PERFLUOROOCTANE SULPHONIC ACID (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) IN ENVIRONMENTAL AND HUMAN SAMPLES FROM GREECE.

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Introduction

Perfluorinated compounds (PFCs) represent a large group of chemicals which are characterised by a completely fluorinated hydrophobic linear carbon chain attached to an hydrophilic head. They exhibit particular physico-chemical characteristics such as chemical and thermal stability, low surface free energy and surface-active properties [1,2,3]. Because of their chemical resistance and surfactant properties, these compounds are used in a wide variety of industrial and consumer applications including adhesives, cosmetics, cleaners, coatings, and electronics [4].

Several studies have shown that perfluorooctane sulphonic acid (PFOS) and perfluorooctanoic acid (PFOA) have moderate acute toxicity, corresponding to a classification as "harmful, if swallowed" [5, 6]. Toxic effects of PFOS and PFOA have been reported, such as peroxisome proliferation in liver cells as well as induction of various enzymes involved in lipid metabolism[7]. These compounds may also cause tumors, immunotoxic, reproductive and/or developmental effects in rodents [8].

Owing to their persistence in the environment and bioaccumulative potential several PFCs are widespread in nearly all areas of ecosystems [9] and have been detected worldwide in the environment [10] and in human blood from a number of regions and countries, with increased levels observed in industrialized areas [11, 12, 13, 14]. The routes of human exposure to perfluorinated compounds have not been well-characterized. Although accumulation and trends of PFCs are still largely unknown, it is well established that in contrast to the classical more lipophilic persistent organic pollutants (POPs) such as dioxins and furans, or polychlorinated biphenyls (PCBs), PFCs do not typically accumulate in lipids [15]. In humans, exposure levels and pathways leading to the presence of PFCs can be better characterized by monitoring these chemicals in blood.

Initially the perfluoroalkane sulphonic acid derivatives, the perfluorinated carboxylic acids and the fluorotelomer alcohols were determined. Today research focuses especially on the PFOS and PFOA since both compounds were produced in highest amounts in the past. Additionally, for both PFCs stable isotope-labelled standards are available, which permit a validated quantification of these bipolar compounds.

The main objective of this study was to obtain, for the first time, representative data on the concentration of PFOS and PFOA of different kinds of samples from Greece. The study included the analyses of different kinds of fish, human blood serum and breast milk and influent and effluent water of municipal wastewater treatment plants.

Methods and materials

Materials

Standards of ^{13}C4-labelled solutions of PFOS and PFOA were purchased from Wellington. Ethyl acetate, methanol, n-hexane, acetonitrile, sulfuric acid 95-97%, and tert-butyl methyl ether were purchased from Merck. For solid phase extraction, Octadecyl (C18) cartridges (500 mg/8 ml) from Altech were used.

Collection of samples

Fish samples (wild, seawater) were collected through the services of the Hellenic Food Authority and were appropriately transported to the laboratory. The samples were kept at -20 ^{o}C until they were processed.

Blood samples were collected at several hospital blood donor facilities. Blood samples were collected in polyethylene recipients. Immediately after sampling, blood samples were processed for serum separation, frozen right after separation and transported to the laboratory.

Breast milk samples were collected in glass vessels by healthy volunteers, living in different areas in Athens. The blood serum samples, as well as the collected breast milk samples, remained frozen until they were analyzed at a temperature of -40 ^{o}C.
Raw influent and final effluent water was collected from three municipal waste water treatment plants (WWTPs). WWTPs 1 and 2 receive and process urban and industrial waste, whereas WWTP 3 receives and processes urban waste.

Sample preparation
Fish
5 g of blended fish were weighed into a beaker of 100 ml, and 200 µl of the internal quantification standard solution (a mixture of 100 ng/ml $^{13}{}^{13}$C4-labelled solutions of PFOS and PFOA in methanol) were added. 50ml of ethyl acetate were added. The sample was then dispersed in the ethyl acetate using a probe homogenizer (Ultra-Turrax Model T-25 equipped with Model S25N-18G shaft, IKA Works, Inc., Wilmington, NC.) until a uniform suspension was obtained and filtered through a glass wool filter. The organic phase was evaporated till dryness in a flash evaporator and the residue obtained was dissolved in 10 ml of phosphate buffer solution (PB) 0.05M, pH: 7.8. 10 ml of hexane were added and stirred. Finally, the sample was centrifuged at approximately 4000 rpm for 5 min and the hexane was discarded.
After conditioning a C18 cartridge with 2.5 ml of methanol and 5 ml of water, the residue dissolved in PBS buffer was passed through the cartridge. The cartridge was then washed with 5 ml of water and PFOS and PFOA were eluted from the cartridge with 5 ml of methanol. The organic phase was evaporated till dryness in a flash evaporator. The dry residue was dissolved in 200 µl of the HPLC mobile phase: MeOH – 5mM ammonium acetate (20:80, v/v).
Blood and breast milk
The method is based on the method applied by Powley et al. [16]. Blood serum and breast milk were extracted by protein precipitation with acetonitrile. 2 ml of blood or breast milk were pipetted into a 50-ml polypropylene centrifuge tube. 200 µl of the internal standard working solution (a mixture of 100 ng/ml ^{13}C4-labelled PFOS and PFOA in methanol) were added. 20 ml of acetonitrile were added and the sample was vortex-mixed for 1 min. Finally, the sample was centrifuged at approximately 4000 rpm for 5 min to clarify the supernatant. The organic phase was evaporated till dryness in a flash evaporator and the residue obtained was dissolved in 5 ml of phosphate buffer solution (PB) 0.05M, pH: 7.8. Solid phase extraction followed as described in the method for fish samples, with an additional step in the case of breast milk of washing with 5 ml of petroleum ether before the elution with methanol.
Influent and effluent water of municipal wastewater treatment plants (WWTPs)
The method is based on the method applied by González-Barreiro et al. [17]. NaCl was added to water samples (400 ml), to give a final concentration of 50 g/L, and the pH was adjusted to 4 with sulfuric acid. 200 µl of the internal standard working solution (a mixture of 100 ng/ml ^{13}C4-labelled PFOS and PFOA in methanol) were added. After this step the target analytes were extracted once (10 min) with 60 ml and twice (2×10 min) with 30 mL tert-Butyl methyl ether (MTBE) by liquid–liquid extraction. The solvent was removed in a flash evaporator to a final volume of 1 mL, with previous exchange of the solvent for MeOH. MeOH was evaporated under a stream of nitrogen. The dry samples were washed with 5 ml of ethyl acetate and 5 ml of hexane to remove remaining impurities, and evaporated once again. The dry residue was dissolved in 200 µl of the HPLC mobile phase: MeOH – 5mM ammonium acetate (20:80, v/v).

