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Perfluoroalkylated compounds in whole blood and plasma from the Swedish population

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Abstract

The levels of five perfluoroalkylated (PFA) compounds, PFOS, PFOA, PFHxS, PFOSA and PFNA were determined in whole blood and plasma from 47 males and 36 females representing the Swedish population. The analytical method developed and used includes solid-phase extraction and liquid chromatography electrospray massspectrometry. PFOS was the PFA found at the highest concentration in whole blood (18.2 pg/ul) followed by PFOSA (4.1 pg/μl), PFOA (2.7 pg/μl), PFHxS (2.3 pg/μl) and PFNA (0.3 pg/μl). Besides the five compounds determined, we were able to detect PFHxA, PFDA, PFDS, PFUnDA, PFDoDA and PFTDA in some of the samples/at low concentrations, the only compound included in this study that we did not detect was PFBuS. This shows that the Swedish population are exposed for a large number of PFAs. The concentrations of PFOS and PFOA correspond to those found in USA. The correlation between PFOS and the other compounds was studied in order to find out if there is a common source of exposure. Only a very weak correlation between PFOS and PFOA could be seen.

1. Introduction

Perfluoroalkylated (PFA) compounds possess unique properties like thermal, chemical and biological stability due to a short C-F bond (1.38 Å), the small size of fluorine and fluorines electronegativity (1). Perfluoroalkylated acids are both hydrophobic and hydrophilic, which are properties that make them frequently used for treatment of carpets, fabric, leather, protection of paper and food packaging and also as performance chemical in plastic production, firefighting foam, polish and cleaners and insecticide (2,3). Since the end of the 1990s perfluorooctanesulfonate (PFOS) has been reported in wildlife all around the world together with perfluorooctanoic acid (PFOA) (4). PFOS is the PFA that has been reported most frequently and in the <1-1656 pg/ul range in human blood from several countries (5,6,7,8,9). Although the world's largest manufacturer of PFOS, 3M, in year 2000 phased out all production of perfluorooctanyl-like compounds (10), PFOS is expected to persist in biota for a long time as it has been shown to bioaccumulate. PFOS main applications were as surfactant and mist suppressant in industrial applications, emulsifier in floor polish, film former in fire fighting foams and active ingredient in insecticides (17). PFOS is suspected to be the stable degradation product from several other perfluorinated compounds, and it has been shown that N-ethyl perfluorooctanesulfonamide (N-EtPFOSA) can be biotransformed to PFOS via perfluorooctanesulfonamide (PFOSA) (11). It has also been suggested that N-EtFOSE (2-N-ethylperfluorooctane sulfonamido ethyl alcohol) used as paper- and packaging protection and corresponding N-MeFOSE (used as surface treatment) can through oxidation and metabolism form PFOSA and therefore also PFOS (12). PFOAs main application is as a polymerisation aid in the manufacture of fluoropolymers and fluoroelastomers (18), of which polytetrafluoroethylene (PTFE) is one of the largest products. PTFE is heavily used in both industrial and household applications because of oil, stain and water rejection, non-sticking properties and thermal resistance.

Potential toxicology effects on humans are not well understood. Listed in the OECD report on PFOS and its salts (2) are effects like hepatotoxicity, postnatal deaths and development effects in animal studies. LD₅₀ and LC₅₀ on rats are in the mg/kg or mg/l range.

Historically, the analysis of PFAs has been limited to mainly PFOS and PFOA but recently a number of other, potentially bioaccumulating, perfluorinated acids were reported in wildlife and in human blood (13,14). Since possible degradation pathways, distribution and exposure routes for this group of compounds are poorly understood the information of exposure to a larger number of PFAs is necessary. The exposure of these compounds to the Swedish population had not been studied before this project was performed, except for 11 samples from Swedish blood banks analysed by 3M Company in 1998 (7). Only PFOS was reported in the 3M study.

