Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere

Kyra Spaan, Carmen van Noordenburg, Merle Plassmann, Lara Schultes, Susan D. Shaw, Michelle Berger, Mads Peter Heide-Jørgensen, Aqqalu Rosing-Asvid, Sandra Granquist, Rune Dietz, Christian Sonne, Frank Rigét, Anna Roos, Jonathan Benskin

Submitted date: 01/11/2019 • Posted date: 07/11/2019
Licence: CC BY-NC-ND 4.0

Citation information: Spaan, Kyra; van Noordenburg, Carmen; Plassmann, Merle; Schultes, Lara; Shaw, Susan D.; Berger, Michelle; et al. (2019): Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.10128653.v1

There is increasing evidence that the ~20 routinely monitored per- and polyfluoroalkyl substances (PFASs) account for only a fraction of extractable organofluorine (EOF) occurring in the environment. To assess whether PFAS exposure is being underestimated in marine mammals from the Northern Hemisphere, we performed a fluorine mass balance on liver tissues from 11 different species using a combination of targeted PFAS analysis, EOF and total fluorine determination, and suspect screening. Samples were obtained from the east coast United States (US), west and east coast of Greenland, Iceland, and Sweden from 2000-2017. Of the 36 target PFASs, perfluorooctane sulfonate (PFOS) dominated in all but one Icelandic and three US samples, where the 7:3 fluorotelomer carboxylic acid (7:3 FTCA) was prevalent. This is the first report of 7:3 FTCA in polar bears (~1000 ng/g, ww) and cetaceans (<6-190 ng/g, ww). In 18 out of 25 samples, EOF was not significantly greater than fluorine concentrations derived from sum target PFASs. For the remaining 7 samples (mostly from the US east coast), 30-75% of the EOF was unidentified. Suspect screening revealed an additional 33 PFASs (not included in the targeted analysis) bringing the total to 59 detected PFASs from 12 different classes. Overall, these results highlight the importance of a multi-platform approach for accurately characterizing PFAS exposure in marine mammals.
Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere

Kyra M. Spaan¹*, Carmen van Noordenburg¹, Merle M. Plassmann¹, Lara Schultes¹, Susan Shaw², Michelle Berger², Mads Peter Heide-Jørgensen³, Aqqalu Rosing-Asvid³, Sandra M. Granquist⁴,⁵, Rune Dietz⁶, Christian Sonne⁶, Frank Rigé⁶, Anna Roos³,⁷, Jonathan P. Benskin¹*

¹Department of Environmental Science and Analytical Chemistry, Stockholm University, Svante Arrhenius Väg 8, 106 91, Stockholm, Sweden.
²Shaw Institute, P.O. Box 1652, Blue Hill, ME 04614
³Greenland Institute of Natural Resources, Nuuk, Greenland
⁴Marine and Freshwater Research Institute, Skúlagata 4, 101 Reykjavik, Iceland.
⁵The Icelandic Seal Center, Brekkugata 2, 530 Hvammstangi, Iceland
⁶Aarhus University, Department of Bioscience, Arctic Research Centre (ARC), Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark
⁷Department of Environmental Research and Monitoring, Swedish Museum of Natural History, Box 50007, 104 05 Stockholm, Sweden

*Corresponding authors:
Kyra.Spaan@aces.su.se
Jon.Benskin@aces.su.se
ABSTRACT

There is increasing evidence that the ~20 routinely monitored per- and polyfluoroalkyl substances (PFASs) account for only a fraction of extractable organofluorine (EOF) occurring in the environment. To assess whether PFAS exposure is being underestimated in marine mammals from the Northern Hemisphere, we performed a fluorine mass balance on liver tissues from 11 different species using a combination of targeted PFAS analysis, EOF and total fluorine determination, and suspect screening. Samples were obtained from the east coast United States (US), west and east coast of Greenland, Iceland, and Sweden from 2000-2017. Of the 36 target PFASs, perfluorooctane sulfonate (PFOS) dominated in all but one Icelandic and three US samples, where the 7:3 fluorotelomer carboxylic acid (7:3 FTCA) was prevalent. This is the first report of 7:3 FTCA in polar bears (~1000 ng/g, ww) and cetaceans (<6-190 ng/g, ww). In 18 out of 25 samples, EOF was not significantly greater than fluorine concentrations derived from sum target PFASs. For the remaining 7 samples (mostly from the US east coast), 30-75% of the EOF was unidentified. Suspect screening revealed an additional 33 PFASs (not included in the targeted analysis) bringing the total to 59 detected PFASs from 12 different classes. Overall, these results highlight the importance of a multi-platform approach for accurately characterizing PFAS exposure in marine mammals.
INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a diverse class of chemicals used throughout society.\textsuperscript{1,2} Perfluoroalkyl chains possess a wide range of unique properties, including extreme stability and combined oil/water repellency. These attributes have led to the use of PFASs in a broad range of products, including fire-fighting foams, textiles, non-stick cookware, food wrapping paper, paints, cosmetics, in addition to many other industrial applications.\textsuperscript{3,4} The most well-studied PFASs are the perfluoroalkyl acids (PFAAs), in particular the perfluoroalkyl carboxylic acids (PFCAs), such as perfluorooctanoic acid (PFOA), and the perfluoroalkyl sulfonic acids (PFSAs), such as perfluorooctanoic sulfonic acid (PFOS). PFSAs and PFCAs are suggested to be the final breakdown products of most PFASs.\textsuperscript{5}

The bioaccumulation potential of PFASs is strongly correlated with perfluoroalkyl chain length; structures containing ≥8 fluorinated carbons for PFCAs and ≥6 fluorinated carbons for PFSAs are considered bioaccumulative.\textsuperscript{6-8} PFAAs are present in the blood of humans and wildlife globally, including remote polar regions.\textsuperscript{9-11} Unlike classical persistent organic pollutants (e.g. polychlorinated biphenyls), PFASs accumulate primarily in protein-rich tissues such as liver and blood.\textsuperscript{12} PFASs have been linked to various toxicological effects, e.g. reproductive deficits,\textsuperscript{13,14} immunotoxicity,\textsuperscript{15,16} thyroid hormone disruption,\textsuperscript{17-19} and disturbance of lipid metabolism.\textsuperscript{20} Due to their persistent, bioaccumulative and toxic properties as their widespread distribution, PFASs have received global attention over the last few decades leading to several regulatory initiatives.\textsuperscript{21-23} However, development and manufacturing of alternative PFASs (which are largely uncharacterized in terms of risks) remain ongoing, despite numerous examples of their environmental occurrence.\textsuperscript{24,25} and therefore hard to detect or often overseen in analyses of environmental samples and wildlife tissue samples.
Recent research by the Organization for Economic Co-operation and Development (OECD) identified 4730 CAS numbers related to PFASs. However, since only a small fraction (<20) of these substances are routinely monitored, PFAS exposure may be underestimated. Indeed, the large quantities of unidentified extractable organofluorine (EOF) in environmental samples (56-100%), cosmetics (68-100%), aqueous film forming foam (AFF; ~50%), human blood (15-67%), and wildlife (68-90%) are cause for considerable concern. Moreover, recent investigations using non-target and suspect-screening analytical workflows have uncovered an unprecedented number of novel PFAS structures, some of which may account for this unidentified organofluorine. However, since standards are unavailable for most of these compounds, the importance of their contribution to overall PFAS exposure remains unclear.

As top predators, marine mammals are vulnerable to persistent and bioaccumulative substances and are among the highest exposed organisms on the planet. Recent investigations in polar bear serum identified 35 additional PFASs that were not included in targeted analyses. This included cyclic or unsaturated PFSAs, ether PFSAs, unsaturated ether-, cyclic ether- or carbonyl PFSAs, and x:2 chlorinated perfluoroalkyl ether sulfonates. The present study builds upon the work of Liu et al. by combining suspect screening with organofluorine mass balance to comprehensively assess PFAS exposure in eleven different marine mammal species from different locations within the Northern Hemisphere (Table S1). To the best of our knowledge, this is the first time organofluorine mass balance combined with suspect screening has been conducted in marine mammals.

MATERIALS AND METHODS

Sample Collection
Marine mammal liver samples included in this study originated from five different locations within the Northern Hemisphere (Table S1). A full list of samples, including information on species (including Latin names), year, age, sex, sampling location, weight, and length are provided in Table S1. A brief overview is provided here. Species from the US Atlantic coast included grey seal, harbor seal, harbor porpoise, and pygmy sperm whale; samples were obtained between the years 2000 and 2012 from stranded animals. Samples from Sweden were collected between 2011 and 2016 from by-caught animals (seals), animals shot during domestic hunting (seals), or from stranded animals (harbor porpoise). Grey and harbor seals as well as harbor porpoise were collected from the south, while ringed seals were collected from the northern Baltic. Samples from Greenland included harp and ringed seals, harbor porpoise, white beaked dolphin, killer whale, humpback whale, minke whale (fetus), and polar bear (including a mother and cub) were collected with help from local Inuit subsistence hunters from 2000-2016. Targeted PFAS data for ringed seal (2012), polar bears (2012), and killer whales (2013) from East Greenland were previously reported in Gebbink et al. but were re-analyzed in the present work. Icelandic seal samples were derived from animals that were by-caught in 2009 and 2010 and included grey, harbor and harp seal. CITES numbers for export and import permissions are provided in the supporting information (SI, Table S2 and S3). Liver tissues were shipped in individual PP-tubes on dry ice, after which they were stored at −20 °C until analysis. The present study was originally designed so that every sample would include a pool of liver tissue from multiple animals, with mixed sexes and ages. However, this was not possible for some species due to low sample availability, and therefore some samples consist of liver tissue from only one animal, while pooled samples consisted of liver tissues from 2-10 animals.

Chemicals and Reagents
Native and isotopically-labelled PFAS standards included in the targeted analysis were purchased from Wellington Labs (Guelph, Canada). Structures and abbreviations of individual PFASs are provided in Table S3. A total of 36 PFASs were targeted in the present work, including 14 perfluoroalkyl carboxylic acids (PFCAs; C_{4-16}, C_{18}), 8 perfluoroalkyl sulfonic acids (PFSAs; C_{4-11}), perfluoroctane sulfonamide (FOSA), 3 perfluoroalkane sulfamidoacetic acids (FOSAA, MeFOSAA, EtFOSAA), 2 chlorinated polyfluorinated ether sulfonates (Cl-PFESAs; 9Cl-PF3ONS, 11Cl-PF3OUdS), ADONA, HFPO-DA, 3 fluorotelomer sulfonates (4:2, 6:2, and 8:2 FTSAs), and 3 fluorotelomer carboxylic acids (3:3, 5:3, and 7:3 FTCAs). Linear (L) and branched (br) isomers were determined separately for some substances (see Table S5). For some target analytes an analogous internal standard (IS) was lacking and these were therefore semi-quantified (see Table S5).

**Overview of Fluorine Mass Balance Approach**

The experimental approach for assessing fluorine mass balance is depicted in Figure S1, and was performed as follows. Three portions of tissue were removed of homogenates of a single liver or pooled sample. The first portion was fortified with an internal standard mix, extracted as described in the next paragraph, and analyzed using both UPLC-MS/MS (targeted analysis) and UPLC-Orbitrap-MS (suspect screening). The second portion was extracted using the same methods but without addition of internal standard, and the resulting extract was analyzed for EOF by combustion ion chromatography (CIC). For comparability to targeted PFAS concentrations, EOF concentrations were recovery-corrected based on the results of a spike-recovery experiment (see QC section). The third portion of tissue was combusted directly on the CIC for determination of total fluorine (TF). Approximately 25% of the samples were run in triplicate. Assuming that all liver tissues display similar instrumental variation, the highest relative standard deviation (RSD)
for each analyte was used to estimate standard deviations for all other samples (i.e. those not run as replicates).

**Sample Preparation**

Liver samples were stored in 13 ml polypropylene (PP) tubes at -20 °C prior to analysis. Sub-sampling was done using a stainless-steel knife of which the blades were pre-cleaned with methanol. For targeted analysis, approximately 0.5 g of liver homogenate was thawed at room temperature and internal standard (IS) solution was added prior to extraction using the procedure described by Powley et al.\(^{42}\) (detailed description is provided in the SI). The final extract was fortified with recovery standards (RSs; \(^{13}\)C\(_8\)-PFOA and \(^{13}\)C\(_8\)-PFOS) and 500 µl of 4mM NH\(_4\)OAc (aq) and then stored at -20 °C until analysis. The extraction procedure for EOF analysis was the same as for target PFAS analysis, with the exception that standards and buffer were not included, and the final extracts were concentrated to ~200 µl under a stream of nitrogen. For TF analysis, 100 mg neat liver was analyzed directly, with no fortification of standards.

**Instrumental Analysis and quality control**

**Targeted analysis**

Targeted analysis was carried out on an Acquity UPLC (Waters) coupled to a triple quadrupole mass spectrometer (Xevo TQS, Waters), equipped with a BEH (Ethylene Bridged Hybrid) C\(_{18}\) column (1.7 µm, 50 × 2.1 mm, Waters), based on a previously described method.\(^{43}\) The gradient program is specified in Table S5. MS source conditions are provided in the SI. Quantification was performed using MassLynx 4.1 (Waters), via a 9-point calibration curve ranging from 0.008 to 150 ng/ml (linear, 1/x weighting). Precursor and product ions are presented in Table S6. Analytes lacking an analogous labeled standard were quantified using the IS with the closest retention time.
and the data quality was defined as semi-quantitative (semiQ). Branched isomers were quantified using the calibration curve of the linear isomer. Limits of quantification (LOQs) are presented in Table S6.

To determine method accuracy and precision, spike/recovery experiments were performed using homogenized seal liver. Seal liver samples (0.5 g) spiked with 10 ng native standard mix showed very good recoveries for most compounds (73-130%; Figure S2). The exceptions were PFHxDA, PFOcDA, 4:2 FTSA, and 8:2 FTSA, which showed very high recoveries (278%, 397%, 212%, and 227%, respectively), while HFPO-DA, 3:3 FTCA, 5:3 FTCA, and 7:3 FTCA showed very low recoveries (22%, 34%, 55%, and 53%, respectively). These deviating recoveries are likely due to matrix effects, which were not accounted for because of the absence of an exactly matching isotopically-labeled internal standard (see detailed discussion in the SI and Figure S2). NIST certified reference material 1957 (CRM 1957) was used for external method validation, and results were generally in good agreement with certified values (see Table S8). Finally, each batch of samples was processed together with three method blanks and control seal liver tissue (spiked and unspiked), and between every 8-10 instrumental injections a standard was included to monitor instrumental drift.

