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Perfluorerade alkylsyror i matkorgsprover och fisk från Vättern och Ålands hav

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Sammanfattning

Perfluorerade alkylsyror (PFAS), dvs perfluoralkylkarboxylater, -sulfonater och en -sulfonamid analyserades i fiskprover från Vättern och Öregrundsgrepen/Dalälvens mynning (Ålands hav) och i matkorgsprover av animaliska livsmedel. Resultaten visade att halterna av många PFAS var högre i fisk från Vättern än i samma fiskarter från Ålands hav. Halterna av perfluorooktan sulfonat (PFOS) i muskel från abborre från Vättern låg endast något lägre än de halter som tidigare uppmätts i abborre fångad i Mälaren. PFOS var den PFAS-substans som förelåg i de högsta halterna i fisk från båda områdena. Halterna av PFOS tycktes vara högre i sötvattensarter såsom abborre, gös och lake än i lax och öring. Samma mönster antyddes för halter av metylkvicksilver (MeHg) i fisk från Ålands hav. I Vättern sågs inga större skillnader i MeHg-halter mellan lake och de två laxfiskarterna. Analyserna av matkorgsproverna visade att halterna av PFOS i animaliska livsmedel i allmänhet är klart lägre än i fisk från Vättern. Det går dock inte att utesluta att enskilda livsmedel i matkorgsproverna kan innehålla förhöjda PFOS-halter. Haltdata saknas också helt för de många livsmedel som inte inkluderades i matkorgsanalyserna. Resultaten antyder dock att fisk från sjöar och vattendrag påverkade av PFAS-utsläpp kan vara en källa till PFOS-exponering. Det går dock ännu inte att dra säkra slutsatser om hur stort bidrag livsmedel ger till den totala PFAS-exponeringen hos den svenska befolkningen.

Perfluorinated alkyl substances in market basket food samples and fish from Lake Vättern and the Baltic Sea

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Summary

Perfluorinated alkyl substances (PFAS), i.e. perfluoroalkyl carboxylates, -sulfonates and a -sulfonamide were analysed in fish muscle from fish species caught in the second largest lake in Sweden, Lake Vättern, and in the brackish water sea the Baltic Sea. Moreover, market basket samples of foods of animal origin were analysed for perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). Concentrations of many of the PFAS were higher in fish from Lake Vättern than in the same fish species from the Baltic Sea. Concentrations in perch (*Perca fluviatilis*) muscle tissue from Lake Vättern were only slightly lower than those recently found in perch from Lake Mälaren, which is situated in the most densely populated area of Sweden. Among all the studied PFAS, PFOS was predominant in both sampling areas. Concentrations of PFOS appeared to be highest in freshwater predatory fish in both areas. In the Baltic Sea these fish species also had higher concentrations of methyl mercury than the salmonid species salmon (*Salmo salar*) and brown trout (*Salmo trutta trutta*). In Lake Vättern no marked difference in MeHg concentrations was found between freshwater predatory fish and the salmonids. PFOS concentrations in the market basket samples were considerably lower than those in fish from Lake Vättern. However, it cannot be excluded that some individual food items in the composite market basket samples contained higher PFOS concentrations. Moreover, data on many foods on the Swedish market are still lacking. Nevertheless, our data suggest that predatory freshwater fish caught in waters affected by PFAS emissions may be a source of human PFOS exposure. It is still uncertain how much food exposure in general contributes to the total PFAS exposure of humans.

Background

Per- and polyfluorinated alkyl substances (PFAS) are a group of anthropogenic chemicals, some of which have been manufactured for more than 50 years. PFAS are used in a multitude of industrial and commercial products such as stain repelling agents, fluoropolymers, pesticides, lubricants, paints and fire-fighting foams (Key et al., 1997; Prevedouros et al., 2006). The exceptional properties that make fluoroorganics so attractive for industrial applications also make them potentially hazardous to organisms and ecological systems. Ionic PFAS repel both water and lipids, and are extremely resistant towards degradation (Faithfull and Weers, 1998). Recently, the development of a new method sequence for analysis (Hansen et al., 2001) revealed relatively high levels of PFAS in the environment. Two of the main groups of PFAS, perfluorinated sulfonates (PFSs, including perfluorooctane sulfonate – PFOS) and perfluorinated carboxylates (PFCAs), were found to be widely distributed over the northern hemisphere, including remote areas such as the Arctic (Bossi et al., 2005; Giesy and Kannan, 2001; Verreault et al., 2005). Unlike legacy persistent organic pollutants, the amphiphilic PFAS have not been shown to accumulate preferentially in adipose tissue. They rather bind to blood proteins and accumulate in the liver of exposed organisms (Jones et al., 2003; Vanden Heuvel et al. 1991a,b). Experimental effect studies have demonstrated the toxicity of a number of perfluorinated substances through impedance of cell-to-cell communication and peroxisome proliferation, which are both mechanisms for hepatocarcinogenesis (Berthiaume and Wallace, 2002; Hu et al., 2002, Upham et al. 1998). Moreover, some PFAS are also suggested to affect lipid metabolism and reproduction (Haugom and Spydevold, 1992; Lau et al., 2003; Thibodeaux et al., 2003).

