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Increasing Concentrations of Perfluoroalkyl Acids in Scandinavian Otters (*Lutra lutra*) between 1972 and 2011: A New Threat to the Otter Population?

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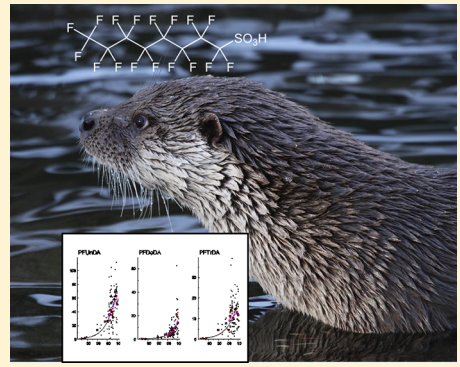
Supporting Information

ABSTRACT: Liver samples from 140 otters *(Lutra lutra)* from Sweden and Norway were analyzed for 10 perfluoroalkyl carboxylic acids (PFCAs; C6−C15), 4 perfluoroalkane sulfonic acids (PFSAs; C4,C6,C8,C10) and perfluorooctane sulfonamide (FOSA). Perfluorooctane sulfonic acid (PFOS) was the dominant compound accounting for approximately 80% of the fluorinated contaminants and showing concentrations up to 16 μg/g wet weight. Perfluorononanoic acid (PFNA) was the dominant PFCA (up to 640 ng/g wet weight) closely followed by the C10 and C11 homologues. A spatial comparison between otters from southwestern Norway, southern and northern Sweden sampled between 2005 and 2011 revealed that the samples from southern Sweden had generally the largest contaminant load, but two PFCAs and FOSA were higher concentrated in the Norwegian samples. A temporal trend study was performed on otters from southern Sweden collected between 1972 and 2011. Seven PFCAs (C8−C14), PFOS and perfluorodecane sulfonic acid (PFDS) showed significantly increasing trends with doubling times between 5.5 and 13 years. The PFCAs also showed significantly increasing trends over the period 2002 to 2011. These findings together with the exceptionally high liver concentrations of PFOS are of great concern for the Scandinavian otter populations.



INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are both oil and water repellent, highly fluorinated, man-made chemicals. Many PFASs have been in use for more than half a century in numerous industrial and consumer product applications,[1] such as textile stain and soil repellents, grease-proofing for food-contact paper, processing aids in fluoropolymer manufacturing and in aqueous film-forming fire fighting foams. There is no known production of PFASs in Scandinavia but there may be (or have been) downstream industries using PFAS formulations and many imported products still contain a variety of PFASs.[2] The PFASs can be divided into two groups: perfluoroalkyl acids (PFAAs) and their environmental or metabolic precursor compounds.[3] PFAAs are resistant to thermal, chemical and biological degradation, thus being extremely persistent in the environment. The two PFAA subgroups of highest concern are the perfluoroalkyl carboxylic acids (PFCAs) and the perfluoroalkane sulfonic acids (PFSAs). Perfluorooctane sulfonic acid (PFOS) is the most widely investigated PFSA. The largest historic producer of PFOS and PFOS-based compounds (i.e., PFOS precursors), the 3M Company, phased out the production on a voluntary basis between 2000 and 2002 after evidence of elevated concentrations of PFOS in blood from their workers and in wildlife.[4,5] PFOS and its precursors have been used in cleaning aids, fire fighting foam and as water and grease/stain proofing in textiles, furniture, paper and carpets. Nowadays PFOS is mainly used in the metal and aircraft industry. PFOS is a PBT chemical; it is persistent, bioaccumulative, and toxic and was included in the Stockholm Convention on Persistent Organic Pollutants in 2009, under Annex B (requiring use restrictions). It was banned in many applications within the EU in June 2008, but was partly replaced by other persistent PFAAs, for example the shorter chain homologue perfluorobutane sulfonic acid (PFBS). Furthermore, PFOS is now produced in southeast Asia. Perfluorooctanoic acid (PFOA) is another persistent PFAA which has received a lot of attention. PFOA is used primarily during the production of fluoropolymers, which have hundreds of various manufacturing and industrial applications.

Adverse health effects following PFAA exposure have been reported from laboratory studies on rodents. The primary target is the liver where PFOS and PFOA cause increased liver weight and hepatocytic hypertrophy[6,7] as well as abnormal

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behavior, weight loss and serious damages in liver and lung[8] and developmental neurotoxic effects.[9] Early pregnancy loss, comprised postnatal survival, delays in general growth and development were also found for mice given PFOA[10] as well as increased mortality, lowered cholesterol and estradiol levels in serum in cynomolgus monkeys given PFOS in different doses.[11] In addition, PFOS and PFOA are potential developmental toxicants and are suspected endocrine disruptors resulting in lower testosterone levels and higher estradiol levels in adult rats.[12]

