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Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls

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ABSTRACT

The comparative accumulation of C_4–C_{15} perfluorinated sulfonates (PFSAs) and carboxylates (PFCAs), and several precursors (e.g., perfluorooctane sulfonamide, N-methyl-FOSA, and fluorotelomer unsaturated acids and alcohols) was examined in tissues (liver, brain, muscle, and adipose), plasma/red blood cells (RBCs) and whole egg clutches (yolk and albumen) of female herring gulls collected in 2010 from Chantry Island, Lake Huron of the Laurentian Great Lakes. Highest mean ∑PFSA concentrations were in yolk, followed by adipose, liver, plasma, muscle, RBCs, and brain. Highest mean ∑PFCA concentrations were in yolk, followed by brain, plasma, liver, RBC, adipose and muscle. PFOS accounted for >88% of ∑PFSA in all samples; the liver, plasma/RBCs, muscle and adipose PFCA patterns were dominated by C_8–C_{11} PFCAs, whereas C_{10}–C_{15} PFCAs in brain and yolk. Among PFSAs and PFCAs there is tissue-specific accumulation, which could be due to a number of pharmacokinetic processes.

1. Introduction

Poly- and perfluorinated compounds (PFCs) are man-made chemicals and have been found globally in wildlife including in birds (Houde et al., 2011). The bioaccumulative properties of perfluoroalkyl acids (PFAAs) such as sulfonates (PFSAs) and carboxylates (PFCAs), and increasing production over previous decades, has led to increasing exposure to birds and fish species to these compounds (Furdui et al., 2008; Holmstrom et al., 2005). Other factors, such as proximity to sources, have been shown to influence the concentrations of PFAAs in wildlife and fish (Furdui et al., 2007; Gebbink et al., 2009).

The distribution of PFCs in tissues within a whole animal has only been studied sporadically and generally in selected tissues and blood, including in harbor seals (*Phoca vitulina*) and harbor porpoises (*Phocoaena phocoena relicta*) from Europe (Ahrens et al., 2009; Van de Vijver et al., 2005, 2007), and ringed (*Pusa hispida*) and bearded seals (*Erignathus barbatus*) from the Canadian Arctic (Powley et al., 2008). In birds, such tissue distribution studies of PFSAs and PFCAs has been limited to only Norwegian glaucous gulls (*Larus hyperboreus*) and Swedish common guillemots (*Uria aalge*) (Holmström and Berger, 2008; Verreault et al., 2005), while Olivero-Verbel et al. (2006) reported on the tissue distribution of just PFOS and PFOSA in Columbian pelicans (*Pelecanus occidentalis*). In these studies, the highest concentrations of either ∑PFSA or ∑PFCA were found in plasma, liver or eggs compared to tissues such as brain, muscle or heart. Regardless, the PFSA and PFCA patterns in Norwegian glaucous gull and Swedish guillemot tissues were dominated by PFOS, and C_{11} (PFUnA) and C_{13} (PFTrA) PFCAs (Holmström and Berger, 2008; Verreault et al., 2005). Although studies are limited in biota, in liver and plasma, and in eggs in the case of birds, the presence of PFOS has been shown to be due to associations with proteins such as the fatty acid binding protein (Jones et al., 2003; Luebker et al., 2002). To our knowledge, the total PFAA amounts in tissue or whole body have only been reported for harbor seals (*P. vitulina*) from Germany (Ahrens et al., 2009), where blood and liver were shown to contain a combined total of 74% of the overall PFSA plus PFCA burden in the whole animal.

The maternal transfer of any PFC from female birds to their eggs has only been reported for PFSAs and PFCAs of varying chain length in Swedish guillemots and Norwegian glaucous gulls (Holmström and Berger, 2008; Verreault et al., 2005). In comparing the PFCA pattern in the Swedish guillemot liver and eggs, there was an enrichment of the longer chain length PFCAs in the egg relative to the liver; however, this was not observed in Norwegian glaucous gulls. PFOS has been associated with yolk proteins (Newsted et al., 2007), however, whether there is chain length dependent binding of PFCAs to yolk proteins has to our knowledge not been studied.

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Herring gull eggs have been used for PFC (including isomers in the case of PFOS) monitoring purposes in the Canadian and Norwegian environment (Gebbink et al., 2009, 2011; Gebbink and Letcher, 2010; Verreault et al., 2007), however, when comparing the PFC pattern in herring gull prey to gull eggs, differences were observed (Gebbink et al., 2009; Martin et al., 2004). The apparent lack of similarities in the PFC patterns are hypothesized to be an indication that pharmacokinetic processes such as selective retention or sequestering in tissues or body compartments (e.g., protein binding) occurs within the female herring gulls and/or selective maternal transfer determines the PFC patterns and levels in eggs. To better understand how PFCs are distributed and accumulatively compartmentalized in the body of Great Lakes female herring gulls, we determined PFSAs and PFCAs and several PFOSA, FTUCA and FTOH precursors in a comprehensive suite of tissues as well as blood components. Furthermore, we examined the maternal transfer of these same PFCs from the female gulls to her entire clutch of eggs, and the albumen and yolk compartments.

