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1st WORLDWIDE INTERLABORATORY STUDY ON PERFLUORINATED COMPOUNDS IN HUMAN AND ENVIRONMENTAL MATRICES
Final report

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Abstract

This first worldwide interlaboratory study on the determination of perfluorinated compounds (PFCs) in environmental and human matrices was conducted in 2005. The main objective was to assess the between-laboratory reproducibility for various PFCs in a number of matrices: fish muscle tissue, freshwater, human plasma and whole blood. In addition, a standard solution and a clean fish extract were included in the study to evaluate instrumental and quantitation performance. Thirty-eight laboratories from 13 countries participated in the analysis of one or several matrices.

There are various challenges in the development of accurate analytical methods for the detection of PFCs. Factors like sample extraction, instrumentation, quantitation and level of experience were taken into consideration when interpreting the results of this study. The participating laboratories agreement with each other and their ability to estimate a true value was evaluated. The data of this study was evaluated by determination of z-scores according to the Cofino model.

In general, the level of agreement between the participating laboratories decreased with increasing complexity of the matrix. Relatively good agreement between the laboratories was obtained for the study standard, the fish extract, the whole blood and plasma sample. On the other hand little agreement was obtained for the fish tissue and the water sample. Extraction and clean-up showed large effects on the results for the more difficult matrices.

Although much information was gained from this first interlaboratory study, a second interlaboratory study is recommended to further improve the PFC data quality. That study will possibly be organised in early 2006.

1. Introduction and aim

Perfluorinated organic compounds (PFCs) are a new class of environmental contaminants which are ubiquitous both in environmental and human samples (Van de Vijver et al., 2003; Kannan et al., 2004; Tomy et al., 2004).
Large knowledge gaps exist in the many aspects of these contaminants such as toxicity, human intake, exposure and so on. To answer these research questions, analytical techniques for the accurate detection of these compounds must be available.
The accurate and reproducible analysis of PFCs in human and environmental tissue is a challenge in many ways (Martin et al., 2004). Extraction and quantification methods, matrices analysed and use of (internal) standards vary between laboratories. Many factors, such as contamination from laboratory materials, incomplete recoveries and leakage from instrumental parts are common problems, and lead to poor accuracy, sensitivity and reproducibility. Due to their physical and chemical properties PFCs may stick to surfaces, and may be lost at any stage of the sample handling. Today LC-MS electrospray is the most common detection technique used in PFC analysis. A known difficulty with this technique is ion-suppression, both from the matrix and from poorly separated PFCs. Branched isomers challenge separation further, and reference standards are needed to assure the specificity of the data.
The accuracy of the methods has only been tested in-house by spike experiments because up to date, no certified reference materials are available and no interlaboratory studies have been organized. The 1st world-wide interlaboratory study reported here was initiated to assess the quality of the analytical techniques applied by laboratories world wide.
The Netherlands Institute for Fisheries Research (RIVO) and Man-Technology-Environment (MTM) Research Centre, Örebro University organized this 1st world-wide interlaboratory study in collaboration with the EU Perforce research project. The aim of this study is to acquire information on the level of agreement of the analytical methods employed for PFCs. This will lead to learn more about the accuracy of laboratories up till now in the detection of PFCs found in environmental and human samples, as well as to try to identify possible limitations of the methods. This study should allow further progress in the field.

The objectives of this interlaboratory study were:

- To determine the level of agreement between different laboratories, for each determinant within a given matrix (laboratory vs other laboratories)

- To determine the level of agreement for different PFCs in the same matrix (determinant vs other determinants).

- To determine the level of agreement for a determinant in different matrices. Hence to identify which matrices presented more difficulties (matrix vs matrix).

Thirty-eight laboratories subscribed to this interlaboratory study out of which 31 indicated that they had the intention to participate in the EM part. Out of these 27 laboratories submitted results for one or more environmental matrices together with results for the standard with undisclosed concentrations. A total of 19 laboratories entered the HM part of which 17 submitted results for the plasma sample. Only seven laboratories analysed and reported results for the whole blood sample. The participating laboratories can be found in Appendix 1. Submission of results from some laboratories was delayed and even withdrawn due to some reported technical problems or tight schedule. Registrations for the participation in environmental samples were from laboratories in Austria Belgium, Canada, Denmark, Germany, Italy, Japan, Norway, Sweden, Switzerland, The Netherlands, U.K. and USA.

2. Materials and methods

2.1 Study design

The analysis of PFCs in environmental (EM) and human matrices (HM) involves an extraction (and clean-up) step, followed by the final determination. These steps all contribute to the total variance associated with the final result. The interlaboratory study was developed in a way for the participants to determine possible sources of error in each step of analysis (extraction, calibration, final determination). This approach has shown to be valuable in earlier interlaboratory studies in e.g. brominated flame retardants and polychlorinated biphenyls (de Boer and Cofino, 2002) leading to improved performance of laboratories in subsequent interlaboratory studies. This approach has also been advocated by the former EU Bureau for Calibration and Reference materials (BCR) (Quevauviller and Maier, 1999).

The following samples were supplied to the participants:

- 1 ampoule with study standard (SS) (4 ml)
- 1 ampoule with a fish liver extract (FLE) (3 ml)
- 1 container with fish tissue (FT) (60 g)
- 1 plastic bottle with water (1 L)
- 1 container with human plasma (HP) (5-8 ml)
- 1 container with human whole blood (HB) (~3 ml)

The study standard with undisclosed concentrations was provided to participants for them to check the calibration of their instrument and the quality of their standards. A cleaned extract of a fish liver was provided in order to evaluate chromatographic separation and detection only (without interference from extraction and clean-up procedures). Finally, a spiked fish muscle tissue and naturally contaminated human whole blood and plasma were provided for evaluation of the complete procedure (including extraction and clean-up procedures).
Laboratories were asked to analyse the samples using their in-house analytical methods.

The following PFCs were targeted in this study:

Full name of determinand	Abbreviation
Perfluorobutanoic acid	PFBA
Perfluorohexanoic acid	PFHxA
Perfluoroheptanoic acid	PFHpA
Perfluorooctanoic acid	PFOA
Perfluorononanoic acid	PFNA
Perfluorodecanoic acid	PFDA
Perfluoroundecanoic acid	PFUnA
Perfluorododecanoic acid	PFDoA
Perfluorobutane sulfonate	PFBS
Perfluorohexane sulfonate	PFHxS
Perfluorooctane sulfonate	PFOS
Perfluorodecane sulfonate	PFDS
Perfluorooctanesulfonamide	PFOSA

Details on the methods used by the participants are discussed in section 2.3. Submission of results was performed by means of electronic mail, pooled together and all converted to equivalent units for sample type. Statistical evaluation was performed using the Cofino Model to obtain the assigned value and z-scores to estimate the agreement between the various participants (see section 2.4).

Samples were prepared in house (see section 2.2) and sent to the participants (at ambient temperature) by courier TNT. Sample delivery time was between 2 to ca. 7 days. The participants were given ca. 3 months to analyze the samples and to submit their results and method details to the coordinator for statistical analysis.

2.2 Materials provided

RIVO was responsible for the production of the test materials of the EM part (water, study standard, FLE and fish muscle tissue), whereas MTM Research Centre was responsible for the HM part (human whole blood and human plasma).

2.2.1 Study standard

The SS was prepared as a solution of methanol to which the target compounds at undisclosed concentrations (in the range of 5-100 ng/ml) had been spiked. After homogenization, 4 ml of the study standard was ampouled in amber glass bottles. The actual concentrations of the targeted PFCs in the study standard are given in Table 2.

2.2.2 Fish samples

The FLE was prepared from livers from flounder (*Platichthys flesus*) originating from the Western Scheldt in The Netherlands. After mincing and homogenization of the liver tissue, the resulting homogenate was extracted batchwise according to a slightly modified method of (Giesy and Kannan, 2001). Subsequently, the lipids were removed from the extract by silica adsorption chromatography. The silica column (1.8 g) was loaded with the sample. The lipids (including PFOSA) were eluted by dichloromethane, whereas the target compounds (PFCAs and PFSAs) retained on the column. The target compounds were eluted by acetone. The acetone was subsequently replaced by methanol and an aliquot of the cleaned extract was analysed to determine the concentrations of the target compounds. The extract was subsequently spiked with the target compounds at concentrations mentioned in Table 2. After homogenization, ca. 3 ml of extract (equivalent to 1.5 gram fish liver) was ampouled.
Stability analysis was carried out on the FLE. The extract was stored at room temperature (ca 20°C) and was analysed at day 1, 18 and 60. The data in Table 1 does not reveal a significant increase or decrease of PFOS and PFOA over time.

Table 1. Stability data (room temperature) of PFOS and PFOA in the FLE.

	Day	PFOA	PFOS
RSD replicates (%, n=5)	1,18,60	11.8	5.4
Average (ng/ml, n=2)	1	12.3	23.4
Average (ng/ml, n=2)	18	11.9	24.9
Single determination	60	12.7	23.0

The FT sample was prepared from fillets of pike perch (*Stizostedion lucioperca*) caught in lake IJssel, The Netherlands. The muscle material was minced and thoroughly homogenized (after addition of butylhydroxytoluene as an antioxidant). Ca. 55 gram of homogenate was packed in a glass jar which is tightly closed to prevent leakage. The material was sterilized at 121°C and 3 bar for 30 minutes.
Because of the very low levels of some of the target compounds in the fish material, some compounds were spiked prior to the homogenization step (see Table 2).
The homogeneity of the material was tested by analysis of 10 lots from the complete batch, in duplicate. The parameters determined were PFOS and PFOA concentrations and moisture content. The relative standard deviation of these determinations was 7.9%, 3.5% and 0.24% respectively. ANOVA statistics revealed no significant difference between the lots and within a lot (i.e. duplicate analysis). The Snedecor F-test did not show a statistically significant variance difference between the duplicates (within-lot homogeneity) and between the different lots (tested at 95 and 99% level). This shows that these PFCs are homogeneously distributed over the pike perch sample material. The relative standard deviation is low compared to the overall standard deviation for PFOS and PFOA found in this interlaboratory study. This shows that this material is very suitable for the use in this interlaboratory exercise.

Table 2. Concentrations of target compounds in the SS, spike concentrations in the FLE and the FT, including details on origin and purity of the compounds.

Determinant	Supplier	Batch nr.	Purity	SS (ng/ml)	FLE* (ng/ml)	FT* (ng/g)
PFBA	ABCR	131726?	99%	4.3	18	50
PFHxA	ABCR	1103-2C18-BS	98%	18	2.7	N.a.
PFHpA	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.
PFOA	Sigma-Aldrich	171468-25G	96%	8.4	11	9.7
PFNA	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.
PFDA	ABCR	1327-1B16	98%	14	14	N.a.
PFUnA	Sigma- Aldrich	00929KB	95%	17	2.1	N.a.
PFDoA	Acros	06623DB	95%	3.8	17	40
PFBS**	Sigma-Aldrich	Not specified	Not specified	47	6.9	21
PFHxS**	Fluka	Unknown	>98%	24	19	22
PFOS**	Fluka	403884/1	>98%	33	45	4.4
PFDS	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.
PFOSA	ABCR	87-10-??	97%	61	6.1	49

* In addition to the traces of PFCs that were already present from natural contamination of the matrix
** Potassium salt

2.2.3 Water sample

Hundred litres of naturally contaminated brackish water has been sampled from the North Sea canal, just outside the locks of IJmuiden, The Netherlands. The water was filtrated over 0.45 µm filter to remove particulate matter and stored in a 100 liter tank. Microbial activity was reduced by lowering the pH to ca. 2 by addition of 0.5% (v/v) formic acid. The water was thoroughly homogenised and was continuously homogenized during dispersion into 1 litre brown HDPE 1 litre bottles. No homogeneity evaluation has been performed as the above mentioned procedure was considered to result in homogeneous samples (both within the bottle as well as between the bottles). Between bottling and dispatch, the samples have been stored at 4°C. The participants were advised, prior to subsampling, to re-homogenise the contents of the bottle by gentle manual shaking.

2.2.4 Human samples

Authentic plasma and whole blood samples, without addition of perfluorinated compounds, representing the current levels in the Swedish general population were used. The samples were provided and administrated by the University Hospital of Örebro (USÖ), Sweden, and released for medical use according to the regulations by the Swedish National Board for Health and Welfare. This includes negative test results for HIV1/2, HBs-Ag, HCV-Ak and Syphilis (VDRL).

The plasma sample was taken by venipuncture. Citrate was added before centrifugation and removal of the cellular elements. The material were homogenized and divided into polypropylene tubes in approximately 7 ml portions and thereafter kept at -20°C before shipment to the participants. The whole blood sample was taken by venipuncture and collected in a heparin treated container. The material were homogenized and divided into polypropylene tubes in approximately 3 ml portions and thereafter kept at -20°C before shipment to the participants.

Short-term stability of the materials at room temperature as well as at elevated (37°) temperature was established. Samples were stored at a laboratory bench at approximately 20°C, and in a temperature regulated cabinet, at 37°C. Whole blood and plasma samples stored at 20°C were analyzed at day 0, 2, 9 and resulted in PFOS variation coefficients (CV) between 1-14%. Corresponding variation for storage at 37° and analysis at day 0, 2, 9 was 13-16%. Also included in Table 3 is the reproducibility of the method used. Concentrations of PFOS, PFOA, PFNA and PFHxS were found stable at room temperature when compared

to method reproducibility and cold storage (-20°C). The CVs are in most cases lower than then the CVs of the method reproducibility, showing that no additional variation is generated as a result of the storage conditions. Unfrozen short-term storage was therefore found acceptable (i.e. shipment). When stored at elevated temperature (37°C) the materials, especially whole blood, became more viscous and an increased variation in concentrations could be seen for all four PFCs. Since volumetric sampling was applied (as part of the analytical method used) it cannot be ruled out that the variation can be due to uncertainty in the sample volume taken. It is therefore not recommended storing the samples at temperatures above room temperature (ca. 20°C), which results in a more viscous sample. Concentrations after three freeze and thaw cycles deviated from replicate determinations on a single occasion with –10% for PFHxS, 5% for PFOA, -3% for PFOS and –4% for PFNA.
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 (Table 3).

Table 3. Coefficient of variation (CV) for 4 PFCs in plasma and whole blood analyzed a) multiple times from the same tube, b) from different tubes, c) stored at 20°C between three analysis, d) stored at 37°C between three analysis, and e) stored at –20°C.

	PFOS	PFOA	PFHxS	PFNA
Method reproducibility[a]	10%	5.9%	6.3%	12%
Interlaboratory whole blood				
a) within tube (n=3)	1.4 %	2.1 %	3.4 %	19 %
b) between tubes (n=8)	4.3 %	3.3 %	4.3 %	6.4 %
c) storage 20°C 0,2,9 days	1.3 %	4.5 %	5.2 %	6.1 %
d) storage 37°C 0,2,9 days	13 %	7.6 %	14 %	42 %
Whole blood, other batch				
e) storage –20°C, 4 months[b]	12%	11%	12%	2%
Interlaboratory plasma				
a) within tube (n=3)	3.0 %	6.4 %	3.1 %	31 %
b) between tubes (n=5)	2.9 %	7.2 %	4.0 %	11 %
c) storage 20°C 0,2,9 days	14 %	7.1 %	1.6 %	3.2 %
Plasma, other batch				
b) between tubes (n=8)	2.9 %	10.6 %	15 %	16 %
c) storage 20°C 0,2,9 days	8.7 %	6.6 %	56 %	-
d) storage 37°C 0,2,9 days	16 %	25 %	45 %	31 %

[a] whole blood (n=13) [b] three analysis during a four months period

2.3 Methods used by participants

2.3.1 Environmental matrices

Table 4 shows method information of the analytical techniques applied for the analysis of the FT as supplied by the participants. Most laboratories applied the ion-pair extraction by Hansen et al. (Hansen et al., 2001), in some cases slightly adjusted for e.g. higher sample intakes. Only 4 laboratories have applied a different method. Lab 13, 33 and 38 have applied a simple methanol extraction, without application of an ion pairing agent. Laboratory 30 has applied acetonitrile extraction, followed by a clean-up step with Envi-Carb. Laboratory 36 applied a clean-up over silica gel (in addition to the ion-pair extraction method) for removal of residual lipids from the extract. No laboratory applied instrumental extraction techniques like accelerated solvent extraction (ASE).
Concerning the separation and detection of the ions by MS, most laboratories applied triple quadrupole MS/MS. One laboratory (22) applied high resolution time of flight MS (TOF-MS), using exact mass determination. Three laboratories (15, 27 and 36) applied ion-trap MS, which can be used in MS/MS mode for the perfluorinated carboxylic acids (PFCAs) and PFOSA. For the perfluorinated sulfonates (PFSAs), the technique can only be applied in single MS mode, although collision energy can be applied to eliminate co-eluting matrix components to a certain extent. Finally, 4 laboratories applied single quadrupole MS.

Nearly all laboratories quantified the levels of the target compounds by application of external (solvent based) calibration curves. Two laboratories applied matrix matched calibration curves. Laboratory 23 applied standard addition quantification by spiking subsamples of the fish tissue at 3 levels prior to extraction of the tissue.
Ten laboratories applied ^{13}C-labeled PFOA or PFDA as procedural internal standard (added prior to extraction). This standard is in most cases used for correction of all compounds that were analysed. Some laboratories have used non labelled perfluorinated compounds such as 7H-PFHpA, PFNA, PFDoA and TH-PFOS. Among others, lab 38 applied 3 different internal standards in order to resemble the native compounds in terms of molecular structure and functional groups: d5-N-ethyl-PFOSA was applied for PFOSA, ^{13}C-PFDA used as internal standard for the PFCAs and TH-PFOS used as internal standard for PFOS. A small group of laboratories have not applied an internal standard at all. One laboratory (8) applied deuterated mecoprop as internal standard, which has a very different molecular structure compared to the target compounds.
Some laboratories have reported blank values for some compounds (e.g. PFOS, PFOA), although only two labs (8 and 23) have corrected their results for the reported blank values.
Nearly all laboratories have reported the sum of the branched and linear isomers.

2.3.2 Human matrices

A summary of the methods used by the participants is given in Table 5. Basically three extraction methods were used SPE, acetonitrile precipitation and ion-pair extraction. Regarding separation and detection technique most laboratories used LC coupled to triple quadrupole MS/MS. Two laboratories used high resolution TOF and single quadrupole MS. One laboratory analysed PFOA and PFNA by GC-NCI-MS. Quantification was performed by either using external standards in solvent or standards spiked to the matrix or a surrogate matrix. Nearly all laboratories used an internal standard. $^{13}C_2$-PFOA was the internal standard used by most laboratories. Other standards used were $^{13}C_2$-PFDA, native 7HPFHpA, PFHpA, PFDoDA, THPFOS, perfluoro-3,7-dimethyloctanoic acid and $^{18}O_2$-labelled PFOS. Five laboratories reported that they experienced blank levels of some target compounds and corrected the results for this. Almost all laboratories quantified resolved isomers together with the peak of the linear compound.

Table 4. Method information for the analysis of the FT.

Lab no	Experience level (years)	Corrted for blank	Average of n replicates	Internal standard (covering the complete procedure)	Syringe standard	Extraction	Mass separation mode	Calibration mode
1	1-3	No	-	N.a.*	na	N.a.	LC-ESI-MS/MS	Unextracted
2	>3	?	-	N.a.	na	N.a.	LC-ESI-MS/MS	MMC
5	?		-	^{13}C-PFOA	-		LC-ESI-MS/MS	Unextracted
6	Recently	No	-	7H-PFHpA	^{13}C-PFOA, ^{13}C-PFDA	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
7	1-3	No	-	^{13}C-PFDA	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
8	1-3	Only PFOS	-	Mecoprop (deuterated)	-	Secret	LC-ESI-MS/MS	Unextracted
9	>3	No	-	-	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
10	>3	No	5	TH-PFOS	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted?
11	Recently	No	-	-	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
13	1-3	No	4 (PFOS), 6 (PFOA)	-	-	MeOH extraction, divinylbenzene SPE clean-up	LC-ESI-MS	Unextracted
15	Recently	No	-	N.a.	na	N.a.	LC-ESI-ITMS(/MS)	Unextracted
16	>3	No	2	^{13}C-PFOA	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
19	1-3	No	3	^{13}C-PFOA	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
20	1-3	No	-	-	-	Ion-pair, MTBE	LC-ESI-MS	Unextracted
21	1-3	No	-	^{13}C-PFOA	-	Ion-pair, MTBE	LC-ESI-MS	Unextracted
22	>3	No	-	7H-PFHpA	$^{13}C_2$-DH-PFOA	Ion-pair, MTBE	LC-ESI-TOF-MS	Unextracted
23	>3	Partly	-	-?	7H-PFHpA	Ion-pair, MTBE	LC-ESI-MS/MS	MMC
25	1-3		-	-?	-?	N.a.	LC-ESI-MS/MS	Unextracted
26	1-3	No	-	?	-?	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
27	1-3	No	-	^{13}C-PFDA	-	Ion-pair, MTBE	LC-ESI-ITMS(/MS)	Unextracted
28	Recently		-	N.a.	na	N.a.	LC-ESI-MS/MS	Unextracted
29	Recently	No	-	PFDoA	PFNA	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted
30	1-3	No	-	^{13}C-PFOA	-	AcN extraction, Envi-Carb clean-up	LC-ESI-MS/MS	Unextracted
33	Recently	No	-	PFNA, ^{13}C-PFOA	-	MeOH extraction	LC-ESI-MS/MS	Unextracted
35	>3	No	3	N.a.	na	N.a.	LC-ESI-MS	Unextracted
36	1-3	No	-	^{13}C-PFOA for PFCAs and 7H-PFHpA for PFSAs	-	Ion-pair, MTBE, Silicagel clean-up	LC-ESI-ITMS(/MS)	Unextracted
38	>3		?	TH-PFOS, ^{13}C-PFDA and d5-N-ethyl-PFOSA	d3-N-ethyl-PFOSA for PFOSA	MeOH extraction	LC-ESI-MS/MS	Unextracted

* N.a.: participant has not analysed fish tissue sample, information shown here concerns the fish liver extract method. MMC = matrix match calibration

Table 5. Method information for the analysis of plasma and whole blood.