The purpose of the performed project was to study the exposure of several perfluoroalkylated compounds to the Swedish population for several reasons; 1) in order to compare the exposure of PFOS and PFOA with other countries, 2) study the extent of other perfluoroalkylated compounds in Swedish blood, 3) study the data for possible information about exposure pattern. For this study, we developed a method for the analysis of 13 perfluoroalkylated compounds, with carbon chain length ranging from 4 to 13, in human whole blood and plasma. The method includes solid-phase extraction (SPE) followed by high performance liquid chromatography electrospray massspectrometry (LC-ES-MS). The challenge in this work was the solid phase extraction of whole blood and analysis with single-quadropol MS, which by our knowledge have not been performed in this research area until now.

2. Experimental section

2.1 Perfluoroalkylated compounds

Of the 13 perfluoroalkylated compounds only 12 were studied because one was selected as internal standard. Information of the compounds studied can be seen in *Table 1*. The complete name and abbreviation of each compound is given in section 2.3.

2.2 Sample collection

Studied samples are control samples (sons and mothers) in a testicular cancer study (MPR 2336), drawn from the Swedish population registry (Stockholm, Sweden), and collected during 1997-2000. The mothers were of age 46-75 and the sons of age 19-46. The sample material consists of both whole blood and plasma samples, initially treated with Heparin, but stored in glass containers without Heparin at -20°C.

2.3 Standards and reagents

Tetrabutylammoniumperfluorobutanesulfonate (PFBuS TBA-salt, $\geq 98\%$), perfluorooctanesulfonic acid potassium salt (PFOS K-salt, $\geq 98\%$), perfluorodecanoic acid (PFDA, $>97\%$), perfluorocaproic acid (PFHxA, $\geq 97\%$) were purchased from Fluka. Perfluoroheptanesulfonic acid (PFHpA, 99%), perfluoronanoic acid (PFNA, 97%), perfluorooctanoic acid (PFOA, 96%), ammonium perfluoro-1-decansulfonate in H₂O/butoxyethanol (PFDS NH₄-salt, 25w%), perfluoroundecanoic acid (PFUnDA, 95%), perfluorododecanoic acid (PFDoDA, 95%), perfluorotetradecanoic acid (PFTDA, 97%) were purchased from Aldrich. Perfluorooctanesulfonamide (PFOSA, 97%), 7H-dodecafluoroheptanoic acid (7HPFHpA, 98%) were purchased from ABCR. 1H,1H,2H,2H-perfluorooctane sulfonate (THPFOS) and perfluorohexane sulfonic acid (PFHxS, 98%) were purchased from Interchim Ammonium acetate ($>99\%$, pa for HPLC) was purchased from Fluka, formic acid (98-100%) from Scharlau and methanol (HPLC) was taken from Labscan. The water used was laboratory produced ultra pure water.

2.4 Extraction and analysis

The frozen blood or plasma sample was allowed to thaw in room temperature and 0.75 ml was then transferred to a 15 ml polypropylene centrifuge tube, previously washed with methanol. An aliquot of 7.5 μ l internal standard solution containing PFHpA and THPFOS was added. After thorough mixing, 3 ml formic acid/water (1:1 by volume) was added and thereafter sonicated for 15 minutes and centrifugated at 10000 x g for 30 minutes. 2.5 ml were extracted on a C18 HF BondElute (Varian) 200 mg/3ml solid phase extraction column that was prepared with 2 ml methanol and 2 ml water. After washing the column with 40v% methanol in water the column was put under vacuum suction until dryness. Elution was performed with 0.5 ml methanol. All solutions including the sample solution were allowed to run through the column material without vacuum. The extract was filtered through 0.2 μ m polypropylene filter into a polypropylene vial and then injected into an Agilent 1100 LC system with a Supelco Discovery HS C18 (50*2.1 mm, 3 μ m) column (Sigma-Aldrich) equipped with a guard column of the same material. The instrument was equipped with a thermostated column department that was kept at 40°C. A water/methanol mobile phase gradient with 2mM ammonium acetate was delivered with a flow rate of 0.3 ml/min. The gradient started at 35% methanol followed by a 20 min ramp to 90% methanol, a 10 min hold followed by a 10 min washing sequence with 100% methanol, and then reverting to initial conditions allowing 7 min stabilisation time. Detection was performed with an Agilent 1100 MSD massspectrometer equipped with an atmospheric electrospray interface operating at negative ion mode with following settings: nebulizer gas temperature 350°C, nebulizer gas pressure 20 psi, drying gas flow 13 ml/min and capillary voltage 3500V. Quantitation was performed with single ion monitoring. Fragmentation settings for each compound can be seen in *Table 1*.

