Observation of emerging per- and polyfluoroalkyl substances (PFASs) in Greenland marine mammals

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The present pilot study examined emerging per- and polyfluoroalkyl substances (PFASs), i.e., a suite of short chain perfluoroalkyl acids (PFAAs), PFAA precursors and replacement chemicals, and legacy PFASs (long chain length PFAAs) in livers from ringed seals, polar bears and, for the first time, killer whales from East Greenland collected in 2012—2013. Among the emerging PFASs, perfluorobutanesulfonic acid (PFBS) and F-53B (a chlorinated polyfluorinated ether sulfonic acid) were detected in Arctic wildlife, albeit at concentrations approximately four orders of magnitude lower compared to perfluorooctanesulfonic acid (PFOS). PFOS was positively correlated with F-53B, but not PFBS in all three species. A total of 17 PFASs were detected in killer whales, including in a mother–fetus pair, demonstrating maternal transfer. PFAS concentrations in killer whales (269 ± 90 ng/g) were comparable to concentrations found in ringed seals (138 ± 7 ng/g), however, an order of magnitude lower compared to concentrations found in polar bear livers (2336 ± 263 ng/g). Patterns of long chain PFAs in killer whales differed from the pattern in ringed seals and polar bears. Of the monitored PFAA precursors, only perfluorooctanesulfonamide (FOSA) was detected in all three species, and FOSA/PFOS ratios and isomer patterns indicated that killer whales have a potential lower metabolic capacity to degrade FOSA compared to polar bears and ringed seals.

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1. Introduction

Per- and polyfluoroalkyl substances (PFASs) — in particular perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) — have been recognized as global environmental contaminants (Buck et al., 2011). Long chain length PFASs (i.e. C6–C14 PFASs) are persistent and bioaccumulative, and have been reported at elevated concentrations in remote Arctic wildlife (Boutilier et al., 2010; Houde et al., 2011). Although the production of several PFASs has been phased-out recently in Europe and North America, e.g. perfluorooctanesulfonic acid (PFOS) and its precursors were phased out in 2002 by 3 M, and emissions of long chain PFASs and their precursors are to be eliminated by 2015 (Martin et al., 2010; US EPA, 2006), manufacturing of other PFASs has been unnoticed (De Silva et al., 2011; Wang et al., 2013a). Recent manufacturing phase-outs have also led to shifts in formulations, often to short-chain or semi-fluorinated substances (Wang et al., 2013b).

While less bioaccumulative than their longer chain homologues (Goeritz et al., 2013), short chain PFASs, such as perfluorobutanesulfonic acid (PFBS), are nevertheless persistent in the environment, and have recently been detected in Swedish herring (Clupea harengus) (Ullah et al., 2014). Physicochemical properties and environmental fate of several PFOS and PFOA replacement chemicals were estimated recently and found to be comparable to PFOS and PFOA itself (Gomis et al., 2015; Wang et al., 2013a). For example, the PFOA replacement chemical F-53B (a chlorinated polyfluorinated ether sulfonic acid) had comparable octanol-water (Kow), air-water (Kow), octanol-air partition coefficient (Koa), acid-dissociation constant (pKa), and bioconcentration and bioaccumulation factor (BCF and BAF) values as PFOS (Gomis et al., 2015; Wang et al., 2013a). F-53B has been detected in Chinese rivers (40 ng/L) and sewage sludge (<2 ng/g) at comparable concentrations to PFOS (Ruan et al., 2015; Wang et al., 2013a). Information on the environmental fate of several PFCA precursors, e.g. fluorotelomer-based perfluoropolyalkyl phosphate esters (PAPs), is limited. Despite the fact that PAPs can be degraded to PFCSs (D’Eon and Mabury, 2011), they have been detected in mussels and fish from Europe, the Great Lakes, and the Indian Ocean (Guo et al., 2012; Zabaleta et al., 2015). Although there is ample information on PFIA exposure (C6–C10 PFASs, FOSA, and C6–C15 PFCA) to Greenland ringed seals (Pusa hispida) and polar bears (Ursus maritimus) (Brossi et al., 2005; Dietz et al., 2008; Riget et al., 2013; Rotander et al., 2012), it remains unknown whether ringed seals, polar bears or other Arctic marine mammals are exposed to other per- and polyfluorinated substances, such as short chain PFASs, alternative replacement chemicals (F-53B), or other PFOS and PFCA precursors. Based on biopsies, killer whales (Orcinus orca) in Alaskan and Norwegian waters have been reported to be most contaminated marine mammal with respect to persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides and polychlorinated diphenyl ethers (PCDEs) (Letcher et al., 2010), however, there are no data on legacy or emerging PFAS exposure to killer whales residing in Greenland waters or any other region. Of the emerging PFASs, the short chain PFBS has been detected in the Arctic environment (sediment and water) (Stock et al., 2007; Yamashita et al., 2008), however, reports on F-53B and other PFIA precursors (e.g. PAPs) in the Arctic are so far non-existing.

