Environment International 59 (2013) 425–430

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Perfluoroalkyl acids in subarctic wild male mink (*Neovison vison*) in relation to age, season and geographical area

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ARTICLE INFO

Article history:
Received 29 March 2013
Accepted 30 June 2013
Available online 6 August 2013

Keywords:
Perfluoroalkyl acids
Perfluorinated
PFOS
Mink

ABSTRACT

This study investigates the influence of biological and environmental factors on the concentrations of perfluoroalkyl acids (PFAAs) in a top predator; the American mink. Perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluoroalkyl carboxylates (PFCAs) with C_8–C_{13} perfluorinated carbon chains were analyzed in livers from wild male mink liver ($n = 101$) from four areas in Sweden representing two inland environments (rural and highly anthropogenic, respectively) and two different coastal environments. Mean PFOS concentrations were 1250 ng/g wet weight and some mink from the urban inland area had among the highest PFOS concentrations ever recorded in mink (up to 21 800 ng/g wet weight). PFBS was detected in 89% of the samples, but in low concentrations (mean 0.6 ng/g ww). There were significant differences in PFAA concentrations between the geographical areas ($p < 0.001$–0.01). Age, body condition and body weight did not influence the concentrations significantly, but there was a seasonal influence on the concentrations of perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUnDA) ($p < 0.01$ and $p < 0.05$, respectively), with lower concentrations in autumn samples than in samples taken in the winter and spring. It is thus recommended to take possible seasonal differences into account when using mink exposure data. The overall results suggest that the mink is a suitable sentinel species for assessing and monitoring environmental levels of PFAAs.

1. Introduction

Perfluoroalkyl acids (PFAAs) have gained considerable attention as environmental pollutants due to their persistence, their bioaccumulative potential (Kelly et al., 2009; Martin et al., 2004b) and their toxic properties. They have been associated with liver toxicity and developmental toxicity in laboratory animals (Lau et al., 2007), and immunotoxicity in both laboratory and wild animals (DeWitt et al., 2012; Kannan et al., 2006). PFAAs are released into the environment, both directly from manufacturing and indirectly through products such as surfactants and surface protectors (Paul et al., 2008; Prevedouros et al., 2006). Due to their unique properties of being both water and oil repellent, perfluoroalkyl and polyfluoralkyl substances are extensively used in a wide range of industrial and consumer applications, such as nonstick coatings on cookware, some waterproof clothes, and in fire-fighting foams. Two fluorinated compound classes, the perfluorinated carboxylic acids (PFCAs) and sulfonic acids (PFSAs) have been studied substantially in recent years. Members of both classes are globally distributed and have been detected in wildlife as well as in humans (Gamberg et al., 2005; Giesy and Kannan, 2001; Houde et al., 2011; Kannan et al., 2001; Kärrman et al., 2007). In addition to direct emission, several precursor compounds have been identified as an indirect source of PFCAs and PFSAs in environmental matrices (Young and Mabury, 2010). So far, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been subjected to most attention as they are among the most toxic PFAAs (Kudo and Kawashima, 2003; Lau et al., 2004) and have been found at relatively high levels (Houde et al., 2006b). In 2009, PFOS was added to the Stockholm convention list of persistent organic pollutants (Stockholm Convention on Persistent Organic Pollutants, 2009) and the largest producer of PFOS-based products, the 3M company, phased out their production by 2002 (3M, 2000). The replacement compound for PFOS is perfluorobutane sulfonate (PFBS) (3M, 2002), which seems to be less potent in rat toxicity tests (Lieder et al., 2009) and has a shorter half-life in human and rat serum (Olsen et al., 2009) than PFOS. However, compared to PFOS and PFOA, the bioaccumulation and toxicity of PFBS have been less investigated, although the literature is increasing.

The wild American mink has been acknowledged as a useful sentinel species for chemical pollution and related health effects (Basu et al.,

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0160-4120
http://dx.doi.org/10.1016/j.envint.2013.06.025

2007; Persson et al., 2012). The arguments are mainly that it is a semi-aquatic top predator with a widespread distribution and it can, especially where it is an invasive species, be captured in large numbers. Also, it has a well-known biology and physiology and can be maintained and studied in captivity. In order to use the mink as a sentinel, it is important that it has the ability to accumulate pollutants. In the literature, data on mink exposure to pollutants such as chlorinated chemicals is quite extensive, especially from North America as reviewed by Basu et al. (2007). However, only a handful of studies have been made regarding exposure of PFAAs to wild mink (Giesy and Kannan, 2001; Kannan et al., 2002b, 2005; Martin et al., 2004a), and among those, only Martin and co-workers (Martin et al., 2004a) analyzed long-chain PFCAs. There is no study on mink addressing the exposure of PFBS. In order to evaluate the mink as a suitable sentinel specifically for PFAAs in the environment, more information is needed regarding the pattern of PFAA contamination in mink.

Environmental and biological factors are important to consider when assessing contamination related effects, temporal and spatial trends and trophic transfer. Taking such factors into account is important in exposure assessment and in study designs. For example, we have shown earlier that, in wild male mink from Sweden, almost half of the variation in the concentrations of polychlorinated biphenyls in fat could be explained by age, sampling area, sampling season and body condition (Persson et al., 2013). Taking such factors into account is therefore needed in any assessment of the exposure, and it could also have implications on sampling regime. Therefore, this study aims to quantify the concentrations of PFBS, perfluorohexane sulfonate (PFHxS), PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA) and perfluorotridecanoic acid (PFTrA) in wild male mink from Sweden, and investigate relationships between the concentrations and age, body condition, body weight, sampling area and sampling season.