Instrumental analysis
All sample extracts were analysed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) with electrospray ionization (ESI) operating in negative mode. The extracts (35 µl injection volume) were chromatographed on a HyPurity Advance C_{18} column (5 µm, 50mm x 2.1mm i.d, Thermo) using an Surveyor MS Pump Plus (Thermo). The gradient operated at a flow rate of 0.25 ml/min starting from 20% MeOH in $AcNH_4$ (A) to 50% MeOH in isopropanol (B) in 6 min. The HPLC was interfaced to a triple quadrupole TSQ QUANTUM ULTRA

(Thermo) equipped with a Ion MAX-S thermoelectrospray source operating in negative ion mode. The source temperature was maintained at 350 °C and the spray voltage at −3500 V. The analyses were performed with a multiple reaction monitoring (MRM) method that monitored two mass transitions (parent ion/product ion) for each analyte. The values of the voltages applied to the tube lense offset and the collision cell were optimized by direct infusion of a solution containing the analytes. Confirmation of analyte identity was based on retention time and on the relative response of the secondary mass transition to the primary mass transition. Quantification of the target compounds was calculated by the sum of areas of the two product ions using a response factor calibration curve vs the ^{13}C4-labelled standard.

Results and discussion

i) Fish samples

6 samples of lean seawater fish were analysed. Lipid content of the samples ranged between 1.13-6.56 %. The results are presented in Table 1 and are in the same range as those reported in studies from Canada and Spain [15, 16].

Table 1. PFOS and PFOA concentrations (ng/g) in fish samples analysed

	PFOS	PFOA
Horse-mackerel	ND	0.95
Cod	0.84	0.18
Cod	ND	ND
Sea bass	ND	0.42
Red mullet	0.30	0.18
Sea bream	ND	0.48

ii) Blood serum samples

3 serum samples from healthy women (general population, range 35-41 years) and 3 serum samples from healthy men (general population, range 33-47 years) living in Athens were analysed. The results are presented in Table 2. No difference is evident between serum samples of women and men. This result is in agreement with data reported in the literature. PFOS (mean value 10.90 ng/ml) and PFOA (mean value 2.43 ng/ml) concentrations measured in this study are at the same level as those reported from other European countries, lower than those reported from East Asia and much lower than those reported from USA[11, 13, 14].

Table 2. PFOS and PFOA concentrations (ng/ml) in blood sera analysed

	Age	PFOS	PFOA		Age	PFOS	PFOA
Female	35	9.82	2.17	Male	45	7.75	2.21
	41	13.65	2.41		47	13.60	2.96
	36	11.27	2.72		33	9.32	3.02

iii) Breast milk samples

4 breast milk samples from healthy volunteers (general population, range 29-40 years), living in Athens were analysed. Results are presented in Table 3. The results from the Greek samples are within the range of those measured in breast milk in Europe and Asia. However, the number of values reported in literature is limited [18, 19].

Table 3. PFOS and PFOA concentrations (ng/l) in milk samples

Age	PFOS	PFOA
29	300	221
37	419	268
38	327	497
40	324	190

iv) Influent and effluent water of WWTPs

In general, PFOA is measured in higher concentrations than PFOS in influent and final effluent water collected from Greek municipal WWTPs. Overall, concentrations are in similar levels as those mentioned in studies of samples from Europe, Asia and U.S.A. [20,21].

Table 4. PFOS and PFOA concentrations (ng/l) in water of WWTPs analysed

		PFOS	PFOA
WWTP 1	Influent	56.53	113.07
	Effluent	78.98	157.96
WWTP 2	Influent	31.82	68.12
	Effluent	42.75	92.29
WWTP 3	Influent	16.02	26.50
	Effluent	14.79	24.95

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