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Perfluorinated compounds in red-throated divers from the German Baltic Sea: new findings from their distribution in 10 different tissues

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Environmental context

Perfluorinated compounds (PFCs) are a widespread, commonly used group of pollutants that are globally detected in all environmental matrices. By investigating red-throated divers, the total body PFC contamination of a marine top predator as well as the occurrence of PFCs in different bird organs were evaluated. This study indicates that the extent of PFC contamination is mainly induced by a diet of fish which is also caught for human consumption and helps us to better understand the behaviour of PFCs in organisms.

Abstract

Twenty poly- and perfluorinated compounds (PFCs) were investigated in four red-throated divers (*Gavia stellata*) from the German Baltic Sea sampled in 2005. Concentrations of five perfluoroalkyl sulfonates (PFSAs), ten perfluoroalkyl carboxylates (PFCAs), two alkylated perfluoroalkyl sulfonamides (FASAs), two alkylated perfluoroalkyl sulfonamidoethanols (FASEs) as well as perfluorooctane sulfonamide (PFOSA) were determined in blood, brain, fatty tissue, gall bladder, heart, kidney, liver, lung, muscle, and spleen by HPLC-MS/MS. For quantification standard addition was applied. Twelve compounds were detected in each of the forty tissue samples with average total PFC concentrations ranging from 42 ng g^{-1} in muscle

and 220 ng g^{-1} in liver samples. Perfluorooctane sulfonate (PFOS) was the major compound in all samples. Except for brain, perfluoroundecanoate (PFUnDA) was the dominant PFCA. In brain samples preferential enrichment of long-chain PFSAs and PFCAs was observed. The total PFC body burden was estimated to 100 µg ± 39 µg or 67 µg kg^{-1} ± 26 µg kg^{-1}. It was supposed that multivariate statistical analyses may support the identification of the preferred accumulation 'location' of individual PFCs in the birds' body.

Additional keywords: PFC, biota, birds, tissue distribution, body burden

Introduction

Due to their remarkable and unique physicochemical properties, poly- and perfluorinated compounds (PFCs) are almost irreplaceable for industry and commerce. [1] Global distribution of ionic perfluorinated compounds such as perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs) is caused by extensive use and persistence of these substances [2, 3] as well as their potential for long-range transport. Depending on the molecule size, perfluorinated acids can be highly soluble in water [4] which explains their transport in the water phase. [5, 6] However, it is suggested that atmospheric transport of neutral, volatile precursor substances and their degradation to PFSAs and PFCAs also is an important long-range transport pathway.[7, 8] PFSAs and PFCAs are persistent, toxic and partly bioaccumulative. [9, 10] Their bioaccumulation potential increases with increasing chain lengths of the molecules. [11] Bioaccumulation of PFSAs starts with a chain length of five [11], for PFCAs with a chain length of seven perfluorinated carbon atoms. [10] As PFSAs and PFCAs biomagnify along the food chain [12], piscivorous top predators possess the highest contamination in marine wildlife [13]. Substance profiles of PFSAs and PFCAs and their distribution in the body of different mammals are quite similar. [12, 14, 15] Mostly, perfluorooctane sulfonate (PFOS) is the dominating compound followed by perfluorononanoate (PFNA). [2, 12] Highest concentrations of PFCs have been detected in liver and kidney tissues. [14, 16, 17] Investigations concerning seabirds reveal different compositions. The dominating PFCA is perfluoroundecanoate (PFUnDA) or sometimes even perfluorotridecanoate (PFTriDA). [12, 18] In northern fulmars and thick-billed murres from Canada, PFCAs presented more than 80 % of the total PFC contamination. [19] Furthermore, this is the only study where PFOS was not observed to be the dominant PFC in biota. Three studies investigated the distribution of PFCs in the body of seabirds. [18, 20, 21] Each study found a different organ to be the most contaminated one: kidney [18], blood [20] and spleen [21].

This means that results concerning substance compositions as well as the major compound and the target organs of contamination in birds are rare and inconsistent.

The red-throated diver is a top predator in the marine food web. It is considered to be an opportunistic feeder and it could be shown that the food spectrum of red-throated divers in the Southern Baltic Proper is dominated by zander (in autumn) and herring (in spring) – two fish species which are part of the human diet as well. [22] Biomagnification of PFSAs and PFCAs in food webs was established in various studies [12, 23] and therefore contamination in red-throated divers is probably related to their diet. In 2005, 21363 metric tonnes (t) of fish including 16554 t herring were caught for human consumption in the fishing grounds of Mecklenburg – West Pomerania in the Southern Baltic Proper. [24] Therefore, PFC concentrations in red-throated divers might indicate a possible contamination source for humans as well.

The objectives of this study were to describe the contamination status of red-throated divers subsisting on herring from the Southern Baltic Proper, a part of the Baltic Sea, as a common fishing ground. Therefore, we determined the concentrations of five PFSAs, ten PFCAs, perfluorooctane sulfonamide (PFOSA), two perfluoroalkyl sulfonamides (FASAs), and two perfluoroalkyl sulfonamidoethanols (FASEs) in these birds. Furthermore, we investigated substance profiles and the organ specific distribution of PFCs in red-throated divers to amplify the state of knowledge and to potentially confirm previous results and estimates. Since the approach covers all available tissues, this dataset enabled the most precise estimate of the PFC body burden in seabirds so far. Besides that, multivariate statistical analyses were used to discover distribution patterns within the dataset.

Experimental

Chemicals and target analytes

Target analytes investigated in this study are listed in Table A1 in the accessory publications. All solvents, reagents, and standards were of high commercial quality and purity. Details are listed in the accessory publications, too (Table A1 and A2).