2. Materials and methods

2.1. Sampling

Mink were collected by local hunters in Sweden each year between 2004 and 2009, from August to the end of April. One hundred and one male mink were sampled in four different areas: two inland areas and two coastal areas. A map of sample area locations can be found in Supplementary data. The Gävle Baltic coast (G; n = 25) is a brackish water environment nearby two towns (70,000 and 12,000 inhabitants), fairly large industries and the mouths of the Dalälven and Ljusnan rivers. The Koster Islands in Skagerrak (K; n = 26) is a sea water environment (partly a national park) about 8 km off the Swedish coast in the North sea, close to the Norwegian border. The Märsta inland region (M; n = 25) with high anthropogenic impact by industrial and agricultural activities located next to a town with 25,000 inhabitants, a large international airport and the former training camp of the Swedish Rescue Services Agency. The inland of Northern Sweden (N; n = 25) is a sparsely populated inland environment with few industries and low agricultural activity. Hunters were instructed to freeze the carcasses at approximately −20 °C as soon as possible after death. The carcasses were thawed just before necropsy. The subcutaneous fat pad between the hind legs was dissected and weighed. Body condition was defined as the weight of the subcutaneous fat (g) divided by total body weight (kg). Liver tissue was removed for chemical analysis and refrozen. Aging was performed by teeth cementum analysis by Matson's laboratory (Milltown, Montana, USA). As the mink kits are born in the beginning of May (Hansson, 1947), a birth date of 1st of May was assumed. The mink were assigned to three different age categories: juvenile (3–12 months old, $n = 51$), one year old (13–24 months, $n = 32$) and two or more years old (older than 24 months, $n = 18$). Hours of daylight at the specific capture date and site for each mink was used to construct three seasonal groups; autumn (from 17 to9 h of daylight before winter solstice, $n = 42$), winter (<9 h daylight, $n = 29$) and spring (from 9 to17 h of daylight after winter solstice, $n = 30$). More detailed information about age, weight of subcutaneous fat, body weight and body length of the mink from the four different areas that were included in this study has been published earlier (Persson et al., 2013).

2.2. Sample preparation and analytical determination

Liver samples were homogenized and a sub-sample of 1 g was transferred to a 50 mL centrifuge tube. The mass-labeled internal standards (see Supplementary data) were added followed by 10 mL acetonitrile. The mixture was vortex mixed and ultrasonicated for 30 min and the supernatant acetonitrile phase was removed after centrifugation (10,000 ×*g*, 30 min). The extraction procedure was repeated once. The acetonitrile fractions were combined and diluted with water. After mixing and centrifugation the solution was put through a WAX solid phase cartridge (Waters, Milford, MA, USA) previously conditioned with 4 mL methanol followed by 4 mL water. After loading the sample, the WAX cartridge was washed with 4 mL 25 mM sodium acetate (pH 4) and 4 mL 40v% methanol in water, followed by drying the SPE cartridge under vacuum. A final wash with 8 mL methanol was employed before the PFAAs were eluted with 2 mL 2% ammonium hydroxide in methanol into a tube with 50 mg ENVI-Carb and 100 μL acetic acid. After mixing and filtration recovery standards, 2 mM ammonium acetate in water was added to the extract. The analysis was performed using an Acquity UPLC coupled to a Quattro Premier XE (Waters Corporation, Milford). Details on the analysis and quantification are presented in the Supplementary data.

2.3. Quality assurance

The analytical method used has previously been evaluated for PFCAs and PFSAs in an interlaboratory study on fish muscle with satisfactory Z-scores ($z < 2$) (van Leeuwen et al., 2009). Low average recovery rates were found for some of the labeled internal standards, most frequently $^{13}C_2$-PFDoDA (Table S1), and are potentially explained by ionization effects caused by interfering components present in the liver matrix. For validation purposes, five liver extracts with low recoveries were diluted up to 1000 times and analyzed on a Xevo TQ-S mass spectrometer (Waters Corporation, Milford, USA), which is a more sensitive instrument compared to the Quattro Premier XE. The recoveries of $^{13}C_4$-PFOS increased from 10–44% to 36–80% in the ×100 and ×1000 diluted samples (Fig. S1, Supplementary data). To compare PFOS concentrations in undiluted (u) and diluted (d) extracts, the mean normalized difference (%) was calculated using the formula: $((u - d) / ((u + d) / 2) \times 100)$. The calculated concentrations of all the diluted extracts, except for one sample, were well in range with the initial concentrations (average mean normalized difference of 18%). Consequently, reliable results can be produced even when recovery rate is low since the internal standard and the native compound are equally suppressed. Recoveries, method reproducibility and method detection limits (MDL) for all samples are presented in Table S1, Supplementary data. One milliliter of ultra pure water was used as procedural blanks and extracted in the same way as the real samples. The MDL was defined as the mean concentration in the procedural blanks plus three standard deviations, and the limit of detection (LOD) for individual samples was calculated as three times the noise level. Overall good recoveries (>50%) of ^{13}C-PFOS and ^{13}C-PFOA were measured for the samples after the replacement of the recovery standard 7H-PFHPA to $^{13}C_8$-PFOS and $^{13}C_8$-PFOA in the middle of the project. One two year old mink caught in autumn in the G area was excluded due to non-reproducible results of the diluted extracts.

