# Perfluorinated compounds in human plasma and blood - 1st worldwide interlaboratory study

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## Introduction

Accurate and reproducible analysis of perfluorinated alkyl compounds (PFCs) in human tissue is a challenge in many ways[1]. An interlaboratory study was initiated to survey the current quality and conformity of results of PFC analysis performed by different laboratories worldwide. Methods, matrices and reference materials used for PFC analysis differ between laboratories. Many factors, such as contamination from laboratory materials, incomplete recoveries and contamination from instrumental parts are common problems, and can lead to poor accuracy, sensitivity or reproducibility. Due to their physical and chemical properties PFCs also tend to adsorb to surfaces, and can be lost at any stage of the sample handling. Today LC-MS electrospray is the most common detection technique used for PFC analysis. This technique is sensitive to ion-suppression, both from the matrix or from poorly separated PFCs. Branched isomers further challenge separation and reference standards are needed to avoid interferences. In addition, new methods for various matrices are continuously being developed. A perfect quality assurance tool to standardize results for PFCs is worldwide interlaboratory studies.

The Netherlands Institute for Fisheries Research and Örebro University organized this interlaboratory study in collaboration with the EU Perforce research project. The study covers environmental matrices, a standard solution, and human plasma and blood. Here the results from the plasma and blood interlaboratory are reported.

## Method and materials

### *Samples*

The human matrices part of the interlaboratory study consisted of a plasma sample and a whole blood sample. Laboratories were asked to use any method they preferred, and to analyze as many PFCs possible. Nineteen laboratories signed up for this part, and 17 of these have returned final results. All 17 laboratories reported results for the plasma sample and 11 laboratories also analyzed the whole blood sample. Authentic blood samples, without addition of PFCs, representing the current levels of PFCs in the Swedish general population were used. The samples were administrated by the University Hospital of Örebro (USÖ), Sweden, and were released for medical use according to the regulations by the Swedish National Board for Health and Welfare. Before shipment to the participants, the materials were homogenized and divided into polypropylene tubes in approximately 3 mL portions for whole blood and 7 mL for plasma.

The stability, including within and between tube homogeneity, of the materials at room temperature as well as at elevated (37°) temperature was established. Homogeneity within and between tubes (n=3-8) varied with a coefficient of variation (CV) between 1-4 % for PFOS concentration in whole blood and plasma. Whole blood and plasma samples stored at 20°C were analyzed at day 0, 2, 9 and resulted in PFOS variation (CV) between 1-14%, corresponding variation for storage at 37° and analysis at day 0, 2, 9 was 13-16%.

### *PFCs*

The PFCs mentioned in this report are perfluorobutanesulfonate (PFBuS), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluorodecanesulfonate (PFDS), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotetradecanoate (PFTDA) and perfluorooctanesulfonamide (PFOSA).

### *Methods used by participants*

Clean-up methods ranged from ion-pair extraction, different types of solid-phase extractions to only protein

precipitation. Analysis and detection methods were for example LC-ESI-MS/MS (triple quadrupole), LC-ESI-MS (single quadrupole), LC-ESI-TOF-MS and GC-MS. Quantification was performed by using extracted or non-extracted standard curves with or without matrix or internal standard present.

**Results and discussion**

Of the total 19 laboratories that entered the human matrices part of the study 89% reported results. This must be considered a good report frequency. Up to the present the most published PFC data in the scientific literature concern plasma or serum levels. This is also reflected in the lower number (58%) of laboratories that analyzed the whole blood sample as well.

In Figure 1 the mean and standard deviation for PFOS determination in plasma based on each laboratory's reported value are given.