A study on wild sea otters (*Enhydra lutris*) from California showed that otters that died from infectious diseases had higher concentrations of both PFOS and PFOA compared to otters that died from noninfectious diseases.[13] The highest concentrations of PFAAs with a dominance of PFOS are usually found in top predators like polar bear (*Ursus maritimus*), mink (*Neovison vison*), river otter (*Lontra canadensis*), and various pinnipeds,[14−17] which demonstrates that some PFAAs do bioaccumulate. The bioaccumulation potential generally increases with increasing chain length of the PFAAs.[18] Therefore most attention in biomonitoring has been given the long-chain PFSAs and PFCAs. PFAAs do not dissolve in lipids but bind to proteins.[19] Thus, they are found in highest concentrations in for example liver, serum, and egg yolks.[20]

The otter (*Lutra lutra*) in Sweden has previously been exposed to high concentrations of organochlorines, showed a low reproduction rate and was only found in few numbers in scattered areas in the 1980s.[21] After the ban of PCBs and DDT in the 1970s the concentrations of these compounds have decreased in Swedish biota.[22,23] In the early 1990s the otter population in Sweden started to increase in numbers and in spatial distribution and otters are now again found in areas that have been abandoned for three decades or more.[24]

Approximately 80% of the otter's diet consists of fish, which makes the otter a target species for waterborne environmental contaminants such as PFAAs. Furthermore, PFAAs are known to have increased in concentrations in the Swedish environment at least up to the late 1990s, for example in guillemot eggs (*Uria aalge*)[25] and in gray seals (*Halichoerus grypus*).[26] Increasing trends were found in biota worldwide including remote areas such as the Arctic[27−29] and Antarctica.[30] However, no comprehensive study on trends of PFAAs in otters has been published to date.

Fifteen PFASs were analyzed in 140 livers of otters from Sweden and Norway in the present study. The aim was to investigate PFAS levels, as well as their temporal trends for southern Sweden and possible spatial differences between areas in Sweden and Norway.

MATERIALS AND METHODS

Samples. Liver samples from otters from Sweden (n = 123, collected between 1972 and 2011) and from southwestern Norway (n = 17, collected in 2010) were included in this study. The sampling areas are depicted in Figure S1 in the Supporting Information (SI). Most of the otters were killed in traffic accidents or drowned in fishing gear. The carcasses were frozen before necropsy and thawed when samples of different tissues were taken for various studies. The liver samples were stored at −25 °C in the Environmental Specimen Bank at the Swedish Museum of Natural History (SMNH) in Stockholm, Sweden, or at the Norwegian Institute for Nature Research (NINA) in Trondheim, Norway. Gender, weight and length of the otters were determined during necropsy and the specimen were grouped into three age groups: juveniles (up to 5 months old, n = 3) if tooth replacement was incomplete, subadults (approximately 5−18 months old, n = 28) when the epiphyseal closure at the proximal ends of the femur was incomplete, and adults (19 months and older, n = 109) when the epiphyseal closure was complete.[31] Two of the samples included in the present study were from a mother and her juvenile cub. The lactiferous tissue from one lactating female was also analyzed, in addition to the liver.

The temporal trend analyses consisted of 97 otters from southern Sweden collected between 1972 and 2011. The geographical study focused on otters collected between 2005 and 2011 and covered three areas: southern and northern Sweden (n = 46 and 16, respectively) and southwestern Norway (n = 17, see Figure S1 in the SI). Most (probably all) of the otters from Sweden came from limnic areas and the otters from Norway were mainly from a marine environment at the southwestern coast of Norway. There was no tendency of including a higher percentage of urban otters in recent years compared to earlier years.

Target Compounds. In this paper we follow the PFAS terminology as suggested by Buck et al.[3] For full compound names, CAS-number and carbon chain length of the analytes see Table S1 in the SI. The target compounds were PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxS, PFOS, PFDS, and FOSA and 80 specimens (including all Norwegian samples) were also analyzed for PFHxA, PFHpA, PFPeDA, and PFBS. For simplicity, perfluorooctane sulfonamide (FOSA, the only precursor compound included in this study) is hereafter included in the generic term PFAAs. Chemical standards were obtained either from Wellington, Aldrich or Fluka.

Chemical Analyses. Prior to analysis, liver samples were thawed and subsampled at SMNH or NIVA. Approximately 1 g of liver was taken for analysis. The analytical method and the number of analytes changed during this study. The first method used up to 2005 was based on ion pair extraction into a nonpolar solvent as described by Ylinen et al.[32] and elaborated by Hansen et al.[33] The method is described in detail in the SI including modifications from the originally published procedure.[33] Instrumental analysis and quantification in the first method was based on high performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) in the negative ion electrospray ionization mode as described in detail by Holmström et al.[25] A more detailed description of the analytical method is given in the SI.

The second method used for analyses performed 2008 or later is described by Berger et al.[34] It was based on solid−liquid extraction with acetonitrile and dispersive cleanup on graphitised carbon. A brief version of the sample preparation including modifications from the procedure described by Berger and co-workers is given in the SI. Instrumental analysis and quantification in the second method was done as described in detail by Holmström and Berger.[35] The same instrumentation as in the first method was employed. Again, the procedure is described in detail in the SI. All concentration values in the present study are given on a sample wet weight (ww) basis.