2.4. Statistical analysis

Using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.02.01), a multiple regression model with the concentrations of PFHxS, PFOS, PFNA, PFDA or PFUnDA as dependent variable and the sample area, sample season, age, body condition, year (of capture) and body weight as independent variables were elaborated on. PFBS, PFOA, PFDoDA and PFTrDA were excluded from this model since the concentrations were consistently low (<17 ng/g). The model was fitted manually, starting with all variables in the model. Variables that were unsignificant ($p > 0.05$) for all dependent variables were removed. Relevant interactions between the effects were tested but none were included in the model due to insignificance or small sample size. The variable age was tested in several ways (different assignments into categories and numerical approaches), but had no significant effect. Area and season were the only variables that had a significant effect and were therefore the only variables kept in the final model:

$$Y = \mu + AREA + SEASON + ERROR.$$

Where: Y is an observed value for concentration of chemicals; μ is the population mean for the concentration; AREA is a fixed effect due to area of sampling; SEASON is a fixed effect due to season of sampling; ERROR is a random residual error term. All dependent data were log-transformed to improve normality of the residuals. Comparisons of least square means (within-group means adjusted for the other effects in the model, i.e. season) were calculated by t-tests. p-Values less than 0.05 were considered significant. Adjustment for correcting for Type I errors (rejection of a null hypothesis that is actually true) was not applied, as any indication of effects of the variables was considered interesting and there was no reason to be overly cautious. Half the LOD value was used in calculations for samples with concentrations < LOD. The number of such samples was none for PFUnDA, and for the other PFAAs only one sample per chemical. Samples for which the analysis did not meet analytical performance criteria were treated as missing values. A principle component analysis (PCA) was performed using the SIMCA P + software (Umetrics, Umeå, Sweden, version 12.0.1). In total one hundred observations (mink samples) and twenty x-variables; the variable area, season and age and body condition and the log-transformed concentrations of contaminants were included in the model. All data were centered and scaled prior to modeling. The value of explained variation (R^2) was calculated and the estimate of the predictive ability of the model (Q^2) was performed by cross validation.

3. Results and discussion

3.1. Concentrations of PFAAs

In this study, the concentrations of PFCAs in mink from subarctic areas (Table 1) were generally higher than those reported in mink from the Canadian arctic, where PFNA was the major contaminant found (mean 16 ng/g), followed by PFOS (8.7 ng/g), PFUnDA (4.3 ng/g), PFDA (3.7 ng/g), PFDoDA and PFTrA (both <0.5 ng/g) (Martin et al., 2004a). The concentrations of PFOS in our study were similar to concentrations previously reported for mink livers from various locations in the USA; on average 2630 ng/g liver (Giesy and Kannan, 2001) and 74–2370 ng/g (Kannan et al., 2002b). However, in our study, some mink had extremely high concentrations of PFOS: four mink from the highly anthropogenic inland area (M) with concentrations ranging from 9640 to 21,800 ng/g ww and five mink with concentrations between 2070 and 3740 ng/g ww. Also, in the Skagerrak coast area (K), two mink contained PFOS concentrations of 2580 ng/g and 4490 ng/g. These high concentrations in Swedish mink are among the highest ever reported for this species in the literature. Higher levels of PFOS were only found in mink from Michigan, USA (Kannan et al., 2005) with concentrations of 1280–59,500 ng/g. Other mammals in which high liver PFOS concentrations have been found are wood mice in Belgium with a range of 470–178,550 ng/g (Hoff et al., 2004), otters in Sweden with a range of 19–16,000 ng/g (Roos, 2013), polar bears in East Greenland with a range of 83–3868 ng/g (Dietz et al., 2008), and harbor seals in the German Bight with concentrations up to 3676 ng/g (Ahrens et al., 2009).

PFBS has not been analyzed in mink prior to this study, where it was found in 89% of the samples, although in low concentrations (Table 1). Reports of PFBS in wild mammalian tissues are relatively uncommon in the international literature and has only recently been found in harbor seals from the Dutch Wadden Sea (1.74–3.28 ng/g spleen) (Van de Vijver et al., 2005), in harbor seals from the German Bight (up

Table 1
Concentrations of PFAAs in wild mink livers from four different areas in Sweden (ng/g wet weight).

	Sample area[a]	N[b]	Mean[c] ± SD[c]	LS means[d]	Median[c]	Range
PFBS		**89**	**0.6 ± 0.9**		**0.2**	**<0.03–4.7**
	G	21	0.2 ± 0.3			<0.03–1.7
	K	26	1.2 ± 1.0			0.04–4.7
	M	20	0.4 ± 0.9			<0.03–4.6
	N	22	0.4 ± 0.7			<0.03–2.8
PFHxS		**99**	**11.0 ± 22.8**		**3.9**	**<0.1–139**
	G	24	4.6 ± 4.3	3.4 a		0.9–16.7
	K	26	6.0 ± 3.9	5.1 a		1.5–20.7
	M	25	32.1 ± 38.4	13.8 b		0.3–139
	N	24	1.1 ± 1.2	0.7 c		<0.1–4.0
PFOS		**99**	**1250 ± 3170**		**520**	**<0.8–21,800**
	G	24	646 ± 390	576 a		68.6–1460
	K	26	867 ± 865	664 a		245–4490
	M	25	3310 ± 5850	1065 a		87.0–21,800
	N	24	170 ± 197	87.6 b		<0.8–854
PFOA		**59**	**2.0 ± 2.1**		**1.2**	**<0.2–9.9**
	G	11	2.8 ± 2.0			<0.3–7.2
	K	19	3.9 ± 2.2			1.0–9.9
	M	18	0.7 ± 0.9			<0.2–3.3
	N	11	0.7 ± 0.9			<0.2–2.8
PFNA		**87**	**49.2 ± 49.1**		**34.0**	**<0.5–269**
	G	21	104 ± 69.4	85.3 a		9.1–269
	K	25	44.2 ± 18.4	40.4 b		17.0–99.0
	M	20	15.4 ± 11.9	12.5 c		3.4–54.0
	N	21	33.8 ± 24.1	21.5 bc		<0.5–84.0
PFDA		**97**	**31.6 ± 21.8**		**28.1**	**<0.3–130**
	G	23	37.8 ± 19.3	36.7 a		8.1–77.6
	K	26	32.2 ± 12.2	29.5 ab		13.0–55.9
	M	24	30.9 ± 30.9	20.6 ab		3.0–130
	N	24	26.0 ± 20.9	16.4 b		<0.3–78.0
PFUnDA		**85**	**28.1 ± 17.5**		**26.0**	**1.8–79.7**
	G	19	36.7 ± 17.2	35.1 a		11.4–70.7
	K	22	30.9 ± 9.3	27.6 a		12.0–48.3
	M	19	15.2 ± 12.6	11.3 b		2.0–46.7
	N	25	29.0 ± 21.5	20.5 a		1.8–79.7
PFDoDA		**55**	**5.6 ± 3.6**		**5.1**	**<0.3–16.4**
	G	16	6.0 ± 2.9			1.9–11.9
	K	13	5.4 ± 1.5			2.3–7.3
	M	12	5.9 ± 4.5			1.9–16.2
	N	14	5.1 ± 4.7			<0.3–16.4
PFTrDA		**57**	**2.8 ± 2.4**		**2.1**	**0.1–11.7**
	G	16	3.2 ± 1.6			1.2–7.2
	K	13	2.2 ± 0.8			1.3–4.1
	M	13	1.6 ± 1.6			0.1–4.6
	N	15	3.8 ± 3.8			0.4–11.7

