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MATERNAL TRANSFER OF PERFLUOROALKYL SUBSTANCES IN HOODED SEALS

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Abstract: The role of milk in the transfer of perfluoroalkyl substances (PFASs) to offspring is not well known in wildlife. Eight PFASs were quantified in plasma and milk in mother–pup pairs of hooded seals (*Cystophora cristata*) during the nursing period, and the role of milk in the transfer process was analyzed. Hooded seal was chosen because of its short lactation period (3–4 d), during which the pup feeds only on milk. Placental or lactation transfer would thus be the only source of PFAS in the pup. Of the 8 PFASs analyzed (Σ_8PFAS), 7 were found in all samples; therefore, milk is a source to PFASs in pups. Perfluorooctane sulfonate was the dominant PFAS in all samples. Mean Σ_8PFAS concentrations were 6.0 ng/g protein (36 ng/g wet wt) in maternal plasma, 0.77 ng/g protein (3.2 ng/g wet wt) in milk, and 12 ng/g protein (66 ng/g wet wt) in pup plasma. Measured concentrations in plasma were within ranges previously reported from other seal species, below known toxicity thresholds for experimental rodents. Individual PFASs differed in transfer efficiency from mother to pup, depending on carbon chain lengths, with the lowest relative transfer for the intermediate-chained PFASs (C_9–C_{10}). The results show maternal transfer of PFASs via both milk and the placenta, of which placental transfer is the dominant pathway. *Environ Toxicol Chem* 2017;36:763–770. © 2016 SETAC

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INTRODUCTION

Perfluoroalkyl substances (PFASs), such as perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs), are globally distributed, persistent contaminants and remain in the environment for decades. They have different physicochemical properties from the legacy persistent organic pollutants; they are amphiphilic, meaning that they tend to bind to proteins, as well as being associated to lipids [1]. This leads to high levels of these substances accumulating in the tissues of wildlife and humans [2,3]. Despite their use since the 1950s, quantification of PFASs in the environment and in wildlife was only made possible around the year 2000 by advances in analytical techniques [4]. Although the understanding of the prevalence of these compounds has increased rapidly, leading to regional and international bans and phasing out of certain long carbon-chained PFASs, there is an urgent need for more knowledge about PFASs in the environment and their mechanisms of exposure, bioaccumulation, and toxicity [5].

Arctic marine ecosystems are exposed to contaminants via long-range transport of pollutants, including PFASs [6]. Marine predators at high trophic levels in the food web, such as marine mammals, are vulnerable to persistent contaminants because biomagnification results in increasing concentrations with increasing trophic level in the food web [2,7].

In mammals, some environmental contaminants are transferred from mothers to their young via the placenta and the milk [8]. Young mammalian neonates are therefore exposed to contaminants both prenatally and postnatally. This is of great concern because developing mammals have a reduced ability to metabolize and excrete xenobiotics and are generally considered to be more susceptible to toxic effects compared with adults [9]. In human studies, PFASs have been detected in cord blood, milk, and blood of nursing children, demonstrating both prenatal and postnatal transfer [8,10]. Several experimental studies on rodents have also reported maternal transfer of PFASs to the offspring via both the placenta and milk [11]. There are, however, no studies on maternal transfer of PFASs in marine mammals, despite the high levels of PFASs found in these animals [12–14].

The aim of the present study was to quantify PFASs in hooded seal (*Cystophora cristata*) mother–pup pairs to determine the occurrence and patterns of maternal transfer of PFASs in this species, including the role of milk in this process. The hooded seal was chosen in the present study because it has the shortest nursing period of any marine mammal (3–4 d), in which the pups have extreme growth rates (4–7 kg/d). The hooded seal milk is extremely energy-rich, with 60% to 70% lipids. The pup does not feed other than milk during the nursing period, and prenatal and postnatal maternal transfer is thus the only source of contaminants for the pup during this period [15]. Additionally, the hooded seal is a high–trophic level predator in the Arctic marine food web and therefore susceptible to high accumulation of contaminants, including PFASs [2]. Occurrence and maternal transfer of several halogenated organic pollutants and their metabolites have previously been reported in hooded seals [16–18].

This article includes online-only Supplemental Data.

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MATERIALS AND METHODS

Sampling

Lactating hooded seal mothers ($n = 15$) and their 1-d-old to 4-d-old pups were live-captured in March 2008 in the West Ice, east of Greenland (approximately 73.3°N,14.5°W; see Gabrielsen et al. [16] for capturing/sampling details). Blood was collected and centrifuged in the field to separate plasma. Milk was collected after an intramuscular injection of 20 IU oxytocin from 9 of the 15 mothers. Estimated pup age based on developmental stage, sex of the pups, and body masses of pups and mothers were recorded [16]. All animal handling was performed following the principles and guidelines and by permit from the Norwegian Animal Research Authority.

PFAS analysis

The PFASs were analyzed at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences. The plasma and milk samples were analyzed for 2 PFSAs (perfluorohexane sulfonate [PFHxS] and perfluorooctane sulfonate [PFOS]) and 6 PFCAs (perfluorooctanoic acid [PFOA], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], perfluoroundecanoic acid [PFUdA], perfluorododecanoic acid [PFDoA], and perfluorotridecanoic acid [PFTrDA]), summarized as Σ_8PFAS.