2. Experimental section

2.1. Standards and chemicals

The PFSAs [C_4 (PFBS), C_6 (PFHxS), C_8 (PFOS) and C_{10} (PFDS)], PFCAs (C_6–C_{14} chain lengths; PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA and PFTeA, respectively), 6:2, 8:2 and 10:2 FTUCA, and 6:2, 8:2 and 10:2 FTOH, and two FOSAs (PFOSA, NMeFOSA) standards, as well as sixteen labeled internal standards (IS) were obtained from Wellington Laboratories (Guelph, ON, Canada). The full chemical names and structures of all PFCs are detailed in Table S1. All solvents used were HPLC grade and purchased from Fisher Scientific (Ottawa, Canada).

2.2. Sample collection

Samples of organs and tissues, i.e., liver, blood, whole brain, muscle and adipose, were collected from female herring gulls ($n = 8$), euthanized by decapitation, from Chantry Island, Lake Huron (44°29′N 81°24′W) in April 2010 (See Fig. S1 for colony location). The blood samples were centrifuged on site to separate plasma from red blood cells (RBCs). All of these samples were frozen in liquid nitrogen on site (within 1 h of death). The complete clutches of eggs ($n = 17$) from the nests from the eight euthanized female herring gulls were collected (seven nests with two eggs, one nest with three eggs). The eggs were kept at ambient temperature, but not incubated, by placing them in a foam padded case. All of the frozen tissue and eggs were transported to Environment Canada's National Wildlife Specimen Bank (EC-NWSB) in Ottawa, Canada within 36 h of collection. Frozen samples were stored in the EC-NWSB at −40 °C prior to chemical analysis. Shortly after arrival at the EC-NWSB, the eggs were processed by separating and homogenizing the yolk and albumen and storing them at −40 °C until chemical analysis. Animal capturing, handling and euthanasia were approved by the Animal Care Committee for NWRC and Ontario region of the Canadian Wildlife Service (Environment Canada).

2.3. Perfluoroalkyl acid and precursor extraction and analysis

The PFC extraction, cleanup and analysis for all tissues, plasma, RBCs, and egg albumen and yolk were the same, and described in Gebbink et al. (2009) and Chu and Letcher (2008). Briefly, samples (0.2–2 g depending on the tissue) were spiked with labeled internal standards (see Table S1 for IS detail), extracted and subsequently cleaned up and fractionated using Waters Oasis WAX solid phase extraction (SPE) cartridges. The first fraction contained FTOHs and FOSAs, the second fraction contained PFSAs, PFCAs and FTUCAs. The separation of the target compounds in both fractions was carried out on a Waters 2695 HPLC equipped with an ACE 3 C_{18} analytical column (50 mm × 2.1 mm I.D., 3 μm particle size, Advanced Chromatography Technologies, Aberdeen, UK), and coupled to the HPLC was a Waters Quattro Ultima triple quadrupole mass spectrometer (Waters, Milford, MA, USA). For neutral PFCs in fraction 1, atmospheric pressure photoionization (APPI) was used in negative mode with krypton UV lamp. An electrospray ionization (ESI) source in negative mode was used for acidic PFCs in fraction 2. Details on mobile phases, source, desolvation, and probe temperature are provided by Gebbink et al. (2009). Quantification was performed using an internal standard approach. The calibration curve of PFTeA was used for PFPA quantification since PFPA standard was unavailable at the time of this study. Where no labeled standards were available, labeled ISs with the closest retention time were used. Since an isotope dilution quantification approach was used, the concentrations were inherently recovery-corrected.

2.4. Quality control and data analysis

The recovery efficiency of all the PFC internal standards was generally greater than 77%. For every block of samples ($n = 10$), an NWRC in-house reference material of double-crested cormorant egg pool (based on eggs collected in 2003) was analyzed to assess reproducibility of the method. For both $\sum$PFSA (C_6, C_8, C_{10}) and $\sum$PFCA (C_8–C_{15}), good reproducibility was obtained with a % relative standard deviation (%RSD) of 20% ($n = 10$).

For the majority of the tissues, blood, and yolk, a normal distribution of PFSA and PFCA concentrations were observed after performing the Shapiro–Wilks test on normal and log-transformed data. Thus, the mean concentration data was reported as arithmetic means. Differences in the pattern of PFCAs in the tissues, plasma, RBCs and yolk were investigated using principal components (PC) analysis using correlation matrices. PC analysis was conducted on percent composition data. For example, percent composition data for individual PFCAs (C_6–C_{15}) were calculated relative to $\sum$PFCA concentrations. Arithmetic mean concentrations were calculated only for samples with >60% detection of individual PFCs in the tissues or yolk. For samples with PFAA concentrations less than method limits of quantitation (MLOQs), a randomly generated value was assigned between zero and MLOQ for statistical purposes. MLOQs for PFSAs and PFCAs were <0.1 and <0.05 ng/g ww, respectively. The differences in patterns and sum concentrations in the tissues and eggs were analyzed using a single factor analysis of variance (ANOVA), followed by a Tukey's Honestly Significantly Different *post hoc* test. A general linear model (GLM) was used to determine the significance and Pearson's coefficient of the PFOS, PFOA, PFNA and PFUnA correlations in the plasma and RBCs. The statistical package utilized was Statistica® (StatSoft, Tulsa, OK, U.S.A.) and α was set at 0.05.