Despite being amphiphilic, PFAS demonstrate bioaccumulation tendencies due to their ability to bind to proteins. Their degree of bioaccumulation generally increases with perfluoroalkyl chain length (Martin et al., 2003a,b) and trophic position (Van de Vijver et al., 2003). The pattern of PFAS contamination in wildlife has been shown to vary greatly among species and geographical locations suggesting multiple emission sources (Houde et al., 2006). A decreasing gradient of PFOS concentration in perch muscle was e.g. found from lake Mälaren to the Stockholm archipelago (Järnberg and Holmström, 2003). Both PFOS and PFCAs are also regularly detected in human blood from all over the globe (Kannan et al., 2004). However, the exposure pathways for humans are scarcely investigated so far. Fish intake might be an important contribution to the ubiquitous levels of PFAS in human blood.

A previous Swedish study showed a significant positive correlation between levels of PFOS and perfluorooctanoate (PFOA), and methyl mercury (MeHg) in human blood (Holmström et al., 2005). MeHg is a well-known neurotoxic environmental contaminant with the highest concentrations found in predatory fish. Since fish is the only exposure source to MeHg in the general population, MeHg in blood can serve as a biomarker of fish intake, and especially of predatory fish species. In the study, blood samples from women with a moderate to high proportion of fish in their diet were collected along with detailed surveys on food habits (food frequency questionnaires). The blood samples were first analysed for MeHg, and subsequently also for PFOS and PFOA. PFOS and PFOA were significantly positively correlated to MeHg in blood ($p=0.02$), and PFOS (not PFOA) to increasing intake of predatory fish species, and to intake of shellfish. Concentrations of PFOS and PFOA were not correlated to intake of fatty fish. No correlation was found to other types of food contained in the survey, such as meat or dairy. The results indicate that predatory freshwater fish species can be a source of exposure to PFAS for humans.

The aim of the present study was to investigate concentrations of PFAS in food with special emphasis on commercially important fish species. Furthermore, differences in PFAS

contamination of certain species living both in freshwater (lake Vättern) and the Baltic Sea were studied. Several predatory freshwater fish species live and reproduce in the brackish water Baltic Sea. MeHg was also analysed in some of the fish samples in order to compare concentrations in fish from Lake Vättern and the Baltic Sea, and to determine if predatory freshwater fish may be a source of MeHg and PFOS exposure to humans.

Materials and methods

Samples

Market basket samples of food of animal origin were used for analysis of PFAS in Swedish food in general (Table 1). Food products consumed at a rate of at least 0.5 kg per person and year were selected from *per capita*-consumption data, derived from Swedish producers and trade statistics (Swedish Board of Agriculture, 2005). Food items were obtained in 2005 at two places of purchase in the City of Uppsala, located close to the Swedish capital Stockholm. Samples of the foods were pooled into the different food groups given in Table 1. One percent of the yearly per capita consumption (by weight) was sampled from each food unit/package for homogenate preparation and subsequent analysis. In case of food items where wastage could be supposed, inedible parts such as bone, skin etc. were removed prior to homogenisation. Samples were thoroughly homogenised using a stainless steel Cut-o-mat® food processor with stainless steel knives. The mixer was thoroughly washed with acetone after mixing of each sample. All other materials used in sampling and preparation of the food samples were also washed in acetone. Homogenised composite samples were kept frozen at minus 20 ºC before analysis.

Table 1. Market basket food samples from 2005 used for analysis of perfluorinated alkyl substances

Sample	Content of composite sample*
Meat and meat products	Smoked and boiled sausage, hotdog, liver paste, german sausage, hamburger, pork chop, stuffed cabbage roll, fried diced meat, chicken breast, beef steak, smoked ham, bacon, moose meat, lamb chop, canned hot dog, canned meat soup
Dairy products	Milk (0.5, 1.5 and 3% fat), fermented milk (0.5, 1.5 and 3% fat), hard cheese (28% fat), fruit yoghurt (0.5 and 3% fat), cream (12 and 40% fat), sour cream (12% fat), cottage cheese, processed cheese (10% fat)
Eggs	Egg
Seafood and seafood products	Cod, farmed salmon, fish balls, pickled herring, shrimp tails, herring, plaice, cod roe spread, fish sticks, canned tuna, pike/perch,

* Food commodities in the composite samples are sorted in order of per capita consumption

In addition to the market basket samples, fish were sampled from two important commercial fishing areas of Sweden, Lake Vättern and the Baltic Sea. Lake Vättern is the second largest freshwater lake in Sweden. Fish from Lake Vättern have relatively high concentrations of environmental pollutants, such as methyl mercury and polychlorinated biphenyls (PCBs). This is due to a combination of current and historical releases of pollutants into the lake and the nutrient-poor state of the lake ecosystem. The Baltic Sea is a brackish water sea that also has been heavily polluted by persistent halogenated organic pollutants, such as PCBs. The methyl mercury levels in fish are, however, generally lower in fish from the Baltic Sea than in the same fish species from Lake Vättern (Emma Ankarberg, Swedish National Food Administration, personal communication).