Briefly, the method was as follows: internal standards (^{13}C-labeled equivalents; Wellington Laboratories) were added prior to extraction with methanol, cleanup was accomplished using active carbon (EnviCarb), and samples were analyzed by separation of compounds on a high-performance liquid chromatograph with a Discovery C18 column connected to a C18 precolumn (Supelco; Sigma-Aldrich) and detection with liquid chromatography–tandem mass spectrometry (API 3000, LC–MS/MS System). The internal standards were used to produce a standard curve from which concentrations of the native ^{12}C PFASs were calculated. Concentrations were calculated from the chromatographic data using the instrument control and data-processing program Analyst Software, Ver 1.6. The limits of detection of the PFAS compounds were calculated using 3 times the signal-to-noise ratio found in the samples, unless a higher signal was recorded in the blank samples. A more detailed description of the method is found in the Supplemental Data.

Quality assurance

For each sample series (1 series with 9 milk samples, 1 series with 30 plasma samples), a relative recovery rate was calculated from samples of low-contaminated material (mixed serum from dog and cat) spiked with known standards. The relative recovery rate for PFASs ranged from 88% to 109% in the milk samples and from 80% to 132% in the plasma samples. For each series, there were 3 controls of serum (Arctic Monitoring and Assessment Program ring test) and 3 blanks, consisting of internal standards and solvents. The mean blanks were subtracted from the samples where positives were detected. The control samples were satisfactory (Supplemental Data, Table S1).

Quantification of lipids and protein in milk and plasma

The lipid content (percentage) was determined gravimetrically in milk (present study) and plasma [16]. Protein content was determined using a modified Lowry's method [19] at the Department of Biosciences, University of Oslo, Oslo, Norway. The protein assay is a 2-step colorimetric assay based on the reaction of proteins with an alkaline copper tartrate solution and a Folin reagent. The blue end product of the reaction was measured at 750 nm on a plate reader, and the protein concentration was calculated using a protein standard to make a standard curve.

Data treatment

In the same way that lipid content of a sample can affect the level of lipophilic contaminants, the protein content can affect the amount of proteinophilic contaminants in a sample [20]. Therefore, protein normalized concentrations (nanograms per gram protein) were used when comparing PFASs across matrices (plasma and milk) because of differences in protein concentrations between milk and plasma (Table 1). Wet weight data were used in the statistical analysis when comparing plasma of mothers and pups, as these did not differ in protein level or lipid content (Tukey's honest significant difference, $p = 0.8$; Table 1).

The ratios of PFAS concentrations between pups and mothers and between plasma and milk were calculated to examine relative concentrations of individual PFASs, related to their carbon chain length.

Statistical analyses

The program R (Ver 3.1.2) was used for all statistical analyses. Data were log-transformed prior to data analyses to reduce deviation from normality and homogeneity of variance. Normal distribution tested significant with Shapiro-Wilk's test after transformation. The α level was set to 0.05, and all tests were 2-tailed.

There was no difference in plasma PFAS concentrations of the mothers from which milk samples were obtained ($n = 9$) compared with all mothers that were sampled ($n = 15$) (Hotelling's T^2, $p = 0.9$; Hotelling's T^2 is the multivariate equivalent of the Student t test [21]). Thus, we assumed that the PFAS concentrations in the milk of the 9 sampled mothers were representative of the whole sample group ($n = 15$). Because there was no difference in PFAS concentrations between male and female pups (Hotelling's T^2, $p = 0.5$), the sexes were pooled for the analyses (data for male and female pups can be found in Supplemental Data, Table S2).

Multivariate principal component analyses were performed to explore relationships in concentrations and patterns of PFASs in the plasma and milk samples. Redundancy analyses were carried out to relate the structure in the PFAS variance to the explanatory variables. Multivariate statistics is a common tool in ecotoxicology [21]. In brief, the indirect ordination method, principal component analysis, extracts ordination axes that minimize the total residual sum of squares among all the response variables and assigns scores to the individual samples that are linear combinations of the variables. Principal component 1 (x axis) accounts for the largest part of the variance among the samples, whereas principal component 2 (y axis) is uncorrelated to principal component 1 and accounts for the largest part of the remaining variance among the samples. Variables are presented as arrows (or loadings), and the direction and length of arrows indicate, respectively, strength and increasing variance. The angles between the arrows indicate correlations (or covariance) between the variables. An angle of 90° means that the variables are not correlated, whereas a small angle indicates a correlation between the variables. The direct ordination method, redundancy analysis, adds further constraint on the

Table 1. Perfluoroalkyl substance concentrations, protein content, and lipid content in maternal plasma, pup plasma, and milk samples of hooded seal mother pup pairs from the West Ice (2008)