Total- and extractable organofluorine analysis

Measurements of TF and EOF were carried out using CIC (Thermo-Mitsubishi) using previously described methods.\textsuperscript{30,44} A detailed description is provided in the SI and the IC gradient program is provided in Table S9. Quantification was performed using a standard calibration curve prepared at 0.05 to 100 µg F/ml ($R^2$>0.98). For EOF measurements the mean fluoride concentration in the method blanks was subtracted from all samples. For TF analysis, instrumental (boat) blank fluoride
concentrations were subtracted. The method quantification limit (LOQ) was defined as the mean concentration plus three times the standard deviation of the method blanks.

Spike/recovery experiments with NaF and PFOS over a range of concentrations revealed that inorganic fluorine was removed efficiently by the extraction procedure, as intended, even at the highest fortification level of 2000 ng F (Figure S3). In contrast, fluorine concentrations increased linearly ($R^2$>0.99) with increasing fortification of PFOS. A comparison of the measured concentration of PFOS using CIC to the amount fortified revealed an average recovery of 69% ± 2% (± standard deviation), which is excellent considering that no internal standard is used for this procedure. This value was used for recovery-correction of all EOF concentrations.

For comparison of sum PFAS concentrations to EOF and TF, concentrations of target PFASs were converted to their corresponding concentration in fluorine equivalents ($C_{F,PFAS}$) according to eqn (1):

\[
C_{F,PFAS} = C_{PFAS} \cdot n_F \cdot A_F / MW_{PFAS}
\]

where $C_{PFAS}$ is the concentration of the target compound, $n_F$ is the number of fluorine atoms in the target compound, $A_F$ is the atomic weight of fluorine (g/mol), and $MW_{PFAS}$ is the molecular weight of the target compound. The sum of known extractable fluorine concentration ($\Sigma C_{F,PFAS}$) was calculated by summing the fluorine concentrations from all individual PFASs. Values <LOQ were set to 0 for calculating $\Sigma C_{F,PFAS}$. EOF concentrations ($C_{F,EOF}$) were corrected using the average PFOS recovery, obtained from spike/recovery experiments. Correction for analyte-specific recoveries would presumably give more accurate results, but this is impossible for unknown PFASs or PFASs lacking standards which contribute to the EOF. Another option is to extract the samples without using ISs, split the final extract and analyze this in both target and total fluorine...
analysis, adding IS to the fraction for targeted analysis only.\textsuperscript{45} Although this approach leads to inaccuracies in the targeted data (since these data would be uncorrected for procedural losses), an additional extraction for targeted analysis with ISs could be included, assuming sufficient sample availability. Overall, correcting the EOF data using PFOS recoveries is reasonable in this case given that a) PFOS is the predominant PFAS in most samples, b) PFOS recoveries are generally representative of recoveries for most perfluoroalkyl acids, and c) targeted results were not compromised using this approach.

Statistical comparisons of $\Sigma C_{F \text{PFAS}}$ and $C_{F \text{EOF}}$ were done with 1-tailed T-tests with unequal variances, assuming that $\Sigma C_{F \text{PFAS}}$ can only be less than or equal to the $C_{F \text{EOF}}$ concentrations. In cases where the $C_{F \text{EOF}}$ appeared to be lower than $\Sigma C_{F \text{PFAS}}$, the fluorine balance was considered closed.

\textit{Suspect screening}

Suspect screening was carried out using a Dionex Ultimate 3000 liquid chromatograph coupled to a Q Exactive HF Orbitrap (Thermo Scientific), based on a previously described method.\textsuperscript{46} Instrumental parameters are provided in the SI. The instrument was run in negative ion, full scan (200-1200 m/z) data dependent acquisition (DDA) MS/MS mode based on an inclusion list derived from a combination of online databases (abbreviated here as EPA,\textsuperscript{47} KemI,\textsuperscript{48} OECD,\textsuperscript{49} and Trier\textsuperscript{50}), literature,\textsuperscript{38,40,51–54} and features identified from PFAS homologue series mining (details below) during pre-screening experiments. The resolution was set to 120 000 (15 000 for MS/MS) and the automatic gain control (AGC) was set to 3e6. Other instrumental parameters are presented in Table S10. Data processing was carried out using Xcalibur 3.1 and Compound Discoverer 3.1\textsuperscript{®} (Thermo Scientific). The workflow included peak retention time alignment, peak integration (using a mass
tolerance of 5 ppm, a minimum signal to noise (S/N) ratio of 30 and a minimum peak intensity of 1e6), grouping and gap-filling (peak integration at S/N = 10 for peaks detected at S/N = 30 in at least one sample). Blank subtraction was carried out by removing all peaks with areas less than 3 times the average peak area in the method blank.

A total of 17973 features remained following data pre-processing. These features were subjected to homologue series mining using the R-package ‘nontarget’ which was used to screen exact masses for homologue series differing by -\( \text{CF}_2^- \) (49.9 Da) and -\( \text{C}_2\text{F}_4^- \) (99.9 Da) fragments, which are characteristic for PFASs. Each homologue series was then checked manually in the extracted ion chromatogram (EIC) for good peak shapes and an increasing retention time with mass-to-charge. At this point, in-source fragments were removed by comparing retention times, exact mass, and MS/MS spectra (if available). The resulting list of exact masses and their MS/MS spectra were annotated through comparison to databases and/or literature. In one case, MS/MS spectra were predicted using the in silico fragmentation predictor MetFrag. Confidence levels (CLs) were assigned according to Schymanski et al. (see SI for details).

RESULTS AND DISCUSSION

Overview of PFAS concentrations in marine mammals

Of the 36 target PFASs analyzed in the present work, 20 were quantifiable in one or more samples: PFHpA, PFOA (L), PFNA, PFDA, PFUnDA, PFDODA, PFTrDA, PFTeDA, PFPeDA, PFHxDA, PFBS, PFHxS (L+Br), PFHpS, PFOS (L+Br), PFDS (L+Br), FOSA (L+Br), 9Cl-PF3OUDS, 5:3 FTCA, 7:3 FTCA, and 6:2 FTSA. Peaks were also observable for FOSAA (L), MeFOSAA (L), EtFOSAA (L), and 11Cl-PF3OUDS, but concentrations were always <LOQ. PFBA, PFPeA, PFHxA, PFOA (Br), PFOcDA, PFPeS, PFNS, PFUnDS, FOSAA (Br), MeFOSAA (Br),
EtFOSAA (Br), ADONA, HFPO-DA, 3:3 FTCA, 4:2 FTSA, and 8:2 FTSA were all below quantification limits in all samples. Both concentrations and PFAS profiles varied widely among species, sampling location, and sampling year (Figure 1). The highest sum PFAS concentrations (i.e. Σ_{36} PFAS) among all species were observed in polar bears (3600-4000 ng/g), which were an order of magnitude higher than most other marine mammals (Figure 1). As apex predators, polar bears are among the most chemically contaminated species on the planet. The three most predominant compounds in polar bears were PFOS, 7:3 FTCA and PFNA, which made up 45-51%, 23-28% and 9-13% of the Σ_{36} PFAS, respectively. 7:3 FTCA has not been reported in polar bears before and it is therefore particularly surprising that this compound makes up such a large fraction of the total PFAS concentration. Σ_{36} PFAS profiles were very similar between all polar bears, Σ_{36} PFAS concentrations were only slightly higher for the female polar bear compared to her cub, which is concerning due to health risks associated with chemical exposure at this early developmental stage.

In cetacean liver samples, the highest Σ_{36} PFAS concentrations were observed in killer whales from East Greenland (614 ± 49 ng/g, ww), while in seals the highest Σ_{36} PFAS concentrations were detected in harbor seals (640 ± 51 ng/g, ww) and ringed seals (536 ± 43 ng/g, ww) from Sweden. PFOS dominated the Σ_{36} PFAS fraction in samples from all locations, except for samples from the US Atlantic coast, where 7:3 FTCA was dominant. For harbor seal and harbor porpoise from the US Atlantic coast, 7:3 FTCA accounted for up to 64 and 71% of Σ_{36} PFAS concentrations, respectively, which may indicate that these animals were located in closer proximity to the source(s) of 7:3 FTCA. Seals from Iceland contained low Σ_{36} PFAS levels compared to the other samples, i.e. 23, 43, and 67 ng/g for grey seal, harp seal, and harbor seal, respectively.
Figure 1. (A) Sum of targeted PFASs (note the separate concentration axis for polar bears) and (B) normalized concentrations for marine mammals sorted according to their sampling location. * = pooled samples (n=2-10). Detailed sample information is available in Table S2.
Inter-species and geographical differences in PFCA distribution

The distribution of PFCA homologues is shown in Figure 2. Among all samples, a characteristic odd/even chain length pattern was observed, wherein the concentration of a given odd chain-length PFCA in most cases exceeds the concentration of its adjacent even chain-length homologues (i.e. PFNA exceeds PFOA and PFDA, PFUnDA exceeds PFDA and PFDoDA, etc). This pattern has been widely reported in the literature,\textsuperscript{41,59–61} and is suggested to occur due to atmospheric oxidation of fluorotelomer alcohols (FTOHs) to corresponding even- and odd-chain length PFCAs, followed by preferential bioaccumulation of the odd (i.e. longer) chain-length homologue.\textsuperscript{62} Despite this consistent pattern, the overall distribution of PFCA homologues was remarkably different among species. Species-specific metabolism may explain these differences.\textsuperscript{63} For example, the dominant PFCA in polar bears from East Greenland was PFNA (C9), while PFUnDA (C11) was dominant in cetaceans (except for the pygmy sperm whale) from Greenland, the US, and Sweden. In comparison, the dominant PFCA in pygmy sperm whale was PFPeDA (C15; 28.0 ng/g, ww). The unique profile in pygmy sperm (\(n=1\)) whale was not explainable by differences in sampling year amongst cetaceans. While C15 has not been quantified in pygmy sperm whales before, long-chain PFCAs (specifically PFTrDA (C13)) were previously reported to make up a large fraction of the total PFAS concentration in pygmy sperm whales.\textsuperscript{64,65} Diet may partly explain this unique pattern, since pygmy sperm whales were one of the few species investigated here (in addition to white-beaked dolphin) that feed offshore on small fish, squid, octopus, and other invertebrates.\textsuperscript{66} However, we cannot be sure that the pattern is representative for the species, since the liver of only one pygmy sperm whale was analyzed. For seals, the PFCA distribution varied among sampling locations, suggesting geographical differences in exposure source (Figure 2). In seals from Sweden (both Baltic Sea and west-coast Skagerrak/Kattegat straits) the most prevalent PFCA homologue
was PFNA (C9), whereas for seals from the Atlantic Ocean (i.e. US, Greenland, Iceland), PFUnDA (C11) represented the highest fraction. These differences (which were not explainable by differences in sampling year), point to a common source of exposure in seals from the US, Greenland, and Iceland that is unique relative to that of the Baltic Sea and Skagerrak/Kattegat straits.

**Figure 2.** Average percent contribution of PFCAs (C8-C15) to \( \Sigma \text{PFCA} \) concentrations (error bars represent standard deviation) in polar bears, seals (grouped by locations with similar patterns), and cetaceans (Pygmy sperm whale and other cetaceans from Sweden/US/Greenland).
Inter-species differences in FOSA concentrations

FOSA:PFOS ratios were generally much higher for cetaceans (0.01-1.28; average 0.33), compared to other marine mammals (0-0.14; average 0.02). The exception was for harbor porpoises, which contained consistently lower FOSA:PFOS ratios (0.02-0.04; average 0.03). Previous studies have observed similar results, with Galatius et al.\(^6\) hypothesizing that smaller cetacean species (i.e. harbor porpoises) might have a higher capacity for transformation. FOSA is the most commonly observed PFOS precursor in wildlife. While FOSA usually occurs at lower concentrations than PFOS, a review of the current literature (see Figure S4) revealed that FOSA:PFOS ratios are higher in cetaceans (0.2-1.0) compared to other marine mammals (ratio <0.005; Figure S4).\(^6\)–\(^7\) This unique pattern is attributed to a phylogenetic difference in the ability of cetacean species to transform FOSA to PFOS.\(^6\)

Elevated concentrations of 7:3 FTCA

The 7:3 FTCA was the second most prevalent PFAS (next to PFOS), and is reported here for the first time in cetaceans and polar bears. FTCAs are not used in consumer products or industrial applications,\(^7\) but may form from biodegradation of fluorotelomer alcohols.\(^7\) 7:3 FTCA has been observed previously in biological samples such as birds (16.2 ng/g, ww in water birds and 0.01-0.84 ng/g, dw in eagle-owl feathers),\(^7\) fish (0.07-0.21 ng/g, ww),\(^7\) human whole blood (from technicians working with ski wax; 3.9 ng/ml)\(^7\) and breast milk (<42 pg/ml),\(^4\) and seals (0.5-2.5 ng/g, ww).\(^7\) However, concentrations are typically much lower than those observed in the present study (e.g. polar bear mother: 1131 ng/g, ww and harbor seal: 192 ng/g, ww). Suspect screening also revealed the presence of other X:3 FTCA homologues (see section on non-targeted and
suspect screening). The origin of FTCAs in marine mammals remains unclear and requires further investigation.