Fish were caught by gill net or other commercial fishing methods in Lake Vättern, which is located in southern Sweden (position: N 58° 00' E 014° 20') and in the Baltic Sea. Fish from the Baltic Sea came from two areas; perch (*Perca fluviatilis*), pike-perch (*Sander lucioperca*), pike (*Esox lucius*), burbot (*Lota lota*) and whitefish (*Coregonus albula*) from Öregrundsgrepen (position: N 60° 27' E 018° 11') and salmon (*Salmo salar*) and brown trout (*Salmo trutta trutta*) from the mouth of the River Dalälven (position: N 60° 35' E 017° 27') (Table 2). Whole skinless fillets were sampled from perch, pike-perch, pike and burbot, and the fish muscle samples were homogenised using a food mixer (Grindmix GM200®) with a polypropylene or glass vessel and stainless steel knives. Muscle samples from salmon and brown trout were taken in the area around the dorsal fin. The mixers were thoroughly washed with acetone after mixing of each sample. All other materials used in sampling and preparation of the food samples were also washed in acetone. Homogenised individual fish muscle samples were kept frozen at -20 ºC before analysis.

Table 2. Fish samples used for analysis of perfluorinated alkyl substances

Fish species	Location	Sampling yr/mo	Age (years)	Length (cm)	Weight (g)
Perch (*Perca fluviatilis*)	Vättern (*n*=5) Baltic Sea (*n*=5)	2001/11 2001/09	 7 (6-8)	28 (28-31) 25 (24-28)	306 (260-330) 185 (166-295)
Pikeperch (*Sander lucioperca*)	Baltic Sea (*n*=5)	2001/09	4 (3-5)	35 (30-47)	327 (207-903)
Pike (*Esox lucius*)	Baltic Sea (*n*=5)	2001/10		59 (48-85)	1270 (590-3570)
Burbot (*Lota lota*)	Vättern (*n*=5) Baltic Sea (*n*=5)	2001/11 2001/11		59 (44-68) 52 (42-65)	2050 (540-2920) 1000 (500-2200)
Salmon (*Salmo salar*)	Vättern (*n*=5) Baltic Sea (*n*=5)	2001/11 2001/09	1 (1-2) 2 (1-3)	68 (54-76) 80 (68-100)	3130 (2850-4550) 4800 (2700-9800)
Brown trout (*Salmo trutta*)	Vättern (*n*=5) Baltic Sea (*n*=5)	2001/11 2001//09	1 (1-2) 1 (1-3)	51 (47-54) 62 (58-69)	1060 (750-1370) 2500 (2100-3600)
Whitefish (*Coregonus lavaretus*)	Vättern (*n*=5) Baltic Sea (*n*=5)	2001/11 2001/09	5 (4-7) 5 (3-6)	40 (38-40) 37 (36-42)	470 (400-650) 390 (360-780)

Chemical analysis

Chemical analyses of PFAS in the fish muscle tissue and the food samples were carried out in the laboratory of the Department of Applied Environmental Science (ITM), Stockholm University in October 2006-January 2007. MeHg was analysed at the Swedish National Veterinary Institute in January-April 2002.

Sample preparation of fish muscle tissue

A 1 g aliquot of homogenized fish muscle tissue was transferred to a polypropylene (PP)-centrifuge tube, and spiked with 5 ng each of the mass-labeled internal standards $^{13}C_4$-perfluorooctanoic acid ($^{13}C_4$-PFOA) and ammonium $^{18}O_2$-perfluorooctane sulfonate ($^{18}O_2$-PFOS). The samples were extracted twice with 5 mL of acetonitrile in an ultrasonic bath. Following centrifugation, the supernatant extract was removed and the combined acetonitrile phases were concentrated to 1 mL under a stream of nitrogen. The concentrated extract underwent dispersive clean-up on graphitized carbon and acetic acid. Approximately 0.5 mL of the cleaned-up extract was added to 0.5 mL of aqueous ammonium acetate. Precipitation occurred and the extract was centrifuged before the clear supernatant was transferred to an autoinjector vial for instrumental analysis. Finally, the volume standard 7H-perfluoroheptanoic acid was added.

Instrumental analysis of fish samples

Aliquots of the final extracts were injected automatically on a high performance liquid chromatography system coupled to high resolution mass spectrometry (HPLC-HRMS for perfluorosulfonates (PFSs) including 6:2 fluorotelomer sulfonate and perfluorooctane sulfonamide) or tandem mass spectrometry (HPLC-MS-MS for perfluorocarboxylates (PFCAs)) (Table 3). The instrumental setup for the HPLC-HRMS was: Acquity Ultra Performance LC (Waters) and Q-ToF Premier (Micromass). For HPLC-MS-MS, an Alliance 2695 pump (Waters) was coupled to a Quattro II triple quadrupole MS (Micromass). Compound separation was achieved for both compound classes on a Discovery HS C18 column (Supelco) with a binary gradient of buffered (4 mM ammonium acetate) methanol and water. Quantification was performed in extracted high resolution mass chromatograms (PFSs) or selected reaction monitoring chromatograms (PFCAs) using the internal standard method. $^{18}O_2$-PFOS and $^{13}C_4$-PFOA were employed as internal standards for the PFSs and PFCAs, respectively.