	Plasma mothers ($n = 15$)				Plasma pups ($n = 15$)			Milk mothers ($n = 9$)			
PFAS (ng/g wet w)	LOD (plasma)	Min–max	Median	Mean[a] ± SD	Min–max	Median	Mean[a] ± SD	LOD	Min–max	Median	Mean[a] ± SD
PFHxS	0.058	0.256–1.89	0.696	0.845 ± 0.49	0.483–5.01	2.60	2.90 ± 1.1	0.263	0.394 ($n = 1$)	n.d.	n.d.
PFOS	0.023	8.51–24.2	12.2	13.4 ± 4.2	6.99–59.8	28.3	30.4 ± 13	0.427	0.540–2.68	1.01	1.24 ± 0.74
PFOA	0.013	0.0250–0.928	0.278	0.312 ± 0.23	0.0410–1.86	0.336	0.537 ± 0.49	0.033	0.0680–0.290	0.189	0.189 ± 0.068
PFNA	0.036	1.09–4.40	1.99	2.29 ± 1.0	0.794–3.86	1.33	1.61 ± 0.81	0.033	0.0830–0.373	0.115	0.167 ± 0.097
PFDA	0.068	1.27–5.50	2.30	2.75 ± 1.2	0.978–3.28	1.46	1.61 ± 0.65	0.030	0.0600–0.301	0.133	0.163 ± 0.080
PFUdA	0.045	5.02–17.9	8.51	9.71 ± 3.4	4.43–22.4	11.1	11.9 ± 4.6	0.026	0.386–1.34	0.611	0.741 ± 0.36
PFDoA	0.120	0.787–2.57	1.41	1.45 ± 0.42	0.905–5.45	3.37	3.38 ± 1.1	0.089	0.108–0.253	0.161	0.182 ± 0.056
PFTrDA	0.130	2.73–9.96	4.72	5.07 ± 1.6	3.33–22.0	12.6	13.1 ± 4.0	0.089	0.336–0.964	0.463	0.512 ± 0.20
Protein (ng/mL)		62.3–89.5	73.1	74.3 ± 7.7	55.9–104	66.4	71.2 ± 14		28.8–80.3	55.2	52.7 ± 17
Lipid (%)		0.490–0.980[b]	0.680[b]	0.698 ± 0.14[b]	0.610–2.92[b]	1.18[b]	1.40 ± 0.69[b]		62.9–78.2	66.5	68.8 ± 4.9

[a]Arithmetic mean.
[b]Published in Gabrielsen et al. [16].
PFAS = perfluoroalkyl substances; LOD = limit of detection; SD = standard deviation; PFHxS = perfluorohexane sulfonate; PFOS = perfluorooctane sulfonate; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFUdA = perfluoroundecanoic acid; PFDoA = perfluorododecanoic acid; PFTrDA = perfluorotridecanoic acid; n.d. = not detected.

analysis by constraining the sample scores to be linear combinations also of the explanatory variables. Thus, it is a powerful tool to test the significance of explanatory variables of the observed structure in the variance of the response variables.

In principal component analysis and redundancy analysis of PFAS concentrations, log-transformed concentrations (wet weight or protein normalized) of PFHxS, PFOS, PFOA, PFNA, PFDA, PFUdA, PFDoA, and PFTrDA were response variables. Explanatory variables (percent lipid, protein concentration, lactation duration [= pup age], body mass, Σ_8PFAS concentration in maternal plasma, and Σ_8PFAS concentration in milk) were entered as passive variables in the principal component analysis plots. Passive variables do not affect the ordination but are projected onto the unconstrained axes, visualizing correlations between response and explanatory variables. Explanatory variables were standardized to unit variance. The significance of the explanatory variables in separating the samples in the multivariate ordination space was analyzed by forward permutation tests.

Based on the results from principal component analysis, paired t tests were used to analyze differences in PFAS concentrations in mother–pup pairs, as these were not independent samples.

RESULTS AND DISCUSSION

The PFASs were detected and quantified in both plasma and milk from hooded seal, documenting for the first time PFASs in seal milk and maternal transfer in mother–pup pairs in marine mammals.

PFAS concentrations

All 8 PFASs analyzed were detected in all plasma samples from both mothers and pups (see Table 1 for wet w; see Supplemental Data, Table S3, for protein normalized concentrations). Seven PFASs were detected in all milk samples, whereas PFHxS was detected in only 1 milk sample. Arithmetic mean ΣPFAS concentrations were 6.0 ng/g protein in maternal plasma, 0.77 ng/g protein in maternal milk, and 12 ng/g protein in pup plasma, demonstrating a substantial transfer from mothers to pups.

Wet weight PFOS concentrations in hooded seal maternal plasma in the present study were approximately 3 times lower than those in harbor seal (*Phoca vitulina*) plasma from Svalbard sampled in 2009 and 2010 [13] and in the lower range of levels in gray seal (*Halichoerus grypus*) plasma from the Canadian Arctic sampled in the 1990s [6]. The plasma PFOS wet weight concentrations in hooded seal mothers were approximately 2 times higher than levels in adult ringed seal (*Pusa hispida*) plasma from the Canadian Arctic sampled in the 1990s and the Norwegian Arctic sampled in 2009 and 2010 [6,22], as well as northern fur seals (*Callorhinus ursinus*) from Alaska sampled in 1995 [23], and 10 times higher than levels in bearded seals (*Erignathus barbatus*) from the Canadian Arctic sampled in 2004 [22]. Because the use and releases of PFOS were reduced after 2000, the variations in the concentrations in the different studies may be related to temporal changes in exposure levels and/or proximity to primary sources. In addition, these other species are mostly mid–trophic level species, whereas the hooded seal is a high–trophic level species; thus, higher PFAS levels would be expected in hooded seal if other parameters were the same.