Quality Assurance. Due to the methodological differences, systematic deviations between the results from the two methods were expected. Therefore, nine liver samples were analyzed with both methods, and the two sets of results were compared for each analyte using regression analysis. All regression analyses were scrutinized and only the statistically

significant ones were used in the conversions. In several of the regressions, potential high leverage effects of single measurements were identified and therefore the ratio between the two methods was used in the conversion which is a more robust way to convert data. For compounds that showed a significant correlation between the two methods a correlation coefficient was calculated. Data from the previous method was then adjusted using the correlation coefficient for each substance so that results from the two methods could be combined for further data treatment. For PFNA, PFTeDA, PFHxS, and FOSA there was no significant correlation between the results of the two methods and therefore only data from the later method was used.

The method detection limits (MDLs) for the first method were around 1 ng/g wet weight for all analytes, while MDLs in the second method were between 0.1 and 1 ng/g. Absolute recoveries of the internal standards (ISTDs) in the second method were calculated for all samples and were between 69 and 91% (average values) with standard deviations <19% for all ISTDs. Duplicate sample extractions were performed with both methods to assess precision. In the first method, quantified concentrations of paired results varied with <10% between the duplicate samples, with one exception of 15% for PFHxS. In the second method the variation of 27 out of 36 paired results was <10%, and the remaining 9 paired values showed deviations between 11 and 19%. A fish sample previously investigated in an international interlaboratory comparison study[36] was analyzed with the second method for accuracy testing. The results for all quantifiable analytes obtained in the present study deviated <24% from the median values obtained in the interlaboratory comparison study.

Statistical and Trend Analyses. Statistical analyses were only performed for PFAAs that had less than 25% nondetects in the sample set under consideration. Values below the MDL (nondetects) were replaced by MDL/$\sqrt{2}$ prior to statistical treatment.[37] Other methods have been suggested that are an improvement over the common practice of recalculating nondetects as MDL/$\sqrt{2}$, and which would increase the statistical power.[38] However, these alternative methods require several values to be above MDL in any particular year and therefore could not be applied in the present study.

The otters from Norway were all collected from the same area and in the same year. Therefore, they were used for testing of a possible correlation of PFAA levels with age and/or sex using students t test. In this material, there were no juveniles, thus only two age classes. Since the Norwegian sample set only consisted of 17 otters, we also used simple regression analysis on the otters from southern Sweden (i.e., the largest group), where the different PFAAs were dependent variables and age, sex, and sampling year were independent variables. Only sampling year turned out to be significant, not sex or age.

Otters collected between 2005 and 2011 were used in a spatial study covering three areas: southwestern Norway, northern Sweden and southern Sweden (Figure S1 in the SI). Kruskal–Wallis non parametric test was used to test for significant differences in medians between the three areas. In case Kruskal–Wallis test was significant, repeated Mann–Whitney U-tests were applied as a post hoc test with the Bonferroni correction.

Temporal trend analyses were only carried out on otters from southern Sweden using ordinary log–linear regression analysis. Prior to statistical analysis the PFAA concentration values were log-transformed in order to approach the assumptions of normal distribution and variance homogeneity. Mean values of at least four individual specimens were calculated to avoid a strong influence of single specimens of a particular year. If the number of measurements were fewer than four in a particular year we fused data from two or more years until we got at least four measurements. Then we calculated the mean value for concentration and year before the linear regression analysis was carried out.

RESULTS AND DISCUSSION

PFAA Concentrations. PFNA, PFOS, and FOSA were detected above their respective MDLs in all samples. PFCAs with 8 and 10 to 13 carbon atoms (PFOA and PFDA-PFTrDA) and PFHxS were detected in >95% of all analyzed samples, whereas PFTeDA, PFPeDA and PFDS were above MDL in 87, 79, and 90% of all samples, respectively. PFHxA was below MDL in all samples (<0.1–<0.5 ng/g ww) and PFHpA was below MDL in all but three samples from 2009 and 2010. PFBS was found in 18 otters from northern and southern Sweden (31%) from the period 2005 to 2011, and in two otters from Norway (12%), in concentrations just above the MDL (0.15–0.3 ng/g ww). Thus, PFHxA, PFHpA, and PFBS were not further evaluated.

