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Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Two Fish Species Collected from the Roter Main River, Bayreuth, Germany

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Abstract Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are widely distributed in the environment. In this study the accumulation potential of PFOA and PFOS in two fish species with different feeding strategies, i.e. chub (*Leuciscus cephalus*) and river goby (*Gobio gobio*) inhabiting a river receiving treated waste waters from a municipal waste water treatment plant, were estimated. PFOS was detected in chub (7–250 μg kg^{-1} wet weight) and river goby (70–400 μg kg^{-1} wet weight) with bioaccumulation factors (BAFs) of 4600 (liver) and 11,000 (organs). PFOA concentrations in both fish were low and in chub mostly below detection limit.

Keywords Perfluorooctanoic acid · Perfluorooctane sulfonate · Fish · Aquatic environment

Perfluorinated surfactants (PFS), especially perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and their derivatives have been widely used in consumer products and industrial applications because of their chemical and thermal stability, and their water- and fat-repellent properties (Kissa 2001). PFS enter the environment in various ways, mainly via waste waters (Becker et al. 2008a, b) as these compounds are used industrially as anti-static agents for surface treatment, moulding or extrusion, as components of fire fighting foams, or as abiotic or biotic degradation products of precursors (Prevendouros et al. 2006) during waste water treatment. Their persistence and potential bioaccumulation have resulted in the ubiquitous presence of PFS in the environment, in wildlife and in humans (Houde et al. 2006; Prevendouros et al. 2006; Becker et al. 2008a, b, c). Recently, fish have been identified as a source of PFS-burden to humans on the Baltic Coast (Falandysz et al. 2006).

The aim of the present study was to determine the accumulation potential of PFOA and PFOS in two fish species with different feeding strategies inhabiting a river with a well quantified source, i.e. a municipal waste water treatment plant (WWTP) (Becker et al. 2008a, b), and to assess their tissue distribution.

Materials and Methods

PFOA (95%, Lancaster, Eastgate, UK), [1,2-$^{13}C_2$]-PFOA (98%, Perkin Elmer, Boston, USA), perfluorooctane sulfonate potassium salt (98%, Fluka, Buchs, Germany), [1,2,3,4-$^{13}C_4$]-perfluorooctane sulfonate sodium salt (99%, 50 μg/mL-solution in methanol, Campro Scientific, Berlin, Germany), acetic acid (100%, Merck, Darmstadt, Germany), ammonium acetate (99.0%, Fluka, Buchs, Germany), potassium hydroxide (KOH, analytical grade, Roth, Karlsruhe, Germany), methanol, and acetonitrile (pico-grade, Promochem, Wesel, Germany) were used as obtained. The equipment was pre-cleaned as described previously (Weremiuk et al. 2006); Teflon equipment was avoided.

On 28 August 2007, two fish species, i.e. chub (*Leuciscus cephalus*) (n = 6) and river goby (*Gobio gobio*) (n = 5), were caught in the river Roter Main by electro-fishing by the employees of the Bavarian Fishery Association. The sampling site was located approximately 3 km downstream of the WWTP of Bayreuth, Upper Franconia, Germany. The river has an average daily flow of 270,000 m^3 and receives a

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daily average of 40,000 m^3 treated waste water of industrial, commercial, and domestic origin of a population of 72,000 inhabitants. The daily loading of the river by WWTP-effluent is 1.2 ± 0.5 g PFOA and 4.7 ± 0.5 g PFOS (Becker et al. 2008a, b).

The chub is a freshwater fish of the family *Cyprinidae* which lives in rivers with slow and moderately fast flowing waters, in canals and still waters of various kinds all over Europe except Scotland, Ireland and Northern Scandinavia. It breeds in flowing waters, with a spawning season from April to June, feeds on water insects, larvae, snails, mussels, worms, and small fishes, and reaches a size of 30–40 cm. The river goby is a bottom-feeding fish of the *Gobiidae* family widespread throughout Europe, sifting through mud and silt of fast to moderately flowing rivers, sucking up invertebrates. It rarely exceeds 15 cm and spawns between Mai and end of June.

