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National Measurement Institute

Proficiency Test Report AQA 16-06 PFOS/PFOA IN FISH, SOIL AND WATER

November 2016

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I would like to thank the management and staff of the participating laboratories for supporting the study. It is only through widespread participation that we can provide an effective service to laboratories.

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SUMMARY

AQA 16-06 was conducted in June 2016. Twenty-four laboratories registered to participate and submitted results.

Six test samples were prepared in the NMI North Ryde laboratory and consisted of:

- Two fish samples, one from a contaminated site (S1) and one formulated to contain 64.9 µg/kg total PFOS and 69.9 µg/kg PFOA (S2).
- Two soil samples, one from a contaminated site (S3) and one formulated to contain 25.7 µg/kg total PFOS and 14.8 µg/kg PFOA (S4).
- Two water samples, one from a contaminated site (S5) and one formulated to contain 7 µg/L total PFOS and 12 µg/L PFOA (S6).

The samples were sufficiently homogeneous and stable for evaluation of participants' performance.

Of a possible 321 numeric results a total of 242 (75%) were submitted.

The assigned values were the robust average of participants results. The associated uncertainties were estimated from the robust standard deviation of the participants' results.

Traceability: The consensus of participants' results is not traceable to any external reference, so although expressed in SI units, metrological traceability has not been established.

The outcomes of the study were assessed against the aims as follows:

- *to compare the performances of participant laboratories and to assess their accuracy in the measurement of total and linear PFOS and PFOA in fish, water and soil matrices;*

  Laboratory performance was assessed using both z-scores and E_n-scores.
  Of 232 z-scores, 213 (92%) were satisfactory with $|z| \leq 2$.
  Of 232 E_n-scores, 174 (75%) were satisfactory with $|E_n| \leq 1$.

  Laboratories **1**, **5**, **8**, **9**, **10**, **15**, **17**, **22** and **24** returned satisfactory z and E_n-scores for all the matrices that they analysed.

  Laboratory **23** performance was unsatisfactory for both z and E_n-scores.

- *evaluate the laboratories' methods;*

  Participants used a variety of methods for extraction. No correlation between results and method was evident. The analytical detection method of choice was LC-MS/MS.

- *develop the practical application of traceability and measurement uncertainty and provide participants with information that will be useful in assessing their uncertainty estimates.*

  Two hundred and thirty of two hundred and forty-two numeric results (95%) were reported with an associated estimate of expanded measurement uncertainty.

  The magnitude of these expanded uncertainties was within the range 0% to 72% of the reported value. Twenty-one were less than 10% relative, which the study coordinator believes is unrealistically small for a routine PFOS/PFOA measurement.

  Laboratory **16** and **18** did not report expanded measurement uncertainties for all the analytes.

1 INTRODUCTION

1.1 NMI Proficiency Testing Program

The National Measurement Institute (NMI) is responsible for Australia's national measurement infrastructure, providing a range of services including a chemical proficiency testing program. Proficiency testing (PT) is: 'evaluation of participant performance against pre-established criteria by means of interlaboratory comparison.'[1] NMI PT studies target chemical testing in areas of high public significance such as trade, environment, law enforcement and food safety. NMI offers studies in:

- pesticide residues in fruit and vegetables, soil and water;
- petroleum hydrocarbons in soil and water;
- metals in soil, water, food and pharmaceuticals;
- controlled drug assay;
- folic acid in flour; and
- allergens in food.

1.2 Study Background

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are synthetic fluorinated chemicals used as a surface-active agent and in a variety of products such as firefighting foams, coating additives and cleaning products.

They are persistent in the environment and resistant to environmental degradation processes. PFOS and PFOA were in 2010 added to the list of chemicals regulated under the international Stockholm Convention for Persistent Organic Pollutants, to which Australia is a signatory.

1.3 Study Aims

The aims of the study were to:

- compare the performances of participant laboratories and assess their accuracy in the measurement of total and linear PFOS and PFOA in fish, soil and water matrices;
- evaluate the laboratories' test methods; and
- develop the practical application of traceability and measurement uncertainty and provide participants with information that will be useful in assessing their uncertainty estimates.

1.4 Study Conduct

The conduct of NMI proficiency tests is described in the NMI Chemical Proficiency Testing Study Protocol.[2] The statistical methods used are described in the NMI Chemical Proficiency Statistical Manual.[3] These documents have been prepared with reference to ISO 17043[1] and The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories.[4]

The study falls within the scope of NMI's accreditation as a proficiency testing provider.

Testing for homogeneity of fish samples was subcontracted to National Research Centre for Environmental Toxicology (ENTOX) from University of Queensland.

2 STUDY INFORMATION

2.1 Study Timetable

The timetable of the study was:

Invitation issued	12 May 2016
Samples dispatched	20 June 2016
Results due	12 August 2016
Interim report issued	24 August 2016

2.2 Participation

One hundred and twenty six Australian and international laboratories were invited to participate. Twenty-four laboratories participated (see Appendix 1) and submitted results by the due date.

2.3 Test Material Preparation

Six test samples were prepared in April-May 2016. Care was taken to avoid any Teflon contamination during sample preparation.

- Two fish samples S1 and S2 each 5 g, one with incurred contaminants and one spiked with a commercial standard of PFOS and PFOA isomers.
- Two soil samples S3 and S4 each 10 g, one from a contaminated site and one spiked with a commercial standard of PFOS and PFOA isomers.
- Two water samples S5 and S6 each 2x50 mL, one from a contaminated site and one spiked with a commercial standard of PFOS and PFOA isomers.

Analytical standards used for spiking Samples S2, S4 and S6 were purchased from Sigma Aldrich. On the analytical report provided with the standards, PFOA (product 33824, batch SZBD308XV) has a stated purity of 99.4% and total PFOS (product 33829, batch SZBE107XV) of 98%. No value was provided for linear PFOS.

2.4 Test Material Homogeneity and Stability Testing

The preparation of the samples and their testing for homogeneity is described in Appendix 2. Sample preparation has been demonstrated to yield sufficiently homogeneous samples for all three matrices.

Stability study for soil and water was conducted in AQA 15-03[5] and found no significant changes in analytes' concentration. No stability study for these matrices was conducted in this PT study. Participants' results gave no reason to question the stability of soil and water test samples.

A stability study of the fish samples was carried out to cover the storage condition during transport, at room temperature, for one month starting 17/06/2016 (before sample dispatch). The stability results for total and linear PFOS and PFOA are given in Appendix 2. Fish samples were demonstrated to be sufficiently stable for use in this PT study.

2.5 Laboratory Code

All laboratories that agreed to participate were assigned a confidential code number.

2.6 Sample Storage, Dispatch and Receipt

Prior to dispatch soil and water samples were refrigerated at 4°C while fish samples were kept in the freezer at -80 °C.

Participants were sent 5 g fish in Greiner tubes for each of Sample S1 and S2, 10 g soil in Greiner tubes for each of Sample S3 and Sample S4 and two 50 mL water in HDPE bottles for each of Sample S5 and Sample S6. The samples were packed in a foam box with a cooler brick and sent by courier on 20 June 2016.

The following items were packaged with the samples:

- a covering letter which included a description of the test samples and instructions for participants; and
- a faxback form for participants to confirm the receipt and condition of the samples.

An Excel spreadsheet for the electronic reporting of results was e-mailed to participants.

2.7 Instructions to Participants

Participants were instructed as follows:

- Quantitatively analyse the samples using your normal test method.
- Report results in units of **µg/kg** on **as received basis** for fish and soil samples
- Report results in units of **µg/L** for water samples
- For each analyte in each sample report a single result expressed as if reporting to a client (ie correct for recovery or not, according to your standard procedure). This figure will be used in all statistical analysis in the study report.
- For each analyte in each sample report the associated expanded measurement uncertainty (eg 0.50 ± 0.02 µg/kg).
- Report any analyte not tested as NT.
- No limit of reporting has been set for this study. Report results as you would to a client, applying the limit of reporting of the method used for analysis.
- Report the basis of your uncertainty estimates (eg uncertainty budget, repeatability precision, long term result variability).
- Return the completed results sheet by e-mail proficiency@measurement.gov.au),
- **Please return completed result sheet by 12 August 2016. Late results cannot be included in the study report.**

2.8 Interim Report

An interim report was emailed to participants on 24 August 2016.

3 PARTICIPANT LABORATORY INFORMATION

3.1 Test Methods Reported by Participants

Table 1 Test methods – Samples S1 and S2 fish

Lab Code	Sample weight (g)	Sample pretreatment	Extraction technique	Extraction solvent(s):	Clean-up	Equipment	Column type	Column Specifications:	Extra column for blank separation
1	1		Sonication	Acetonitrile / ammonium hydroxide	Weak anion	Low resolution LC-MSMS	C18	5cm x 2.0mm x 1.6µm	Yes
2	1		Solvent	Acetonitrile		LC-MSMS	C18	5cm x 2.1mm x 1.8µm	No
3	1	Frozen	QuEChERS	Acetonitrile	SPE	LC-MSMS (Qtrap)	C18	4.6cm x 100mm x 2.6µm	Yes
4	1		Sonication	Methanol	Filtration 0.2 um cellulose	LC-MSMS	C18 BEH	5cm x 2.1mm x 1.7µm	No
7	0.3 0.23		Sonication	Methanol		High resolution LC-MSMS	Nucleodur Sphinx	3 µm 100/2	No
9	1	200 mM NaOH	Sonication	Acetonitrile	Active Carbon	Low resolution LC-MSMS	C18 Gemini NX	5cm x 2.0mm x 3.0µm	No
10	0.5		LLE	Acetonitrile	SPE-WAX	LC-MSMS	C18	15cm x 2.0mm x 3.0µm	Yes
11	4.3 4.7		Mechanical agitation	Methanolic potassium hydroxide	SPE	LC-MSMS	C18	10cm x 2.1mm x 3.5µm	No
12	0.05	Enzyme treatment, hexane extraction	Ion Pairing	MTBE		Low resolution LC-MSMS	ODS AQ	15cm x 2.0mm x 3µm	Yes

Lab Code	Sample weight (g)	Sample pretreatment	Extraction technique	Extraction solvent(s):	Clean-up	Equipment	Column type	Column Specifications:	Extra column for blank separation
14	1			Buffered MTBE		LC-MSMS	C18	10cm x 3.0mm x 2.6µm	No
15	0.5	Alkalinization	SPE	50 mM NaOH MeOH	SPE	LC-MSMS	HSS PFP	15cm x 2.1mm x 1.8µm	Yes
16	0.5			MTBE/TBAS ion pairing		Low resolution LC-MSMS	C18	5cm x 2.0mm x 1.6µm	No
18	1		Solid/Liquid	Acetonitrile	C18, Carbon	Low resolution LC-MSMS	C18	10cm x 2.1mm x 1.8µm	No
24*	0.087/0.435 0.051/0.255 0.076/0.38 0.087/0.435	Enzyme treatment, hexane extraction	Ion pairing, LLE	MTBE		LC-MSMS	ODS AQ	15cm x 2.0mm x 3µm	Yes

*Laboratory 24 sample weight represents sample/water (g/mL).

Table 2 Test methods – Samples S1 and S2 fish (cont'd)

Lab code	Internal std*	Recovery std**	Recovery correction	If yes, which method?	Standard Method used	Blank corrected
1	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	^{13}C Int. std	No	No
2	$^{13}C_4$PFOS; $^{13}C_4$PFOA	PFOS, PFOA PFNA	No		04-068	No
3	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std	No	No
4	$^{13}C_4$PFOA		No		No	No
7	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOA	Yes	Isotope dilution	No	Yes
9	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	Isotope dilution	No	No
10	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{18}O_2$PFOS; $^{13}C_8$PFOA	Yes	^{13}C Int. std	No	Yes
11	$^{13}C_4$PFOS; $^{13}C_2$PFOA	$^{13}C_2$PFOUEA; $^{13}C_4$PFOA	Yes	^{13}C Int. std Isotope dilution	No	No
12	$^{13}C_4$PFOS; $^{13}C_4$PFOA		No		No	No
14	$^{13}C_4$PFOS; $^{13}C_4$PFOA		No		No	No
15	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std Isotope dilution	No	Yes
16	$^{13}C_8$PFOS; $^{13}C_4$PFOA	$^{13}C_4$PFOS	No		No	No
18	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std	No	No
24	$^{13}C_4$PFOS; $^{13}C_4$PFOA		No		No	No

Note: * Internal standard refers to a labelled compound which is spiked directly into samples prior to extraction.

** Recovery standard refers to a labelled compound which is added to the final sample extract prior to instrumental analysis.[5]

Table 3 Test methods – Samples S3 and S4 soil

Lab Code	Sample weight (g)	Sample pretreatment	Extraction technique	Extraction solvent(s):	Clean-up	Equipment	Column type	Column Specifications:	Extra column for blank separation
1	0.5		Sonication	Methanol / ammonium hydroxide	Carbon SPE	Low resolution LC-MSMS	C18	5cm x 2.0mm x 1.6µm	Yes
2	1		Solvent	Acetonitrile		LC-MSMS	C18	5cm x 2.1mm x 1.8µm	No
3	1	Cool		Acetonitrile/methanol	SPE	LC-MSMS (Qtrap)	C18	4.6cm x 100mm x 2.6µm	Yes
4	1		Sonication	Methanol	Filtration 0.2 um cellulose	LC-MSMS	C18 BEH	5cm x 2.1mm x 1.7µm	No
5	1		Sonication	Methanol	DSPE (active carbon)	High resolution LTQ-Orbitrap	C18	15cm x 2.1mm x 3µm	No
7	0.02		Sonication	Methanol		High resolution LC-MSMS	Nucleodur Sphinx	3µm 100/2	No
8	0.1/1 0.1		Sonication	Methanol	SPE	LC-MSMS	C8	3cm x 100mm x 3.5µm	No
9	1		Sonication	Methanol / ammonium hydroxide	Active Carbon	Low resolution LC-MSMS	C18 Gemini NX	5cm x 2.0mm x 3.0µm	No
11	21.2 23.3	Acidification	Mechanical agitation	Methanolic potassium hydroxide	SPE	LC-MSMS	C18	10cm x 2.1mm x 3.5µm	No
13	5 5			Methanol/water		LC-MSMS	C18 Zorbax extended	5cm x 2.1mm x 1.8µm	

Lab Code	Sample weight (g)	Sample pretreatment	Extraction technique	Extraction solvent(s):	Clean-up	Equipment	Column type	Column Specifications:	Extra column for blank separation
14	5			1% NH_4OH in methanol		LC-MSMS	C18	10cm x 3.0mm x 2.6µm	No
16	2			MTBE/TBAS ion pairing		Low resolution LC-MSMS	C18	5cm x 2.0mm x 1.6µm	No
17	2			Methanol/acetonitrile		Low resolution LC-MSMS	C18	10cm x 2.1mm x 1.7µm	Yes
18	1	Digestion with base	Solid/Liquid	Methanol	Carbon	Low resolution LC-MSMS	C18	10cm x 2.1mm x 1.8µm	No
19	1		Tumble	Methanol in 0.1%acetic acid and methanol in 0.1% ammonium hydroxide	Filter	LC-MSMS	C18	5cm x 2.0mm x 1.6µm	Yes
20	1			Methanol		LC-MSMS	C18	7.5cm x 3.0mm x 2.7µm	No
21	10.22 10.15	0.67 N NaOH mixed with sample, pH adjusted to ≤ 2	Leach	Methanol	Filtration	Low resolution LC-MSMS	C18	15cm x 2.0mm x 15µm	No
22	5.02 5.04		Shaker/sonication	Methanol/KOH	SPE	LC-MSMS	C18	15cm x 3.0mm x 1.7µm	

Table 4 Test methods – Samples S3 and S4 soil (cont'd)