2.5. Burden estimates of perfluorinated compounds in herring gull tissues and eggs

To determine the extent of maternal transfer of PFCs and amount burdens in tissues, amounts of $\sum$PFSA and $\sum$PFCA were estimated in the eggs, liver, blood, and brain. Mean masses of the whole body, eggs, and brain were 946 ± 15 g, 82 ± 2 g (29% yolk, 71% albumen), and 5.7 ± 0.1 g, respectively. Livers mass was not recorded; however, a significant relationship ($n = 109$, $r = 0.478$, $p < 0.0001$) has been observed between body mass and liver mass for breeding female herring gulls (Mr. Glen A. Fox, personal communication). This relationship was used to estimate liver mass

(25.3 ± 0.5 g, 2.7% of body mass) for the eight female herring gulls. Blood samples were separated into plasma (~60% v/v) and red blood cells (~40% v/v). Herring gull whole blood volume was estimated at 60 mL/kg body mass (Clark et al., 1987; Hoysak and Weatherhead, 1991), resulting in a blood volume estimate of 56.8 ± 0.9 mL for the present herring gulls. The total burdens of PFAAs in eggs, liver, blood and brain were calculated by multiplying the tissue-specific concentrations (ng/g ww) by the estimated weight of the tissue (g).

3. Results and discussion

3.1. Body compartment-specific PFSA and PFCA patterns and concentrations

Among tissues and blood, the highest mean ∑PFSA concentrations (PFHxS, PFOS, PFDS) were detected in adipose tissue at 171 ± 83 ng/g ww, followed by liver > plasma > muscle > RBCs > brain (Table 1). In studies that reported on other breeding seabirds, in Swedish guillemots, a similar ∑PFSA distribution was found where concentrations in the liver was greater than in muscle (Holmström and Berger, 2008); however, in Norwegian glaucous gulls the concentration in plasma was greater than in liver and brain (Verreault et al., 2005). In Columbian brown pelicans, Olivero-Verbel et al. (2006) reported PFOS concentration in the liver was greater than brain and muscle. In the adipose tissue of the present herring gulls, ∑PFSA concentrations ranged from 3 to 577 ng/g ww. No previous studies have reported PFSAs in adipose tissue from a bird species; however, Van de Vijver et al. (2005) measured PFOS in the blubber of harbor seals and found comparable concentrations compared to the liver.

The PFSA pattern was dominated by PFOS in all tissues, ranging from 88.4% (brain) to 99.9% (adipose) of the ∑PFSA concentration (Table 1). Shorter chain PFHxS was detected in all the tissues, although in RBCs, brain, muscle, and adipose detection frequency in samples was <60%. ∑PFSA concentrations in the plasma contained the highest proportion of PFHxS among compartments (8%), which was significantly higher relative to other tissues where the next highest was liver (0.8%). PFDS was detected in all tissues with the exception of adipose. In plasma, RBCs, and muscle, PFDS detection frequency in samples was <60%. Brain contained the highest proportion of PFDS in the ∑PFSA concentration, 11.4%, followed by the liver (0.5%). The preferential accumulation of PFHxS in plasma and PFDS in brain observed in the present herring gulls was not greater in harbor seal (Ahrens et al., 2009). PFBS was below detection limit in all the samples.

Among tissues and blood, the highest mean ∑PFCA concentrations (C_6–C_{15}) were detected in the brain at 22 ± 2 ng/g ww, followed by the plasma > liver > RBC > adipose > muscle (Table 1). In Swedish guillemot, ∑PFCA concentrations were reported to be highest in the liver followed by muscle (Holmström and Berger, 2008), and Verreault et al. (2005) reported the highest ∑PFCA concentrations in plasma followed by the liver and brain in Norwegian glaucous gulls. None of the existing tissue distribution studies of PFAAs in any species have reported such high ∑PFCA concentrations in the brain compared to other tissues as we found in the present herring gulls. Ahrens et al. (2009) reported that ∑PFCA concentrations in the brain of harbor seals collected in German Bight (North Sea) were higher than muscle and blubber, which is consistent with the present results.

The PFCA pattern was highly variable among the tissues (Table 1, Fig. 1). The PFCA patterns in liver, RBC, muscle, and adipose were dominated by the C_8–C_{11} PFCAs, although in muscle and adipose the detection of these compounds was infrequent. The liver also contained PFDoA, PFTrA, and PFTeA, although detection was <60% in individual samples. In plasma, the C_6–C_{14} PFCAs were present; however, the pattern was dominated by C_8–C_{11} PFCAs and PFTrA (83% of ∑PFCA). In brain, the PFCA pattern consisted of C_{10}–C_{15} PFCAs but was dominated by PFTrA, PFTeA, and PFPA (88% of ∑PFCA). To our knowledge, tissue-specific accumulation of PFCAs as a function of chain length has not as yet been reported in birds. In both Swedish guillemots and Norwegian glaucous gulls, the PFCA pattern in liver, kidney, muscle, and plasma was reported to be dominated by PFUnA and PFTrA, while individual PFCAs in the glaucous gull brain were not reported (Verreault et al., 2005). PFCAs with chain length < C_{11} dominated patterns in harbor seal (German Bight) liver and plasma (Ahrens et al., 2009), which is consistent with the patterns in the present herring gull liver and plasma. However, the shorter PFCAs also dominated the pattern in the harbor seal brain, unlike the herring gull brain pattern. When combining the PFCA pattern in all the tissues and blood, the pattern was dominated by PFTrA, PFTeA, and PFPA (64% of ∑PFCA), which was mainly driven by the abundance of these longer chain length PFCAs in the brain (Fig. 2). The PFCA pattern to which the herring gulls are exposed to remains unknown, although in another study the C_8–C_{14} PFCAs were detected in prey fish from Lake Ontario (alewife and rainbow smelt) (Martin et al., 2004), which suggests

Table 1
Arithmetic mean percent (±SE) of detectable perfluorinated sulfonates (PFSAs) and carboxylic acids (PFCAs) and arithmetic mean concentrations of ∑PFSA and ∑PFCA (±SE ng/g ww) in female herring gull eggs and tissues collected in 2010 from Chantry Island, Lake Huron.