PFOS was found in all samples in concentrations ranging from 19 to 16 000 ng/g ww. It was by far the dominant compound (approximately 80% of the analyzed PFAAs was PFOS), which is in agreement with numerous other wildlife studies.[22,23,34,39,40] The concentrations of PFOS were higher or much higher in otters from Sweden compared to the otters from Norway, as well as in other mammals and birds of prey from Scandinavia.[26,41–43]

PFNA was the second most abundant PFAA and the dominant PFCA in our study with liver concentrations ranging between 0.51 and 637 ng/g ww. Among the PFCAs, the concentrations decreased gradually with increasing chain length from PFNA to PFPeDA. This pattern is different from the typical PFCA pattern often observed in marine mammals and birds, where the odd carbon chain homologues clearly dominate over the adjacent even carbon chain homologues.[35,44] However, the Norwegian otters in the present study did show a tendency of this "marine" pattern (see Spatial trends section below). Compared to the otters in our study, the concentrations of PFCAs were lower in arctic fox (*Alopex lagopus*), ringed seal (*Pusa hispida*), mink, birds, and fish from the Canadian Arctic, but the concentrations in polar bears were generally similar or only somewhat lower.[44] Polar bears from East Greenland had generally similar or higher mean concentrations of most PFAAs compared to the otters in the present study.[45]

FOSA was the only nonpersistent precursor compound included in this study. It was found in all samples analyzed (0.7–92 ng/g). The FOSA concentrations in otters were somewhat lower compared to harbor porpoise from Danish waters.[43] River otter and mink from the United States had higher (Illinois) or similar (Massachusetts, South Carolina and Louisiana) concentrations of FOSA as the otters in our study.[15]

Age and Gender. No significant differences in PFAA concentrations between subadults and adults were found in the Norwegian otters apart from PFNA, where adults had slightly higher concentrations compared to the subadults (t test, p = 0.008). Also, no significant difference in concentrations between males and females was found for any of the compounds. Age and sex had no significance in the multiple

Table 1. PFAA Liver Concentration Ratios Cub/Mother in an Otter Pair That Was Killed in Traffic in Central Sweden in 2009 and Concentration Ratios in Lactiferous Tissue/Liver in a Lactating Female

	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFPeDA	PFHxS	PFOS	PFDS	FOSA
ratio cub/mother	0.79	0.31	0.42	0.59	0.80	0.99	0.89	0.55	0.72	0.61	0.62	0.38
ratio lactiferous tissue/liver	0.09	0.03	0.03	0.07	0.14	0.23	0.23	0.27	0.38	0.07	0.20	0.14

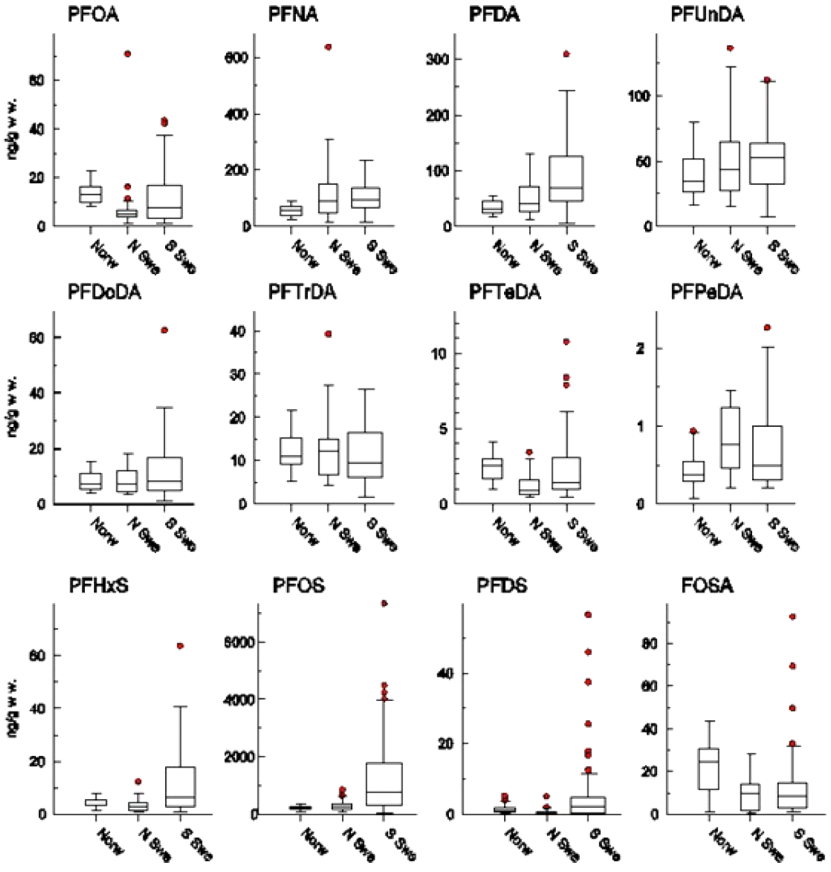

Figure 1. PFAA concentrations in otters (ng/g ww) collected between 2005 and 2011 from three areas (southwestern Norway, northen Sweden, and southern Sweden). The results are presented in boxplots, showing the median in a box delimited by the lower and upper quartiles. A vertical line is drawn from the bottom and top of the box to the lowest and largest observation within 1.5 interquartile ranges, respectively. All observations beyond these limits are plotted individually. The width of each box is proportional to the number of analyses for that group.

regression analyses either, carried out on otters from southern Sweden (including two juveniles). Therefore, otters of different age and/or sex were treated as one group in further statistical analysis.