Table 3. Acronyms and compound-specific method detection limits (MDLs) (pg/g wet weight) of perfluorinated alkyl substances in fish muscle tissue.

	Acronyms	Internal standard used	MDLs
6:2 Fluorotelomer sulfonate	6:2 FTS	$^{18}O_2$-PFOS	200
Perfluorooctane sulfonamide	PFOSA	$^{18}O_2$-PFOS	250
Perfluorosulfonates	*PFSs*		
Perfluorobutane sulfonate	PFBS	$^{18}O_2$-PFOS	50
Perfluorohexane sulfonate	PFHxS	$^{18}O_2$-PFOS	20
Perfluorooctane sulfonate	PFOS	$^{18}O_2$-PFOS	250
Perfluorodecane sulfonate	PFDcS	$^{18}O_2$-PFOS	200
Perfluorocarboxylates	*PFCAs*		
Perfluorohexanoate	PFHxA	$^{13}C_4$-PFOA	100
Perfluoroheptanoate	PFHpA	$^{13}C_4$-PFOA	120
Perfluorooctanoate	PFOA	$^{13}C_4$-PFOA	100
Perfluorononanoate	PFNA	$^{13}C_4$-PFOA	80
Perfluorodecanoate	PFDcA	$^{13}C_4$-PFOA	80
Perfluoroundecanoate	PFUnA	$^{13}C_4$-PFOA	80
Perfluorododecanoate	PFDoA	$^{13}C_4$-PFOA	80
Perfluorotridecanoate	PFTriA	$^{13}C_4$-PFOA	100
Perfluorotetradecanoate	PFTeA	$^{13}C_4$-PFOA	150
Perfluoropentadecanoate	PFPeDA	$^{13}C_4$-PFOA	300

Quality control - fish samples

Method detection limits (MDLs) for all compounds were determined on the basis of five blank extraction experiments. MDLs were defined for each compound as mean plus three standard deviations of the blank experiments. A complete list of the compound acronyms and specific MDLs can be found in Table 3. The stable isotope mass-labelled internal standards $^{18}O_2$-PFOS and $^{13}C_4$-PFOA were used as surrogate standards for quantification of the PFSs and PFCAs, respectively. Recoveries of the internal standards were on an average (± 1 standard deviation) 74 ± 10% (n=60) for $^{18}O_2$-PFOS and 74 ± 11% (n=60) for $^{13}C_4$-PFOA.

Two of the low-contaminated samples (X 281 and X 332) were analysed in duplicate in two different batches at different days. Maximum deviation for the duplicate analyses was 27 % for PFDcA in X 281. Furthermore, a fish tissue sample used in an international inter-laboratory comparison (ILC) study in 2005 was analysed along with the samples. The obtained concentrations deviated by <20% from the median concentration in the ILC for all three detected PFSs and five detected PFCAs. Only for PFOSA the here obtained concentration (43 ng/g ww) deviated from the median in the ILC (28 ng/g ww) by 53%. However, due to high variability of results for PFOSA the mean value in the ILC was 60 ng/g ww and the median might not reflect the real value well for this compound.

Sample preparation of market basket samples

The extraction method for the food samples was based on ion pairing as described by Hansen et al. (2001). Samples were first homogenized in Milli-Q purified water (sample/water ratio 1:5). A 1 mL sub-sample of the homogenate was transferred to a polypropylene (PP) tube and spiked with the mass-labelled internal standards $^{13}C_4$-perfluorooctanoic acid ($^{13}C_4$-PFOA) and ammonium $^{18}O_2$-perfluorooctane sulfonate ($^{18}O_2$-PFOS). A volume of 2 mL of a sodium carbonate buffer (1 M) and 1 mL of tetrabutylammonium (TBA) hydrogensulfate solution (10 mM at pH 10) were added. The resulting aqueous suspension was vortex mixed, and extracted twice with 5 mL of methyl-*tert*-butyl ether (MTBE). The two MTBE fractions were combined and gently evaporated until dryness using dry nitrogen. Methanol (500 µL) was added to dissolve the residues, and the extract was filtered through a 0.46-µm PP-filter into a PP vial. Finally, the volume standard 7H-perfluoroheptanoic acid was added.

Instrumental analysis - market basket samples

Aliquots of 10 µL of the final extracts were injected automatically on a high performance liquid chromatography system coupled to tandem mass spectrometry (HPLC-MS-MS). The instrumental setup for HPLC-MS-MS was an Alliance 2695 pump (Waters) coupled to a Quattro II triple quadrupole MS (Micromass). The analytes of interest were separated using a Discovery HS C18 column (Supelco) with a binary gradient of buffered (10 mM ammonium acetate) methanol and water. Quantification was performed in selected reaction monitoring chromatograms using the internal standard method. $^{18}O_2$-PFOS and $^{13}C_4$-PFOA were employed as internal standards for PFOS and PFOA, respectively.