Fluorine mass balance

An overview of the fluorine mass balance including the sum target PFAS ($\Sigma C_{F\_PFAS}$), EOF ($C_{F\_EOF}$), and TF ($C_{F\_TF}$) concentrations is presented in Figure 3. A total of seven out of 25 samples displayed significantly (i.e. $p<0.05$ or $p<0.1$) higher $C_{F\_EOF}$ compared to $\Sigma C_{F\_PFAS}$ concentrations (Figure 3A). This included the pooled polar bear sample from East Greenland from 2012 (32% unidentified EOF); pooled East Greenland killer whale from 2013 (35% unidentified EOF); pooled ringed seal from Sweden from 2015 (45% unidentified EOF); and finally, the pooled harbor porpoise, pooled grey seal, pooled harbor seal, and the pygmy sperm whale (all sampled 2000-2012) from the US Atlantic coast (30-75% unidentified EOF). These results show that exposure of these species to organofluorine is indeed underestimated in some cases. Animals sampled from the US Atlantic coast contained the largest fraction of unidentified EOF, which may indicate that these animals are closer to the source(s) of unidentified organofluorine. Notable, however, is the fact that the US samples also tended to be older than those sampled at other sites. $C_{F\_EOF}$ and $\Sigma C_{F\_PFAS}$ concentrations were not significantly different in 9 of the samples, indicating a closed EOF mass balance. Another 9 samples displayed slightly lower $C_{F\_EOF}$ than their respective $\Sigma C_{F\_PFAS}$ concentrations, likely caused by under-reporting of $C_{F\_EOF}$ due to recovery-correction using PFOS (see methods section). While we considered the EOF mass balance to be closed for these samples, the source of this under-reporting requires further investigation. TF concentrations were consistently higher than EOF and target PFASs for all samples, which may be attributed to the presence of inorganic and/or- non-extractable organic fluorine in the tissues. Overall, percentage of unknown TF ranged from 10-93% (average 58%).
Sum target PFAS concentrations, EOF and TF were natural log (ln)-linearly correlated with one another (Figure 4; p<0.001; R² 0.58-0.77), which can be expected since the organofluorine mass balance was closed or nearly closed in most samples. The unidentified fraction of the EOF could consist of novel PFASs, metabolites and/or transformation products of PFASs. Fluorinated pharmaceuticals and/or pesticides may also accumulate in marine mammals, but given their low percentage of fluorine (i.e. these substances typically only contain a few fluorine atoms), they are not expected to make a significant contribution to EOF or TF concentrations unless they are present in very high abundance. Trifluoroacetic acid (TFA) was also considered since it occurs naturally in sea water at high concentrations (up to 17-190 ng/L in the Northern Atlantic) and is ubiquitous throughout the entire aquatic environment. However, this was ultimately ruled out since TFA is non-bioaccumulative and therefore not expected to occur in marine mammals.
Figure 3. (A) Sum target PFAS and unidentified extractable organofluorine (EOF) concentrations in ng F/g, ww. Significantly higher EOF concentrations are denoted by asterisks (*p<0.1; **p<0.05, 1-sided T-test, unequal variance). (B) Concentrations of target PFASs, EOF, and total fluorine (TF) in ng F/g, ww. Error bars indicate the standard deviation. Note the separate concentration axis for polar bears. • = pooled samples (n=2-10). Detailed sample information is available in Table S2.
Figure 4. Natural log (ln)-linear correlations between sum target PFAS, EOF and TF concentrations. Data <LOQ were excluded. P-values were < 0.001 in all cases.

PFAS suspect screening

Figure 5 summarizes the PFASs that were identified via suspect screening along with the relative abundance of each suspect in individual samples. Classes 1-7 (PFCAs, PFSAs, FTCAs, FTSAs, FASAs, FASAAs, an Cl-PFESAs) were present in our target list, but additional homologues from some of these classes were identified through homologue series mining. Classes 8-11 (PFECAs, d/c PFSAs, ether PFSAs, and enol-ether/cyclic ether or carbonyl PFSAs) were identified by matching exact masses (and MS/MS fragments when available) to those in literature. Finally, class 12 was flagged through homologue series mining; thereafter we attempted structural elucidation through database matching and comparison of MS/MS spectra to in silico fragmentation predictions.

Among the FTCAs, 5 additional homologues were detected that were not present in our target list (i.e. 6:3 and 8:3 – 11:3 FTCAs; < 2 ppm mass error). These substances displayed a similar fragmentation pattern to target FTCAs; thus a high degree of confidence (CL=2a) is ascribed to
their assignment, despite an absence of standards (Figure S5). 5:3, 6:3, and 7:3 FTCAs showed highest abundancies in polar bears, while 8:3-11:3 FTCAs showed highest abundancy in harbor porpoise and ringed seals from the US, when comparing peak areas to other samples. All three samples from the US contained significant quantities of unidentified EOF. We posit that quantification of the full suite of FTCA homologues may account for a large portion of the missing EOF in these samples.

10:2 and 12:2 FTSAs (class 4) and C4-C7 FASAs (class 5) were not included in our target list and were identified through a combination of homologue series mining and by comparing their MS/MS fragments to those homologues for which standards were available (i.e. 6:2 and 8:2 FTSAs, and FOSA; see Figures S6-7). Notably, the peak area of 10:2 FTS was elevated in all polar bear samples and the US harbor seal sample compared to other samples, suggesting that this target may contribute to the missing EOF observed in these samples. Among FASA homologues, perfluorobutane sulfonamide (FBSA) is particularly notable as this substance is a degradation product of a wide range of substances derived from perfluorobutanesulfonyl fluoride, which replaced PFOS-precursors in the early 2000s. FBSA was present mainly in cetaceans and in all animals from Sweden. FBSA has previously been reported in several fish species in Canada and The Netherlands and one study even reported FBSA in polar bear liver at concentrations of 0.4 ng/g ww.

Perfluoroalkyl ether carboxylates (PFECAs; class 8, C8-11) were identified by matching the exact mass of multiple homologues to those reported previously in water, and particulate matter. While C3-C8 and C10-C15 PFECAs have been reported previously, to the best of our knowledge this is the first report of C9 PFeca homologue in the environment. Similarly, a homologue series of double bond or cyclic PFSAs (d/c PFSAs; class 9, C8-C10) were identified
by first matching the parent mass and MS/MS spectrum for perfluoroethylcyclohexanesulfonate (PFECHS; C8; Figure S10) to those reported previously in polar bear serum.\textsuperscript{40} Notably, PFECHS was prevalent in both ringed seals and harbor seals from Sweden relative to other samples, the former of which was found to have a significant quantity of missing EOF.

MS/MS data was not available for either C6-C9 ether-PFSAs (class 10) and C7-C9 enol-ether/cyclic-ether/carbonyl PFSAs (class 11) due to low peak intensities. Therefore, tentative identification (i.e. CL=3-4) was carried out by matching the exact mass of the precursor ions to those reported previously in polar bear serum.\textsuperscript{40} For class 11, peaks for the C10 homologue eluted both at retention time 5.03 and 5.55 suggesting a mixture of structures (e.g. both an enol ethers and a cyclic ether).

Finally, one of the compounds of the “unknown” class (class 12; \( \text{C}_n\text{F}_{2n+1}\text{H}_{10-5}\text{SO}_4\text{N} \)) was originally matched with a methyl ester structure listed in both the OECD and KemI lists (CAS# 87988-69-0; mass error = 0.456 ppm). However, methyl esters are generally non-detectable by ESI-MS so this structure was ruled out.\textsuperscript{86} Alternatively, this substance may be an isomer or in-source fragment of a neutral compound. This feature displayed the highest peak areas in the harbor porpoise and pygmy sperm whale from the US (which had a large fraction of unidentified EOF). Ultimately, confirming the identity of this substance and quantifying it is necessary to assess how much it contributed to the unidentified EOF fraction.

Overall, an additional 33 PFASs were identified through our suspect screening workflow, which were not included in the targeted analysis, bringing the total number of substances detected at a CL of 1-4 to 59 substances from 12 different PFAS classes (not including isomers). We note that the highest peak areas for suspects were not always in samples containing significant unknown
EOF. This should not be surprising, considering that EOF measurements are based on fluorine equivalents, rather than molecular weight-based concentrations, and because the contribution to EOF from a few dominant substances (e.g. PFOS) may dwarf that of some important novel PFAS. Thus, while EOF remains an important tool for prioritizing samples for closer scrutiny; suspect screening (and ultimately quantification) of novel PFASs is clearly needed to obtain a complete picture of PFAS exposure in wildlife.
Figure 5. Heatmap showing relative abundance of PFASs identified by suspect screening across all samples. Data are normalized row-wise based on the maximum response observed across all samples for a given substance. Green indicates high abundance, red indicates low abundance. Pink shading indicates suspects identified by manual inspection of the data. Bold font indicates samples where a significant gap in EOF was identified.
ACKNOWLEDGMENT

The Danish Cooperation for Environment in the Arctic (DANCEA) is acknowledged for financial support and local subsistence hunters are for collection of East Greenland samples, respectively. BONUS BALTHEALTH that has received funding from BONUS (Art. 185), funded jointly by the EU, Innovation Fund Denmark (grants 6180-00001B and 6180-00002B), Forschungszentrum Jülich GmbH, German Federal Ministry of Education and Research (grant FKZ 03F0767A), Academy of Finland (grant 311966) and Swedish Foundation for Strategic Environmental Research (MISTRA) is acknowledged for support for the Baltic sampling and support for the work of BONUS BALTHEALTH collaborators engaged in the present study.

SUPPORTING INFORMATION

Further information on chemicals and reagents, sample preparation, instrumental analysis, along with results of spike/recovery experiments (targeted and CIC analysis), literature review of FOSA:PFOS ratios, EICs for suspects, detailed sampling information, CITES permits, target PFASs, LC mobile phase gradient, MS and RTs for target PFASs, LOQs, NIST results, eluent programs for CIC analysis, HRMS parameters.
REFERENCES

(1) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; Voogt, P. De; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. J. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integr. Environ. Assess. Manag.* **2011**, 7 (4), 513–541. https://doi.org/10.1002/ieam.258.

(2) OECD. Toward a New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs). *Ser. Risk Manag.* **2018**, No. 39, 1–24.

(3) Kissa, E. *Fluorinated Surfactants and Repellents*, Second Edi.; CRC Press: New York (NY), 2001; Vol. 97.

(4) Kemikalieinspektionen. *Kemikalier i Textilier*. **2014**.

(5) Jahnke, A.; Berger, U. Trace Analysis of Per- and Polyfluorinated Alkyl Substances in Various Matrices-How Do Current Methods Perform? *J. Chromatogr. A* **2009**, 1216 (3), 410–421. https://doi.org/10.1016/j.chroma.2008.08.098.

(6) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Dietary Accumulation of Perfluorinated Acids in Juvenile Rainbow Trout (*Oncorhynchus Mykiss*). *Environ. Toxicol. Chem.* **2003**, 22 (1), 189–195. https://doi.org/10.1002/etc.5620220125.

(7) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Bioconcentration and Tissue Distribution of Perfluorinated Acids in Rainbow Trout (*Oncorhynchus Mykiss*). *Environ. Toxicol. Chem.* **2003**, 22 (1), 196–204. https://doi.org/10.1002/etc.5620220126.

(8) Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C. Are PFCAs
Bioaccumulative? A Critical Review and Comparison with Regulatory Criteria and Persistent Lipophilic Compounds. *Environ. Sci. Technol.* **2008**, *42* (4), 995–1003. https://doi.org/10.1021/es070895g.

(9) Giesy, J. P.; Kannan, K. Global Distribution of Perfluorooctane Sulfonate in Wildlife. *Environ. Sci. Technol.* **2001**, *35* (7), 1339–1342. https://doi.org/10.1021/es001834k.

(10) Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G. Biological Monitoring of Polyfluoroalkyl Substances: A Review. *Environ. Sci. Technol.* **2006**, *40* (11), 3463–3473. https://doi.org/10.1021/es052580b.

(11) Houde, M.; De Silva, A. O.; Muir, D. C. G.; Letcher, R. J. Monitoring of Perfluorinated Compounds in Aquatic Biota: An Updated Review. *Environ. Sci. Technol.* **2011**, *45* (19), 7962–7973. https://doi.org/10.1021/es104326w.

(12) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of Perfluorinated Fatty Acids to Serum Proteins. *Environ. Toxicol. Chem.* **2003**, *22* (11), 2639–2649.

(13) Lau, C.; Butenhoff, J. L.; Rogers, J. M. The Developmental Toxicity of Perfluoroalkyl Acids and Their Derivatives. *Toxicol. Appl. Pharmacol.* **2004**, *198* (2), 231–241. https://doi.org/10.1016/j.taap.2003.11.031.

(14) Apelberg, B. J.; Goldman, L. R.; Calafat, A. M.; Herbstman, J. B.; Kuklenyik, Z.; Heidler, J.; Needham, L. L.; Halden, R. U.; Witter, F. R. Determinants of Fetal Exposure to Polyfluoroalkyl Compounds in Baltimore, Maryland. *Environ. Sci. Technol.* **2007**, *41* (11), 3891–3897. https://doi.org/10.1021/es0700911.
(15) Loveless, S. E.; Hoban, D.; Sykes, G.; Frame, S. R.; Everds, N. E. Evaluation of the Immune System in Rats and Mice Administered Linear Ammonium Perfluorooctanoate. *Toxicol. Sci.* **2008**, *105* (1), 86–96. https://doi.org/10.1093/toxsci/kfn113.

(16) Sunderland, E. M.; Hu, X. C.; Dassuncao, C.; Tokranov, A. K.; Wagner, C. C.; Allen, J. G. A Review of the Pathways of Human Exposure to Poly- and Perfluoroalkyl Substances (PFASs) and Present Understanding of Health Effects. *J. Expo. Sci. Environ. Epidemiol.* **2019**, *29* (2), 131–147. https://doi.org/10.1038/s41370-018-0094-1.

(17) Dallaire, R.; Dewailly, É.; Pereg, D.; Dery, S.; Ayotte, P. Thyroid Function and Plasma Concentrations of Polyhalogenated Compounds in Inuit Adults. *Environ. Health Perspect.* **2009**, *117* (9), 1380–1386. https://doi.org/10.1289/ehp.0900633.

(18) Weiss, J. M.; Andersson, P. L.; Lamoree, M. H.; Leonards, P. E. G.; Van Leeuwen, S. P. J.; Hamers, T. Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin. *Toxicol. Sci.* **2009**, *109* (2), 206–216. https://doi.org/10.1093/toxsci/kfp055.

(19) Ji, K.; Kim, S.; Kho, Y.; Paek, D.; Sakong, J.; Ha, J.; Kim, S.; Choi, K. Serum Concentrations of Major Perfluorinated Compounds among the General Population in Korea: Dietary Sources and Potential Impact on Thyroid Hormones. *Environ. Int.* **2012**, *45* (1), 78–85. https://doi.org/10.1016/j.envint.2012.03.007.

(20) Nelson, J. W.; Hatch, E. E.; Webster, T. F. Exposure to Polyfluoroalkyl Chemicals and Cholesterol, Body Weight, and Insulin Resistance in the General U.S. Population. *Environ. Health Perspect.* **2010**, *118* (2), 197–202. https://doi.org/10.1289/ehp.0901165.
(21) Environment and Climate Change Canada - PFCAs and their precursors in perfluorinated
products  https://www.ec.gc.ca/epe-epa/default.asp?lang=En&n=AE06B51E-1  (accessed
Oct 1, 2019).

(22) United States Environmental Protection Agency, Fact Sheet: 2010/2015 PFOA Stewardship
Program  https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-
20102015-pfoa-stewardship-program (accessed Oct 1, 2019).

(23) Stockholm Convention on Persistent Organic Pollutants
http://www.pops.int/TheConvention/Overview/tabid/3351/Default.aspx (accessed Oct 1, 2019).

(24) Gebbink, W. A.; Van Asseldonk, L.; Van Leeuwen, S. P. J. Presence of Emerging Per- and
Polyfluoroalkyl Substances (PFASs) in River and Drinking Water near a Fluorochemical
Production Plant in the Netherlands. Environ. Sci. Technol. 2017, 51 (19), 11057–11065.
https://doi.org/10.1021/acs.est.7b02488.