The caught fish were cooled immediately and transported in polypropylene (PP)-bags to the laboratory. The chubs were dissected; heart, liver, kidneys, gonads, and muscle tissue were stored separately in 50-mL PP-centrifuge tubes at −20°C. Of the river gobies, due to their small size, only muscles and organs were separated (Table 1).

The tissue samples were extracted according to a slightly modified, published method (So et al. 2006). For example, 5 g thawed muscle tissue was homogenised in a 50-mL PP-bottle with a mechanical homogeniser (Ultra-Turrax, Janke & Kankel GmbH, Staufen, Germany) without addition of solvent. After homogenisation of the tissue of one fish, the homogeniser was thoroughly washed with tap water, bidistilled water, and methanol; the washes were discarded. Each sample was extracted in triplicate: 1.0 g homogenate was weighed into a new 50-mL PP-centrifuge tube and 150 µL standard solution containing 100 µg L^{-1} each of ^{13}C-PFOA and ^{13}C-PFOS was added. After addition of 30 mL methanolic KOH solution (0.01 N), the mixture was shaken at room temperature for 16 h (22 rpm, Shaker, GFL 3040, Burgwegel, Germany) and centrifuged (High-Performance Centrifuge, Avanti J-25, Beckman, USA, 3000 rpm, 20°C, 10 min); 2 mL of the supernatant was transferred to a 250-mL PP-bottle, diluted with 100 mL deionised water and mixed thoroughly. The analytes were preconcentrated and precleaned by solid phase extraction (SPE).

The organs (Table 1) were mechanically homogenised with 5 mL each of methanolic KOH solution (0.01 N) in a 50-mL PP-bottle. Residues of the sample sticking to the homogeniser were recovered by five times washing with 5 mL methanolic KOH solution each and combining the washes with the homogenised sample. Between samples, the homogeniser was cleaned as above. Standard solution, 150 µL, containing 100 µg L^{-1} each of ^{13}C-PFOA and ^{13}C-PFOS, was added to each 30-mL sample of tissue homogenate, and the mixture was shaken at room temperature for 16 h (22 rpm). Upon centrifugation (3000 rpm, 20°C, 10 min), three aliquots, 2 mL each, of the supernatant were transferred to three 250-mL PP-bottles containing 100 mL deionised water. The bottles were thoroughly shaken.

The analytes were preconcentrated by SPE as previously described (Weremiuk et al. 2006) but without applying vacuum and omitting the washing of the cartridge to avoid losses. Analysis and quantification was done by HPLC–ESI–MS/MS.

Table 1 Animal size and organ or aliquot (muscles) weights for PFS-analysis, Roter Main, 27 Aug. 2007

Fish	Length cm	Weight g	Age year	Organ weight, g				Aliquot, g
				Liver	Kidneys	Gonads	Heart	Muscles
Chub (*Leuciscus Cephalus*)								
1	26	172.5	4	2.77	0.22	3.71	0.21	1.00
2	25	161.1	4	1.80	0.16	3.23	0.17	1.00
3	22	110.8	4	1.51	0.21	1.63	0.18	1.00
4	24	129.2	4	1.07	0.73	1.43	0.17	1.00
5	24	129.7	4	2.31	1.10	2.27	0.20	1.00
6	32	367.5	4	4.55	2.48	7.13	0.49	1.00

Fish	Length cm	Weight g	Age year	Organs weight, g	Aliquot, g Muscles
River Goby (*Gobio gobio*)					
1	13.0	19.05	3	2.44	1.00
2	13.0	22.05	3	2.12	1.00
3	14.5	23.85	3	2.18	1.00
4	12.0	13.40	3	1.74	1.00
5	13.5	17.00	3	1.67	1.00

For calibration, stock solutions of ^{13}C-PFOA, ^{13}C-PFOS, PFOA, PFOS and three working standard solutions containing: a) 100 μg L^{-1} each of both ^{13}C-PFOA and ^{13}C-PFOS, b) 20 μg L^{-1} each of both labelled standards, and c) 10 μg L^{-1} each of both non-labelled standards were prepared as described previously (Becker et al. 2008b).