[a] Results in bold are results from all areas. The Baltic coast (G), the Koster Islands in Skagerrak (K), the anthropogenic inland region (M) and the rural inland of Northern Sweden (N). See Section 2.1 for detailed description.
[b] Number of samples that met the analytical performance criteria and had concentrations above LOD (of total 100 samples, G n = 24, K n = 26, M n = 25, N n = 25).
[c] Arithmetic mean, standard deviation and median. For samples with concentration < LOD half the LOD value was used for calculations.
[d] Anti-logarithm of least square means (within-group means adjusted for the other effects in the model, i.e. season). For each compound, rows sharing the same letters are not significantly different ($p > 0.05$).

to 3.1 ng/g liver) (Ahrens et al., 2009) and in gray seals from the Baltic Sea (up to 3.5 ng/g liver) (Kratzer et al., 2011). The concentrations were approximately the same as in the mink in our study (Table 1), although PFBS was only found in 27–55% of the samples (compared to 89% in our study). In addition, PFBS has been found in sea turtles from the east coast of USA (<0.02–0.846 and <0.01–0.195 ng/g serum) (Keller et al., 2012; O'Connell et al., 2010). In contrast, PFBS was below detection limit in all samples of Arctic and North Atlantic pilot whale, ringed seal, minke whale, harbor porpoise, hooded seal, white-sided dolphin and fin whales (Rotander et al., 2012). Also, PFBS was not detected in ringed seal populations in the Canadian Arctic (Butt et al., 2007, 2008), nor in common guillemot from the Baltic Sea (Berger, 2008) or harbor porpoise in the North and Baltic Sea (Huber et al., 2012). PFBS is persistent (Quinete et al., 2010), but not expected to be as bioaccumulative as PFAAs with longer carbon chains (Conder et al., 2008). However, as a replacement for PFOS, the use of this compound will probably increase in the future. Mink, with its wide geographical distribution and the proximity of its habitat to human activities, could be a suitable sentinel species for monitoring PFBS exposure to mammals.

3.2. Differences in PFAA patterns in relation to geographical area

The sampling areas in this study were selected because of their assumed differences in contamination and this was confirmed by the multiple regression model, which showed that area of sampling was significantly influencing the concentrations of PFHxS, PFOS, PFNA, PFDA and PFUnDA ($p = <0.001–0.01$). The multiple regression models explained 18–53% of the variation in the tissue concentrations. Pairwise comparisons of least squares between the areas are given in Table 1. To visualize the variation in contaminant concentrations in the four areas, a PCA model ($R^2 = 0.52$, $Q^2 = 0.119$) containing 3 significant principal components according to cross validation was calculated. Scores and loadings plots of component 1 versus component 2 are given in Figs. 1 and 2, explaining 23% and 15% of the variance, respectively. The scores plot is a summary of the relationships among the observations (mink). The loadings plot can be used to interpret the patterns seen in the scores plot, as the plots are superimposable. Plots of component 2 versus 3, the descriptive data for the components and the R^2 and Q^2 calculated for each variable are found in the Supplementary data.

In the PCA scores plot (Fig. 1), the mink from the Baltic coast area (G) and Skagerrak area (K) are grouped tightly together, indicating a large similarity in concentration pattern in these coastal mink. The mink from the rural inland area (N) and the more anthropogenic inland area (M) are not grouped as tightly together as the two coastal areas (G and K), indicating a relatively large variation in contaminant concentration patterns in mink from the M and N areas. In contrast to mink from the inland areas, higher concentrations of PFAAs, e.g. PFBS, PFOA, PFDA PNFA, PFUnDA, PFDoDA and PFTrDA were seen for the coastal (G and K) mink. The pairwise comparisons of least squares (Table 1) revealed that the Baltic coast area (G) had significantly higher concentrations of PFNA than all the other areas ($p = <0.001–0.01$). In comparison to gray seals from the Baltic Sea, the mink from the Baltic coast area (G) had similar PFNA and PFOS concentrations but somewhat higher concentrations of PFHxS, PFDA and PFUnDA (Kratzer et al., 2011).

Most mink from the anthropogenic inland area (M) are located in the lower part of the scores plot (Fig. 1). Some mink from this area are plotted in the lower right corner of the scores plot, which indicates that they tend to have higher concentrations of PFHxS and PFOS than mink from the other areas, as these compounds are located in lower right corner of the corresponding loadings plot (Fig. 2). The pairwise comparisons of least squares in the multiple regression model confirmed that mink from the M area had significantly higher concentrations of PFHxS than the other three areas ($p = <0.001–0.01$). The pattern in the inland area (M), with high PFOS and PFHxS levels, can be explained by the fact that these mink were caught in the vicinity of the Swedish Rescue Services former training camp which was closed down as recently as 2009. Also, some mink were caught in a stream which carries PFAA contaminated water from an international airport (IVL, 2010). PFOS is used as a surfactant additive in aqueous film forming foam (AFFF) used to fight petroleum fires (Moody and Jennifer, 2000; Paul et al., 2008). Although PFOS has been phased out from fire-fighting foam, it is expected to be present in the environment for a long time due to its persistence. The observed co-variation between PFHxS and PFOS suggests a common source and is likely a result from PFHxS being an impurity in the PFOS formulation. Low concentrations of PFHxS have been found to originate from AFFFs (Olsen et al., 2003).