A mother and her juvenile cub were analyzed and the liver concentration ratio cub/mother was below 1 for all PFAAs, indicating a limited transfer from mother to cub (Table 1). The highest ratios were found for the long-chain PFCAs (PFDoDA, PFTrDA, and PFTeDA), with values between 0.80 and 0.99. The exception was PFPeDA having a ratio of only 0.55. PFHxS showed the highest ratio among the PFSAs with 0.72. The ratio of PFAA concentrations in lactiferous tissue/liver from a lactating female was $\leq$0.2 for most compounds (Table 1). Only long-chain PFCAs (PFTrDA, PFTeDA, and PFPeDA) and PFHxS displayed higher ratios, which can explain the elevated ratios of these PFAAs compared to the other analytes in the cub/mother comparison. However, only one pair cub/mother was analyzed so the data should be treated with caution. Since subadults had similar liver concentrations as adults for all compounds, it seems likely that a steady state is reached relatively early in an otters life.

Male polar bears showed a significant increase in concentrations of PFCAs only up to an age of six years even though the oldest male in the study was 28 years old.[45] Similarly to the present study on otters, no difference in PFAA concentrations between adult male and female polar bears was found. This could indicate that lactation is not a major pathway of PFAA elimination among otters and polar bears. No gender or age differences in PFAA levels were seen in ringed seal populations in Canada. The authors suggested that the lack of a concentration trend with age could be due to relatively rapid depuration rates.[17] A half-life of 21 weeks was estimated for PFOS in bottlenose dolphins (*Tursiops truncatus*), and urine was suggested to be an important depuration pathway for PFAAs.[40]

However, harbor seal pups (*Phoca vitulina concolor*) in the northwestern Atlantic had higher concentrations of PFOS and PFDS compared to adults, and it was suggested that maternal transfer is an important route for PFAAs to pups among harbor seals. No gender difference in concentrations was seen among adult seals.[46] Similar patterns were observed for bottlenose dolphins from Florida[40] and harbor porpoises in Danish