Methyl mercury analysis of fish samples

MeHg in fish (Table 2) was analysed as total Hg. Homogenised samples were wet digested in concentrated nitric acid/perchloric acid (1/1) in an open system. Digested samples were diluted with 0.5 M hydrochloric acid and analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES). The limit of quantification was 7.5 ng Hg/g wet weight . A certified reference material for trace metals (dogfish liver, DOLT-3) from the National Research Council, Canada, with a high Hg level was analysed together with the samples. Analysis of 5 certified samples gave results (3.40±0.13 µg Hg/g dry weight; mean±standard deviation) that were similar to the certified value (3.37±0.14 µg Hg/g dry weight). A certified reference material with low Hg level (oyster tissue, SMR 1566b) from the National Institute of Standards and Technology was also analysed once and the result (36 pg Hg/g dry weight) was similar to the certified value (37±1 pg Hg/g dry weight).

Statistical analysis

The Kruskal-Wallis test was used when results from more than two study groups were compared. The Mann-Whitney U-test was used for comparison of results of two groups.

Results

PFOS and PFOA concentrations in the market basket food samples (including seafood products) were below the quantification limit of 2.2 and 3.2 ng/g fresh weight, respectively, for PFOS and PFOA (Table 4). Concentrations of PFOA in fish from both Lake Vättern and the Baltic Sea were also in many cases below LOQ (<0.10 ng/g fresh weigh) (Table 5). Concentrations of PFOS were, however, higher in fish from Lake Vättern and the Baltic Sea than in the market basket samples. Moreover fish from Lake Vättern exhibited significantly higher PFOS concentrations than fish from the Baltic Sea, except in the cases of whitefish and brown trout. Whitefish deviated from the other fish species in that the median concentrations of all PFAS in the whitefish muscle were similar in the two study areas (Table 5).

Table 4. PFOS and PFOA concentrations in pooled food samples from two grocery store chains in Uppsala Sweden sampled in a market basket study 2005.

Food	PFOS (ng/g fresh weight)	PFOA (ng/g fresh weight)
Meat and meat products (*n*=2)	<2.2	<3.2
Dairy products (*n*=2)	<2.2	<3.2
Eggs (*n*=2)	<2.2	<3.2
Fish and fish products (*n*=2)	<2.2	<3.2

Concentrations of most of the other analysed PFAS in fish were low and often close to or below LOQ, which made statistical analysis difficult. Nevertheless, as in the case of PFOS, concentrations of PFNA, PFDcA, PFDoA, PFTriA, and PFTeA were higher in perch and burbot from Lake Vättern than in the same species from the Baltic Sea (Table 5). A similar concentration pattern was suggested for salmon and brown trout, although statistical analysis could not be performed for some compounds since all the samples had concentrations below the LOQ in the Baltic Sea salmon and trout.

The median concentrations of PFOS differed significantly between the studied fish species both in Lake Vättern (Kruskal-Wallis test: p=0.014) and the Baltic Sea (p=0.005) (Table 5). In Lake Vättern median concentrations changed in the order burbot≈perch>salmon>brown trout>whitefish (Table 5). In the Baltic Sea whitefish had the highest median concentrations followed by perch, burbot, brown trout and salmon. Pike and pikeperch from the Baltic Sea were also analysed and pikeperch had a median level of PFOS in the same range as the species with the highest concentrations (1.95 ng/g fresh weight (1.84-2.93); median (min-max)), whereas pike had a median concentration in the range of those with the lowest concentrations (0.70 ng/g fresh weight (0.55-1.26)). Also for the other PFAS with enough results above the LOQ the concentrations differed between species (Kruskal-Wallis test, $p \leq 0.05$).

Among fish species with median concentrations above LOQ the pattern of PFAS contamination differed both between study areas and between fish species (Table 6). Perch, burbot, salmon and brown trout from the Baltic Sea had lower median quotients of PFOS and PFNA than the same species from Lake Vättern, whereas no difference was seen for whitefish. Similar patterns were observed for PFOS/PFDcA and PFOS/PFUnA quotients. In the cases of PFOS/PFDoA (perch) and PFOS/PFTriA (perch and burbot) quotients no differences were observed between the sampling areas (Table 6).

Table 5. Concentrations of perfluorinated chemicals in fish from Lake Vättern and the Baltic Sea (ng/g fresh weight)