Lab no	Experience level (years)	Corrected for blank levels	Average of n replicates	Internal standard (covering the complete procedure)	Syringe standard	Extraction	Mass/ separation mode	Calibration mode
2	>3	-	-	^{13}C-PFOA, ^{13}C-PFDA	^{13}C-PF(hexyl-ethanoic)A	SPE (copolymer)	LC-ESI-MS/MS	MMC
3	1-3	PFOA,PFNA, PFOS	-	PF(3,7-dimethyl-octanoic)A	-	Ion-pair, MTBE	LC-ESI-MS/MS	Rabbit matrix
5	?	?	?	?	?	?	?	?
10	>3	-	2	THPFOS	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted,
12	>3	All compounds	-	$^{13}C_2$-PFOA	-	PFOA&PFNA=Ion-pair, derivatization PFOS&PFBS=AcN precipitation	PFOA&PFNA = GC-NCI-MS PFOS&PFBS = LC-ESI-MS/MS	Unextracted,
16	>3	-	2	^{13}C-PFOA	-	Ion-pair, MTBE	LC-ESI-MS/MS	Unextracted,
17	?	?	6	?	?	?	LC-ESI-MS/MS	?
22	>3	-	-	7H-PFHpA	$^{13}C_2$-DH-PFOA	Ion-pair, MTBE	LC-ESI-TOF-MS	Unextracted,
23	>3	PFHpA,PFOA, PFDA, PFUnDA	2	-	7H-PFHpA	Ion-pair, MTBE	LC-ESI-MS/MS	MMC
24	1-3	-	-	PFHpA	7H-PFHpA	SPE (C18)	LC-ESI-MS	Unextracted,
25	1-3	All compounds	-	^{13}C-PFOA	11H-PFUnA	AcN precipitation	CapLC-ESI-MS/MS	Unextracted,
30	1-3	-		^{13}C-PFOA	-	AcN precipitation	LC-ESI-MS/MS	Unextracted,
31[a]	1-3	All compounds	-	^{13}C-PFOA $^{18}O_2$-PFOS	-	SPE (copolymer)	LC-ESI-MS/MS	Unextracted,
32	>3	-	2	^{13}C-PFOA	-	AcN precipitation	LC-ESI-MS/MS	Rabbit matrix
34	>3	-	-	$^{13}C_2$-PFOA $^{18}O_2$-PFOS	-	SPE (copolymer)	LC-ESI-MS/MS	MMC
35	>3	-	-	^{13}C-PFOA	-	AcN precipitation	LC-ESI-MS/MS	Rabbit matrix

[b] Lab 31 method denoted "off". MMC = matrix match calibration

2.4 Statistical analysis of the data

Interlaboratory study data can be evaluated by various tools. The data of this study is evaluated by determination of z-scores. Z-scores provide valuable information on the performance of the laboratories compared to others. A z-score gives an indication on the accuracy of a laboratory. Z-scores do not provide information on a laboratorys' precision (repeatability or reproducibility) as this study was not designed to answer that question.

For calculation of a z-score, a target (or assigned) value is required. In this study this value is statistically derived from the submitted data and is based on the majority of the submitted data. Classical statistics are not considered to provide a close estimation of the true value for a set of data with a large variance, as it is unable to counteract the contribution of outlying data. For example, for a pool of data with a number of outlying data, robust statistics has been widely employed to calculate the assigned (or true) value. However, this tool has the disadvantage of insufficient correction for outliers.

In the early 2000's, the Cofino Model Statistics has been developed which circumvents this problem (Wells et al., 2004). It is based on normal distribution assumptions and has the ability to identify clusters of values with a high degree of affinity. Less than values can also be taken into account when present in a dataset. This model does not eliminate or down-weight, but rather illustrates the distribution of the data. Employing the Cofino Model Statistics, clusters of data are being identified. The assigned value is calculated from the cluster that represents the greatest percentage of the data.

The calculation of the z-scores of each participant is carried out according to the following formula:

$$Z = \frac{(x_l - X)}{\delta}$$

Z = Z-score value
x_l = Laboratory mean (if applicable, otherwise single determination)
X = Assigned value
δ = 0.125

The variance value is set at 0.125. A z-score of 2 corresponds to a variance of 25%, which is considered a target variance for analytical methods for organic micropollutants in e.g. environmental samples. As discussed above, setting the δ at 0.125 prevents the z-scores to be effected by a possible high variety (due to considerable outlying values in the dataset). This method is routinely applied in interlaboratory studies such as those organized by QUASIMEME (www.quasimeme.org).

Z-scores have been calculated for PFOS, PFHxS, PFOA, PFHxA, PFDA, PFDoA and PFOSA, providing that >5 observations from laboratories were submitted. For the remaining compounds, the data as submitted is listed, including basic statistics in Appendix 2 to Appendix 6. Z-score and distribution plots can be also found there. The result of a laboratory is considered satisfactory when the z-score is ≤|2|, doubtful when the z-score is between |2| and ≤|3| and unsatisfactory when outside that range. The scale of the y-axis in the plots showed here is limited from z=-6 to z=+6. Some participants have reported the results in another unit than was requested in the report forms. In those cases, the results were recalculated in order to match with the requested units.

3. Results and discussion

The submitted results from the participants can be found in Appendix 2 to Appendix 6. For all sample matrices almost all laboratories reported values for PFOS and PFOA and a lower number of participants reported levels for the other PFCs.
The results and discussion will be focused on PFOA, PFOS and PFOSA. The remaining compounds will be only briefly discussed. Not all laboratories that submitted results for the determinands in the study standard submitted results for the other matrices. Thus the number of data for the determinands in the e.g. the plasma or fish extract was lower than that for the study standard and lowest for the whole blood and fish tissue.

3.1 PFOS

Figure 1 to Figure 6 show the z-score plots for PFOS in all matrices. The graphical representations clearly indicate that the level of agreement of most laboratories is fairly good when analysing PFOS in a simple matrix such as the study standard, but decreases with increasing complexity of the matrix. Table 6 shows the summary of performance of laboratories. It can be seen that the largest number of z-scores exceeding |2| were obtained for the fish tissue.
Some laboratories have been producing consistent results. Lab 29 obtained negative z-scores for all matrices of –1.4 in the SS, a slightly worse –1.8 in the FLE, –5.8 in the FT, -5.3 for the water sample, -4.0 for the HP and -6.5 for the HB. Lab 10 showed more variety: a z-score of 5.5 for the SS, 2.9 for the FLE, -3.9 for the FT, -6.1 for water, and -5.4 for HP.

Table 6. Summary of performance of laboratories for PFOS in various matrices.

Matrix*	SS	FLE	FT	Water	HB	HP
Spiked/assigned value	33.3/28.6 ng/ml	45**/19.3 ng/ml	4.4**/36.5 ng/g ww	N.a./19.5 ng/ml	N.a./10.4 ng/ml	N.a./22 ng/ml
Satisfactory |z|<2	22/29***	12/22	3/18	5/16	6/9	10/16
Questionable 2<|z|<3	1/29	4/22	0/18	2/16	1/9	-
Unsatisfactory |z|>3	6/29	6/22	15/18	9/16	2/9	6/16

* SS: study standard; FLE: fish liver extract; FT: fish tissue; HB: human blood and HP: human plasma
** Spike in addition to the natural contamination
*** Number of labs/total submitted datasets

Calibration and use of internal standards
Concerning calibration, most laboratories employed unextracted curves, meaning that no matrix was involved in their calibration standards. Obviously, this works well for the SS, where 22 out of 29 laboratories are capable of good calibration (Figure 1). The assigned value (28.6 ng/ml) is slightly lower than the theoretical (spiked) concentration (33.3 ng/ml) in the SS. This shows that the majority of the laboratories are close to the theoretical value. However, individual laboratories such as lab 9 and 6 show a considerable negative bias, whereas lab 26, 10, 3 and 5 show a positive bias. Apart from general analytical variance due to the instrument, and dilution or concentration errors, there can be other reasons for these z-scores including not using an internal standard. Also, a different isomer pattern in the lab's standard compared to the pattern in the SS may cause a deviating result. Laboratory 29, as discussed earlier, obtained consistently negative z-scores in all matrices of -1.4 to -6.5 which may be an indication for a systematic calibration error. Also, laboratories 9 and (to a lesser extent) 6 consequently show low z-scores for the SS, FLE and FT. Matrix matched calibration (MMC) instead of unextracted curves (or solvent based calibration: by standards dissolved in e.g. methanol) is regarded essential for an accurate quantification when matrix effects due to ion suppression or mass interference occur (Martin et al., 2004). This is shown by the FLE where the assigned value (19.3 ng/ml) is considerably lower than the spiked addition of PFOS (44.7 ng/ml). It should be noted that the actual level of PFOS is even higher as the FLE already contained PFOS prior to the additional spike of 44.7 ng/ml. This shows that the majority of the laboratories have not been capable of accurate quantification of PFOS (using unextracted curves) in the FLE due to matrix effects.

For all matrices, laboratory 23 applied MMC. The PFOS level reported by this lab in the FLE is 66 ng/ml, which may be closer to the true value than the level reported by the remaining laboratories. Concerning the HP and HB samples, lab 23 used MMC and reported high PFOS values for both matrices. The same extraction method was used by laboratory 2 who was also assigned a high z-score for the HP but a negative z-score for HB. Laboratories 3 and 35 used surrogate rabbit matrix for the plasma determination resulting in a high z-score for laboratory 3 and a z-score close to zero for laboratory 35. The inconsistent results using MMC and surrogate matrix calibration when quantifying HP and HB in this study together with a relatively good agreement between the laboratories might indicate that human blood is less sensitive to matrix effects in electrospray ionisation compared to the FLE (Karrman et al., 2005).
One-third of the participants (of the EM part) has used isotopically labelled PFCAs as internal standards. From the z-scores, it cannot be concluded that these laboratories have more accurate results than other laboratories. This may partially be caused by the fact that a PFCA type of labelled standard will behave differently during extraction (and clean-up) compared to PFOS. Therefore, the availability of a (^{13}C-) labelled PFOS internal standard is very desirable to minimize (by correction) the effect of matrix effects on the final result. It should be noted however, that electro spray suppression may even be enhanced by application of such an internal standard.
Some laboratories used native PFCs like TH-PFOS, PFDoA and PFNA as internal standard to correct for their target PFCs. However, these compounds can be found in real environmental samples and the application of these internal standards can lead to considerable analytical errors. TH-PFOS was detected at considerable amounts in the water sample (see Appendix 5), which has led to the negative bias of laboratory 10 which used TH-PFOS as internal standard. Laboratories are therefore advised to check their samples for the absence of these (or other native) PFCs prior to the application of these internal standards.

Extraction and clean-up
Concerning the FT, most laboratories have applied the ion-pairing method originally published by (Hansen et al., 2001). Although individual laboratories may have obtained good recoveries in their internal validation experiments, in the case of the FT, the agreement between the participating laboratories is rather poor with only 27% of the laboratories obtaining a satisfactory z-score less than 2 (absolute). This is merely an effect of their extraction (and the absence of a) clean-up procedure since the overall performance for only the instrument calibration is much better (75% having a satisfactory z-score). The methods used for human matrices seem acceptable in the perspective of around 60% satisfactory z-scores.

Level of experience
The participants were asked to provide information on the number of years experience (recently started, 1-3 years of >3 years of experience). Principal component analysis (PCA) of the data did not reveal a relation between the level of experience and reported PFC levels in FLE, FT or WS.

MS/MS versus MS
Most laboratories used MS/MS for analysis of PFOS in the EM samples, which enables the detection of the daughter-ion m/z 80 and 99. Laboratories using single quadrupole MS (lab 20, 21 and 35) and those using ion-trap MS (15, 27 and 36) don't have the ability of detecting a daughter ion and may suffer from mass interferences. The FLE data (Figure 2) does not show a specific bias of laboratories applying MS. These laboratories are not grouped together but are distributed over the dataset and have z-scores (except lab 21) of <|2|. This does not *exclude* the occurrence of a mass interference, but other sources may have a stronger effect on the total analytical error. Concerning the FT, a single MS bias may have occurred as lab 27, 36 and 21 have a positive bias compared to the assigned value (although lab 20 has a negative z-score). However, several MS/MS laboratories have considerable z-scores of >6, which may be due to the matrix effect discussed above. The only laboratory using MS for the human matrices obtained z-scores <|2| for both plasma and whole blood. Mass interferences are not expected for the study standard. The MS laboratories are distributed over the dataset as are the MS/MS laboratories. As a general recommendation, MS laboratories are advised to check for possible bias due to mass and/or matrix interference.

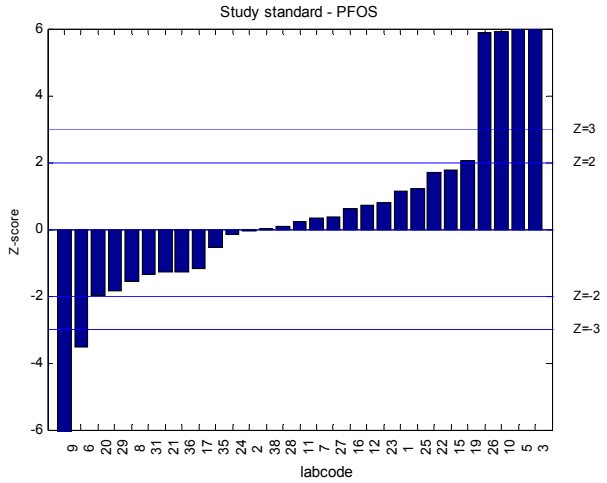

Figure 1. z-score plot of PFOS in the SS.

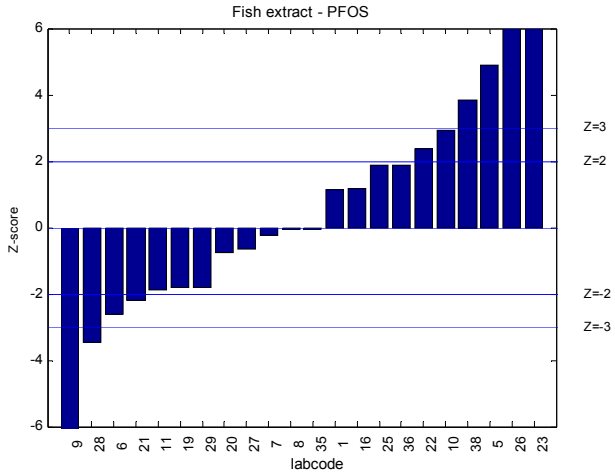

Figure 2. z-score plot of PFOS in the FLE.

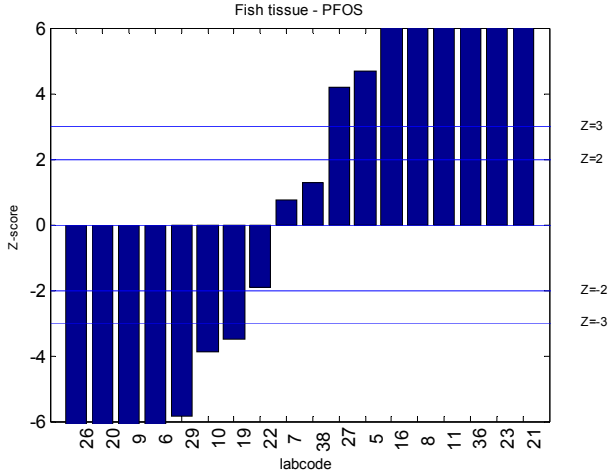

Figure 3. z-score plot of PFOS in the fish tissue.

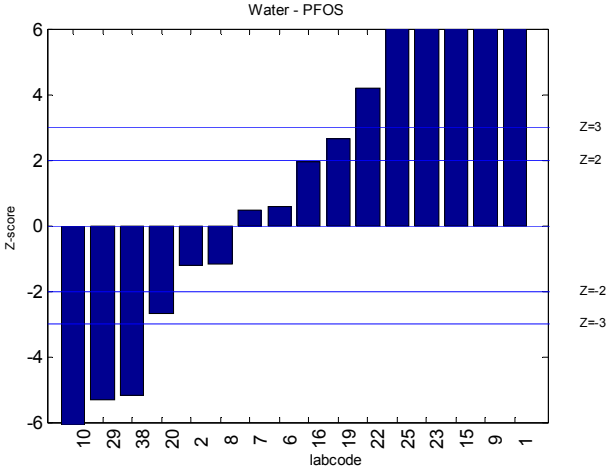

Figure 4. z-score plot of PFOS in water.

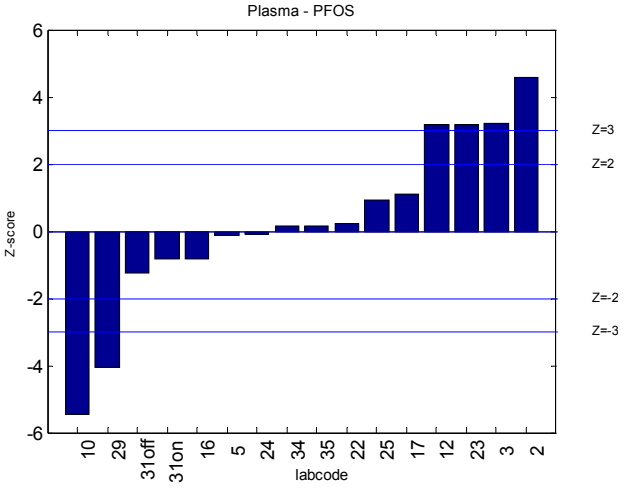

Figure 5. z-score plot of PFOS in HP.

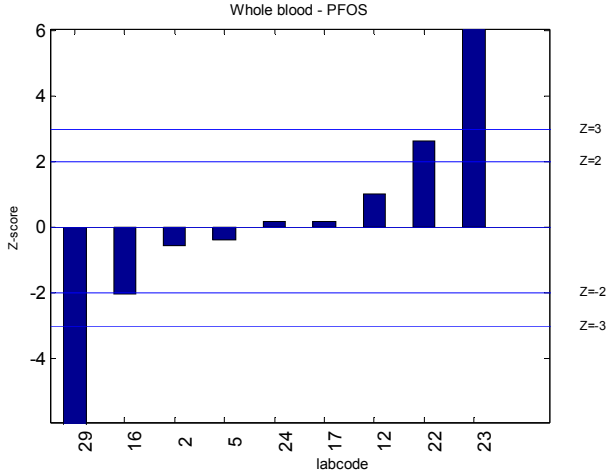

Figure 6. z-score plot of PFOS in HB.

3.2 PFOA

Similar to PFOS, the number of submitted datasets reduces by increasing complexity of the matrix. Also, the agreement between laboratories decreases as more laboratories have obtained unsatisfactory z-scores with the more complex matrices (Figures 7-12).
Table 7 shows the summary of performance of laboratories. The assigned value of PFOA in the SS is 7.8 ng/ml, which is very close to the theoretical value of 8.4 ng/ml, showing that the calibration of the majority of the laboratories is under control. However, 10 laboratories have unsatisfactory z-scores and should critically study their calibration. Concerning the FLE, and the FT, assigned values are also close to the spiked values, although it should be noted that the true value is not exactly known as little amounts of PFOA may have been present in FLE and FT prior to the spiking. The agreement between laboratories for plasma and human blood is relatively good with a percentage of satisfactory z-scores similar or better compared to the study standard.

Table 7. Summary of performance of laboratories for PFOA in various matrices.

Matrix	SS	FLE	FT	Water	HB	HP
Spiked/assigned value	77 / 8.4 ng/ml	11.3* / 12.3 ng/ml	9.7* / 10.2 ng/g ww	N.a. / 19.4 ng/ml	N.a./1.0 ng/ml	N.a./2.0 ng/ml
Satisfactory |z|<2	21/33**	10/25	5/20	4/18	8/11	11/18
Questionable 2<|z|<3	2/33	5/25	6/20	1/18	0/11	2/18
Unsatisfactory |z|>3	10/33	10/25	9/20	13/18	3/11	5/18

* Spike in addition to the natural contamination
** Number of labs/total submitted datasets

Like with PFOS, laboratories tend not to show a consistent performance. Lab 15 obtained a z-score of –0.59 in the SS but 9.0 in the FLE and 4.8 for water. Lab 9 obtained z-scores of 5.0 in the SS, –5.1 in the FLE and 40 in the water sample. Lab 30 has a large positive bias for whole blood, plasma and FLE but satisfactory/questionable results for SS and FT. Contrary to this, lab 38 has high z-scores of >6 for the SS, FLE and FT and 4.1 for water. In the same way obtained lab 32 high z-scores for SS, plasma and blood. This may suggest a systematical (calibration) error. In the long-term experience of QUASIMEME (www.quasimeme.org), a z-score of >6 is often caused by errors in units, concentration/dilution or calculation errors rather than intrinsic analytical methodology errors. Factors of 2, 5, 10 or 1000 are common for these type of extreme values (Wells et al., 2004).
Compared to PFOS, more laboratories may be capable of producing satisfactory results for the fish tissue. This may be associated with the fact that many laboratories apply a PFCA type of internal standard (^{13}C-labelled PFOA, -PFDA, PFDoA or 7H-PFHpA) which resembles more closely the target compound. Laboratories using no internal standard at all have shown similar performance as those using an internal standard. It is not known if these laboratories applied some way of correction for losses during analysis.