2.5 Quantification

Quantification was performed using the internal standard method. Since no C-13 labelled PFA standard was commercial available at the time for this project, other compounds similar in character to the studied PFAs were investigated for that purpose. As a result, both PFHpA and THPFOS were used as internal standard but it turned out that THPFOS were less appropriate, and after assessing possible concentration of PFHpA in the blood studied, concentrations calculated with PFHpA as an internal standard were reported. The quantification ion used for each compound can be seen in *Table 1*.

2.6 Method performance

Recoveries were calculated by standard addition to a whole blood sample at three concentration levels. Results are presented in *Table 2*. Detection limits, defined as three times the noise level, were calculated with both plasma and whole blood matrix and can be seen in *Table 3*. Method repeatability was controlled by extracting a control sample with every extraction batch. The control sample was prepared by adding a known amount of each compound, except for PFOS that was already in the blood at suitable concentration. After homogenisation small portions were then stored at -20°. Instrument repeatability was evaluated by adding the same vial of whole blood extract to every LC-MS sequence. A method blank with water instead of blood was extracted with every extraction batch confirming that nothing could be extracted out of the materials used. Methanol injections were carried out at regular intervals during the LC-MS analysis. In order to measure the recovery of the internal standard, which was 63-112%, 7HPFHpA was added the extract prior LC-MS analysis.

2.7 Method accuracy/suitability confirmation

An intercalibration study was performed with one reference solution in methanol in order to study the accuracy of the single-quadropol massspectrometer compared to an MS/MS system

(Micromass Quattro II). The deviations were between 1-35% except for PFBuS, which is somewhat difficult to analyse. In addition, one whole blood sample was extracted and analysed according to the method described and compared with ion-pair extraction according to Hansen et.al. (15) and LC-MS/MS analysis. Good agreement between the two methods could be shown with deviations 7-20% except for some compounds that occurred at low concentrations.

3. Results and discussion

The SPE-LC/MS method was applied to 66 whole blood samples and 17 plasma samples, a total of 83 samples which can be further divided into females (36) and males (47). Of the 12 PFAs studied, 5 were found in almost all of the samples and these results are presented in *Table 4*. The concentrations are not related to lipid content since PFAs do not distribute to fat tissue like other halogenated persistent organic pollutants. It is suggested that PFOS binds to serum albumin (16) which makes it important to distinguish whole blood and plasma results from each other and as can be seen in *Table 4* the average PFOS concentration in the plasma samples (33.4 pg/ μ l) is a factor of 1.8 higher compared to the concentration of the whole blood samples (18.2 pg/ μ l). Also the concentration of PFOA is higher in the plasma samples (factor 1.5) but for the remaining compounds this relationship is not as distinct. However, since the whole blood and plasma samples are independent from each other, that is not from the same person, no conclusions can be drawn. PFOS was the PFA found at the highest concentration in whole blood (18.2 pg/ μ l) followed by PFOSA (4.1 pg/ μ l), PFOA (2.7 pg/ μ l), PFHxS (2.3 pg/ μ l) and PFNA (0.3 pg/ μ l). Regarding the plasma samples, the average PFOS concentration is highest (33.4 pg/ μ l) followed by PFOA (4.0 pg/ μ l), PFHxS (2.1 pg/ μ l), PFOSA and PFNA (both 0.6 pg/ μ l). Since the material consists of mothers (age 46-75) and sons (age 19-46) the difference between males and females is also the age difference. The