In contrast to the pattern in the M area, mink from the rural inland area N area are mostly situated in the upper left part of the scores plot, showing a general pattern of low concentrations of both PFOS and PFHxS, which was confirmed by comparisons of least squares (Table 1). There were also a few mink from this area plotted in the upper right corner of the scores plot, indicating relatively high concentrations of PFNA, PFUnDA and PFTrDA in these mink.

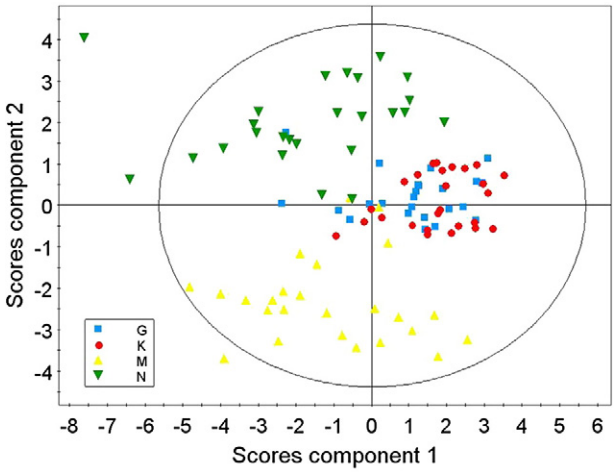

Fig. 1. Scores plot (from the principal component analysis) with individual mink ($n = 100$) from the Baltic coast (G), the Koster Islands in Skagerrak (K), the anthropogenic inland region (M) and the rural inland of Northern Sweden (N). The circle shows Hotellings T^2 95% confidence ellipse.

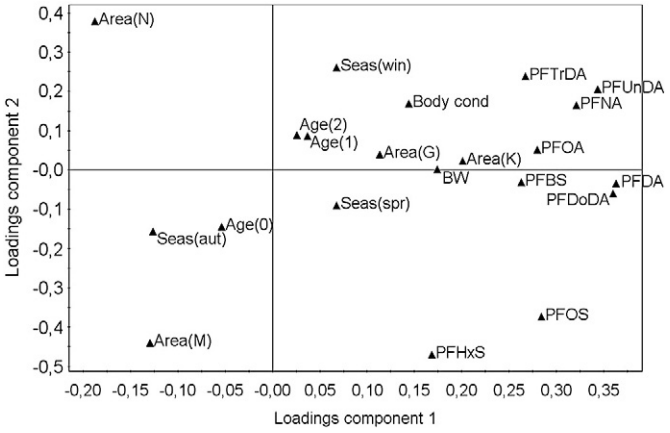

Fig. 2. Loadings plot (corresponding to the scores plot, Fig. 1) Areas are: the Baltic coast (G), the Koster Islands in Skagerrak (K), the anthropogenic inland region (M) and the rural inland of Northern Sweden (N). Seasons are: autumn (aut), winter (win) and spring (spr). Age is: juveniles (0), one year old (1) and two or more years old (2). Body cond is the weight of the subcutaneous fat (g) divided by total body weight (kg) and BW is total body weight. For detailed description of variables, see Section 2.

The geographical differences likely reflect the local contamination, but could also to some extent mirror the possible different diets between the mink in these areas. Mink generally feeds on fish, birds, rodents and frogs (Gerell, 1967). They are generalist predators and tend to feed on available prey (Clode and Macdonald, 2009) and the composition of the diet has been seen to differ between coastal and riverine mink (Ben-David et al., 1997) as well as between mink with habitats along rivers and mink with habitats near lakes (Gerell, 1967; Jedrzejewska et al., 2001). In our experience, coastal mink in Sweden has a higher frequency of fish in their stomach compared to inland mink (unpublished data).

3.3. PFAA concentrations in relation to biological and environmental factors

Age did not influence any of the concentrations of PFAAs in the multiple regression models. The same was found in a study by Kannan et al. (2002b), where there were no age-related differences in PFOS concentrations between juvenile and adult mink. This underlines the possible difference in accumulation patterns in mink between PFAAs and lipophilic compounds such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), as many PCB and PBDE congeners have been found to increase with age in wild male mink (Persson et al., 2013). In wild animals in general, there are contradictory reports of associations between PFAAs and age. For instance, there were indications of a lack of age related differences in PFOS concentrations in a study of adult and subadult Alaskan polar bears (Kannan et al., 2001), as well as in a study of ringed and gray seal in the Baltic sea (Kannan et al., 2002a). Regarding PFAAs with longer chain lengths, a study of Danish harbor seals found no age relationship between age and concentrations of PFAAs with carbon chain length 6–11 (Dietz et al., 2012). In contrast, in a study on polar bears from East Greenland, age significantly influenced the summarized concentrations of perfluorinated acids (Sonne et al., 2008). In an earlier study on polar bears from the same area, concentrations of PFCAs with carbon chain length 10–14 significantly increased up to six years of age in a subset of six polar bears, but there was no significant difference in concentrations between all adults and all subadults for any of the analyzed chemicals (Smithwick et al., 2005).