Lab code	Internal std	Recovery std	Recovery correction	If yes, which method?	Method used	Blank corrected
1	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	^{13}C Int. std	No	No
2	$^{13}C_4$PFOS; $^{13}C_4$PFOA	PFOS, PFOA PFNA	No		04-068	No
3	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std	No	No
4	$^{13}C_4$PFOA		No		No	No
5	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	13C Int Std	No	No
7	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOA	Yes	Isotope Dilution	No	Yes
8	$^{13}C_8$PFO; $^{13}C_8$PFOA	[$^{13}C_7$]-PFUdA; [$^{13}C_3$]-PFHxS	No		No	No
9	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	Isotope Dilution	No	No
11	$^{13}C_4$PFOS; $^{13}C_2$PFOA	$^{13}C_2$PFOUEA; $^{13}C_4$PFOA	Yes	^{13}C Int. std, Isotope dilution	No	No
13	$^{13}C_8$PFOS $^{13}C_8$PFOA, $^{13}C_2$PFHxA, ^{13}C2-6:2FTS, d_5-N-EtFOSAA	$^{13}C_4$PFOS, $^{13}C_2$PFOA, d_3-N-MeFOSAA	Yes	Isotope Dilution	USEPA 537	No
14		$^{13}C_4$PFOS; $^{13}C_4$PFOA	No		USEPA 537	No
16	$^{13}C_8$PFOS; $^{13}C_4$PFOA	$^{13}C_4$PFOS	No		No	No
17	$^{13}C_4$PFOS; $^{13}C_4$PFOA		No		DIN38414	No
18	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std	No	No
19	$^{13}C_4$PFOS	$^{13}C_8$PFOS; $^{13}C_4$PFOA	Yes	^{13}C Int. std	ASTMD 7969-14	No
20		PFOS; PFOA	No		EPA	No
21	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	Isotope Dilution	No	No
22	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	Isotope Dilution	No	No

Note: * Internal standard refers to a labelled compound which is spiked directly into samples prior to extraction.** Recovery standard refers to a labelled compound which is added to the final sample extract prior to instrumental analysis.[5]

Table 5 Test methods – Samples S5 and S6 water

Lab Code	Sample weight (mL)	Sample pretreatment	Extraction technique	Extraction solvent(s):	Clean-up	Equipment	Column type	Column Specifications:	Extra column for blank separation
1	1 (50 mL SPE)		Direct injection SPE (for PFOA)	Dilution Methanol Methanol / NH_4OH	RC filter SPE	Low resolution LC-MSMS	C18	5cm x 2.0mm x 1.6µm	Yes
	1		Direct injection	Dilution Methanol	RC filter				
2	10		SPE	Methanol	SPE	LC-MSMS	C18	5cm x 2.1mm x 1.8µm	No
3	100		Filter through glass microfibre		SPE	LC-MSMS (Qtrap)	C18	4.6cm x 100mm x 2.6µm	Yes
5	10		DLLME	Octanol		High resolution LTQ-Orbitrap	C18	15cm x 2.1mm x 3µm	No
6	1		Direct injection			Low resolution LC-MSMS	C18 BEH	5cm x 2.1mm x 1.7µm	No
7	30		SPE	Methanol		High resolution LC-MSMS	Nucleodur Sphinx	3µm 100/2	No
	5								
9	50/1		SPE/Direct injection		SPE	Low resolution LC-MSMS	C18 Gemini NX	5cm x 2.0mm x 3.0µm	No
11	66.8		SPE	Methanol	SPE	LC-MSMS	C18	10cm x 2.1mm x 3.5µm	No
	65.9								

Lab Code	Sample weight (mL)	Sample pretreatment	Extraction technique	Extraction solvent(s):	Clean-up	Equipment	Column type	Column Specifications:	Extra column for blank separation
13	50	0.5 g Trizma	SPE		SPE	LC-MSMS	C18 Zorbax extended	5cm x 2.1mm x 1.8µm	
14	0.5		Direct injection			LC-MSMS	C18	10cm x 3.0mm x 2.6µm	No
16	10		SPE			Low resolution LC-MSMS	C18	5cm x 2.0mm x 1.6µm	No
18	10		SPE	Methanol/water		Low resolution LC-MSMS	C18	10cm x 2.1mm x 1.8µm	No
19	0.01		Direct injection	MeOH to stabilise	Filter	LC-MSMS	C18	5cm x 2.0mm x 1.6µm	Yes
20	50		SPE	Methanol	SPE	LC-MSMS	C18	7.5cm x 3.0mm x 2.7µm	No
21	131.8 133.5		SPE	10% Methanol/NH_4OH		Low resolution LC-MSMS	C18	15cm x 2.0mm x 15µm	No
22	66.3 66.5		SPE	Methanol/NH_4OH	SPE	LC-MSMS	C18	15cm x 3.0mm x 1.7µm	
23	0.05 0.05		Direct injection			High resolution Orbitrap	C18	10cm x 2.1mm x 1.7µm	No

Table 6 Test methods – Samples S5 and S6 water (cont'd)

Lab code	Internal std*	Recovery std**	Recovery correction	If yes, which method?	Method used	Blank corrected
1	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	^{13}C Int. std	No	No
2	$^{13}C_4$PFOS; $^{13}C_4$PFOA	PFOS, PFOA PFNA	No		04-068	No
3	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std	EPA 537	No
5	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	13C Int Std	No	No
6		PFOS and PFOA spikes	No		No	No
7	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOA	Yes	Isotope Dilution	ISO	Yes
9	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	Isotope Dilution	No	No
11	$^{13}C_4$PFOS; $^{13}C_2$PFOA	$^{13}C_2$PFOUEA; $^{13}C_4$PFOA	Yes	^{13}C Int. std Isotope dilution	No	No
13						
14		$^{13}C_4$PFOS; $^{13}C_4$PFOA	No		USEPA 537	No
16	$^{13}C_8$PFOS; $^{13}C_4$PFOA	$^{13}C_4$PFOS	No		No	No
18	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std	No	No
19	$^{13}C_4$PFOS	$^{13}C_8$PFOS; $^{13}C_4$PFOA	Yes	^{13}C Int. std	USEPA 537	No
20		PFOS; PFOA	No		EPA	No
21	$^{13}C_4$PFOS; $^{13}C_4$PFOA	$^{13}C_8$PFOS; $^{13}C_8$PFOA	Yes	Isotope Dilution	No	No
22	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	Isotope Dilution	No	No
23	$^{13}C_4$PFOS; $^{13}C_4$PFOA		Yes	^{13}C Int. std	ISO 17025	No

Note: * Internal standard refers to a labelled compound which is spiked directly into samples prior to extraction.

** Recovery standard refers to a labelled compound which is added to the final sample extract prior to instrumental analysis.[5]

3.2 Basis of Participants' Measurement Uncertainty Estimates

Table 7 Basis of Participants' Uncertainty Estimate

Lab Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision	Method Bias	
1	Top Down - precision and estimates of the method and laboratory bias	Control samples Duplicate analysis	Recoveries of sample spike Standard Purity	Nata Technical Note 33
2	Method validation data	Control samples Duplicate analysis Instrument calibration	Instrument calibration Recoveries of sample spike Standard Purity	NMI Uncertainty Course
3	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram)	Control samples Duplicate analysis	CRM	Eurachem/CITAC Guide
4	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Recoveries of sample spike	ISO/GUM
5	Top Down - precision and estimates of the method and laboratory bias	Control samples	Recoveries of sample spike	Eurachem/CITAC Guide
6	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Recoveries of sample spike Standard Purity	Nata Technical Note 33
7	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples		ISO/GUM
8	Top Down - precision and estimates of the method and laboratory bias	Duplicate analysis	Recoveries of sample spike Standard Purity	Eurachem/CITAC Guide
9	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples Duplicate analysis Instrument calibration	Instrument calibration Recoveries of sample spike	Eurachem/CITAC Guide
10	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis		In-house SOP
11	Top Down - precision and estimates of the method and laboratory bias	Control samples Duplicate analysis Instrument calibration	CRM Recoveries of sample spike	ISO/GUM
12	Standard deviation of replicate analyses multiplied by 2 or 3	Duplicate analysis	Recoveries of sample spike	
13	QC DATA - Control Chart	Control samples Duplicate analysis Instrument calibration	CRM Instrument calibration Recoveries of sample spike	Within-laboratory data on bias and precision has been calculated as per the procedures outlined in ASTM E2554-13
14		Control samples Duplicate analysis Instrument calibration	Instrument calibration Recoveries of sample spike	

Lab Code	Approach to Estimating MU	Information Sources for MU Estimation		Guide Document for Estimating MU
		Precision	Method Bias	
15	Bottom Up (ISO/GUM, fish bone/ cause and effect diagram)	Duplicate analysis Instrument calibration	Instrument calibration Standard Purity	ISO/GUM
16	Top Down - precision and estimates of the method and laboratory bias	Control samples Duplicate analysis Instrument calibration	Laboratory bias from PT studies Recoveries of sample spike	Nata Technical Note 33
17	Top Down - precision and estimates of the method and laboratory bias			DIN38414
18	Standard deviation of replicate analyses multiplied by 2 or 3	Control samples		
19	Top Down - precision and estimates of the method and laboratory bias	Control samples Duplicate analysis	Recoveries of sample spike	Nata Technical Note 33
20	Top Down - precision and estimates of the method and laboratory bias	Control samples	Instrument calibration Recoveries of sample spike Standard Purity	ISO/GUM
21	Top Down - precision and estimates of the method and laboratory bias	Control samples	Recoveries of sample spike	Laboratory QA Manual
22	Top Down - precision and estimates of the method and laboratory bias	Control samples	Recoveries of sample spike	Standard practice for laboratories utilizing US EPA's SW-846 document.
23	Professional judgment		Instrument calibration Recoveries of sample spike	
24	Standard deviation of replicate analyses multiplied by 2 or 3	Instrument calibration	Recoveries of sample spike	

3.3 Participants' Comments

The study co-ordinator welcomes comments or suggestions from participants about this study or possible future studies. Participants' comments are reproduced in Table 6.

Table 8 Participants' Comments

Lab Code	Sample	Participants' Comments	Study manager response
1	All	Total PFOS is quantified using linear stds.	
2	All	Both total and linear PFOS were quantified using linear PFOS calibration standards. PFOA and PFOS results are calculated using PFOA-C_4 and PFOS-C_4 Internal standards respectively. For Sample S3 PFOS results are out side the range of the validated method. The method was modified for sample weights.	
3	S3, S5 and S6	A dilution was performed on PFOS, reported recovery was from the non-diluted data.	
5	All	Total PFOS and linear PFOS were quantified with the linear PFOS standard. Average results of 6 replicates for Samples S3 and S4 with RSD<13% and <11% and average results of 3 replicates for Samples S5 and S6 with RSD<6% and <2%.	
9	All	Calculation of Total PFOS based on linear PFOS standard	
10	S1 and S2	Linear PFOS contains branched isomer #2 (Potassium 1-trifluoromethylperfluorohepatanesulfonate)	
11	All	Samples were received above our method specifications at ambient temperature.	Stability studies for soil and water were performed in AQA 15-03. Analytes stability in fish samples was checked in this study. Samples were confirmed stable at room temperature for at least one month.
12	S2	The concentrations of PFOS and PFOA in the unknown fish sample were too high compared to the real samples.	The PT study was designed to cover a range of concentrations based on our participants' capabilities.
13	All	Should include the PFHxS as per recent enHealth advice.	PFHxS will be included in the next PT study.
14	All	Measured against non-matrix matched standards. Internal standard used for instrument correction only for samples S3 to S6.	
15	S1	Total PFOA is not detected.	
	All	Reported Total PFOA result is linear PFOA isomer only. Branched PFOA isomers not tested. Reported Total PFOS (sum of di, mono and linear isomers) used linear PFOS standard for calculation. Total PFOS using br-PFOS standard for calculation is S1 16 ug/Kg, S2 35 ug/Kg, S3 200 ug/Kg, S4 18 ug/Kg, S5 1.9 ug/L and S6 3.6 ug/L.	
20	S3, S4, S5 and S6	PFOA data calculated on the anion-basis and not calculated on the salt basis.	

Lab Code	Sample	Participants' Comments	Study manager response
21	All	Date analyzed above was date of last sample analysed. Total PFOA results are based on Linear PFOA standard. For Samples S3 and S4 two aliquots of the sample were received and were composited prior to taking sample aliquot for processing. Uncertainty is sd from calculated LCS measured amount. (Spike amount 20 ug/kg; n=53) Laboratory evaluates IS recovery but does not report percent recovery for IS. For Sample S3, the IS recoveries were outside of acceptance limits; sample showed signs of matrix interference For Samples S5 and S6 standard uncertainty is sd from calculated LCS measured amount. (Spike amount 0.2 ug/L; n=63) Laboratory evaluates IS recovery but does not report percent recovery for IS For Sample S6 results reported from the analysis of a diluted extract (5x) due to high concentration of the target analyte in the analysis of the undiluted extract. As a result of dilution, IS did not meet criteria.	
22	S3, S4, S5 and S6	The laboratory reports only total PFOS for client data. The Total PFOS value is above the upper calibration limit for the instrument for S3, S5 and S6.	

4 PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

4.1 Results Summary

Participant results are listed in Tables 6 to 12 with the summary statistics robust average, mean, median, maximum, minimum, robust standard deviation (SD_{rob}) and robust coefficient of variation (CV_{rob}). Bar charts of results and performance scores are presented in Figures 2 to 8.

An example chart with interpretation guide is shown in Figure 1.

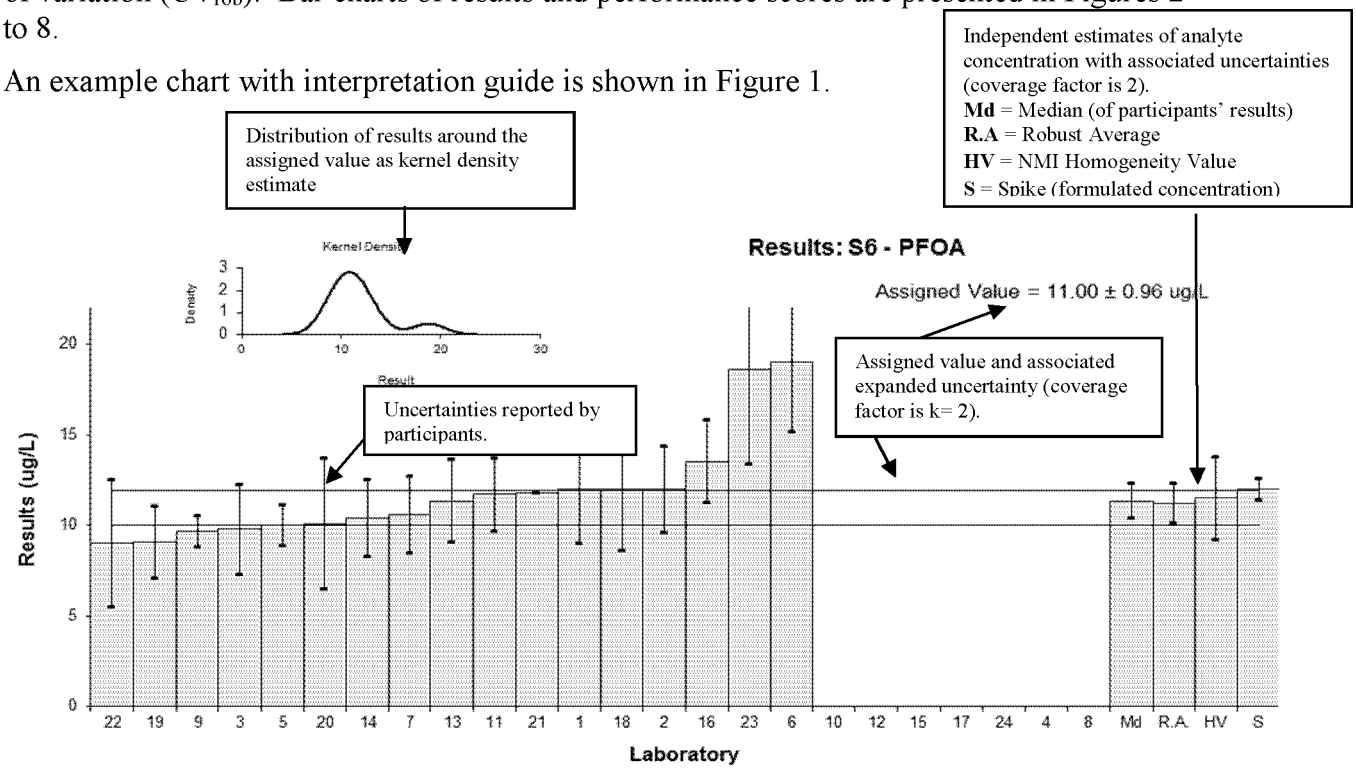


Figure 1 Guide to Presentation of Results

4.2 Assigned Value

The assigned value is defined[1] as: 'value attributed to a particular property of a proficiency test item.' In this study property is the mass fraction of analyte. Assigned values were the robust average of participants' results; the expanded uncertainties were estimated from the associated robust standard deviations.