PFAA[a]	Egg compartment (n = 17)		Tissue (n = 8)				Blood (n = 8)	
	Yolk	Albumen	Liver	Brain	Muscle	Adipose	Plasma	RBC
%PFHxS	0.8 ± 0.2	0[b]	0.8 ± 0.3	0–1.5[c]	0–2.1	0–0.2	7.9 ± 3.1	0–7.9[c]
%PFOS	96.8 ± 0.4	0	98.8 ± 0.3	88.4 ± 1.7	99.3 ± 0.4	99.9 ± 0.1	91.7 ± 3.1	98.9 ± 1.0
%PFDS	2.4 ± 0.2	0	0.5 ± 0.1	11.4 ± 1.5	0–1.7	0	0.4 ± 0.2	0–0.4
∑PFSA (ng/g ww)	258 ± 39	<0.1	85 ± 16	5.0 ± 0.9	9.2 ± 4.2	171 ± 83	29 ± 8	6.2 ± 1.8
%PFHxA	0–0.3	0	0	0	0	0	0–1.3	0
%PFHpA	0–0.3	0	0	0	0	0	3.7 ± 1.2	0
%PFOA	2.1 ± 0.2	0	14.2 ± 4.5	0–0.3	0–100	0	22.1 ± 3.4	50.0 ± 7.5
%PFNA	5.1 ± 0.2	0	28.3 ± 4.5	0	0–31.3	0–23.2	15.5 ± 1.8	7.9 ± 4.8
%PFDA	11.2 ± 1.0	0	28.4 ± 2.7	1.4 ± 0.3	0–41.0	0	12.5 ± 2.3	0–17.4
%PFUnA	13.3 ± 0.8	0	23.9 ± 2.4	3.7 ± 0.8	0–90.6	0–100	16.1 ± 2.5	36.9 ± 8.9
%PFDoA	17.1 ± 0.7	0	0–1.0	7.2 ± 0.9	0	0	6.5 ± 1.8	0
%PFTrA	29.8 ± 1.0	0	0–10.9	37.1 ± 1.6	0	0	18.7 ± 3.4	0
%PFTeA	15.9 ± 0.7	0	0–15.3	26.8 ± 1.4	0	0	4.5 ± 1.4	0–24.5
%PFPA	5.4 ± 0.3	0	0	23.8 ± 1.7	0	0	0	0
∑PFCA (ng/g ww)	88 ± 9	<0.05	5.2 ± 3.8	22 ± 2	0.2 ± 0.1	0.3 ± 0.1	8.8 ± 2.0	0.7 ± 0.3

[a] PFAA denotes perfluoroalkyl acid. See Table S1 for the full chemical names of all PFAAs.
[b] Zero percent means that a calculation was not possible since the individual PFSA or PFCA were below the method limit of quantification (MLOQ; see Experimental section).
[c] Where detection of the individual PFSAs or PFCAs was <60% in individual eggs or tissues, a min.–max. percent range is given.

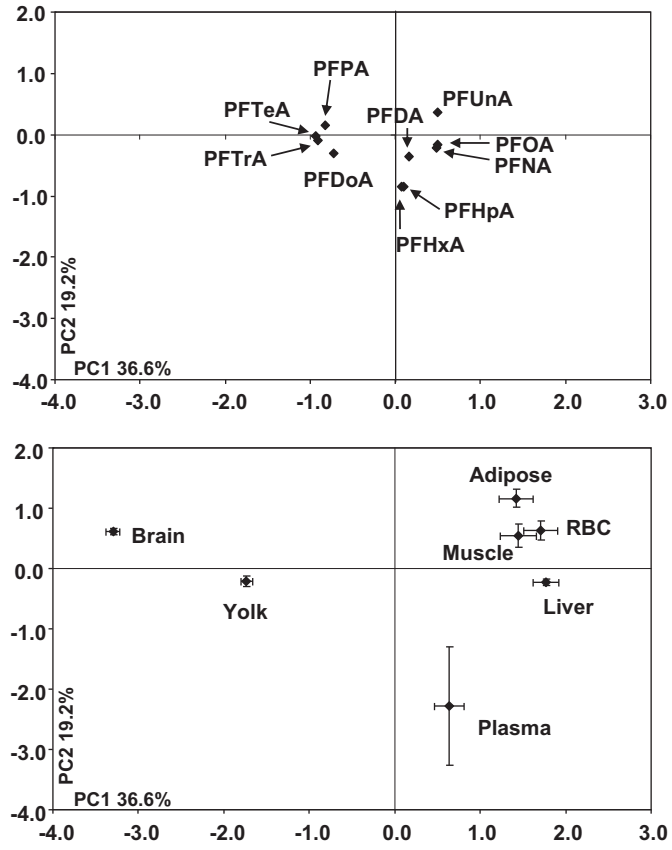

Fig. 1. Proportions of C_6–C_{15} PFCA to ∑PFCA concentrations plotted using the first two principal components (PCs), PC1 and PC2. Mean (±SE) factor scores (bottom biplot) are shown for the tissues and yolk. The percent variability explained by PC1 and PC2 is provided.

that prey fish are likely a source of PFCAs for the herring gulls from Chantry Island.