In addition, there are reports of higher concentrations of some PFAAs in pups compared to adults in harbor seals (Ahrens et al., 2009; Shaw et al., 2009), Baikal seals (Ishibashi et al., 2008) and Northern Sea otters (Hart et al., 2009), and it has been discussed that maternal transfer could be an important source of exposure. Notably, in an analysis of a subset of our data, concentrations of PFHxS and PFOS were significantly lower in 3–5 month old mink ($n = 6$, K area) than in the older mink ($n = 20$, K area, $p < 0.01$), but no significant differences were found for PFNA, PFDA or PFUnDA. This challenges the idea of a significant maternal transfer of PFAAs in mink. A recent study on polar bear mother-cub pairs concluded that the maternal transfer of PFAAs was substantial, but relatively low when compared to maternal transfer of PCB (Bytingsvik et al., 2012).

Results indicated no effect of body weight or body condition on PFAA concentrations in this study. Similar results have been found in sea otters from California, USA (Kannan et al., 2006). As PFOS and PFOA have been found to mainly bind to serum albumins (Han et al., 2003; Jones et al., 2003), it is not surprising that lipid dynamics does not affect the concentration of PFAAs.

The general linear model revealed a significant effect of season for PFDA and PFUnDA ($p < 0.05$ and $p < 0.01$ respectively). The concentrations were significantly lower during autumn than spring for both PFDA and PFUnDA ($p < 0.01$ and $p < 0.05$, respectively). Autumn concentrations of PFDA and PFUnDA were also significantly lower than the concentrations during winter ($p < 0.05$ and $p < 0.01$, respectively). These results could be explained by the fact that the mink may change diet seasonally (Gerell, 1967; Jedrzejewska et al., 2001). Another possible contribution to the seasonal pattern could be that some mink may shift the use of their habitat seasonally (Gerell, 1970). For instance, a hunter in the G area reported that during winter mink often abandon the small archipelago along the coast in favor for streams in the coastal mainland (S-A, Ängwald, personal communication). There could also be intrinsic factors affecting the elimination of PFDA and PFUnDA. Organic anion transport proteins in the kidney have been shown to be important for PFCA elimination, depending on sex, species and fluorocarbon chain length (Han et al., 2012). For example, the renal clearance of PFOA is lower in male than in female rats due to an inhibitory effect of testosterone (Kudo et al., 2002; Van den Heuvel et al., 1992). As the testosterone level is very seasonal in the male mink (Pilbeam et al., 1979) it could be speculated that this contributes to seasonal variation in the concentrations of these chemicals. In other species, there are only a limited number of studies that have investigated season as source of variation. No seasonal differences for PFOS and PFOA were found in sea otters from California, USA (Kannan et al., 2006), which is in line with the findings in our study, and no seasonal differences were found in the total sum of perfluoroalkyl compounds in plasma from bottlenose dolphins (Houde et al., 2006a).

In summary, the high concentrations of PFOS found in mink from the highly anthropogenic inland sampling area in this study are among the highest ever reported in the literature. In addition, PFBS was found in most mink samples, indicating that it is present in the environment at levels that allow detection/quantitation in top predators. Mink seem to readily accumulate both short and long chain PFAAs. Differences in the pattern of PFAA contamination were seen between the coastal and inland mink, but also between the rural and highly anthropogenic sampling sites.

The data suggest that age, body condition and body weight do not influence the concentrations to any larger extent. However, sampling season should be regarded as a possible source of variation in exposure data. Taken together the results from this study indicate that the wild mink is a species suitable for sensitive and cost efficient environmental monitoring of PFAA exposure.

Acknowledgments

This work was funded by the Environmental Monitoring Program at the Swedish University of Agricultural Sciences. The mink hunters B. Almberg, G. Anttila, Å. Degermark, B. Engström, M. Eriksson, W. Eriksson, T Hall, J. Karlsson, S. Karlsson, S-G. Lunneryd, M. Nilsson, B. Nyberg, A. Olofsson, E. Olsson, S. Sundin and S-A. Ängwald are thanked for their work of providing mink carcasses for this study.

Appendix A. Supplementary data

Additional information on chemicals, recoveries, method reproducibility, method detection limits and recovery rates. Descriptive data on principal component analysis, model overview, scores and loadings plots for component 2 versus 3. Map of sample area locations. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.06.025.

References

3M. Phase-out plan for PFOS-based products. US EPA Docket AR 226-0588; 2000.
3M. Environmental, health, safety, and regulatory (EHSR) profile of perfluorobutane sulfonate (PFBS). Technical data bulletin; 2002.
Ahrens L, Siebert U, Ebinghaus R. Temporal trends of polyfluoroalkyl compounds in harbor seals (*Phoca vitulina*) from the German Bight, 1999–2008. Chemosphere 2009;76:151–8.
Basu N, Scheuhammer AM, Bursian SJ, Elliott J, Rouvinen-Watt K, Chan HM. Mink as a sentinel species in environmental health. Environ Res 2007;103:130–44.
Ben-David M, Hanley T, Klein D, Schell D. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. Can J Zool 1997;75:803–11.
Berger U. Tissue distribution of perfluorinated surfactants in common guillemot (*Uria aalge*) from the Baltic Sea. Environ Sci Technol 2008;42:5879–84.
Butt CM, Muir DCG, Stirling I, Kwan M, Mabury SA. Rapid response of Arctic ringed seals to changes in perfluoroalkyl production. Environ Sci Technol 2007;41:42–9.

