

Occurrence of Perfluoroalkyl Substances in Fish and Water from the Svitava and Svatka Rivers, Czech Republic

Jana Kovarova · Petr Marsalek · Jana Blahova ·
Jana Jurcikova · Barbora Kasikova ·
Zdenka Svobodova

Received: 30 June 2011 / Accepted: 24 November 2011 / Published online: 7 December 2011
© Springer Science+Business Media, LLC 2011

Abstract Perfluoroalkyl substances (PFHxS, FHUEA, PFOA, PFOS, FOSA, *N*-methyl FOSA and PFNA) from seven sites on the Svitava and Svatka rivers in the Brno conurbation (Czech Republic) were determined in fish blood plasma and water. Concentrations of PFHxS, FHUEA, FOSA, and *N*-methyl FOSA were below detection limits. Major compound in fish blood was PFOS (38.9–57.8 ng mL⁻¹), followed by PFNA and PFOA. In water, the major compound detected was PFOA (1.7–178.0 ng mL⁻¹), followed by PFOS and PFNA. A significant ($p < 0.05$) correlation for PFOA concentration in blood plasma and water was found ($r = 0.74$).

Keywords Perfluorinated compounds · POCIS · Aquatic ecosystem · Chub

Perfluoroalkyl substances (PFAS) comprise a family of emerging environmental pollutants that are ubiquitous, persistent, and bioaccumulative. They have wide commercial applications because of their chemical and thermal stability (Prevedouros et al. 2006). Toxicity tests in animals have demonstrated numerous negative effects of PFAS (Kovarova and Svobodova 2008). Unlike other classical persistent organic pollutants, these chemicals are primarily

emitted into water. They accumulate in surface waters, and water is the major reservoir of PFAS in the environment, as well as the most important medium for their transport (Prevedouros et al. 2006).

In the Czech Republic, a pilot monitoring study was conducted by Jandova (2006) in selected fish from the Vltava and Labe rivers. PFAS content has also been studied in the vicinity of Brno city on the Svitava and Svatka rivers (Czech Republic). This project is focused on the assessment of aquatic pollution by environmental endocrine disruptors at seven selected locations upstream and downstream of Brno city using analyses of fish biomarkers and pollutant content in several *a/biotic* matrices. In the first phase of the project (years 2006 and 2007), the PFAS content was studied only in water using passive samplers (Grabic et al. 2010). The following year, monitoring was extended to determination of these analytes in fish blood plasma. Results are summarized in the presented study. Seven most widespread PFAS, perfluorohexansulfonate (PFHxS), 2H-perfluoro-2-octenoic acid (FHUEA), perfluorooctanoic acid, perfluorocyclopentane sulfonate, perfluoro-1-octanesulfonamide (FOSA), *N*-methylperfluoro-1-octanesulfonamide (*N*-methyl FOSA), and perfluorononanoic acid (PFNA), were assessed on the Svitava and Svatka rivers using chub plasma. Results were correlated with water samples data acquired from passive samplers.