The objective of this study was to investigate whether Arctic marine mammals (ringed seals, polar bears, and killer whales) were exposed to short chain PFASs, the PFOA replacement F-53B, and PAPs and to determine their importance relative to legacy (longer chain) PFASs. Additionally, killer whale exposure and maternal transfer of PFASs were investigated and concentrations and patterns (including isomers) were compared to ringed seals and polar bears.

2. Materials and methods

2.1. Chemicals and reagents

Targeted PFASs in the present study included: C6,8,10 PFASs (including a mixture of branched and linear PFOs), C4–14 PFCSs, FOSA and its methyl and ethyl derivatives (Me- and EtFOSA), FOSAA and its methyl and ethyl derivatives (Me- and EtFOSAA), four monopAPs (4:2, 6:2, 8:2, 10:2), 11 dipAPs (4:2/4:2, 4:2/6:2, 6:2/6:2, 6:2/8:2, 8:2/8:2, 6:2/10:2, 8:2/10:2, 10:2/10:2, 8:2/12:2, 6:2/14:2), and 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFES, with trade name F-53B). A total of 19 isotopically-labeled standards were included in this study and details on the isotopically-labeled and native standards can be found in Table S1 in the Supplementary material. All solvents and reagents were of the highest commercial purity and employed as received.

2.2. Sample collection and preparation

All sampling was conducted in cooperation with local Inuit subsistence hunters in Greenland in 2012 and 2013. Liver samples from ringed seals (n = 10) and polar bears (n = 8) were collected in Iltqortoortoimit/Scoresby Sound area, while samples of killer whale livers (n = 6) were collected in the Tasilaq/Ammassalik area (Fig. S1 and Table S2). The killer whale samples included liver samples from a mother–fetus pair. Samples from ringed seals and polar bears were collected <1 h post mortem while sampling of killer whales could take as long as 12 h due to transport at sea and appropriate tidal time for dissection. After sampling, liver tissues were stored at −20 °C prior to processing. The extraction and cleanup was based on published methods (Gebbink et al., 2013, 2015). Briefly, homogenized liver samples (0.5 g) were spiked with isotopically-labeled internal standards and extracted three times with acetone (3 mL) by sonication. Concentrated extracts (<1 mL) were loaded on preconditioned (with methanol and water) weak anion exchange (WAX) cartridges (150 mg, 6 mL, Waters) and subsequently washed with aqueous formic acid and water. Neutral compounds were eluted with 3 mL methanol (fraction 1) and ionic compounds were eluted with 4 mL of a solution of 1% ammonium hydroxide in methanol (fraction 2). Both fractions were dried under a stream of nitrogen at 35 °C and the residuals were re-dissolved in methanol. The extracts were filtered using centrifugal filters (modified nylon 0.2 μm, 500 μL) and 13C8-PFOA and 13C8-PFOS were added as recovery standards prior to ultra-performance liquid chromatography–tandem mass spectrometry (UPLC/MS/MS) analysis.