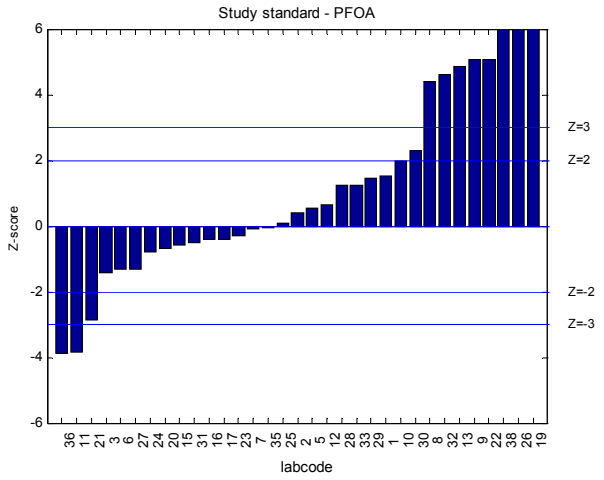

Figure 7. Z-score plot of PFOA in the SS.

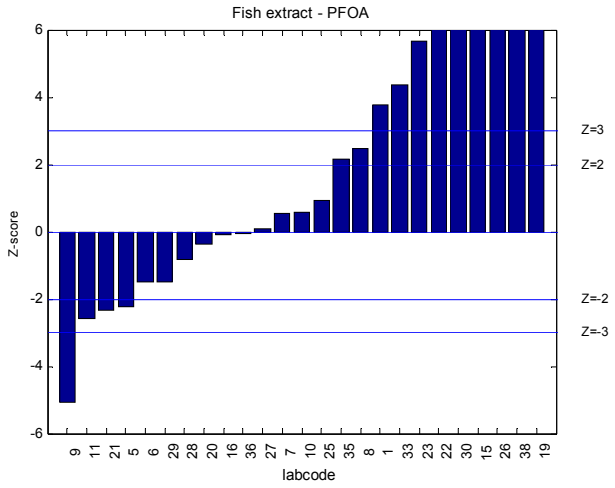

Figure 8. Z-score plot of PFOA in the FLE

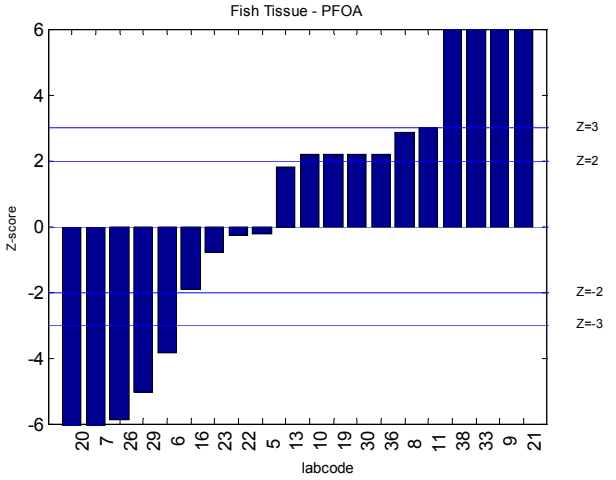

Figure 9. Z-score plot of PFOA in the FT.

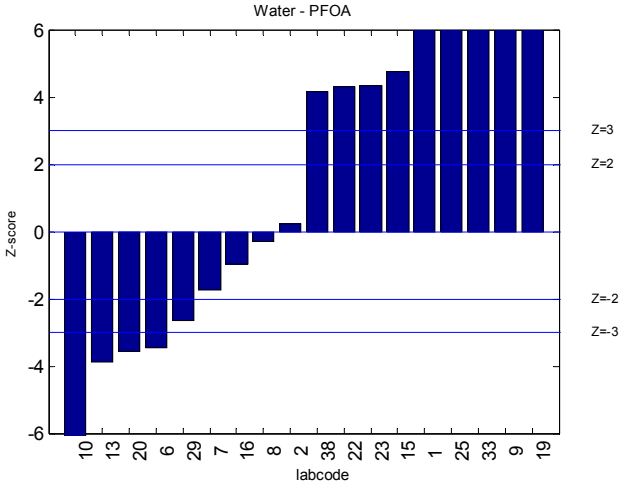

Figure 10. Z-score plot of PFOA in the water.

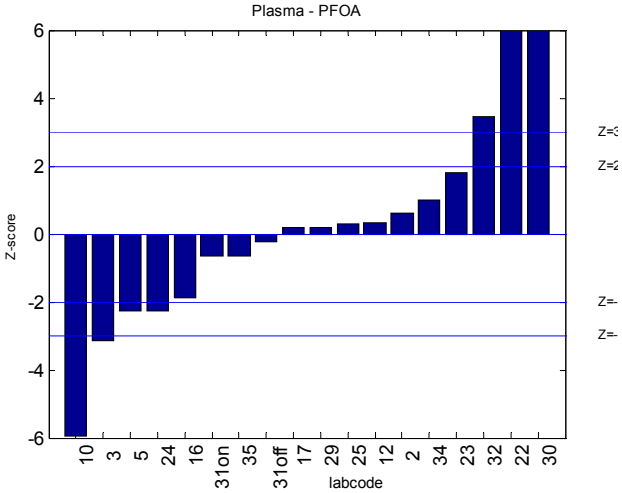

Figure 11. Z-score plot of PFOA in the HP

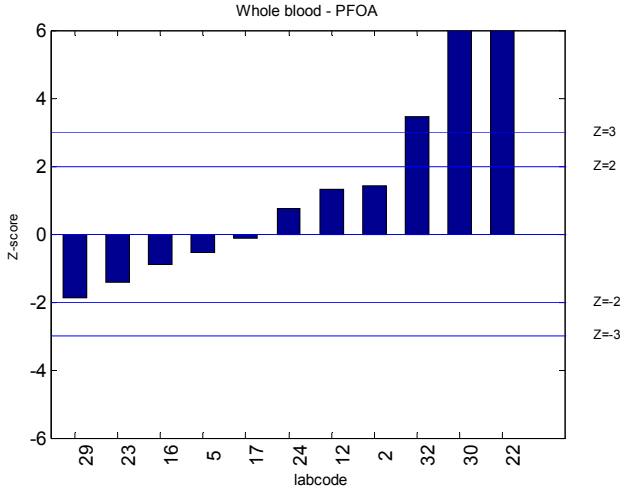

Figure 12. Z-score plot of PFOA in the HB

3.3 PFOSA

In the case of the SS and the FLE, the assigned value is close to the spiked value. Some laboratories have values reported close to this value, whereas laboratories with outlying values have reported values up to almost 10 times the assigned value (FLE, lab 9). For the FT, many laboratories are underreporting the PFOSA concentration. The spiked value is 49, whereas the assigned value is 20 ng/g ww. Lab 5 was closest to the assigned value (41.3 ng/g ww).

A summary of the performance of laboratories is given in Table 8 and the z-scores are shown in Figure 13 to Figure 17. Some laboratories (lab 19, 23 and 38) report consistently high z-scores of z>3 for all matrices. It is currently not known what the reason could be for the systematically high z-scores. The three laboratories are experienced; apply regular instrumentation, methods and internal standards (see Table 4). Lab 38 is the only lab applying a non-ionic internal standard (d5-N-ethyl-PFOSA), which may be an advantage (although not clear from the z-scores). Laboratory 23 has consistently high values for a wide range of compounds including PFOSA. This may be due to the fact that this laboratory used MMC. On the other hand, a systematic error leading to high values cannot be excluded.

Table 8. Summary of performance of laboratories for PFOSA in various matrices.

Matrix	SS	FLE	FT	Water	HB
Spiked/assigned value	61/52 ng/ml	6.1/5.6 ng/ml	49*/20 ng/g ww	N.a/1.0 ng/ml	N.a./0.39 ng/ml
Satisfactory |z|<2	10/24**	6/18	3/14	4/10	3/5
Questionable 2<|z|<3	4/24	0/18	1/14	0/10	1/5
Unsatisfactory |z|>3	10/24	12/18	10/14	6/10	1/5

* Spike in addition to the natural contamination
**: Number of labs/total submitted datasets

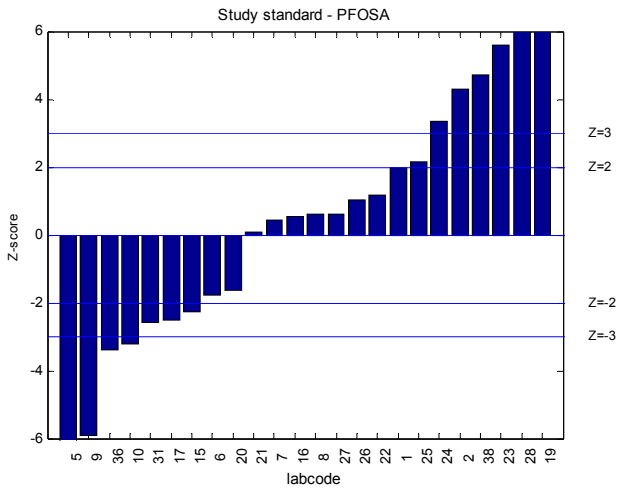

Figure 13. Z-score plot of PFOSA in the SS.

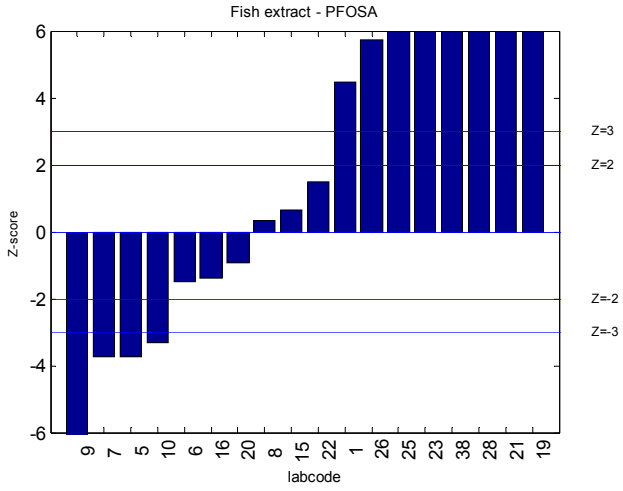

Figure 14. Z-score plot of PFOSA in the FLE

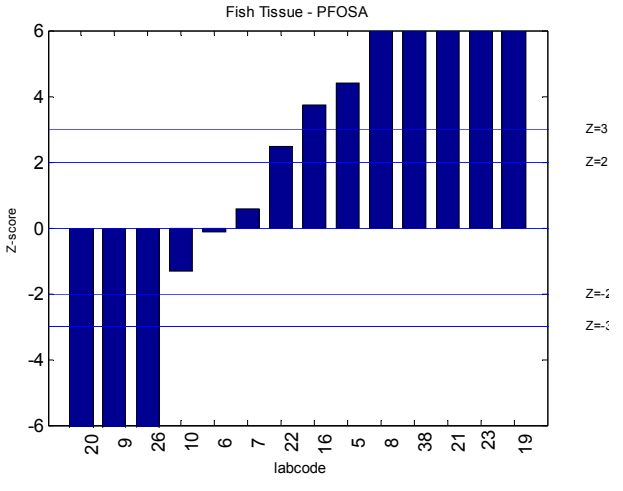

Figure 15. Z-score plot of PFOSA in the FT

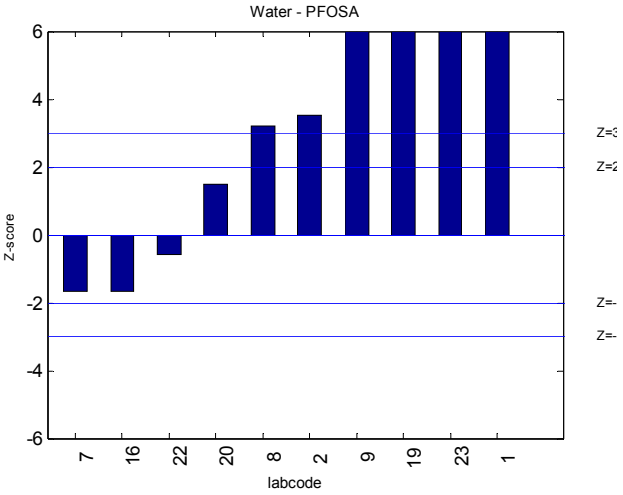

Figure 16. Z-score plot of PFOSA in the water.

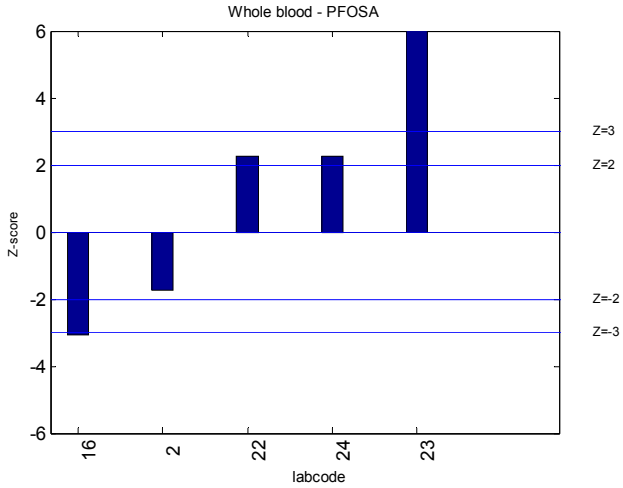

Figure 17. Z-score plot of PFOSA in the HB

3.4 Other PFCs

Considerable number of laboratories has submitted data on other PFCs such as PFHxS, PFHxA, PFNA, PFDA and PFDoA. Data of these compounds have been analysed statistically and z-scores have been calculated which can be found in Appendix 2 to Appendix 6.
Very limited data was provided for PFBA and PFDS. No (commercial) fully characterized standard exists for PFDS, which hampers the quantification of this compound. Therefore, these results should be regarded as indicative.
For all PFCs with >5 observations basic statistical analysis has been performed, which can be found in above mentioned appendices. From these data, it is clear that in the water and FT sample generally the highest RSD values are observed for the PFCAs (114 to 236%), and the PFSAs (47-250%) as compared to the other matrices. It should be noted however that outliers have not been removed from the basic statistics (e.g. lab 21 in the FT sample). A factor of 100 between the lowest and the highest reported value is sometimes observed (e.g. PFNA and PFDA in the water sample). Since no addition of PFCs was made to the human matrices the result is limited to the natural occurring compounds in the blood samples. Except PFOS and PFOA, PFNA and PFHS were frequently found and reported with an agreement between laboratories comparable to the discussed PFOS and PFOA (RSD 29-64%).

4. Conclusions

This first world-wide interlaboratory study has successfully evaluated the quality of the data reported by the participants in a range of environmentally relevant matrices.
Considering the recent development of the methods there is a fair agreement of the laboratories for PFOA, PFOS and PFOSA in the study standard, the fish liver extract, the whole blood and plasma sample. On the other hand there is little agreement for these three compounds in fish tissue and water. Concerning the fish tissue, the extraction and (absence of) clean-up is a considerable source of error contributing significantly to the overall variance of the dataset.

The results show that the interpretation of the z-scores should be carefully made. The z-score definitely identifies the laboratories with the highest level of agreement, but does not necessarily point out those laboratories which obtained the closest estimate to the true value. Some of the datasets might be skewed and consequently the assigned values obtained are not necessarily the closest estimates of the true values for these PFCs. This has been observed for PFOS and PFOSA in the FLE, where the assigned value was considerably lower than the spiked value. As a result, laboratories with a somewhat elevated z-score could actually have been closer to the theoretical value.
One should be careful relating performance to (parts of) analytical methodology. The error of a final result is often the sum of errors from various sources. In other words, a bias can have multiple origins such as extraction and clean-up, use of internal standards, unextracted calibration curves and the (non) use of (isotope labelled) internal standards. Given the spread in the dataset, it is highly likely that in most laboratories more than one analytical condition is not under control.

Sample pre-treatment, extraction and cleanup procedures of the more complex matrices like the fish tissue are probably the most important sources of error. Laboratories are therefore advised to carefully check the efficiency and selectivity of their extraction and clean-up methods. However, interference during analysis due to matrix effects (electrospray suppression, mass interference) can also be an important factor in causing variability and bias in the final result.

The type of calibration may have a considerable effect on the final result. This may have been the cause for the low results compared to the spiked amount for PFOS in the fish liver extract. For the whole blood and plasma dataset, there are no indications that quantification with unextracted calibration curves produce different results from matrix matched curves.

The development of certified reference materials of different matrices is urgently required as a complementary QA tool.

This study has shown that an interlaboratory study for PFCs is an essential aspect of today's QA methods for evaluation of the performance of various laboratories. Laboratories are encouraged to have a critical look at their method performance and use this opportunity for improving their analytical methods. This will certainly have a positive effect on the quality of the results reported for monitoring and environmental and human risk assessment.

5. Recommendations

The participants of this study are advised to improve their performance through critically assess the quality of their methods.

Authors of peer viewed publications generally have limited the analytical methodology part of a publication in order to save space for results and discussion. However, a detailed discussion on the quality assurance will certainly improve the quality of the publication.

The organization of the 2nd world-wide interlaboratory study is recommended in order to continuously improve the PFC data quality.

Acknowledgements

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Appendix 1: List of participants

Given in table A1 is the participants affiliations and contact information. Also seen is what matrices the laboratories participated in.

Table A1. Participants in the 1st worldwide interlaboratory study

Contact person	Laboratory name	P.O. Box	Visiting address	Postal code	City	Country	Email	Telephone	Fax	Environ.	Human
Berger, Urs	Norwegian Institute for Air Research (NILU)		Hjalmar Johansensgt 14	9296	Tromsø	Norway	urs.berger@NILU.org	+47 77750385	+47 77750376	X	X
Bersuder, Philippe	CEFAS Laboratory		Remembrance Avenue	CM0 8HA	Burnham-on-Crouch	U.K.	p.bersuder@cefas.co.uk	+44 1621787212	+44 16217894989	X	
Bossi, Rossanna	National Environmental research Institute	358	Frederiksborgvej 399	4000	Roskilde	Denmark	rbo@dmu.dk	+45 46301357	+45 46301114	X	
Calafat, Antonia M.	Centers for Disease Control & Prvention	4770	Buford HWY, MS F17	30341	Atlanta, GA	USA	acalafat@cdc.gov	+1 7704887891	+1 7704884609		X
Connolly, Paul	Exygen Research		3058 Research Drive	PA-16801	State College	USA	Paul.Connolly@exygen.com	+1 8142721039	+1 8142311580		X
Crozier, Patrick	Ontario Ministry of the Environment, Lab. Services Branch			M9P 3V6	Etobicoke, Ontario	Canada	Patrick.crozier@ene.gov.on.ca	+1 4162355824	+1 416 2355744	X	
de Felip, Elena	National Institute of Health		Vale Regina Elena 299	00161	Rome	Italy	defelip@iss.it	+39 649902904	+39 649387139		X
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di Domenico, Alessandro	Italian Institute for Health, dept. Environ. Primary Prevention		Viale Regina Elena 299	00161	Rome	Italy	Addeke@iss.it	+39 649902826	+39 649387139	X	
Dymerski, Mark	STL Denver		4955 Yarrow Str.	80002	Arvada, Colorado	USA	mdymerski@stl-inc.com	+1 3037360116	+1 3034317171	X	
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Hoover, Dale	AXYS Analytical services Ltd	2219	2045 Mills Road West	V8L 3S8	Sidney, BC	Canada	dhoover@axys.com	+1 2506555800	+1 2506555811	X	X
Hoppe, Hans-Wolfgang	Medical Lab Bremen		Haferwende 12	D-28357	Bremen	Germany	Hans-wolfgang.hoppe@mlhb.de		+49 42120727251		X
Järnberg, Ulf	ITM Stockholm University			SE 106 91	Stockholm	Sweden	Ulf.jarnberg@itm.su.se	+46 86747152	+46 86747637	X	X
Kimura, Yoshitaka	Sumika chemical Analysis service Ltd		2-1 Kitasode, Sodegaura-shi	299-0833	Chiba-ken	Japan	y.kimura@scas.co.jp	+81 438636176	+81 438636921	X	
Langlois, Ingrid	University of Basel Organic Analytical Chemistry		Neuhausstrasse 31	CH-4057	Basel	Switzerland	Ingrid.langlois@unibas.ch	+41 616392302	+41 616392300	X	
Lindström, Gunilla	Örebro University MTM Research Centre		Dept. of Natural Sciences - Örebro University	SE-701 82	Örebrö	Sweden	Gunilla.lindstrom@nat.oru.se	+46 19301098	+46 19303169	X	
Martin, Jonathan W.	University of Alberta, Environmental Health Sciences		10-102 Clinical Sciences Building	T6G 2G3	Edmonton, Alberta	Canada	Jon.martin@ualberta.ca	+1 7804921190	+1 7804927800	X	