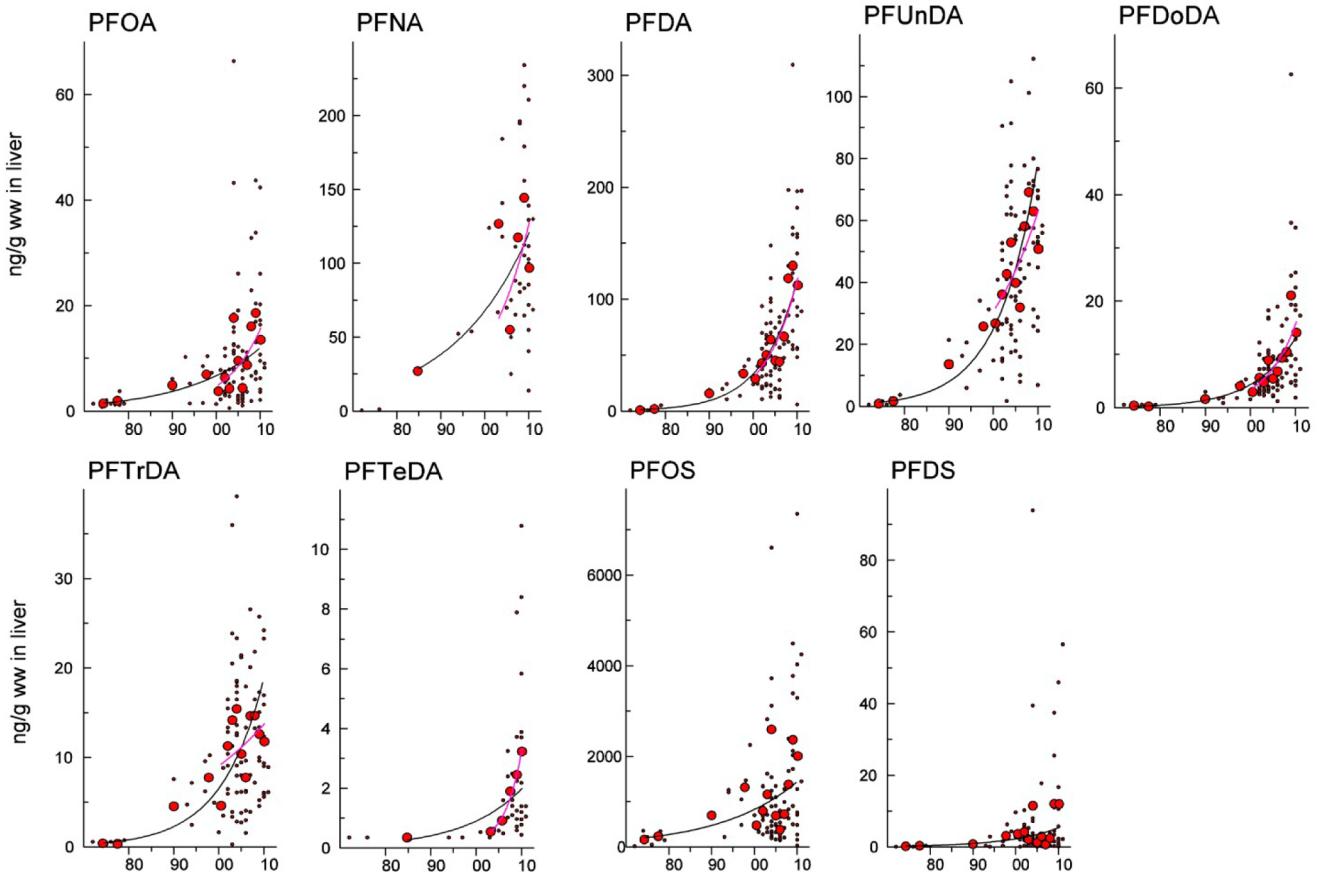

Figure 2. Time trends of PFAA concentrations (ng/g ww) in livers from otters from southern Sweden collected between 1972 and 2011. Red circles represent annual geometric means. Black solid lines show the results of linear regression on log-transformed annual means for PFAAs displaying significantly increasing levels, and red solid lines show the results from the last 10 years, if the linear regression analysis was significant. Some extreme individual values are outside the ranges of the graphs.

waters.[43] Clearly, the toxicokinetic behavior of PFAAs in otters and bears is different from that in pinnipeds and cetaceans.

Spatial Trends. In the sample set used for the spatial trend analysis the percentage nondetects was below 11% for all analytes (apart from PFHxA, PFHpA, and PFBS, see above). The results of the spatial trend comparison are visualized with boxplots in Figure 1. Table S2 in the SI gives a numeric summary of the concentration data from the spatial trend study. Much smaller concentration ranges were observed for the PFAAs in otters from Norway compared to otters from Sweden. This was probably due to the fact that the otters in Norway were all sampled within a confined marine area during a short time period (2010), whereas otters from Sweden were sampled between 2005 and 2011 from larger areas feeding from a limnic food chain (Figure S1 in the SI).

The general pattern (i.e., relative proportions) of PFAAs in otters was similar for all three sampling regions. Among the PFCAs, PFNA dominated (33−40%) followed by PFDA and PFUnDA (20−30%) (Figure S2 in the SI). The other homologues did not contribute much to the sum of PFCAs. The Norwegian otters showed a tendency of a pattern of domination of odd carbon number PFCAs compared to adjacent even carbon chains (Table S2 in the SI). Such a pattern has often been associated with the marine food web (see PFAA concentrations section above), which is in accordance with the fact that the Norwegian otters originated from a marine environment. As for the PFSAs and FOSA, PFOS dominated in otters from Sweden (95−98%) as well as in otters from Norway (86%). Other PFSAs contributed with 1−3%. FOSA contributed with 11% in otters from Norway to the sum of PFSAs plus FOSA, and to a lesser extent in Swedish samples (Figure S2 in the SI).

Significant concentration differences between the three regions were found for PFOA ($p < 0.01$), PFNA ($p < 0.008$), PFDA ($p < 0.001$), PFTeDA ($p < 0.004$), PFOS ($p < 0.001$), and FOSA ($p < 0.008$). Median values of PFOA, PFTeDA, and FOSA in otters from Norway were higher compared to those in otters from the two areas in Sweden (Figure 1). The median concentration of PFOA in otters from Norway was 14 ng/g ww, compared to 9.0 and 5.3 ng/g ww in otters from southern and northern Sweden, respectively. However, the PFOA concentration ranges were larger in Sweden, especially in the north (Table S2 in the SI). The median concentrations of PFTeDA (2.5 ng/g ww in Norway, 1.2 and 0.9 ng/g ww in southern and northern Sweden, respectively) and the median levels of FOSA (25 ng/g ww in Norway, 8.6 and 12 ng/g ww in southern and northern Sweden, respectively) were twice to three times as high in otters from Norway compared to Sweden. Otters from southern Sweden had higher median concentrations of PFNA (86 ng/g ww) and PFDA (71 ng/g ww) compared to otters from the other two areas (Table S2 in the SI).

The median concentration of PFOS in otters from southern Sweden (803 ng/g ww) was significantly higher than that in

Table 2. Results from Log-Linear Regression Analysis of PFAAs in Otters from Southern Sweden Collected Between 1972 and 2011[a]