(25) Newton, S.; McMahen, R.; Stoeckel, J. A.; Chislock, M.; Lindstrom, A.; Strynar, M. Novel
Polyfluorinated Compounds Identified Using High Resolution Mass Spectrometry
Downstream of Manufacturing Facilities near Decatur, Alabama. Environ. Sci. Technol. 2017, 51 (3), 1544–1552. https://doi.org/10.1021/acs.est.6b05330.

(26) Miyake, Y.; Yamashita, N.; Rostkowski, P.; So, M. K.; Taniyasu, S.; Lam, P. K. S.; Kannan,
K. Determination of Trace Levels of Total Fluorine in Water Using Combustion Ion
Chromatography for Fluorine: A Mass Balance Approach to Determine Individual
Perfluorinated Chemicals in Water. J. Chromatogr. A 2007, 1143 (1–2), 98–104.
(27) Wang, P.; Wang, T.; Giesy, J. P.; Lu, Y. Perfluorinated Compounds in Soils from Liaodong Bay with Concentrated Fluorine Industry Parks in China. *Chemosphere* 2013, 91 (6), 751–757. https://doi.org/10.1016/j.chemosphere.2013.02.017.

(28) Yeung, L. W. Y.; De Silva, A. O.; Loi, E. I. H.; Marvin, C. H.; Taniyasu, S.; Yamashita, N.; Mabury, S. A.; Muir, D. C. G.; Lam, P. K. S. Perfluoroalkyl Substances and Extractable Organic Fluorine in Surface Sediments and Cores from Lake Ontario. *Environ. Int.* 2013, 59 (2013), 389–397. https://doi.org/10.1016/j.envint.2013.06.026.

(29) Tan, B.; Wang, T.; Wang, P.; Luo, W.; Lu, Y.; Romesh, K. Y.; Giesy, J. P. Perfluoroalkyl Substances in Soils around the Nepali Koshi River: Levels, Distribution, and Mass Balance. *Environ. Sci. Pollut. Res.* 2014, 21 (15), 9201–9211. https://doi.org/10.1007/s11356-014-2835-6.

(30) Schultes, L.; Vestergren, R.; Volkova Hellström, K.; Westberg, E.; Jacobson, T.; Benskin, J. P. Per- and Polyfluoroalkyl Substances and Fluorine Mass Balance in Cosmetic Products from the Swedish Market: Implications for Environmental Emissions and Human Exposure. *Environ. Sci. Process. Impacts* 2018. https://doi.org/10.1039/C8EM00368H.

(31) Weiner, B.; Yeung, L. W. Y.; Marchington, E. B.; D’Agostino, L. A.; Mabury, S. A. Organic Fluorine Content in Aqueous Film Forming Foams (AFFFs) and Biodegradation of the Foam Component 6:2 Fluorotelomermercaptoalkylamido Sulfonate (6:2 FTSAS). *Environ. Chem.* 2013, 10 (6), 486–493. https://doi.org/10.1071/EN13128.

(32) Yeung, L. W. Y.; Miyake, Y.; Taniyasu, S.; Wang, Y.; Yu, H.; So, M. K.; Wu, Y.; Li, J.;
Giesy, J. P.; Yamashita, N.; et al. Perfluorinated Compounds and Total and Extractable Organic Fluorine in Human Blood Samples from China. *Environ. Pollut.* **2008**, *42* (21), 8140–8145. https://doi.org/10.1021/es800631n.

(33) Yeung, L. W. Y.; Yamashita, N.; Taniyasu, S.; Lam, P. K. S.; Sinha, R. K.; Borole, D. V.; Kannan, K. A Survey of Perfluorinated Compounds in Surface Water and Biota Including Dolphins from the Ganges River and in Other Waterbodies in India. *Chemosphere* **2009**, *76* (1), 55–62. https://doi.org/10.1016/j.chemosphere.2009.02.055.

(34) Loi, E. I. H.; Yeung, L. W. Y.; Taniyasu, S.; Lam, P. K. S.; Kannan, K.; Yamashita, N. Trophic Magnification of Poly- and Perfluorinated Compounds in a Subtropical Food Web. *Environ. Sci. Technol.* **2011**, *45* (13), 5506–5513. https://doi.org/10.1021/es200432n.

(35) Place, B. J.; Field, J. A. Identification of Novel Fluorochemicals in Aqueous Film-Forming Foams Used by the US Military. *Environ. Sci. Technol.* **2012**, *46* (13), 7120–7127. https://doi.org/10.1021/es301465n.

(36) D’Agostino, L. A.; Mabury, S. A. Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates. *Environ. Sci. Technol.* **2014**, *48* (1), 121–129. https://doi.org/10.1021/es403729e.

(37) Barzen-Hanson, K. A.; Roberts, S. C.; Choyke, S.; Oetjen, K.; McAlees, A.; Riddell, N.; McCrindle, R.; Ferguson, P. L.; Higgins, C. P.; Field, J. A. Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater. *Environ. Sci. Technol.* **2017**, *51* (4), 2047–2057. https://doi.org/10.1021/acs.est.6b05843.
(38) Wang, Y.; Yu, N.; Zhu, X.; Guo, H.; Jiang, J.; Wang, X.; Shi, W.; Wu, J.; Yu, H.; Wei, S. Suspect and Nontarget Screening of Per- and Polyfluoroalkyl Substances in Wastewater from a Fluorochemical Manufacturing Park. *Environ. Sci. Technol.* **2018**, *52* (19), 11007–11016. https://doi.org/10.1021/acs.est.8b03030.

(39) Liu, Y.; Pereira, A. D. S.; Martin, J. W. Discovery of C5-C17 Poly- and Perfluoroalkyl Substances in Water by in-Line Spe-HPLC-Orbitrap with in-Source Fragmentation Flagging. *Anal. Chem.* **2015**, *87* (8), 4260–4268. https://doi.org/10.1021/acs.analchem.5b00039.

(40) Liu, Y.; Richardson, E. S.; Derocher, A. E.; Lunn, N. J.; Lehmler, H.-J.; Li, X.; Zhang, Y.; Cui, J. Y.; Cheng, L.; Martin, J. W. Hundreds of Unrecognized Halogenated Contaminants Discovered in Polar Bear Serum. *Angew. Chemie Int. Ed.* **2018**, *201809906* (Figure 1), 1–7. https://doi.org/10.1002/anie.201809906.

(41) Gebbink, W. A.; Bossi, R.; Rigét, F. F.; Rosing-Asvid, A.; Sonne, C.; Dietz, R. Observation of Emerging Per- and Polyfluoroalkyl Substances (PFASs) in Greenland Marine Mammals. *Chemosphere* **2016**, *144*, 2384–2391. https://doi.org/10.1016/j.chemosphere.2015.10.116.

(42) Powley, C. R.; George, S. W.; Ryan, T. W.; Buck, R. C. Matrix Effect-Free Analytical Methods for Determination of Perfluorinated Carboxylic Acids in Environmental Matrixes. *Anal. Chem.* **2005**, *77* (19), 6353–6358. https://doi.org/10.1021/ac0508090.

(43) Nyberg, E.; Awad, R.; Bignert, A.; Ek, C.; Sallsten, G.; Benskin, J. P. Inter-Individual, Inter-City, and Temporal Trends of per- and Polyfluoroalkyl Substances in Human Milk from Swedish Mothers between 1972 and 2016. *Environ. Sci. Process. Impacts* **2018**, *20*
(8), 1136–1147. https://doi.org/10.1039/c8em00174j.

Schultes, L.; Peaslee, G. F.; Brockman, J. D.; Majumdar, A.; McGuinness, S. R.; Ngwenyama, R. A.; Wilkinson, J. T.; Sandblom, O.; Ngwenyama, R. A.; Benskin, J. P.

Total Fluorine Measurements in Food Packaging: How Do Current Methods Perform?

*Environ. Sci. Technol. Lett.* **2019**, *6*, 73–78. https://doi.org/10.1021/acs.estlett.8b00700.

Kärrman, A.; Wang, T.; Kallenborn, R.; Langseter, A. M.; Grønhovd, S. M.; Ræder, E. M.; Lyche, J. L.; Yeung, L.; Chen, F.; Eriksson, U.; et al. *PFASs in the Nordic Environment*; 2019.

Miaz, L. T. Development and Application of UHPLC-Orbitrap Method for Quantitative and Suspect Screening of PFASs in Human Serum, Master’s thesis, Stockholm University, 2018.

Environmental Protection Agency. Chemistry Dashboard | PFAS: Cross-Agency Research List https://comptox.epa.gov/dashboard/chemical_lists/EPAPFASRL (accessed Oct 1, 2019).

Fischer, S. S14 | KEMIPFAS | PFAS Highly Fluorinated Substances List: KEMI. 2017. https://doi.org/10.5281/ZENODO.2621525.

Wang, Z. S25 | OECDPFAS | List of PFAS from the OECD. *Zenodo* 2018. https://doi.org/10.5281/ZENODO.2648776.

Trier, X.; Lunderberg, D. S9 | PFASTRIER | PFAS Suspect List: Fluorinated Substances. 2015. https://doi.org/10.5281/ZENODO.2621989.
Liu, Y.; Agostino, L. A. D.; Qu, G.; Jiang, G.; Martin, J. W. High-Resolution Mass Spectrometry (HRMS) Methods for Nontarget Discovery and Characterization of Poly- and Perfluoroalkyl Substances (PFASs) in Environmental and Human Samples. *Trends Anal. Chem.* **2019**, No. xxxx. https://doi.org/10.1016/j.trac.2019.02.021.

Yu, N.; Guo, H.; Yang, J.; Jin, L.; Wang, X.; Shi, W.; Zhang, X.; Yu, H.; Wei, S. Non-Target and Suspect Screening of Per- and Polyfluoroalkyl Substances in Airborne Particulate Matter in China. *Environ. Sci. Technol.* **2018**, **52** (15), 8205–8214. https://doi.org/10.1021/acs.est.8b02492.

Liu, Y.; Qian, M.; Ma, X.; Zhu, L.; Martin, J. W. Nontarget Mass Spectrometry Reveals New Perfluoroalkyl Substances in Fish from the Yangtze River and Tangxun Lake, China. *Environ. Sci. Technol.* **2018**, **52** (10), 5830–5840. https://doi.org/10.1021/acs.est.8b00779.

Mejia-Avendaño, S.; Munoz, G.; Vo Duy, S.; Desrosiers, M.; Benolt, P.; Sauvé, S.; Liu, J. Novel Fluoroalkylated Surfactants in Soils Following Firefighting Foam Deployment during the Lac-Mégantic Railway Accident. *Environ. Sci. Technol.* **2017**, **51** (15), 8313–8323. https://doi.org/10.1021/acs.est.7b02028.

Loos, M.; Singer, H. Nontargeted Homologue Series Extraction from Hyphenated High Resolution Mass Spectrometry Data. *J. Cheminform.* **2017**, **9** (1), 1–11. https://doi.org/10.1186/s13321-017-0197-z.

Ruttkies, C.; Schymanski, E. L.; Wolf, S.; Hollender, J.; Neumann, S. MetFrag Relaunched: Incorporating Strategies beyond in Silico Fragmentation. *J. Cheminform.* **2016**, **8** (1), 1–16. https://doi.org/10.1186/s13321-016-0115-9.
(57) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097–2098. https://doi.org/10.1021/es5002105.

(58) Routti, H.; Atwood, T. C.; Bechshoft, T.; Boltunov, A.; Ciesielski, T. M.; Desforges, J.-P.; Dietz, R.; Gabrielsen, G. W.; Jenssen, B. M.; Letcher, R. J.; et al. State of Knowledge on Current Exposure, Fate and Potential Health Effects of Contaminants in Polar Bears from the Circumpolar Arctic. *Sci. Total Environ.* **2019**, *664*, 1063–1083. https://doi.org/10.1016/j.scitotenv.2019.02.030.

(59) Bossi, R.; Dam, M.; Rigét, F. F. Perfluorinated Alkyl Substances (PFAS) in Terrestrial Environments in Greenland and Faroe Islands. *Chemosphere* **2015**, *129* (June 2008), 164–169. https://doi.org/10.1016/j.chemosphere.2014.11.044.

(60) Martin, J. W.; Smithwick, M. M.; Braune, B. M.; Hoekstra, P. F.; Muir, D. C. G.; Mabury, S. A. Identification of Long-Chain Perfluorinated Acids in Biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004**, *38* (2), 373–380. https://doi.org/10.1021/es034727+.

(61) Shaw, S.; Berger, M. L.; Brenner, D.; Tao, L.; Wu, Q.; Kannan, K. Specific Accumulation of Perfluorochemicals in Harbor Seals (Phoca Vitulina Concolor) from the Northwest Atlantic. *Chemosphere* **2009**, *74* (8), 1037–1043. https://doi.org/10.1016/j.chemosphere.2008.10.063.

(62) Ellis, D. A.; Martin, J. W.; De Silva, A. O.; Mabury, S. A.; Hurley, M. D.; Sulbaek Andersen, M. P.; Wallington, T. J. Degradation of Fluorotelomer Alcohols: A Likely
Atmospheric Source of Perfluorinated Carboxylic Acids. *Environ. Sci. Technol.* 2004, 38 (12), 3316–3321. https://doi.org/10.1021/es049860w.

Letcher, R. J.; Chu, S.; McKinney, M. A.; Tomy, G. T.; Sonne, C.; Dietz, R. Comparative Hepatic in Vitro Depletion and Metabolite Formation of Major Perfluorooctane Sulfonate Precursors in Arctic Polar Bear, Beluga Whale, and Ringed Seal. *Chemosphere* 2014, 112, 225–231. https://doi.org/10.1016/j.chemosphere.2014.04.022.

Reiner, J. L.; O’Connell, S. G.; Butt, C. M.; Mabury, S. A.; Small, J. M.; De Silva, A. O.; Muir, D. C. G.; Delinsky, A. D.; Strynar, M. J.; Lindstrom, A. B.; et al. Determination of Perfluorinated Alkyl Acid Concentrations in Biological Standard Reference Materials. *Anal. Bioanal. Chem.* 2012, 404 (9), 2683–2692. https://doi.org/10.1007/s00216-012-5943-5.

Fujii, Y.; Kato, Y.; Kozai, M.; Matsuishi, T.; Harada, K. H.; Koizumi, A.; Kimura, O.; Endo, T.; Haraguchi, K. Different Profiles of Naturally Produced and Anthropogenic Organohalogens in the Livers of Cetaceans from the Sea of Japan and the North Pacific Ocean. *Mar. Pollut. Bull.* 2018, 136 (August), 230–242. https://doi.org/10.1016/j.marpolbul.2018.08.051.