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Peter, Robert	CIBA Specialty Chemicals	R-1047.1.12		CH-4002	Basel	Switzerland	Robert.peter@cibasc.com	+41 616365057	+41 616362906	X	X
Powley, Charles R.	DuPont Haskell Laboratories	50	1090 Elkton Road	19714	Newark, Delaware	USA	Chuck.powley@usa.dupont.com	+1 3024514502	+1 3023665003	X	X
Reagen, William	3M Environmental Laboratory		Environmental, Health and Safety Operations, Bldg 2-3E-09. 935 Busch Avenue	55106	St Paul. Minnesota	USA	wkreagen@mmm.com	+1 6517786565		X	X
Rose, Martin	Central Science Laboratory		Sand Hutton	YO41 IL7	York	U.K.	m.rose@csl.gov.uk	+44 1904462655	+44 1904462158	X	X
Schroeder, Horst F.	Environmental Analytical Laboratory, dpt Environmental Engineering	D-52056	Krefelder Strasse 299	D-52070	Aachen	Germany	Hf.schroeder@post.rwth-aachen.de	+49 241153252	+49 2418092500	X	
Scott, Brian F.	National Water Research Institute	5050	867 Lakeshore Rd	L7A 4A6	Burlington, Ontario	Canada	Brian.scott@ec.gc.ca	+1 9053364934	+1 9053364989	X	X
Takasuga, Takumi	Shimadzu Techno-research Inc.		1 Nishinokyo-Shimoaicho, Nkagyo-Ku	604-8436	Kyoto	Japan	T_takasuga00@shimadzu-techno.co.jp	+81 758113182	+81 758113278	X	
Theobald, Norbert	Bundesamt für Saeeschiffahrt und Hydrographie		Wüstland 2	22589	Hamburg	Germany	Norbert.theobald@bsh.de	+49 4031903340	+49 4031905000	X	
Tittlemier, Sheryl	Health Canada-Health Product: Food Branch		1 Ross Ave Banting Ridg	K1A OL2	Ottawa Ontario	Canada	Sheryl-tittlemier@hc-sc.gc.ca	+1 9415603	+1 9414775		X
Tomy, Gregg	Department of Fisheries and Oceans		501 University Crescent	R3T 2N6	Winnipeg, Manitoba	Canada	tomyG@DFO-MPO.GC.CA	+1 2049835167	+1 2049842403	X	
Tuckermann, Rudolf	GKSS Research Center, Institute for Coastal Research		Max-Planck Strasse 1	D-21802	Geesthacht	Germany	Rudolf.tuckermann@gkss.de	+49 4152872346	+49 4152872332	X	
van Leeuwen, Stefan	Netherlands Institute for Fisheries Research, RIVO		Haringkade 1	1976 CP	IJmuiden	The Netherlands	Stefan.vanleeuwen@wur.nl	+ 31 255 564735	+ 31 255 564 64	X	
Wolf, Lee	Columbia Analytical Services	419	1317 South 13th Avenue,	98626	Kelso, WA	USA	lwolf@kelso.caslab.com	+1 3605777222	+1 3606361068	X	X
Yamashita, Nobuyoshi	EMTECH Aist Japan		16-1 Onogawa	305-8569	Tsukaba, Ibaraki	Japan	Nob.yamashita@aist.go.jp	+81 298618335	+81 298618355	X	X

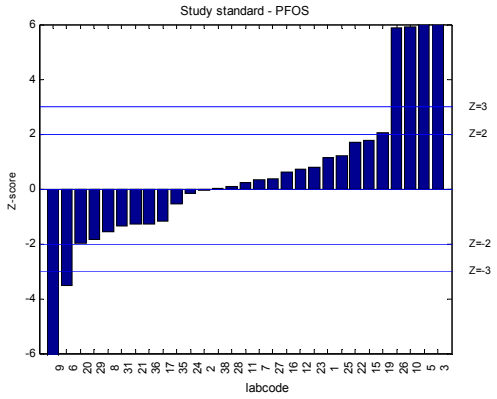
Appendix 2: Results for study standard

Study standard results in ng/ml are presented as they were reported from each laboratory. Average, median and standard deviation was calculated after removing “non detect” values and with as many digits the laboratories reported. Distribution figures are given for the compounds that were added to the standard solution.

NObs > LOQ	Number of observations above limit of quantification
NObs < LOQ	Number of observations below limit of quantification
Assigned value	True value obtained using Cofino statistics
-	Not analysed
ND	Not detected
NQ	Not quantified
<	Less than

Table A2. Study standard results (ng/ml)

Lab. code	PFOS	PFOA	PFNA	PFBS	PFOSA	PFHxS	PFHxA	PFHpA	PFDA	PFUnA	PFDoA	PFBA	PFDS
1	32.70	9	<1	-	64.2	-	21.60	3	8.3	17	4.3	-	<2
2	28.4	8.18	<0.130	37.0	79.2	25.1	11.0	<0.105	7.70	14.9	3.23	-	-
3	65	6.4	ND	-	-	-	15	ND	6.8	16	4.5	-	-
5	59.1	8.3	ND	-	8.4	-	-	-	10.9	21.7	6.6	-	-
6	16	6.5	< 0.4	-	40	15	-	< 0.4	7.5	8.0	3.0	-	-
7	29.8	7.7	ND	-	54.4	23.9	-	ND	7.3	13.1	-	-	-
8	23.02	12.05	ND	-	55.44	22.22	8.67	ND	8.86	-	-	-	-
9	6.2	12.7	2.7	15.3	13.5	16.5	11.9	-	7.1	12.3	7.3	-	0.5
10	49.8	9.7	-	-	30.9	17.3	11.8	-	-	-	-	-	-
11	29.43	4.05	-	-	-	-	-	-	-	-	-	-	-
12	31.2	8.4	<0.2	35.2	-	-	-	-	-	-	-	-	-
13	NQ	12.5	ND	-	-	-	-	-	ND	ND	ND	-	-
15	35	7.2	< LOQ	53	37	-	4.2	< LOQ	9.4	-	3.5	-	-
16	30.85	7.37	0.08	35.15	54.97	25.21	8.49	0.13	6.41	10.54	3.08	4.97	-
17	24.4	7.4	ND	-	35.3	-	-	-	7.1	-	-	-	-
19	36	46	-	7.1	690	31	-	-	-	-	5.1	-	-
20	21.5	7.1	0.1	33.8	40.9	21.5	5.0	0.2	7.2	10.8	3.5	2.1	-
21	24	5	<1.6	25	52	26	7	1.4	7	11	3	-	-
22	34.7	12.7	0.11	42.9	59.1	26.2	15.4	0.21	13.2	24.7	6.74	5.07	<0.01
23	31.5	7.5	<0.5	48	87.5	24	9	<0.5	10	17	6.3	-	0.8
24	28	7	0.3	36	73	20	7	-	8	11	<0.3	-	<0.5
25	32.9	7.88	0.18	44.1	65.4	23.6	8.69	-	9.53	18.4	8.2	-	-
26	49.7	40.8	38	38.2	58.2	34.4	15	LOD	13.8	56.6	19.9	17.8	9.2
27	29.9	6.5	-	35.7	55.5	23.3	10.4	-	4.7	8.1	12.3	5.3	-
28	29	9	<2	38	387	32	8	<2	9	46	65	-	-
29	22	9.2	-	53	-	30	12	0.58	6.4	15	-	-	-
30	-	10	ND	-	-	-	6.6	-	9.3	14	2.8	-	-
31	23.8	7.3	<0.1	22.1	35	10.5	-	<0.4	6.8	14.5	4.3	-	-
32	-	12.27	-	-	-	-	-	-	-	-	-	-	-
33	-	9.0	-	-	-	-	-	-	-	-	-	-	-
35	26.7	7.73	<0.50	-	-	-	-	-	-	-	-	-	-
36	24	4	<3.7	37.2	29.8	21.4	8	-	5.9	6	<6.5	-	-
38	28.7	20.8	ND	-	81.8	-	-	-	10.6	6	4.5	-	-
NObs > LOQ	29	33	7	18	24	20	20	6	25	22	20	5	3
NObs < LOQ	1		19					10	1	1	3		3
Spiked amount	33.3	8.4	-	47.3	60.9	24	17.8	-	13.5	17.3	3.8	4.3	-
Assigned value	28.6	7.8	-	-	51.5	23.6	9.0	-	7.7	-	4.1	-	-
Min	6.2	4.1	0.08	7.1	8.4	10.5	4.2	0.13	4.7	5.8	2.7	2.1	0.5
max	65	46	38	53	690	34	22	2.8	14	57	65	18	9
Average	32	11	5.2	35	91	23	10	0.76	8.3	17	8.8	7.1	
Median	29	8.2	0.15	37	55	24	8.9	0.21	7.7	14	4.5	5.1	
St. dev.	12	9.0	13	12	146	5.9	4.2	1.0	2.2	12	14	8.2	
RSD (%)	38	83	256	34	160	25	40	134	26	72	156	87	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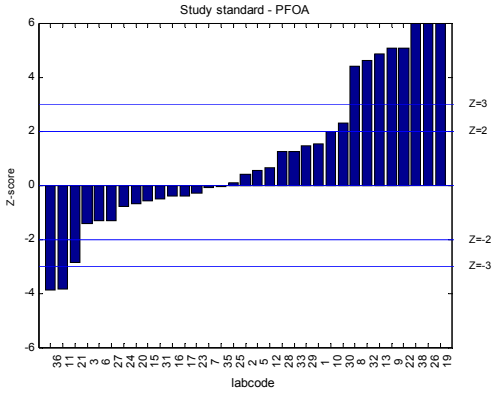
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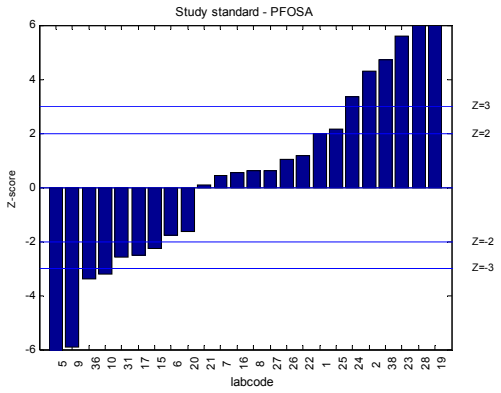
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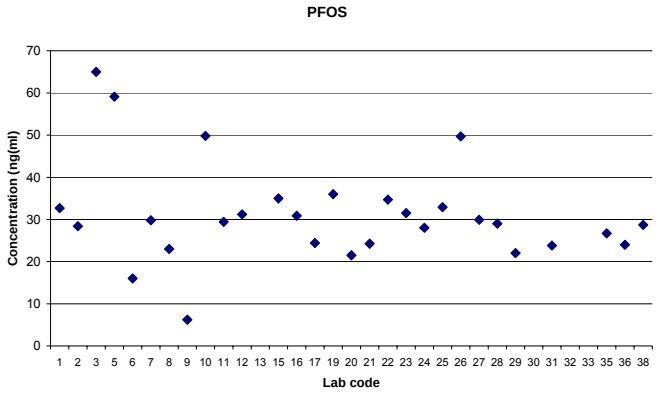
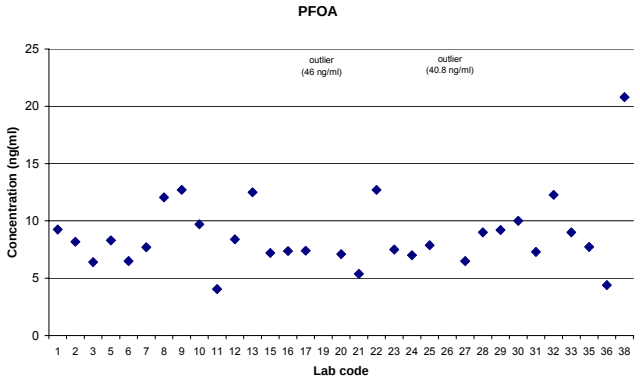

PFOS
Concentration (ng/ml)
Lab code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outlier
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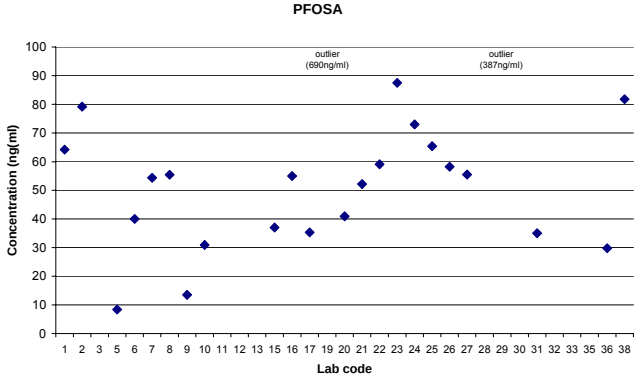
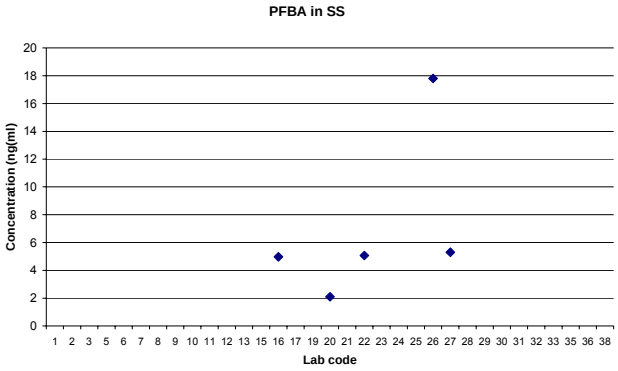
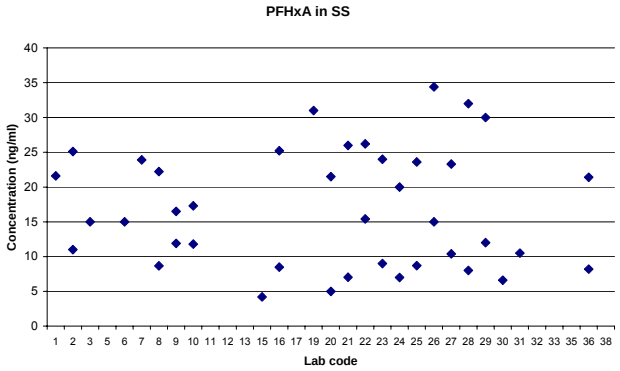
outlier
(40.8 ng/ml)
Concentration (ng/ml)
Lab code


PFOSA
outlier
(690ng/ml)
outlier
(387ng/ml)
Concentration (ng/ml)
Lab code


PFBA in SS
Concentration (ng/ml)
Lab code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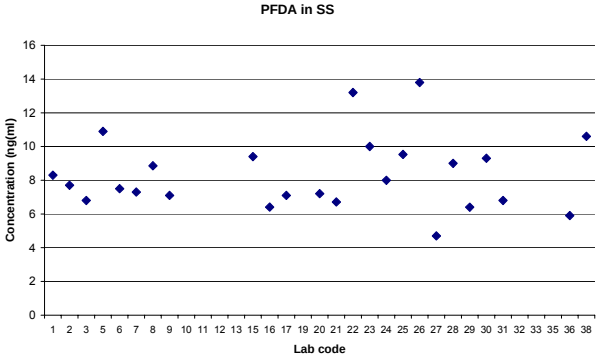
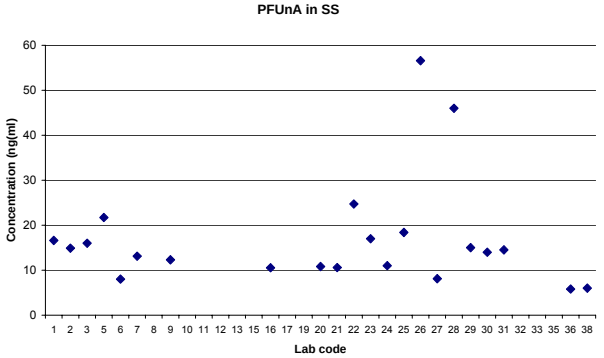
Concentration (ng/ml)
Lab code


PFDA in SS
Concentration (ng/ml)
Lab code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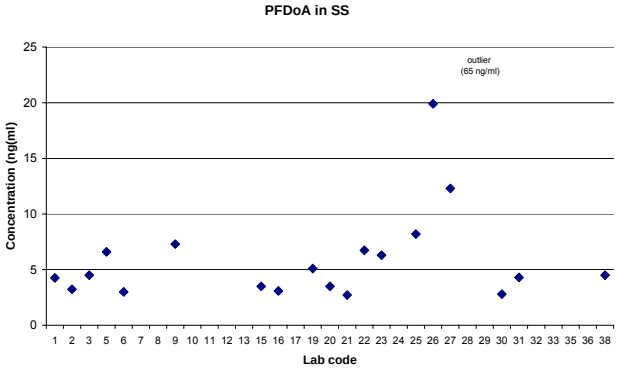
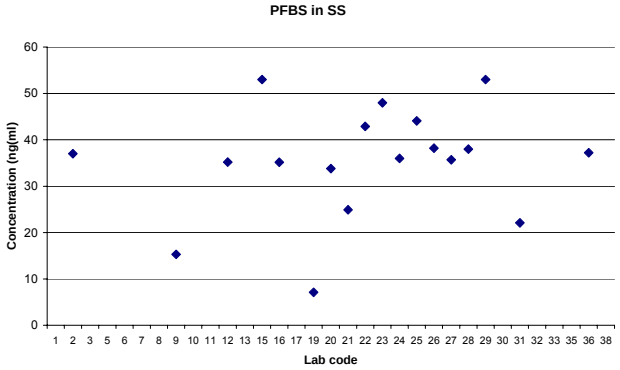
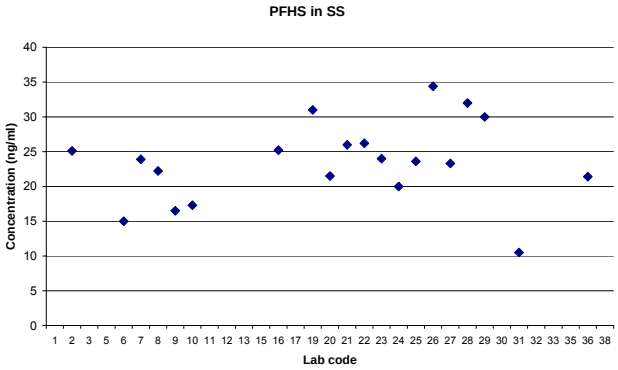
Concentration (ng/ml)
Lab code


PFDoA in SS
outlier
(65 ng/ml)
Concentration (ng/ml)
Lab code


PFBS in SS
Concentration (ng/ml)
Lab code


PFHS in SS
Concentration (ng/ml)
0
5
10
15
20
25
30
35
40
1 2 3 5 6 7 8 9 10 11 12 13 15 16 17 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 35 36 38
Lab code

Appendix 3: Results for fish liver extract

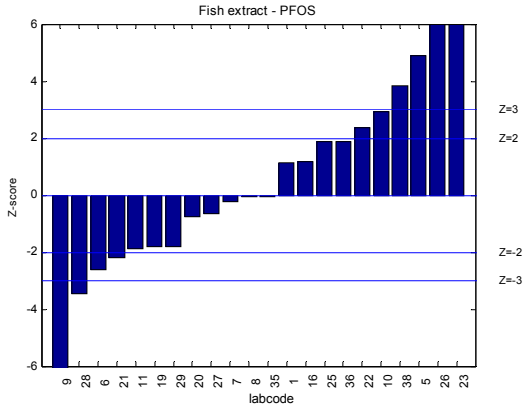
Fish liver extract results in ng/ml are presented as they were reported from each laboratory. Average, median and standard deviation was calculated after removing “non detect” values and with as many digits the laboratories reported. Distribution figures are given.