For the other PFASs, there are fewer studies for comparison. However, the concentrations in the present study were within the range of wet weight concentrations in adult harbor seal females from Svalbard in 2009 and 2010 for PFHxS, PFOA, PFNA, PFDA, and PFTrDA [13], whereas concentrations of PFNA and PFDA in adult hooded seals in the present study were approximately 2 to 3 times higher than concentrations in ringed seals from the Canadian Arctic sampled in 2004 [22]. It should be noted that the wet weight concentrations of all PFASs analyzed in the present study, except for PFDA and PFTrDA, were 17 to 38 times higher in the plasma of female polar bears (*Ursus maritimus*) sampled in Svalbard in 2008 than in the hooded seal mothers [12]. This demonstrates the high biomagnification potential of these substances, except for PFDA and PFTrDA, within the Arctic marine food chain.

To our knowledge, there is only 1 other published study of PFAS concentrations in blood from seal pups in the Arctic [23]; that study sampled northern fur seals from Alaska in 1995 and reported approximately 5 times lower PFOS concentrations than the hooded seal pups from the present study. The increase in PFOS levels to the late 2000s may reflect the increased use of

these substances until phasing out and regulations were implemented; however, no temporal PFAS trend studies of Alaskan northern fur seal or Greenland hooded seal are presently known.

Developmental effects have been linked to PFAS exposure; however, the levels reported in the present study were lower than levels of reported effects in exposure studies on rats and mice [24]. Still, developmental effects of PFASs are often associated with reduced birth weight; and because the hooded seal is so dependent on an intense weight gain during the 3 d to 4 d of lactation to be able to survive the long fasting period [15], a reduction in birth weight could be especially detrimental to this species.

PFASs in plasma and milk

Concentrations (nanograms per gram protein) of PFOS, PFNA, PFDA, PFUdA, PFDoA, and PFTrDA were higher in plasma than in milk; for PFOA, however, there was no difference between maternal plasma, pup plasma, and milk samples (Figure 1; Supplemental Data, Table S3). The generally high concentrations in maternal plasma, compared with those milk, are in accordance with previous studies in humans [10] and support that PFASs bind with high affinity to blood proteins, which probably limits their ability to partition into milk [25]. This emphasizes the importance of protein normalizing PFAS concentrations when comparing matrices with different protein content [20]. The comparable levels of PFOA in maternal plasma, pup plasma, and milk samples are in accordance with previous studies, showing that the postnatal exposure of PFOA through lactation was higher compared with exposure of other PFASs [26]; that is, the partitioning to milk is higher for PFOA compared with other PFASs, and thus so is the postnatal exposure to the pups.

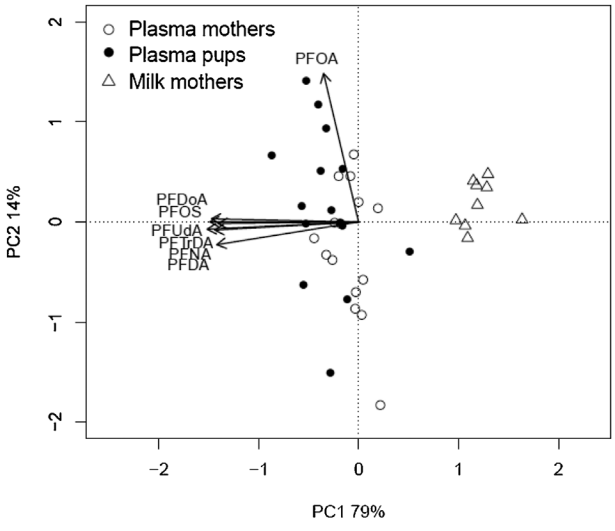

Figure 1. Biplot of perfluoroalkyl substance (PFAS) concentrations (ng/g protein) in maternal plasma ($n = 15$), pup plasma ($n = 15$), and milk ($n = 9$) samples from hooded seals. Sample scores (equal numbers are mother–pup pairs) and PFAS loadings are extracted on the principal components with the percentage of the total variance explained by each principal component. The first 2 principal components extracted (PC1 and PC2) accounted for 93% of the total variance among samples. Perfluorohexane sulfonate was not included in the principal component analysis because of low detection in milk samples (less than the limit of detection in 8 of 9 samples). Direction and length of arrows indicate, respectively, strength and increasing variance of loading. PFDA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate; PFTrDA = perfluorotridecanoic acid; PFUdA = perfluoroundecanoic acid.

The PFHxS concentration was below the limit of detection in 8 of the 9 milk samples. This is in accordance with results from a human study in South Korea, where PFHxS was detected in only 6% of the milk samples [27]. In a study on breast-feeding as an exposure pathway for PFASs in humans, PFHxS was the only PFAS analyzed that did not increase in concentration in infants during the breast-feeding period [8]. In general, PFHxS is infrequently detected in biological tissues in wildlife studies [28] and has a lower bioaccumulation potential compared to most of the other PFASs reported in the present study [29]. This might explain the relatively low concentrations of PFHxS in the plasma samples of hooded seals in the present study and the low detection frequency in the milk samples.