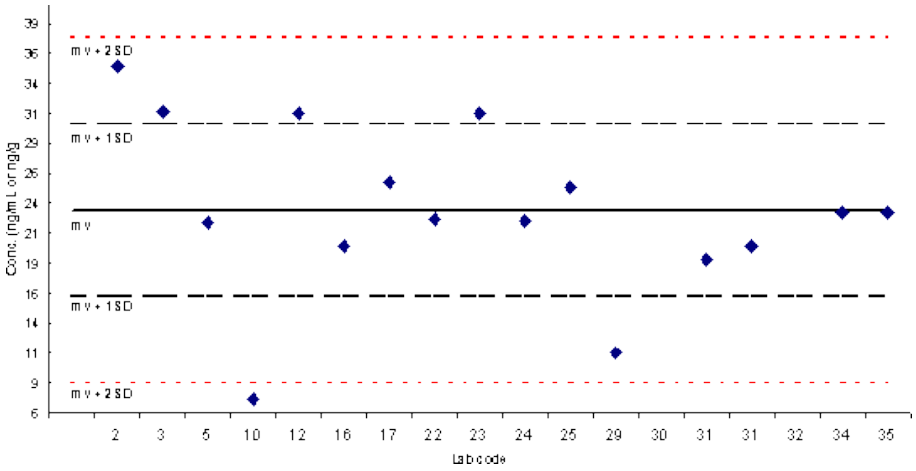


**Figure 1.** Mean (mv), standard deviation limits (SD) and individual results for PFOS concentration in plasma reported by 18 laboratories.

No processing of data or removal of outliers have been performed on the data presented, and consequently no consensus value is given here. Tables 1 and 2 show the results for a number of PFCs in the plasma and whole blood sample reported from the different laboratories. Mean and variability (CV) are given if sufficient number of participants (>50%) reported above the detection limit. Since not all participants reported the exact limit of detection all non-detect values were excluded from the calculations. The number of PFCs reported (found values or detection limits) varied between laboratories.

**Table 1**. Plasma results in ng/mL or ng/g (given in italic) presented as they were reported from each laboratory.

| Code | PFOS | PFOA | PFNA | PFHxS | PFOSA | PFBS | PFDS | PFHpA | PFDA | PFUnDA |
|---|---|---|---|---|---|---|---|---|---|---|
| Lab 2 | 34.9 | 2.11 | 0.909 | 0.616 | <0.029 | <0.342 | NA | <0.043 | 0.281 | 0.309 |
| Lab 3 | 31.1 | 1.19 | 0.77 | NA | NA | NA | NA | ND | ND | ND |
| Lab 5 | 21.9 | 1.5 | <0.4 | NA | <0.7 | NA | NA | NA | <0.4 | <0.1 |
| Lab 10 | 7.1 | 0.5 | NA | <0.05 | <0.05 | NA | NA | NA | NA | NA |
| Lab 12 | 31 | 2.04 | 0.52 | NA | NA | 0.29 | NA | NA | NA | NA |
| Lab 16 | 19.92 | 1.5 | 0.38 | 0.78 | <0.01 | 0.02 | NA | <0.05 | <0.05 | 0.14 |
| Lab 17 | 25.3 | 2.0 | 0.62 | NA | ND | NA | NA | NA | ND | NA |
| *Lab 22* | *22.2* | *3.63* | *1.14* | *1.38* | *<0.16* | *<0.01* | *0.22* | *0.09* | *0.31* | *0.22* |
| *Lab 23* | *31* | *2.4* | *0.72* | *1.9* | *0.62* | *<2.4* | *1.7* | *0.68* | *0.59* | *0.44* |
| Lab 24 | 22 | 1.4 | 0.5 | 1.6 | 0.6 | <2 | <0.5 | NA | <0.2 | <0.1 |
| Lab 25 | 24.8 | 2.03 | 0.29 | 1.7 | ND | ND | NA | NA | 0.63 | 0.14 |
| Lab 29 | 11 | 2 | NA | 4 | NA | <0.2 | NA | 0.55 | <0.2 | <0.1 |
| Lab 30 | NA | 5.2 | NQ | NA | NA | NA | NA | NA | NQ | NQ |
| Lab 31[a] | 18.8 | 1.9 | NQ | 1.1 | <0.2 | 0.5 | NA | <0.3 | <0.3 | <0.3 |
| Lab 31[a] | 19.9 | 1.8 | 0.4 | 0.8 | 0.1 | 0.5 | NA | <0.4 | 0.2 | 0.2 |
| Lab 32 | NA | 2.8 | NA | NA | NA | NA | NA | NA | NA | NA |
| Lab 34 | 22.7 | 2.2 | NA | 1.3 | NA | <1.0 | NA | NA | NA | NA |
| Lab 35 | 22.7 | 1.8 | 1.25 | NA | NA | NA | NA | NA | NA | NA |
| MEAN[b] | 22.9 | 2.11 | 0.68 | 1.52 | | | | | | |
| SD | 7.2 | 1.02 | 0.31 | 0.97 | | | | | | |
| CV | 32 % | 48 % | 46 % | 64 % | | | | | | |

NA=not analysed, ND=not detected, NQ=not quantified.