frequency distributions of five PFAs in whole blood and plasma are displayed in *Figure 1*. Besides the five compounds determined, we were able to detect PFHxA, PFDA, PFDS, PFUnDA, PFDODA and PFTDA in some of the samples/at low concentrations, the only compound included in this study that we did not detect was PFBuS. This shows that the Swedish population are exposed for a large number of PFAs.

The concentrations of PFOS and PFOA correspond to those found in USA. The geometric mean from 645 serum samples from six American Red Cross blood banks throughout the United States was 34.9 ppb for PFOS, 4.6 ppb for PFOA and 1.9 ppb for PFHxS (8). Another study performed by Kannan and colleagues (9) compares human exposure in USA, Italy and India. The mean PFOS concentrations for the different countries were 32.5, 4.4 and 2.3 ng/ml. The same trend, were USA>Italy>India, could also be seen for PFOA (4.7, <3, <3), PFHxS (3.6, 1.3, 1.6) and PFOSA (3.7, 1.7, <3). In Japan (6), whole blood from 26 people resulted in an average of 8.1 ng/ml PFOS and 1.7 ng/ml PFOSA. No PFOA (<3.35 ng/ml) was detected. It seems like the exposure for these compounds are about the same in Sweden and USA, but lower in Italy, Japan and India. One reason for the elevated concentrations in USA has been said to be the presence of industrial production of this type of chemicals, however Sweden does not have this kind of industries. Higher concentrations can also be a result of longer and more frequent use of textile, carpets, leather and paper protectors.

The correlation between PFOS and the other compounds was studied in order to find out if there is a common source. Only a very weak correlation between PFOS and PFOA could be seen (*Figure 2*).

Table 1. Structural information on the perfluoroalkylated target compounds. The three compounds at the bottom are internal standards (IS) and recovery standard (RS).

Compound	Molecular formula	Molecular weight	Quantification ion	Fragmentation voltage	CAS (salt)
PFBuS	C ₄ F ₉ SO ₃ ⁻	541.6	C ₄ F ₉ SO ₃ ⁻	95	108427-52-7 (TBA)
PFHxA	C ₅ F ₁₁ COOH	314.0	C ₅ F ₁₁ ⁻	65	307-24-4
PFHxS	C ₆ F ₁₃ SO ₃ ⁻	438.2	C ₆ F ₁₃ SO ₃ ⁻	130	3871-99-6 (K)
PFOA	C ₇ F ₁₅ COOH	414.1	C ₇ F ₁₅ ⁻	75	335-67-1
PFNA	C ₈ F ₁₇ COOH	469.0	C ₈ F ₁₇ ⁻	75	375-95-1
PFOS	C ₈ F ₁₇ SO ₃ ⁻	538.2	C ₈ F ₁₇ SO ₃ ⁻	150	2795-39-3 (K)
PFDA	C ₉ F ₁₉ COOH	514.1	C ₉ F ₁₉ ⁻	83	335-76-2
PFDS	C ₁₀ F ₂₁ SO ₃ ⁻	617.2	C ₁₀ F ₂₁ SO ₃ ⁻	150	67906-42-7 (NH ₄)
PFOSA	C ₈ F ₁₇ SO ₂ NH ₂	498.9	C ₈ F ₁₇ SO ₂ NH ⁻	113	
PFUnDA	C ₁₀ F ₂₁ COOH	564.1	C ₁₀ F ₂₁ ⁻	85	2058-94-8
PFDoDA	C ₁₁ F ₂₃ COOH	614.1	C ₁₁ F ₂₃ ⁻	90	307-55-1
PFTDA	C ₁₃ F ₂₇ COOH	714.1	C ₁₃ F ₂₇ ⁻	93	376-06-7
IS PFHpA	C ₆ F ₁₃ COOH	364.1	C ₆ F ₁₃ ⁻	70	375-85-
IS THPFOS	C ₈ F ₁₃ H ₄ SO ₃ H	428	C ₈ F ₁₃ H ₄ SO ₃ ⁻	110	
RS 7H-PFHpA	HC ₆ F ₁₂ COOH	346.1	HC ₆ F ₁₂ COO ⁻	75	1546-95-8