Although all the tissues and the egg compartments were analyzed for PFOS and PFCA precursors [PFOSA, NMeFOSA, FTOHs (6:2, 8:2, 10:2), and FTUCAs (6:2, 8:2, 10:2)], none of these compounds were quantifiable. Gebbink et al. (2009) reported low concentrations (0.03 ng/g ww) of PFOSA in herring gull eggs collected from Chantry Island in 2007. The absence of PFOSA in the present eggs (collected in 2010) could be related to the phase out of C_8 PFOSF chemistry in North America by the 3M Company in 2002. This could have resulted in decreased environmental concentrations of PFOS precursors, or "PreFOS" as has been coined by Martin et al. (2010), which refers to the complexity of fluorinated precursors that can degrade and give rise to PFOS. PFOSA has been detected in herring gull prey fish collected from Lake Ontario (Martin et al., 2004). The absence of PFOSA in herring gull tissues and eggs could also be an indication that PFOSA is degraded to PFOS if obtained through their diet. Degradation of PFOSA has been shown in *in vitro* experiments using rat liver slices (Xu et al., 2004). FTOHs and FTUCAs have not been reported in the herring gull diet; however, FTUCAs have been found in lake trout from the Great Lakes, indicating their presence in the aquatic environment (Furdui et al., 2007). If exposed through their diet, we hypothesize that gulls metabolize these precursors to PFCAs, similar to what has been shown in *in vitro* experiments using rat hepatocytes (Martin et al., 2005).

3.2. Tissue distribution based on concentrations and amount burdens

In birds, as well as other species, in limited tissue distribution studies the liver is often the body compartment or tissue with the highest PFSA and PFCA concentrations, although, in the case of the birds studies, adipose tissue was not analyzed (Ahrens et al., 2009; Holmström and Berger, 2008; Olivero-Verbel et al., 2006). Although adipose tissue in the present herring gulls contained highest PFSA concentrations, the liver concentrations were higher compared to other tissues, which was consistent with tissue distribution of other bird studies (Table 1). Regardless, protein binding/association of PFAAs likely plays a key role in the accumulation of these classes of PFAAs in the liver. Luebker et al. (2002) reported on the binding of PFOS and PFOA to the liver fatty acid binding protein (L-FABP) isolated from rats. Chain length-dependant binding to such proteins might also explain the selective accumulation of PFOA, PFNA, PFDA, and PFUnA in the present herring gull livers. In relation to protein-associated mediation of PFOS deposition into and out of the liver, Yu et al. (2011) reported that female Wistar rats exposed to PFOS (3.0 mg/kg of PFOS) showed enhanced hepatic mRNA expression of the organic anion transport (OAT) protein OATP2, and increased hepatic expression of multidrug resistance-assisted protein MRP2. They concluded that PFOS-induced hepatic expression of OAPT2 and MRP2, which could potentially enhance hepatic uptake, efflux and metabolism of thyroxine (T4) in rats. Unconjugated T4 has to interact with OATP1, OATP2 and MRP2 in order to gain access into and out of liver cells.

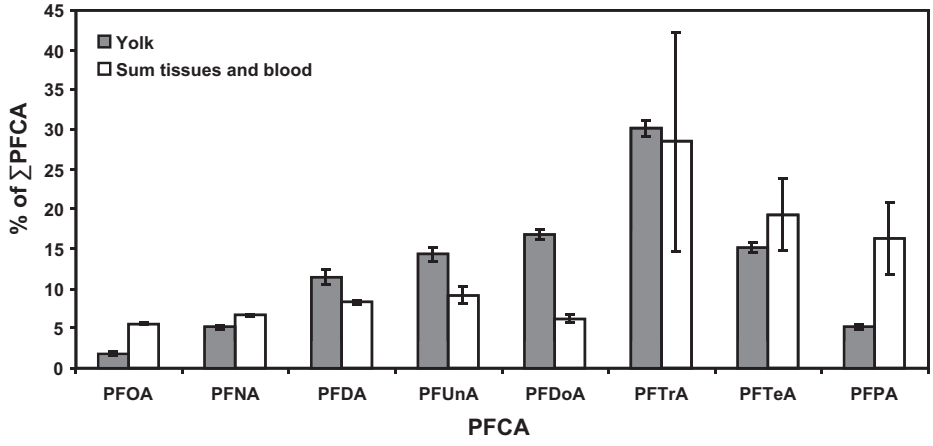

Fig. 2. Arithmetic mean percent (±SE) of C_8–C_{15} PFCAs to ∑PFCA in the yolk and combined herring gull tissues and blood.