4.3 Between-Laboratory Coefficient of Variation

The between laboratory coefficient of variation is a measure of the between laboratory variation that in the judgement of the study coordinator would be expected from participants given the analyte concentration. It is important to note this is a performance measure set by the study coordinator; it is not the coefficient of variation of participant results.

4.4 Target Standard Deviation

The target standard deviation (σ) is the product of the assigned value (X) and the between-laboratory coefficient of variation (CV). This value is used for calculation of participant z-score.

$$\sigma = X * CV \qquad \text{Equation 1}$$

4.5 z-Score

For each participant result a z-score is calculated according to Equation 2 below:

$$z = \frac{(\chi - X)}{\sigma} \qquad \textit{Equation 2}$$

where:

z is z-score
χ is participant result
X is the study assigned value
σ is the target standard deviation from equation 1

A z-score with absolute value (|z|):

- $|z| \leq 2$ is satisfactory;
- $2 < |z| \leq 3$ is questionable;
- $|z| > 3$ is unsatisfactory.

4.6 E_n-Score

The E_n-score is complementary to the z-score in assessment of laboratory performance. E_n-score includes measurement uncertainty and is calculated according to Equation 3 below:

$$E_n = \frac{(\chi - X)}{\sqrt{U_\chi^2 + U_X^2}} \qquad \textit{Equation 3}$$

where:

E_n is E_n-score
χ is a participant's result
X is the assigned value
U_χ is the expanded uncertainty of the participant's result
U_X is the expanded uncertainty of the assigned value

An E_n-score with absolute value ($|E_n|$):

- $|E_n| \leq 1$ is satisfactory;
- $|E_n| > 1$ is unsatisfactory.

4.7 Traceability and Measurement Uncertainty

Laboratories accredited to ISO/IEC Standard 17025:2015[6] must establish and demonstrate the traceability and measurement uncertainty associated with their test results.

Guidelines for quantifying uncertainty in analytical measurement are described in the Eurachem /CITAC Guide.[7]

4.8 Robust Average

The robust averages and associated expanded measurement uncertainties were calculated using the procedure described in 'ISO13528:2015(E), Statistical methods for use in proficiency testing by interlaboratory comparisons'.[8]

5 TABLES AND FIGURES

Table 9

Sample Details

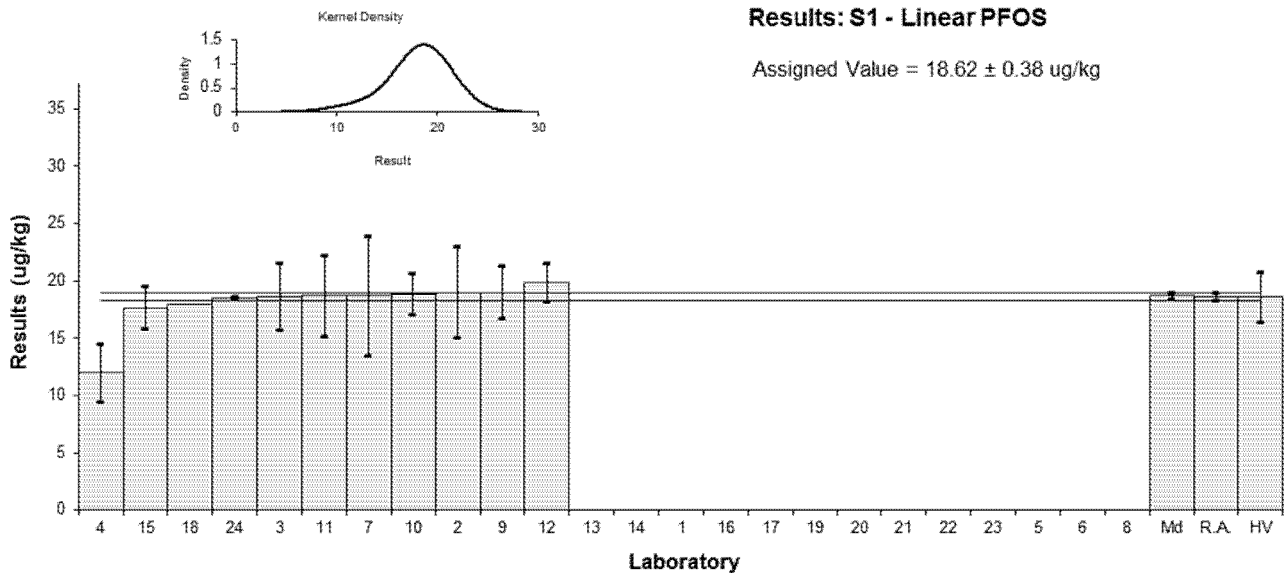
Sample No.	S1
Matrix.	Fish
Analyte.	Linear PFOS
Units	ug/kg

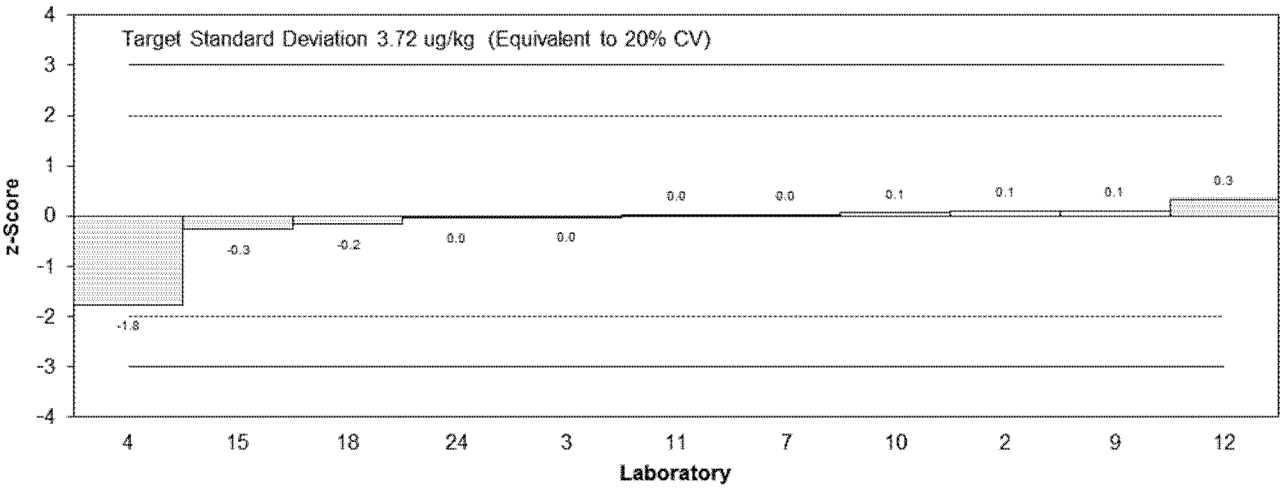
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	NT	NT	NT		
2	19	4.0	NR	0.10	0.09
3	18.6	2.9	84	-0.01	-0.01
4	12	2.514	77.9	-1.78	-2.60
5	NT	NT	NT		
6	NT	NT	NT		
7	18.7	5.2	42	0.02	0.02
8	NT	NT	NT		
9	19	2.3	90	0.10	0.16
10	18.841	1.839	96.8	0.06	0.12
11	18.7	3.5	69.5	0.02	0.02
12	19.86	1.66	86.05	0.33	0.73
13	NR	NR	NR		
14	NR	NR	NR		
15	17.642	1.844	92.67	-0.26	-0.52
16	NT	NT	NT		
17	NT	NT	NT		
18	18	NR	101	-0.17	-1.63
19	NT	NT	NT		
20	NT	NT	NT		
21	NT	NT	NT		
22	NT	NT	NT		
23	NT	NT	NT		
24	18.49	0.14	77	-0.03	-0.32

Statistics

Assigned Value	18.62	0.38
Spike	Not spiked	
Homogeneity Value	18.6	2.2
Robust Average	18.62	0.38
Median	18.70	0.30
Mean	18.08	
N	11	
Max.	19.86	
Min.	12	
Robust SD	0.50	
Robust CV	2.7%	


Kernel Density
Density
Result
Results: S1 - Linear PFOS
Assigned Value = 18.62 ± 0.38 ug/kg
Results (ug/kg)
Laboratory
4 15 18 24 3 11 7 10 2 9 12 13 14 1 16 17 19 20 21 22 23 5 6 8 Md R.A. HV


z-Scores: S1 - Linear PFOS
Target Standard Deviation 3.72 ug/kg (Equivalent to 20% CV)
z-Score
Laboratory
4 15 18 24 3 11 7 10 2 9 12
-1.8 -0.3 -0.2 0.0 0.0 0.0 0.0 0.1 0.1 0.1 0.3

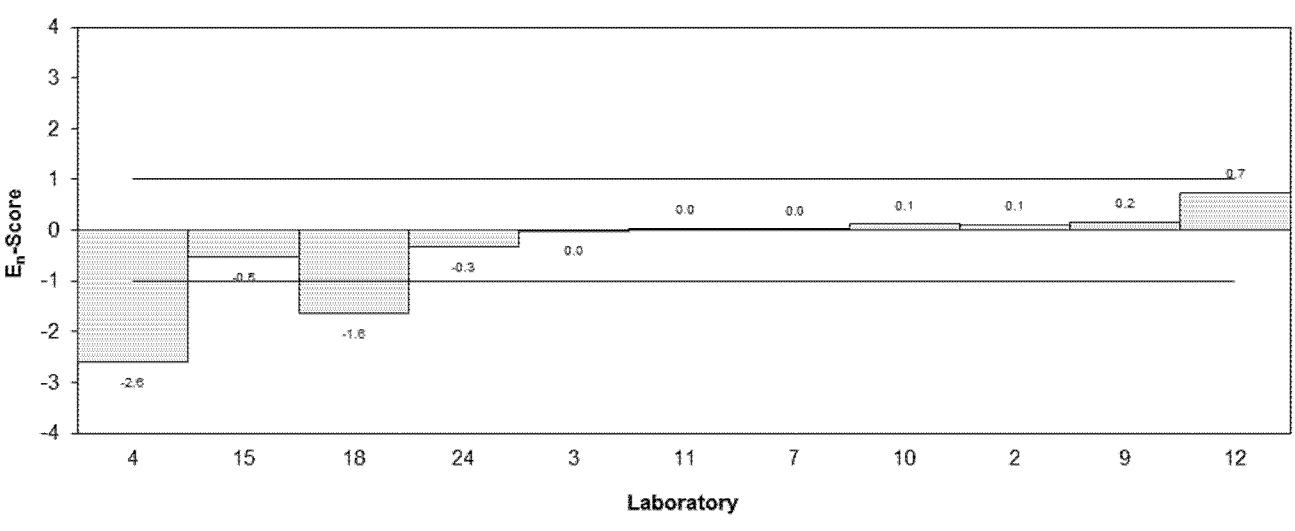

En-Scores: S1 - Linear PFOS
En-Score
Laboratory
4 15 18 24 3 11 7 10 2 9 12
-2.6 -0.5 -1.6 -0.3 0.0 0.0 0.0 0.1 0.1 0.2 0.7

Figure 2

Table 10

Sample Details

Sample No.	S1
Matrix.	Fish
Analyte.	PFOA
Units	ug/kg

Participant Results

Lab Code	Result	Uncertainty	Recovery
1	<0.2	NR	80
2	<1	NR	NR
3	<0.10	NR	136
4	< 5	1.048	77.9
5	NT	NT	NT
6	NT	NT	NT
7	<0.148	0.03	50.4
8	NT	NT	NT
9	< 0.05	NR	NR
10	0.714	0.259	103.0
11	<1.2	1.2	78.7
12	0.06	0.00	65.22
13	NR	NR	NR
14	<2	NR	9
15	<0.010	NR	93.38
16	<1	NR	91
17	NT	NT	NT
18	<1	NR	87
19	NT	NT	NT
20	NT	NT	NT
21	NT	NT	NT
22	NT	NT	NT
23	NT	NT	NT
24	0.022	0.00015	152

Statistics*

Assigned Value	Not Set
Spike	Not Spiked
N	3

*Not enough results to calculate statistics on this analyte.

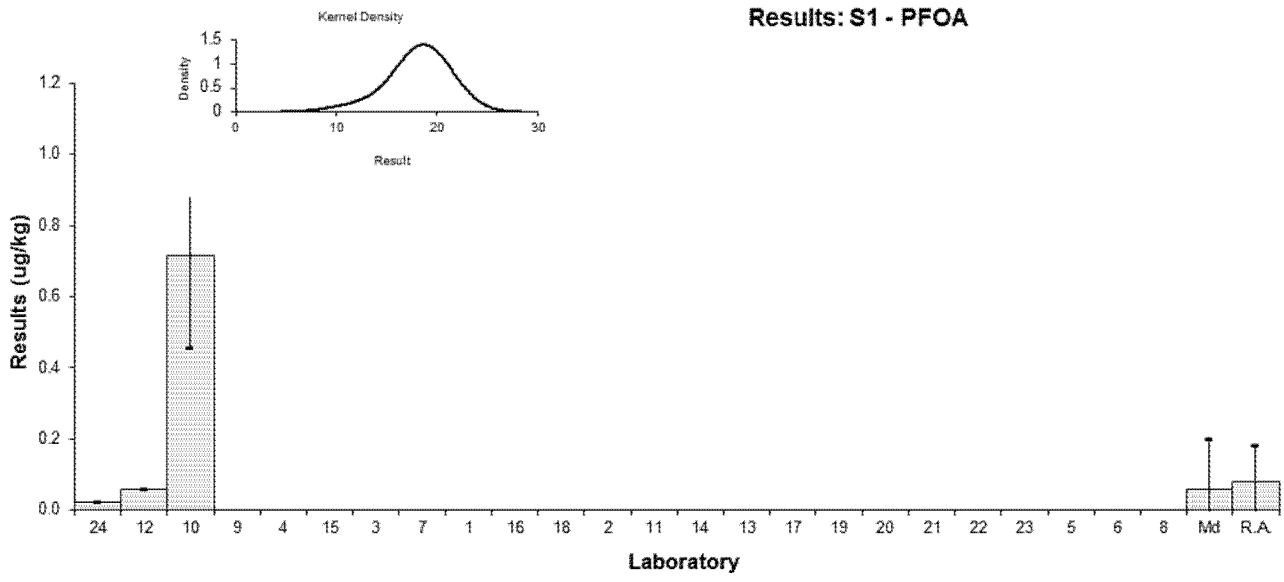

Results: S1 - PFOA
Kernel Density
Density
Result
Results (ug/kg)
Laboratory
24 12 10 9 4 15 3 7 1 16 18 2 11 14 13 17 19 20 21 22 23 5 6 8 Md R.A.