Table 2. Recoveries of 12 PFA compounds determined by standard addition to whole blood and SPE-LC/MS analysis.

	n	Recovery % 1-8 pg/μl	Recovery % 5-13 pg/μl	Recovery % 10-27 pg/μl
PFBuS	3	26±3	27±2	27±1
PFHxA	3	98±24	90±7	92±1
PFHxS	3	112±5	110±0.5	108±2
PFOA	3	79±5	97±2	96±1
PFNA	3	81±10	84±4	90±4
PFOS	3	99±3	98±4	103±7
PFDA	3	75±1	80±7	85±2
PFDS	3	69±5	83±6	87±11
PFOSA	3	81±10	na	na
PFUnDA	3	82±6	79±7	84±7
PFDoDA	3	64±14	na	na
PFTDA	3	75±24	nan	na

na not analysed

Table 3. Detection limits (pg/μl) of 12 PFA compounds defined as signal to noise ratio 3.

	Plasma	Whole blood
PFBuS	2*	2*
PFHxA	0.3	0.3
PFHxS	0.1	0.1
PFOA	0.3	0.5
PFNA	0.1	0.1
PFOS	0.1	0.1
PFDA	0.3	0.1
PFDS	0.5	0.5
PFOSA	0.1	0.1
PFUnDA	0.1	0.1
PFDoDA	0.4	0.2
PFTDA	0.3	0.1

* Due to low recovery

Concentrations (pg/μl) of 5 individual PFA compounds including sums of PFCA¹, PFSA² and PFA³ in food and plasma collected in Sweden, 1997-2000.

Whole blood	HxS	DA	DS	NA	OSA	FCA ¹	FSA ²	FA ³
All, N=66								
Range	-28.4	-12.4	-37.0	1-1.9	1-22.9			
Arithmetic mean			2		1		6	6
Median			1		7			
Males, N=40								
Range	-28.4	-12.4	-37.0	1-1.9	3-22.9			
Arithmetic mean			4		5		0	3
Median			7		7			
Females, N=26								
Range	-2.5	-4.1	-32.8	1-1.0	1-9.5			
Arithmetic mean			8		3		3	0
Median			9		7			
Plasma								
All, N=17								
Range	-3.5	-8.4	1-90.9	1-1.4	1.1-2.9			
Arithmetic mean			4		5		8	3
Median			0		2			
Males, N=7								
Range	-3.5	-8.4	5-90.9	1-0.6	2-2.9			
Arithmetic mean			6		3		6	8
Median			0		2			
Females, N=10								
Range	-2.9	-7.4	1-54.7	1-1.4	1.1-0.9			
Arithmetic mean			1		4		1	0
Median			2		3			

Sum of perfluorocarboxylic acids. ² Sum of perfluorosulfonic acids incl. PFOSA. ³ Sum of ¹ and ².