Sampling

Red-throated divers (n = 4) were collected near Usedom, Mecklenburg – West Pomerania, Germany in March and April 2005. All red-throated divers were by-caught in set net fisheries in the Pomeranian Bight, a small part of the Southern Baltic Proper. The set nets followed the 10 m depth line along the coast of Usedom and were checked twice to four times a week.

While looking for food red-throated divers were caught by the nylon nets and drowned. Between collection and dissection in November 2009 the birds were stored at -20 °C at the Research and Technology Centre Westcoast (FTZ). All tissue samples were taken with clean stainless steel instruments and weighed prior to the sub-sampling. Parameters like wing length or nutritional conditions were logged as well. The dissection protocols are listed in the accessory publications (Table A3). Depending on their size the whole organs or parts of them were stored in polypropylene containers, which had been rinsed with acetonitrile before, at -20 °C until preparation. The investigated tissue samples comprised blood, brain, fatty tissue, gall bladder (without bile), heart, kidney, liver, lung, muscle tissue, and spleen. The remaining parts of the birds, e.g. skin, feathers, beak, feet, or gastrointestinal tract were not sampled for this study.

Sample preparation

Samples were prepared by a modified method of Powley et al [25]. All tissue samples were homogenised using a disperser (T 25 basic Ultra-Turrax, IKA, Staufen, Germany). To avoid heating up while homogenising, tissue samples were cooled in a water bath. Depending on the tissue weight, up to 1 g of the homogenised sample or 2 mL of blood were loaded into a pre-cleaned 15 mL polypropylene centrifuge tube. 5 mL of acetonitrile were added to the homogenised tissue sample. After careful vortex-mixing (REAX top, Heidolph, Schwabach, Germany) for 30 s, the sample was placed in an ultrasonic bath at a temperature of about 30 °C for 30 min. The dispersion was centrifuged at 5000 rpm (Universal 320, Hettich, Tuttlingen, Germany) for 30 min. The clear supernatant was transferred to another polypropylene centrifuge tube and the extraction was repeated once. Since standard addition was used for quantification (see below), the combined supernatants were carefully vortex-mixed and separated in three aliquots of 3 mL each. The first and the second aliquot were spiked with 50 µL and 100 µL of a standard solution containing native PFCs (standard solution 1), respectively. Composition and compound concentrations of standard solution 1 are listed in Table A4 in the accessory publications. 30 µL of a standard solution containing mass-labelled PFCs only (standard solution 2) were added to the third aliquot. Concentrations complied with those of standard solution 1. Each aliquoted tissue extract was concentrated to approximately 1 mL under a gentle stream of nitrogen (vapotherm mobil, Barkey, Leopoldshöhe, Germany). For clean-up, the extract was transferred to a 1.7 mL polypropylene centrifuge tube filled with 30 mg of activated carbon and 50 µL glacial acetic acid. After vortex-mixing and 20 min of centrifugation at 5000 rpm the supernatant was transferred to a

glass vial. The activated carbon was rinsed with 1 mL of acetonitrile, centrifuged and the second supernatant was combined with the first one. Each extract was concentrated to exactly 150 µL under a gentle N_2 stream (flowtherm optocontrol, Barkey, Leopoldshöhe, Germany). Finally, the first and the second aliquot were transferred to 200 µL HPLC auto sampler vials filled with 20 µL of nanopure water (MilliQ Integral 10 TOC, Millipore, Schwalbach/Taunus, Germany) and 30 µL of methanol, each. The third aliquot was transferred to a 200 µL HPLC auto samples vial filled with 20 µL of nanopure water and 30 µL of standard solution 2. The contents in each vial were carefully mixed.

Instrumental analysis

Samples were analysed using high performance liquid chromatography (HPLC; Agilent 1100 Series HP, Agilent, Waldbronn, Germany) coupled to a tandem mass spectrometer (MS/MS; API 3000, Applied Biosystems/MDS Sciex Triple Quadrupol, Darmstadt, Germany) interfaced with an electrospray ionisation source in a negative ionisation mode ((-)ESI). As HPLC column a Phenomenex Synergi, 4 µm packing, Hydro-RP 80A, 150 mm x 2 mm (Phenomenex, Aschaffenburg, Germany) was used. A Phenomenex Synergi, 2.5 µm packing, Hydro-RP Mercury, 20 mm x 2 mm was used as precolumn. The mobile phase consisted of buffered methanol and nanopure water (10 mM ammonium acetate, each) and started at 70 % methanol. The linear gradient was increased to 90 % methanol at 4 min, to 100 % at 30 min and was held there until 10 min before changing to equilibration conditions. The equilibration took 8 min at 30 % methanol. Injection volumes of 10 µL were used, with a flow rate of 0.2 mL/min and a column temperature of 30 °C. The precursor ion to fragment transitions for all target analytes are listed in Table A5 in the accessory publications.

Quantification

As observed previously [26] sample matrix may result in alterations of the ionisation efficiency such as signal suppressions. Application of a solvent calibration to quantify a spiked standard mixture in bird extracts confirmed this assumption: all calculated concentrations were much lower than the true values. [27] Thus, this matrix effect-related bias needs to be corrected. However, only twelve mass-labelled internal standards were available for the twenty target analytes. To assure that both substance and mass-labelled standard behave equally during analyses, relative recovery rates (recovery ratios of native substance and corresponding mass-labelled standard) ought to be calculated in each of the ten different matrices as suggested previously. [28] However, due to the small sample quantities involved in the present study, this

kind of validation was not feasible. Another quantification option, matrix assisted calibration, was also limited by the small sample quantities. Furthermore, in previous tests the use of a 'comparable' matrix (turkey liver) for the matrix assisted calibration caused bias concentrations of a spiked standard mixture. Therefore, the method of standard additions was used for quantification. According to preliminary findings in red-throated divers target analytes were classified into three groups with different concentrations (Table A4). Standard addition was performed with two spike levels. The procedure of standard addition complies with the requirements of DIN standards. [29]

QA/QC

Sample preparation was performed in a Varipro clean lab system (class 10.000, Daldrop+Dr. Ing. Huber, Neckartailfingen, Germany). Perfluorinated materials or fluorinated polymers such as teflon were avoided during sampling, sample preparation and instrumental analysis. The glassware was machine-washed, heated at 250 °C for 12 h, and washed with acetonitrile before use.