[a] Lab 31 reported two results using two different methods.

[b] ng/g results have been used for calculation of the mean without recalculation on a ng/mL basis.

Almost all laboratories reported values for PFOS and PFOA. The CV for PFOS was 30% for plasma and 59% for whole blood. Corresponding results for PFOA were 50 and 41%. A lower number of participants reported levels for PFNA and PFHxS and the variations were larger compared to PFOS and PFOA. Included in Tables 1 and 2 are low concentrations of other PFCs reported within this study.

Considering that this is the first interlaboratory study on a larger worldwide scale and the number of different methods used, the variation between participating laboratories is satisfactory. Concentrations of approximately 10-20 ng/ml for PFOS and 2 ng/ml for PFOA in whole blood and plasma can be determined reasonably well by most laboratories, and the coefficient of variation will further improve after removing obvious outliers and possible erroneous numbers reported. In the next round of PFC interlaboratory studies it would be preferable to use human samples with higher concentrations of other PFCs that have frequently been reported in human blood, for instance PFOSA.

**Table 2.** Whole blood results in ng/mL or ng/g (given in italic) presented as they were reported from each laboratory.

| Code | PFOS | PFOA | PFNA | PFHxS | PFOSA | PFBS | PFDS | PFHpA | PFDA | PFUnDA |
|---|---|---|---|---|---|---|---|---|---|---|
| Lab 2 | 9.09 | 2.15 | 0.49 | 1.63 | 0.305 | <0.006 | NA | 0.116 | 0.239 | 0.275 |
| Lab 5 | 9.3 | 1.7 | <0.4 | NA | <0.7 | NA | NA | NA | <0.4 | <0.1 |
| Lab 12 | 10.6 | 2.13 | 0.19 | NA | NA | <2 | NA | NA | NA | NA |
| Lab 16 | 7.3 | 1.62 | 0.29 | 0.66 | 0.24 | <0.02 | NA | <0.1 | 0.11 | <0.2 |
| Lab 17 | 10.0 | 1.8 | 0.53 | NA | ND | NA | NA | NA | ND | NA |
| *Lab 22* | *11.8* | *4.06* | *0.87* | *1.17* | *0.45* | *<0.01* | *0.02* | *0.22* | *0.47* | *0.26* |
| *Lab 23* | *24* | *1.4* | *0.5* | *1.4* | *1.6* | *<2.4* | *<0.5* | *<0.6* | *0.5* | *<0.3* |
| Lab 24 | 10 | 2 | 0.4 | 1.5 | 0.5 | <2 | <0.5 | NA | <0.1 | <0.1 |
| Lab 29 | 1.8 | 1.4 | NA | 0.99 | NA | <0.2 | NA | 0.54 | <0.2 | <0.1 |
| Lab 30 | NA | 3.6 | NQ | NA | NA | NQ | NA | NA | NQ | ND |
| Lab 32 | NA | 2.62 | NA | NA | NA | NA | NA | NA | NA | NA |
| MEAN[a] | 10.4 | 2.23 | 0.47 | 1.14 | | | | | | |
| SD | 5.85 | 0.88 | 0.22 | 0.34 | | | | | | |
| CV | 56 % | 39 % | 46 % | 29 % | | | | | | |

NA=not analysed, ND=not detected, NQ=not quantified.

[a] ng/g results have been used for calculation of the mean without recalculation on a ng/mL basis.

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**References**

1. Martin, J. W.; Kannan, K.; Berger, U.; de Voogt, P.; Field, J.; Franklin, J.; Giesy, J. P.; Harner, T.; Muir, D. C.; Scott, B.; Kaiser, M.; Järnberg, U.; Jones, K. C.; Mabury, S. A.; Schroeder, H.; Simcik, M.; Sottani, C.; van Bavel, B.; Kärrman, A.; Lindström, G.; van Leeuwen, S. (2004) *Environmental Science and Technology*. 248A-255A.