Butt CM, Mabury SA, Kwan M, Wang X, Muir DCG. Spatial trends of perfluoroalkyl compounds in ringed seals (*Phoca hispida*) from the Canadian Arctic. Environ Toxicol Chem 2008;27:542–53.
Bytingsvik J, van Leeuwen SPJ, Hamers T, Swart K, Aars J, Lie E, et al. Perfluoroalkyl substances in polar bear mother–cub pairs: a comparative study based on plasma levels from 1998 and 2008. Environ Int 2012;49:92–9.
Clode D, Macdonald D. Evidence for food competition between mink (*Mustela vison*) and otter (*Lutra lutra*) on Scottish islands. J Zool 2009;237:435–44.
Conder JM, Hoke RA, Wolf W, Russell MH, Buck RC. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ Sci Technol 2008;42:995–1003.
DeWitt JC, Peden-Adams MM, Keller JM, Germolec DR. Immunotoxicity of perfluorinated compounds: recent developments. Toxicol Pathol 2012;40:300–11.
Dietz R, Bossi R, Riget FF, Sonne C, Born E. Increasing perfluoroalkyl contaminants in East Greenland polar bears (*Ursus maritimus*): a new toxic threat to the Arctic bears. Environ Sci Technol 2008;42:2701–7.
Dietz R, Riget FF, Galatius A, Sonne C, Teilmann J, Bossi R. Spatial trends of perfluorochemicals in harbor seals (*Phoca vitulina*) from Danish waters. Sci Total Environ 2012;414:732–7.
Gamberg M, Braune B, Davey E, Elkin B, Hoekstra PF, Kennedy D, et al. Spatial and temporal trends of contaminants in terrestrial biota from the Canadian Arctic. Sci Total Environ 2005;351:148–64.
Gerell R. Food selection in relation to habitat in mink (*Mustela vison* Schreber) in Sweden. Oikos 1967;18:233–46.
Gerell R. Home ranges and movements of the Mink (*Mustela vison* Schreber) in southern Sweden. Oikos 1970;21:160–73.
Giesy JP, Kannan K. Global distribution of perfluorooctane sulfonate in wildlife. Environ Sci Technol 2001;35:1339–42.
Han X, Snow TA, Kemper RA, Jepson GW. Binding of perfluorooctanoic acid to rat and human plasma proteins. Chem Res Toxicol 2003;16:775–81.
Han X, Nabb DL, Russell MH, Kennedy GL, Rickard RW. Renal elimination of perfluorocarboxylates (PFCAs). Chem Res Toxicol 2012;25:35–46.
Hansson A. The physiology of reproduction in mink (*Mustela vison*, Schreb.) with special reference to delayed implantation. Acta Zool 1947;28:1–136.
Hart K, Gill VA, Kannan K. Temporal trends (1992–2007) of perfluorinated chemicals in northern sea otters (*Enhydra lutris kenyoni*) from South-Central Alaska. Arch Environ Contam Toxicol 2009;56:607–14.
Hoff PT, Scheirs J, Van de Vijver K, Van Dongen W, Esmans EL, Blust R, et al. Biochemical effect evaluation of perfluorooctane sulfonic acid-contaminated wood mice (*Apodemus sylvaticus*). Environ Health Perspect 2004;112:681–6.
Houde M, Balmer BC, Brandsma S, Wells RS, Rowles TK, Solomon KR, et al. Perfluoroalkyl compounds in relation to life-history and reproductive parameters in bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, Florida, USA. Environ Toxicol Chem 2006a;25:2405–12.
Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DCG. Biological monitoring of polyfluoroalkyl substances: a review. Environ Sci Technol 2006b;40:3463–73.
Houde M, De Silva AO, Muir DCG, Letcher RJ. Monitoring of perfluorinated compounds in aquatic biota: an updated review PFCs in aquatic biota. Environ Sci Technol 2011;45:7962–73.
Huber S, Ahrens L, Bårdsen BJ, Siebert U, Bustnes JO, Víkingsson GA, et al. Temporal trends and spatial differences of perfluoroalkylated substances in livers of harbor porpoise (*Phocoena phocoena*) populations from Northern Europe, 1991–2008. Sci Total Environ 2012;419:216–24.
Ishibashi H, Iwata H, Kim EY, Tao L, Kannan K, Amano M, et al. Contamination and effects of perfluorochemicals in Baikal Seal (*Pusa sibirica*). 1. Residue level, tissue distribution, and temporal trend. Environ Sci Technol 2008;42:2295–301.
IVL Swedish Environmental Research Institute. Årsrapport 2009 för projektet RE-PATH (risks and effects of the dispersion of PFAS on aquatic, terrestrial and human populations in the vicinity of International Airports); 2010.
Jedrzejewska B, Sidorovich VE, Pikulik MM, Jedrzejewski W. Feeding habits of the otter and the American mink in Bialowieza Primeval Forest (Poland) compared to other Eurasian populations. Ecography 2001;24:165–80.
Jones PD, Hu W, De Coen W, Newsted JL, Giesy JP. Binding of perfluorinated fatty acids to serum proteins. Environ Toxicol Chem 2003;22:2639–49.
Kannan K, Koistinen J, Beckmen K, Evans T, Gorzelany JF, Hansen KJ, et al. Accumulation of perfluorooctane sulfonate in marine mammals. Environ Sci Technol 2001;35:1593–8.
Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. Environ Sci Technol 2002a;36:3210–6.
Kannan K, Newsted J, Halbrook RS, Giesy JP. Perfluorooctanesulfonate and related fluorinated hydrocarbons in mink and river otters from the United States. Environ Sci Technol 2002b;36:2566–71.
Kannan K, Tao L, Sinclair E, Pastva SD, Jude DJ, Giesy JP. Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. Arch Environ Contam Toxicol 2005;48:559–66.
Kannan K, Perrotta E, Thomas NJ. Association between perfluorinated compounds and pathological conditions in southern sea otters. Environ Sci Technol 2006;40:4943–8.
Kärrman A, Ericson I, van Bavel B, Darnerud PO, Aune M, Glynn A, et al. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden. Environ Health Perspect 2007;115:226–30.