In plasma, ∑PFSA and ∑PFCA concentrations were significantly higher compared to concentrations in RBCs (Table 1). To our knowledge, there has been no reported examination of the comparative compartmental accumulation of any PFC in blood, e.g., plasma versus RBCs, from any wildlife species. However, Ehresman et al. (2007) suggested that PFHxS, PFOS, and PFOA are present in human plasma or serum and not in RBCs, which was based on plasma and whole blood measurements with no specific measurement in the RBC component. PFAAs have been found to bind to proteins such as albumin and sex-hormone binding globulins in birds, fish and humans (Chen and Guo, 2009; Jones et al., 2003). The fact that these proteins are found in the plasma and not in the RBCs might explain the observed preferred accumulation in the plasma. In the gull RBCs, PFCA/PFSA patterns were skewed toward PFOS, PFOA, PFNA, and PFUnA, although concentrations were lower. However, the PFCA/PFSAs patterns are basically the same as significant positive correlations were found between the plasma and RBC for the dominant PFOS, PFOA, and PFUnA concentrations (Fig. 3). For PFUnA and PFOA in Fig. 3, the presence or absence of the apparent outliers made no difference in that the RBC and plasma concentration correlations were still significant ($p < 0.05$). Relative abundances of individual PFSAs or PFCAs were all within 1% when comparing the plasma and the estimated whole blood patterns (combined plasma and RBC patterns). Thus, plasma patterns are reflective of the patterns in whole blood.

∑PFSA concentrations in herring gull brains were one order of magnitude lower compared to the liver concentrations; which was consistent with other limited bird studies (Olivero-Verbel et al., 2006; Verreault et al., 2005). Contrastingly, the ∑PFCA concentrations in the brain were higher compared to the liver, and brain was the most contaminated tissue or body compartment studied (Table 1). This preferential accumulation of PFCAs in the brain has not been observed in any species. Furthermore, the brain PFCA and PFSA pattern relative to all other tissues and blood components was heavily skewed to the longer chain PFTrA, PFTeA and PFPA, and favored PFDS (Table 1, Fig. 1). Given that the whole brain was sampled, there is evidence of selective transfer of longer chain PFCAs and PFSAs through the blood brain barrier and into the brain. The mechanism of this longer chain PFCA passage into and retention in the brain is unclear.

The presence of these PFAAs in the gull brain could be of concern from a toxicological standpoint. Vongphachan et al. (2011) reported variable transcriptional changes in herring gull embryonic neuronal cells exposed to shorter chain length PFSAs (PFBS, PFHxS) and PFCAs (PFHxA, PFHpA), but PFTrA, PFTeA and PFPA were not studied. It has been shown in several toxicological studies with monkeys, rats and mice that serum thyroid hormone (TH) deficiency, especially for total T4, is induced by PFOS exposure (Chang et al., 2008). In mammals TH deficiency is known to be associated with structural brain damage, neurological defects, developmental delay, and behavioral problems (Zoeller et al., 2002).

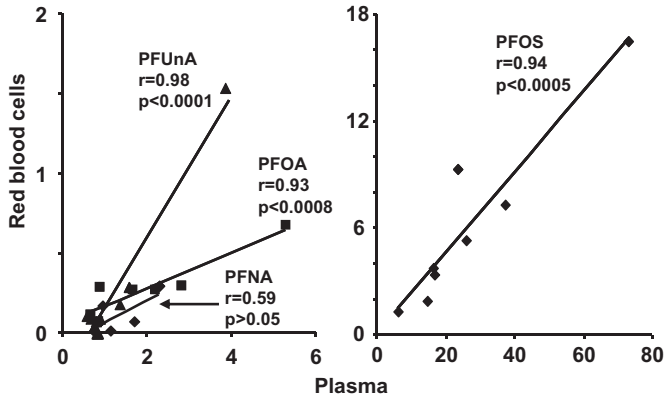

Fig. 3. Linear correlations between the plasma and red blood cell concentrations (ng/g ww) for PFOS, PFOA, PFNA and PFUnA. Detection of other individual PFSAs and PFCAs was <60% in plasma and/or red blood cells and were not included.

In limited reports for birds as well as other species, muscle and adipose tissue were reported to be the less contaminated with PFCA and PFSA than liver (Ahrens et al., 2009; Holmström and Berger, 2008; Olivero-Verbel et al., 2006). In the present herring gulls, muscle generally had the lowest PFSA and PFCA concentrations among other tissues and blood components (Table 1). However, adipose tissue contained the highest ∑PFSA (99.9% PFOS) concentration, approximately 2-fold higher than liver. In contrast, ∑PFCA concentrations were very low at 0.3 ng/g ww and comparable to muscle. Regardless, the mean PFOS concentration in adipose tissue was highly variable among individuals, ranging from 3 to 577 ng/g ww. Although PFAAs have been found to bind to proteins (Jones et al., 2003) and have low accumulation potential in fatty tissues (Ahrens et al., 2009), the present results on the accumulation of PFOS in adipose tissue is difficult to explain. No correlations were observed between ∑PFSA concentrations in adipose and any other tissues, which, although speculative, may suggest divergent types of proteins that can influence tissue-specific accumulation in herring gulls.

The burdens of ∑PFSA in liver, blood and brain were estimated at 2.2, 1.1, and 0.03 μg, respectively, totaling 3.4 μg (Fig. 4). In liver, blood and brain, ∑PFCA burdens were estimated at 0.13, 0.32, and 0.13 μg, respectively, and totaling 0.6 μg. As PFC burden in the muscle and adipose were not estimated (due to unknown percent of the body mass), and PFCs have been reported in other avian tissues such as kidney, lung, heart as well as feathers (Holmström and Berger, 2008; Meyer et al., 2009; Olivero-Verbel et al., 2006), the combined liver, blood, and brain burden underestimate whole body burdens in gulls. However, the combined liver, blood, and brain burdens in harbor seals (German Bight), represented 75% of the ∑PFAA body burden (Ahrens et al., 2009).

