TISSUE DISTRIBUTION OF PERFLUOROOCTANSULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) IN FISH

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Abstract
Perfluorooctansulfonate (PFOS) and perfluorooctanoic acid (PFOA) are well-known organohalogen compounds in fish and have been accepted as key indicators for a contamination with perfluorinated compounds (PFC). However, the knowledge about their distribution in different fish tissues is scare, especially with respect to muscle tissue. The latter is of significant importance for the risk assessment of PFC entering the human food chain via fish dishes.

Therefore, 19 fish samples were caught from different locations. Following pressurized liquid extraction and SPE clean-up, muscle tissues, blood and livers were separately analyzed by HPLC-ESI-MS/MS.

PFOA concentrations (range: <0.5 – 15 ng/g wet weight) were significantly lower than the corresponding PFOS levels (range: 3.4 – 898 ng/g wet weight). However, for both, PFOA and PFOA, levels in liver and blood were significantly higher than the corresponding concentrations in muscle tissue. Additionally, it could be shown that levels of PFOS and PFOA in muscle tissue, liver and blood are closely correlated to each other.

Introduction
Perfluorooctansulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been identified as key indicators of perfluorinated compounds (PFC) in aquatic biota samples throughout the world.[1, 2] Falandysz et al. reported an increase of blood levels of PFOS and PFOA in humans with increased fish consumption.[3] Thus, fish contamination might be a severe issue for the risk assessment of the human exposure to PFC via food.

However, knowledge about uptake of PFC from water and food chain as well as the subsequent tissue distribution in fish is scare. With rainbow trout in a laboratory environment Martin et al. revealed low bioconcentration factors for PFOA (27 L/kg into blood and 8 L/kg into liver), but reported an effective bioconcentration of PFOS (BFC of 4300 L/kg into blood and 5400 L/kg into liver).[4] Jones et al. disclosed that PFOS and other PFC have been shown to bind with high affinity to the serum protein albumin[5] and several authors reported an accumulation of PFC in liver tissues.[2, 6] Nevertheless, we found no comprehensive literature data on concentrations in muscle tissues, the most important part of fish with respect to human consumption.

Thus, it was the aim of the present study to investigate PFOS and PFOA levels in fish samples from several fresh water sites. Therefore, a series of fish species was caught and at least three tissues of each fish, i.e. muscle, liver and blood, were subjected to the analysis of PFOS and PFOA.

Materials and Methods
Samples: 40 fishes were caught from several sites and anaesthetized in ethylenglycol monophenylether (Merck, Darmstadt) at a concentration of 1:1000 and subsequently killed by decapitation. Tissue specimens of the liver, muscle tissue and blood were collected from each fish. Tissue samples were filled in PP vial and kept frozen until analysis.

The analytical method based on a pressurized liquid extraction (ASE 200, Dionex) as described elsewhere in this volume.[7] Briefly, wet samples of fish muscle or fish liver were mixed with silica, spiked with $^{13}C_4$-PFOS and $^{13}C_4$-PFOA (Wellington) and filled into an ASE cartridge. Extraction was performed with methanol/water (1/1;v/v) at 100°C and 100 bar in three static cycles of 15 minutes each. Extracts were diluted with the 3-fold amount of water and passed through a syringe filter before further clean-up. Blood samples were spiked with $^{13}C_4$-PFOS and $^{13}C_4$-PFOA and treated with formic acid prior to centrifugation and solid phase extraction (SPE).

Weak anion exchange SPE cartridges were preconditioned with 2 ml of methanol and water, respectively, and the diluted extracts as well as the pretreated blood samples were passed through the preconditioned cartridges. The cartridges were then washed with methanol/water (1/1; v/v) and eluted with 1% NH_4OH in methanol. SPE eluates were evaporated under a gentle stream of nitrogen and diluted with water to a final volume of 1 ml.

Identification and quantification of perfluorinated substances was performed on a Surveyor Plus HPLC connected to a Quantum Ultra AM mass spectrometer (both Thermo, Dreieich, Germany). Chromatographic separation was achieved by a Fusion RP phase (20 x 2 mm, 2 µm, Phenomenex, Aschaffenburg, Germany). Gradient HPLC was performed with methanol and 5mM ammonia acetate in water (pH 3.5), increasing methanol from 20 to 100% within 10 minutes. Mass spectrometry was performed by electron spray ionization in the negative ion mode and subsequent single reaction monitoring (MS/MS).

Results and Discussion

Here we present the result of tissues of 19 fish samples. As expected from the data of Martin et al.[4], PFOA concentrations (range: <0.5 – 15 ng/g wet weight) were significantly lower than the corresponding PFOS levels (range: 3.4 – 898 ng/g wet weight). For both, PFOA and PFOA, levels in liver and blood were significantly higher than the corresponding concentrations in muscle tissue.

