in harbor seals (*Phoca vitulina*) from the German Bight.