PERFLUORINATED SUBSTANCES (PFOS, PFOA) IN BLOOD OF AN ADULT POPULATION IN GERMANY

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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 a hydrophilic head. A prominent example is the Perfluorooctane Sulfonate (PFOS), which contains a sulfonyl group as the hydrophilic head. PFCs have been produced for several decades and are widely used for many industrial and consumer applications such as the impregnation of carpets, textiles and leather, coatings in paper, cardboards, food packing materials and electronic and photographic devices, surfactant in diverse cleaning agents and the use in fire-fighting foams.

In the meantime, several PFCs can be detected in different environmental media and biota reflecting the widespread global pollution in all parts of the ecosystem.[1] Their occurrence and persistence in the environment and their potential to bioaccumulate in organisms has caused concern among toxicologists as well as the public.

The occurrence of PFCs in human blood of occupationally exposed groups, but also of the general population not occupationally exposed, although in much lower concentrations, has been demonstrated.[2,3] Because PFOS-salts are poorly water soluble and less volatile, the sources of human exposure are currently not well understood.

The purpose of the present study was to quantify the internal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in the general population using human plasma of adult blood donors.

Materials and Methods

Study population

Blood samples were derived from two blood banks. All together 308 plasma samples (142 females, 166 males) were obtained from healthy adult donors (18–67 years of age) in summer 2005. The blood donors were living in the southern part of Bavaria, Germany, including the city of Munich and some rural areas.

Sampling and laboratory analysis
After venipuncture each sample was centrifuged to obtain the plasma fraction (3-5 ml) and then was stored without any preservatives at -20°C until laboratory analysis. Extensive measures for quality control were implemented to ensure accuracy and reliability of measurements.

The analytical method consisted of an offline protein precipitation with acetonitrile followed by HPLC and MS/MS detection. Briefly, the internal standard solution was added to 250 µl of plasma/serum aliquots. After addition of 700 µl acetonitrile, the samples were centrifuged at 1500 rpm for 15 minutes. For chromatographic separation 30 µl of the supernatant were injected into the HPLC system which comprised an Eclipse Zorbax XDB-C8 analytical column (Agilent Technologies, 4.5 x 150 mm). Elution was performed with a gradient described as follows: with a constant percentage of 1 % acetic acid (v/v in water) the initial flow rate was 0.3 ml/min with 75 % acetonitrile (A) and 24 % water (B) until 2.0 min, then raised to 99 % A and 0 % B at 7.0 min; 99% A and 0 % B was held with an increased flow rate of 1.0 ml/min until 11 min; then the composition was changed to 75 % A and 24 % B at 11.1 min until 13 min. After that the flow rate was again decreased to 0.3 ml/min at 13.1 min and held until 15 min, which was the total run time. Retention times were 6.5 and 6.0 min for PFOS and PFOA, respectively.

For detection, an Applied Biosystems API 2000 triple quadrupole mass spectrometer (Foster City, CA, USA) was employed which operated in the negative ionization MS/MS mode and used multiple reaction monitoring (MRM). The following transitions were monitored: PFOS 499 -> 80, 1,2,3,4-$^{13}C_4$-PFOS 503 -> 80, PFOA 413 -> 169, and 1,2-$^{13}C_2$-PFOA 415 -> 370, which generated the most intensive signals and interference free peaks.
Lower limits of quantification (LOQ) based on a 10-fold peak-to-noise ratio were 0.5 µg/l for both PFOS and PFOA.

Statistical analysis
For the estimation of correlations the non-parametric Spearman rank correlation coefficient was derived, the difference in median was tested with the Wilcoxon rank sum test for two groups and with the Kruskal-Wallis test for more than two groups, respectively.

Results and Discussion
Overall, we were able to detect the target analytes in all plasma samples. The distribution of the PFCs levels of all subjects, stratified by sex are given in Table 1, with generally higher PFOS concentrations than PFOA levels. In female participants, the levels of PFOS and PFOA fluctuated between 2.5 and 30.7 µg/l and 1.5 and 16.2 µg/l, respectively. In males, measurements ranged between 2.1 and 50.3 µg/l for PFOS and 0.5 and 17.7 µg/l for PFOA.

A significant positive correlation ($r = 0.64$, $p < 0.001$) between the PFOS and PFOA concentration was observed and the median PFOS and PFOA levels were significantly increased in male compared to female participants ($p < 0.001$; $p < 0.05$). Furthermore, levels of PFOS and PFOA differed significantly by age group, with the oldest age group showing

higher levels than participants of age 18-34 or 35-51 (PFOS: p = 0.01; PFOA: p = 0.001). If looking on both genders separately, the association with age reached statistical significance in women for PFOS and in men for PFOA (see Figure 1).