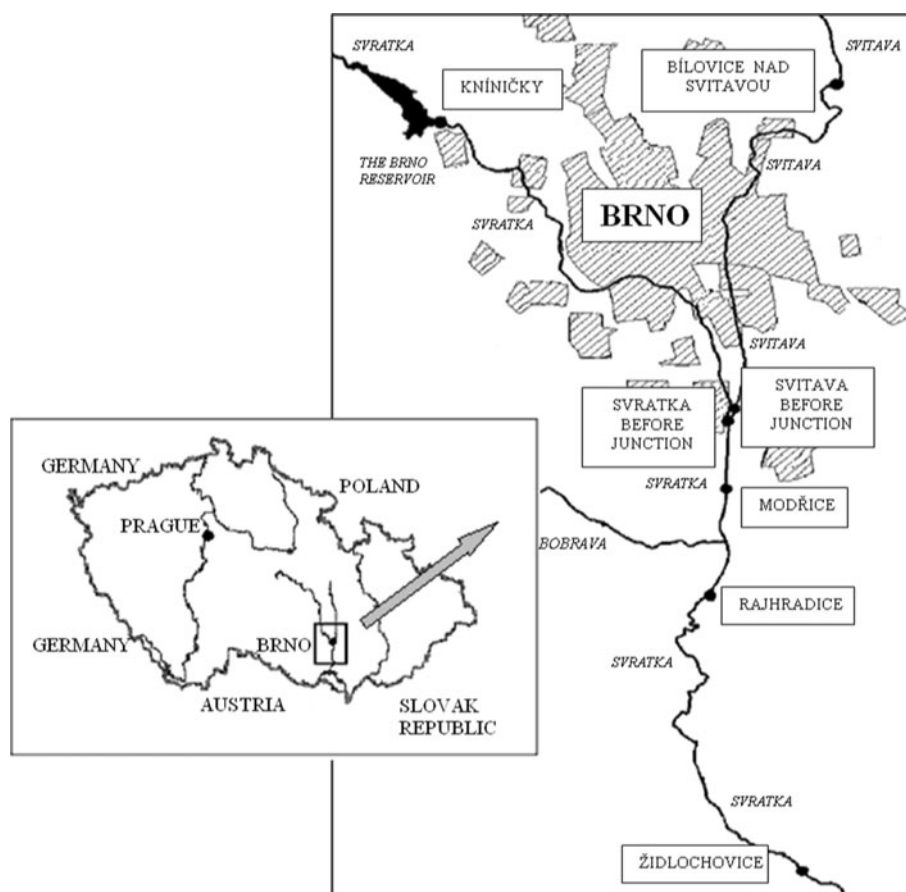
J. Kovarova · P. Marsalek (✉) · J. Blahova · Z. Svobodova
Department of Veterinary Public Health and Toxicology,
University of Veterinary and Pharmaceutical Sciences Brno,
Palackého 1-3, 612 42 Brno, Czech Republic
e-mail: marsalekp@vfu.cz

J. Jurcikova · B. Kasikova
Institute of Public Health Ostrava, Partyzánské nám. 7,
702 00 Ostrava, Czech Republic

Materials and Methods

Monitoring was carried out upstream and downstream of Brno city (Fig. 1), the second-largest city (population 370,000) in the Czech Republic. Brno city is located in the southeastern part of the country, at the confluence of the Svitava and Svatka rivers, and is an important industrial

Fig. 1 Locations of sampling sites around Brno (Czech Republic)



city with highly developed chemical, engineering, textile, and food-processing industries.

A total of 32 adult fish were caught by electrofishing at the seven locations in June and July 2008. Chub (*Leuciscus cephalus* L.) was selected as the most suitable indicator species. The biometric characteristics of fish are given in Table 1. Blood samples were collected into heparinized tubes from the heart and/or caudal vein. The blood samples were stored at 4°C and transported to the laboratory, where they were centrifuged (800×g for 10 min). The plasma samples were stored at −85°C until PFAS analysis.

Passive samplers of a standard dimension (2.5 × 91.4 cm filled with 1 mL triolein) manufactured with duality assurance and quality control for the highest reproducibility were used (Exposmeter AB, Sweden). A single polar organic chemical integrative sampler (POCIS) was installed at each site for 30 days twice a year (May and September, 2008). Following exposure, membranes were briefly rinsed with distilled water and placed on ice for transport to the laboratory. They were stored in the laboratory at −20°C until analysis. Passive samplers spiked with performance reference compounds (PRC) were used for in situ calibration of the

Table 1 Basic characteristics of chub captured at the sampling localities

Locality (river km)	Fish (n)	Mean age (years) (min–max)	Mean weight ± SD (g)
Svitava River			
Bílovice nad Svitavou (18.0)	5	3.9 (2.5–4.5)	138.0 ± 32.7
Svitava before junction (0.6)	4	3.5 (3.5–3.5)	200.0 ± 36.7
Svratka River			
Kníničky (56.2)	5	4.3 (2.5–5.5)	309.0 ± 106.7
Svratka before junction (40.9)	4	4.3 (3.5–5.5)	335.0 ± 215.2
Modřice (38.7)	5	4.5 (3.5–5.5)	285.0 ± 142.0
Rajhradice (35.0)	4	3.0 (2.5–3.5)	193.8 ± 71.2
Židlochovice (30.0)	5	3.7 (3.5–4.5)	301.0 ± 66.2

sampling rates. Results obtained in units of nanograms per membrane were recalculated to water concentration by using in situ PRC calibration of each membrane.