	1972−2011					2002−2011				
	n	slope (95% CI)	r^2	sign. level	doubling time (yr) (95% CI)	*n*	Slope (95% CI)	r^2	sign. level	doubling time (yr) (95% CI)
PFOA	97	5.8% (3.4−8.3)	0.69	$p < 0.001$	12 (8.4−20)	76	12% (7.2−17)	0.81	$p < 0.001$	5.8 (4.2−9.7)
PFNA	41	5.7% (0.57−11)	0.70	$p < 0.037$	12 (6.4−121)	36	10% (4.0−16)	0.90	$p < 0.017$	6.8 (4.2−18)
PFDA	97	13% (11−14)	0.96	$p < 0.001$	5.5 (4.9−6.3)	76	14% (9.9−17)	0.90	$p < 0.001$	5.1 (4.0−7.1)
PFUnDA	97	11% (9.9−13)	0.95	$p < 0.001$	6.1 (7.1−5.3)	76	7.1% (4.5−9.7)	0.83	$p < 0.001$	9.8 (7.2−15)
PFDoDA	97	11% (9.4−13)	0.94	$p < 0.001$	6.3 (5.4−7.4)	76	15% (12−18)	0.95	$p < 0.001$	4.6 (3.8−5.6)
PFTrDA	97	11% (8.3−13)	0.89	$p < 0.001$	6.6 (5.4−8.4)	76	4.1% (0.13−8.1)	0.42	$p < 0.043$	17 (8.6−516)
PFTeDA	41	7.8% (0.8−15)	0.70	$p < 0.038$	8.9 (4.7−90)	36	27% (21−32)	0.99	$p < 0.003$	2.6 (2.2−3.4)
PFHxS	41	ns	ns	ns	ns	36	ns	ns	ns	ns
PFOS	97	5.5% (2.5−8.6)	0.57	$p < 0.002$	13 (8.1−28)	76	ns	ns	ns	ns
PFDS	97	8.6% (4.0−13)	0.58	$p < 0.002$	8.1 (5.3−17)	76	ns	ns	ns	ns
FOSA	41	ns	ns	ns	ns	36	ns	ns	ns	ns

[a]Number of individuals (*n*), slope and 95% confidence interval (CI), r^2, significance level and doubling time (with 95% CI) are shown for the whole time period (left) as well as for the last 10 years only (right). ns = not statistically significant.

otters from northern Sweden (266 ng/g ww) and Norway (201 ng/g ww). A similar geographical pattern was seen in fish from 27 Swedish lakes, where the highest concentrations of PFOS were found in fish from Southern Sweden.[23] No significant difference between concentrations of PFOS in otters from northern Sweden and Norway was observed, although some very high (up to 3660 ng/g ww) concentrations were also found in otters from northern Sweden, but not in Norway.

Since southern Sweden is the most densely populated of the three regions, it could be expected that otters from southern Sweden would have the highest concentrations of all PFAAs, but this was not the case. An explanation might be that many otters from this area feed in eutrophic lakes, which might have a lower contaminant burden in the fish compared to the other areas, due to dilution effects.[47] However, the ranges in PFAA concentrations were large. Some otters showed very high concentrations of PFOS, whereas other otters found nearby might have low concentrations. This is an indication for locally confined contamination hot spots (such as e.g. firefighting training sites), which make it difficult to find plausible explanations for the observed spatial trends. Nevertheless, a general tendency among the PFCA homologue series was observed, with significant concentration differences between the regions for PFOA to PFDA, and a quite homogeneous distribution of the longer chain compounds PFUnDA to PFPeDA (with the exception of PFTeDA). This indicates that direct sources and waterborne transport may be more important for spatial contamination patterns of PFOA to PFDA (as well as PFOS), whereas indirect atmospheric long-range transport via precursor compounds (such as fluorotelomer alcohols) may lead to a more uniform distribution of longer chain homologues throughout Scandinavia.

Time Trends. PFPeDA was excluded from statistical temporal trend analysis due to high proportion of nondetects in the corresponding sample set. The other analytes were above MDL in >75% (PFTeDA) or even >87% (all other PFAAs) of the samples. The temporal trend analyses of PFAA concentrations in otters from southern Sweden revealed a significant increase between 1972 and 2011 for 9 of the 11 investigated compounds: PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFOS, and PFDS (Figure 2 and Table 2). The statistical power of the time trends is given in SI Table S3. The yearly increase for the different PFAAs was in the range 5.5−13%, resulting in doubling times for the whole time series between 5.5 and 13 years (Table 2). Four compounds were only analyzed in 41 out of the 97 temporal trend samples (PFNA, PFTeDA, PFHxS, and FOSA). This lower sample number affected the power to detect trends substantially. Of these PFAAs only PFNA and PFTeDA showed a significant increase over time. Although the mean concentrations of PFHxS were higher during the last years of the time trend compared to earlier, the range was large and no statistically significant time trend for PFHxS was seen.

When looking only at the last 10 years of the time trend (2002−2011), the PFCAs still showed increasing trends and most PFCAs (with the exception of PFUnDA and PFTrDA) increased even at a faster rate during recent years compared to the whole study period (Figure 2 and Table 2). We have no indication of a change in the otter's diet during this time that could explain the increasing concentrations. No significant upward or downward trend of any of the PFSAs was detected between 2002 and 2011 (Table 2). This might be due to a combination of the production phase-out of the long chain PFSAs by the 3M Company in 2002 and their extraordinary environmental persistence.