For quality assurance accuracy, precision, and linearity of the instrumental analysis as well as instrumental detection limits (IDLs) and instrumental quantification limits (IQLs) were ascertained. IDLs on the basis of signal to noise (S/N) ratios of 3 were between 3 ng L^{-1} (perfluorooctanoate (PFOA)) and 22 ng L^{-1} (perfluoroheptane sulfonate (PFHpS)). IQLs were evaluated on the basis of an S/N ratio of 10. The complete method was validated by evaluating linearity, homoscedasticity, precision (including and excluding the quantification), accuracy (estimating the recovery rates of every single step of the sample preparation as well as of the complete method and comparing a calculated concentration of a spiking experiment with the true value (Student t-Test)), as well as method detection limits (MDLs) and method quantification limits (MQLs). For the determination of these validation parameters a 'comparable' matrix (extract of unpolluted turkey liver samples) was spiked with a solvent-based standard solution. MDLs on the basis of S/N ratios of 3 were between 0.12 µg L^{-1} (perfluorotetradecanoate (PFTeDA)) and 4.2 µg L^{-1} (PFOSA). MQLs were evaluated on the basis of an S/N ratio of 10. MDLs and MQLs were calculated for each of the ten different tissue samples. For each sample, recovery rates of mass-labelled internal standards were calculated. Recovery rates were dependent on the tissue investigated. On average, they were lowest in blood (60 %) and highest in brain samples (102 %). Recovery rates as well as MDLs and MQLs in real samples are listed in the accessory publications (Table A6 and A7). Analytes in the real tissue samples were considered as 'detected' if the calculated

concentration were above the MDL. To validate the standard addition method, the number of spikes and the time of spiking were evaluated, additionally.

A method blank (1 mL of acetonitrile) was extracted with each sample batch consisting of four tissue samples. Method blanks were only sporadically contaminated with PFOS and PFOA. Because analyte concentrations observed in blank samples were below the MQLs, results were not corrected for blank values.

Data treatment and statistical analyses

Datasets were tested for normal distribution (David test), outliers (Grubbs test) and trend (Neumann test). Student t-Test and F-Test ($p = 0.01$ or $p = 0.05$) were used to evaluate if differences between certain parameters were significant. For evaluating the linearity of the standard addition method, Mandel test was applied. For precision of sample processing and standard addition method the relative standard deviation was calculated. Additionally, their accuracy was evaluated using the Student t-Test or calculating the recovery rates.

For multivariate statistical analyses only concentrations of twelve compounds which were detected in more than 50 % of all samples were used: perfluorohexane sulfonate (PFHxS), PFHpS, PFOS, perfluorodecane sulfonate (PFDS), PFOA, PFNA, perfluorodecanoate (PFDA), PFUnDA, perfluorododecanoate (PFDoDA), PFTriDA, PFTeDA, and PFOSA. Due to the possible decomposition of PFOSA to PFOS [30], these two variables are not mutually independent. Therefore, they were combined to one combined parameter. Concentrations below the detection limit were calculated using Eqn 1:

$$c_{\mathrm{mod}} = 0{,}95 \cdot c_{MDL} + 0{,}1 \cdot c_{MDL} \cdot rnd$$

where c_{mod} is the modified concentration, c_{MDL} is concentration of the method detection limit, and *rnd* is a random number between 0 and 1. Concentrations of the completed dataset were standardised using Eqn 2:

$$z = \frac{c - \bar{c}}{s}$$

where z is the standardised concentration, c is the concentration, $\bar{c}$ is the mean concentration, and s is the standard deviation.

Cluster analysis was performed for concentrations of the tissue samples where the tissue samples present the objectives and the PFC concentrations present the variables. The analysis was performed on the basis of average total PFC contents in each tissue ($n = 4$; nine objects). Due to the uncertainties concerning PFC contamination in the lungs (see below), lung tissues

were excluded from cluster analyses. Clustering occurred using the Ward agglomeration method and the euclidean squared distance.

Factor analysis was performed on individual PFC concentrations using the Kaiser criterion so that only eigenvalues greater than 1 were applied. To facilitate the interpretation of the factors, the varimax rotation was applied.