2.3. Instrumental analysis and quantification

For all instrumental analyses, chromatographic separation was carried out on an Acquity UPLC system (Waters) equipped with a BEH C18 (50 × 2.1 mm, 1.7 μm particle size, Waters) analytical column. A trapping column was installed prior to the injector to delay any contamination from the UPLC system and solvents. Mobile phases, gradient programs and flow rates for the different UPLC methods can be found in Tables S3 and S4. Connected to the UPLC system was a Xevo TQ-S triple quadrupole mass spectrometer (Waters) operated in negative ion electrospray ionization (ESI–) mode. The capillary voltage was set at 2.0 kV, and 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. Compound-specific optimized
cone voltages and collision energies are listed in Table S1.

Quantification was performed using an isotope dilution approach. Analytes lacking an analogous labeled standard were quantified using the internal standard with the closest retention time (Table S1). Quantification was performed using the precursor – product ion multiple reaction monitoring (MRM) transitions reported in Table S1. PFPeDA was quantified using the PFPeDA calibration curve. For diPAPs for which no authentic standards were available, a technical mixture was used to optimize MRM channels and for confirmation of retention times (Gebbink et al., 2013). Quantification of these diPAPs was based on calibration curves of authentic diPAP standards with similar chain length (Table S1). Results for these compounds should be considered semi-quantitative. Calibration curves were linear over the whole concentration ranges with r values greater than 0.99 for all compounds. For PFOS and FOSA, linear and sum-branched isomers were chromatographically separated and quantified individually. Sum-branched and linear PFOS were quantified using a standard mixture containing branched and linear isomers using m/z 99 as product ion. Branched FOSA isomers were quantified using the linear isomer calibration curve.

2.4. Quality control and data analysis

In each batch of samples three method blanks were included to monitor for background contamination. For compounds where blank contamination (PFOS an PFPOA) was observed the method quantification limits (MQLs) were determined as the mean plus three times the standard deviation of the quantified procedural blank signals. A blank correction was performed by subtracting the average quantified concentration in the blanks from PFAS concentrations in the samples. For other compounds the MQL was determined as the concentration in a liver sample giving a peak with a signal-to-noise ratio of 10. Table S5 lists all compound-specific MQLs. Recoveries of the labeled internal standards in the liver samples are listed in Table S6, and ranged between 42% and 145% with the exception of 16% for d3-MeFOSA and 20% for d5-EtFOSA. Arithmetic mean concentrations were only determined for individual PFASs with >60% detection in the liver samples per species. Concentrations below the MQL were replaced with MQL/√2 for statistical purposes. Shapiro–Wilks’ W test indicated a normal distribution of PFOS and F-53B concentrations and Pearson’s correlation coefficients were used to examine the relationship between PFOS and F-53B concentrations. Differences in PFOS and FOSA isomer pattern (i.e. percent linear isomer) and FOSA/PFOS concentration ratios among the three species were analyzed using one way analysis of variance (ANOVA), followed by a Tukey’s HSD post hoc test. The statistical package utilized was Statistica® (StatSoft, Tulsa, OK, U.S.A.).

3. Result and discussion

3.1. Emerging PFASs in Greenland marine mammals

The ringed seal, polar bear, and killer whale liver samples were analyzed for a range of emerging PFASs (short chain PFAs, F-53B and PAPs). While F-53B and PFBS were detected in these marine mammals, short chain PFCAs and mono- and diPAPs were consistently below the detection limit. Although PAPs were found in mussels and fish species collected from Europe, the Great Lakes and the Indian Oceans (Guo et al., 2012; Zabaleta et al., 2015), these PFCA precursors have not been reported in the abiotic or biotic Arctic environment. The absence of PAPs in the present samples could be due to several factors, e.g. biotransformation by the species included in this study or by lower trophic level species in their respective food chains or due to the absence of long-range transfer to the Arctic region. F-53B was detected in liver tissue from all ringed seals and polar bears and in five of the six killer whales (details on the method optimization for F-53B are provided in the Supplementary material). The F-53B concentrations were highest.
in polar bears (0.27 ± 0.04 ng/g), followed by ringed seals and killer whales (0.045 ± 0.004 and 0.023 ± 0.009 ng/g, respectively) (Table 1). PFBS was detected in all polar bear livers at 0.032 ± 0.008 ng/g and in 67% of the killer whale livers at 0.0052 ± 0.0017 ng/g; but was below detection limit in all the ringed seal livers (Table 1). Although F-53B concentrations were 3–4 orders of magnitude lower compared to PFOS concentrations in the liver of all three species, positive correlations were observed between F-53B and PFOS, which were significant in ringed seal and killer whale livers (r = 0.827, p = 0.003 and r = 0.969, p = 0.002, respectively) but not in polar bear livers (r = 0.498, p = 0.21) (Fig. 1). No significant (p > 0.05) relationship was observed between PFBS and PFOS concentrations in polar bears and killer whales. Although reported in Chinese water and sludge samples (Ruan et al., 2015; Wang et al., 2013a), F-53B has previously not been detected in wildlife. PFBS, on the other hand, has been reported in sediment and water from the Arctic region (Stock et al., 2007; Yamashita et al., 2008), however, there is, to our knowledge, no reporting on Arctic wildlife exposure to PFBS.