The concentrations of PFHxS, PFOS, PFDoA, PFUdA, and PFTrDA were higher in pup plasma than maternal plasma, whereas concentrations of PFDA and PFNA were higher in maternal plasma than pup plasma (all paired t test, $p < 0.001$). Previous findings of PFASs in mammalian mother–offspring studies are scarce and variable. In an experimental rodent study, PFAS concentrations were generally higher in mothers than in neonates [11]. However, a similar pattern to that found in the present study, with lower PFOS concentrations in adult females than young pups, was reported in various pinniped and cetacean species [23,30,31]. Thus, experimental rodents were not representative of the maternal transfer process in hooded seal, whereas data from marine mammals were in line with the present study. This illustrates the importance of considering the species' different adaptations with regard to physiological and reproductive life history traits.

Higher concentrations of PFHxS, PFOS, PFDoA, PFUdA, and PFTrDA in pups compared with mothers imply that the elimination capacity of these PFASs for adult hooded seal females may be relatively high. This includes metabolism and excretion as well as placental transfer in utero and postnatal milk transfer; pups, however, lack urinary and fecal excretion capabilities during the fetal stage [32]. Furthermore, the hooded seal mother fasts during the entire lactation period and is therefore not exposed to additional dietary PFAS during this period, in contrast with opposed to the pups, who are the recipients of PFASs from the milk (>10 L/d). Consequently, the hooded seal pups may be exposed to large amounts of contaminants both prenatally and postnatally [18], which the findings in the present study confirm for PFASs.

PFAS patterns

The predominant PFAS in all matrices was PFOS, which comprised 37% of Σ_8PFAS in maternal plasma, 39% of Σ_7PFAS in milk, and 45% of Σ_8PFAS in pup plasma (Supplemental Data, Figure S1). This is in accordance with the high relative occurrence of PFOS compared with other PFASs in previous wildlife studies [2,3], including studies on pinnipeds [13,22]. Although PFOS was the predominant PFAS in the present study, the relative contribution of PFOS to Σ_8PFAS was lower than for most other studies. Perfluorooctane sulfonate comprised 75% to 80% of ΣPFAS in ringed seal and white whale (*Delphinapterus leucas*) liver [7] and 60% to 90% in plasma of harbor seals [13,33]. Because these studies included a higher number of PFASs than the present study, the lower contribution of PFOS to ΣPFAS in the present hooded seals was not explained by the number of congeners. The present data might be the result of the phasing out of PFOS around the year 2000, although several of the mentioned studies

used samples collected at approximately the same time as the present study and still reported a higher relative occurrence of PFOS. This might indicate that there are species-specific differences in toxicokinetics and accumulation for different PFASs, leading to different relative patterns, as also suggested for differences in pattern between seals and whales [34]. The pattern may also reflect differences in the PFAS composition in the diet or the local environment where the animals reside.

Maternal transfer of PFASs

All PFASs detected in maternal plasma were also detected in pup plasma. Because the samples were collected during the nursing period, when the pups spend the entire lactation period on ice floes without entering the water [15], maternal transfer during lactation or prenatally via the placenta was the only source of PFASs to the pups. Thus, PFASs in pup plasma confirm maternal transfer of PFASs in hooded seals, which is in accordance with previous findings from other mammalian wildlife studies on polar bears [12].

Constrained multivariate analyses (redundancy analyses) were performed separately for maternal plasma, pup plasma, and milk samples to analyze the variance of PFAS concentrations of each matrix to explanatory variables. In maternal plasma, none of the explanatory variables (lactation duration, body mass, protein, lipid) were found to have a significant effect on the overall PFAS variation (redundancy analysis, $p > 0.05$; Figure 2a).

In pup plasma, PFAS concentrations increased with the Σ_8PFAS concentration in the maternal plasma, which was the only significant explanatory variable and explained 31% of the total variation in PFAS concentration (redundancy analysis, $p = 0.004$; Figure 2b). The finding that neither ΣPFAS concentration in milk nor lactation duration was an additional significant predictor for the PFAS concentrations in the plasma of pups suggests that placental transfer is of higher importance for PFAS exposure in pups. Thus, although the ordination diagram suggests that PFAS concentrations in pups are negatively correlated to lactation duration and body mass of the pup, these variables did not significantly add to the explanation of the PFAS concentrations in pups, and growth dilution and lactational transfer are thus interpreted as negligible. This is because of low PFAS concentrations in milk and the fact that the body mass increase during the short lactation period is mainly blubber and not muscle/protein gain. These findings were not surprising since the hooded seals have a high energetic prenatal investment during the gestation period, which results in a highly precocial pup. Hooded seal pups are considered to be the most precocial of all Phocidae pups, and the energy density in newborn hooded seal pups is the highest of any neonatal mammal [15]. All of the above favors prenatal transfer as a dominant source of PFASs to the pups.

The PFAS concentrations in milk increased with ΣPFAS concentrations in the mothers' plasma, the lactation duration, and the protein concentration in the milk, which together explained 83% of the total variance (redundancy analysis, $p < 0.001$; Figure 2c). This indicates that the magnitude of contaminant transfer via the milk increased with contaminant body burden of the mother and nursing duration and is in accordance with human maternal transfer of PFASs [8] and other persistent organic pollutants such as polychlorinated biphenyls and polybrominated diphenyl ethers [18]. In domesticated animals with suckling offspring, the amount of protein mobilization from the liver and other tissues increases as a response to the increased nutritional demand to uphold the ongoing milk production [35]. Thus, mobilization of proteins during the lactation period may explain the increase in PFASs and proteins in the mothers' milk.