Keller JM, Ngai L, McNeill JB, Wood LD, Stewart KR, O'Connell SG, et al. Perfluoroalkyl contaminants in plasma of five sea turtle species: comparisons in concentration and potential health risks. Environ Toxicol Chem 2012;31:1223–30.
Kelly BC, Ikonomou MG, Blair JD, Surridge B, Hoover D, Grace R, et al. Perfluoroalkyl contaminants in an Arctic marine food web: trophic magnification and wildlife exposure. Environ Sci Technol 2009;43:4037–43.
Kratzer J, Ahrens L, Roos A, Bäcklin BM, Ebinghaus R. Reprint of: temporal trends of polyfluoroalkyl compounds (PFCs) in liver tissue of grey seals (*Halichoerus grypus*) from the Baltic Sea, 1974–2008. Chemosphere 2011;85:253–61.
Kudo N, Kawashima Y. Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. J Toxicol Sci 2003;28:49–57.
Kudo N, Katakura M, Sato Y, Kawashima Y. Sex hormone-regulated renal transport of perfluorooctanoic acid. Chem Biol Interact 2002;139:301–16.
Lau C, Butenhoff JL, Rogers JM. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol Appl Pharmacol 2004;198:231–41.
Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci 2007;99:366–94.
Lieder PH, York RG, Hakes DC, Chang SC, Butenhoff JL. A two-generation oral gavage reproduction study with potassium perfluorobutanesulfonate (K + PFBS) in Sprague Dawley rats. Toxicology 2009;259:33–45.
Martin JW, Smithwick MM, Braune BM. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. Environ Sci Technol 2004a;38:373–81.
Martin JW, Whittle DM, Muir DCG, Mabury SA. Perfluoroalkyl contaminants in a food web from Lake Ontario. Environ Sci Technol 2004b;38:5379–85.
Moody CA, Jennifer A. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. Environ Sci Technol 2000;34:3864–70.
O'Connell SG, Arendt M, Segars A, Kimmel T, Braun-McNeill J, Avens L, et al. Temporal and spatial trends of perfluorinated compounds in juvenile loggerhead sea turtles (*Caretta caretta*) along the east coast of the United States. Environ Sci Technol 2010;44:5202–9.
Olsen GW, Church TR, Miller JP, Burris JM, Hansen KJ, Lundberg JK, et al. Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. Environ Health Perspect 2003;111:1892.
Olsen GW, Chang SC, Noker PE, Gorman GS, Ehresman DJ, Lieder PH, et al. A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. Toxicology 2009;256:65–74.
Paul AG, Jones KC, Sweetman AJ. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. Environ Sci Technol 2008;43:386–92.
Persson S, Brunstrom B, Backlin B-M, Kindahl H, Magnusson U. Wild mink (*Neovison vison*) as sentinels in environmental monitoring. Acta Vet Scand 2012;54:S9.
Persson S, Rotander A, van Bavel B, Brunström B, Bäcklin B-M, Magnusson U. Influence of age, season, body condition and geographical area on concentrations of chlorinated and brominated contaminants in wild mink (*Neovison vison*) in Sweden. Chemosphere 2013;90:1664–71.
Pilbeam TE, Concannon PW, Travis HF. The annual reproductive cycle of mink (*Mustela vison*). J Anim Sci 1979;48:578–84.
Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol 2006;40:32–44.
Quinete N, Orata F, Maes A, Gehron M, Bauer KH, Moreira I, et al. Degradation studies of new substitutes for perfluorinated surfactants. Arch Environ Contam Toxicol 2010;59:20–30.
Roos A. The otter (*Lutra lutra*) in Sweden: contaminants and health. Acta Universitatis Upsaliensis Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology, 1051. ; 2013. [47 pp.].
Rotander A, Kärrman A, Bavel B, Polder A, Rigét F, Auðunsson GA, et al. Increasing levels of long-chain perfluorocarboxylic acids (PFCAs) in Arctic and North Atlantic marine mammals, 1984–2009. Chemosphere 2012;86:278–85.
Shaw S, Berger ML, Brenner D, Tao L, Wu Q, Kannan K. Specific accumulation of perfluorochemicals in harbor seals (*Phoca vitulina concolor*) from the northwest Atlantic. Chemosphere 2009;74:1037–43.
Smithwick M, Muir DCG, Mabury SA, Solomon KR, Martin JW, Sonne C, et al. Perflouroalkyl contaminants in liver tissue from East Greenland polar bears (*Ursus maritimus*). Environ Toxicol Chem 2005;24:981–6.
Sonne C, Bossi R, Dietz R, Leifsson PS, Rigét FF, Born EW. Potential correlation between perfluorinated acids and liver morphology in East Greenland polar bears (*Ursus maritimus*). Toxicol Environ Chem 2008;90:275–83.
Stockholm Convention on Persistent Organic Pollutants (POPs). Adoption of amendments to annexes A, B and C. C.N.524.2009.Treaties-4 2009. chm.pops.int/Portals/0/download.aspx?d=UNEP-POPS-TREATY-NOTIF-CN524-2009.En.pdf, 2009.
Van de Vijver KI, Hoff P, Das K, Brasseur S, Van Dongen W, Esmans E, et al. Tissue distribution of perfluorinated chemicals in harbor seals (*Phoca vitulina*) from the Dutch Wadden Sea. Environ Sci Technol 2005;39:6978–84.
Van den Heuvel JP, Davis II JW, Sommers R, Peterson RE. Renal excretion of perfluorooctanoic acid in male rats: inhibitory effect of testosterone. J Biochem Toxicol 1992;7:31–6.
van Leeuwen SPJ, Swart CP, van der Veen I, de Boer J. Significant improvements in the analysis of perfluorinated compounds in water and fish: results from an interlaboratory method evaluation study. J Chromatogr A 2009;1216:401–9.
Young CJ, Mabury SA. Atmospheric perfluorinated acid precursors: chemistry, occurrence, and impacts. Rev Environ Contam Toxicol 2010;208:1–109.