PERFLUORINATED COMPOUNDS IN CZECH AQUATIC ENVIRONMENT: FISH AS BIOINDICATOR ORGANISM

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Introduction

Since the end of 20th century, the interest in perfluorinated compounds (PFC) has been exponentially increasing. Studies concerned with PFC levels in various environmental compartments, their fate as well as distribution patterns and temporal trends, have been initiated worldwide in the recent decade. With regard to a wide range of uses in many industrial products, and their exceptional stability in the environment, PFCs have "emerged" as a global pollution problem. Perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA) together with a major precursor, perfluorooctane sulfonamide (FOSA), are the most investigated representatives of these persistent environmental pollutants (POPs). Due to the occurrence of low levels of PFOS in food chains, the process of health risk assessment associated with consumers´ dietary exposure was initiated by European Food Safety Authority (EFSA) in 2004. It was pointed out, that not many occurrence data in food, in particular from Europe, are available.

PFOS is considered toxic through experiments done on rats. Currently, most of studies are focused on aquatic food web (typically, PFOS has been reported as a dominating PFC representative in all types of examined environmental samples) [1-4].

In general, some similarity exists in distribution patterns of PFC and hydrophobic persistent organohalogenated pollutants (POPs) with regard to global distribution, bioaccumulation and biomagnification. However, whereas most of these organohalogenated compounds are typically accumulated in lipid-rich tissue, PFC are bound to blood proteins and accumulated in liver and gall bladder. On this account, analytical methods used for the determination of "classic" POPs are not applicable for the PFC analysis.

It should be noted that in most studies realized until now procedure introduced by Hansen et al.[5] has been employed of polar PFCs analysis. In the first step, hydrophobic ion pairs of target analytes with tetrabutylammonium (TBA) are formed in an alkalinized matrix homogenate. In the next step, they are transferred into a methyl-tert-butylether (MTBE) layer. Under these conditions – ion-pair extraction (IPE), some lipids and other less polar matrix components are co-isolated into the organic phase and thus they may interfere with an instrumental determinative step. To avoid these problems, some modification or novel sample preparation strategy has been introduced. Unfortunately, analytical methods, mentioned above, are laborious and time consuming [6].

This study was focused on the assessment of pollution extent of the Czech aquatic ecosystem by PFCs. For this purpose, PFOS, PFOA and FOSA, occurring in bioindicator matrix - liver samples of chub collected at various sampling sites located at Czech rivers were determined. A novel, simple and fast analytical procedure based on a clean-up of a crude extract by activated charcoal and its examination by a high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed and validated.

Materials and methods

Samples. Chub livers (*Leuciscus cephalus*) were used for presented monitoring study. Samples were collected in years 2006 and 2007 by electro-fishing in 11 sampling sites located mainly in the lower reaches of several rivers in the Czech Republic, see Figure 1.

Extraction and clean-up. 2 g of homogenized fish liver was transferred into 50 mL disposable polypropylene (PP) centrifuge tube and mixed with 6 mL of methanol using an Ultra Turrax homogenizer. Then 340 mg of activated charcoal were added to this suspension. After 1 min vortexing, the sample was centrifuged (10000 rpm, 5 min). The supernatant was filtered through 0.45 μm PP syringe filter and ca. 500 μL of filtrate were transferred into a PP LC-vial prior to an instrumental analysis.

HPLC-MS/MS. Samples were analyzed by a high performance liquid chromatography (HPLC) carried out with a Waters Alliance 2695 HPLC instrument (USA). Separations were conducted using LiChroCART Purospher Star RP-18e analytical column (125 mm x 4 mm i.d., 5-μm particle) equipped with the guard column (4 mm x 4 mm

i.d., 5-µm particle). The column temperature was 40°C and the flow rate was 0.3 mL min^{-1}. The mobile phase gradient (10 mM aqueous ammonium acetate /A/ and methanol /B/) was as follows: 60% B changed linearly to 95% B in 2.5 min, hold for 8 min, before a reversion to original conditions (post run 5.5 min).
The identification/detection of target analytes was performed employing the Waters Quattro Premier XE tandem-quadrupole mass spectrometer (USA) equipped with a Z-spray interface. Nitrogen was used as a drying and nebulizing gas, argon was used as a collision gas. A capillary potential was held at 0.5 kV and a cone potential ranged between 15 and 50 V, depending on the target compound. A source block and desolvation temperatures were maintained at 120°C and 350°C, respectively. Desolvation gas and cone gas flows were 700 and 70 L h^{-1}, respectively. The instrument was operated in negative electrospray ionization multiple reaction monitoring (MRM) mode for a quantification of each compound. The identity of target analytes was confirmed by comparison of its retention times with standard and by monitoring of daughter ions in MS/MS. Quantification to confirmation in ratio was used to this purpose. Retention times and MRM transitions are listed in Table 1.
The precision of the analytical method represent as repeatability ranged from 2 to 4% (expressed as relative SD). The matrix-matched standard calibration curve exhibited a good linearity within the given range between 0.5 ng mL^{-1} for PFOS and PFOA and 0.2 ng mL^{-1} for FOSA. The limits of quantitation (LOQs) were 2 ng g^{-1} for PFOS and PFOA and 0.5 ng g^{-1} for FOSA.

