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

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Abstract
Perfluorinated compounds such as PFOS and PFOA have been used in a wide variety of consumer applications for many years and are now found in many environmental matrices. The exposure of humans to these compounds has led to their presence in human blood. We undertook a study of the concentrations of PFOS and PFOA in blood serum samples from 56 healthy adults belonging to the general population of Athens. The results showed the presence of both analytes in all samples measured. Their levels were in accordance with similar results from other countries. A significant difference was observed between the values of both PFOS and PFOA between male and female participants, while only in PFOS concentrations was there a difference among the three age groups studied.

Introduction

Perfluorinated compounds (PFCs) are characterized by a number of unique physical and chemical properties[1,2] such as thermal and chemical stability, amphiphilicity, non-flammability and surface-active properties that render them ideal for a number of industrial applications. The most industrially useful perfluorinated compounds are considered to be the eight-carbon members, and especially POSF ($C_8F_{17}SO_2F$). This compound is known to degrade or metabolize to PFOS (perfluorooctane sulphonic acid). Besides being an end product of several POSF based fluorochemicals, PFOS has also been used as a surfactant in fire-fighting preparations. Another member of the PFC family, PFOA (perfluorooctanoic acid), has been produced as an emulsifier in a variety of industrial applications [3], but may also have been present as a manufacturing impurity in other POSF-based industrial products [4]. It is also possible that it is formed instead of PFOS under abiotic or hydrolytic conditions during the last step of the degradation pathway [5].

The presence of perfluorinated compounds (PFCs) has been identified in a variety of environmental matrices, including surface waters, sludge, soil, air, and ice caps [6,7]. The exposure to these environmental sources of PFCs, as well as consumer use of related products, has led to the presence of these compounds in human blood, through mechanisms not yet elucidated. Several studies of human biomonitoring for PFCs have included reports of PFOS and PFOA levels in blood, including whole blood, plasma and serum of the general population as well as occupationally and environmentally exposed individuals [8,9]. Comparative studies of blood levels of PFOS and PFOA have also been performed in people belonging to different age groups or sex, or living in different locations (rural or agricultural), showing in some cases significant differences between groups [10,11].

In Greece, a prelimimary study conducted by our group [12] measured the concentration of PFOS and PFOA of a limited number of different kinds of samples. The study included the analyses of different kinds of fish, human blood serum and breast milk as well as influent and effluent water of municipal wastewater treatment plants. In this study we report the measurement of a larger number of blood samples from adult Greeks belonging to the general population.

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. Blood samples were collected at the Medical Care Centre of NCSR "Demokritos", in Athens, Greece. 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.

Sample preparation. The method is based on the method applied by Powley et al. [13]. Blood serum was extracted by protein precipitation with acetonitrile. 2 ml of blood 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 was performed as follows. 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).

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.

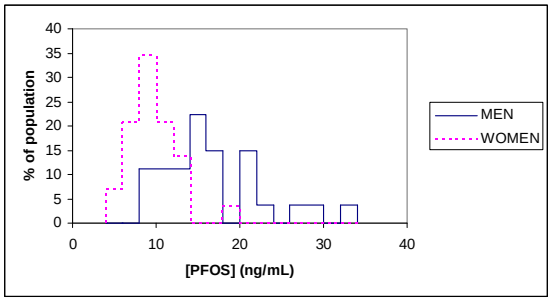
Results and discussion

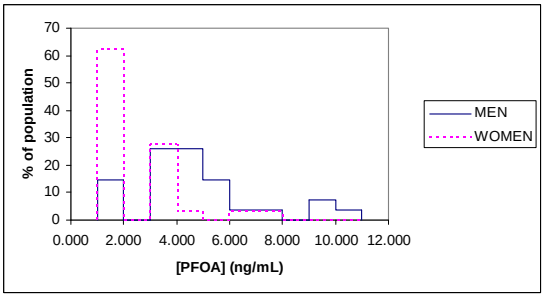
Blood serum was collected from 56 healthy adults living in Athens, Greece, with ages raging between 24 and 66 years. The donors were anonymous and there was no information about their past exposure histories to fluorochemicals. The only known demographic factors were age, gender, and date and location of blood collection. It was therefore assumed that the donors were representative of the general population of Athens.