Five microlitres of isotope labeled internal standard containing a mixture of perfluoro-*n*-(1,2,3,4,5-13C5) nonanoic acid, perfluoro-*n*-(1,2,3,4-13C4) octanoic acid, and sodium perfluoro-1-(1,2,3,4-13C4) octanesulfonate was added to 0.5 mL thawed plasma. Seven hundred microliters of acetonitrile was added and samples were centrifuged at $800\times g$ for 15 min. The supernatant was diluted with 300 μL water and used for LC/MS determination. Description of the elution of analytes from passive samplers was described by Alvarez et al. (2004). An aliquot of eluates (100 μL) was used for LC/MS determination. PFAS contents were measured by means of a Quantum AccessTM (Thermo Fisher Scientific, USA) triple quadrupole-mass spectrometer with Rheos2000 (Flux) HPLC and an HTC PAL autosampler (CTC Analytics AG). Elution was performed on a 50×2 mm, 5 μm Phenomenex Aqua C18 125A column. Gradient elution was performed with a methanol/water mixture with the addition of sodium formate. Limits of detection for PFHxS, FHUEA, PFOA, PFOS, FOSA, *N*-methyl FOSA and PFNA were 0.49, 0.43, 0.21, 0.65, 0.19, 0.18 and 0.33 ng mL^{-1} , respectively.

Statistical analysis was performed using Statistica 8.0 for Windows (StatSoft, Inc., Tulsa, OK). A normality check of all of the datasets of results obtained for the parameters investigated was conducted with the Kolmogorov–Smirnov test. An analysis of variance (ANOVA) was used to evaluate results of PFAS content in fish samples. When significant differences were found, a conservative Tukey's test was conducted as a post hoc test to determine differences among sampling sites and also among contents of PFAS compounds. In the case of non-significant results obtained by Tukey's test, a less conservative Fisher's LSD test was used to evaluate differences between sampling sites. Non parametric Spearman rank correlation was applied to prove the relationships between PFAS content of fish blood and POCIS samples. Significance was accepted at $p < 0.05$.

Results and Discussion

Concentrations of PFHxS, FHUEA, FOSA, and *N*-methyl FOSA in fish blood plasma were below detection limits in all samples. The main PFAS detected was PFOS, with the concentration of 38.9–57.8 ng mL^{-1} , followed by PFNA (0.88–7.1 ng mL^{-1}). The lowest detected compound was PFOA with concentration of 0.10–0.63 ng mL^{-1} . The results are summarized in Fig. 2. Our data indicated that PFOS is the predominant PFAS compound in biota. Identical results were reported by Berger et al. (2009) and

(Senthilkumar et al. 2007). This is supported by the hypothesis that PFOS is a biotransformation product of fluorotelomer alcohols, major products of PFAS synthesis. Recently published data provided PFOS levels detected in fish worldwide. Yoo et al. (2009) presented a mean blood PFOS level of 93 ng mL^{-1} in the Flathead grey mullet (*Mugil cephalus*). Li et al. (2008) reported a mean PFOS concentration of 9.88 ng mL^{-1} in white semiknife carp (*Hemiculter leucisculus*), 5.74 ng mL^{-1} in the Nile tilapia (*Oreochromis niloticus*), 12.9 ng mL^{-1} in leather catfish (*Clarias lazera*), 32.2 ng mL^{-1} in common carp (*Cyprinus carpio*), and 64.2 ng mL^{-1} in Crucian carp (*Carassius auratus*) in China. Other studies have focused on measurements made from whole body homogenates. The examination of whole body homogenates is useful, as it represents how fish are consumed by piscivorous predators. However, from a human exposure perspective, liver data and whole body homogenates are less relevant (Houde et al. 2006). The other PFAS determined in fish samples were generally at least an order of magnitude lower than PFOS, and the levels of PFHxS, FHUEA, FOSA, and *N*-methyl FOSA were under detection limits. Similar results were obtained in a study by Taniyasu et al. (2003), who investigated the level of PFOS, PFHxS, and perfluorobutane sulfonate in surface water and fish blood collected in Japan. The dominant compound was PFOS, with PFHxS detected only in approximately 33% of the fish blood samples (in range 1–121 ng L^{-1}). Perfluorobutane sulfonate was under detection limit in all samples.

Fish sampling showed the highest level of PFAS contamination at Kníničky and Bílovice nad Svitavou. These sites are situated upstream of the Brno agglomeration, and these results might be unexpected. Sampling site Kníničky is downstream of Brno's dam and probably is indicative of the concentration of PFAS pollutants in the artificial reservoir, which presents a significant risk factor for PFAS storage in the local surface waters. Bílovice nad Svitavou reflects the state of surface water before entering the Brno conurbation. Although it was chosen as a control location, the content of PFAS in fish from this site was higher than Modřice situated downstream of Brno. This may be associated with the waste water treatment plant (WWTP) in Bílovice nad Svitavou (150 m upstream of the target site) and by chemical and engineering facilities in the nearby cities Adamov and Blansko.