Partitioning of PFASs into milk may be a result of binding either to milk proteins or to the surface of lipids [36]. The former mechanism may explain why the protein concentration in milk was correlated with several of the PFASs: the more milk proteins, the more incorporation of PFASs into the milk. Indeed, evidence is accumulating that the toxicokinetics of PFASs within an organism is affected by several proteins [20], including albumin [25]. Still, milk was not the major source of PFASs to the pups.

Transfer efficiency for different PFASs

The maternal plasma-to-cord blood contaminant concentration has been suggested to be a good measure of transplacental transfer efficiency [37]. In the present study, the ratios of plasma concentrations in pups to those in mothers ($plasma_{pup}$:$plasma_{mother}$) were used as a measure of maternal transfer efficiency because cord blood was not obtainable. Note that this ratio is not only a measure of transplacental transfer efficiency; the PFASs in pup plasma are also the result of lactational transfer during the 1 d to 4 d since birth.

The $plasma_{pup}$:$plasma_{mother}$ ratio was higher for C_6-sulfonates than for C_8-sulfonates (Figure 3a). For the carboxylates, a *U*-shaped pattern was found in the maternal transfer efficiency, with a decreasing $plasma_{pup}$:$plasma_{mother}$ ratio with increasing chain length from C_8 to C_{10} and an increasing ratio with increasing chain length from C_{10} to C_{13} (Figure 3a). The differences in maternal transfer efficiency among carboxylates with different carbon chain lengths are in accordance with previous studies [27], including a study on polar bears [12]. In mammals, binding affinities of PFASs to proteins are known to increase with increasing carbon chain length [25]. Since binding to proteins may limit the transfer across barriers, this could explain the differences in $plasma_{pup}$:$plasma_{mother}$ ratio for the sulfonates but not the *U* shape for the carboxylates. However, when protein binding affinities of PFASs were tested, binding affinities to blood proteins increased from C_2 to C_8 and decreased from C_9 to C_{13} [38]. The decrease in protein binding from C_9 to C_{13} is likely the result of steric hindrances associated with longer and more rigid perfluoroalkyl chains. Thus, the *U*-shaped trend of transfer efficiency for the carboxylates observed in the present study may be related to binding affinities to proteins. Another explanation may be a selective excretion through elimination routes for different PFASs or differences in distribution to various organs [39]. In Baikal seals (*Pusa sibirica*), higher concentrations of PFNA (C_9) and PFDA (C_{10}) were reported in liver than in serum [30]. The aqueous solubility of C_6 to C_8 PFCAs appears to facilitate rapid urinary excretion, whereas the relative hydrophobicity of the longer-chained carboxylates appears to favor biliary enterohepatic recirculation [40]. This suggests that PFNA and PFDA are more efficiently retained in the liver and less stable in serum than, for instance, PFOS (C_8) and, hence, that a compound-specific persistence and retention of PFASs in liver is plausible. Retention of PFNA and PFDA in liver may explain why these mid–chain length PFASs seem to be less efficiently transferred via the placenta from mother to pup in hooded seals.

The similar pattern between the $plasma_{pup}$:$plasma_{mother}$ ratio (Figure 3a) and the ratio of concentrations in the mothers' milk to concentration the mothers' plasma ($milk_{mother}$:$plasma_{mother}$) (Figure 3b) indicates that the PFASs that are efficiently