Each sample was analyzed for the determination of PFOS and PFOA and statistical analysis was applied to the results in order to assess the distribution of these analytes according to two demographic attributes (i.e. sex and age).

Both analytes were detected in all samples. Measurements showed that serum samples from male participants had significantly higher mean value for PFOA (*t-test:* $p<0.001$) and PFOS (*t-test*: $p<0.001$) than those from female participants, as seen in the following table:

Group	**Men (aged 24-66)**		**Women (aged 25-51)**	
N	27		29	
	PFOA	**PFOS**	**PFOA**	**PFOS**
Range (ng/mL)	1.681-10.214	6.974-30.36	0.57-6.57	2.276-16.636
Median (ng/mL)	3.144	13.695	1.707	7.033
Mean (ng/mL)	3.885	14.936	2.083	7.491





Our findings also showed a significant difference of PFOS values (ANOVA: $p<0.01$) among the three age groups studied. More specifically, significant difference was observed between the age groups up to 30 and over 40 (*t-test*: $p<0.001$), as well as between the age groups of 31-40 and over 40 (*t-test*: $p<0.03$), while no significant difference exists between age groups up to 30 and 31-40 (*t-test*: $p<0.058$) On the contrary, no significant difference was observed between the three age groups in PFOA (ANOVA: $p=0.420$). The results are presented below:

Ages	**Up to 30**		**31-40**		**Over 40**	
N	**18**		**19**		**19**	
	PFOA	**PFOS**	**PFOA**	**PFOS**	**PFOA**	**PFOS**
Range (ng/mL)	0.758-6.57	4.85-13.888	0.94-10.214	3.993-27.802	0.57-8.051	2.276-30.36
Median (ng/mL)	2.516	8.164	2.033	7.897	2.778	14.665
Mean (ng/mL)	2.569	8.928	2.852	9.803	3.415	14.398

Our findings are generally in agreement with previous human data. Samples have been measured from numerous countries including the United States [10], Japan [14, 15], China [16], Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia, Korea [8], Australia [17], Canada [18], Germany [19]. Although different analytical methodologies have been applied in these studies, and the blood matrices used were not always the same (whole blood, plasma or serum has been used), in every case PFOS and PFOA have been detected, in varying levels. In general, average PFOS levels are about 20-30 ng PFOS/mL, though the results suggest that there are wide geographical fluctuations, with levels rising as high as about 1500 ng PFOS/mL in a sample from an American Red Cross donor [10], to levels near the detection limit from samples in India [17]. Other European countries such as the Netherlands, Germany and Belgium, had PFOS levels in pooled serum samples between these two extreme values, i.e. 53, 37 and 17 ng/mL, respectively, similar to the levels found in Greece. Most possibly, the use of carpets and various kinds of packaging materials and other paper products containing sulfonated perfluorochemicals is more widespread in developed countries such as the United States, Canada and European countries, and minimal India. The levels of PFOA, though also always detectable, are somewhat lower than those of PFOA.

It would be difficult to explain the difference in values obtained from male and female donors, since the pathways leading to the presence of PFOS and PFOA in human blood are not clear. Similar differences have also been observed in previous studies [10,16] while other studies have found these two groups without difference [3]. On the other hand, the higher PFOS values observed in older age groups could be attributed to the bioaccumulation of PFOS, whose half-life of serum elimination has been estimated to be approximately 9 years [20].

In conclusion, the present study assessed the concentration trends of PFOS and PFOA in blood samples from individuals in the general population of Athens, following our preliminary findings.

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