Results and discussion

Occurrence of the target analytes

Of twenty determined PFCs, twelve (PFHxS, PFHpS, PFOS, PFDS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA and PFOSA) were detected in almost all samples. Their concentrations and proportions in the different tissues are described below. Two target compounds were rarely detected in individual samples (perfluoroheptanoate (PFHpA), six samples; perfluorodecahexanoate (PFHxDA), eight samples). Six target analytes (perfluorobutane sulfonate (PFBS), and perfluorooctadecanoate (PFOcDA), N-methyl perfluorooctane sulfonamide (N-MeFOSA), N-ethyl perfluorooctane sulfonamide (N-EtFOSA), N-methyl perfluorooctane sulfonamidoethanol (N-MeFOSE), N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE)) were not detected in any of the forty tissue samples. Due to the expected degradation of N-MeFOSA, N-EtFOSA, N-MeFOSE, and N-EtFOSE under environmental and biological conditions [30, 31], the lack of FASAs and FASEs was not surprising. Non detects of PFBS and rare detects of PFHpA (only six samples) were probably caused by the nonexistent bioaccumulation of these compounds. Bioaccumulation was observed for PFSAs containing more than four and for PFCAs containing more than six perfluorinated carbon atoms. [10, 11] Previous studies demonstrated increasing PFC bioconcentration factors with increasing chain length. [11] Additionally, relatively low bioconcentration factors for PFTeDA suggested a possible limitation of bioaccumulation for long-chain PFCAs. [11] In compliance with this assumption, in the present study PFTeDA was detected in 70 %, PFHxDA in 20 % and PFOcDA in none of the forty tissue samples.

PFC concentrations

Total PFC concentrations in all tissue samples are presented in Figure 1. Individual concentrations and standard deviations are listed in Table A8 and A9 in the accessory publications. The good congruence of median and mean suggests normally distributed data as well as a lack of outliers. Due to standard addition as quantification method as well as concentrations occurring in trace levels, calculated confidence intervals were quite high (up to

300 %). Results of previously conducted validations confirmed the accuracy of the complete method (absolute recovery rates calculated on the basis of a matrix assisted calibration were between 85 % (PFOcDA) and 115 % (PFHxS)) as well as the high standard deviation (Table A10). Relative standard deviations increased in the order of instrumental detection (1.6 % (PFTeDA) - 4.4 % (PFHxS)) < sample preparation + instrumental detection (1.6 % (PFOSA) - 12 % (PFHpS)) < complete method including the quantification using standard additions (29 % (PFOA) - 58 % (PFDS)). Although the absolute concentrations bear the comparison with the results of previous studies determining PFC concentrations and tissue distribution in birds [18, 20, 21], they are rather to be considered as 'concentration levels' in this study.

In this study, the most contaminated bird tissue on the ng g^{-1} basis is liver followed by kidney, lung, gall bladder and blood. Lowest concentrations were observed in fatty and muscle tissue. Except for kidney and gall bladder, total PFC concentrations in liver were significantly higher than in the other tissue samples ($p < 0.05$). Previous studies investigating biota samples found liver to be the most highly contaminated tissue in mammals [16, 32] whereas in fish [11] and birds [18, 20, 21] highest concentrations were observed in plasma/blood, kidney, or spleen. As in the present study, these studies observed lowest concentrations in muscle and fatty tissue.

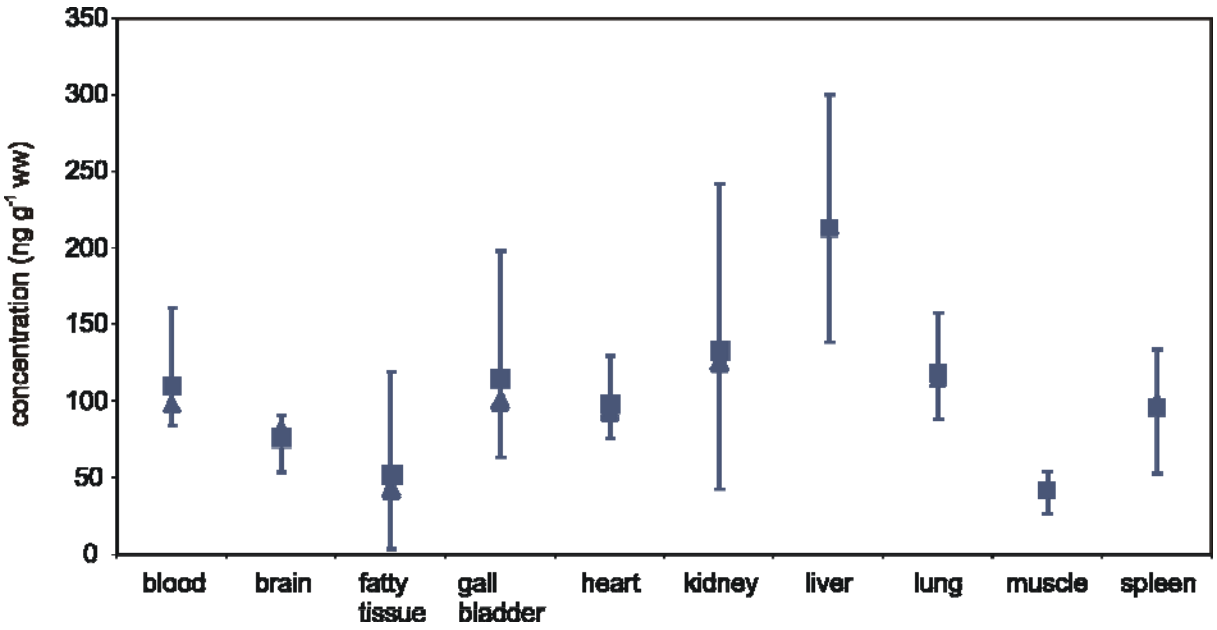

Fig. 1. Total perfluorinated compound concentrations (ng g^{-1} wet weight) in ten different tissue samples of red-throated divers (n = 4). ■ : mean concentration, ▲ : median concentration, bars: minimum and maximum concentrations.