Figure 3

Table 11

Sample Details

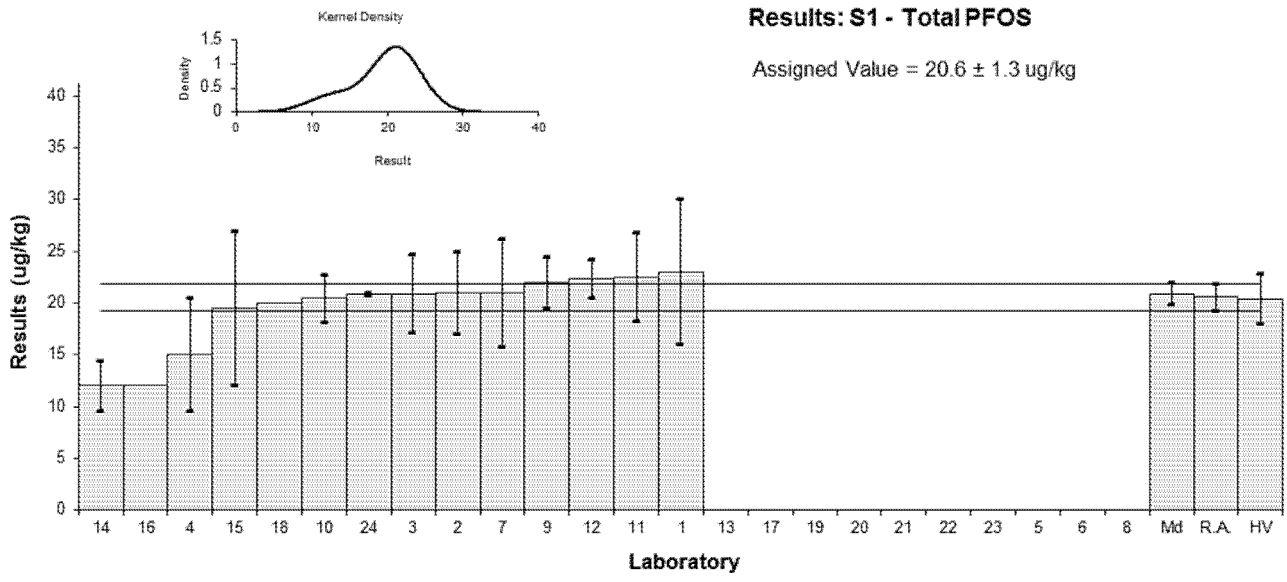
Sample No.	S1
Matrix.	Fish
Analyte.	Total PFOS
Units	ug/kg

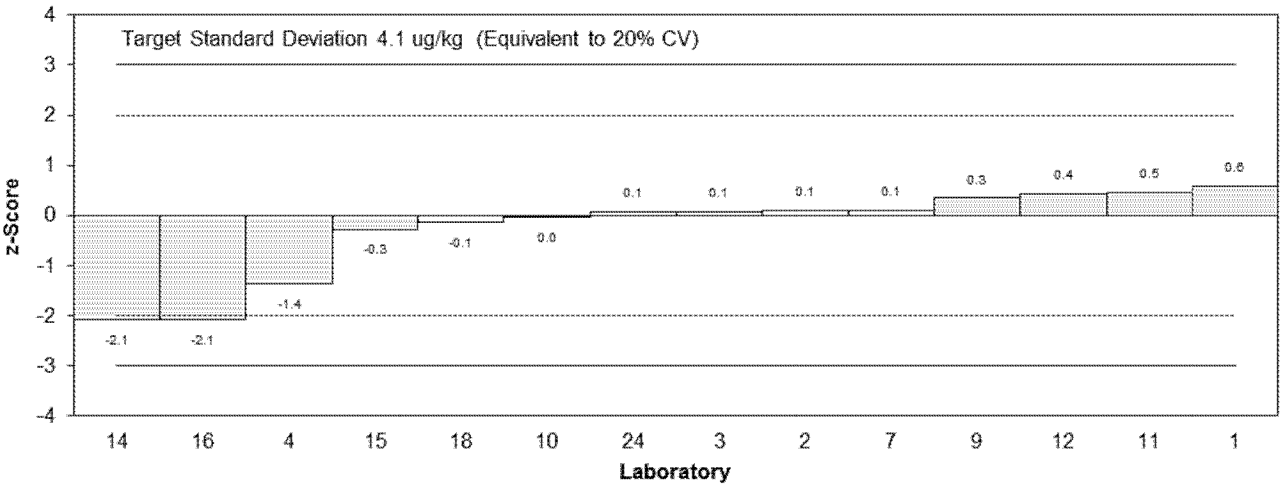
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	23	7	67	0.58	0.34
2	21	4.0	NR	0.10	0.10
3	20.9	3.8	84	0.07	0.07
4	15	5.493	77.9	-1.36	-0.99
5	NT	NT	NT		
6	NT	NT	NT		
7	21	5.2	42	0.10	0.07
8	NT	NT	NT		
9	22	2.5	90	0.34	0.50
10	20.453	2.308	96.8	-0.04	-0.06
11	22.5	4.3	69.5	0.46	0.42
12	22.35	1.81	86.05	0.42	0.79
13	NR	NR	NR		
14	12	2.4	30	-2.09	-3.15
15	19.463	7.422	92.67	-0.28	-0.15
16	12	NR	100	-2.09	-6.62
17	NT	NT	NT		
18	20	NR	NR	-0.15	-0.46
19	NT	NT	NT		
20	NT	NT	NT		
21	NT	NT	NT		
22	NT	NT	NT		
23	NT	NT	NT		
24	20.86	0.12	77	0.06	0.20

Statistics

Assigned Value	20.6	1.3
Spike	Not Spiked	
Homogeneity Value	20.4	2.4
Robust Average	20.6	1.3
Median	20.9	1.1
Mean	19.4	
N	14	
Max.	23	
Min.	12	
Robust SD	1.90	
Robust CV	9.2%	


Kernel Density
Density
Result
Results: S1 - Total PFOS
Assigned Value = 20.6 ± 1.3 ug/kg
Results (ug/kg)
Laboratory
14 16 4 15 18 10 24 3 2 7 9 12 11 1 13 17 19 20 21 22 23 5 6 8 Md R.A. HV


z-Scores: S1 - Total PFOS
Target Standard Deviation 4.1 ug/kg (Equivalent to 20% CV)
z-Score
-2.1 -2.1 -1.4 -0.3 -0.1 0.0 0.1 0.1 0.1 0.1 0.3 0.4 0.5 0.6
Laboratory
14 16 4 15 18 10 24 3 2 7 9 12 11 1

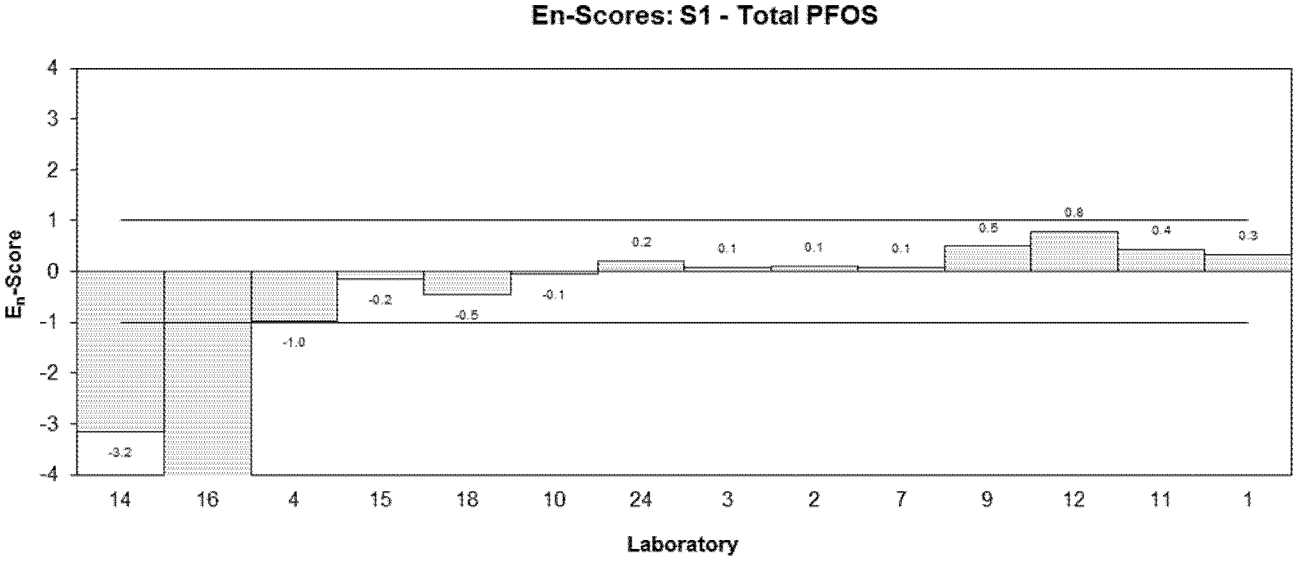

En-Scores: S1 - Total PFOS
En-Score
-3.2 -1.0 -0.2 -0.5 -0.1 0.2 0.1 0.1 0.1 0.5 0.8 0.4 0.3
Laboratory
14 16 4 15 18 10 24 3 2 7 9 12 11 1

Figure 4

Table 12

Sample Details

Sample No.	S2
Matrix.	Fish
Analyte.	Linear PFOS
Units	ug/kg

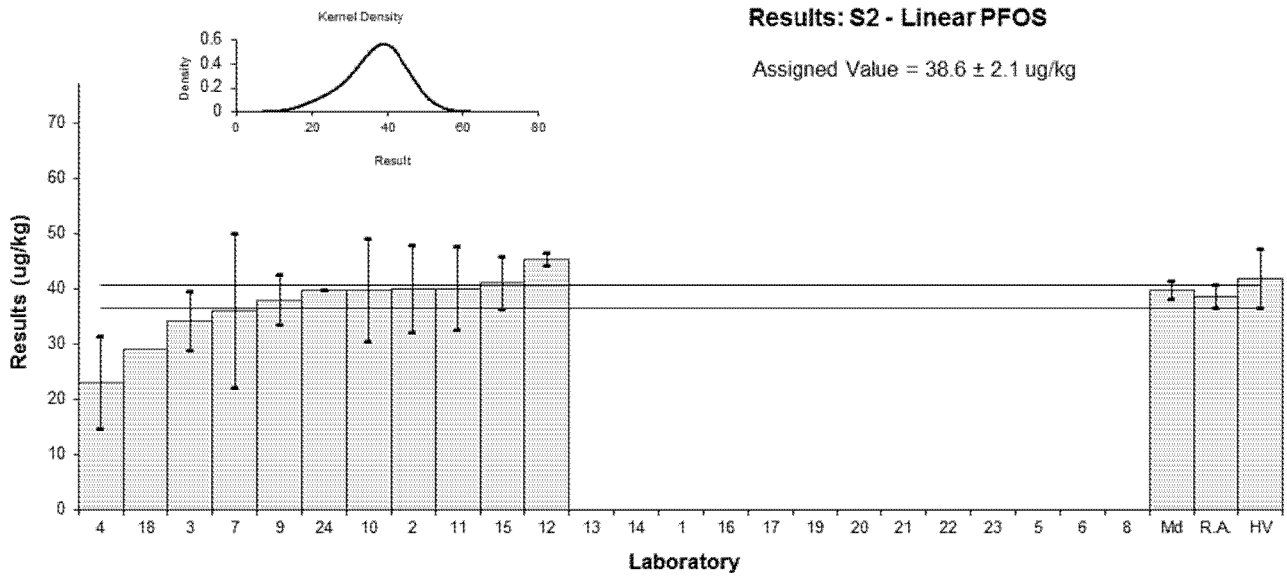
Participant Results

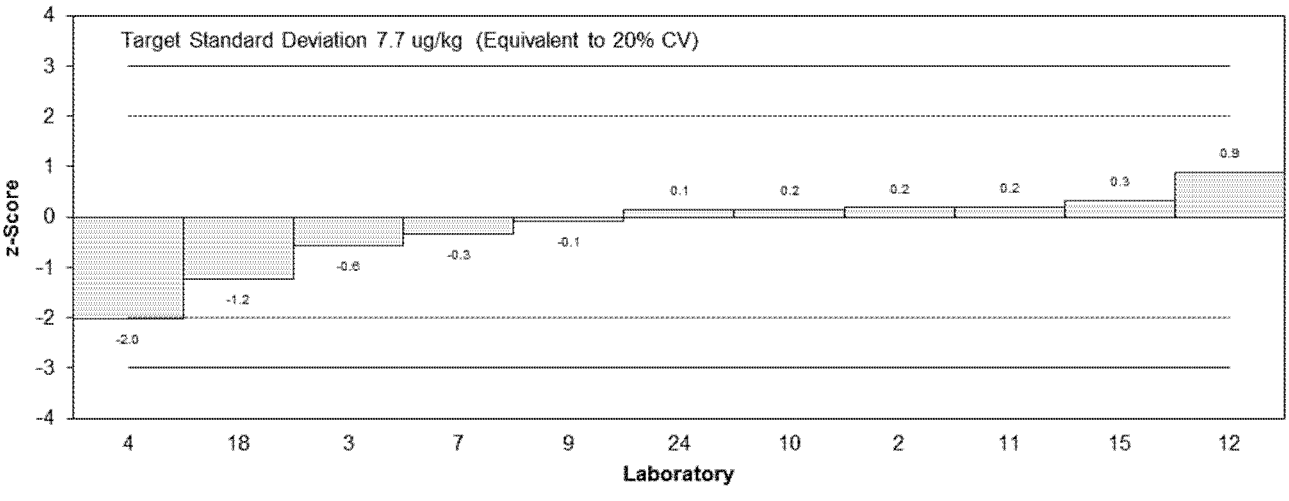
Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	NT	NT	NT		
2	40	8	NR	0.18	0.17
3	34.2	5.3	71	-0.57	-0.77
4	23	8.423	79.8	-2.02	-1.80
5	NT	NT	NT		
6	NT	NT	NT		
7	36	14	53.6	-0.34	-0.18
8	NT	NT	NT		
9	38	4.5	95	-0.08	-0.12
10	39.768	9.240	93.9	0.15	0.12
11	40.1	7.6	88.5	0.19	0.19
12	45.40	1.18	75.83	0.88	2.82
13	NR	NR	NR		
14	NR	NR	NR		
15	41.056	4.852	89.33	0.32	0.46
16	NT	NT	NT		
17	NT	NT	NT		
18	29	NR	105	-1.24	-4.57
19	NT	NT	NT		
20	NT	NT	NT		
21	NT	NT	NT		
22	NT	NT	NT		
23	NT	NT	NT		
24	39.75	0.017	78	0.15	0.55

Statistics

Assigned Value	38.6	2.1
Spike*	NA	
Homogeneity Value	41.8	5.4
Robust Average	38.6	2.1
Median	39.8	1.7
Mean	36.9	
N	11	
Max.	45.4	
Min.	23	
Robust SD	2.8	
Robust CV	7.3%	

*Sample was spiked with a technical mixture, linear PFOS value has not been certified.