Kannan, K.; Koistinen, J.; Beckmen, K.; Evans, T.; Gorzelany, J. F.; Hansen, K. J.; Jones, P. D.; Helle, E.; Nyman, M.; Giesy, J. P. Accumulation of Perfluorooctane Sulfonate in Marine Mammals. *Environ. Sci. Technol.* 2001, 35 (8), 1593–1598. https://doi.org/10.1021/es001873w.

Galatius, A.; Bossi, R.; Sonne, C.; Rigét, F. F.; Kinze, C. C.; Lockyer, C.; Teilmann, J.;
Dietz, R. PFAS Profiles in Three North Sea Top Predators: Metabolic Differences among Species? *Environ. Sci. Pollut. Res.* **2013**, *20* (11), 8013–8020. https://doi.org/10.1007/s11356-013-1633-x.

Benskin, J. P.; Holt, A.; Martin, J. W. Isomer-Specific Biotransformation Rates of a Perfluorooctane Sulfonate (PFOS)-Precursor by Cytochrome P450 Isozymes and Human Liver Microsomes. *Environ. Sci. Technol.* **2009**, *43* (22), 8566–8572.

D'eon, J. C.; Mabury, S. A. Exploring Indirect Sources of Human Exposure to Perfluoroalkyl Carboxylates (PFCAs): Evaluating Uptake, Elimination, and Biotransformation of Polyfluoroalkyl Phosphate Esters (PAPs) in the Rat. *Environ. Health Perspect.* **2011**, *119* (3), 344–350. https://doi.org/10.1289/ehp.1002409.

Peng, H.; Zhang, S.; Sun, J.; Zhang, Z.; Giesy, J. P.; Hu, J. Isomer-Specific Accumulation of Perfluorooctanesulfonate from (N-Ethyl Perfluorooctanesulfonamido)Ethanol-Based Phosphate Diester in Japanese Medaka (Oryzias Latipes). *Environ. Sci. Technol.* **2014**, *48* (2), 1058–1066. https://doi.org/10.1021/es404867w.

Xu, L.; Krenitsky, D. M.; Seacat, A. M.; Butenhoff, J. L.; Anders, M. W. Biotransformation of N-Ethyl-N-(2-Hydroxyethyl)Perfluorooctanesulfonamide by Rat Liver Microsomes, Cytosol, and Slices and by Expressed Rat and Human Cytochromes P450. *Chem. Res. Toxicol.* **2004**, *17* (6), 767–775. https://doi.org/10.1021/tr034222x.

Eriksson, U.; Haglund, P.; Kärrman, A. Contribution of Precursor Compounds to the Release of Per- and Polyfluoroalkyl Substances (PFAs) from Waste Water Treatment Plants (WWTPs). *J. Environ. Sci.* **2017**, *61*, 80–90.
(73) Wang, N.; Szostek, B.; Buck, R. C.; Folsom, P. W.; Sulecki, L. M.; Gannon, J. T. 8-2
Fluorotelomer Alcohol Aerobic Soil Biodegradation: Pathways, Metabolites, and
Metabolite Yields. Chemosphere 2009, 75 (8), 1089–1096.
https://doi.org/10.1016/j.chemosphere.2009.01.033.

(74) Dahlberg Persson, M. J. Levels of Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs)
in Feathers of Eurasian Eagle-Owls (Bubo Bubo) in Norway, Norwegian University of
Science and Technology, 2017.

(75) Loi, E. I. H.; Yeung, L. W. Y.; Taniyasu, S.; Lam, P. K. S.; Kannan, K.; Yamashita, N.
Trophic Magnification of Poly- and Perfluorinated Compounds in a Subtropical Food Web.
Environ. Sci. Technol. 2011, 45 (13), 5506–5513. https://doi.org/10.1021/es200432n.

(76) Nilsson, H.; Kärrman, A.; Rotander, A.; van Bavel, B.; Lindström, G.; Westberg, H.
Biotransformation of Fluorotelomer Compound to Perfluorocarboxylates in Humans.
Environ. Int. 2013, 51 (2013), 8–12. https://doi.org/10.1016/j.envint.2012.09.001.

(77) Powley, C. R.; George, S. W.; Russell, M. H.; Hoke, R. A.; Buck, R. C. Polyfluorinated
Chemicals in a Spatially and Temporally Integrated Food Web in the Western Arctic.
Chemosphere 2008, 70 (4), 664–672. https://doi.org/10.1016/j.chemosphere.2007.06.067.

(78) Yeung, L. W. Y.; Miyake, Y.; Wang, Y.; Taniyasu, S.; Yamashita, N.; Lam, P. K. S. Total
Fluorine, Extractable Organic Fluorine, Perfluorooctane Sulfonate and Other Related
Fluorochemicals in Liver of Indo-Pacific Humpback Dolphins (Sousa Chinensis) and
Finless Porpoises (Neophocaena Phocaenoides) from South China. Environ. Pollut. 2009,
Scott, B. F.; Macdonald, R. W.; Kannan, K.; Fisk, A.; Witter, A.; Yamashita, N.; Durham, L.; Spencer, C.; Muir, D. C. G. Trifluoroacetate Profiles in the Arctic, Atlantic, and Pacific Oceans. *Environ. Sci. Technol.* **2005**, *39* (17), 6555–6560. https://doi.org/10.1021/es047975u.

Voogt, P. De. *Reviews of Environmental Contamination and Toxicology Perfluorinated Alkylated Substances*; 2010; Vol. 208. https://doi.org/10.1007/978-1-4419-6880-7_4.

Chu, S.; Letcher, R. J. In Vitro Metabolic Formation of Perfluoroalkyl Sulfonamides from Copolymer Surfactants of Pre- and Post-2002 Scotchgard Fabric Protector Products. *Environ. Sci. Technol.* **2014**, *48* (11), 6184–6191. https://doi.org/10.1021/es500169x.

Chu, S.; Letcher, R. J.; McGoldrick, D. J.; Backus, S. M. A New Fluorinated Surfactant Contaminant in Biota: Perfluorobutane Sulfonamide in Several Fish Species. *Environ. Sci. Technol.* **2016**, *50* (2), 669–675. https://doi.org/10.1021/acs.est.5b05058.

Boisvert, G.; Sonne, C.; Rigét, F. F.; Dietz, R.; Letcher, R. J. Bioaccumulation and Biomagnification of Perfluoroalkyl Acids and Precursors in East Greenland Polar Bears and Their Ringed Seal Prey. *Environ. Pollut.* **2019**, *252*, 1335–1343. https://doi.org/10.1016/j.envpol.2019.06.035.

Strynar, M.; Dagnino, S.; McMahan, R.; Liang, S.; Lindstrom, A.; Andersen, E.; McMillan, L.; Thurman, M.; Ferrer, I.; Ball, C. Identification of Novel Perfluoroalkyl Ether Carboxylic Acids (PFECAs) and Sulfonic Acids (PFESAs) in Natural Waters Using Accurate Mass Time-of-Flight Mass Spectrometry (TOFMS). *Environ. Sci. Technol.* **2015**, *49* (19), 11622–
Sun, M.; Arevalo, E.; Strynar, M.; Lindstrom, A.; Richardson, M.; Kearns, B.; Pickett, A.; Smith, C.; Knappe, D. R. U. Legacy and Emerging Perfluoroalkyl Substances Are Important Drinking Water Contaminants in the Cape Fear River Watershed of North Carolina. Environ. Sci. Technol. Lett. 2016, 3 (12), 415–419. https://doi.org/10.1021/acs.estlett.6b00398.

D’eon, J. C.; Mabury, S. A. Exploring Indirect Sources of Human Exposure to Perfluoroalkyl Carboxylates (PFCAs): Evaluating Uptake, Elimination, and Biotransformation of Polyfluoroalkyl Phosphate Esters (PAPs) in the Rat. Environ. Health Perspect. 2011, 119 (3), 344–350. https://doi.org/10.1289/ehp.1002409.
Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere

Supporting Information

Kyra M. Spaan¹ *, Carmen van Noordenburg¹, Merle M. Plassmann¹, Lara Schultes¹, Susan Shaw², Michelle Berger², Mads Peter Heide-Jørgensen³, Aqqalu Rosing-Asvid³, Sandra M. Granquist⁴,⁵, Rune Dietz⁶, Christian Sonne⁶, Frank Rigét⁶, Anna Roos³,⁷, Jonathan P. Benskin¹ *

¹Department of Environmental Science and Analytical Chemistry, Stockholm University, Svante Arrhenius Väg 8, 106 91, Stockholm, Sweden.
²Shaw Institute, P.O. Box 1652, Blue Hill, ME 04614
³Greenland Institute of Natural Resources, Nuuk, Greenland
⁴Marine and Freshwater Research Institute, Skúlagata 4, 101 Reykjavik, Iceland.
⁵The Icelandic Seal Center, Brekkugata 2, 530 Hvammstangi, Iceland
⁶Aarhus University, Department of Bioscience, Arctic Research Centre (ARC), Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark
⁷Department of Environmental Research and Monitoring, Swedish Museum of Natural History, Box 50007, 104 05 Stockholm, Sweden

*Corresponding authors:
Kyra.Spaan@aces.su.se
Jon.Benskin@aces.su.se

Number of Pages: 36
Number of Figures: 13
Number of Tables: 9
Chemicals and Reagents

Methanol (99.8%, LiChrosolv®) and ammonium acetate (98%) were purchased from Merck (Darmstadt, Germany). Acetonitrile (≥99.9%, Chromasolv™) was obtained from Honeywell (France). Water was purified by a millipore water purification system and had a resistance <18 MΩ/cm (Milli-Q water). Fluoride standard (1000 mg/L) was obtained from Thermo Scientific. EnviCarb (Supelclean™) was obtained from Sigma Aldrich. Stainless steel beads (4.8 mm) were purchased from Next Advance©. Certified NIST serum (SRM 1957) was used for quality control. For CIC analysis, argon and oxygen gases were of purity grade 5.0 and the certified reference material (CRM) clay (BCR-461) was purchased from Sigma Aldrich.

Sample preparation

Targeted analysis

The extraction method was based on the method described by Powley et al. (2005) and was performed as follows. For each sample about 0.5 g of liver was thawed at room temperature and 50 μl of internal standard (IS) solution (20 pg/μl) was added to each sample prior to extraction. Extraction was performed by adding 4 ml acetonitrile (ACN) together with 7-8 beads (stainless steel ø 4.8 mm); thereafter the samples were homogenized in a bead blender (SPEX SamplePrep 1600 MiniG®) for 5 min at 1500 rpm. The samples were then centrifuged at 2000 rpm for 5 min (Centrifuge 5810, Eppendorf, Hamburg) and the supernatant was transferred to a new 13 ml PP-tube. The precipitate was extracted one more time by adding another 4 ml ACN, and vortexing, blending, and centrifuging again. The supernatant was added to the existing tube with the previous supernatant. The combined extracts were concentrated to ~1 ml under a stream of nitrogen in a water bath at 40 °C (TurboVap LV Evaporator, Biotage). The concentrated extracts were weighed and added to a 1.7 ml Eppendorf tube containing 25 mg EnviCarb and 50 μl acetic acid. The tubes were vortexed and centrifuged for 10 min at 10 000 rpm (Galaxy 14D, Microcentrifuge, VWR). Then 500 μl of the supernatant were transferred to another Eppendorf tube. To this 50 μl recovery standard (RS) solution (20 pg/μl 13C8-PFOA and 13C8-PFOS) and 500 μl NH₄OAc (4 mM in water) were added and the extracts were stored at -20 °C until analysis. On the day of analysis, the extract was adjusted to room temperature, vortex-mixed and transferred to an LC vial.

Clean-up step test
Two clean-up steps were evaluated for their potential to remove inorganic fluorine and recovery of target analytes: 1) a solid phase extraction (SPE)-based clean-up, and 2) an EnviCarb-based clean-up. The SPE extraction method was based on Miyake et al.\(^2\) and the EnviCarb extraction on Powley et al.\(^1\) Fish muscle samples were spiked with 250 ng PFOS (~162 ng F) and 500 ng NaF. Method blanks showed high concentrations for the SPE clean-up with high variation, while the method blanks for the EnviCarb clean-up step were rather low and consistent. The unspiked samples showed similar concentrations for both methods, however EnviCarb showed a bit higher deviation. PFOS and NaF recoveries were calculated according to the following formula:

\[
\text{Recovery (\%) = } \frac{\text{Measured spike (ng F)} - \text{Measured no spike (ng F)}}{\text{Spiked concentration (ng F)}} \times 100\%
\]

PFOS recovery was high for both methods, i.e. 96% and 92% for SPE and EnviCarb, respectively. Both extraction methods aim to remove inorganic fluorine, such as NaF, and get as low recovery as possible. NaF recovery was 12.5% and -0.2% for SPE and EnviCarb, respectively. Only the EnviCarb method was able to remove the inorganic fluorine effectively. After this extraction method comparison, EnviCarb was found to be the best suitable to use for analysis of the real samples, since this approach resulted in lower method blanks and more efficient removal of inorganic fluorine and was therefore considered the most suitable clean-up method. The results of this comparison experiment are presented in Figure S1.

Total and extractable organofluorine

A similar extraction procedure was applied to the liver samples prior to analysis with the CIC. Since the CIC measures the total fluorine concentration, no internal standards were added, also no NH\(_4\)OAc was added in the end. The final extracts (~ 1 ml) were split into two parts, in order to have a replicate of each sample. Also, since the sample boats have limited sample space, the final split extracts were concentrated to ~200 μl under a stream of nitrogen.

Instrumental Analysis

Targeted analysis

The system was operated in negative ion electrospray ionization (ESI-) mode. The source and desolvation temperatures were set at 150 °C and 350 °C, respectively. The desolvation and cone gas flows (nitrogen) were set at 650 L/h and 150 L/h, respectively. The capillary voltage was set...
Qualification and quantification were carried out using MassLynx 4.1 (Waters). Quantification was performed using internal standards via a 9-point calibration curve ranging from 0.008 to 150 ng/ml (linear, 1/x weighting). Precursor and product ions are presented in Table S7. Analytes lacking an analogous labeled standard were quantified using the IS with the closest retention time and the data quality was defined as semi-quantitative (semiQ). Branched isomers were quantified using the calibration curve of the linear isomer.