PFC profiles in tissue samples

PFC profiles in the different tissue samples are presented in Figure 2. Average proportions of PFOS and its precursor substance PFOSA accounted for about 90 % of the total PFC amount in eight of ten tissues. Except for brain and lung, profiles of the twelve detected PFCs of the remaining tissues were quite similar. Previous studies determined similar relative distributions. [14, 18, 20] Only in one study sampling seabirds from the Canadian Arctic, the percentage of total PFCAs and total PFSAs was reversed. [19]

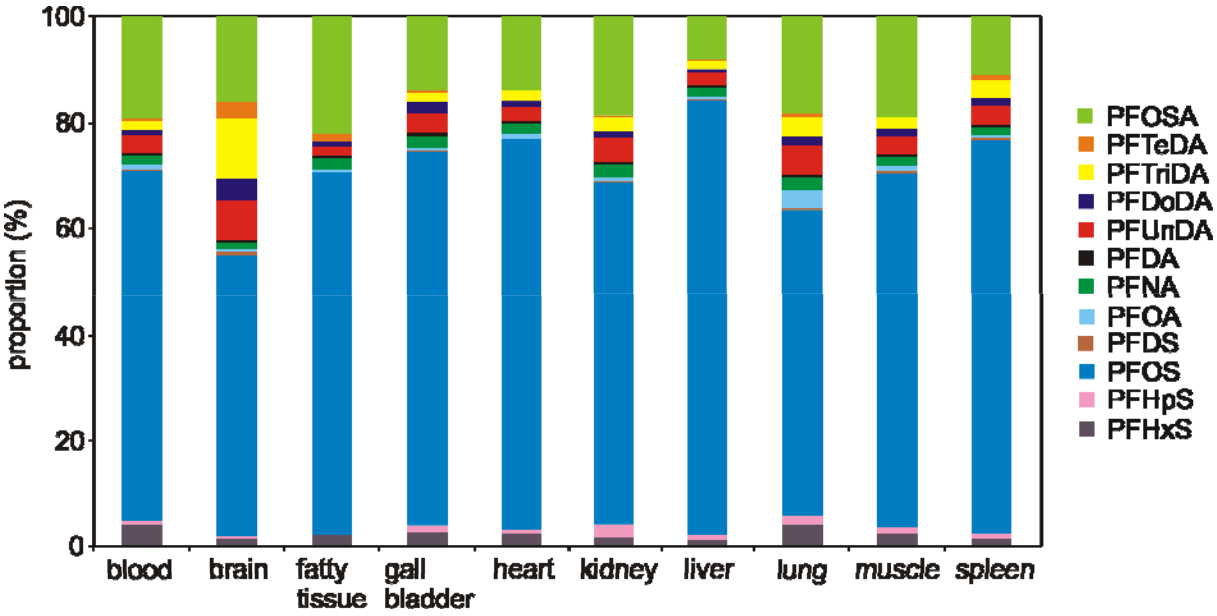

Fig. 2. Average substance profiles (%) of twelve perfluorinated compounds mainly detected in ten different tissue samples of red-throated divers (n = 4). PFHpA and PFHxDA were not presented due to their spare and irregular detection (six and eight samples, respectively).

In comparison to other tissue (0.4 and 0.9 %), lung samples were characterised by a rather high PFOA contribution (3.5 %) although its bioaccumulation is the lowest within the group of perfluorinated carboxylates. [11] Hence, PFOA was detected only sporadically and in small amounts in biota samples including the main feed of red-throated divers. [14, 18] The exact reason for finding relatively high PFOA concentrations in lung tissue samples remained unclear. One reason might be the birds' dead by drowning in seawater. Seawater in this part of the Baltic Sea contains PFOA concentrations of about 1 ng L^{-1}. [33] Therefore, PFOA contaminated water may have entered the birds' lungs. However, as rough estimate 100 L of seawater would have been necessary to reach the contamination level observed in the lung tissue. Another hypothesis may comprise the inhalation of marine aerosols by the red-throated divers. Recently, it was discussed that PFOA concentrations may be up to 80 times higher in aerosols than in the parent water body (ocean water). [34-36] Enriched in marine aerosols,

PFOA might have reached the birds' lungs as a part of the breathable air. This is supported by medical studies using perfluorocarbon aerosol to improve the gas exchange and the mechanical lung function. [37] After having entered the lungs, PFOA, as a main compound in Baltic Sea water, may preferentially accumulate in the lung tissue, probably as a result of interaction with hydrophobic parts of the lipid shares or with surfactant proteins of the cells. Due to the hypothetical character of these interpretations, we decided to exclude lung samples from statistical discussions.

Except for the kidney, PFCA proportions in brain samples were significantly higher than in the other tissue samples ($p < 0.05$). Besides PFUnDA, PFDoDA, PFTriDA, and PFTeDA, the PFSA with the longest chain length determined in this study (PFDS) was observed in elevated concentrations in brain samples, too. Hence, the question arises whether perfluorinated carboxylates only or long-chain PFCs including perfluorinated carboxylates as well as sulfonates accumulate preferentially in the brain. Verreault et al. [20] did not determine a preferential accumulation of PFCAs or long-chain PFCs in general in brain samples.