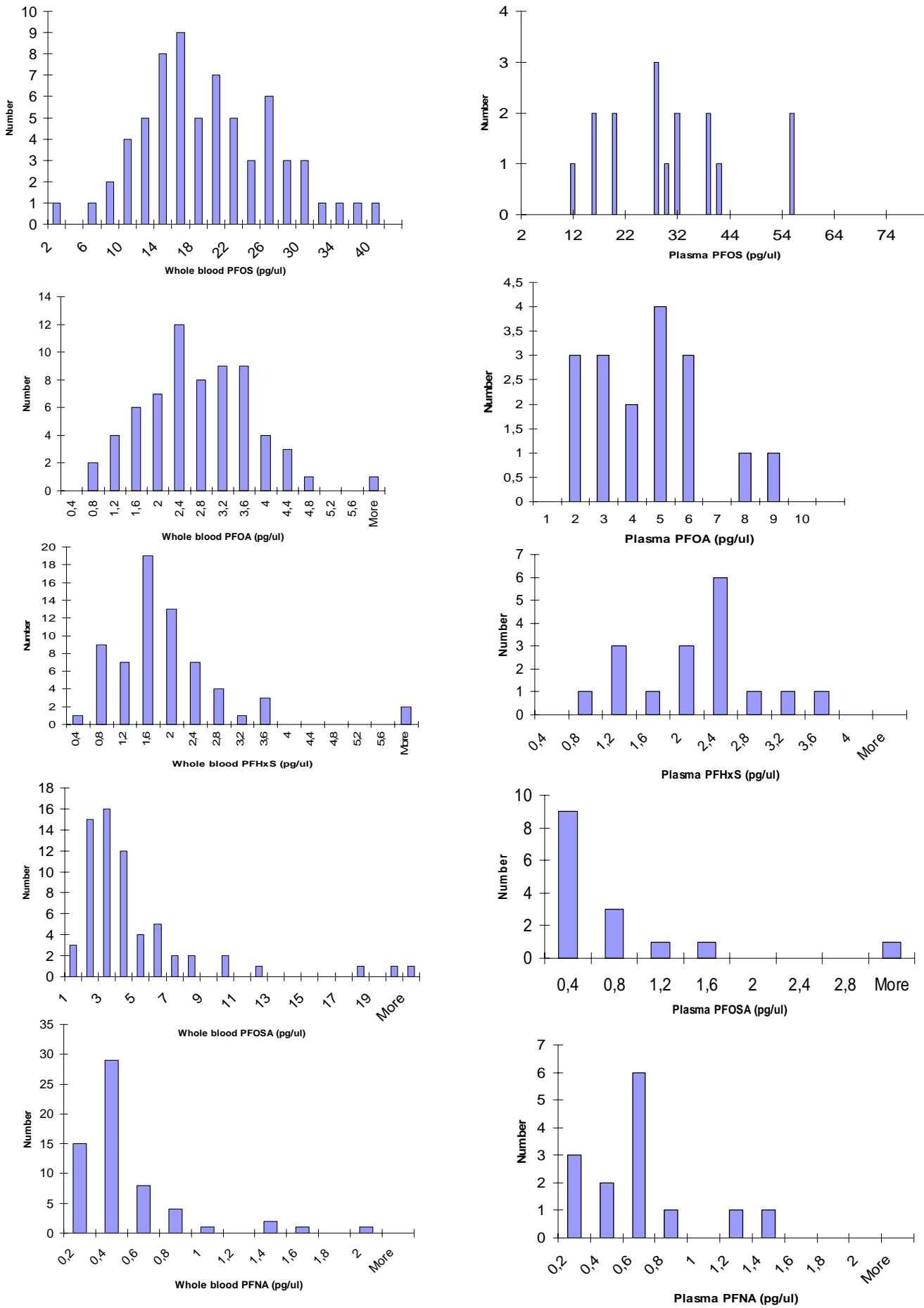


Figure 1. Population distribution of five perfluoroalkylated compounds in whole blood (n=66) and plasma (n=17) from Sweden, 1997-2000.