Kernel Density
Density
Result
Results: S2 - Linear PFOS
Assigned Value = 38.6 ± 2.1 ug/kg
Results (ug/kg)
Laboratory
4 18 3 7 9 24 10 2 11 15 12 13 14 1 16 17 19 20 21 22 23 5 6 8 Md R.A. HV


z-Scores: S2 - Linear PFOS
Target Standard Deviation 7.7 ug/kg (Equivalent to 20% CV)
z-Score
Laboratory
4 18 3 7 9 24 10 2 11 15 12
-2.0 -1.2 -0.6 -0.3 -0.1 0.1 0.2 0.2 0.2 0.3 0.9

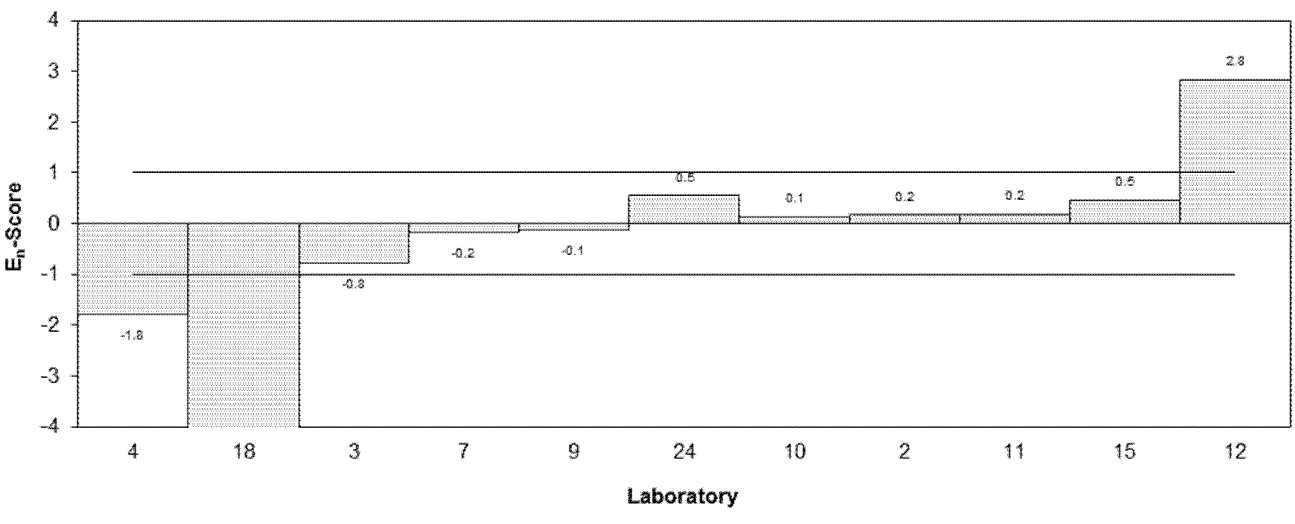

En-Scores: S2 - Linear PFOS
En-Score
Laboratory
4 18 3 7 9 24 10 2 11 15 12
-1.8 -0.8 -0.2 -0.1 0.5 0.1 0.2 0.2 0.5 2.8

Figure 5

Table 13

Sample Details

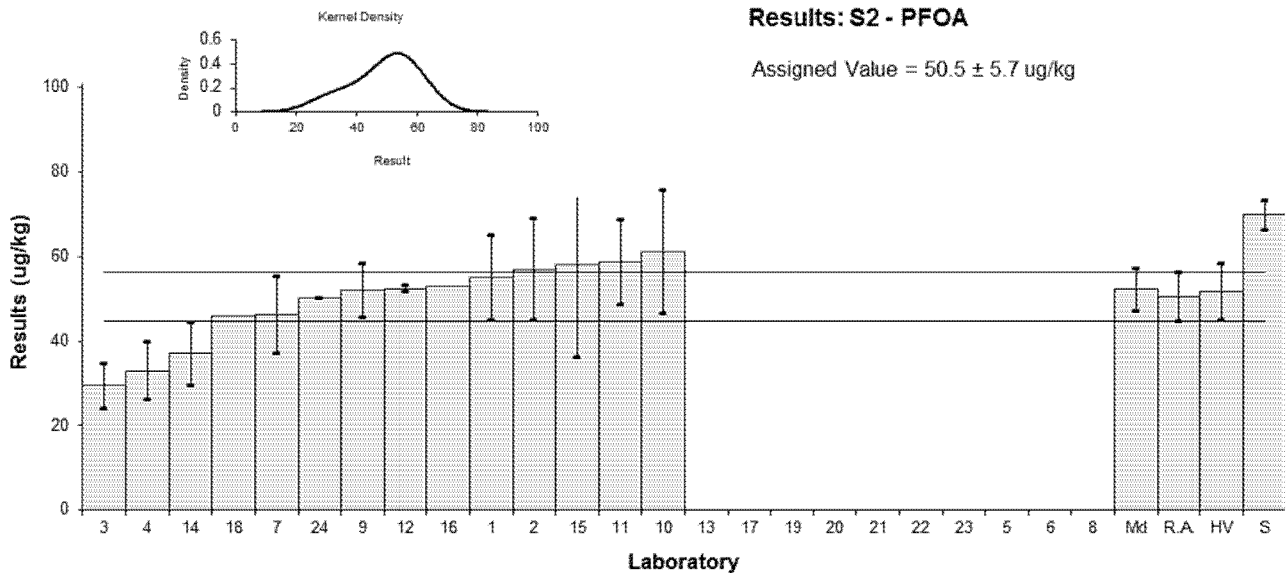
Sample No.	S2
Matrix.	Fish
Analyte.	PFOA
Units	ug/kg

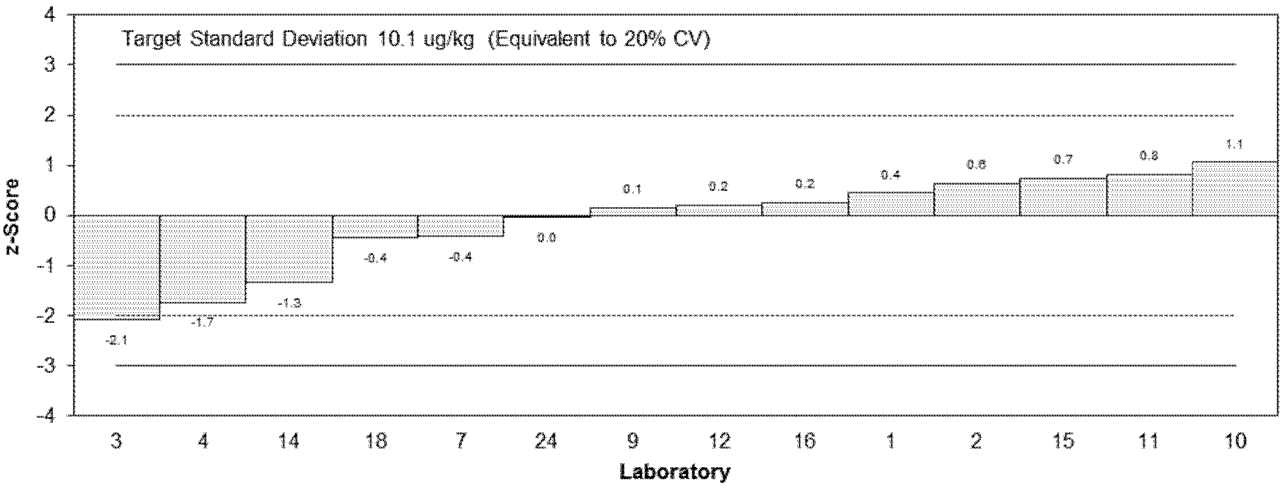
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	55	10	80	0.45	0.39
2	57	12	NR	0.64	0.49
3	29.5	5.3	90	-2.08	-2.70
4	33	6.914	79.8	-1.73	-1.95
5	NT	NT	NT		
6	NT	NT	NT		
7	46.2	9.2	57.7	-0.43	-0.40
8	NT	NT	NT		
9	52	6.5	92	0.15	0.17
10	61.190	14.672	88.6	1.06	0.68
11	58.8	10.0	76.3	0.82	0.72
12	52.42	0.79	84.06	0.19	0.33
13	NR	NR	NR		
14	37	7.4	145	-1.34	-1.45
15	58.003	21.852	94.26	0.74	0.33
16	53	NR	99	0.25	0.44
17	NT	NT	NT		
18	46	NR	75	-0.45	-0.79
19	NT	NT	NT		
20	NT	NT	NT		
21	NT	NT	NT		
22	NT	NT	NT		
23	NT	NT	NT		
24	50.19	0.074	113	-0.03	-0.05

Statistics

Assigned Value	50.5	5.7
Spike	69.9	3.5
Homogeneity Value	51.8	6.7
Robust Average	50.5	5.7
Median	52.2	5.1
Mean	49.2	
N	14	
Max.	61.19	
Min.	29.5	
Robust SD	8.6	
Robust CV	17%	


Kernel Density
Density
Result
Results: S2 - PFOA
Assigned Value = 50.5 ± 5.7 ug/kg
Results (ug/kg)
Laboratory
3 4 14 18 7 24 9 12 16 1 2 15 11 10 13 17 19 20 21 22 23 5 6 8 Md R.A HV S


z-Scores: S2 - PFOA
Target Standard Deviation 10.1 ug/kg (Equivalent to 20% CV)
z-Score
Laboratory
3 4 14 18 7 24 9 12 16 1 2 15 11 10
-2.1 -1.7 -1.3 -0.4 -0.4 0.0 0.1 0.2 0.2 0.4 0.6 0.7 0.8 1.1

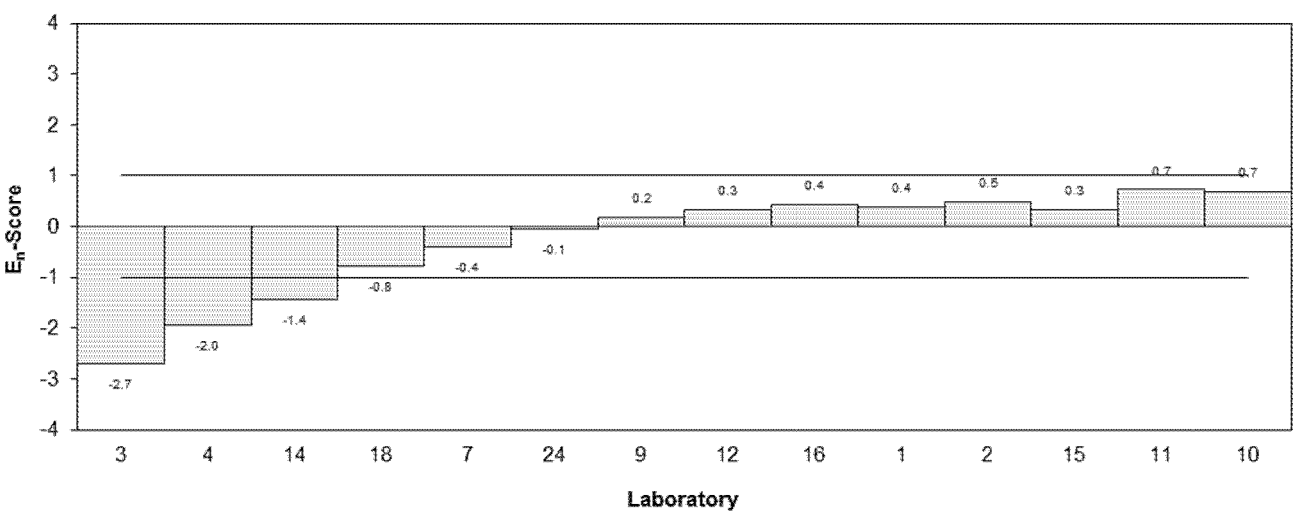

En-Scores: S2 - PFOA
En-Score
Laboratory
3 4 14 18 7 24 9 12 16 1 2 15 11 10
-2.7 -2.0 -1.4 -0.8 -0.4 -0.1 0.2 0.3 0.4 0.4 0.5 0.3 0.7 0.7

Figure 6

Table 14

Sample Details

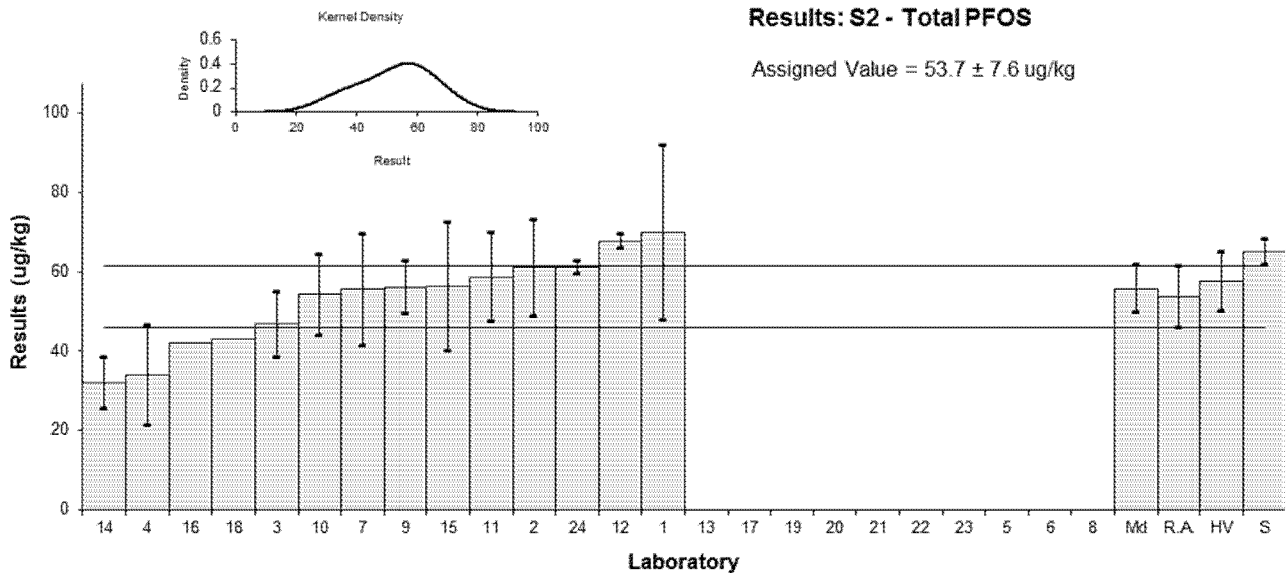
Sample No.	S2
Matrix.	Fish
Analyte.	Total PFOS
Units	ug/kg

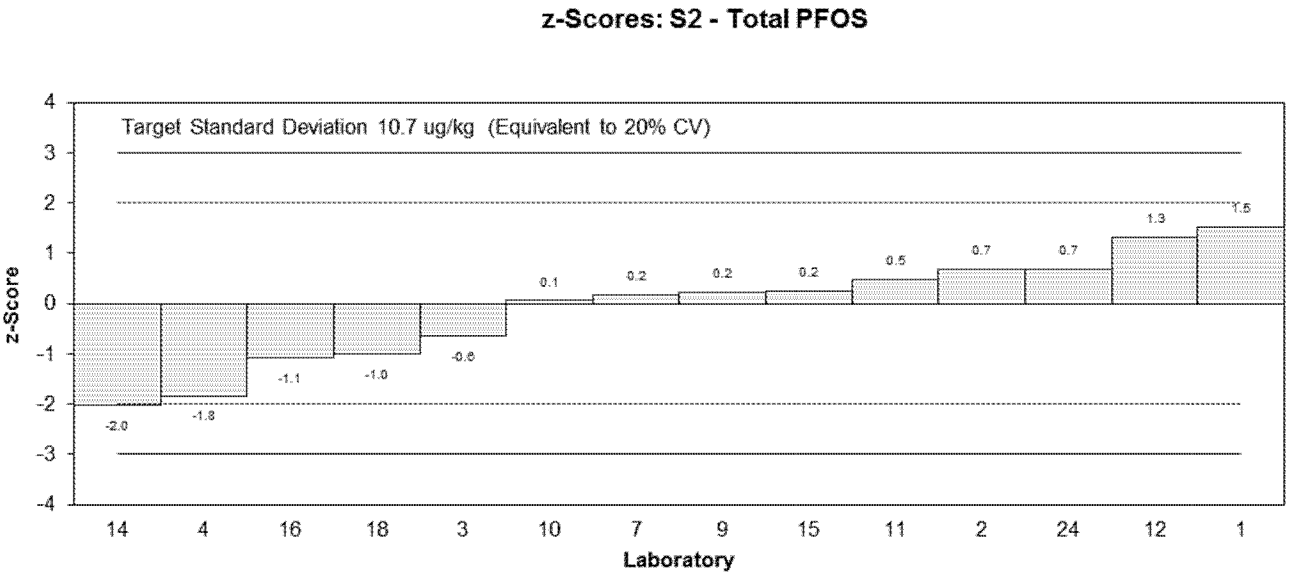
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	70	22	70	1.52	0.70
2	61	12	NR	0.68	0.51
3	46.8	8.3	71	-0.64	-0.61
4	34	12.451	79.8	-1.83	-1.35
5	NT	NT	NT		
6	NT	NT	NT		
7	55.5	14	53.6	0.17	0.11
8	NT	NT	NT		
9	56	6.6	95	0.21	0.23
10	54.273	10.174	93.9	0.05	0.05
11	58.7	11.1	88.5	0.47	0.37
12	67.75	1.76	75.83	1.31	1.80
13	NR	NR	NR		
14	32	6.4	19	-2.02	-2.18
15	56.320	16.064	89.33	0.24	0.15
16	42	NR	98	-1.09	-1.54
17	NT	NT	NT		
18	43	NR	NR	-1.00	-1.41
19	NT	NT	NT		
20	NT	NT	NT		
21	NT	NT	NT		
22	NT	NT	NT		
23	NT	NT	NT		
24	61.09	1.55	78	0.69	0.95