Compound	Site	Perch	Burbot	Whitefish	Salmon	Brown trout
6:2 Fluorotelomer sulfonate	Vättern	<0.20 (<0.20-0.42)	0.22 (<0.20-0.36)	0.34 (0.30-1.01)	0.33 (<0.20-0.64)	<0.20 (<0.20-0.34)
	Baltic Sea	<0.20 (<0.20-0.26)	0.23 (<0.20-0.58)	<0.20 (<0.20-0.54)	0.25 (<0.20-0.29)	0.21 (<0.20-0.29)
PFOSA	Vättern	1.02 (0.42-1.41)	1.59 (1.02-2.21)	0.64 (<0.25-1.46)	0.69 (0.62-0.74)	0.53 (0.41-0.68)
	Baltic Sea	**<0.25 (<0.25-0.45)**	0.71 (0.47-3.30)	<0.25 (<0.25-0.52)	<0.25	0.71 (0.44-0.72)
Perfluorosulfonates						
PFBS	Vättern	<0.10	<0.10	<0.10	<0.10	<0.10
	Baltic Sea	<0.10	<0.10	<0.10	<0.10	<0.10
PFHxS	Vättern	0.05 (0.03-0.14)	0.73 (0.50-0.80)	0.03 (<0.02-0.12)	0.08 (0.05-0.10)	0.04 (0.02-0.06)
	Baltic Sea	<0.02	**0.03 (<0.02-0.20**)	<0.02 (<0.02-0.03)	**<0.02**	0.04 (0.02-0.05)
PFOS	Vättern	11.2 (5.85-23.1)	12.0 (7.45-15.2)	2.86 (1.21-10.1)	8.49 (6.86-10.1)	5.73 (0.97-6.87)
	Baltic Sea	**2.13 (1.95-2.92)**	**1.69 (0.60-2.66)**	2.51 (0.73-3.34)	**0.98 (0.47-1.24)**	1.08 (0.58-1.38)
PFDcS	Vättern	<0.20	<0.20 (<0.20-0.25)	<0.20	<0.20	<0.20
	Baltic Sea	<0.20	<0.20	<0.20	<0.20	<0.20
Perfluorocarboxylates						
PFHxA	Vättern	<0.10	<0.10	<0.10	<0.10	<0.10
	Baltic Sea	<0.10	<0.10	<0.10	<0.10	<0.10
PFHpA	Vättern	<0.12	<0.12	<0.12	<0.12	<0.12
	Baltic Sea	<0.12	<0.12	<0.12	<0.12	<0.12
PFOA	Vättern	0.11 (<0.10-0.13)	0.25 (0.15-0.25)	<0.10 (<0.10-0.15)	<0.10 (<0.10-0.13)	0.10 (<0.10-0.11)
	Baltic Sea	<0.10	0.20 (<0.10-0.39)	<0.10	0.12 (<0.10-0.22)	0.12 (<0.10-0.16)
PFNA	Vättern	0.31 (0.22-0.51)	0.68 (0.33-0.10)	0.24 (<0.08-0.71)	0.15 (0.12-0.20)	0.21 (0.08-0.26)
	Baltic Sea	**0.16 (0.14-0.22)**	**0.17 (0.14-0.47)**	0.26 (0.18-0.38)	0.11 (0.10-0.19)	0.16 (<0.08-0.18)
PFDcA	Vättern	0.35 (0.28-0.75)	0.57 (0.39-0.81)	0.15 (0.13-0.37)	0.27 (0.23-0.32)	0.33 (0.15-0.41)
	Baltic Sea	**0.15 (0.12-0.20)**	**0.17 (0.10-0.27**)	0.15 (0.15-0.34)	**0.08 (<0.08-0.12)**	<0.08
PFUnA	Vättern	0.37 (0.28-0.89)	0.61 (0.45-0.75)	0.15 (0.12-0.89)	0.43 (0.41-0.58)	0.32 (0.09-0.41)
	Baltic Sea	0.30 (0.18-0.37)	**0.17 (0.13-0.34)**	0.20 (0.12-0.61)	<0.08	0.08 (<0.08-0.18)
PFDoA	Vättern	0.32 (0.17-0.53)	0.31 (0.23-0.32)	<0.08 (<0.08-0.39)	0.29 (0.24-0.38)	0.15 (<0.08-0.25)
	Baltic Sea	**0.09 (<0.08-0.10)**	**<0.08 (<0.08-0.12**)	<0.08 (<0.08-0.15)	<0.08	<0.08
PFtriA	Vättern	1.26 (0.69-1.60)	1.01 (0.86-1.32)	0.19 (<0,10-1.23)	1.44 (1.09-1.83)	0.69 (0.12-1.10)
	Baltic Sea	**0.23 (0.16-0.34)**	**0.19 (0.14-0.38)**	0.19 (<0.10-0.33)	<0.10	<0.10
PFTeA	Vättern	0.34 (0.31-0.48)	0.24 (0.21-0.36)	<0.15 (<0.15-0.29)	0.47 (0.27-0.65)	<0.15 (<0.15-0.34)
	Baltic Sea	**<0.15**	**<0.15**	<0.15	**<0.15**	<0.15
PFPeA	Vättern	<0.30 (<0.30-0.36)	<0.30	<0.30	0.44 (0.31-0.74)	<0.30
	Baltic Sea	<0.30	<0.30	<0.30	**<0.30**	<0.30

No statistical analysis could be performed in cases when all samples for a certain species had concentrations below the limit of quantification in one sampling location. Baltic Sea results in **bold** are lower than those for the same fish species from Lake Vättern (Mann-Whitney U-test, $p \leq 0.05$, $n=5$)