F-53B is used as a PFOS replacement in the electroplating industry in China and has been produced for over 30 years (Wang et al., 2013a) with a reported usage of 20–30 tonnes in 2009 (Wang et al., 2013b). Besides the manufacturing in China, it remains unclear whether there are other emission sources that could contribute to the environmental presence of F-53B. Two recently published studies estimated the physicochemical properties and the environmental fate of F-53B and compared it to PFOS, as these two chemicals are structurally similar (Comis et al., 2015; Wang et al., 2013a). These studies estimated physicochemical properties (such as pK_a, KOW, KAW, and KOM) and BCF and BAF for F-53B and found them to be comparable to the physicochemical properties of PFOS (summarized in Table S7). Overall the persistence of F-53B was estimated to be comparable to PFOS, which was corroborated by the fact that F-53B was resistant to photodegradation and oxidation (Wang et al., 2013a). Production of PFBS (and its precursors) increased after the PFOS phase-out in Europe and North America in 2002, and although it is as persistent as longer chain homologues, it has a lower bioaccumulation potential compared to PFOS (Goeritz et al., 2013).

Transportation pathways of F-53B and PFBS into the Arctic region are not well defined. F-53B, having similar estimated physicochemical properties to PFOS, would be present in the environment in ionic form. Therefore oceanic transport is likely a dominant transportation pathway, although atmospheric transport through binding to particles cannot be ruled out. Besides oceanic transportation of PFBS, atmospheric transportation of volatile PFBS precursors could be an additional source of PFBS to the Arctic region following precursor degradation. Although not reported for the Arctic region specifically, PFBS precursors (Me-FBSA and Me-FBSE) have been reported in air samples from remote locations, i.e. the Antarctic (Del Vento et al., 2012).

F-53B BCF and BAF values in fish were recently estimated and found to be comparable to PFOS (Wang et al., 2013a), while PFBS has lower BCF in fish compared to PFOS (Zhou et al., 2013). F-53B and PFBS (and/or PFBS precursors) likely bioaccumulate into lower trophic level species, being an exposure source to higher trophic level species such as ringed seals and/or killer whales. Greenland ringed seal feed mainly on polar cod (Boreogadus saida) and on invertebrates such as Parathemisto libellula (Labansens et al., 2011), while North Atlantic killer whales feed on fish such as herring and mackerel (Scomber scombrus) (Foote et al., 2009). Killer whales have also been reported to prey on other marine mammals (Foote et al., 2009), which was corroborated by the finding of harp seal (Pagophilus groenlandicus) and minke whale (Balaenoptera acutorostrata) remains in the stomach of a killer whale included in the present study. For both seals and whales, the presence of F-53B and PFBS in any of their prey species has not been studied. Ringed seal are a main prey species for polar bears, although the proportion of ringed seals in East Greenland polar bear diet has declined over the last 30 years in favor of harp and hooded seals (Cystophora cristata) (McKinney et al., 2013). Based on the present data, ringed seals are a source of F-53B to polar bears, however, it is unclear how much ringed seals contribute to overall exposure of polar bears to F-53B. As PFBS was below the detection limit in ringed seals, polar bear exposure to PFBS (or its precursors) could have resulted through consumption of harp and hooded seals. Dietary exposure of F-53B and PFBS to ringed seals, polar bears, and killer whales potentially originates from various prey species; therefore future research should focus on investigating the presence of these chemicals in multiple or key prey species. This would allow for a better understanding of the bioaccumulation potential of F-53B and PFBS from prey to predator.