Whereas PFUnDA was the major PFCA in the remaining tissue samples, PFTriDA was the most highly concentrated carboxylate in brain samples indicating a shift of the concentration maximum within the PFCAs up to long-chain molecules. As observed in the present study's investigated tissues (except for brain), previous studies analysing bird tissue samples discovered PFUnDA as most highly concentrated PFCA, too. [18, 20] In contrast to birds, PFNA was the dominating compound among PFCAs found in mammals. [38] In fish, PFUnDA and PFTriDA were observed in highest concentrations. [38] Therefore, it was supposed that high concentrations of PFUnDA and PFTriDA in tissue samples of piscivorous seabirds reflect the PFC pattern of their food. [38] As well as in previous studies [2, 38] , results of these observations confirmed odd PFCAs (PFNA, PFUnDA, PFTriDA) to be higher concentrated than the even ones (PFDA, PFDoDA, PFTeDA). Ellis et al. [7] determined FTOHs as an important source for PFCAs. Additionally, they confirmed that the previous observed even-odd pattern in biota samples [38] can be probably be attributed to the degradation of FTOHs. [7]

Cluster analysis

Results of the cluster analysis are presented in the dendrogram in Figure 3 and confirm the above mentioned findings. The first clustering divided the objects in two clusters segregating brain, kidney, and liver from the other tissues. In both clusters a substructure is obvious forming two sub-clusters, each. Kidney and liver, the two tissues of highest PFC contamination of the dataset, form one cluster. Brain contains a medium PFC concentration

but reveals the highest concentrations of long-chain molecules and is thus forming a separate sub-cluster. In cluster 2, fatty and muscle tissues are separated from blood, gall bladder, heart, and spleen, probably due to their very low PFC concentrations.

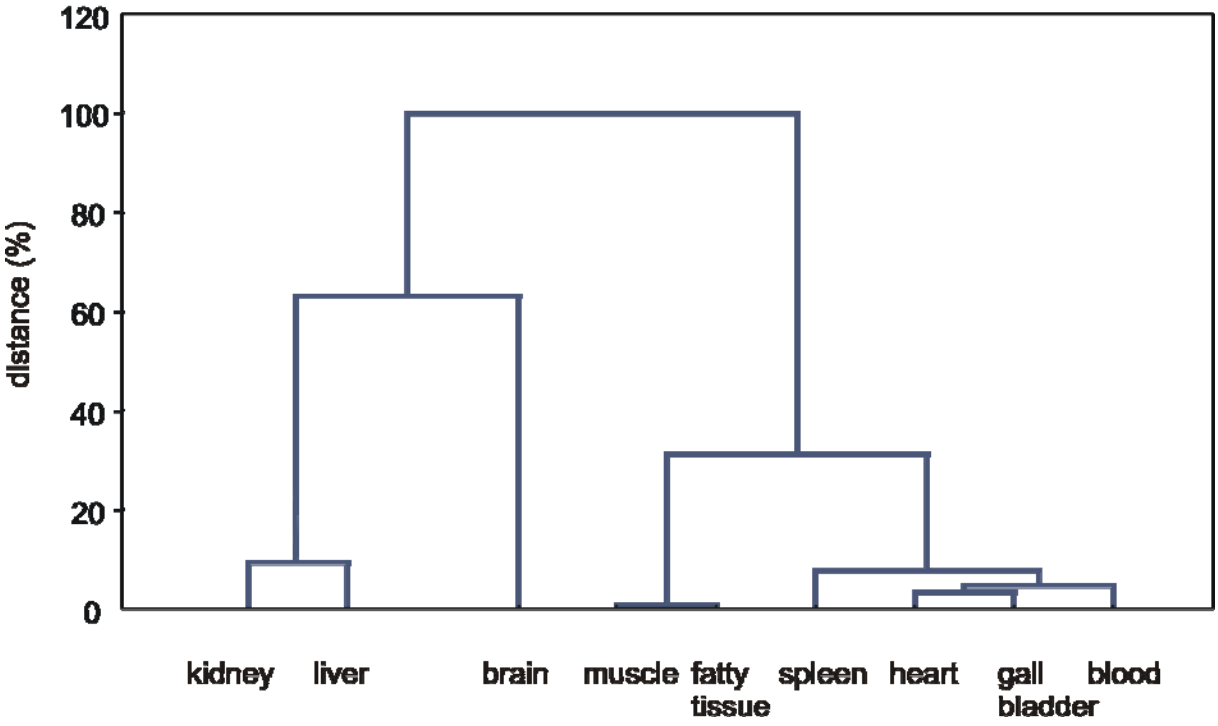

Fig. 3. Dendrogram of the cluster analysis. The analysis was performed on the basis of the average total PFC content in each tissue (n = 4; nine objects). Lung samples were excluded. The clustering occurred using Ward's agglomeration method and the Euclidean squared distance.

Distribution of PFCs in tissues and whole body burden

By multiplying the organs' PFC concentrations by the weights of the organs themselves, the absolute PFC content of each organ was calculated. The relative distribution of total PFC amounts in the ten tissues as well as the tissue distribution (i.e. the fraction of the individual tissue mass related to the birds' body mass) is given in Figure 4. More than 65 % of the seabirds' total mass is fatty and muscle tissue. Therefore, fatty and muscle tissue contain more than 50 % of the total PFC amount despite their low PFC concentrations. Thus, although the accumulation of PFCs in these two tissues is rather marginal, they do not seem to be insignificant reservoirs of PFCs. Figure 4 clearly illustrates the elevated PFC concentrations in liver samples. Even though the liver accounts for 5 % of the bird's total body mass only, it contains nearly 20 % of the total PFC amount.