Result and Discussion
Similarly to approaches employed in the monitoring of other halogenated POPs, fish liver and muscle tissue were the matrices analyzed in this survey aimed at assessment of aquatic environment pollution by perfluorinated compounds. Chub, an omnivorous fish, widely occurring in Czech rivers, was used as a bioindicator organism. The levels of PFOS and FOSA determined in all monitored localities are summarized in Table 2 (PFOA was not detected in any of examined samples).
As shown here, PFOS was the dominating fluoro-chemical, it was present in 8 and 6 from 11 monitored localities in the years 2006 and 2007, respectively; its concentrations in positive samples ranged from 10.1 to 198.5 ng g^{-1} wet tissue weight. Based on bioindicator examination, the highest pollution was observed in the locality Děčín at the Elbe River, close to the Germany border. Relatively extensive pollution was also found in Zelčín at the Vltava River, downstream from Prague industrial area. However, the result obtained along the Vltava River did not suggest unequivocal localization of an emission source. Comparing the results of two consecutive years 2006 and 2007, some decrease of PFOS levels in chub livers occurred in most of sampling sites, the most pronounced drop (from 96.6 µg kg^{-1} below LOQ) of this highly persistent pollutant was observed in the locality Louny (Ohře River). In line with this time trend, also a distinct decline of FOSA took place, no residues exceeding LOQ were found in the second monitoring year.
The generated data in this study documented relatively extensive pollution of the Elbe River basin where most of industrial emission sources is located. On the other hand, no occurrence PFCs was found in small rivers, such as Svratka and Otava, flowing through unpolluted rural areas.

The levels of PFC, especially PFOS, found in livers of chub from Czech rivers were comparable to those reported in other similar monitoring studies concerned with PFCs in freshwater fish. For instance PFOS in salmon liver from the Great Lakes ranged from 33 to 170 ng g^{-1} wet weight [1]. Levels of PFC found in most European samples were lower as compared to our results; nevertheless, localities with extreme contamination were also identified in Europe. Levels of PFOS in fish collected in localities in the neighbourhood of a fluoro-chemical factory in Belgium were as high as 9030 ng/g wet weight [7]. The results of FOSA were comparable with previously reported contamination of trout (***Salvelinus namaycush***) from the Great Whale River in Canada which were in range from 2.8 to 6.8 ng g^{-1} wet weight [3]. Unfortunately, we could not compare exactly our results with other published data from Japan, countries of South America or Australia because, to our knowledge, there were examined mainly sea food [8,9].

To our knowledge, this was the first, pilot study conducted in the Czech Republic assessing the pollution of aquatic ecosystem by PFC. Based on these results, follow-up research focused on transfer of PFCs into human food chains is planned. For the purpose of humans´ dietary exposure estimation, contamination of fish and fish products available at the Czech market will be examined.

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Figure 1 Overview of sampling sites in which chub was collected

Table 1 Ion transitions used for multiple reactions monitoring analysis in LC-MS/MS of target analytes (the quantitation ions are bolded)

Compound	Abbreviation	t_R(min)	Transition (m/z)
Perfluorooctanoic acid	**PFOA**	9.0	412.9 → 412.9
			412.9 → 368.9
			412.9 → 168.9
Perfluorooctane sulfonate	**PFOS**	9.3	498.8 → 498.8
			498.8 → 129.9
			498.8 → 98.7
			498.8 → 79.7
Perfluorooctane sulfonamide	**FOSA**	9.8	497.9 → 497.9
			497.9 → 77.7

Table 2 Levels of PFOS and FOSA in chub liver samples (μg kg^{-1}) from monitoring localities in 2006 and 2007

River - locality	COMPOUND			
	2006	2007	2006	2007
	PFOS		FOSA	
Elbe – Děčín	198.5	89.1	6.7	n.d.
Dyje – Pohansko	19.7	24.6	5.6	n.d.
Morava – Lanžhot	49.0	22.8	5.8	n.d.
Lužnice – Bechyně	23.0	n.d.	6.1	n.d.
Svratka – Ždilochovice	n.d.	n.d.	n.d.	n.d.
Otava – Topělec	n.d.	n.d.	n.d.	n.d.
Vltava – Zelčín	78.2	72.8	7.3	n.d.
Berounko – Srbsko	n.d.	13.4	n.d.	n.d.
Sázava – Nespeky	10.1	n.d.	5.7	n.d.
Ohře – Louny	96.6	n.d.	8.3	n.d.
Odra – Bohumín	25.1	19.3	6.2	n.d.

n.d. – not detected