**Total and extractable organofluorine analysis**

Measurements of total fluorine (TF) and extractable organofluorine (EOF) were carried out using a Thermo-Mitsubishi CIC using previously described methods. Briefly, extracts (~200 µl for samples and 100 µl for standards) were placed in a ceramic sample boat containing glass wool (for fluid dispersion), while neat liver material (~100 mg) was weighed directly into the sample boat. The samples were combusted slowly in a horizontal furnace (HF-210, Mitsubishi) at 1100 °C under a flow of oxygen (400 ml/min), argon (200 ml/min), and argon mixed with water vapor (100 ml/min) for approximately 5 minutes. Combustion gases were absorbed in MilliQ water during the entire length of the combustion process using a gas absorber unit (GA-210, Mitsubishi). A 200 µl aliquot of the absorption solution was subsequently injected onto an ion chromatograph (Dionex Integrion HPIC, Thermo Fisher Scientific) equipped with an anion exchange column (2 × 50 mm guard column (Dionex IonPac AS19-4µm) and 2 × 250 mm analytical column (Dionex IonPac AS19-4µm) operated at 30 °C. Chromatographic separation was achieved by running a gradient of aqueous hydroxide mobile phase ramping from 8 mM to 100 mM at a flow rate of 0.25 ml/min (Table S8), and fluoride was detected using a conductivity detector. Quantification was carried out using a standard calibration curve prepared at 0.05 to 100 µg F/ml. The calibration curve showed very good linearity with R²>0.98. The mean fluoride concentration in the method blanks was subtracted from the samples. The method detection limit (MDL) was defined as the mean concentration plus three times the standard deviation in the method blanks.

**Suspect screening**

Suspect screening was carried out using a Dionex Ultimate 3000 liquid chromatograph coupled to a Q Exactive HF Orbitrap (Thermo Scientific), based on a previously described method. The flow rate was held constant at 0.4 ml per minute throughout the run. The mobile phases and eluent
program used for non-target/suspect screening were the same as those used for target analysis (i.e.
by UPLC-MS/MS). The instrument was run in negative ion, full scan (200-1200 m/z) data
dependent acquisition (DDA) MS/MS mode (50-1200 m/z). The resolution was set to 120 000 (15
000 for MS/MS), the automatic gain control (AGC) was set to 3e6, and other instrumental
parameters are presented in Table S11. Briefly, CL = 5 is assigned when only the exact mass is
known. CL = 4 is used when the unknown analyte ion can be assigned an unambiguous formula,
but no structural information is available. CL = 3 represents tentative candidates whose possible
structure can be proposed but lack sufficient information to assign an exact structure. CL = 2a
represents probable structures by comparing to library spectra where spectrum-structure is
unambiguous. CL = 2b can be assigned when no standard or literature information is available for
confirmation and there is only diagnostic evidence. Finally, CL =1 represents confirmed structures,
that match a reference standard with MS, MS/MS and RT.

Quality Control

Targeted analysis

Limits of quantification (LOQs) were determined by the lowest calibration concentration that
showed a well-shaped peak with intensity >1e3 and signal-to-noise (S/N) >3. For compounds that
were not present in the calibration standard, but that were detected in the samples (PFPeDA,
PFHpS, and branched isomers), the LOQ from the corresponding standard was used. For
compounds where method blank contamination was observed (PFBS, PFOS, and FOSAA), the
LOQ was determined as the average of the quantified concentrations in the method blanks plus ten
times the standard deviation. The compound-specific LOQs are listed in Figure S10.

Method accuracy and precision for most substances was very good, with percent recoveries
ranging from 73-130% and standard deviations ranging from 3-30% (Figure S3). The exceptions
were for PFHxDA, PFOcDA, 4:2 FTSA, and 8:2 FTSA, which showed very high recoveries
(278%, 397%, 212%, and 227%, respectively), while HFPO-DA, 3:3 FTCA, 5:3 FTCA, and 7:3
FTCA showed very low recoveries (22%, 34%, 55%, and 53%, respectively). These deviating
recoveries are likely due to matrix effects, which were not accounted for because of the absence
of an exactly matching isotopically-labeled internal standard. Nevertheless, the targets with very
high recoveries were included in the analysis, since their concentrations in the samples were so
low (<1 ng/g, ww). The targets with low recoveries were also included in the analysis, albeit measured concentrations may be underreported. Finally, the method was externally validated by analyzing a standard reference material (SRM) sample of NIST serum 1957. Results are presented in Table S9 and were generally in good agreement with certified values and prior measurements of this material by other researchers.

157

**Total and extractable organofluorine**

158 All boats were baked out prior to sample combustion to minimize background contamination. Each run started and ended with a calibration curve and after every 8-10 samples, a blank and a mid-level calibration standard were analyzed for quality control. The removal efficiency of inorganic fluoride was tested by spiking a range of known concentrations of NaF (0.25, 0.5, 0.75, 1, and 2 µg) into liver tissue followed by extraction (Figure S4). Furthermore, recovery of organic fluoride was determined by spiking PFOS (0.08, 0.13, 0.25, 0.5, and 1 µg/ml) to liver tissue and performing the extraction (Figure S4). CIC analysis of both the extracted liver residue and the EnviCarb used for clean-up showed that the inorganic fluoride remained in the extracted liver; in other words, it was not extracted during the initial acetonitrile extraction step. The obtained recovery for PFOS was used to correct the measured concentrations of EOF in real samples. In theory, since the recovery is different for each target analyte, the recovery should be determined for each individual compound. However, practically this would mean a large number of experiments and therefore only the recovery for the most abundant compound, PFOS, was assessed here.
Figure S1. Diagram of the experimental design.
Figure S2. Recovery ± standard deviation (%) of native compounds spiked in seal samples \((n=4)\). Right panel shows severe over-recovery of four targets, attributable to matrix-induced ionization enhancement.
Figure S3. Results from spike/recovery experiments for CIC analysis. Comparison between PFOS- and NaF-spiked samples.
Figure S4. Overview on the FOSA:PFOS ratio for cetaceans and other marine mammals from literature as well as from the present study. a Fujii et al., b Tomy et al., c Kelly et al., d Present study, e Yeung et al., f Gebbink et al., g Shaw et al., h.
Figure S5. Above the EICs for x:3 FTCAs (class 3) observed in harbor seal from the US are shown. MS/MS spectra including the molecular formulas belonging to the most common peaks.
Figure S6. EICs of x:2 FTSA (class 4) in polar bear cub sample. MS/MS spectra from 6:2 FTSA (grey seal from Sweden), 8:2 FTSA (calibration standard), and 10:2 FTSA (polar bear cub) are shown with molecular formulas assigned to the most common peaks.
**Figure S7.** EICs of FASAs (class 5) in minke whale from Greenland. MS/MS spectra for FBSA, FHxSA, and FOSA (harbor porpoise from Sweden) are shown.
Figure S8. EICs for PFECAs (class 8) in polar bear cub sample. No MS/MS spectra were available.
Figure S9. EICs for PFECAs (class 8) in polar bear cub sample. No MS/MS spectra were available.
Figure S10. EICs for d/c PFSAs (class 9), as well as the MS/MS spectrum for 4-PFECHS in harbor seal from Sweden.
Figure S11. EICs of ether-PFSAs (class 10) in polar bear cub sample. No MS/MS spectra were available.
Figure S12. EICs of enol-ether-, cyclic- ether- or carbonyl- PFSAs (class 11) in (A) harbor seal from Sweden and (B) polar bear cub. No MS/MS spectra were available.
Figure S13. EICs for unknowns (class 12) in pygmy sperm whale. MS/MS spectra for four compounds within class 12 with molecular formulas assigned to the most common fragments.
Table S1. Detailed overview on the marine mammals that were assessed in this study.

| Sampled by                  | IDs            | Specie                  | Latin name       | Year | Age     | Sex (M/F) | Location                     | Weight (kg) | Length (cm) | Pooled |
|-----------------------------|----------------|-------------------------|------------------|------|---------|-----------|-------------------------------|-------------|-------------|--------|
| Susan Shaw and Michelle Berger | MH 00670 HG, #7 | Grey seal               | Halichoerus grypus | 2000 | Adult   | M         | Narragansett Bay, RI, US     | 136         | 190         |        |
|                             | MH 01830 HG, #17 | Grey seal               | Halichoerus grypus | 2001 | Pup     | M         | Narragansett Bay, RI, US     | 22.73       | 99          |        |
|                             | NY 308404 HG, #245 | Grey seal              | Halichoerus grypus | 2004 | Pup     | F         | E Long Island, NY, US        | 21.7        | 97.2        |        |
|                             | MH 02637 HG, #314 | Grey seal              | Halichoerus grypus | 2002 | Pup     | F         | S Massachusetts, US          | 18.2        | 96          |        |
|                             | CCSN 02243 HG, #320 | Grey seal              | Halichoerus grypus | 2002 | Subadult | M         | Massachusetts, US            | 136.4       | 181         |        |
|                             | MH 00543F PV, 21 | Harp seal              | Phoca vitulina    | 2000 | Fetus   | F         | Massachusetts Bay, US       | 6.4         | 64          |        |
|                             | COA020730PV, #133 | Harp seal              | Phoca vitulina    | 2002 | Adult   | F         | Midcoast, ME, US            | 54.55       | 140         |        |
|                             | COA060622PV, #285 | Harp seal              | Phoca vitulina    | 2006 | Adult   | M         | Midcoast, ME, US            | 63.6        | 157         |        |
|                             | COA060705PV, #333 | Harp seal              | Phoca vitulina    | 2006 | Pup     | F         | E Maine, US                  | 14.2        | 85          |        |
|                             | COA080717PV-01, #352 | Harp seal          | Phoca vitulina    | 2008 | Yearling | M         | Midcoast, ME, US            | 16.6        | 100         |        |
|                             | COA060619PP, #287 | Harbor porpoise       | Phocoena phocoena | 2006 | Calf   | F         | E Maine, US                  | 9.9         | 81          |        |
|                             | COA060713PP, #289 | Harbor porpoise       | Phocoena phocoena | 2006 | Adult   | F         | Midcoast, ME, US            | 54.5        | 145         |        |
|                             | COA060905PP, #340 | Harbor porpoise       | Phocoena phocoena | 2006 | Calf   | M         | E Maine, US                  | 11.8        | 97          |        |
|                             | COA101005PP, #392 | Harbor porpoise       | Phocoena phocoena | 2010 | Juvenile | M         | E Maine, US                  | 18.2        | 116.5       |        |
|                             | COA121030PP, #410 | Harbor porpoise       | Phocoena phocoena | 2012 | Juvenile | M         | Midcoast, ME, US            | 23.3        | 126.8       |        |
|                             | COA071003KB, #358 | Harbor porpoise       | Phocoena phocoena | 2009 | -      | M         | Maniitsoq, West Greenland   | -           | -           |        |
|                             | COA081030PP, #359 | Ringed seal           | Pusa hispida     | 2013 | -      | -         | Illulisat (North-West Greenland) | -          | -           |        |
|                             | COA101030PP, #359 | Ringed seal           | Pusa hispida     | 2013 | -      | -         | Illulisat (North-West Greenland) | -          | -           |        |
|                             | COA151030PP, #359 | Ringed seal           | Pusa hispida     | 2013 | -      | -         | Illulisat (North-West Greenland) | -          | -           |        |
|                             | COA121030PP, #359 | Ringed seal           | Pusa hispida     | 2013 | -      | -         | Illulisat (North-West Greenland) | -          | -           |        |
|                             | PAX16/0331      | Harp seal              | Pagophilus groenlandicus | 2016 | Adult   (pregnant) | F | Nuuk (Kobbejford) (West Greenland) | 93         | 145         |        |
| Anna Roos, Mads Peter Heide-Jørgensen, Aqqalu Rosing-Asvid, Kristin Laidre | PAX16/0329 | Ringed seal           | Pusa hispida     | 2013 | -      | -         | Illulisat (North-West Greenland) | -          | -           |        |
|                             | PAX16/0329      | Ringed seal           | Pusa hispida     | 2013 | -      | -         | Illulisat (North-West Greenland) | -          | -           |        |
|                             | PAX16/0329      | Ringed seal           | Pusa hispida     | 2013 | -      | -         | Illulisat (North-West Greenland) | -          | -           |        |
|                             | PAX16/0327      | Harbor porpoise       | Phocoena phocoena | 2009 | -      | -         | Manitsoq, West Greenland    | -           | -           |        |
|                             | PAX16/0327      | Harbor porpoise       | Phocoena phocoena | 2009 | -      | -         | Manitsoq, West Greenland    | -           | -           |        |
|                             | PAX16/0327      | Harbor porpoise       | Phocoena phocoena | 2009 | -      | -         | Manitsoq, West Greenland    | -           | -           |        |
|                             | PAX16/0327      | Harbor Porpoise       | Phocoena phocoena | 2009 | -      | -         | Manitsoq, West Greenland    | -           | -           |        |
| Code       | Species                  | Nature of Sample | Location                          | Sex | Year | Coordinates |
|------------|--------------------------|------------------|-----------------------------------|------|------|--------------|
| PAX16/0328 | Humpback whale           | Pooled           | Nuuk (West Greenland)             |      | 2011 |              |
|            | Humpback whale           |                  | Nuuk (West Greenland)             |      | 2013 |              |
| PAX15/0326 | Minke whale              | Pooled           | Qasigiannguit (West Greenland)    | F    | 2000 |              |
|            | Minke whale              |                  | Arsuk (West Greenland)            |      |      |              |
| PAX16/0330 | White beaked dolphin     |                  | Tasilaq-Kulusuc (East Greenland)  |      | 2016 |              |
|            | Minke whale              |                  |                                    |      | 2017 |              |
|            | Killer whale             |                  | Tasilaq, East Greenland            |      | 2017 |              |
| Sandra     | Grey seal                |                  | Iceland                           |      |      |              |
| Granquist  | Grey seal                |                  | Iceland                           |      |      |              |
| 060709-L-LB3 | Grey seal               |                  | Iceland                           |      |      |              |
| 060709-L-LB4 | Harbor seal             |                  | Barðaströnd (Iceland)             | F    | 2009 | 29.5         |
| 180609-L-SH1 | Harbor seal             |                  | Skagaströnd (Iceland)             | F    | 2009 | 42           |
| 080510-L-SH1 | Harbor seal             |                  | Húnaflói (Iceland)                | M    | 2010 | 25.5         |
| 080510-L-SH2 | Harbor seal             |                  | Húnaflói (Iceland)                | M    | 2010 | 32.5         |
| 080510-L-SH3 | Harbor seal             |                  | Barðaströnd (Iceland)             | M    | 2009 | 36.5         |
| 080510-V-GP3 | Harp seal               |                  | Pístifiðjörður (Iceland)           | F    | 2009 | 104.5        |
| 140410-V-ELS8 | Harp seal              |                  | Iceland                           |      | 2010 |              |
| 200409-V-JSH4 | Harp seal              |                  | Págufjörður (Iceland)             | F    | 2009 | 82.5         |
| 140410-V-ELS4 | Harp seal              |                  | Iceland                           |      | 2010 |              |
| 080510-V-SH2 | Harp seal               |                  | Iceland                           |      | 2010 |              |
| Rune Dietz, Frank Riget, Christian | Ringed seal        |                  | Ittoqq/Scoresby Sound, E Greenland |      | 2012 |              |
| 46701      | Ringed seal              |                  | Ittoqq/Scoresby Sound, E Greenland |      | 2012 |              |
| 46702      | Ringed seal              |                  | Ittoqq/Scoresby Sound, E Greenland |      | 2012 |              |
| 46703      | Ringed seal              |                  | Ittoqq/Scoresby Sound, E Greenland |      | 2012 |              |
|  |  |  |  |  |
|---|---|---|---|---|
| **Sonne and Aqqalu Rosing-Asvid** |  |  |  |  |
| 46706 | Ringed seal | *Pusa hispida* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46709 | Ringed seal | *Pusa hispida* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46711 | Ringed seal | *Pusa hispida* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46712 | Ringed seal | *Pusa hispida* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46714 | Ringed seal | *Pusa hispida* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46717 | Ringed seal | *Pusa hispida* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46726 | Ringed seal | *Pusa hispida* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 35143 | Killer whale | *Orcinus orca* | 2013 | Tasilaq/Ammassalik, East Greenland |
| 35144 | Killer whale | *Orcinus orca* | 2013 | Tasilaq/Ammassalik, East Greenland |
| 48732 | Killer whale | *Orcinus orca* | 2013 | Tasilaq/Ammassalik, East Greenland |
| 48733 | Killer whale | *Orcinus orca* | 2013 | Tasilaq/Ammassalik, East Greenland |
| 48734 | Killer whale | *Orcinus orca* | 2013 | Tasilaq/Ammassalik, East Greenland |
| 48735 | Killer whale | *Orcinus orca* | 2013 | Tasilaq/Ammassalik, East Greenland |
| 46752 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46753 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46754 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46755 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46756 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46758 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46759 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |
| 46760 | Polar bear | *Ursus maritimus* | 2012 | Ittoqq/Scoresby Sound, E Greenland |