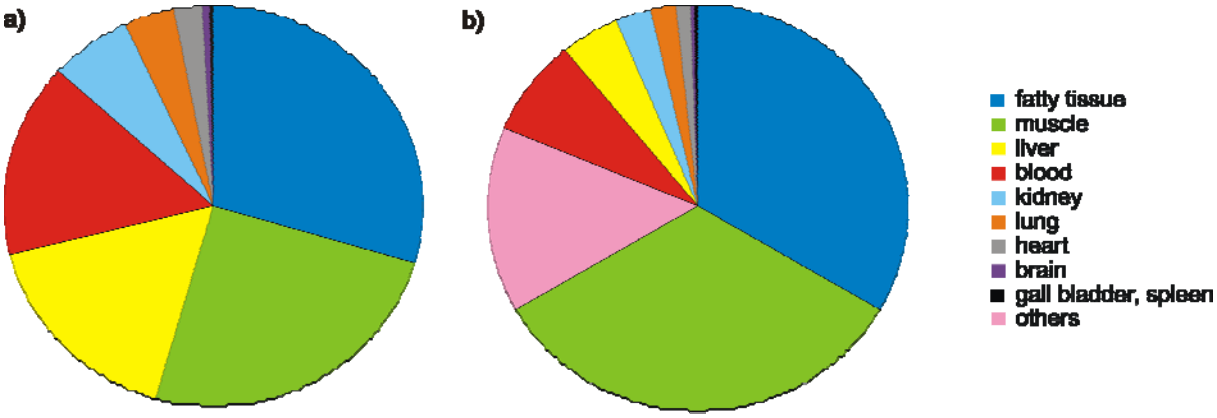

Fig. 4. Relative distribution (%) of total PFC amount (85 µg) in the ten tissues (a) and tissue distribution (i.e. fraction of the individual tissue mass related to the birds' body mass; %) in red-throated divers (b). The sampled tissues included 85 % of the total birds' mass. The term 'others' comprises the remaining 15 % including beak, feet, feathers or skin.

To the best of our knowledge only three more studies investigating PFCs in animals (seals) determined the amount of PFCs in each organ including fatty tissue. [14, 16, 17] Two studies estimated the relative distribution of PFC amounts in the observed tissues [16, 17], the third study only calculated PFC concentrations of tissue samples [14]. Sturman et al. [17] found concentrations in seal blubber to be highly variable along the seasons. Whilst in spring blubber contained only 1 % of the total PFC amount (n = 10, Nain, Canada), in fall this percentage increased up to 10 % (n = 5, Nain, Canada). [17] Therefore, a relation between PFC loadings and blubber contents was suggested. [17] Ahrens et al. [16] analysed PFCs in one to two year old harbour seals sampled in wintertime in the German Wadden Sea. They observed 2 % of the total PFC amount in blubber and 36 % in liver. [16] They found highest concentrations in liver as well. Compared to a previously published study investigating harbour seals in the Dutch Wadden Sea (n = 17) [14], PFC liver concentrations calculated by Ahrens et al. [16] were one order of magnitude higher and PFC blubber concentrations were one order of magnitude lower. [16] This may be due to differences in species, sampling location, season, age, and physical conditions which hamper direct inter-study comparisons of PFC concentrations as described above. In the present study, fatty tissue contained about 33 % of the total PFC amount with a suggested fat content of the red-throated divers of approximately 30 % of the whole body mass. [39] However, in the present as well as in the published studies [14, 16, 17] the total mass of fatty tissue was only estimated on the basis of literature data. Even though the estimated percentage of 33 % of total PFC amount in fatty tissue in the present study is relatively high, it indicates the potential relevance of fatty tissues

403 as PFC reservoirs for species exhibiting high contents of fat. This is in contrast to several
404 studies where fatty tissue was not identified as important reservoir for PFCs. [e. g. 11, 40]
405 The summation of organ-specific PFC amounts enables the estimation of the whole PFC body
406 burden. In the present study 'whole body' only includes the ten sampled tissues. Other organs
407 such as beak, feet, feathers or skin are not included. Their amount is estimated to be
408 approximately 15 % of the whole body mass. The total PFC amount of the sampled bird (Σ10
409 tissues) is 85 µg ± 33 µg. This corresponds to a concentration of 67 µg kg^{-1} ± 26 µg kg^{-1}.
410 Assuming that the remaining 15 % of the bird's tissue are equally contaminated [41], the total
411 PFCs body burden can be estimated to be 100 µg or 67 µg kg^{-1}.
412
413 **Factor analysis**
414 The lipophilicity of PFCs increases with increasing perfluorinated carbon chain length and
415 correlates positively with the bioaccumulation potential. A common criterion for lipophilicity
416 is the K_{OW}. Because the K_{OW} cannot determined for ionic surfactants [42] and the critical
417 micelle concentrations (CMC) is suggested not to be a suitable surrogate for surfactant
418 lipophilicity [23], the number of perfluorinated carbons was used to estimate the
419 bioaccumulation of PFSAs and PFCAs. [10] Martin et al. [11, 23] reported that bioaccumulation
420 factors of PFSAs and PFCAs with an equal number of perfluorinated carbon atoms are higher
421 for PFSAs than for PFCAs. Therefore, they suggested not only the lipophilicity but also the
422 acid function being responsible for different bioaccumulation potentials. [23] The sulfonate
423 moiety contains two double bonds. Therefore, the negative loading of a molecule containing a
424 sulfonate moiety can possibly be delocalised more effectively than molecules containing a
425 carboxylic moiety with one double bond only. Thus, perfluorinated sulfonates are most likely
426 slightly less polar and therefore slightly more lipophilic than carboxylates of equal
427 perfluorinated chain length as also assumed previously by Higgins et al.. [43]
428 Detailed results of the factor analysis are given in Table A11 of the accessory publications.
429 They imply a possible dependence of lipophilicity and the 'location' of PFC accumulation.
430 Long-chain PFCAs (PFTeDA, PFTriDA, and PFDoDA) as well as long-chain PFSA (PFDS)
431 correlated with the first factor. PFCs of a medium chain length (PFDA, PFOS, and PFNA)
432 correlated with second factor. The shortest investigated PFCs (PFOA and PFHxS) correlated
433 with the third factor. This classification which can be attributed to the molecules' chain length
434 is potentially linked to the lipophilicity, as well. The factor scores, presented in Figure 5,
435 reveal the degree of influence induced by the factors and their correlating variables on the
436 objectives (tissue samples). Positive factor scores may indicate an influence of the substances

correlating with the individual factor on the tissue. Negative factor scores suggest that the substances which correlate with the individual factor do not influence the respective tissue.