NObs > LOQ	Number of observations above limit of quantification
NObs < LOQ	Number of observations below limit of quantification
Assigned value	True value obtained using Cofino statistics
-	Not analysed
ND	Not detected
NQ	Not quantified
<	Less than

Table A3. Overview of submitted results and statistical data for the FLE (ng/ml)

Lab code	PFBA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFOS	PFDS	PFOSA
1	-	17	11	18	1.7	9.8	4.8	20	-	-	22	17	8.8
5c*	-	-	-	8.9	0.79	9.7	4	17.2			31.2		3
6	-	-	< 0.4	10	0.6	8.8	1.5	16	-	13	13	-	4.6
7	-	-	<LOD	13.1	<LOD	8.8	3.8	-	-	17.4	18.8	-	3
8	-	1.36	0	16.07	0.66	11.77	-	-	-	17.74	19.18	-	5.87
9	-	-	-	4.5	0.7	2.4	1	5.5	4	5.4	2.7	0.5	1
10	-	3	-	13.2	-	-	-	-	-	12.3	26.4	-	3.3
11	-	-	-	8.32	-	-	-	-	-	-	14.83	-	-
15c	-	-	-	26.1	-	< LOD	-	-	< LOD	-	< LOD	-	6.1
16	22.06	1.3	0.11	12.18	0.81	8.19	2.48	12.75	5.4	19.16	22.16	-	4.67
19	-	-	-	77	-	-	-	22	<0.39	13	15	-	53
20	9.3	1.6	0.2	11.7	0.4	9.2	2.3	18.2	5.3	17.3	17.5	-	5
21	-	<7	1.4	8.7	<1.6	5.8	1.5	13	<15	20	14	-	27
22	18.6	2.58	0.23	22.9	2.48	21.3	6.64	34.2	7.01	19	25.1	0.24	6.69
23	-	0.63	<0.5	21	1.6	20	4.8	27	11	27	62	1.2	11
25	-	0.84	-	13.7	1.08	28.6	11.2	54	3.61	16.6	23.9	-	10.4
26	9.3	2.1	LOD	29.1	-	31.9	12.4	95.7	13.7	36.6	49.5	<LOD	9.7
27	18.1	ND	-	12.4	-	6.2	14.5	15.1	<LOD	18.2	17.8	-	<LOD
28	-	<2	<2	11	<2	11	11	337	<5	15	11	-	24
29	-	1.6	<0.2	10	VS**	7.6	1.9	IS**	5.5	16	15	-	-
30c		2.1	-	24.6	1.4	20.6	5.4	19	-	-	-	-	-
33	-	-	-	19	-	-	-	-	-	-	-	-	-
35	-	-	-	15.6	0.61	-	-	-	-	-	19.2	-	-
36	-	<3.5	-	12.2	<3.7	15.7	<3.5	24.1	<3.0	16.6	23.9	-	-
38c	-	-	-	40.6	-	8.4	3.2	38.8	-	-	28.6	-	14.7
NObs > LOQ	5	11	6	25	12	19	17	17	8	17	22	4	18
NObs < LOQ	-	4	6	-	4	1	1	-	6	-	1	1	1
Spiked amount	18	2.7	-	11	-	14	2.1	17	6.9	19	45	-	6.1
Assigned value	-	1.7	-	12.3	-	8.9	-	18.1	-	17.0	19.3	-	5.6
Min	9.3	0.63	0	4.5	0.4	2.4	1	5.5	3.61	5.4	2.7	0.24	1
Max	22.06	17	11	77	2.48	31.9	14.5	337	13.7	36.6	62	17.325	53
Average	15.5	3.1	2.2	18.4	1.1	12.9	5.4	45.3	6.9	17.7	22.4		11.2
Median	18.1	1.6	0.2	13.2	0.8	9.7	4.0	20.4	5.5	17.3	19.2		6.4
St. dev.	5.8	4.7	4.4	14.6	0.6	8.0	4.2	78.1	3.6	6.6	12.7		12.6
RSD	38	150	202	79	57	62	78	172	51	37	57		112

* Submitted data converted to ng/ml, ** VS: volume standard, IS: internal standard


Fish extract - PFOS
Z-score
labcode
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Z=2
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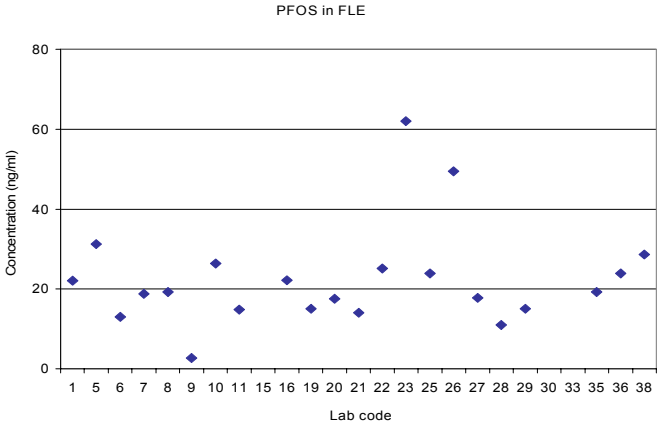
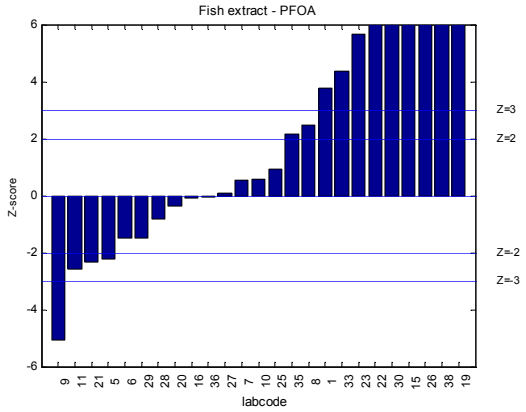
Z=-3


PFOS in FLE
Concentration (ng/ml)
Lab code


Fish extract - PFOA
Z-score
labcode
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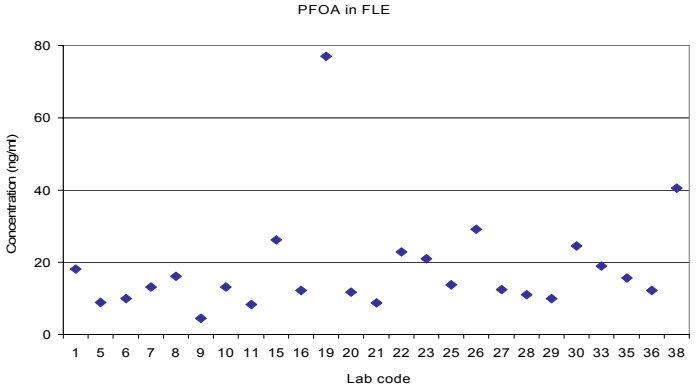
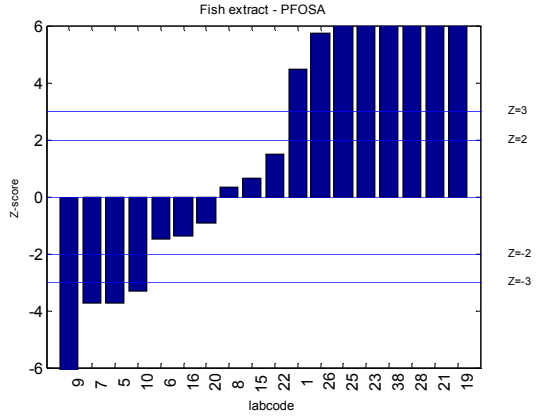

PFOA in FLE
Concentration (ng/ml)
Lab code


Fish extract - PFOSA
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labcode
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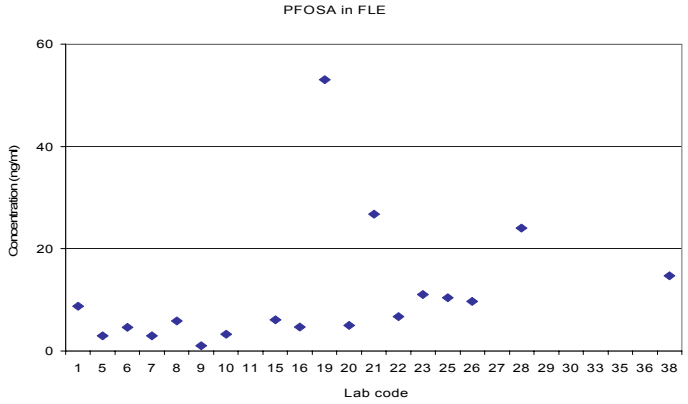
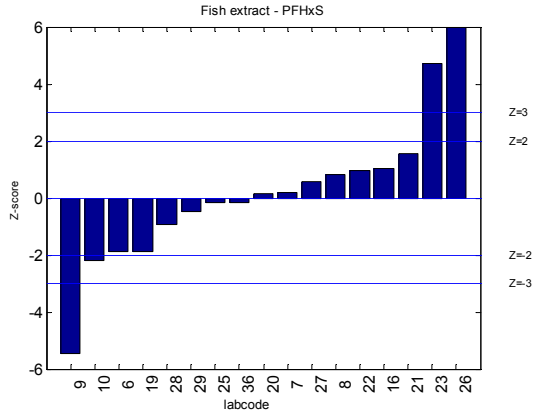

PFOSA in FLE
Concentration (ng/ml)
Lab code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Z-score
labcode
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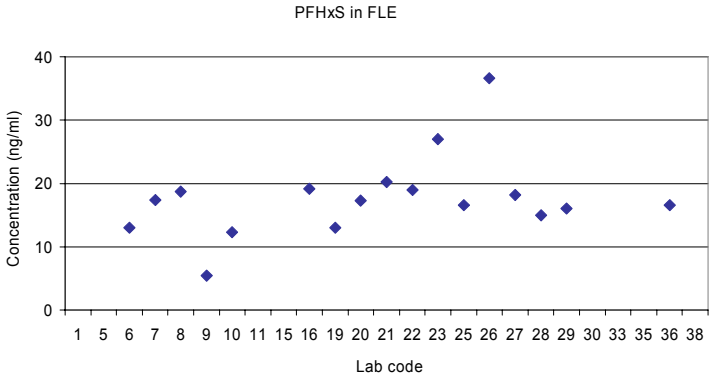
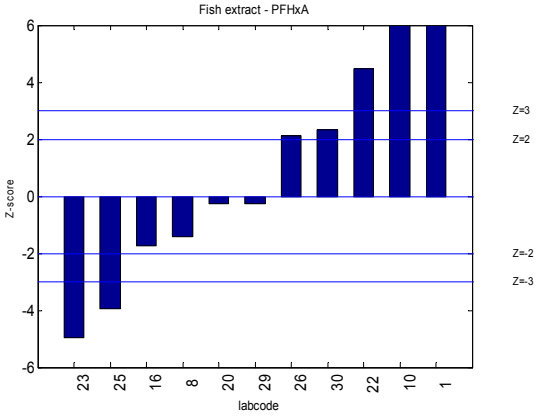
Z=-3


PFHxS in FLE
Concentration (ng/ml)
Lab code


Fish extract - PFHxA
Z-score
labcode
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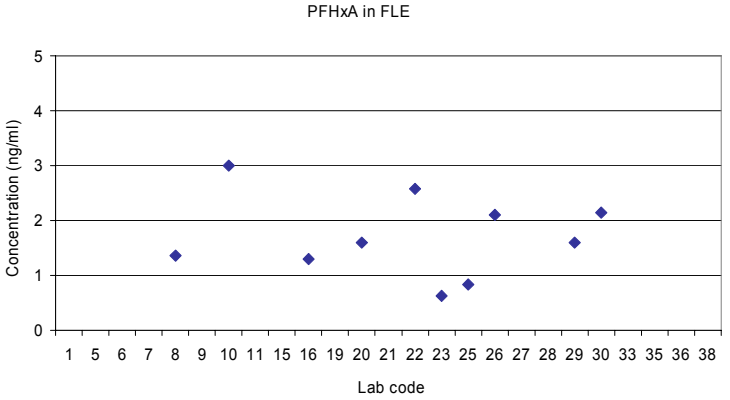
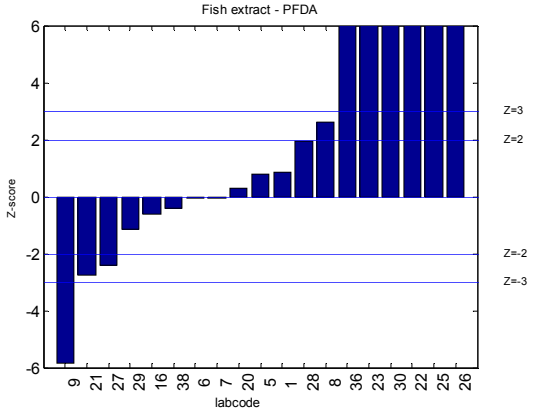
Z=-3


PFHxA in FLE
Concentration (ng/ml)
Lab code


Fish extract - PFDA
Z-score
labcode
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Z=2
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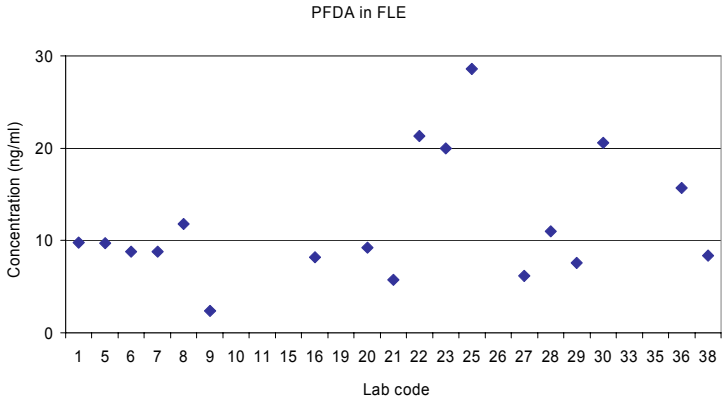
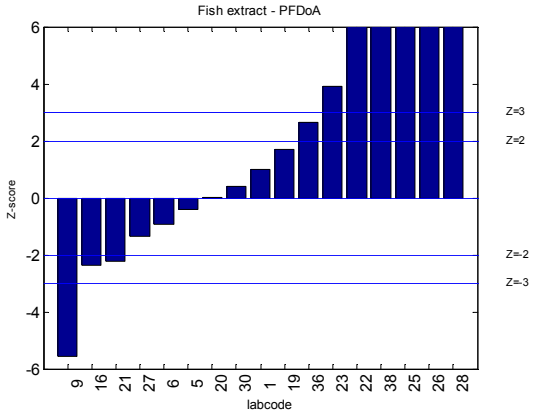
Z=-3


PFDA in FLE
Concentration (ng/ml)
Lab code


Fish extract - PFDoA
Z-score
labcode
Z=3
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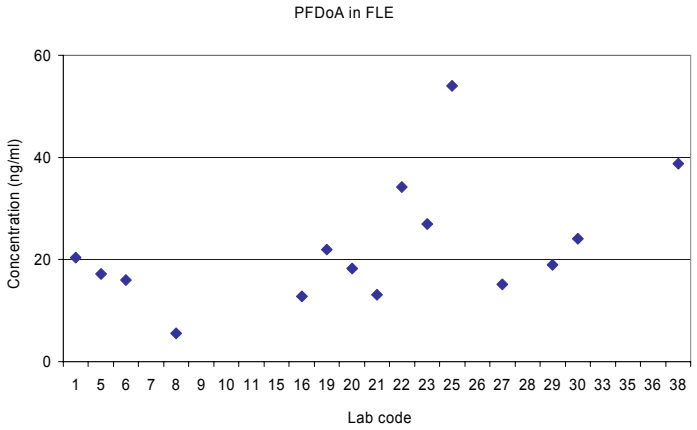
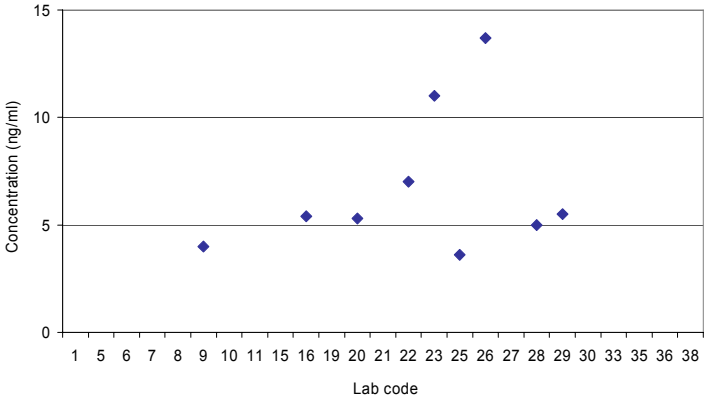
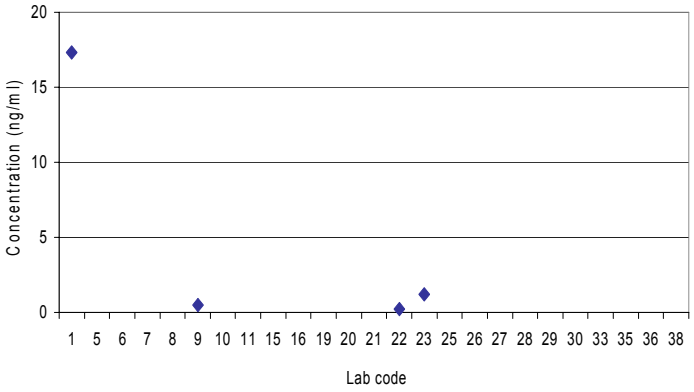
Z=-3


PFDoA in FLE
Concentration (ng/ml)
Lab code

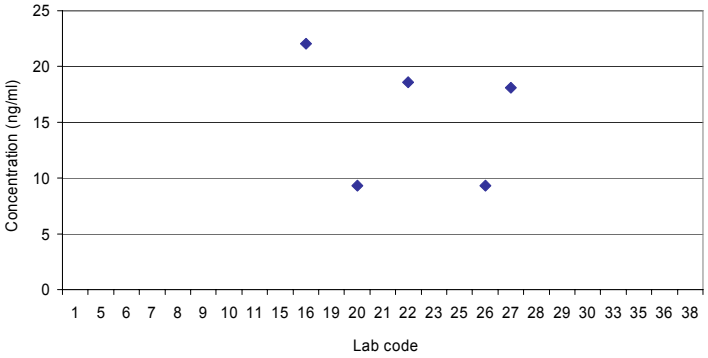
PFBS in FLE



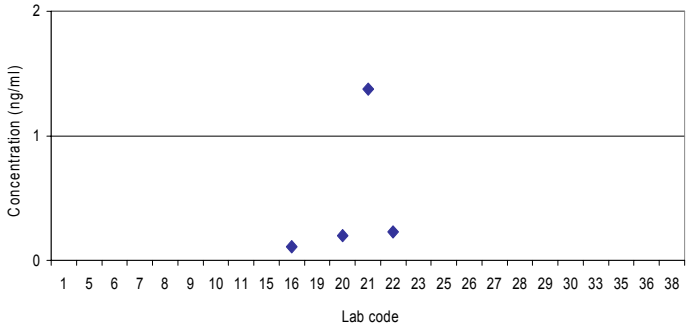
PFDS in FLE

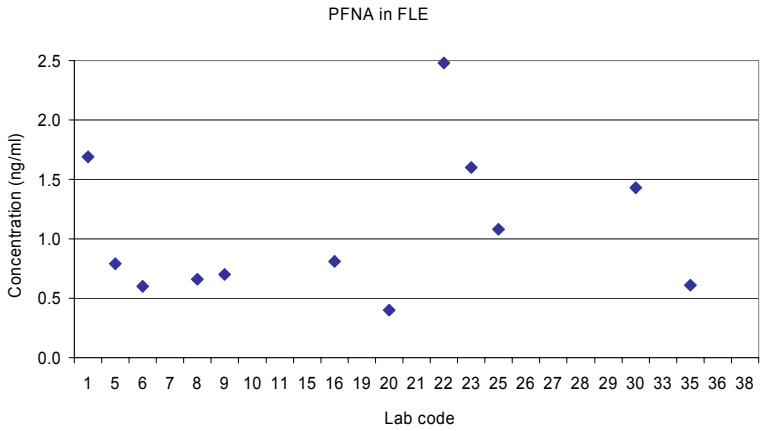


PFBA in FLE



PFHpA in FLE




PFNA in FLE
Concentration (ng/ml)
2.5
2.0
1.5
1.0
0.5
0.0
1 5 6 7 8 9 10 11 15 16 19 20 21 22 23 25 26 27 28 29 30 33 35 36 38
Lab code

Appendix 4: Results for fish tissue

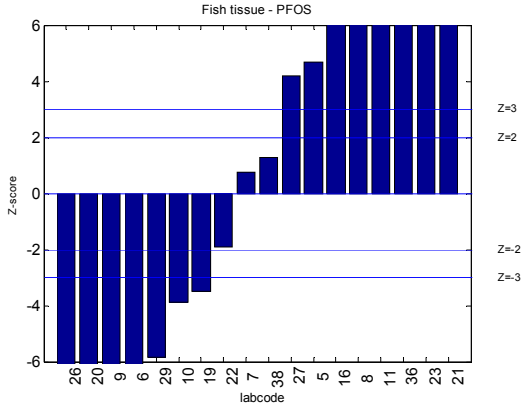
Fish tissue results in ng/g ww are presented as they were reported from each laboratory. Average, median and standard deviation was calculated after removing "non detect" values and with as many digits the laboratories reported. Distribution figures are given.