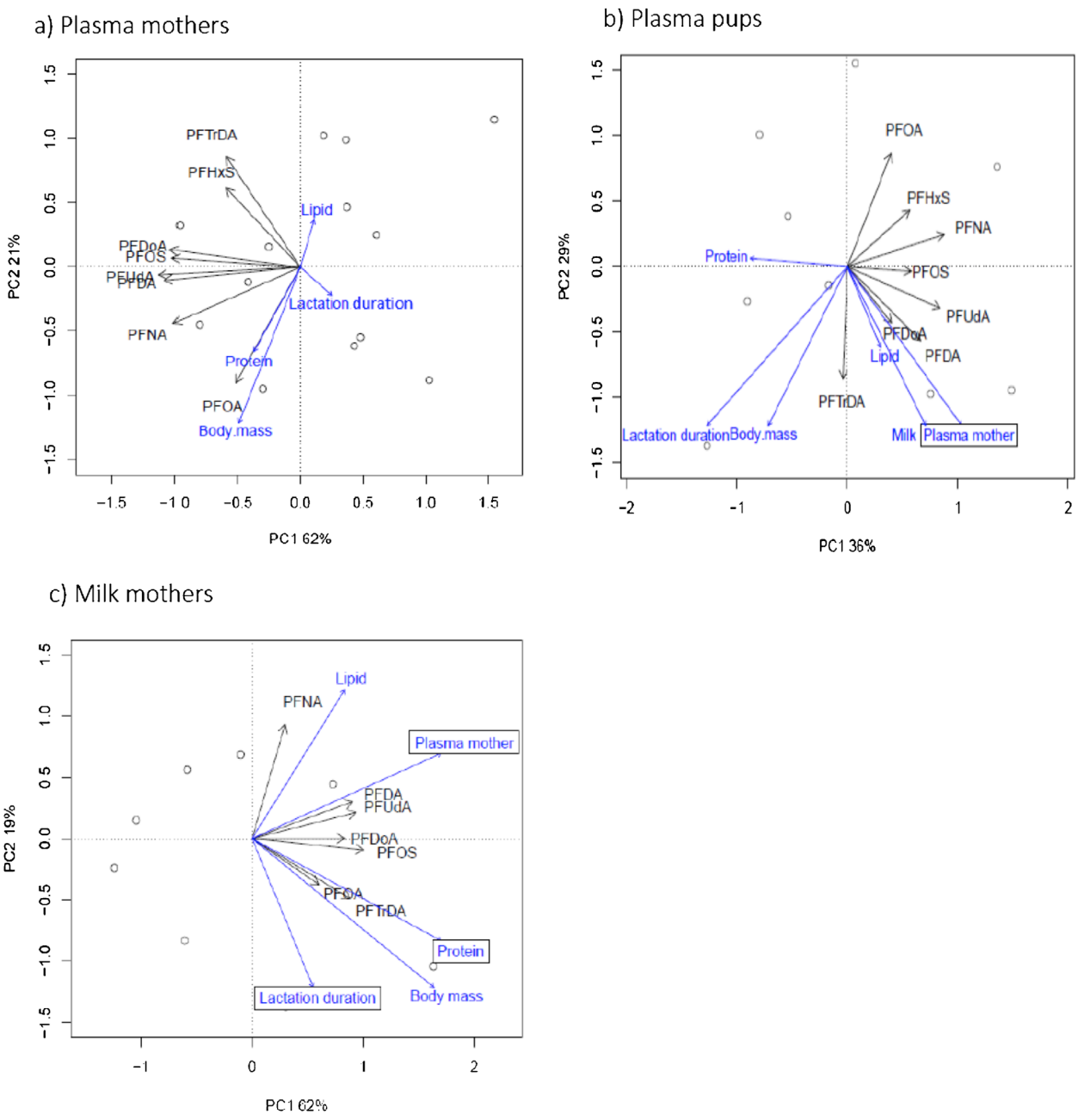

Figure 2. Biplot of perfluoroalkyl substance concentrations (ng/g wet wt) in hooded seal (**a**) maternal plasma samples ($n = 15$), with the explanatory variables lactation duration, body mass, protein, lipid); (**b**) pup plasma samples ($n = 9$), with explanatory the variables lactation duration, maternal plasma (plasma mother), milk, body mass, protein, lipid; and (**c**) milk samples ($n = 9$), with the explanatory variables lactation duration, plasma mother, body mass, protein, lipid. Explanatory variables are projected as passive arrows (blue arrows), and significant explanatory variables (test = permutation test) are marked with boxes. The percentage of the total variance explained by each principal component is given on each axis. Principal component analyses were based on logarithmically transformed concentrations. Direction and length of arrows indicate respective strength and increasing variance of loading. PC1 and PC2 = principal components 1 and 2; PFDA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate; PFTrDA = perfluorotridecanoic acid; PFUdA = perfluoroundecanoic acid.

transferred from the mother to the pup are also more efficiently transferred from the maternal plasma to the milk. This indicates similarity in transfer efficiency or, in other words, a similar limitation of transfer from maternal plasma to milk and from maternal plasma to pup plasma via the placenta. The different patterns in transfer ratios between $plasma_{pup}$:$plasma_{mother}$ (Figure 3a) and $plasma_{pup}$:$milk_{mother}$ (Figure 3c) along with elevated $plasma_{pup}$:$milk_{mother}$ ratios emphasize the insignificance of lactational PFAS transfer.

When comparing the 2 groups of PFASs, the sulfonates and the carboxylates, there was a higher relative occurrence of sulfonates (PFHxS and PFOS) in pup plasma compared with maternal plasma and milk (Figure 4). This might indicate a more efficient maternal transfer of PFASs containing a sulfonate group compared with PFASs with a carboxylic group. In mother–pup pairs of harbor seals from the northwest Atlantic, a similar pattern was found with a higher transfer efficiency for the sulfonates compared with the carboxylates [31]. However, previous studies on blood protein binding affinities show that sulfonates have stronger binding affinity than carboxylates with equivalent chain lengths [38]; and if protein binding inhibits transfer across the placenta, one would expect an opposite