Fig. 1. Relationships between PFOS and F-53B concentrations (ng/g) in East Greenland ringed seal, polar bear, and killer whale liver samples.
3.2. Legacy PFASs in Greenland marine mammals

3.2.1. Concentrations and patterns

Long chain PFASs and PFCAs and FOSA are commonly reported PFASs in Arctic marine mammals. Although killer whales are among the most contaminated marine mammals with POPs [polychlorinated biphenyls (PCBs), organochlorine pesticides and polybrominated diphenyl ethers (PBDEs), based on lipid weight concentrations] as previously mentioned, PFAS exposure to killer whales has not yet been studied. The present killer whale livers contained 125 ± 43 ng/g ∑PFSA (Table 1), which was comprised primarily by PFOS (97% of ∑PFSA), followed by PFHxS (1.7%), PFDS (1.1%), and PFBS (<0.01%). ∑PFCAs concentrations in livers were 139 ± 48 ng/g, with PFDA, PFUnDA, and PFTdDA being the dominant PFCAs (75% of ∑PFCAs) (Fig. 2), while FOSA was the only detected PFOS precursor in the liver samples at 5.3 ± 0.6 ng/g (Me- and EtFOSA and FOSAA and its methyl- and ethyl-derivatives were below detection limit in all the samples). The ∑PFSA and ∑PFCAs concentrations in killer whales were comparable to concentrations found in ringed seals (94 ± 6 ng/g and 43 ± 2 ng/g for ∑PFSA and ∑PFCAs, respectively), however, at least an order of magnitude lower compared to concentrations found in polar bear livers (1825 ± 219 ng/g and 506 ± 55 ng/g for ∑PFSA and ∑PFCAs, respectively) (Table 1). Concentrations of the only detected PFOS precursor in the livers of the seals and bears, FOSA, were 0.80 ng/g and 4.3 ng/g, respectively. Like in killer whales, the PFSA patterns in seals and bears were dominated by PFOS (>98% of ∑PFSA, respectively) followed by PFHxS (<0.8% of ∑PFSA) and PFBS (<0.2% of ∑PFSA), while PFBS contributed <0.01% to the ∑PFCAs in polar bears. The PFCA patterns in seals and bears were comparable (PFNA, PFDA, and PFUnDA contributed 90%–93% of ∑PFCAs), but differed from the pattern observed in killer whales (Fig. 2). Concentrations and patterns of PFSA, FOSA, and PFCAs in ringed seals and polar bears were comparable to earlier studies on these East Greenland marine mammals (Greaves et al., 2012; Rigét et al., 2013).

Linear and the sum of all branched isomers of PFOS and FOSA were chromatographically separated and quantified in all ringed seal, polar bear, and killer whale samples (Table 1, Fig. S5). For PFOS, the isomer pattern in polar bears contained 88.4% ± 0.8% linear isomer, this was significantly less (p < 0.001) compared to ringed seals (92.1% ± 0.4%) while there was no significant difference compared to killer whales (89.8% ± 0.6%) (Fig. 3). The FOSA isomer pattern in polar bears contained significantly more (p < 0.002) linear isomer (98.5% ± 0.31%) compared to ringed seals and killer whales (95.0% ± 0.5% and 94.4% ± 1.1%, respectively) (Fig. 3). PFOS isomer patterns have been reported in Greenland ringed seals and polar bears and were found. A more diverse diet the present study (Greaves and Letcher, 2013; Rotander et al., 2012). Data on FOSA isomer patterns in marine mammals is, to our knowledge, non-existent. Comparing precursor/PFOS concentration ratios has previously been used as an indication of potential species-specific differences in precursor degradation (Galatius et al., 2013). No significant difference was observed between the FOSA/PFOS concentration ratios of branched isomers, linear isomer, and total isomers in ringed seals and polar bears. However, the branched, linear, and total isomer FOSA/PFOS concentration ratios in both these species were significantly (p < 0.002) lower compared to the ratios in killer whales (Fig. 3). Species-specific differences in FOSA/PFOS concentration ratios have previously been reported for marine mammals, i.e. ratios found in white-beaked dolphin (Lagenorhynchus albirostris) and harbor porpoise (Phocoena phocoena) were higher compared to harbor seal (Phoca vitulina) (Galatius et al., 2013). In combination with the values obtained from the literature, Galatius et al. (2013) expanded their conclusions to claim that carnivor species including pinnipedia have a much higher capacity of transforming PFOS to perfluorooctane sulfonic acid (PFOS) compared to cetacean species. Comparing FOSA/PFOS concentration ratios among the three species included in the present study further strengthen the evidence that cetaceans (in this case killer whales) have lower metabolic capacity to degrade precursors compared to other species such as ringed seals and polar bears (Letcher et al., 2014).