**Anna Roos**

|  |  |  |  |  |
|---|---|---|---|---|
| A2012/05463 | Grey seal | *Halichoerus grypus* | 2012 | Sweden |
| A2013/05230 | Grey seal | *Halichoerus grypus* | 2013 | Sweden |
| A2015/05571 | Grey seal | *Halichoerus grypus* | 2015 | Sweden |
| A2015/05614 | Grey seal | *Halichoerus grypus* | 2015 | Sweden |
| A2016/05270 | Grey seal | *Halichoerus grypus* | 2016 | Sweden |
| A2015/05387 | Harbor seal | *Phoca vitulina* | 2015 | Sweden |
| A2015/05390 | Harbor seal | *Phoca vitulina* | 2015 | Sweden |
| A2016/05109 | Harbor seal | *Phoca vitulina* | 2015 | Sweden |
| A2016/05167 | Harbor seal | *Phoca vitulina* | 2015 | Sweden |
| A2016/05316 | Harbor seal | *Phoca vitulina* | 2015 | Sweden |
| Code     | Species         | Year | Type  | Sex | Area          | Age | Weight | Pooled |
|----------|-----------------|------|-------|-----|---------------|-----|--------|--------|
| A2014/05650 | Ringed seal  Phoca hispida | 2014 | 23    | F   | Northern Baltic | -   | -      | -      |
| A2015/05591 | Ringed seal  Phoca hispida | 2015 | Adult | M   | Northern Baltic | -   | -      | -      |
| A2016/05110 | Ringed seal  Phoca hispida | 2015 | Yearling | M | Northern Baltic | 31.8 | 100.5  | -      |
| A2016/05126 | Ringed seal  Phoca hispida | 2015 | Adult | F   | Northern Baltic | 41.6 | 132    | -      |
| A2016/05133 | Ringed seal  Phoca hispida | 2015 | Yearling | F | Northern Baltic | 37.1 | 108    | -      |
| A2015/05283 | Harbor porpoise Phocoena phocoena | 2011 | Adult | M   | Southern Baltic | -   | 145    | -      |
| A2016/05526 | Harbor porpoise Phocoena phocoena | 2016 | Subadult | M | Southern Baltic | -   | 124    | -      |
| A2016/05528 | Harbor porpoise Phocoena phocoena | - | Juvenile | F | Southern Baltic | -   | 114    | -      |
| A2016/05637 | Harbor porpoise Phocoena phocoena | 2016 | Adult | M   | Southern Baltic | 43   | 141.5  | -      |
| C2012/00009 | Harbor porpoise Phocoena phocoena | 2011 | Adult | M   | Southern Baltic | 45   | 154.5  | -      |
| A2016/05633 | Grey seal Halichoerus grypus | 2016 | (pregnant) | F | Sweden - Gävleborgs län | -   | -      | -      |
Table S2. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) numbers for export permissions.

| CITES nr     | Species                          | Name                |
|--------------|----------------------------------|---------------------|
| 17GL1167082  | Polar bear                       | Anna Roos           |
| 17GL1167083  | Harbor porpoise                  | Anna Roos           |
| 17GL1167084  | Humpback whale                   | Anna Roos           |
| 17GL1167085  | Minke whale                      | Anna Roos           |
| 17GL1167088  | Killer whale                     | Anna Roos           |
| 17GL1167090  | Humpback whale                   | Anna Roos           |
| 17GL1167098  | White beaked dolphins, East Greenland | Anna Roos     |
| 17US18692C/9 | Pygmy sperm whale                | Susan Shaw and Michelle Berger |
| 17US18692C/9 | Harbor porpoise                  | Susan Shaw and Michelle Berger |
Table S3. Target compounds according to their compound class, acronyms, and molecular formula.

| Name                              | Acronym | Molecular formula |
|-----------------------------------|---------|-------------------|
| Perfluorobutanoic acid            | PFBA    | C₄F₇O₂H           |
| Perfluoropentanoic acid           | PFPeA   | C₅F₉O₂H           |
| Perfluorohexanoic acid            | PFHxA   | C₆F₁₃O₂H          |
| Perfluoroheptanoic acid           | PFHpA   | C₇F₁₃O₂H          |
| Perfluoroctanoic acid             | PFOAₗ⁺Br| C₈F₁₅O₂H          |
| Perfluorononanoic acid            | PFNA    | C₉F₁₇O₂H          |
| Perfluorodecanoic acid            | PFDA    | C₁₀F₁₉O₂H         |
| Perfluoroundecanoic acid          | PFUnDA  | C₁₁F₂₁O₂H         |
| Perfluorododecanoic acid          | PFDnDA  | C₁₂F₂₃O₂H         |
| Perfluorotridecanoic acid         | PFTDA   | C₁₃F₂₅O₂H         |
| Perfluorotetradecanoic acid       | PFTeDA  | C₁₄F₂₇O₂H         |
| Perfluoropentadecanoic acid       | PFPeDA  | C₁₅F₂₉O₂H         |
| Perfluorohexadecanoic acid        | PFHxDA  | C₁₆F₃₁O₂H         |
| Perfluorooctadecanoic acid        | PFDoDA  | C₁₈F₃₅O₂H         |
| Perfluorobutane sulfonic acid     | PFBS    | C₄F₅SO₃H          |
| Perfluoropentane sulfonic acid    | PFPeS   | C₅F₇SO₃H          |
| Perfluorohexane sulfonic acid     | PFHxAₗ⁺Br| C₆F₁₃SO₃H       |
| Perfluoroheptane sulfonic acid    | PFHpS   | C₇F₁₅SO₃H         |
| Perfluoroctane sulfonic acid      | PFOSₗ⁺Br| C₈F₁₇SO₃H         |
| Perfluorononane sulfonic acid     | PFNS    | C₉F₁₉SO₃H         |
| Perfluorodecane sulfonic acid     | PFDSₗ⁺Br| C₁₀F₂₁SO₃H        |
| Perfluoroundecane sulfonic acid   | PFUnDS  | C₁₁F₂₃SO₃H        |
| Perfluoroctane sulfonamide        | FOSAₗ⁺Br| C₈F₁₇SO₂NH₂       |
| Perfluoroctane sulfonamidoacetic acid | FOSAAₗ⁺Br| C₁₀F₁₈SO₄NH₅     |
| N-Methyl perfluoroctane sulfonamidoacetic acid | MeFOSAAₗ⁺Br| C₁₁F₁₇SO₄NH₇ |
| N-Ethyl perfluoroctane sulfonamidoacetic acid | EtFOSAAₗ⁺Br| C₁₂F₁₇SO₄NH₉ |
| 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate | 9Cl-PF3ONS| C₈F₁₆SO₃ClH     |
| PFECAs                                      | 11-chloroeicosfluoro-3-oxaundecane-1-sulfonate | 11Cl-PF3OUdS | C<sub>10</sub>F<sub>20</sub>SO<sub>4</sub>ClH |
|--------------------------------------------|-----------------------------------------------|---------------|----------------------------------|
| Ammonium dodecafluoro-3H-4,8-dioxanonoate | ADONA                                         | C<sub>7</sub>F<sub>13</sub>NO<sub>4</sub>H<sub>5</sub> |
| 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid | HFPO-DA (GenX)                                | C<sub>6</sub>F<sub>14</sub>O<sub>3</sub>H    |
| n<sub>3</sub> FTCAs                           | 3:3 fluorotelomer carboxylic acid             | 3:3 FTCA      | C<sub>6</sub>F<sub>7</sub>O<sub>2</sub>H<sub>5</sub> |
| n<sub>5</sub> FTCA                            | 5:3 fluorotelomer carboxylic acid             | 5:3 FTCA      | C<sub>8</sub>F<sub>11</sub>O<sub>2</sub>H<sub>5</sub> |
| n<sub>7</sub> FTCA                            | 7:3 fluorotelomer carboxylic acid             | 7:3 FTCA      | C<sub>10</sub>F<sub>13</sub>O<sub>2</sub>H<sub>5</sub> |
| n<sub>2</sub> FTSA                            | 1H,1H,2H,2H-perfluorohexane sulfonate         | 4:2 FTSA      | C<sub>6</sub>F<sub>9</sub>SO<sub>3</sub>H<sub>5</sub> |
|                                           | 1H,1H,2H,2H-perfluorooctane sulfonate         | 6:2 FTSA      | C<sub>8</sub>F<sub>13</sub>SO<sub>3</sub>H<sub>5</sub> |
|                                           | 1H,1H,2H,2H-perfluorodecane sulfonate         | 8:2 FTSA      | C<sub>10</sub>F<sub>17</sub>SO<sub>3</sub>H<sub>5</sub> |

L+B = both linear and branched isomers are analyzed.
Table S4. Mobile phase gradient program for targeted analysis. Flow rate was 0.4 ml/min, column temperature 50°C, injection volume 5 µl.

| Time (min) | Mobile phase A¹ (%) | Mobile phase B² (%) |
|------------|---------------------|---------------------|
| 0.0        | 90                  | 10                  |
| 0.5        | 90                  | 10                  |
| 5.0        | 20                  | 80                  |
| 5.1        | 0                   | 100                 |
| 8.0        | 0                   | 100                 |
| 10.0       | 90                  | 10                  |

¹ Mobile phase A: 90% water and 10% acetonitrile containing 2 mM ammonium acetate. ² Mobile phase B: 99% acetonitrile and 1% water containing 2 mM ammonium acetate.
Table S5. Target analytes with their quantification and qualifications ions as well as the internal standard used for quantification. L indicates linear ions, and br indicates branched ions. All ISs were purchased from Wellington Laboratories (Guelph, Canada).