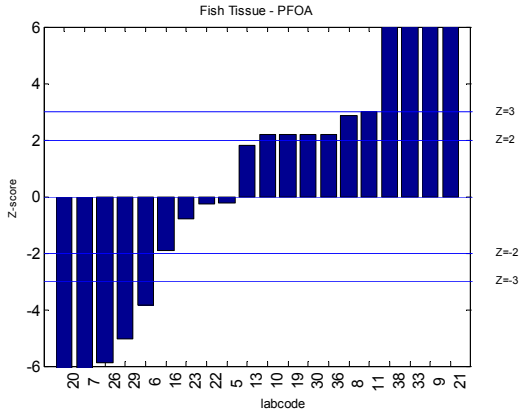
NObs > LOQ	Number of observations above limit of quantification
NObs < LOQ	Number of observations below limit of quantification
Assigned value	True value obtained using Cofino statistics
-	Not analysed
ND	Not detected
NQ	Not quantified
<	Less than

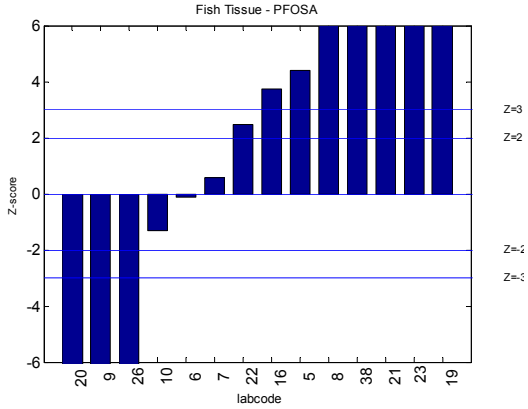
Table A4. Overview of submitted results and statistical data for FT (ng/g ww)

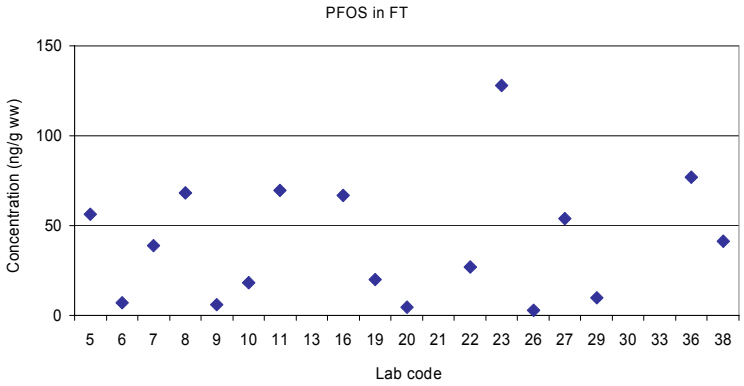
Lab code	PFBA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFOS	PFDS	PFOSA
5	-	-	-	9.9	0.9	1.5	1.6	5.7	-	-	56.3	-	31.5
6	-	-	<0.4	5.3	0.6	1.3	0.4	14	-	0.6	7.1	-	20
7	-	-	-	1.7	<LOD	<LOD	<LOD	-	-	12.6	38.8	-	21.8
8	-	0.18	0.24	13.85	0.68	2.99	-	-	-	22.01	63.32	-	30.2
9	-	36.2	-	44.2	48.2	44.1	42.6	53.2	8.1	7.1	5.9	35.3	3.4
10c*	-	4.2	-	13	-	-	-	-	-	4.7	18.3	-	17.0
11	-	-	-	14.02	-	-	-	-	-	-	69.64	-	-
13	-	-	-	12.5	<LOD	<LOD	<LOD	<LOD	-	-	"detected"	-	-
16	-	<0.25	<0.25	7.78	0.99	1.9	1.09	26.11	16.46	16.36	66.76	-	29.83
19	-	-	-	13	-	-	-	17	<0.54	3.0	20	-	230
20	1.91	0.01	0.06	0.54	0.15	0.19	0.07	1.50	0.95	1.13	4.41	-	1.97
21	-	337	13	204	272	<0.5	90	54	23	54	295	-	112
22	29.7	<0.67	0.32	9.85	1.17	2.64	1.03	18.3	16.0	13.0	27.0	0.08	26.6
23	-	<3	1.3	9.2	1.4	3.8	2	191	22	26	128	<0.5	207
26	'blank"	<LOD	1.9	2.7	70.8	0.63	0.39	7.9	2.3	29.5	2.8	<LOD	3.6
27	41	<LOD	-		-	29	<LOD	74	<LOD	21	54	-	<LOD
29	-	1	3.1	3.8	VS**	<1.0	<0.5	IS**	7.7	6.3	9.63	-	-
30	-	<LOD	-	13	1.8	3.7	2	47	-	-	-	-	-
33	-	-	-	24	-	-	-	-	-	-	-	-	-
36	-	<1.7	-	13	<1.9	4.0	<1.8	21.0	22	24	77	-	-
38	-	-	-	21.3	0.9	1.5	2.1	26.9	-	-	41.1	-	110.2
NObs > LOQ	3	6	7	20	12	13	11	14	9	15	18	2	14
NObs < LOQ	-	7	2	1	3	4	5	1	2	-	-	2	1
Spiked amount	50	-	-	9.7	-	-	-	40	21	22	4.4	-	49
Assigned value		1.0		10.2		2.2	-	19.6	-	13.2	36.5	-	20.3
Min	1.91	0.01	0.06	0.54	0.15	0.19	0.07	1.5	0.95	0.6	2.8	0.08	1.97
Max	41	337	13	204	272	44.1	90	191	23	54	295	35.3	230
Average		63.2	2.8	21.8	33.3	7.5	13.1	39.9	13.2	16.1	54.7		60.4
Median		2.6	1.3	12.8	1.1	2.6	1.6	23.6	16.0	13.0	40.0		28.2
St. dev.		135.1	4.5	43.9	78.7	13.3	28.5	48.4	8.6	14.2	68.5		75.5
RSD %		214	161	201	236	178	218	121	65	88	125		125

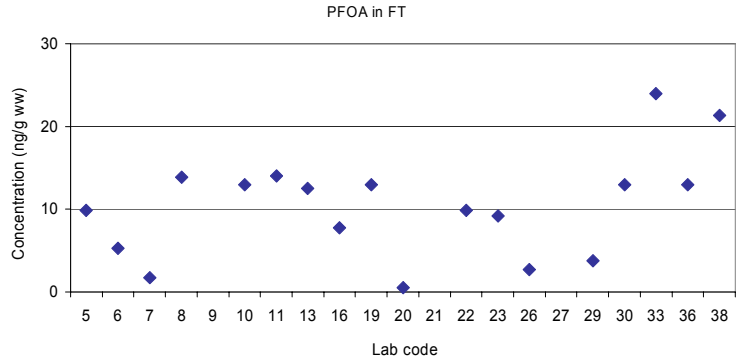
* Converted to ng/g ww, ** VS: volume standard IS: internal standard

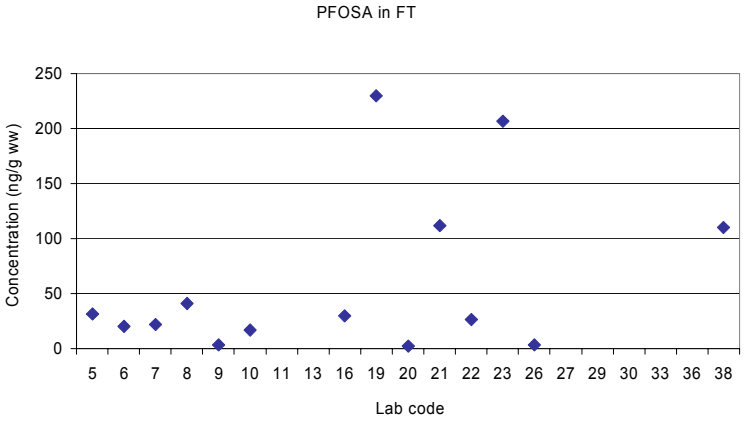

Fish tissue - PFOS
Z-score
labcode
Z=3
Z=2
Z=-2
Z=-3

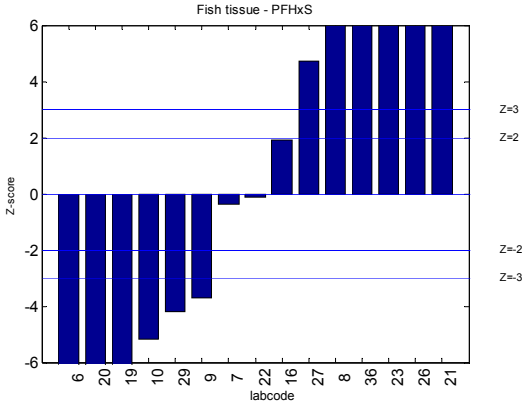

Fish Tissue - PFOA
Z-score
labcode
Z=3
Z=2
Z=-2
Z=-3

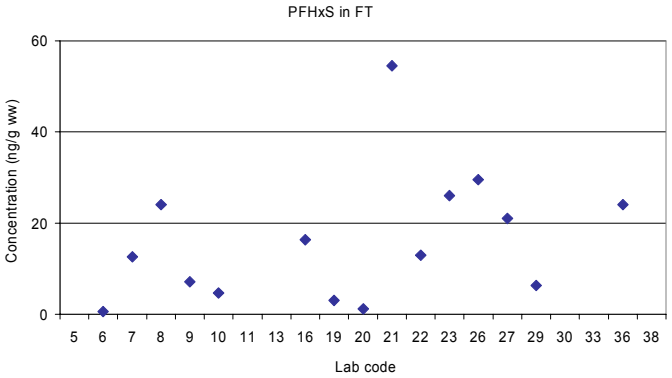

Fish Tissue - PFOSA
Z-score
labcode
Z=3
Z=2

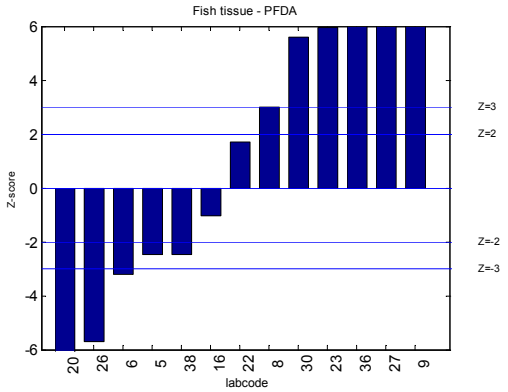

PFOS in FT
Concentration (ng/g ww)
Lab code


PFOA in FT
Concentration (ng/g ww)
Lab code


PFOSA in FT
Concentration (ng/g ww)
Lab code


Fish tissue - PFHxS
Z-score
labcode
Z=3
Z=2
Z=-2
Z=-3
6 20 19 10 29 9 7 22 16 27 8 36 23 26 21


PFHxS in FT
Concentration (ng/g ww)
Lab code
5 6 7 8 9 10 11 13 16 19 20 21 22 23 26 27 29 30 33 36 38


Fish tissue - PFDA
Z-score
labcode
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Z=2
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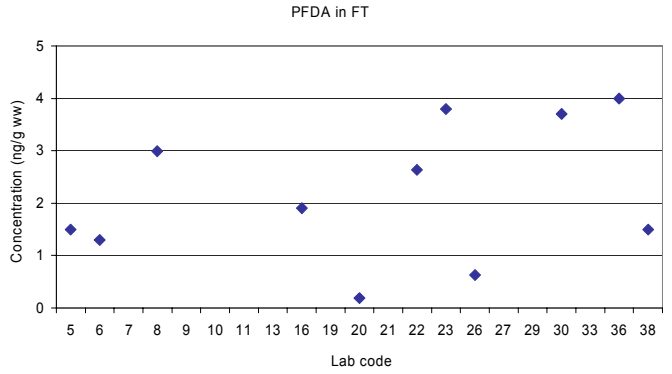
Z=-3
20 26 6 5 38 16 22 8 30 23 36 27 9


PFDA in FT
Concentration (ng/g ww)
Lab code
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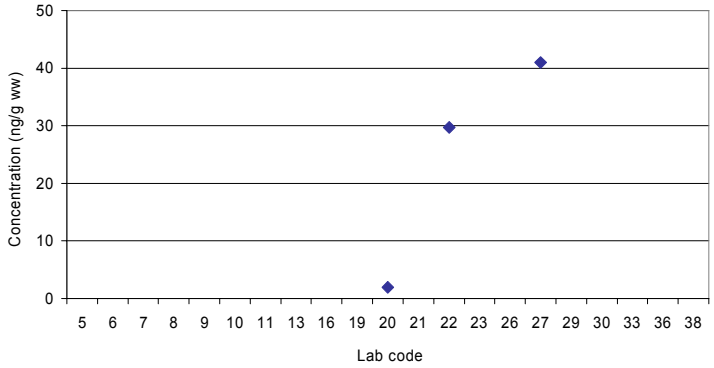
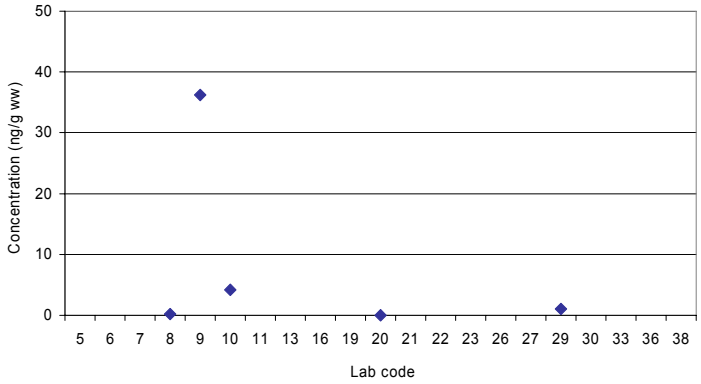
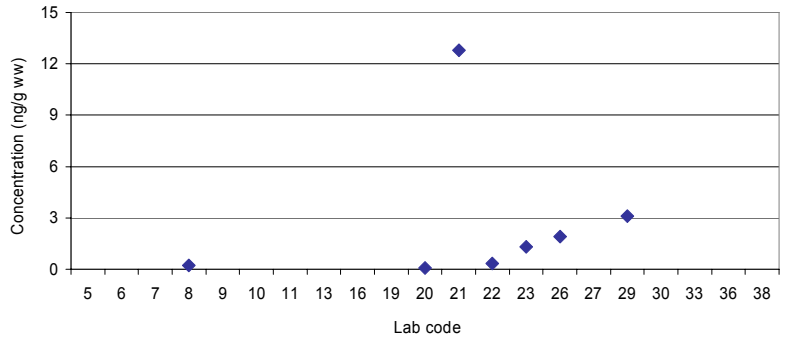
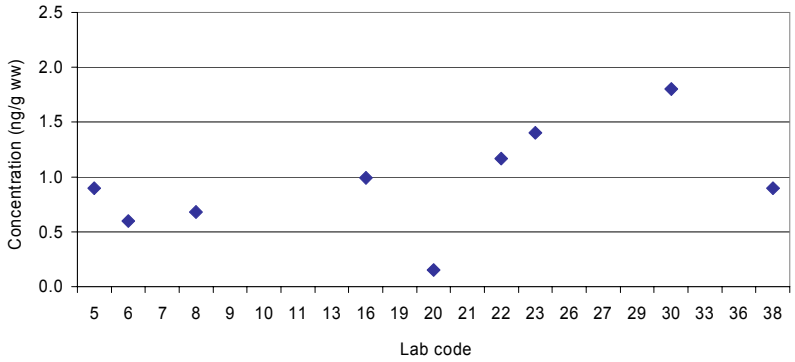

PFBA in FT
Concentration (ng/g ww)
Lab code
5 6 7 8 9 10 11 13 16 19 20 21 22 23 26 27 29 30 33 36 38


PFHxA in FT
Concentration (ng/g ww)
Lab code
5 6 7 8 9 10 11 13 16 19 20 21 22 23 26 27 29 30 33 36 38

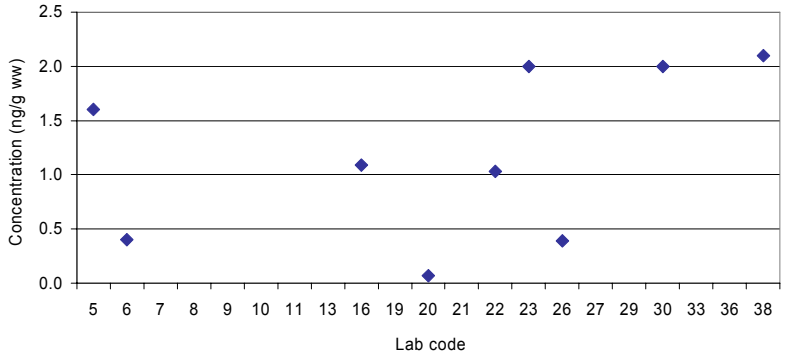
PFHpA in FT



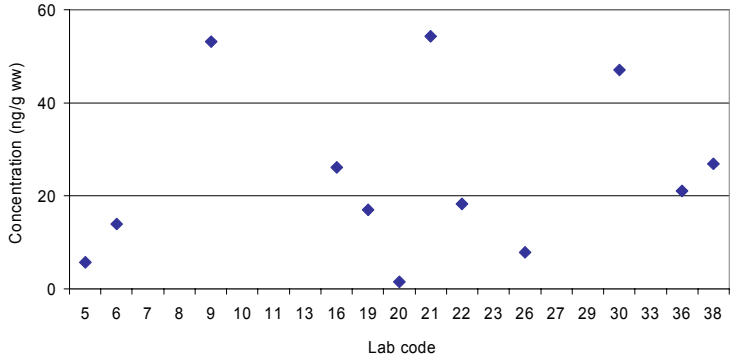
PFNA in FT



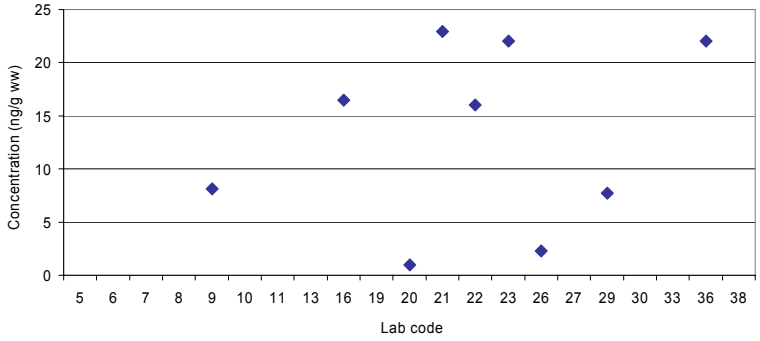
PFUnA in FT



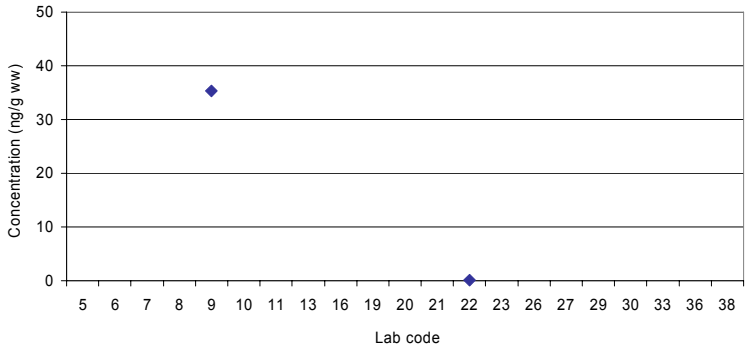
PFDoA in FT



PFBS in FT



PFDS in FT



Appendix 5: Results for water

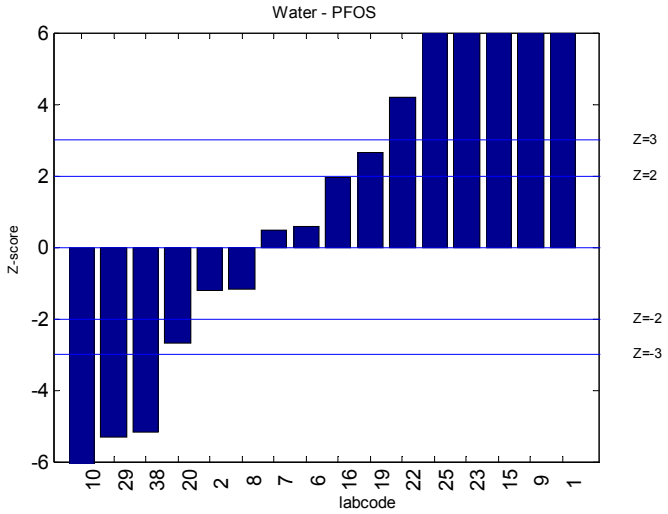
Water results in ng/l are presented as they were reported from each laboratory. Average, median and standard deviation was calculated after removing “non detect” values and with as many digits the laboratories reported. Distribution figures are given for all compounds.