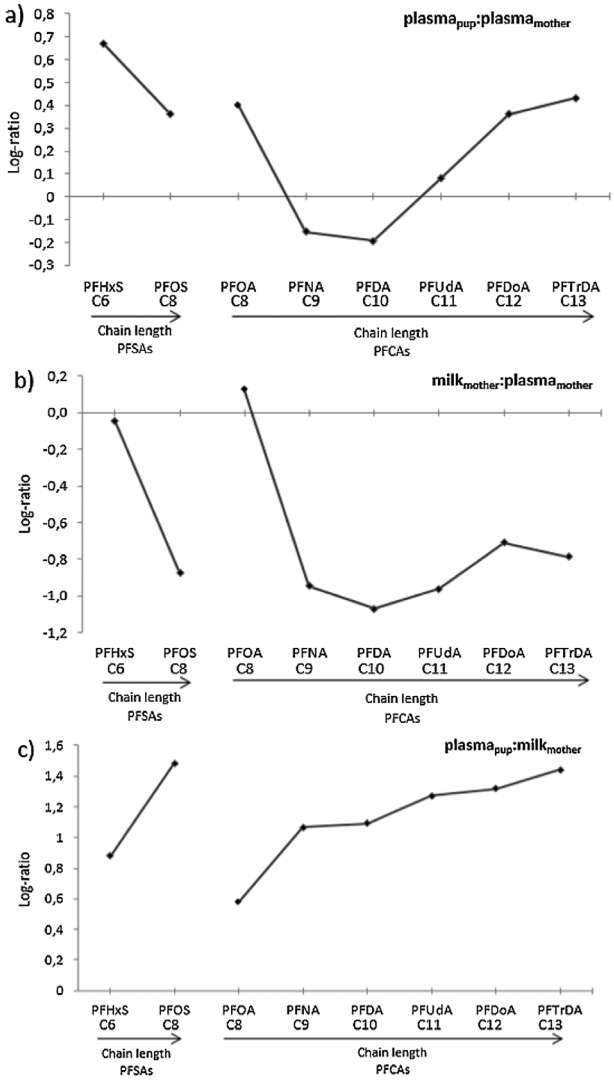

Figure 3. Log ratios of (**a**) perfluoroalkyl substance (PFAS) concentrations in pups' plasma to concentrations in mothers' plasma (plasma$_{pup}$:plasma$_{mother}$), (**b**) PFAS concentrations in mothers' milk to concentrations in mothers' plasma (milk$_{mother}$:plasma$_{mother}$), (**c**) and PFAS concentration in pups' plasma to concentrations in mothers' milk (plasma$_{pup}$:milk$_{mother}$) in hooded seals. Ratios were based on mean concentrations (ng/g wet wt for plasma:plasma ratios, ng/g protein for plasma:milk ratios). The C6-C13 labeling indicates the various PFAS compounds' carbon chain length. 0 indicates 1:1 relationship. PFCA = perfluoroalkyl carboxylate; PFDA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate; PFSA = perfluoroalkyl sulfonate; PFTrDA = perfluorotridecanoic acid; PFUdA = perfluoroundecanoic acid.

pattern. On the other hand, compound-specific binding to carrier proteins, such as transthyretins, which are involved in the transfer of thyroid hormones from mothers to the fetus across the placenta, could favor transplacental transfer of sulfonates with high binding affinity to transthyretin, such as PFHxS and PFOS [41]. This means that the greater binding affinity of specific sulfonates would in fact increase the transplacental transfer rate if they bind to this protein complex, as the results in the present study appear to indicate.

The present study is one of the first to report maternal transfer of PFASs in Arctic pinnipeds. Furthermore, we report the first values for PFASs in plasma from hooded seal mothers and pups

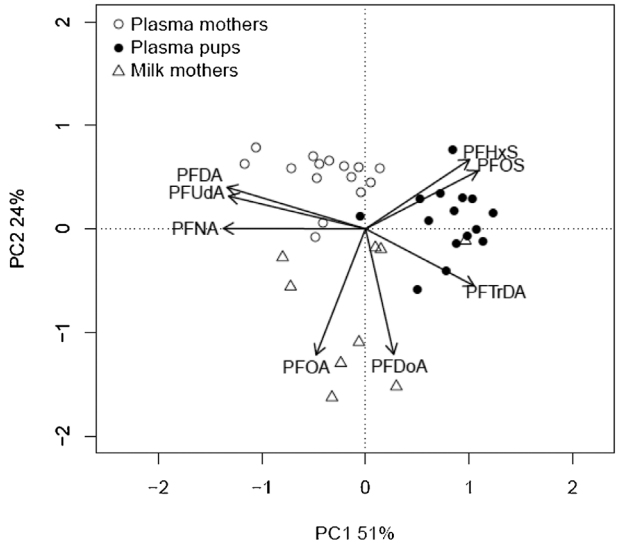

Figure 4. Biplot of perfluoroalkyl substance (PFAS) pattern (relative occurrence) in maternal plasma samples ($n = 15$), pup plasma samples ($n = 15$), and milk samples ($n = 9$) from hooded seals. Sample scores (equal numbers are mother–pup pairs) and PFAS loadings are extracted on the principal components with the percentage of the total variance explained by each principal component. Principal components 1 and 2 (PC1 and PC2) accounted for 75% of the total variance. The principal component analysis was based on the percentage of ΣPFAS concentration. Direction and length of arrows indicate respective strength and increasing variance of loading. PFDA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonate; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate; PFTrDA = perfluorotridecanoic acid; PFUdA = perfluoroundecanoic acid.

and the first PFAS levels in milk from any seal species. The results show maternal transfer of PFASs from hooded seal mothers to pups via both the placenta and the milk. There were different transfer ratios for PFASs with different chain lengths, most likely the result of protein binding affinity related to carbon chain length, as well as specific binding affinities. Plasma concentrations were generally higher in pups than in mothers and higher in plasma than in milk, supporting that binding to plasma proteins limits the partitioning into milk. Although the PFASs were found in milk, the results suggest lactational transfer as a minor source compared with placental transfer of PFASs.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3623.

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Data availability—The data and metadata are contained in the present article, and the statistical methods described and used are standard packages in the R project, freely available online.

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