The present data shows killer whale exposure to 17 PFASs. However, concentrations of individual PFASs, PFCA patterns, and isomer patterns differed in whales compared to ringed seals and polar bears. Moreover, differences in ∑PFSA/∑PFCAs concentration ratios among the species were observed. Significant relationships (p < 0.02) were found between ∑PFSA and ∑PFCAs concentrations in the livers of all three species, however, where in killer whales ∑PFSA and ∑PFCAs concentrations were comparable (slope = 0.9), ∑PFSA concentrations in ringed seals and polar bears were higher compared to ∑PFCAs concentrations (slope = 2.5 and 3.1, respectively). The observed difference (concentrations and patterns) in killer whale exposure to PFASs compared to seals and bears could be due to several factors. A more diverse diet of killer whales (feeding on fish and other marine mammals) (Foote et al., 2009) compared to ringed seal (fish and crustaceans) (Labansen et al., 2011) and polar bears (seals) (McKinney et al., 2013) could have resulted in these concentration and pattern differences, although...
PFASs in killer whale prey have not been studied. Also, it is uncertain to what extent the East Greenland killer whales are conducting longer migrations than ringed seals and polar bears. Long range migration has been reported for killer whales, i.e., in the eastern Canadian Arctic (Matthews et al., 2011), but it remains unclear whether this migratory behavior impacts PFAS exposure. Finally, species-specific differences in toxicokinetics such as uptake, distribution, biotransformation of precursors, and elimination of PFASs could also have contributed to the observed differences. In order to get a better insight into killer whales exposure to PFASs future research should address these knowledge gaps.

3.2.2. Maternal transfer in Killer Whales

The killer whale samples included in the present study contained liver samples from a mother–fetus pair. A significant relationship (p < 0.0001) was observed between individual PFAS concentrations in mother and fetus, indicating maternal transfer from the mother whale to the fetus (Fig. S6). Individual PFAS concentrations in the fetus were systematically higher in the fetus liver compared to the mother’s liver (slope 1.7; Fig. S6), this was also reported in melon-headed whales (Peponocephala electra) when concentrations in fetus and mother livers were compared (Hart et al., 2008). Based on body length measurements, body and liver weights were estimated for the mother killer whale and her fetus (Table S8) in order to determine the liver burdens and transfer rate from mother to fetus for individual PFASs. PFAS burdens in the mother and fetus liver were estimated at 2000 and 97 mg for PFSA, respectively and 3100 and 130 mg for PFCA, respectively (Table 2). Transfer rates for individual PFASs from mother to fetus were estimated to range between 2.2% (PFHpA) and 11% (PFOA).

Table 2

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*Liver burden = liver weight × individual PFAS concentration. See Table S8 for liver weight estimations.

*Transfer rate (%) = burden in fetus/(burden in mother + burden in fetus) × 100%.

*Hart et al. (2008), based on two mother–fetus pairs.

*Sharpe et al. (2010), based on fish to eggs burdens.

*∑PFSA = PFBS, PFHxS, PFOS, PFDS.

*∑PFCA = PFHpA, PFDA, PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFTeDA, PPeDA.
Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.10.116.

References