Levels of PFOS and PFOA in muscle tissue, liver and blood are closely correlated to each other. This is shown in the graphs of Fig. 1 and is apparent from the Pearson correlation coefficients between 0.75 and 0.9 listed in Table 1.

Table 1: Pearson correlation coefficients calculated for the concentrations of PFOS and PFOA in the dedicated tissues.

	liver / muscle	blood / muscle	blood / liver
PFOS	0.82	0.75	0.86
PFOA	0.90	0.85	0.79

However, the convincing dependencies of the concentrations in the three tissues might increase when further influencing factors are included in the investigation. Amongst others these might be fish species and the contamination level of the corresponding surface water and the related food chain.

These aspects will be addressed in our future work, which will also include additional fish samples as well as residues within gonadal tissue, which might be of significance to future fish generations.

Acknowledgements

We thank the Bavarian State Ministry of the Environment, Public Health and Consumer Protection for their financial support.

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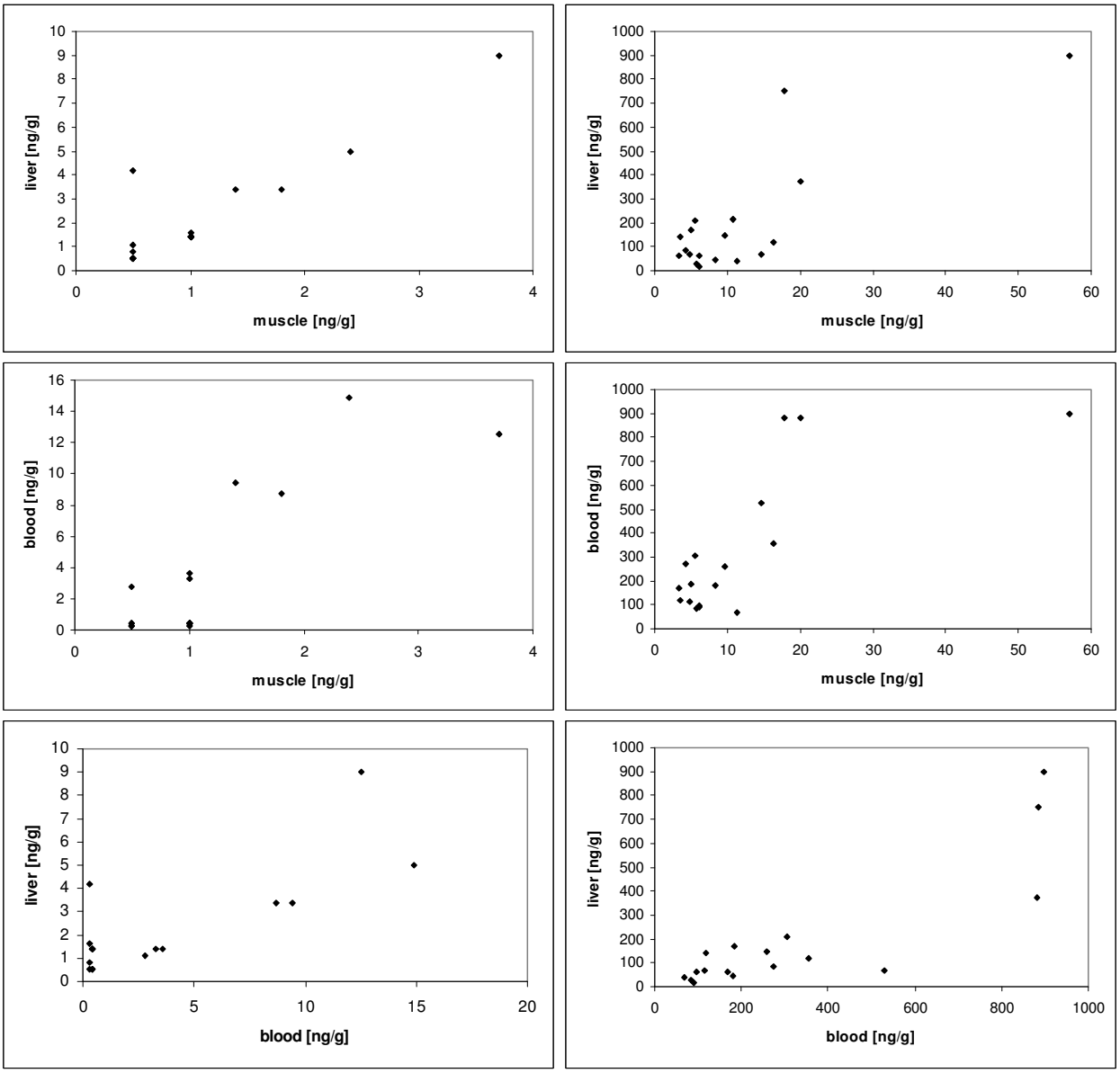

Fig. 1: Dependencies of tissue concentrations observed in 19 fishes. The three graphs on the left show levels of PFOA, whereas the three graphs on the right show PFOS levels.