3.3. Maternal transfer of PFCAs and PFSAs to eggs

The egg yolk contained 258 ng/g ww ∑PFSA (PFHxS, PFOS, PFDS) and 88 ng/g ww ∑PFCA (C_6–C_{15}), while in the albumen, PFSAs and PFCAs were below detection limits. Information on the order that the present eggs were laid within a clutch was not available. Concentrations of ∑PFSA and ∑PFCA in the yolk were higher (3-fold and 17-fold higher, respectively) than liver (Table 1). This is consistent with results for Norwegian glaucous gulls although whole eggs were analyzed in that study (Verreault et al., 2005). In the present yolks, C_6–C_{15} PFCAs were detected and the pattern was dominated by PFTrA > PFDoA > PFTeA > PFUnA, making up 76% of ∑PFCA. This PFCA pattern, as well as the PFSA pattern, were comparable to the patterns found in gull eggs collected in 2007 from Chantry Island (Gebbink et al., 2009). The present gull eggs were consistently comprised of ~30% yolk and ~70% albumen, thus if concentrations were adjusted for yolk only, the ∑PFSA in the whole egg would be about half the concentration of ∑PFSA reported by Gebbink et al. (2009) in eggs collected in 2007, while ∑PFCA concentrations would be comparable. When using whole egg homogenates to monitor for PFSAs and PFCAs, the albumen dilutes the absolute localization of these PFAAs in the yolk. Although both the yolk and the albumen contain proteins, absolute accumulation of PFCAs and PFSAs in the yolk is most likely a function of specific proteins transferred *in ovo* and into the yolk. Newsted et al. (2007) found that PFOS was associated with very low density lipoproteins (VLDL) and to a lesser extent phosvitin and lipovitellin in the yolk of quails and mallards.

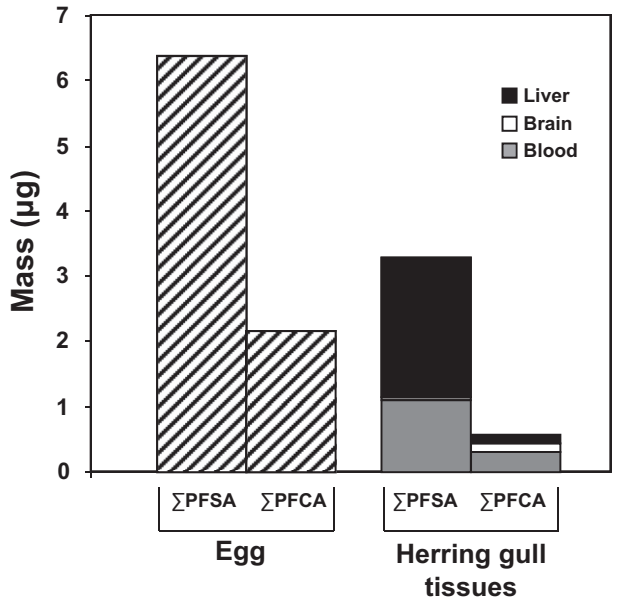

Fig. 4. Tissue burden mass of ∑PFSA and ∑PFCA (µg) in herring gull egg, liver, brain, and blood collected from Chantry Island in 2010.

Not only were the sum PFAA concentrations different between the yolk and liver, but the PFCA pattern in the yolk and liver differed as well, showing preferential accumulation depending of longer chain length PFCAs in the yolk (this was not observed for the PFSA pattern) (Table 1, Fig. 1). Holmström and Berger (2008) assessed the maternal transfer of PFAAs in common guillemots and related it to the transfer of proteins produced in the liver to the egg. Yolk proteins, such as vitellogenin and VLDL, are produced in the liver and transported from the liver to the ovaries via the blood. The PFCA pattern in the liver was dominated by C_8–C_{11} PFCAs (95% of the total), whereas the pattern in yolk was dominated by C_{10} and longer chain length PFCAs (93% of the total). As has been shown for PFOS (Newsted et al., 2007), binding of PFCAs to these proteins could serve as a transport mechanism from the liver to the eggs. The preferential accumulation of the longer chain length PFCAs in the egg relative to the liver was also seen for the guillemot; however, not for glaucous gulls (Fig. 5) (Holmström and Berger, 2008; Verreault et al., 2005).

As the present data show, the PFAA profile, and especially the PFCA pattern in the egg does not reflect the PFCA pattern in other herring gull tissues. Using the PFCA pattern in the yolk as a reference point, the PFCA pattern in the liver (source of PFAAs to the egg), blood (transport of PFAA-protein from the liver to the egg) and whole body (combined pattern in all the tissues and blood) were compared (Fig. 6). Higher proportions of C_8–C_{11} PFCAs in the liver were observed compared to the yolk. As mentioned earlier, selective binding of the longer chain length PFAAs to proteins and subsequent transfer to the egg might explain this. In the blood, PFCA pattern increases were seen for C_8–C_{11} and decreases for C_{12}–C_{14} compared to the yolk pattern. The blood transports PFAA-protein complexes from the liver to the egg. The observed pattern change might be related to binding to blood proteins (e.g., albumin) in addition to the binding to liver protein (e.g., OAT and MRP proteins (Yu et al., 2011)). Although the PFCA pattern in individual tissues showed differences in the pattern compared to the yolk due to tissue-specific accumulation, the combined PFCA pattern in all the tissues and blood showed a greater resemblance to the yolk pattern (Fig. 2). The enrichment of PFTeA and PFPA in the whole body pattern is due to the high abundance of these two PFCAs in the brain. Declines of PFDoA in the PFCA pattern in the whole body compared to the yolk might be due to selective transfer to the yolk.