For quantitative analysis, standard solutions containing non-labelled PFOA and PFOS in the range from 0.5 to 15 μg L^{-1}, and 2 μg L^{-1} each of both ^{13}C-labelled standards were used for calibration. Calibration curves were constructed by plotting analyte and internal standard peak area ratios versus analyte concentrations; regression coefficients were higher than 0.995.

Recoveries from fish samples relative to the ^{13}C-labelled standard were 88% (±10% rsd) for PFOA and 86% (±10% rsd) for PFOS. The limits of quantification (LOQ, signal to noise ratio 7) for PFOA and PFOS were 1.5 and 3 ng, respectively, divided by the sample weight. In procedural blanks consisting of 50-mL PP-tubes filled with 30 mL methanolic KOH solution and spiked with 150 μL of a standard solution containing 100 μg L^{-1} each of ^{13}C-PFOA and ^{13}C-PFOS, both analytes were below detection limit.

Results and Discussion

PFOA-concentrations in chub were above LOQ only occasionally (Table 2), i.e. in the heart of chub 1, the liver of chub 6, the kidneys of chubs 2 and 3, and the gonads of chubs 2, 4, 5, and 6. In the river gobies, they were generally higher and ranged from <0.6 to 3.0 μg kg^{-1} wet weight (ww) in the organs, and from 2.0 and 9.8 μg kg^{-1} ww in the muscles (Table 3).

Overall, PFOA concentrations found in both species were lower than in the liver of jack mackerel purchased on a Japanese market originating from a fish farm (Senthilkumar et al. 2007), or in muscles (6.4–53 μg kg^{-1}) and livers (2.6–840 μg kg^{-1}) of eel, barb, carp, nase, greyling from the river Alz (Federal Office for Environment 2007a) where PFOA concentrations up to 7.5 μg L^{-1} (Federal Office for Environment 2007b) have been found in the water. Concentrations were higher than in muscle tissue of largemouth bass or smallmouth bass from the River Raisin, St. Clair and Calumet (<2 μg kg^{-1}), and in whole body homogenate of trout from Lake Ontario (1 ± 0.1 μg kg^{-1}, Houde et al. 2006).

PFOS concentrations in river water are higher in comparison to PFOA, (March–June 2007, median: 25 ng L^{-1}, mean: 31 ± 18 ng L^{-1}, Becker et al. 2008a) and sediment (October 2006, mixed sample collected 1 km downstream of the WWTP: 240 ng kg^{-1} dry weight, Becker et al. 2008a). Correspondingly, higher PFOS-levels in chub (Table 2), especially in liver (123 ± 15 μg kg^{-1} ww), kidneys (100 ± 30 μg kg^{-1} ww), gonads (52 ± 1 μg kg^{-1} ww) and heart (57 ± 27 μg kg^{-1} ww), lowest values (13 ± 3 μg kg^{-1} ww) in muscle tissue. These concentrations are in agreement with previous findings (Houde et al. 2006), and are similar to those in smallmouth bass liver from New York State lakes (10–140 μg kg^{-1}, Sinclair et al. 2006), chinook salmon (30–170 μg kg^{-1}) or whitefish (33–81 μg kg^{-1}) of the Great Lakes (Houde et al. 2006), but lower than in the livers of eel, perch, roach from the river Main or Alz (15–4300 μg kg^{-1}) (Federal Office for Environment 2007a) or in carp or gibel carp from Flanders (Belgium) (10–9030 μg kg^{-1}, Houde et al. 2006). Concentrations in muscles were comparable to those in fish from other Bavarian rivers (Federal Office for Environment 2007a).