Table 6. Quotient of concentrations of PFOS, PFOSA, PFNA and PFTriA

Compounds	Location	Perch	Burbot	Salmon	Brown trout	Whitefish
PFOS/PFOSA	Vättern		7 (5-10)		10 (2-14)	
	Baltic Sea		2 (1-2)*		2 (1-2)*	
PFOS/PFHxS	Vättern		15 (14-20)		109 (41-176)	
	Baltic Sea		54 (13-85)		31 (23-33)*	
PFOS/PFOA	Vättern		49 (48-61)		64 (10-114)	
	Baltic Sea		8 (2-46)*		12 (4-25)	
PFOS/PFNA	Vättern	39 (26-45)	17 (15-23)	51 (49-58)	27 (12-32)	13 (11-53)
	Baltic Sea	14 (10-20)*	7 (2-15)*	6 (4-10)*	7 (3-31)*	10 (3-14)
PFOS//PFDcA	Vättern	31 (21-38)	19 (15-27)	31 (29-32)		19 (8-27)
	Baltic Sea	15 (10-20)*	8 (5-20)*	12 (4-27)*		12 (4-22)
PFOS/PFUnA	Vättern	26 (21-36)	20 (17-24)		17 (10-21)	12 (10-19)
	Baltic Sea	8 (6-13)*	7 (4-13)*		12 (6-31)	9 (4-19)
PFOS/PFDoA	Vättern	38 (34-45)				
	Baltic Sea	29 (23-53)				
PFOS/PFTriA	Vättern	9 (8-17)	10 (8-18)			
	Baltic Sea	9 (6-13)	7 (3-15)			

Comparisons were only made when median concentration for a fish species was above LOQ in both study areas
*$p \leq 0.05$, Mann Whitney U-test (n=5)

MeHg was not measured in all fish samples. However, similarly as in the case of PFOS, Lake Vättern burbot, salmon and brown trout had significantly higher MeHg concentrations than the same species caught in the Baltic Sea (Table 7).

Table 7. Concentrations of MeHg in fish from Lake Vättern and the Baltic Sea (median, min-max).

Fish species	Site	MeHg concentration (µg/g fresh weight)
Burbot	Vättern	0.51 (0.22-0.63)
	Baltic Sea	0.20 (0.17-0.33)*
Salmon	Vättern	0.25 (0.17-0.28)
	Baltic Sea	0.09 (0.08-0.18)*
Brown trout	Vättern	0.33 (0.25-0.64)
	Baltic Sea	0.08 (0.07-0.14)*

*$p \leq 0.05$, Mann Whitney U-test (n=5)

A plot of PFOS and MeHg concentrations in the different fish species suggests that PFOS concentrations were higher in freshwater predatory fish, such as perch an burbot, than in fat-rich fish (salmon and brown trout) from both sampling locations (Figure 1). Whitefish, however, was among the fish with the lowest concentrations in Lake Vättern, whereas in the Baltic Sea concentrations of PFOS in this salmonid species were among the highest. Pike, which similarly to perch and burbot is a lean predatory fish, had low concentrations of PFOS (only sampled in the Baltic Sea). Pikeperch from the Baltic Sea had among the highest concentrations of PFOS in this study area (Figure 1).

In Lake Vättern the concentrations of MeHg were similar among burbot, salmon and brown trout, whereas salmon and brown trout had lower concentrations than perch, pike and burbot in the Baltic Sea (Figure 1).

