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

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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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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.