NObs > LOQ	Number of observations above limit of quantification
NObs < LOQ	Number of observations below limit of quantification
Assigned value	True value obtained using Cofino statistics
-	Not analysed
ND	Not detected
NQ	Not quantified
<	Less than

Table A5. Overview of submitted results and statistical data for the water sample (ng/l)

Lab code	PFBA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFOS	PFDS	PFOSA	TH-PFOS
1c*	-	94.40	17.10	44.10	9.40	6.40	14.10	6.50	-	-	112.00	16.40	8.10	
2	-	4.02	2.48	20.0	1.45	1.09	<0.506	<0.361	16.3	5.02	16.6	-	1.46	
6	-	-	< 4	11	< 2	< 2	< 2	< 2	-	< 4	21	-	< 1	
7	-	-	IS**	15.2	n.d.	0.3	0.3	-	-	5.6	20.7	-	0.8	
8	-	4.2	2.11	18.75	0.55	0.78	-	-	-	4.45	16.66	-	1.42	
9	-	233.5	-	117.5	62.0	29.6	37.0	43.8	-	232.0	105.0	39.4	2.31	
10	-	< 0,5	-	3.4	-	-	-	-	-	< 0,5	4.7	-	< 0,5	
13	-	-	-	10	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	
15	-	-	-	31	-	< LOD	-	-	16	-	56	-	< LOD	
16	22.86	5.04	2.09	17.1	0.46	0.31	<0.04	<0.04	13.18	6.63	24.3	-	0.8	
19	-	-	-	190	-	-	-	LQL	8.4	7.6	26	-	4.0	
20	1.2	10.7	1.9	10.8	0.5	0.4	<LOD	<LOD	30.6	4.3	13.0	-	1.2	
22	<0.02	8.23	3.74	29.9	2.76	1.50	0.13	<0.03	17.0	8.01	29.8	0.23	0.94	186
23		-	-	30	0.9	2.5	0.5	<0,2	-	7.7	47	3.1	4	100
25	-	44.7	-	44.5	5.9	10.7	< LOD	< LOD	27.4	10.7	41.7	-	< LOD	
28c	-	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<5000	<5000	<5000	-	<20000	
29	-	6.1	4.2	13	VS**	<1.0	<1.0	IS**	8.7	6.8	6.6	-	-	
30	-	-	-	-	<LOD	<LOD	<LOD	<LOD	-	-	-	-	-	
33c	-	-	-	110	-	-	-	-	-	-	-	-	-	
38	-	-	-	29.5	2.6	<LOD	<LOD	<LOD	-	-	6.9	-	-	
NObs > LOQ	2	9	7	18	10	10	5	2	8	11	16	4	10	2
NObs < LOQ	1	2	2	1	4	7	10	11	1	3	1	-	5	-
Spiked amount	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Assigned value	-	5.0	-	19.4	-	0.42	-	-	-	6.3	19.5	-	1.0	-
Min	1.2	4.02	1.9	3.4	0.46	0.3	0.13	6.5	8.4	4.3	4.7	0.23	0.8	100
Max	22.86	233.5	17.1	190	62	29.6	37	43.8	30.6	232	112	39.4	8.1	186
Average		45.7	4.8	41	8.7	5.4	10.4		17.2	27.2	34.2		2.5	
Median		8.2	2.5	24.8	2.0	1.3	0.5		16.2	6.8	22.7		1.4	
St. dev.		76.6	5.5	49	19.0	9.2	16.0		8.0	68.0	32.4		2.3	
RSD		168	114	118	219	171	154		47	250	95		92	

* Submitted data converted to ng/l, ** VS: volume standard, IS: internal standard


Water - PFOS
Z-score
labcode
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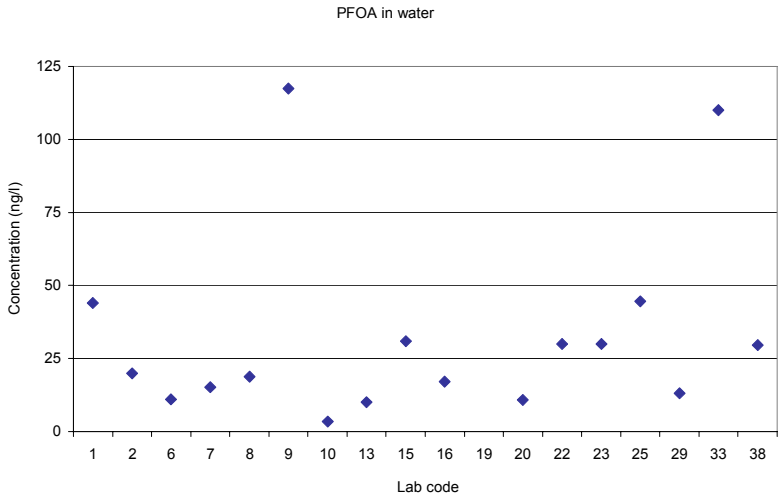
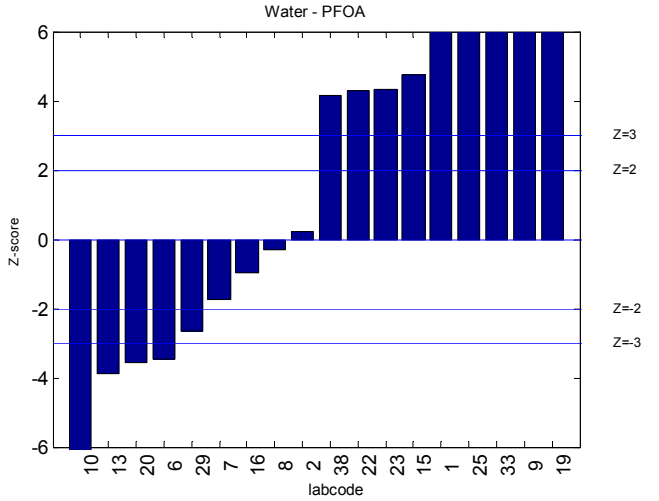
Z=2
Z=-2
Z=-3
6
4
2
0
-2
-4
-6
10 29 38 20 2 8 7 6 16 19 22 25 23 15 9 1


PFOA in water
Concentration (ng/l)
Lab code
125
100
75
50
25
0
1 2 6 7 8 9 10 13 15 16 19 20 22 23 25 29 33 38


Water - PFOA
Z-score
labcode
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4
2
0
-2
-4
-6
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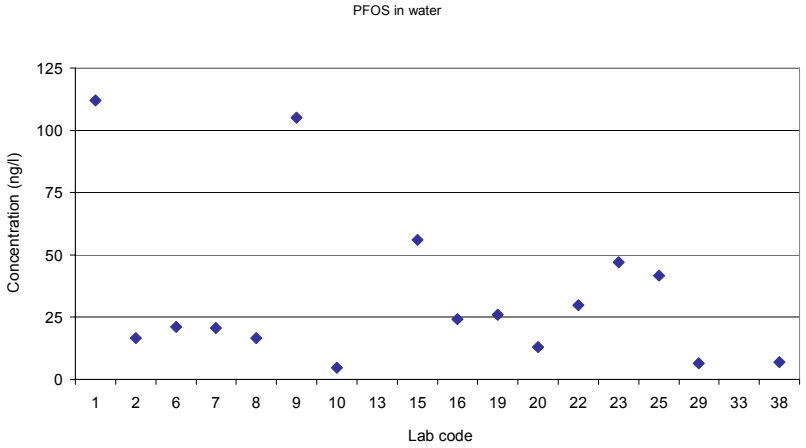
PFOS in water
Concentration (ng/l)
Lab code
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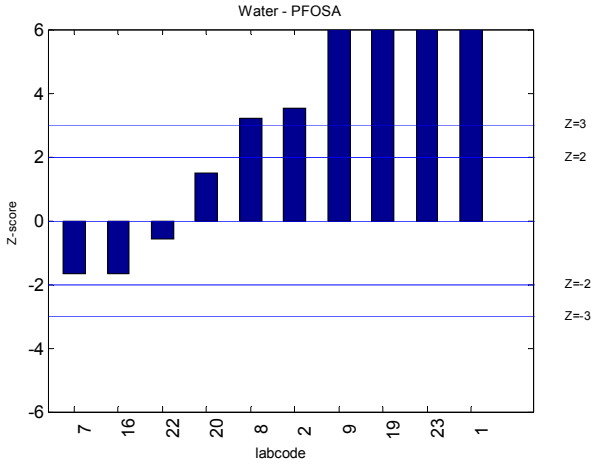
100
75
50
25
0
1 2 6 7 8 9 10 13 15 16 19 20 22 23 25 29 33 38


Water - PFOSA
Z-score
labcode
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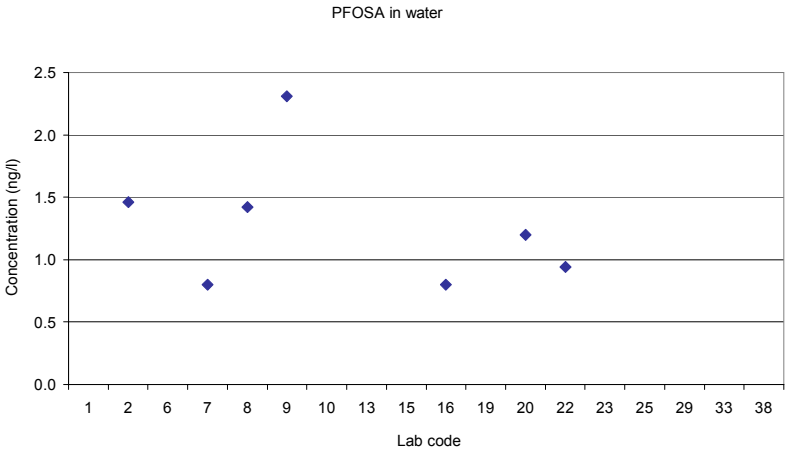
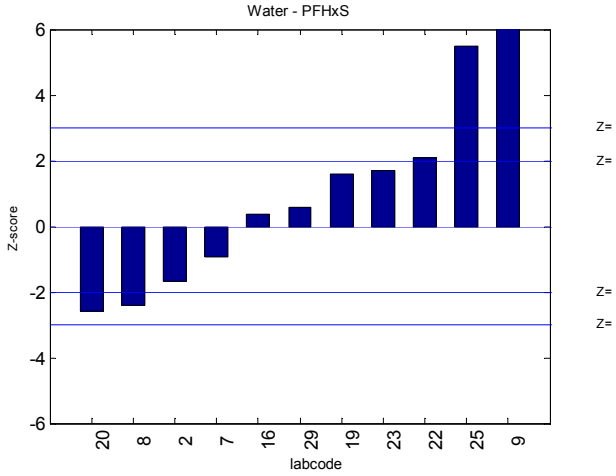
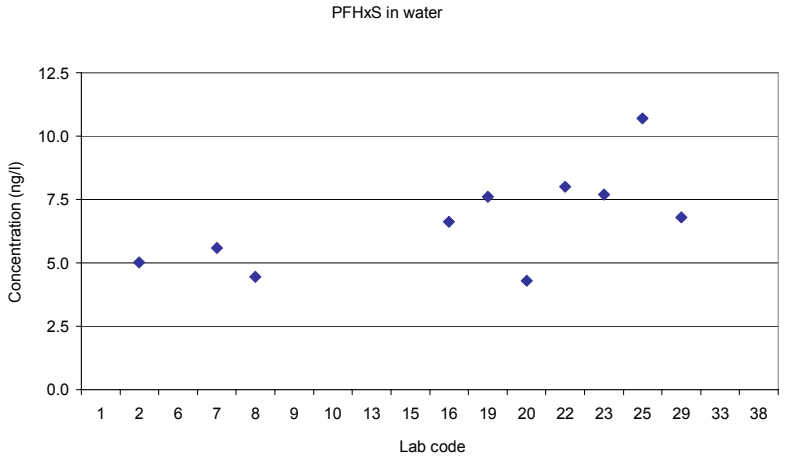
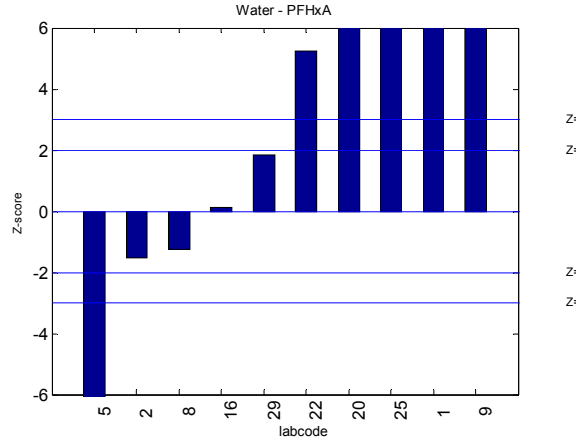
Z=-3


PFOSA in water
Concentration (ng/l)
Lab code


Water - PFHxS
Z-score
labcode


PFHxS in water
Concentration (ng/l)
Lab code


Water - PFHxA
Z-score
labcode
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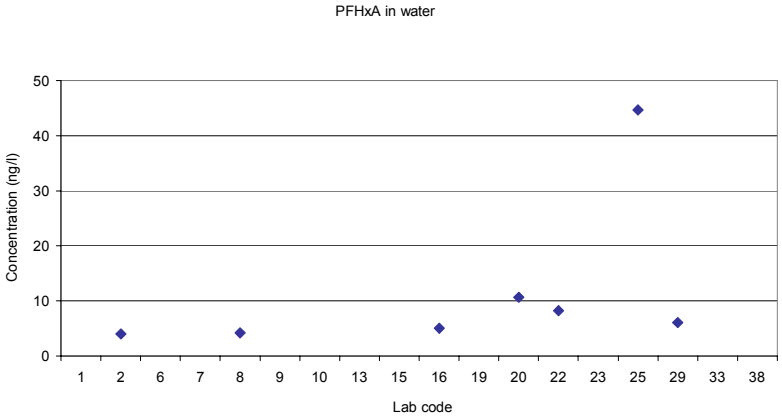
2
8
16
29
22
20
25
1
9


PFHxA in water
Concentration (ng/l)
Lab code
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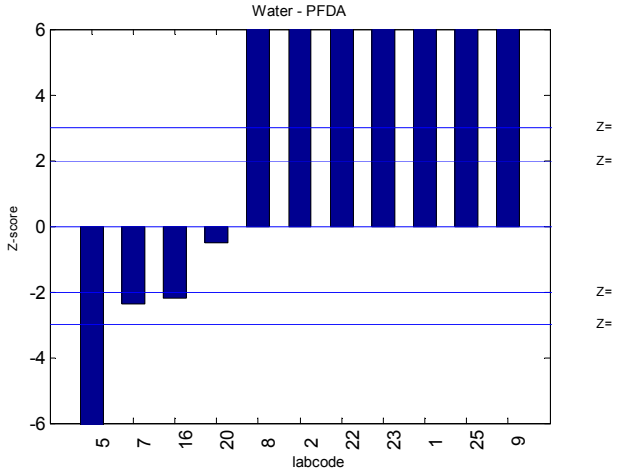
2
6
7
8
9
10
13
15
16
19
20
22
23
25
29
33
38


Water - PFDA
Z-score
labcode
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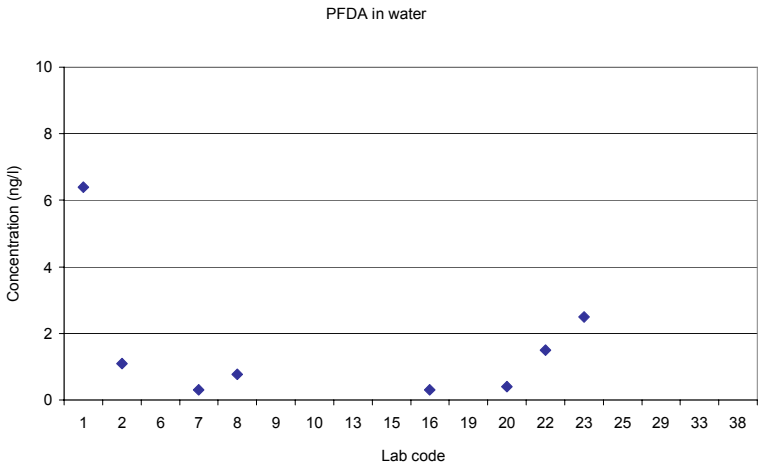
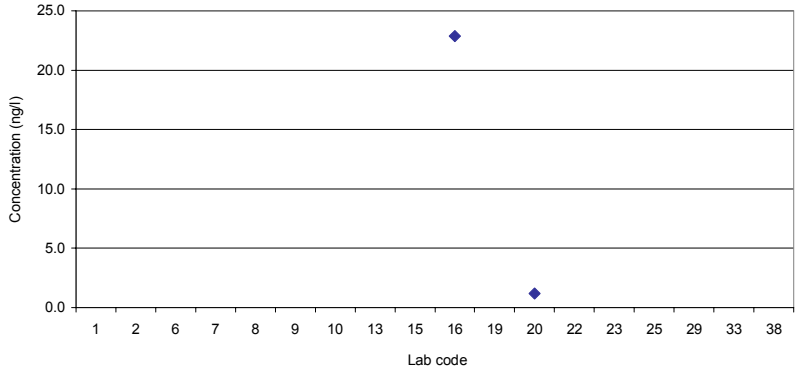
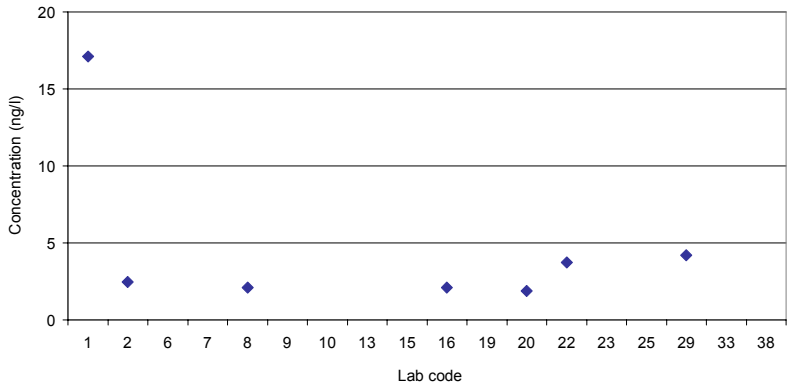
7
16
20
8
2
22
23
1
25
9


PFDA in water
Concentration (ng/l)
Lab code
1
2
6
7
8
9
10
13
15
16
19
20
22
23
25
29
33
38

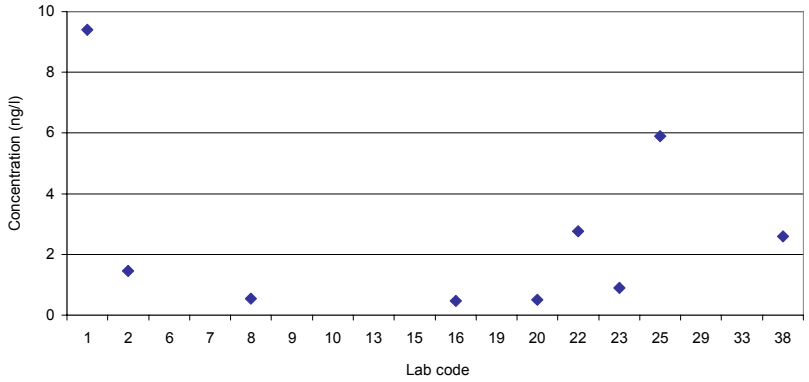
PFBA in water



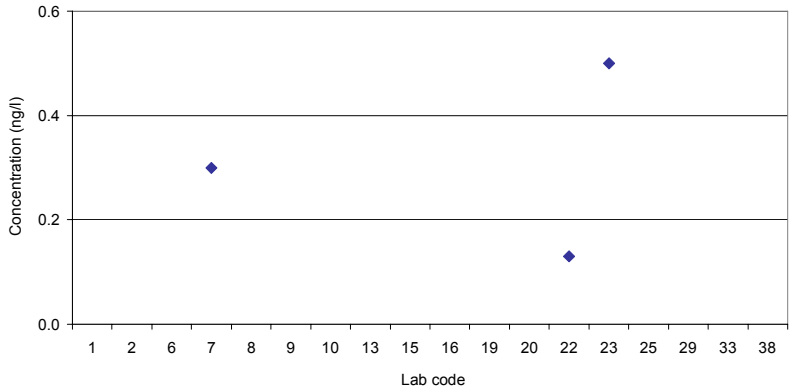
PFHpA in water



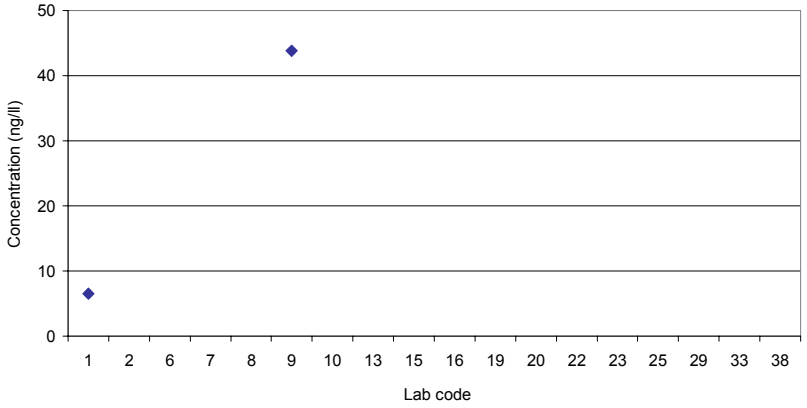
PFNA in water



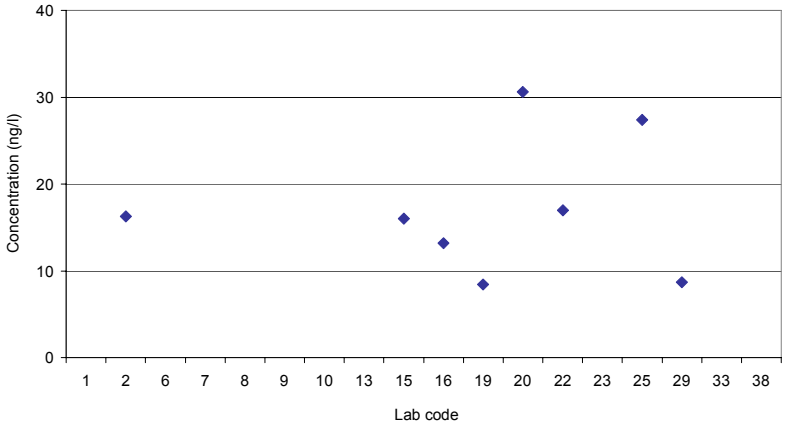
PFUnA in water



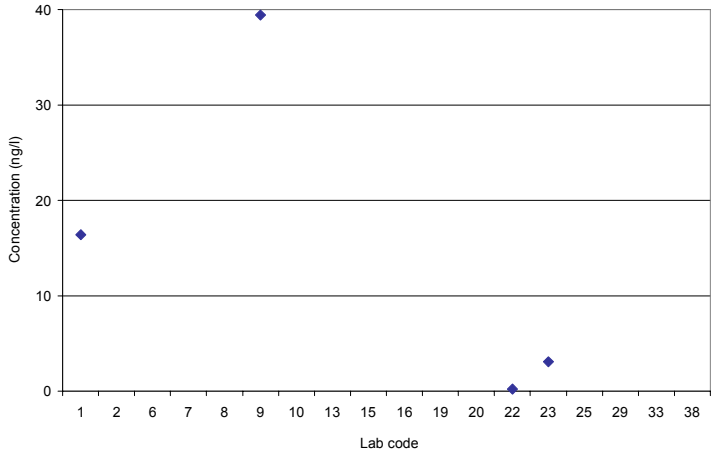
PFDoA in water



PFBS in water



PFDS in water



Appendix 6: Results for human matrices

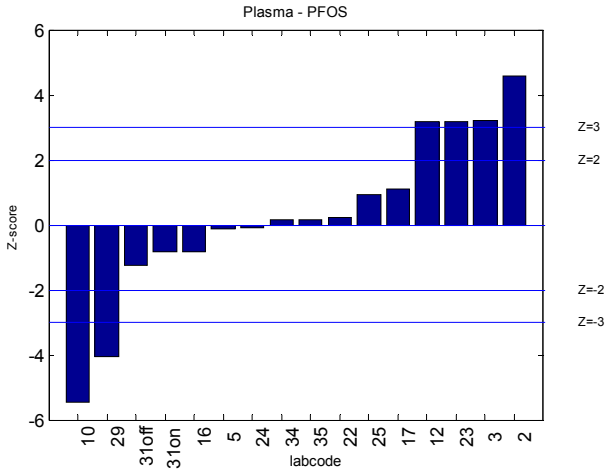
Plasma and whole blood results in ng/ml are presented as they were reported from each laboratory. Statistics and distribution figures are given for a compound if ≥5 laboratories submitted results above their limit of quantification. Average, median and standard deviation were calculated after removing "non detect" values and with as many digits the laboratories reported.