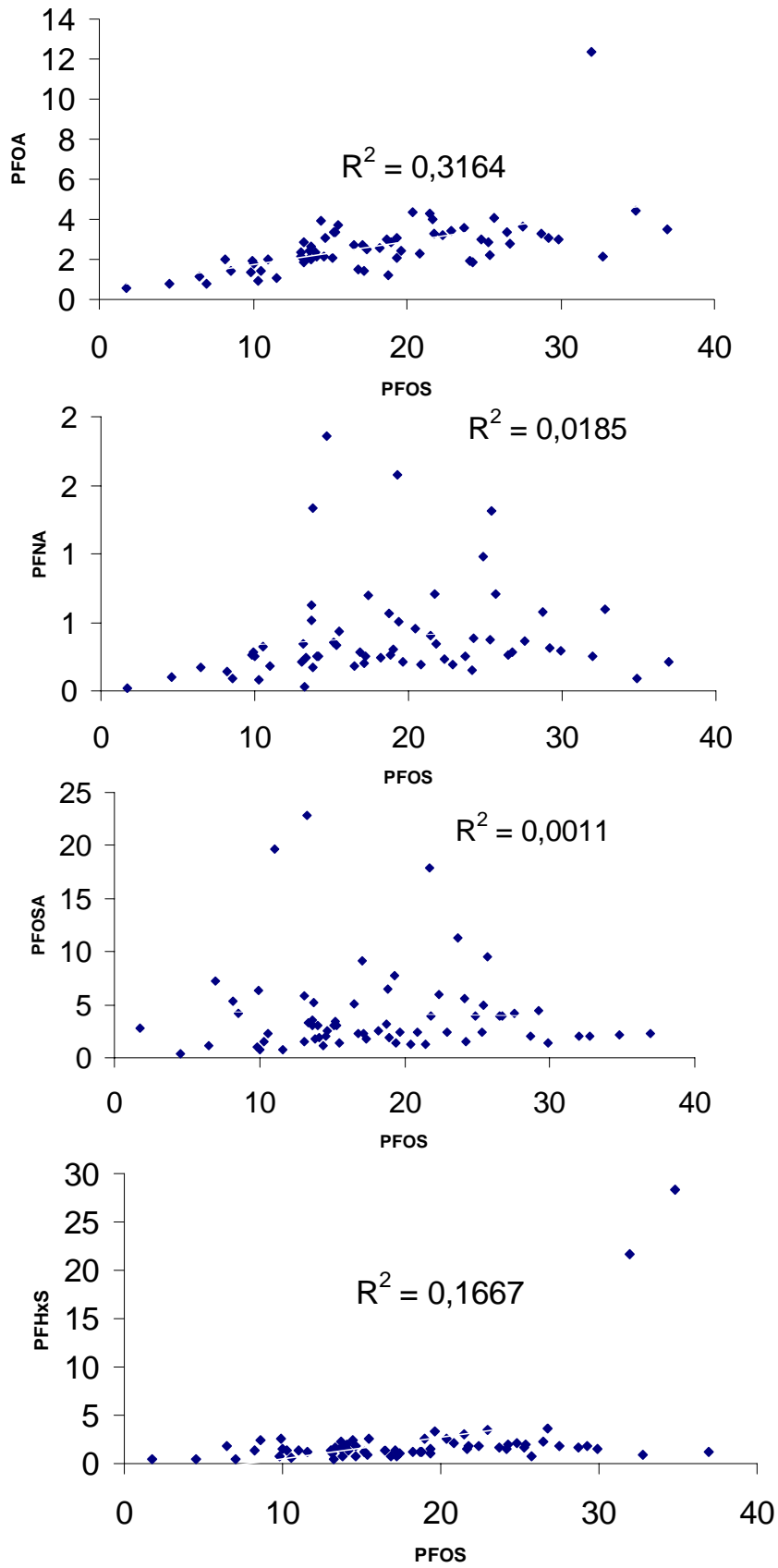


Figure 2. Correlation between PFOS and 4 PFA compounds in whole blood from Sweden.

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Appendix: Data table