Statistics

Assigned Value	53.7	7.6
Spike	64.9	3.2
Homogeneity Value	57.7	7.4
Robust Average	53.7	7.6
Median	55.8	6.1
Mean	52.8	
N	14	
Max.	70	
Min.	32	
Robust SD	11.3	
Robust CV	21%	


Kernel Density
Density
Result
Results: S2 - Total PFOS
Assigned Value = 53.7 ± 7.6 ug/kg
Results (ug/kg)
Laboratory
14 4 16 18 3 10 7 9 15 11 2 24 12 1 13 17 19 20 21 22 23 5 6 8 Md R.A HV S


z-Scores: S2 - Total PFOS
Target Standard Deviation 10.7 ug/kg (Equivalent to 20% CV)
z-Score
-2.0 -1.8 -1.1 -1.0 -0.6 0.1 0.2 0.2 0.2 0.5 0.7 0.7 1.3 1.5
Laboratory
14 4 16 18 3 10 7 9 15 11 2 24 12 1

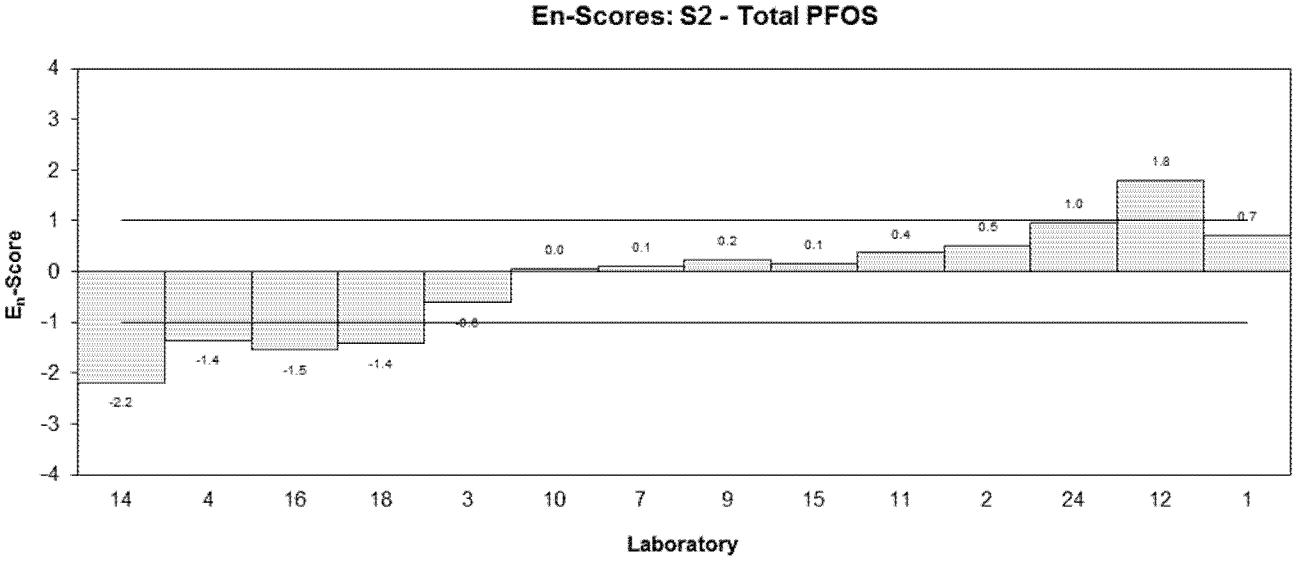

En-Scores: S2 - Total PFOS
En-Score
-2.2 -1.4 -1.5 -1.4 -0.6 0.0 0.1 0.2 0.1 0.4 0.6 1.0 1.8 0.7
Laboratory
14 4 16 18 3 10 7 9 15 11 2 24 12 1

Figure 7

Table 15

Sample Details

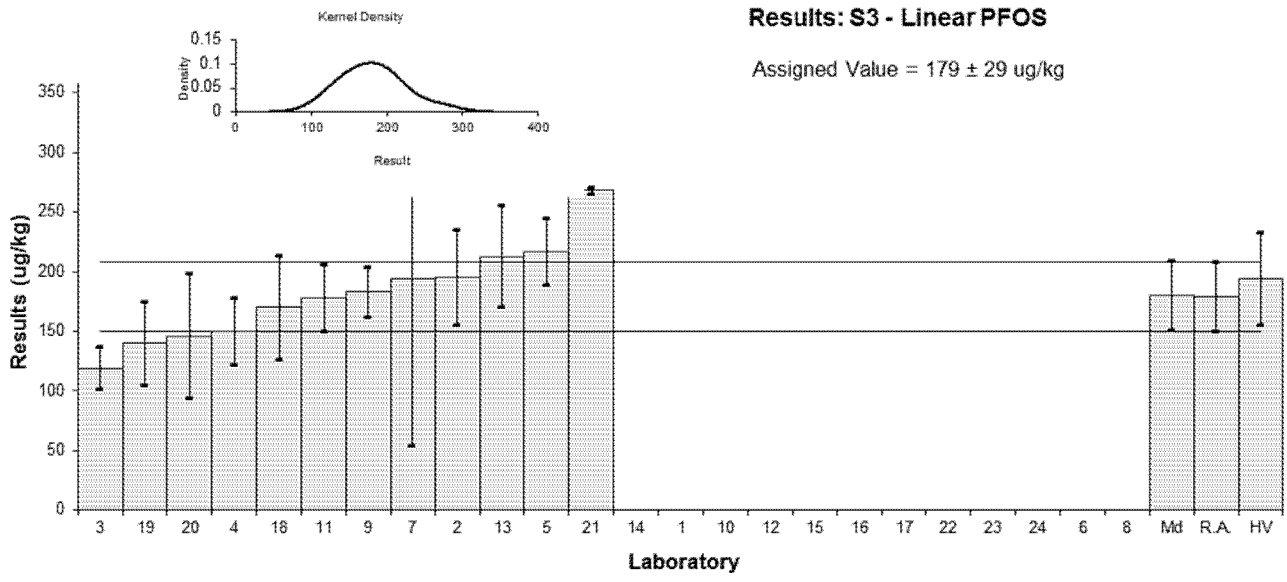
Sample No.	S3
Matrix.	Soil
Analyte.	Linear PFOS
Units	ug/kg

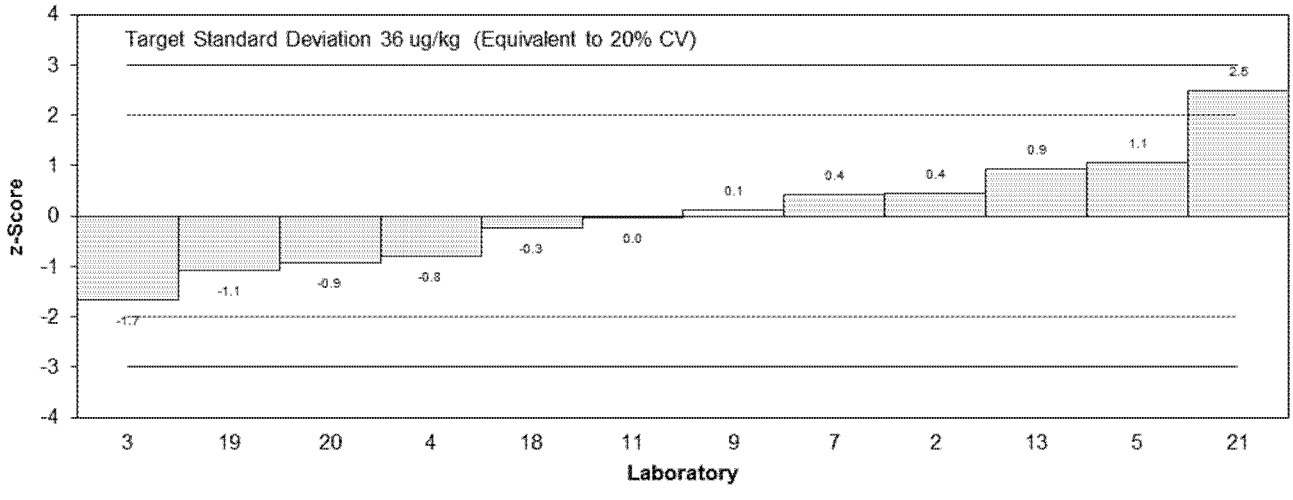
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	NT	NT	NT		
2	195	40	NR	0.45	0.32
3	119	18.0	50	-1.68	-1.76
4	150	28.110	83.5	-0.81	-0.72
5	217	28	74.7	1.06	0.94
6	NT	NT	NT		
7	194	140	76.1	0.42	0.10
8	NT	NT	NT		
9	183	21	85	0.11	0.11
10	NT	NT	NT		
11	178	28	60.1	-0.03	-0.02
12	NT	NT	NT		
13	212.6	42.5	NR	0.94	0.65
14	NR	NR	NR		
15	NT	NT	NT		
16	NT	NT	NT		
17	NT	NT	NT		
18	170	44	90	-0.25	-0.17
19	140	35	85	-1.09	-0.86
20	146	52.6	NR	-0.92	-0.55
21	268	2.6	NR	2.49	3.06
22	NT	NT	NT		
23	NT	NT	NT		
24	NT	NT	NT		

Statistics

Assigned Value	179	29
Spike	Not Spiked	
Homogeneity Value	194	39
Robust Average	179	29
Median	180	29
Mean	181	
N	12	
Max.	268	
Min.	119	
Robust SD	40	
Robust CV	22%	


Kernel Density
Density
Result
Results: S3 - Linear PFOS
Assigned Value = 179 ± 29 ug/kg
Results (ug/kg)
Laboratory
3 19 20 4 18 11 9 7 2 13 5 21 14 1 10 12 15 16 17 22 23 24 6 8 Md R.A. HV


z-Scores: S3 - Linear PFOS
Target Standard Deviation 36 ug/kg (Equivalent to 20% CV)
z-Score
Laboratory
3 19 20 4 18 11 9 7 2 13 5 21
-1.7 -1.1 -0.9 -0.8 -0.3 0.0 0.1 0.4 0.4 0.9 1.1 2.5

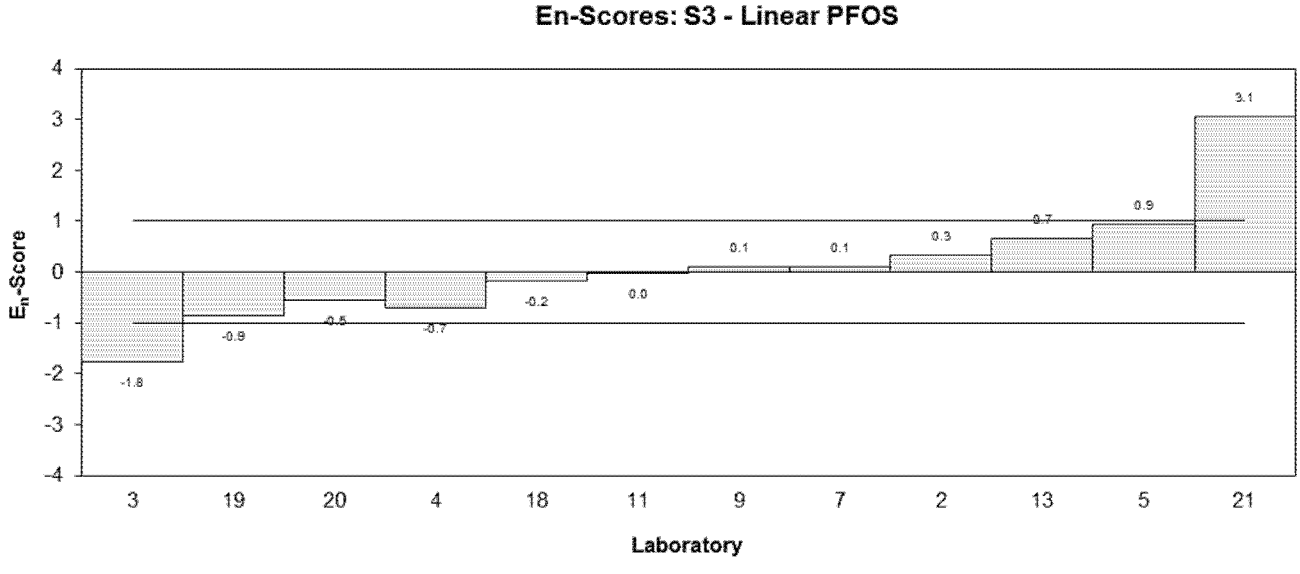

En-Scores: S3 - Linear PFOS
En-Score
Laboratory
3 19 20 4 18 11 9 7 2 13 5 21
-1.8 -0.9 -0.5 -0.7 -0.2 0.0 0.1 0.1 0.3 0.7 0.9 3.1

Figure 8

Table 16

Sample Details

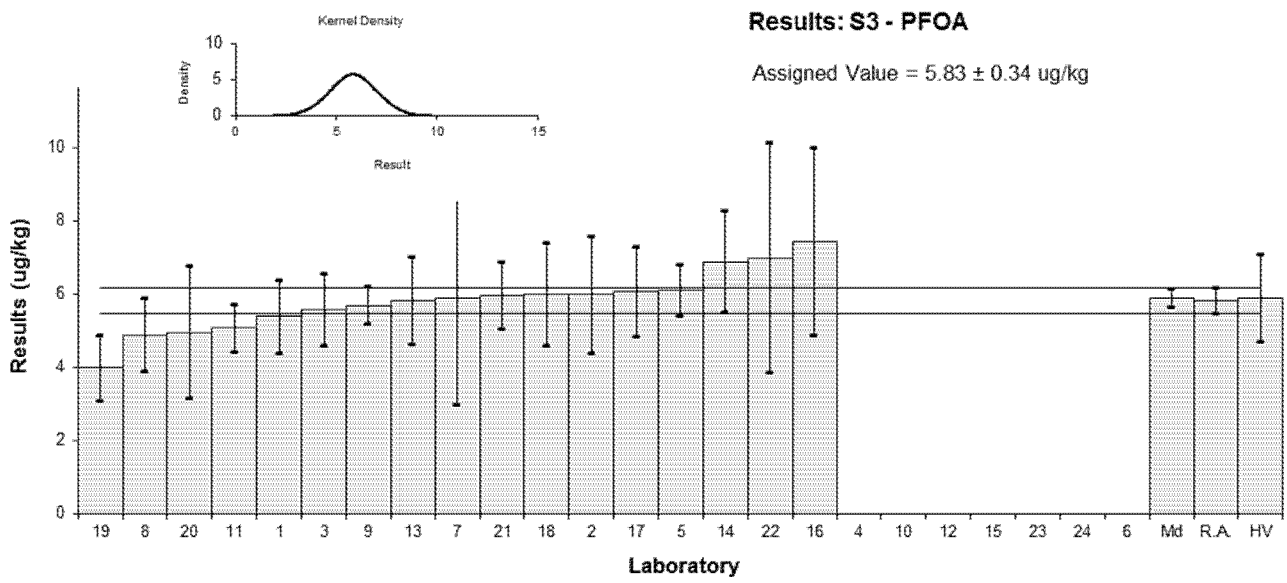
Sample No.	S3
Matrix.	Soil
Analyte.	PFOA
Units	ug/kg