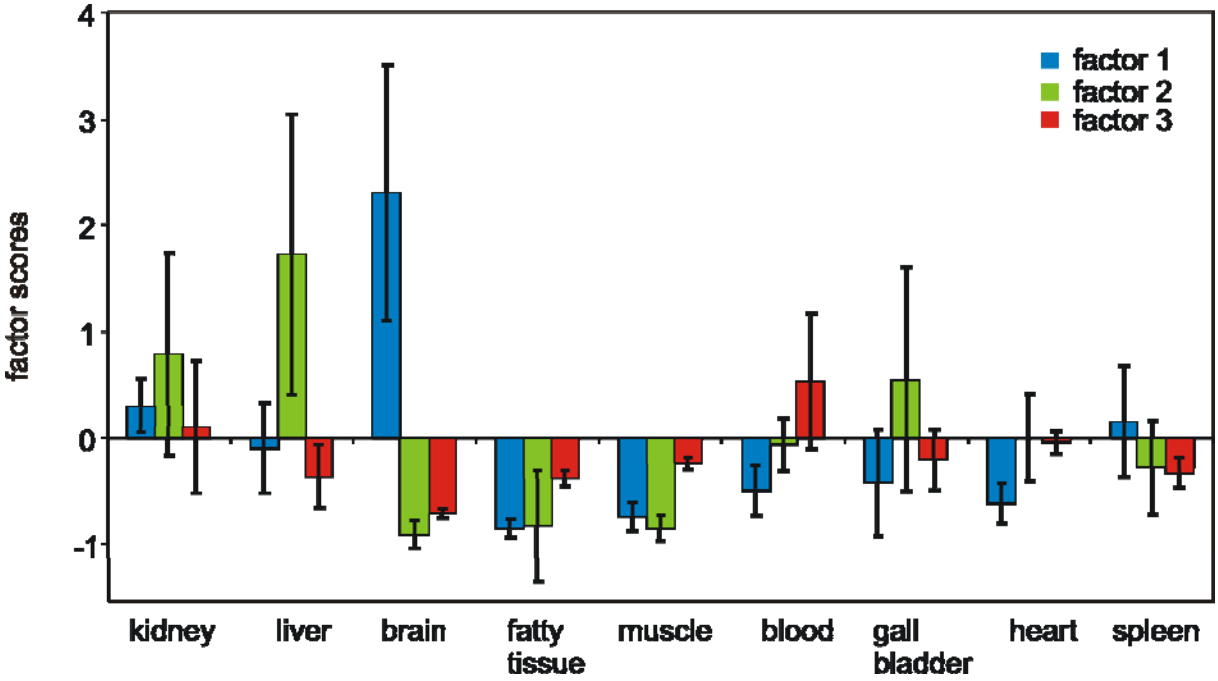

Fig. 5. Average factor scores (columns) and absolute standard deviation (error bars) of nine different tissue samples of red-throated divers (n = 4). For the factor analysis the Kaiser criterion was used so that only eigenvalues greater than 1 were applied and varimax rotation was performed. Lung tissue was excluded from this analysis (please refer to the main text). A detailed graph is presented in accessory publications (Figure A1).

Confirming the observations on the basis of the substance composition which was discussed above, brain samples are mainly influenced by long-chain PFCAs and PFDS. Factor scores of the second factor (including PFOS) were highest for liver samples. This corroborates previous studies reporting liver as the target organ of PFOS accumulation. [40, 44] For blood samples, all factor scores of the third factor were positive. This means that the shortest investigated PFCs (PFOA and PFHxS) which correlate with the third factor might have an influence on the birds' blood. Kidney as an excretory organ was influenced by all investigated substances. Fatty and muscle tissue, gall bladder, heart, and spleen revealed no significant presence of any substance groups as factor scores were mainly negative or evenly distributed for all factors. It is well known that lipophilic substances are able to pass the blood-brain barrier. [45] To the best of our knowledge, this is the first time that a preferential accumulation of long-chain PFCAs (dominance of factor 1) in brain was determined. Austin et al. [44] detected PFOS in rats' brains and concluded its potential to cross the blood-brain barrier. They suggested that PFOS affects central and neuroendocrine functions. [44] The effect of PFCAs in the brain is not known so far.

Conclusions and Outlook

This study corroborates many results of previous investigations for birds. Liver was found to contain the highest PFC concentrations followed by kidney. The similarity of these two organs concerning the degree of PFC contamination was confirmed by the results of the cluster analysis. PFOS represented the major PFC in all tissue samples and PFOS and PFOSA accounted for approximately 90 % of total PFC amount in eight of ten organ samples. Confirming a previously published characteristic of bird samples, in nine of ten organ samples PFUnDA was found to be the major PFCA. Additionally, the previously observed even-odd pattern of PFCAs was proven. But also new findings were discussed. Fatty tissue was found to be a PFC reservoir for species having a high fat content that was probably neglected in former studies. Preferential enrichment of long-chain PFSAs and PFCAs in brain samples was observed and confirmed by results of cluster and factor analysis. Owing to the results of the factor analysis a possible relationship between PFC lipophilicity and their preferred accumulation 'location' within the organism was derived. However, all of these assumptions need to be confirmed by additional data of future studies in order to understand resulting consequences. Particularly the potential of several long-chain PFCs to cross the blood-brain barrier needs further investigation, also in terms of toxicological effects of long-chain PFSAs and PFCAs in the brain itself.

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