NObs > LOQ	Number of observations above limit of quantification
NObs < LOQ	Number of observations below limit of quantification
Assigned value	True value obtained using Cofino statistics
-	Not analysed
ND	Not detected
NQ	Not quantified
<	Less than

Table A6.1 Plasma results (ng/ml)

Lab. code.	PFOS	PFOA	PFNA	PFHxS	PFOSA	PFBS	PFDS	PFHpA	PFDA	PFUnA
2	34.9	2.11	0.909	0.616	<0.029	<0.342	-	<0.043	0.281	0.309
3	31.	1.19	0.77	-	-	-	-	ND	ND	ND
5	21.9	1.5	<0.4	-	<0.7	-	-	-	<0.4	<0.1
10	7.1	0.5	-	<0.05	<0.05	-	-	-	-	-
12	31	2.04	0.52	-	-	0.29	-	-	-	-
16	19.92	1.5	0.38	0.78	<0.01	0.02	-	<0.05	<0.05	0.14
17	25.3	2.0	0.62	-	ND	-	-	-	ND	-
22[a]	22.2	3.63	1.14	1.38	<0.16	<0.01	0.22	0.09	0.31	0.22
23[a]	31	2.4	0.72	1.9	0.62	<2.4	1.7	0.68	0.59	0.44
24	22	1.4	0.5	1.6	0.6	<2	<0.5	-	<0.2	<0.1
25	24.8	2.03	0.29	1.7	ND	ND	-	-	0.63	0.14
29	11	2	-	4	-	<0.2	-	0.55	<0.2	<0.1
30	-	5.2	NQ	-	-	-	-	-	NQ	NQ
31[b]	18.8	1.9	NQ	1.1	<0.2	0.5	-	<0.3	<0.3	<0.3
31[c]	19.9	1.8	0.4	0.8	0.1	0.5	-	<0.4	0.2	0.2
32	-	2.8	-	-	-	-	-	-	-	-
34	22.7	2.2	-	1.3	-	<1.0	-	-	-	-
35	22.7	1.8	1.25	-	-	-	-	-	-	-
NObs > LOQ	16	18	11	10	3	4	2	3	5	6
NObs < LOQ	-	-	1	1	8	7	1	5	7	5
Spiked amount	-	-	-	-	-	-	-	-	-	-
Assigned value	22.2	2.0	-	1.2	-	-	-	-	-	-
Min	7.1	0.50	0.29	0.62	0.1	0.02	0.22	0.09	0.2	0.14
max	34.9	5.2	1.25	4	0.62	0.5	1.7	0.68	0.63	0.44
Average	23	2.1	0.62	1.5					0.43	0.23
Median	23	2.0	0.57	1.3					0.45	0.20
St. dev.	7.2	1.1	0.26	0.97					0.21	0.12
RSD (%)	32	51	42	64					49	54

[a] Reported values transformed from ng/g to ng/ml using average density of human plasma 1.03 g/ml (Benson, Katherine. MCAT review. Emory University.1999).
[b] Lab 31 method denoted “off”
[c] Lab 31 method denoted “on”


Plasma - PFOS
Z-score
labcode
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Z=2
Z=-2
Z=-3
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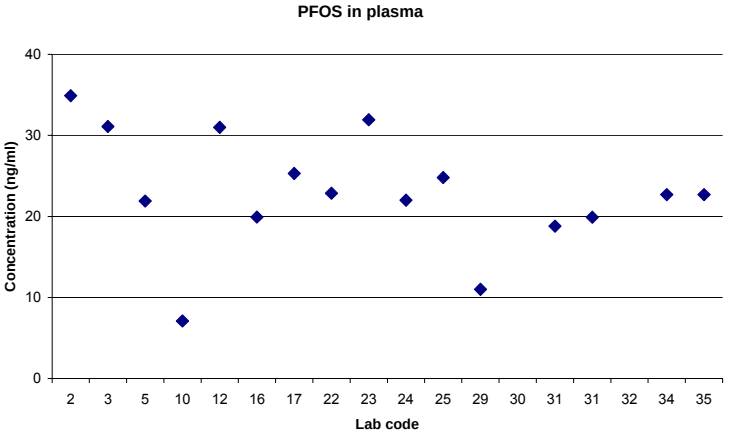
PFOS in plasma
Concentration (ng/ml)
Lab code
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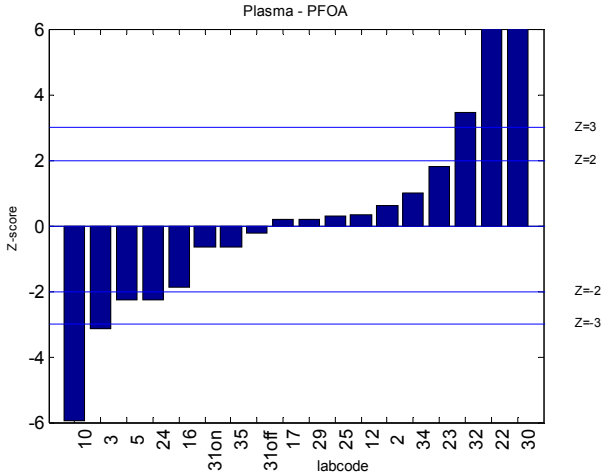
Plasma - PFOA
Z-score
labcode
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Z=2
Z=-2
Z=-3
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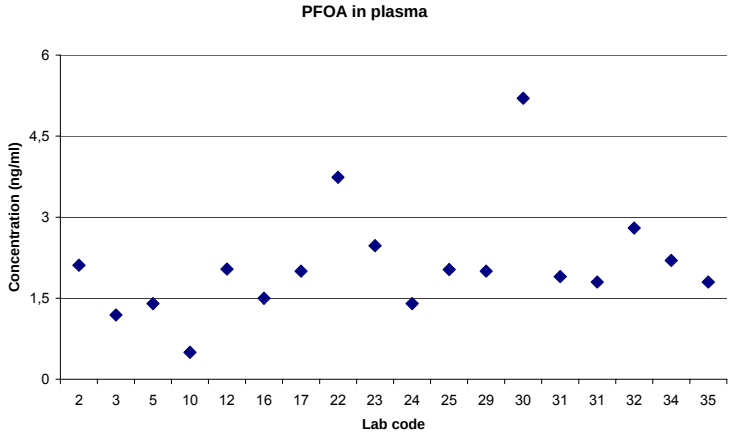
PFOA in plasma
Concentration (ng/ml)
Lab code
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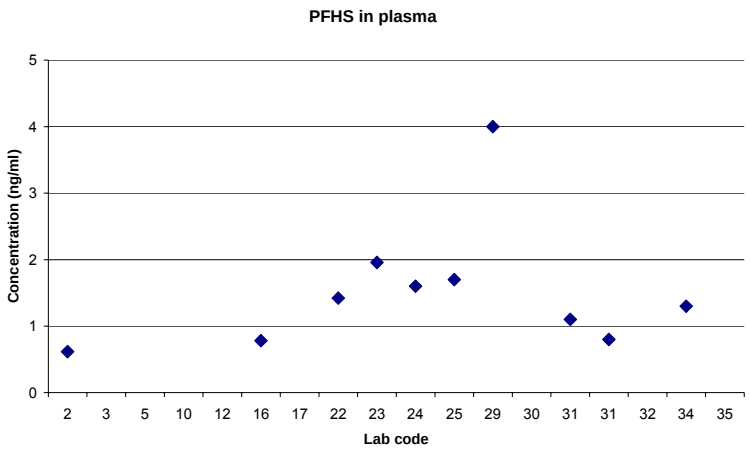
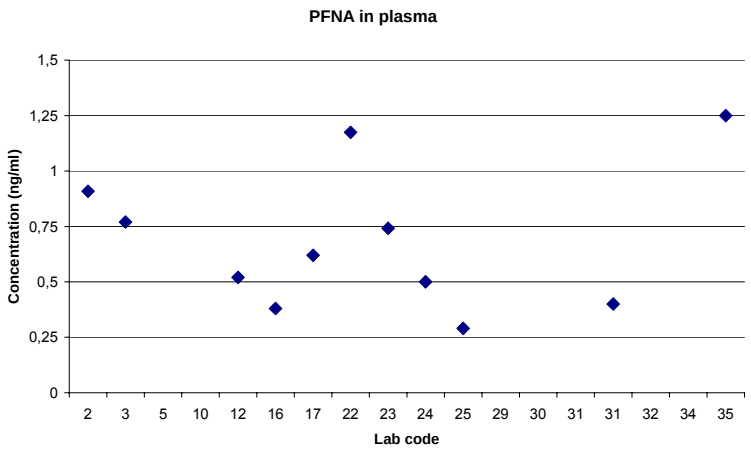

PFHS in plasma
Concentration (ng/ml)
0
1
2
3
4
5
2 3 5 10 12 16 17 22 23 24 25 29 30 31 31 32 34 35
Lab code


PFNA in plasma
Concentration (ng/ml)
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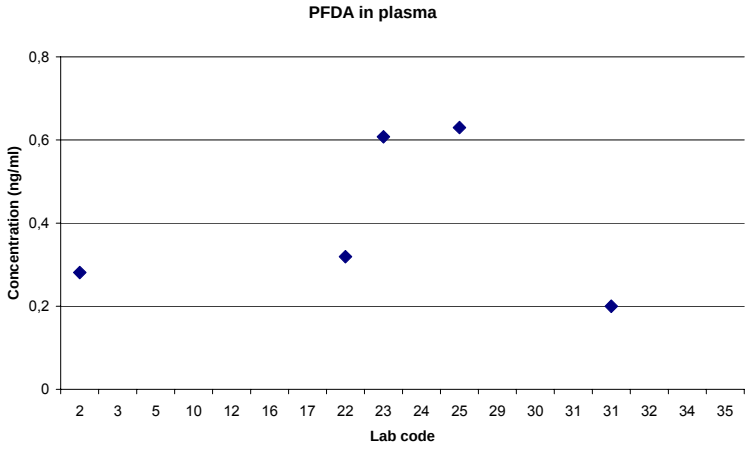
0,5
0,75
1
1,25
1,5
2 3 5 10 12 16 17 22 23 24 25 29 30 31 31 32 34 35
Lab code


PFDA in plasma
Concentration (ng/ml)
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0,4
0,6
0,8
2 3 5 10 12 16 17 22 23 24 25 29 30 31 31 32 34 35
Lab code

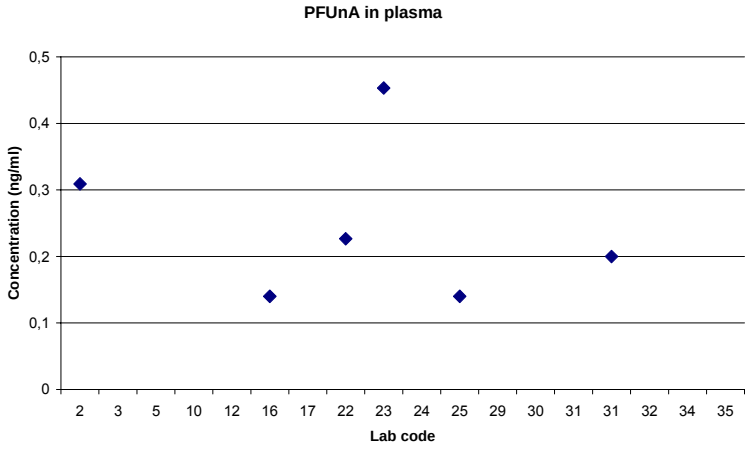
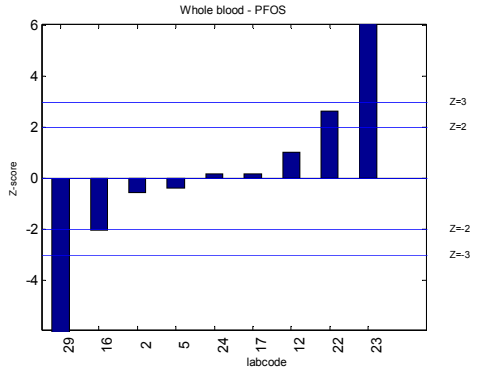

PFUnA in plasma
Concentration (ng/ml)
0
0,1
0,2
0,3
0,4
0,5
2 3 5 10 12 16 17 22 23 24 25 29 30 31 31 32 34 35
Lab code

Table A.6.2 Whole blood results (ng/ml)

Lab. code.	PFOS	PFOA	PFNA	PFHxS	PFOSA	PFBS	PFDS	PFHpA	PFDA	PFUnA
Lab 2	9.09	2.15	0.49	1.63	0.305	<0.006	-	0.116	0.239	0.275
Lab 5	9.3	1.7	<0.4	-	<0.7	-	-	-	<0.4	<0.1
Lab 12	10.6	2.13	0.19	-	-	<2	-	-	-	-
Lab 16	7.3	1.62	0.29	0.66	0.24	<0.02	-	<0.1	0.11	<0.2
Lab 17	10.0	1.8	0.53	-	ND	-	-	-	ND	-
Lab 22[a]	11.8	4.06	0.87	1.17	0.45	<0.01	0.02	0.22	0.47	0.26
Lab 23[a]	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	-	<0.1	<0.1
Lab 29	1.8	1.4	-	0.99	-	<0.2	-	0.54	<0.2	<0.1
Lab 30	-	3.6	NQ	-	-	NQ	-	-	NQ	ND
Lab 32	-	2.62	-	-	-	-	-	-	-	-
NObs > LOQ	9	11	7	6	5	0	1	3	4	2
NObs < LOQ	-	-	1		2	7	2		4	6
Spiked amount	-	-	-	-	-	-	-	-	-	-
Assigned value	9.8	1.8	-	1.4	0.39	-	-	-	-	-
Min	1.8	1.4	0.19	0.66	0.24			0.12	0.11	0.26
max	24	4.1	0.87	1.63	1.6			0.54	0.5	0.28
Average	10.4	2.2	0.47	1.23	0.62					
Median	10.0	1.9	0.49	1.29	0.45					
St. dev.	5.9	0.91	0.22	0.36	0.56					
RSD (%)	56	42	46	29	90					

[a] Reported values transformed from ng/g to ng/ml using average human whole blood density 1.060 g/ml (“Blood” Funk and Wagnalls Encyclopedia. 1985:157).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Z-score
labcode
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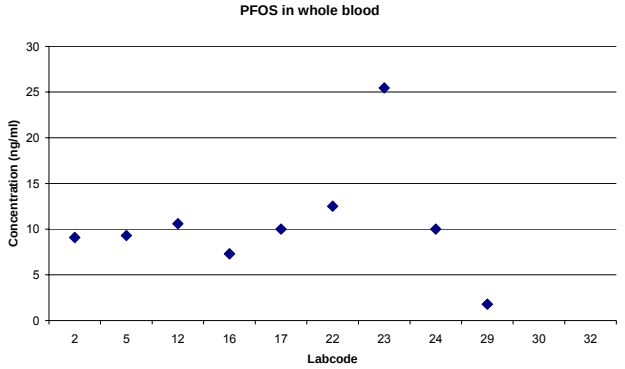
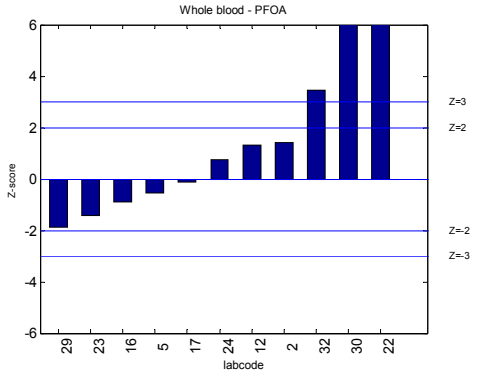
Z=-3


PFOS in whole blood
Concentration (ng/ml)
Labcode


Whole blood - PFOA
Z-score
labcode
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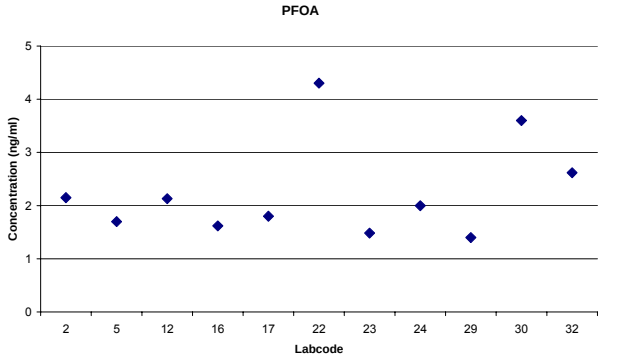
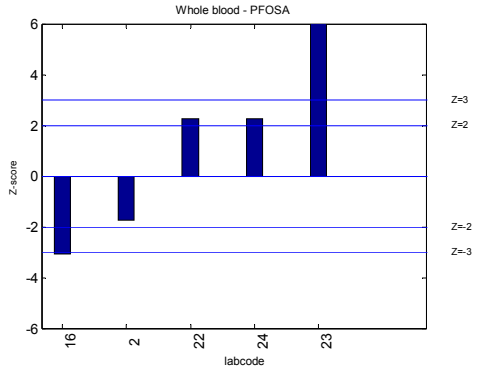
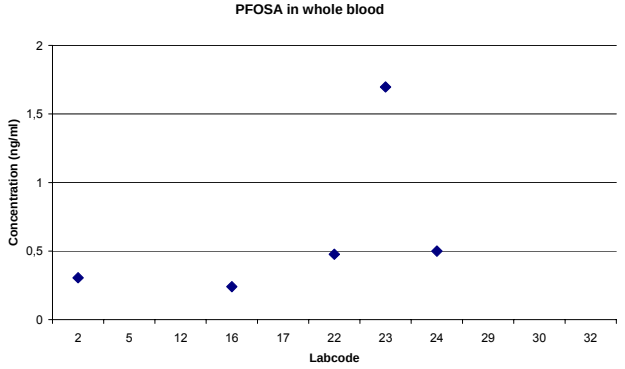
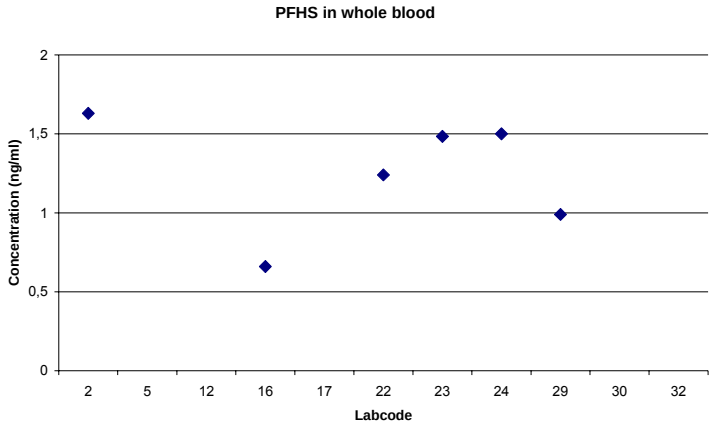
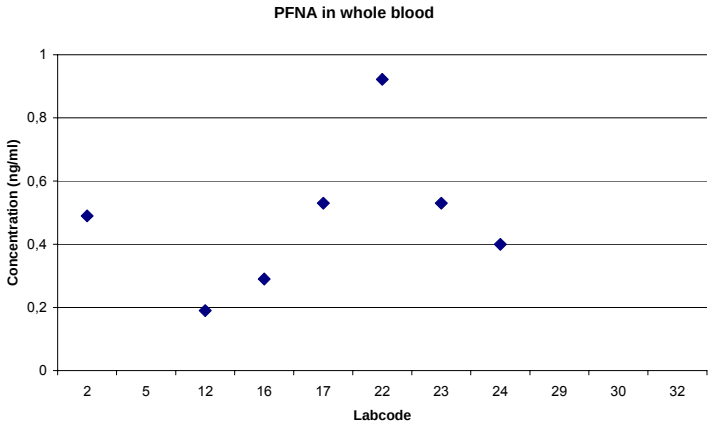
Z=2
Z=-2
Z=-3


PFOA
Concentration (ng/ml)
Labcode


Whole blood - PFOSA
Z-score
labcode
Z=3
Z=2
Z=-2
Z=-3


PFOSA in whole blood
Concentration (ng/ml)
Labcode


PFHS in whole blood
Concentration (ng/ml)
2
1,5
1
0,5
0
2 5 12 16 17 22 23 24 29 30 32
Labcode


PFNA in whole blood
Concentration (ng/ml)
1
0,8
0,6
0,4
0,2
0
2 5 12 16 17 22 23 24 29 30 32
Labcode