| Target Analyte | Average RT (min) | Precursor ion | Quantitative product ion | Qualitative product ion | IS | IS transition | Data quality¹ |
|----------------|------------------|---------------|--------------------------|-------------------------|----|----------------|----------------|
| PFBA           | 0.66             | 213           | 169                      | 149                     | $^{13}$C₁₂-PFBA | 217/172        | Q              |
| PFPeA          | 1.46             | 263           | 219                      | 169                     | $^{13}$C₁₂-PFHxA | 266/222        | Q              |
| PFHxA          | 2.19             | 313           | 269                      | 119                     | $^{13}$C₁₂-PFHxA | 315/270        | Q              |
| PFHpA          | 2.63             | 363           | 319                      | 169                     | $^{13}$C₁₂-PFOA | 367/322        | Q              |
| L-PFOA         | 2.98             | 413           | 369                      | 169                     | $^{13}$C₁₂-PFOA | 417/372        | Q              |
| br-PFOA        | 2.92             | 413           | 369                      | 169                     | $^{13}$C₁₂-PFOA | 417/372        | semiQ          |
| PFNA           | 3.30             | 463           | 419                      | 219                     | $^{13}$C₁₂-PFNA | 468/423        | Q              |
| PFDA           | 3.58             | 513           | 469                      | 269                     | $^{13}$C₁₂-PFDA | 515/470        | Q              |
| PFUnDA         | 3.87             | 563           | 519                      | 269                     | $^{13}$C₁₂-PFUnDA | 565/520        | Q              |
| PFDaDA         | 4.14             | 613           | 569                      | 169                     | $^{13}$C₁₂-PFDaDA | 615/570        | Q              |
| PFTrDA         | 4.40             | 662.9         | 619                      | 169                     | $^{13}$C₁₂-PFTrDA | 615/570        | semiQ          |
| PFTeDA         | 4.65             | 712.9         | 669                      | 169                     | $^{13}$C₁₂-PFTeDA | 615/570        | semiQ          |
| PFPeDA         | 4.90             | 762.9         | 719                      | 169                     | $^{13}$C₁₂-PFPeDA | 615/570        | semiQ          |
| PFHxDA         | 5.15             | 813           | 769                      | 169                     | $^{13}$C₁₂-PFHxDA | 615/570        | semiQ          |
| PFOcDA         | 5.59             | 913           | 869                      | 169                     | $^{13}$C₁₂-PFOcDA | 615/570        | semiQ          |
| PFBS           | 2.12             | 298.9         | 80                       | 99                      | $^{18}$O₂-PFHxS | 403/84         | Q              |
| PFPeS-80       | 2.57²            | 348.9         | 80                       | 99                      | $^{18}$O₂-PFHxS | 403/84         | semiQ          |
| PFPeS-99       | 2.57²            | 348.9         | 99                       | 80                      | $^{18}$O₂-PFHxS | 403/84         | semiQ          |
| L-PFHxS        | 3.02             | 398.9         | 80                       | 99                      | $^{18}$O₂-PFHxS | 403/84         | Q              |
| Compound       | m/z | 99% TIC | 80% TIC | 99% Area | 18\(^{O}_2\)-PFHxS | 403/84 | semiQ |
|----------------|-----|---------|---------|----------|-------------------|--------|-------|
| br-PFHxS       | 2.97| 399     | 80      | 99       |                   | 403/84 | semiQ |
| PFHpS-80       | 3.36| 448.9   | 80      | 99       | 18\(^{O}_2\)-PFHxS | 403/84 | semiQ |
| PFHpS-99       | 3.36| 448.9   | 99      | 80       | 18\(^{O}_2\)-PFHxS | 403/84 | semiQ |
| L-PFOS-80      | 3.66| 498.9   | 80      | 99       | 13\(^{C}_4\)-PFOS | 503/80 | Q     |
| br-PFOS-80     | 3.57| 498.9   | 80      | 99       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| L-PFOS-99      | 3.66| 498.9   | 99      | 80       | 13\(^{C}_4\)-PFOS | 503/80 | Q     |
| br-PFOS-99     | 3.57| 498.9   | 99      | 80       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| PFNS-80        | 3.83| 548.9   | 80      | 99       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| PFNS-99        | 3.83| 548.9   | 99      | 80       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| L-PFDS         | 4.23| 598.9   | 80      | 99       | 13\(^{C}_4\)-PFOS | 503/80 | Q     |
| br-PFDS        | 4.17| 598.9   | 80      | 99       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| PFUnDS-80      | 4.63 \(^2\) | 648.9 | 80      | 99       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| PFUnDS-99      | 4.63 \(^2\) | 648.9 | 99      | 80       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| L-FOSA         | 4.40| 497.9   | 78      | 169      | 13\(^{C}_8\)-FOSA | 506/78 | Q     |
| br-FOSA        | 4.34| 497.9   | 78      | 169      | 13\(^{C}_8\)-FOSA | 506/78 | semiQ |
| L-FOSAA        | 3.49| 555.9   | 498     | 419      | D\(_3\)-MeFOSAA   | 573/419 | Q     |
| br-FOSAA       | 3.44| 555.9   | 498     | 419      | D\(_3\)-MeFOSAA   | 573/419 | semiQ |
| L-MeFOSAA      | 3.62| 570     | 419     | 483      | D\(_3\)-MeFOSAA   | 573/419 | Q     |
| br-MeFOSAA     | 3.56| 570     | 419     | 483      | D\(_3\)-MeFOSAA   | 573/419 | semiQ |
| L-EtFOSAA      | 3.74| 584     | 419     | 526      | D\(_5\)-EtFOSAA   | 589/419 | Q     |
| br-EtFOSAA     | 3.69| 584     | 419     | 526      | D\(_5\)-EtFOSAA   | 589/419 | semiQ |
| 9Cl-PF3ONS     | 3.86| 531     | 351     | 83       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| 11Cl-PF3OuDS   | 4.42| 631     | 451     | 83       | 13\(^{C}_4\)-PFOS | 503/80 | semiQ |
| Compound  | t (min) | m/z  | m/z  | % R | Isotope | Process | Detection |
|-----------|---------|------|------|-----|---------|---------|-----------|
| ADONA     | 2.75    | 377  | 251  | 85  | ^13C4-PFOS | 503/80 | semiQ     |
| HFPO-DA   | 2.36    | 329  | 169  | 185 | ^13C4-PFOA | 417/372 | semiQ     |
| 3:3 FTCA  | 0.95    | 241  | 117  | 177 | ^13C4-PFOA | 417/372 | semiQ     |
| 5:3 FTCA  | 2.50    | 341  | 237  | 217 | ^13C4-PFOA | 417/372 | semiQ     |
| 7:3 FTCA  | 3.30    | 441  | 337  | 148 | ^13C4-PFOA | 417/372 | semiQ     |
| 4:2 FTSA  | 2.03    | 327  | 307  | 80.6| ^13C2-6:2 FTSA | 429/409 | semiQ     |
| 6:2 FTSA  | 2.86    | 427  | 407  | 80.6| ^13C2-6:2 FTSA | 429/409 | Q         |
| 8:2 FTSA  | 3.46    | 527  | 507  | 80.6| ^13C2-6:2 FTSA | 429/409 | semiQ     |
| 13C8-PFOA | 2.98    | 421  | 376  |     |         |         |           |
| 13C8-PFOS | 3.66    | 507  | 80   |     |         |         |           |

1 Q = quantitative, semiQ = semi-quantitative for compounds lacking analogous ISs.
2 Estimated retention time, based on retention times of adjacent PFSAs.
3 13C8-PFOA and 13C8-PFOS were used as recovery internal standards.
Table S6. Limit of quantification (LOQ) for all compounds determined by the lowest calibration concentration.

| Compound     | LOQ (ng/g) | Compound     | LOQ (ng/g) | Compound     | LOQ (ng/g) |
|--------------|------------|--------------|------------|--------------|------------|
| PFBA         | 0.814      | PFOcDA       | 15.1       | L-FOSAA<sup>a</sup> | 1.16       |
| PFPeA        | 0.290      | PFBS<sup>a</sup> | 2.37       | L-MeFOSAA    | 0.826      |
| PFHxA        | 0.290      | L-PFHxS      | 0.014      | L-Et-FOSAA   | 0.296      |
| PFHpA        | 0.290      | br-PFHxS<sup>b</sup> | 0.014     | 9Cl-PF3ONS   | 0.840      |
| L-PFOA       | 0.290      | PFHpS<sup>b</sup> | 0.014      | 11Cl-PF3OUdS | 0.042      |
| PFNA         | 0.290      | L-PFOS-80<sup>a</sup> | 5.81       | ADONA        | 0.816      |
| PFDA         | 0.042      | br-PFOS-80<sup>a</sup> | 5.81       | HFPO-DA      | 40.6       |
| PFUnDA       | 0.290      | L-PFOS-99<sup>a</sup> | 6.28       | 3:3 FTCA     | 105        |
| PFDoDA       | 0.290      | br-PFOS-99<sup>a</sup> | 6.28       | 5:3 FTCA     | 106        |
| PFTrDA       | 0.290      | L-PFDS       | 0.040      | 7:3 FTCA     | 5.61       |
| PFTeDA       | 0.814      | br-PFDS<sup>b</sup> | 0.040      | 4:2 FTSA     | 0.820      |
| PFPeDA<sup>b</sup> | 0.290 | L-FOSA      | 0.302      | 6:2 FTSA     | 0.826      |
| PFHxDA       | 0.814      | br-FOSA<sup>b</sup> | 0.302      | 8:2 FTSA     | 40.9       |

<sup>a</sup>Compounds that were present in the method blanks and for these the LOQ was determined alternatively by calculating the average contamination concentration plus ten times the standard deviation.  
<sup>b</sup>Compounds that were not present in the calibration curve, but that were present in the samples.
Table S7. Comparison of NIST serum standard reference material (SRM) 1957, reported reference values, and results from method used in the present study.

| Compound | NIST certificate values (ng/g) | Gebbink et al.\textsuperscript{12} (ng/g) | Yeung et al.\textsuperscript{13} (ng/g) | Present study (ng/g) |
|----------|-------------------------------|----------------------------------------|----------------------------------------|---------------------|
| PFHpA    | 0.305 ± 0.036                 | 0.2 ± 0.02                             | 0.2 ± 0.1                              | <0.29               |
| PFOA     | 5 ± 0.4                       | 3.86 ± 0.13                            | 4.1 ± 0.3                              | 5.0 ± 0.1           |
| PFNA     | 0.88 ± 0.068                  | 0.72 ± 0.04                            | 0.8 ± 0.1                              | 0.8 ± 0.2           |
| PFDA     | 0.39 ± 0.1                    | 0.24 ± 0.01                            | 0.3 ± 0.0                              | 0.3 ± 0.1           |
| PFUnDA   | 0.174 ± 0.031                 | 0.11 ± 0.01                            | 0.1 ± 0.1                              | <0.29               |
| PFDaDA   | -                             | 0.017 ± 0.003                          | -                                      | -                   |
| PFTrDA   | -                             | 0.009 ± 0.004                          | -                                      | -                   |
| PFHxS    | 4 ± 0.75                      | 3.25 ± 0.06                            | 4.1 ± 0.5                              | 4.0 ± 0.2           |
| PFOS     | 21.1 ± 1.2                    | 18.5 ± 0.7                             | 19.3 ± 1.2                             | 18.4 ± 0.5          |
| FOSA     | -                             | 0.029 ± 0.007                          | -                                      | -                   |

\textsuperscript{a}“-” = not detected
Table S8. Eluent program for the ion chromatography part of the CIC analysis.

| Time (min) | Concentration OH⁻ (mM) |
|------------|-------------------------|
| 0.0        | 8.0                     |
| 4.0        | 8.0                     |
| 9.9        | 45.0                    |
| 10.0       | 100.0                   |
| 14.0       | 100.0                   |
| 14.1       | 8.0                     |
| 20.0       | 8.0                     |
**Table S9.** Set-up parameters for the HRMS Orbitrap.

| Scan parameters          | HESI source               |
|--------------------------|---------------------------|
| **Scan typ**             | Sheath gas flow rate      | 30          |
| **Scan range**           | Aux gas flow rate         | 10          |
| 200-1200 m/z             | Sweep gas flow rate       | 0           |
| **Fragmentation**        | Spray voltage (kV)        | 3.70        |
| None or NCE(35) (z=1)    | Capillary temp. (°C)      | 350         |
| **Resolution**           | S-lens RF level           | 55.0        |
| 120000                   | Aux gas heater temp. (°C) | 350         |
| **Polarity**             |                           |             |
| Negative                 |                           |             |
| **Maximum inject time**  |                           |             |
| 30/250                   |                           |             |
REFERENCES

(1) Powley, C. R.; George, S. W.; Ryan, T. W.; Buck, R. C. Matrix Effect-Free Analytical Methods for Determination of Perfluorinated Carboxylic Acids in Environmental Matrixes. *Anal. Chem.* **2005**, *77* (19), 6353–6358. https://doi.org/10.1021/ac0508090.

(2) Miyake, Y.; Yamashita, N.; Rostkowski, P.; So, M. K.; Taniyasu, S.; Lam, P. K. S.; Kannan, K. Determination of Trace Levels of Total Fluorine in Water Using Combustion Ion Chromatography for Fluorine: A Mass Balance Approach to Determine Individual Perfluorinated Chemicals in Water. *J. Chromatogr. A* **2007**, *1143* (1–2), 98–104. https://doi.org/10.1016/j.chroma.2006.12.071.

(3) Schultes, L.; Vestergren, R.; Volkova Hellström, K.; Westberg, E.; Jacobson, T.; Benskin, J. P. Per- and Polyfluoroalkyl Substances and Fluorine Mass Balance in Cosmetic Products from the Swedish Market: Implications for Environmental Emissions and Human Exposure. *Environ. Sci. Process. Impacts* **2018**. https://doi.org/10.1039/C8EM00368H.

(4) Schultes, L.; Peaslee, G. F.; Brockman, J. D.; Majumdar, A.; McGuinness, S. R.; Ngwenyama, R. A.; Wilkinson, J. T.; Sandblom, O.; Ngwenyama, R. A.; Benskin, J. P. Total Fluorine Measurements in Food Packaging: How Do Current Methods Perform? *Environ. Sci. Technol. Lett.* **2019**, *6*, 73–78. https://doi.org/10.1021/acs.estlett.8b00700.

(5) Miaz, L. T. Development and Application of UHPLC-Orbitrap Method for Quantitative and Suspect Screening of PFASs in Human Serum, Master’s thesis, Stockholm University, 2018.

(6) Fujii, Y.; Kato, Y.; Kozai, M.; Matsuishi, T.; Harada, K. H.; Koizumi, A.; Kimura, O.; Endo, T.; Haraguchi, K. Different Profiles of Naturally Produced and Anthropogenic Organohalogens in the Livers of Cetaceans from the Sea of Japan and the North Pacific Ocean. *Mar. Pollut. Bull.* **2018**, *136* (August), 230–242. https://doi.org/10.1016/j.marpolbul.2018.08.051.

(7) Tomy, G. T.; Budakowski, W.; Halldorson, T.; Helm, P. A.; Stern, G. A.; Friesen, K.; Pepper, K.; Tittlemier, S. A.; Fisk, A. T. Fluorinated Organic Compounds in an Eastern Arctic Marine Food Web. *Environ. Sci. Technol.* **2004**, *38* (24), 6475–6481. https://doi.org/10.1021/es049620g.

(8) Kelly, B. C.; Ikonomou, M. G.; Blair, J. D.; Surridge, B.; Hoover, D.; Grace, R.; Gobas, F. A. P. C. Perfluoroalkyl Contaminants in an Arctic Marine Food Web: Trophic...
Magnification and Wildlife Exposure. *Environ. Sci. Technol.* **2009**, *43* (11), 4037–4043. https://doi.org/10.1021/es9003894.

(9) Yeung, L. W. Y.; Miyake, Y.; Li, P.; Taniyasu, S.; Kannan, K.; Guruge, K. S.; Lam, P. K. S.; Yamashita, N. Comparison of Total Fluorine, Extractable Organic Fluorine and Perfluorinated Compounds in the Blood of Wild and Perfluorooctanoate (PFOA)-Exposed Rats: Evidence for the Presence of Other Organofluorine Compounds. *Anal. Chim. Acta* **2009**, *635* (1), 108–114. https://doi.org/10.1016/j.aca.2009.01.004.

(10) Gebbink, W. A.; Bossi, R.; Rigét, F. F.; Rosing-Asvid, A.; Sonne, C.; Dietz, R. Observation of Emerging Per- and Polyfluoroalkyl Substances (PFASs) in Greenland Marine Mammals. *Chemosphere* **2016**, *144*, 2384–2391. https://doi.org/10.1016/j.chemosphere.2015.10.116.

(11) Shaw, S.; Berger, M. L.; Brenner, D.; Tao, L.; Wu, Q.; Kannan, K. Specific Accumulation of Perfluorochemicals in Harbor Seals (Phoca Vitulina Concolor) from the Northwest Atlantic. *Chemosphere* **2009**, *74* (8), 1037–1043. https://doi.org/10.1016/j.chemosphere.2008.10.063.

(12) Gebbink, W. A.; Glynn, A.; Darnerud, P. O.; Berger, U. Perfluoroalkyl Acids and Their Precursors in Swedish Food: The Relative Importance of Direct and Indirect Dietary Exposure. *Environ. Pollut.* **2015**, *198*, 108–115. https://doi.org/10.1016/j.envpol.2014.12.022.

(13) Yeung, L. W. Y.; De Silva, A. O.; Loi, E. I. H.; Marvin, C. H.; Taniyasu, S.; Yamashita, N.; Mabury, S. A.; Muir, D. C. G.; Lam, P. K. S. Perfluoroalkyl Substances and Extractable Organic Fluorine in Surface Sediments and Cores from Lake Ontario. *Environ. Int.* **2013**, *59* (2013), 389–397. https://doi.org/10.1016/j.envint.2013.06.026.