The proportion of PFOA in relation to the sum of all PFCAs decreased from 25% in the 1970s to 5% in the 2000s (Figure 3). This means that the temporal increase in concentrations was not as steep for PFOA as it was for the longer chain PFCAs, which can also be seen from the slopes and doubling times given in Table 2. This could be due to differences in elimination half-lives between PFOA and longer chain PFCAs, or due to differences in exposure sources and/or pathways. During the whole study period, PFOS was the dominant PFSA. The relative contribution of FOSA to the sum of PFSAs was 15−30% in the beginning of the study period and decreased to less than 5% in the end of the study period (Figure 3). However, this finding should be interpreted with caution due to the low number of available samples from early years. The proportions of the other PFSAs were below 5%.

Most PFAA temporal trend studies have so far been conducted on humans or wildlife from marine areas. Decreasing trends for some PFAAs such as PFOA and PFOS were often reported starting from the beginning of the 2000s.[48−50] This applies also to temporal trends from Sweden, such as for human serum[51] as well as Baltic gray seals[26] and guillemots.[52]

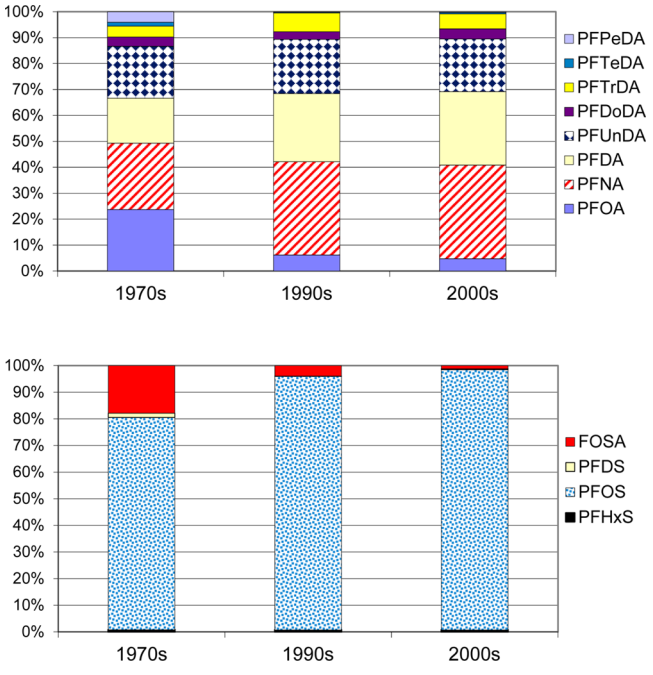

Figure 3. PFCA patterns (top) and PFSA patterns (bottom) in otters from three decades, 1970s, 1990s, and 2000s.

In the otters of the present study, however, all significant trends (including PFOA and PFOS) point upward. The reason for this discrepancy is not known. It could possibly be due to an extraordinary long elimination half-life of PFAAs in otters. This would explain both the potential to accumulate very high levels of PFOS as well as the continuously increasing trends of PFOA and PFOS despite potentially decreasing trends or unchanged concentrations in the otter's environment and diet. However, there was no correlation with age among the otters in this study. Concentrations of PFOS in guillemot eggs collected annually since 1968 showed an increasing trend up to 2011.[52] However, when only looking at the last 10 years a decreasing trend is observed, which is not observed in the otters of the present study. Time trends in Swedish freshwater fish (1980–2011) from two lakes, one from the north and one from the south of Sweden, show similar patterns. PFOS increased up to year 2000 in arctic char in the north and perch from the south. Thereafter no clear trend is seen in arctic char but a significantly decreasing trend over the last 10 years was detected in perch.[23]

Terrestrial and also limnic animals, such as the Swedish otters in the present study, are mainly exposed to airborne pollutants and contamination from land-based point sources, whereas marine animals live in an environment that also accumulates pollutants transported via water currents. Therefore, differences in time trends in terrestrial or limnic species as compared to marine biota could be expected. However, a study based on eggs from peregrine falcon collected in Sweden between 1974 and 2007[41] showed a different pattern compared to our study. The concentrations of PFOS and PFHxS increased initially during the study period but started leveling off already after the mid-1980s. Also in a study on roe deers (*Capreolus capreolus*) from Germany decreasing trends of PFAAs were detected in recent years. The sum PFAA concentration decreased from 11.2 μg/kg in 2000 to 4.2 μg/kg in 2010, which was primarily a reflection of decreasing PFOS concentrations.[53] Why the concentrations of PFOS do not decrease in otters from the present study and why otters accumulate such high levels of PFOS is not known but it is of great concern. Furthermore, also the fact that the concentrations of most PFCAs are still increasing (at a faster rate than before 2000) is alarming. From the results of the present study, negative effects from PFAA contamination on the Scandinavian otter populations cannot be ruled out. Regulatory action to stop future emissions of PFAAs and their precursor compounds to the environment is urgently needed. Additionally, further studies should investigate patterns and levels of PFAAs in the feed of otters as well as PFAA toxicokinetics within otters, in order to shed light on the unique exposure that these animals are experiencing.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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