An important aspect of our study was to understand the extent of the maternal transfer of bioaccumulative PFCAs and PFSAs and overall burden in the egg. As female herring gulls usually lay three eggs in a clutch, a clutch of three eggs would contain 19.2 µg of ∑PFSA and 6.6 µg of ∑PFCA (6.4 µg of ∑PFSA and 2.2 µg of ∑PFCA per egg) (Fig. 4). Compared to the combined tissue burden (liver, blood, brain; 3.4 µg of ∑PFSA and 0.6 µg of ∑PFCA), female herring gulls are clearly transferring a considerable proportion of the estimated ∑PFSA and ∑PFCA body burden to their eggs, thus *in ovo* transfer is a very substantial elimination route for PFAAs for female herring gulls. To our knowledge, this is the first study showing the extent of PFAA elimination via egg depuration. Although Holmström and Berger (2008) did not compare PFAA burdens between Swedish guillemot eggs and tissues, the higher liver concentrations of PFCAs/SAs in chicks compared to adult birds was suggested to be the result of the transfer of high amounts to eggs. This may have toxicological implications. However, O'Brien et al. (2009) showed that chicken eggs injected with comparable amounts of PFOS showed no effect on the expression of PPARα-regulated genes. Only after exposure to PFOS concentrations two

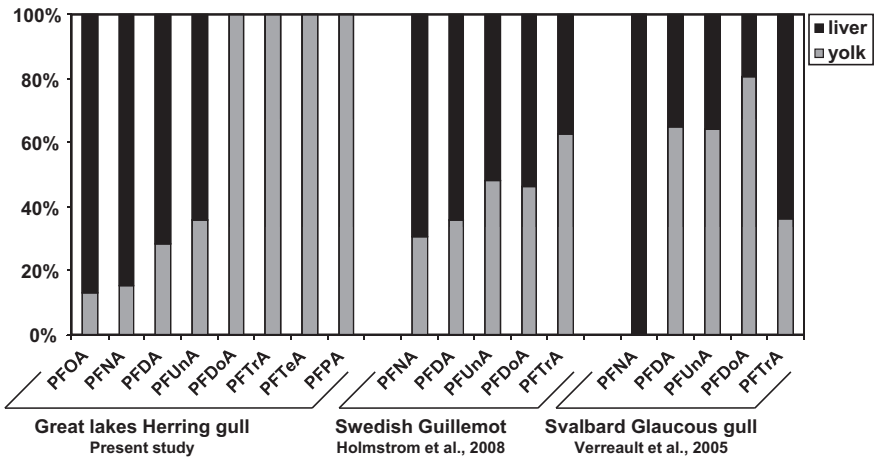

Fig. 5. Relative percent composition of individual PFCA concentrations in liver versus egg yolk in female herring gulls and eggs, respectively, collected in 2010 from the Chantry Island (Lake Huron) colony site. Detection of PFDoA, PFTrA, and PFTeA in the liver was <60% in individual samples and a comparison was not possible. Guillemot and glaucous gull liver and egg data were adapted from Holmström and Berger (2008) and Verreault et al. (2005).

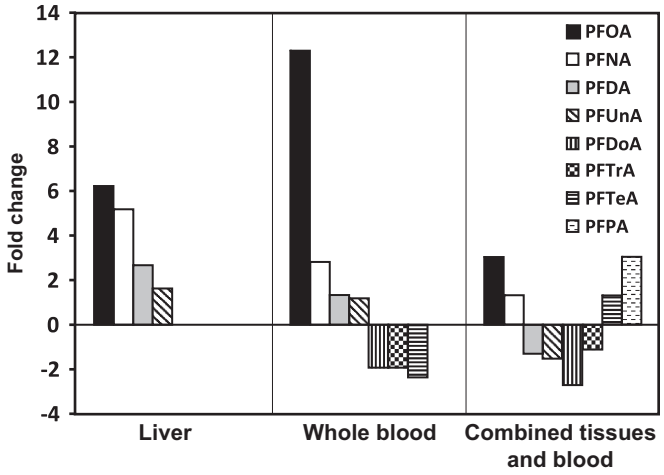

Fig. 6. Fold change of PFCA to ∑PFCA concentration ratio in herring gull liver, whole blood, and combined tissues and blood compared to the yolk. Only PFCAs with >60% detection are included.

orders of magnitude higher than observed here was embryo pippability decreased.

4. Conclusions

In the present study, regardless of maternal transfer, PFCAs and PFSAs were detected in the liver, plasma, RBC, brain, muscle, and adipose, and tissue-specific accumulation appears to be related to protein-associated factors, which appear to be chain length dependent. Although the PFCA pattern was highly variable among tissues, the PFCA pattern in combined tissues and blood resembled the yolk pattern. For monitoring purposes, this is important as the egg PFCA pattern represents the PFCA pattern in the female herring gull. However, when investigating tissue-specific effects of PFAAs, information on tissue PFAA pattern and concentrations is essential.

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Appendix. Supplementary information

Supplementary information associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2011.10.011.

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