With analysis of POCIS samples, the major PFAS detected was PFOA, followed by PFNA and PFOS (Table 2). Levels of PFHxS, FHUEA, FOSA, and *N*-methyl FOSA were under detection limits in all samples. Predominance of PFOA was particularly observed in locations downstream of the Brno agglomeration (Modřice and Rajhradice). This could suggest a local source of PFOA in Brno, since high levels of PFOA was also found

Fig. 2 The content of PFAS, PFOS, PFOA and PFNA in fish blood plasma samples (B–Bílovice nad Svitavou, S–Svitava before junction, K–Kníničky, V–Svratka before junction, M–Modřice, R–Rajhradice, P–Židlochovice). Within localities, bars that do not have a common superscript differ, $p < 0.05$. For PFAS, PFOS and PFNA was used Fisher LSD test, for PFOA was used Tukey HSD test

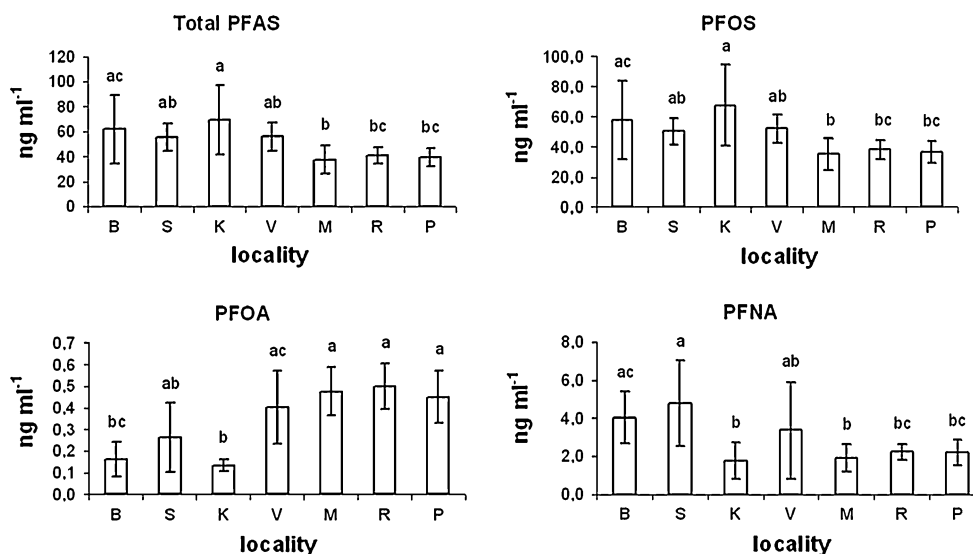


Table 2 Concentrations of PFAS in water samples obtained by POCIS

Locality	Sum of PFAS (ng mL ⁻¹)		PFOS (ng mL ⁻¹)		PFNA (ng mL ⁻¹)		PFOA (ng mL ⁻¹)	
	May	Sept.	May	Sept.	May	Sept.	May	Sept.
Bílovice nad Svitavou	a	28.2	a	12	a	8.1	a	8.1
Svitava b. junction	24.3	a	9.5	a	5.7	a	9.1	a
Kníničky	2.6	4.4	0.65	0.6	0.24	0.68	1.7	3.1
Svratka b. junction	a	2.7	a	0.6	a	0.17	a	1.9
Modřice	144	186	12.3	6.6	4.5	2.0	128	178
Rajhradice	13.9	33.1	3.6	6.6	0.5	2.5	9.8	24.0
Židlochovice	a	a	a	a	a	a	a	a