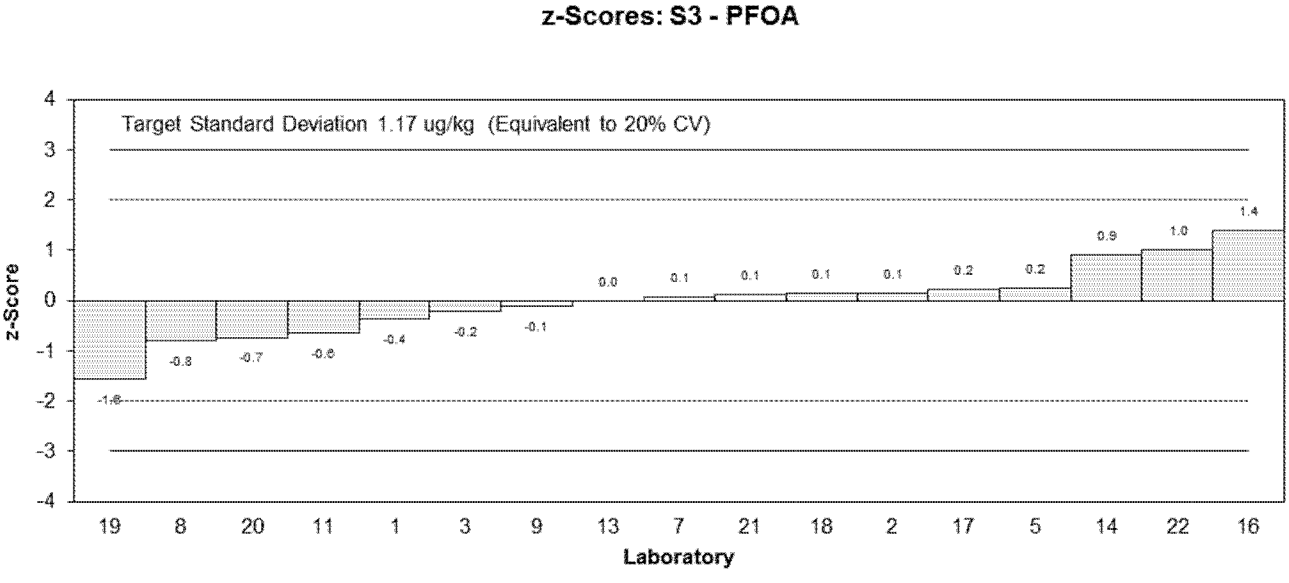
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	5.4	1.0	83	-0.37	-0.41
2	6.0	1.6	NR	0.15	0.10
3	5.58	0.99	93	-0.21	-0.24
4	< 5	1.051	83.5		
5	6.11	0.69	99.3	0.24	0.36
6	NT	NT	NT		
7	5.9	2.9	64.8	0.06	0.02
8	4.9	1.0	87.8	-0.80	-0.88
9	5.7	0.5	87	-0.11	-0.22
10	NT	NT	NT		
11	5.08	0.66	79.9	-0.64	-1.01
12	NT	NT	NT		
13	5.83	1.2	97	0.00	0.00
14	6.9	1.4	120	0.92	0.74
15	NT	NT	NT		
16	7.45	2.563	102	1.39	0.63
17	6.08	1.22	75	0.21	0.20
18	6.0	1.4	100	0.15	0.12
19	4.0	0.9	85	-1.57	-1.90
20	4.97	1.8	NR	-0.74	-0.47
21	5.97	0.91	NR	0.12	0.14
22	7.00	3.15	98	1.00	0.37
23	NT	NT	NT		
24	NT	NT	NT		

Statistics

Assigned Value	5.83	0.34
Spike	Not Spiked	
Homogeneity Value	5.9	1.2
Robust Average	5.83	0.34
Median	5.90	0.24
Mean	5.82	
N	17	
Max.	7.45	
Min.	4	
Robust SD	0.56	
Robust CV	9.6%	


Kernel Density
Density
Result
Results: S3 - PFOA
Assigned Value = 5.83 ± 0.34 ug/kg
Results (ug/kg)
19 8 20 11 1 3 9 13 7 21 18 2 17 5 14 22 16 4 10 12 15 23 24 6 Md R.A. HV
Laboratory


z-Scores: S3 - PFOA
Target Standard Deviation 1.17 ug/kg (Equivalent to 20% CV)
z-Score
-1.6 -0.8 -0.7 -0.6 -0.4 -0.2 -0.1 0.0 0.1 0.1 0.1 0.1 0.2 0.2 0.9 1.0 1.4
19 8 20 11 1 3 9 13 7 21 18 2 17 5 14 22 16
Laboratory

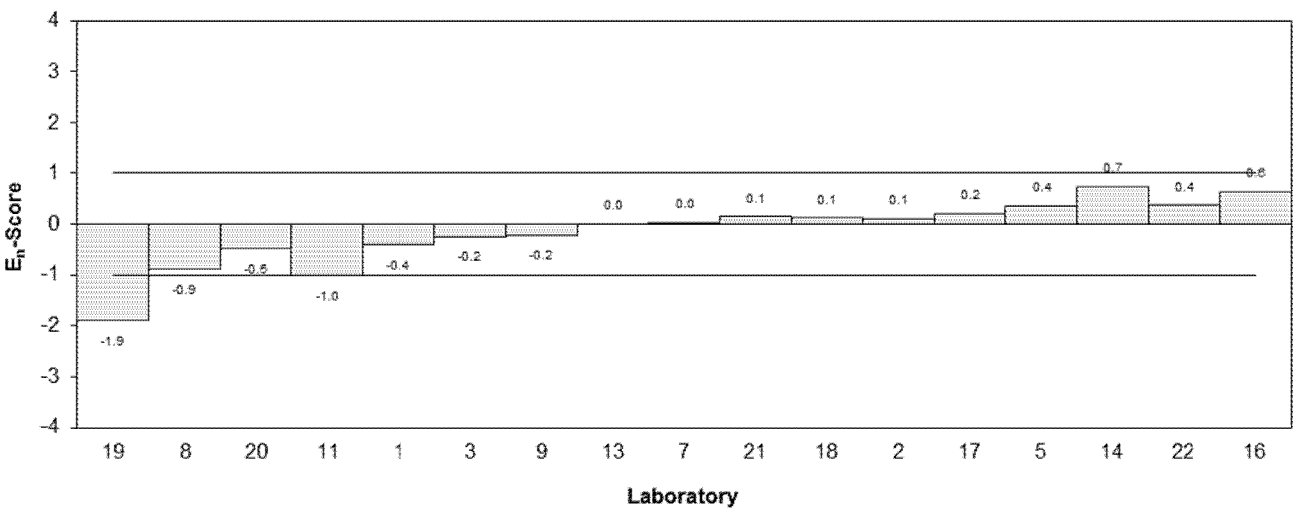

En-Scores: S3 - PFOA
En-Score
-1.9 -0.9 -0.5 -1.0 -0.4 -0.2 -0.2 0.0 0.0 0.1 0.1 0.1 0.2 0.4 0.7 0.4 0.6
19 8 20 11 1 3 9 13 7 21 18 2 17 5 14 22 16
Laboratory

Figure 9

Table 17

Sample Details

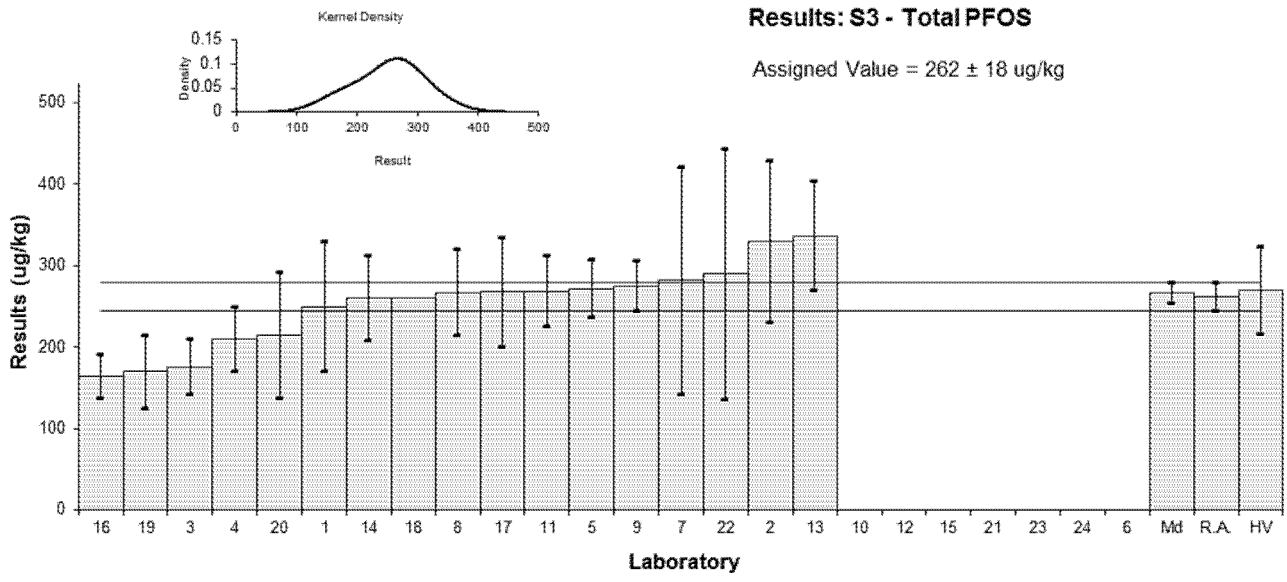
Sample No.	S3
Matrix.	Soil
Analyte.	Total PFOS
Units	ug/kg

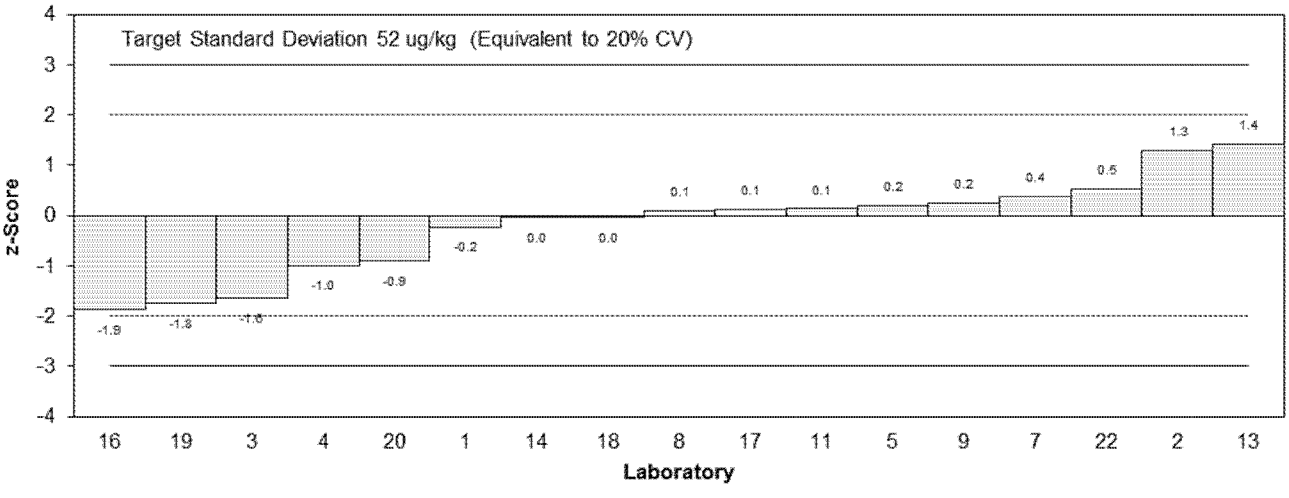
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	250	80	100	-0.23	-0.15
2	330	99	NR	1.30	0.68
3	176	34.4	50	-1.64	-2.22
4	210	39.354	83.5	-0.99	-1.20
5	272	35	74.7	0.19	0.25
6	NT	NT	NT		
7	282	140	76.1	0.38	0.14
8	267	53	88.1	0.10	0.09
9	275	31	85	0.25	0.36
10	NT	NT	NT		
11	269	43	60.1	0.13	0.15
12	NT	NT	NT		
13	336.6	67.3	94	1.42	1.07
14	260	52	107	-0.04	-0.04
15	NT	NT	NT		
16	164	27.2	97	-1.87	-3.00
17	267.74	66.94	86	0.11	0.08
18	260	NR	NA	-0.04	-0.11
19	170	45	85	-1.76	-1.90
20	214.4	77.2	NR	-0.91	-0.60
21	NT	NT	NT		
22	290	154	51	0.53	0.18
23	NT	NT	NT		
24	NT	NT	NT		

Statistics

Assigned Value	262	18
Spike	Not Spiked	
Homogeneity Value	270	54
Robust Average	262	18
Median	267	13
Mean	253	
N	17	
Max.	336.6	
Min.	164	
Robust SD	29	
Robust CV	11%	


Kernel Density
Density
Result
Results: S3 - Total PFOS
Assigned Value = 262 ± 18 ug/kg
Results (ug/kg)
Laboratory
16 19 3 4 20 1 14 18 8 17 11 5 9 7 22 2 13 10 12 15 21 23 24 6 Md R.A. HV


z-Scores: S3 - Total PFOS
Target Standard Deviation 52 ug/kg (Equivalent to 20% CV)
z-Score
Laboratory
16 19 3 4 20 1 14 18 8 17 11 5 9 7 22 2 13

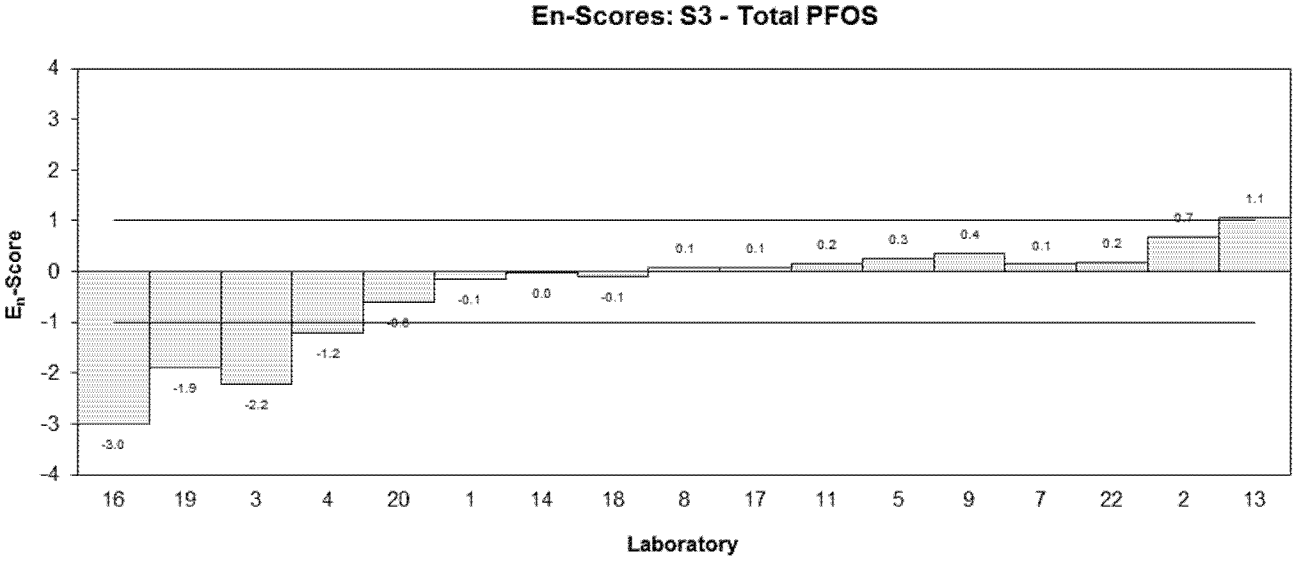

En-Scores: S3 - Total PFOS
En-Score
Laboratory
16 19 3 4 20 1 14 18 8 17 11 5 9 7 22 2 13