Table 1: Descriptive statistics of perfluorinated compounds in blood plasma (µg/l)

	PFOS			**PFOA**		
	all	**female**	**male**	**all**	**female**	**male**
N	308	142	166	308	142	166
Mean	13.1	11.4	14.5	5.5	5.2	5.7
Standard deviation	6.4	5.3	6.8	2.3	2.1	2.4
Minimum	2.1	2.5	2.1	0.5	1.5	0.5
10^{th} percentile	6.5	5.1	7.6	3.0	2.8	3.1
25^{th} percentile	8.9	7.2	10.4	4.0	3.8	4.1
Median	11.9	10.3	12.8	5.1	4.8	5.4
75^{th} percentile	16.1	14.4	16.7	6.6	6.1	6.8
90^{th} percentile	21.0	18.0	22.9	8.5	7.7	9.0
Maximum	50.3	30.7	50.3	17.7	16.2	17.7

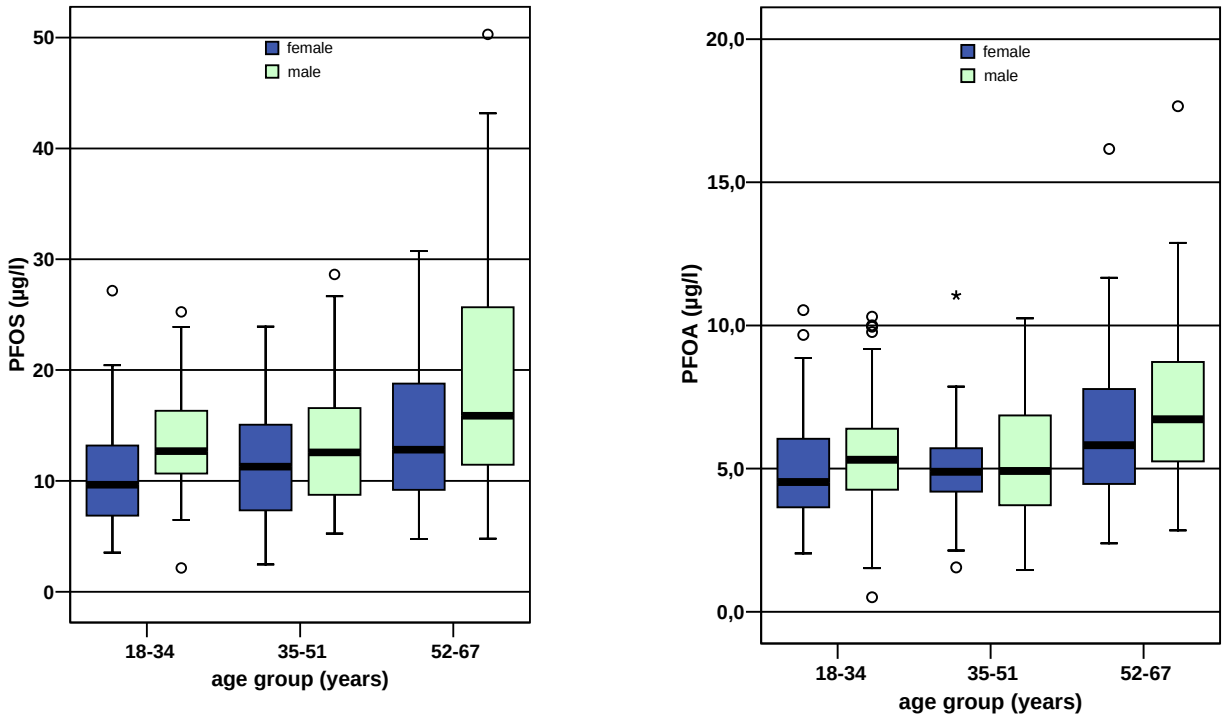

Figure 1: Distribution of PFOS (left) and PFOA (right) in men and women stratified by age group

No difference was found in PFOS-level between participants residing in urban locations in comparison to participants residing in the countryside, but PFOA-levels were significantly decreased in urban living subjects (median = 4.6 µg/l) in comparison to subjects living in the countryside (median = 5.4 µg/l).

Our results are in line with findings from other studies conducted in Europe in which median PFOS concentrations of between 4.4 µg/l and 18.2 µg/l and median PFOA concentrations of between 2.7 µg/l and 6.5 µg/l were observed. Considerably higher concentrations were found only in a study described in the OECD review, in which a median of 37 µg/l was observed in 6 pooled samples from a German blood bank in 1999 and in one study in Poland in which median values of 34 µg/l (males) and 41 µg/l (female) were reported.[4]

Investigations conducted in North America resulted in higher levels in comparison to European studies. Here, the median concentration of PFOS and PFOA ranged from 18 µg/l to 81 µg/l and 2.2 µg/l to 25 µg/l, respectively. In Asia concentrations of PFOS and PFOA comparable to those observed in Europe were found.

Our results indicate that increased concentrations of PFOS and PFOA are associated with male sex, higher age (partly), and rural residence (only PFOA). Increased concentrations of PFCs in males have been observed in many studies already,[5,6,7,8,9,10,11] however, in some studies this association was not found.[4,12] In the interpretation of the results of many previous studies, however, it has to be considered that the PFOA levels usually were low and in many cases did not reach the detection limit.

In previous studies a relation between PFC concentration in blood with age was not reported [4,5,6] or only reported for a subgroup of blood donors of a special sampling year.[13]

Our data suggest that the exposure to PFOS and PFOA in the adult German general population is similar to the exposure in other European countries and Asian countries but low compared to the exposure in North America.

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