^a Because of technical problems POCIS did not produce samples

in fish samples from sites downstream from Brno. Moreover, a significant positive correlation ($r = 0.74$) between PFOA concentrations in fish and water was observed. In contrast, total PFAS concentration in fish showed a significant negative correlation ($r = -0.81$) with that in passive samplers. The Modřice site (Svratka River) is below the WWTP in Modřice, which treats wastewater conveyed by a system of sanitary sewers from the city of Brno and, increasingly, by a system of pumping stations that connects other municipalities to the WWTP. The Rajhradice site (Svratka River), is situated below the confluence of the two rivers and the effluent from the municipal WWTP downstream from Brno. This site is adversely influenced by contamination from domestic wastes, i.e. from the WWTP in Modřice (4 km upstream from Rajhradice). Our results are in agreement with study by Senthilkumar et al. (2007), who reported that PFOS is the predominant compound in biota and that PFOA is predominant in environmental matrices. On the other hand, Naile et al. (2010) reported PFOS as the major perfluorinated compound in water in

estuarine and coastal areas of Korea. Passive sampling methods for monitoring of perfluorinated compounds are often used for assessment of air contamination (Chaemfa et al. 2010; Dreyer et al. 2010). Most aquatic monitoring programs are based on conventional sampling of water and sediments and use of passive samplers is not widespread (Grabic et al. 2010).

Although the production of some PFAS by its major manufacturer was phased out at the end of 2002, the occurrence of these compounds was confirmed in fish plasma and POCIS samples from the Svitava and Svratka rivers. The results of the present study, along with data from previously published studies, showed that PFAS contamination is present in fish in fresh waters worldwide. It is suggested that fish can be a significant source of human dietary PFAS exposure and may continue to be for many years or decades to come. In the present study, we confirmed that PFOS is the predominant perfluorinated compound in biota while PFOA is in abiotic matrices. Results suggest possible sources of PFOA in the Brno agglomeration.

Acknowledgments This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (MSM No. 6215712402 and 2B06093) and IGA 197/2009/FVHE.

References

- Alvarez DA, Petty JD, Huckins JN, Jones-Lepp TL, Getting DT, Goddard JP, Manahan SE (2004) Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. *Environ Toxicol Chem* 23:1640–1648
- Berger U, Glynn A, Holmstrom KE, Berglund M, Ankarberg EH, Tornkvist A (2009) Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden—analysis of edible fish from Lake Vattern and the Baltic Sea. *Chemosphere* 76:799–804
- Chaemfa C, Barber JL, Huber S, Breivik K, Jones KC (2010) Screening for PFOS and PFOA in European air using passive samplers. *J Environ Monit* 12:1100–1109
- Dreyer A, Shoeib M, Fiedler S, Barber J, Harner T, Schramm KW, Jones KC, Ebinghaus R (2010) Field intercomparison on the determination of volatile and semivolatile polyfluorinated compounds in air. *Environ Chem* 7:350–358
- Grabic R, Jurcikova J, Tomsejova S, Ocelka T, Halirova J, Hypr D, Kodes V (2010) Passive sampling methods for monitoring endocrine disruptors in the Svratka and Svitava rivers in the Czech Republic. *Environ Toxicol Chem* 29:550–555
- Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DCG (2006) Biological monitoring of polyfluoroalkyl substances—a review. *Environ Sci Technol* 40:3463–3473
- Jandova V (2006) Statistical environmental yearbook of the Czech Republic. MŽP ČR, Prague
- Kovarova J, Svobodova Z (2008) Perfluorinated compounds: occurrence and risk profile. *Neuro Endocrinol Lett* 29:599–608
- Li XM, Yeung LWY, Xu MQ, Taniyasu S, Lam PKS, Yamashita N, Dai JY (2008) Perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish blood collected near the outfall of wastewater treatment plant (WWTP) in Beijing. *Environ Pollut* 156:1298–1303
- Naile JE, Khim JS, Wang TY, Chen CL, Luo W, Kwon BO, Park J, Koh Ch, Jones PD, Lu YL, Giesy JP (2010) Perfluorinated compounds in water, sediment, soil and biota from estuarine and coastal areas of Korea. *Environ Pollut* 158:1237–1244
- Prevedouros K, Sousins IT, Buck RC, Korzeniowski SH (2006) Sources, fate and transport of perfluorocarboxylates. *Environ Sci Technol* 40:31–44
- Senthilkumar K, Ohi E, Sajwan K, Takasuga T, Kannan K (2007) Perfluorinated compounds in river water, river sediment, market fish, and wildlife samples from Japan. *Bull Environ Contam Toxicol* 79:427–431
- Taniyasu S, Kannan K, Horii Y, Hannari N, Yamashita N (2003) A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds and humans from Japan. *Environ Sci Technol* 37:2634–2639
- Yoo H, Yamashita N, Taniyasu S, Lee KT, Jones PD, Newsted JL, Khim JS, Giesy JP (2009) Perfluoroalkyl acids in marine organisms from Lake Shihwa, Korea. *Arch Environ Contam Toxicol* 57:552–560