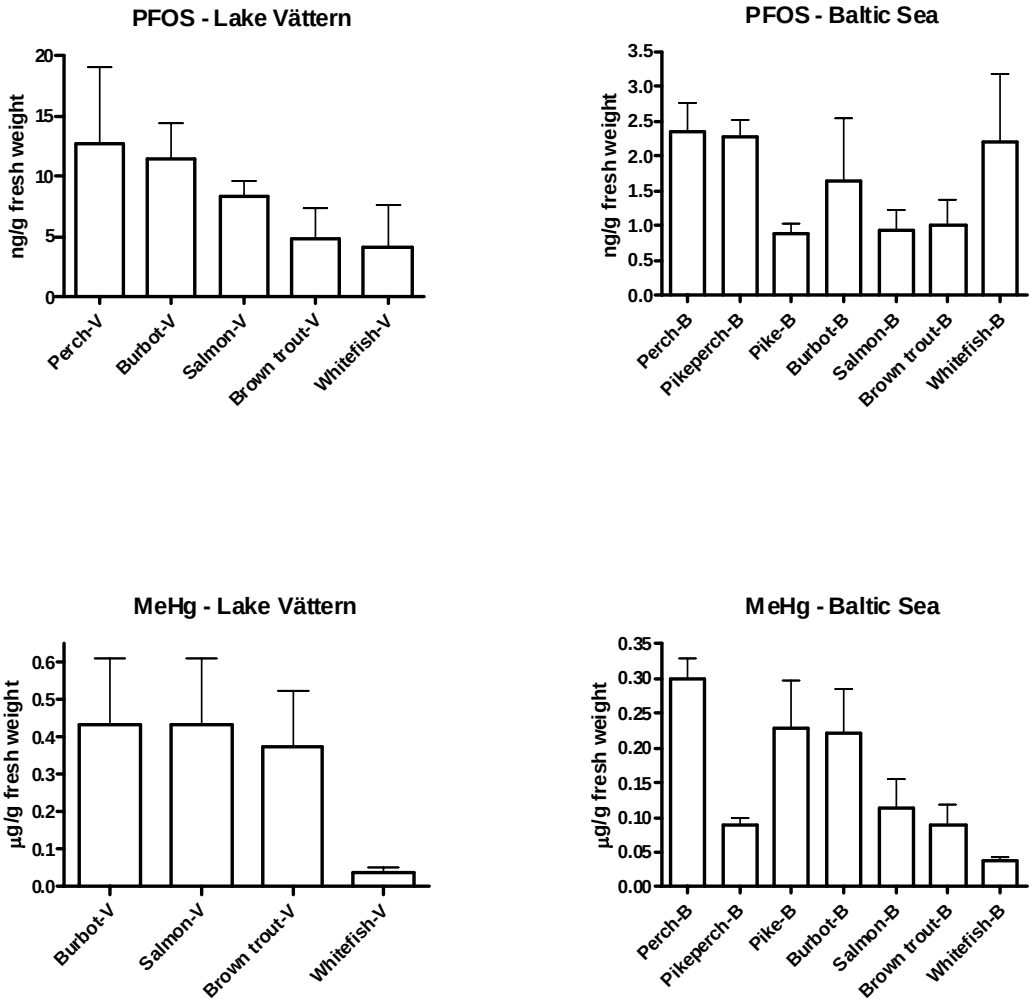

Figure 1. PFOS and MeHg concentrations in fish from Lake Vättern and the Baltic Sea

Discussion

Our results show that the concentrations of PFAS in commercial fish species varies depending both on the study area and the fish species studied. We analysed PFAS in fish muscle from both lean and fat-rich fish species from Sweden´s second largest lake, Lake Vättern, and from the brackish water sea the Baltic Sea. Concentrations of several PFAS were clearly higher in fish from Lake Vättern than in the same species from the Baltic Sea. PFOS was the compound that was present at highest concentrations among the PFAS at both sampling locations.

The analysis of the market basket food samples showed that concentrations of PFOS and PFOA were low in the foods of animal origin that contributes most to the per capita consumption of foods of animal origin in Sweden (<3.2 ng/g fresh weight). It cannot be ruled out, however, that certain foods contain higher levels of PFAS than shown in the market

basket study, since the use of composite samples may have masked high concentrations in individual food items. Moreover, many food items and food groups were not included in the analysis. In a total diet study in the UK most of the composite samples had PFOS concentrations below LOQ, which ranged from 0.5 ng/g fresh weight in milk and beverages to 20 ng/g in bread (FSA, 2006). PFOS was detected in composite samples of eggs (average 1 ng/g fresh weight), sugar and preserves (1 ng/g fresh weight), canned vegetables (2 ng/g fresh weight) and potatoes (10 ng/g fresh weight) (FSA, 2006). In another total diet study 49 composite food samples from Canada were analysed including samples of fast food and snacks, such as popcorn (Tittlemier et al., 2007). Seven composite samples, including beef steak, ground beef, cold cuts, marine fish, freshwater fish, and microwave popcorn, contained detectable concentrations of PFOS in the range 0.5 to 2.7 ng/g fresh weight.

PFOS concentrations in the Swedish market basket sample of seafood and seafood products were lower (<2.2 ng/g fresh weight) than concentrations in most of the fish samples from Lake Vättern and in some fish samples from the Baltic Sea. A comparison with fish from the Baltic Sea was difficult since the limit of detection was much higher in the market basket study. The market basket samples of sea food and seafood products mainly contained marine fish, such as cod, herring, plaice and tuna fish. Moreover, the fish products in the samples (fish balls and fish sticks) contained fish meat from marine fish species. Studies on PFOS concentrations in seafood from China showed that PFOS concentrations generally were low in muscle tissue from the marine fish species sampled (<3 ng/g fresh weight) (Gulkowska et al., 2006). Our finding of considerably higher PFOS concetrations in predatory fish from Lake Vättern than in the other foods analysed, at least partially, supports the hypothesis that predatory freshwater fish species may be a significant source of human PFOS exposure in Sweden. It is however currently not possible to draw firm conclusions about the contribution of PFOS in food to the total exposure of the compound. Drinking water, dust and air (via volatile PFOS precursors) in homes and commercial buildings may be other significant sources of human PFOS exposure (Skutlarek et al., 2006, Kubwabo et al., 2005; Moriwaki et al., 2003, Shoeib et al., 2005).

There could be several reasons behind the higher concentrations of certain PFAS in Lake Vättern than in the same fish species in the Baltic Sea. Apart from the large difference in water volume between Lake Vättern and the Baltic Sea, the Lake Vättern eco-system is nutrient-poor in comparison with the Baltic Sea eco-system. Consequently, environmental pollutants that are released into Lake Vättern are not "diluted" by the biomass to the same extent as in nutrient-rich aquatic systems (Lindell et al., 2001). Some of the differences in contamination levels and contamination patterns between the two study areas could also be due to differences in contribution of sewage treatment effluents to the PFAS emissions (Posner and Järnberg, 2004).

Elevated concentrations of PFOS have been found in muscle from perch caught in the vicinity of municipal sewage treatment plants and in waterways both upstream and downstream the City of Stockholm (Järnberg and Holmström, 2003). PFOS concentrations in fish from our study area of the Baltic Sea were in the same range as background concentrations reported for perch muscle (Järnberg and Holmström, 2003). Concentrations in perch muscle from Lake Vättern were slightly lower than those found in perch from Lake Mälaren (Järnberg and Holmström, 2003), which is located in the most densely populated area of Sweden. Obviously, there is a dilution gradient in PFOS (and in general PFAS) concentrations from lakes in populated, urban areas with several point and diffuse sources towards the open sea.

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