Table 2 Concentrations of PFOA and PFOS [μg kg^{-1} ww] in chub tissue

Fish	Liver	Kidneys	Gonads	Heart	Muscles
PFOA[a]					
1	<0.5	<6.8	<0.4	021 ± 0.7	<1.5
2	<0.8	206,0 ± 18	9.7 ± 0.5	<9	<1.5
3	<1.0	008.2 ± 01	<0.9	<8	<1.5
4	<1.4	<2.1	5.8 ± 0.4	<9	<1.5
5	<0.7	<1.3	2.0 ± 0.1	<8	<1.5
6	3.6 ± 0.2	<0.6	2.7 ± 0.2	<3	<1.5
PFOS					
1	110 ± 12	66 ± 8	52 ± 1[b]	23 ± 3	7.5 ± 0.5
2	120 ± 9	83 ± 1	66 ± 2[b]	49 ± 1	14.5 ± 0.5
3	113 ± 1	102 ± 8	56 ± 2[b]	103 ± 1	14.6 ± 0.5
4	152 ± 13	137 ± 6	67 ± 8[b]	66 ± 3	11.3 ± 0.9
5	117 ± 9	133 ± 4	57 ± 3[b]	59 ± 5	15.6 ± 0.6
6	123 ± 12	100 ± 1	247 ± 15[b]	40 ± 1	12.2 ± 0.7
Mean PFOS	**123 ± 15**	**103 ± 28**	**60 ± 7**[b]	**57 ± 27**	**13.0 ± 3.0**

[a] Mean concentration in individual organs was not calculated due to low values

[b] Outlier, not included in mean calculation

Table 3 Concentrations of PFOA and PFOS [μg kg^{-1} ww] in river goby

Fish	PFOA		PFOS	
	Organs	Muscles	Organs	Muscles
1	<0.6[a]	4.5 ± 0.3	290 ± 09	69 ± 6
2	2.4 ± 0.3	7.8 ± 0.1	230 ± 16	76 ± 5
3	3.0 ± 0.2	9.8 ± 0.5	345 ± 40	65 ± 4
4	1.2 ± 0.2	2.0 ± 0.4	205 ± 02	85 ± 6
5	<0.9[a]	5.2 ± 0.8	406 ± 40	108 ± 9
Mean	**1.5 ± 1.2**	**5.9 ± 0.8**	**295 ± 80**	**80 ± 17**

[a] Mean is calculated using half of the LOQs

In the muscles of river gobies, PFOS levels were between 65 and 108 μg kg^{-1} ww (average: 80 ± 17 μg kg^{-1} ww), in pooled organs between 205 and 406 μg kg^{-1} ww (average: 300 ± 80 μg kg^{-1} ww, n = 5). Obviously, PFOS in river goby muscles is about 6-fold higher than in chub. Although exposure to PFOS via water has been suggested to be more important than dietary intake (Martin et al. 2003), higher values for river goby reflect the fact that they feed mainly on benthic invertebrates living in the sediment with relatively high PFOS concentrations (October 2006, mixed sample collected 1 km downstream the WWTP: 35 ± 9 ng kg^{-1}; sediment/water concentration ratio = 22, Becker et al. 2008b). Higgins and Luthy (2006) suggested that PFS in sediments are readily bioavailable, can be bioaccumulated and contribute to the bioaccumulation of PFOS in the food chain.

Under consideration of the mean PFOA concentration in river water of mean: 9 ± 4 ng L^{-1} (March–June 2007, Becker et al. 2008a) a bioaccumulation factor (BAF) of 740 is found for muscle tissue of river gobies. Houde et al. (2006) reported a BAF of 4 for rainbow trout under laboratory conditions. It was observed that field BAFs tend to be greater than BAFs estimated from laboratory experiments due to a number of factors, i.e. organism size, unknown contributions from non-quantified precursors present in environment and metabolised to PFOA, temporal and spatial variability in surface water concentrations (Houde et al. 2006; Moody et al. 2002).

Comparison of the PFOS-concentrations in chubs' livers and gobies' pooled organs (123 and 295 μg kg^{-1} ww, respectively) with the average water concentration of 27 ng L^{-1} (Roter Main, 1 km downstream the WWTP, March–June 2007; personal communication) gave a liver-based BAF of 4600 for chub and an organ-based BAF 11,000 for river goby; it can be assumed that a liver-BAF of the latter species would even be higher. The values are in agreement with those determined for coastal fish from Japan (8540) or the Niagara River (8850, Houde et al. 2006).

This study shows that PFOS released to the river from a municipal WWTP can be accumulated in the liver of exposed fish by a factor of 10^4 or higher, PFOA by a factor of less than 10^3.

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