Figure 10

Table 18

Sample Details

Sample No.	S4
Matrix.	Soil
Analyte.	Linear PFOS
Units	ug/kg

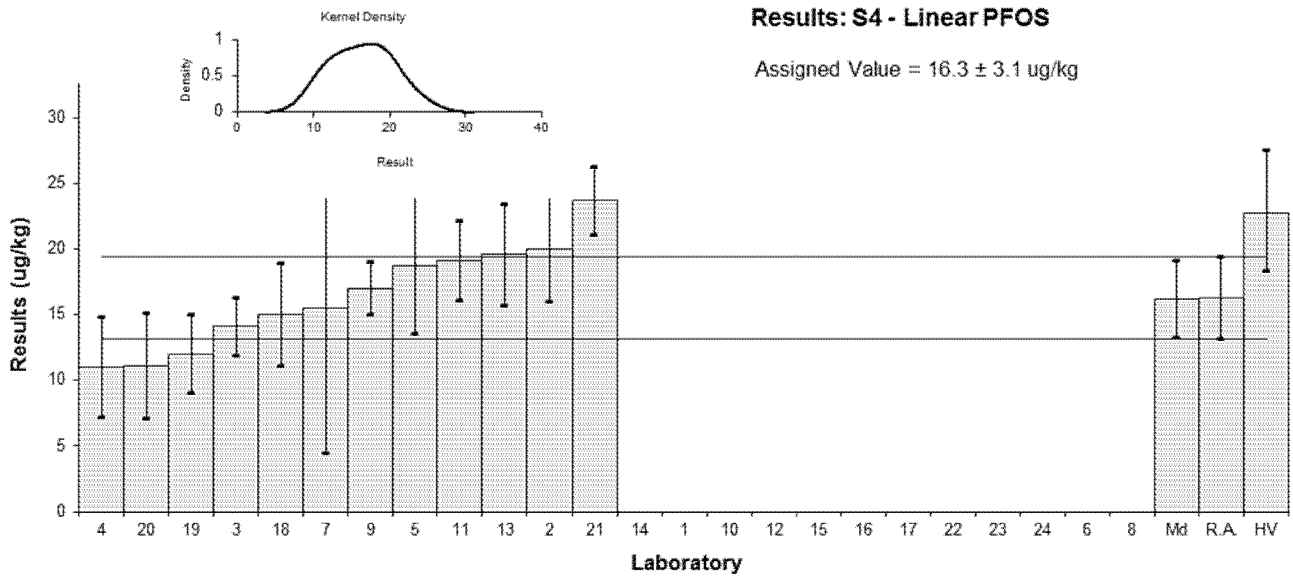
Participant Results

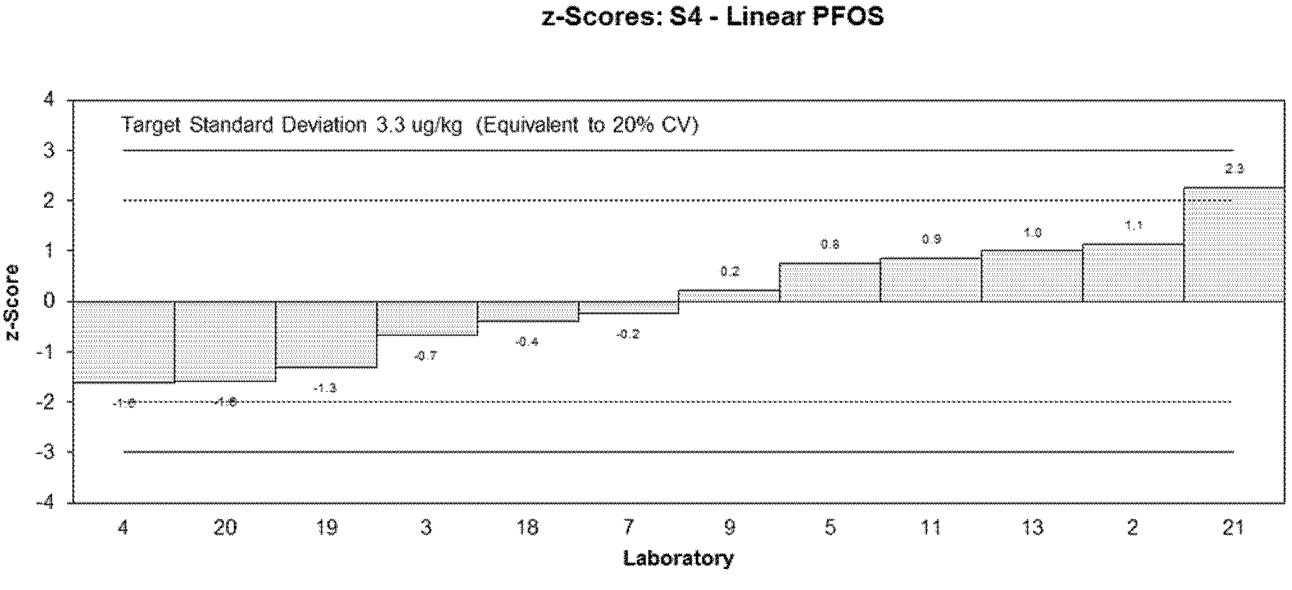
Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	NT	NT	NT		
2	20	4.0	NR	1.13	0.73
3	14.1	2.2	94	-0.67	-0.58
4	11	3.804	70.1	-1.63	-1.08
5	18.75	5.24	103	0.75	0.40
6	NT	NT	NT		
7	15.5	11	57.5	-0.25	-0.07
8	NT	NT	NT		
9	17	2	87	0.21	0.19
10	NT	NT	NT		
11	19.1	3.0	77.7	0.86	0.65
12	NT	NT	NT		
13	19.56	3.9	NR	1.00	0.65
14	NR	NR	NR		
15	NT	NT	NT		
16	NT	NT	NT		
17	NT	NT	NT		
18	15	3.9	96	-0.40	-0.26
19	12	3	86	-1.32	-1.00
20	11.1	4	NR	-1.60	-1.03
21	23.7	2.6	NR	2.27	1.83
22	NT	NT	NT		
23	NT	NT	NT		
24	NT	NT	NT		

Statistics

Assigned Value	16.3	3.1
Spike*	NA	
Homogeneity Value	22.7	4.5
Robust Average	16.3	3.1
Median	16.2	2.9
Mean	16.4	
N	12	
Max.	23.7	
Min.	11	
Robust SD	4.4	
Robust CV	27%	

*Sample was spiked with a technical mixture, linear PFOS value has not been certified.


Kernel Density
Density
Result
Results: S4 - Linear PFOS
Assigned Value = 16.3 ± 3.1 ug/kg
Results (ug/kg)
Laboratory
4 20 19 3 18 7 9 5 11 13 2 21 14 1 10 12 15 16 17 22 23 24 6 8 Md R.A. HV


z-Scores: S4 - Linear PFOS
Target Standard Deviation 3.3 ug/kg (Equivalent to 20% CV)
z-Score
Laboratory
4 20 19 3 18 7 9 5 11 13 2 21
-1.6 -1.6 -1.3 -0.7 -0.4 -0.2 0.2 0.8 0.9 1.0 1.1 2.3

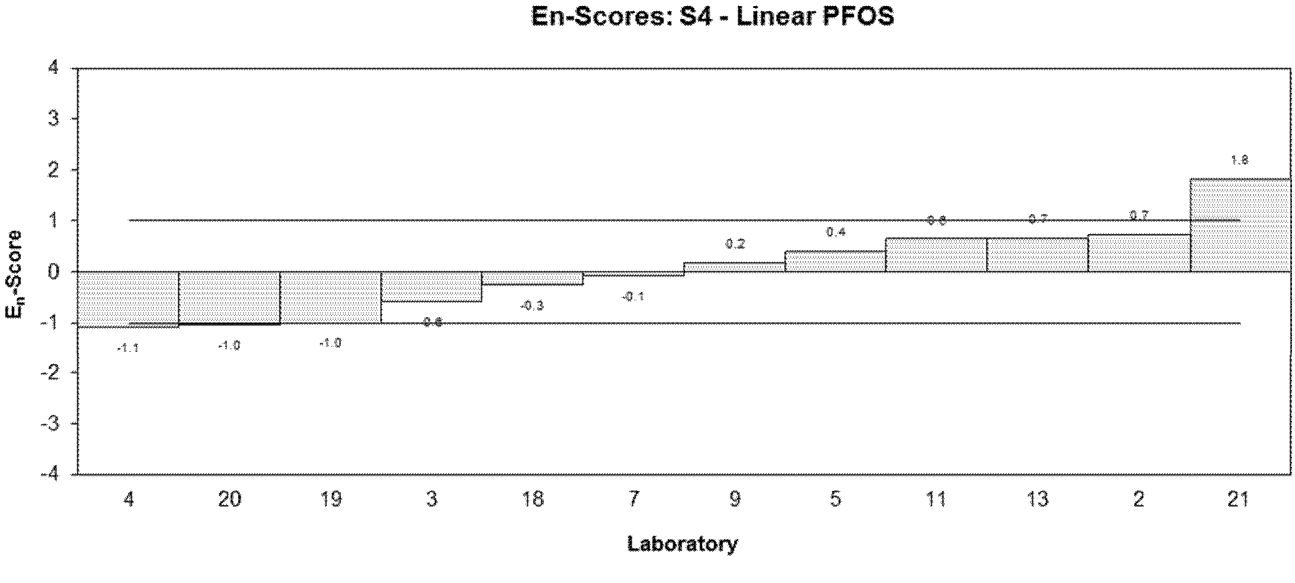

En-Scores: S4 - Linear PFOS
En-Score
Laboratory
4 20 19 3 18 7 9 5 11 13 2 21
-1.1 -1.0 -1.0 -0.6 -0.3 -0.1 0.2 0.4 0.6 0.7 0.7 1.8

Figure 11

Table 19

Sample Details

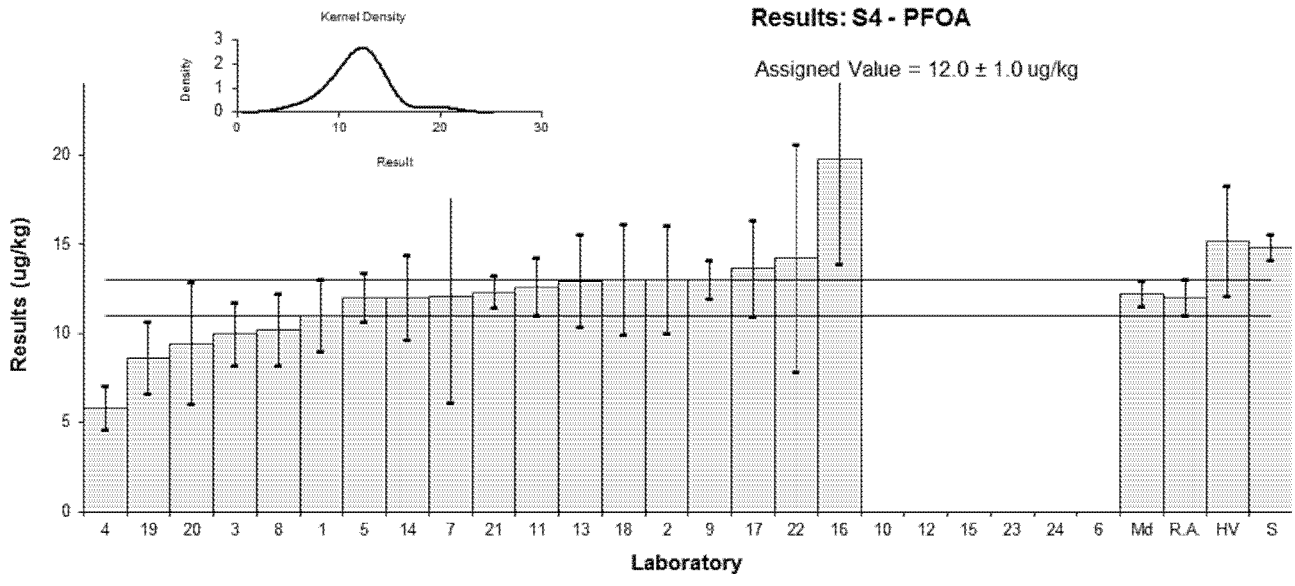
Sample No.	S4
Matrix.	Soil
Analyte.	PFOA
Units	ug/kg

Participant Results

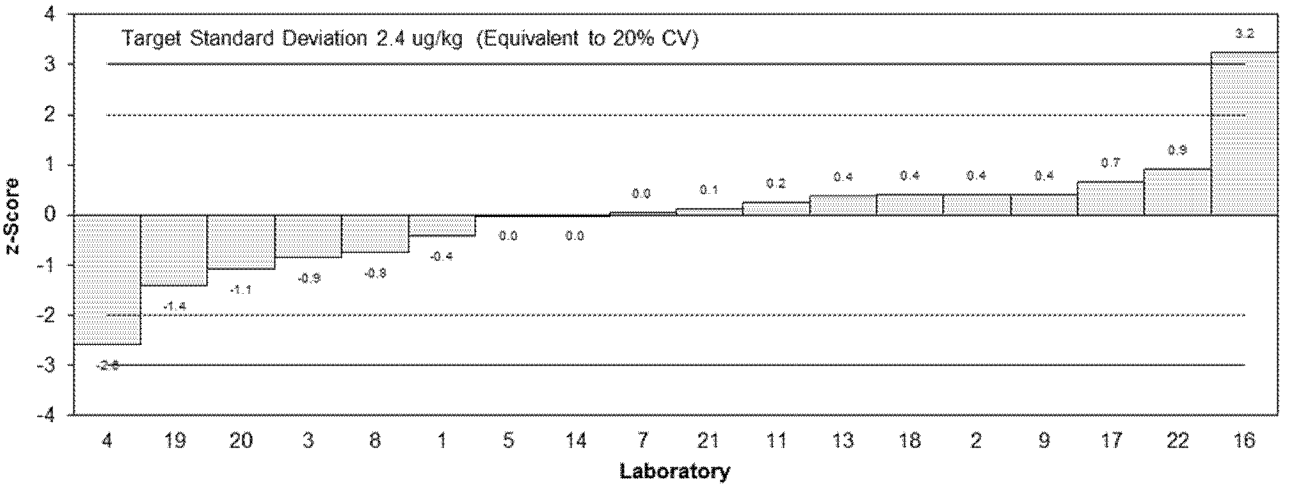
Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	11	2	79	-0.42	-0.45
2	13	3.0	NR	0.41	0.31
3	9.96	1.78	111	-0.85	-1.00
4	5.8	1.219	70.1	-2.59	-3.94
5	11.98	1.35	93.3	-0.01	-0.02
6	NT	NT	NT		
7	12.1	6	69.1	0.04	0.01
8	10.2	2.0	86.3	-0.75	-0.81
9	13	1.1	82	0.41	0.67
10	NT	NT	NT		
11	12.6	1.6	97.4	0.25	0.31
12	NT	NT	NT		
13	12.92	2.6	101	0.38	0.33
14	12	2.4	120	0.00	0.00
15	NT	NT	NT		
16	19.8	5.93	95	3.24	1.30
17	13.62	2.72	60	0.67	0.56
18	13	3.1	103	0.41	0.30
19	8.6	2	86	-1.42	-1.52
20	9.43	3.4	NR	-1.07	-0.73
21	12.3	0.91	NR	0.12	0.21
22	14.2	6.39	91	0.91	0.34
23	NT	NT	NT		
24	NT	NT	NT		

Statistics

Assigned Value	12.0	1.0
Spike	14.80	0.70
Homogeneity Value	15.2	3.0
Robust Average	12.0	1.0
Median	12.20	0.74
Mean	11.97	
N	18	
Max.	19.8	
Min.	5.8	
Robust SD	1.7	
Robust CV	14%	



z-Scores: S4 - PFOA



En-Scores: S4 - PFOA

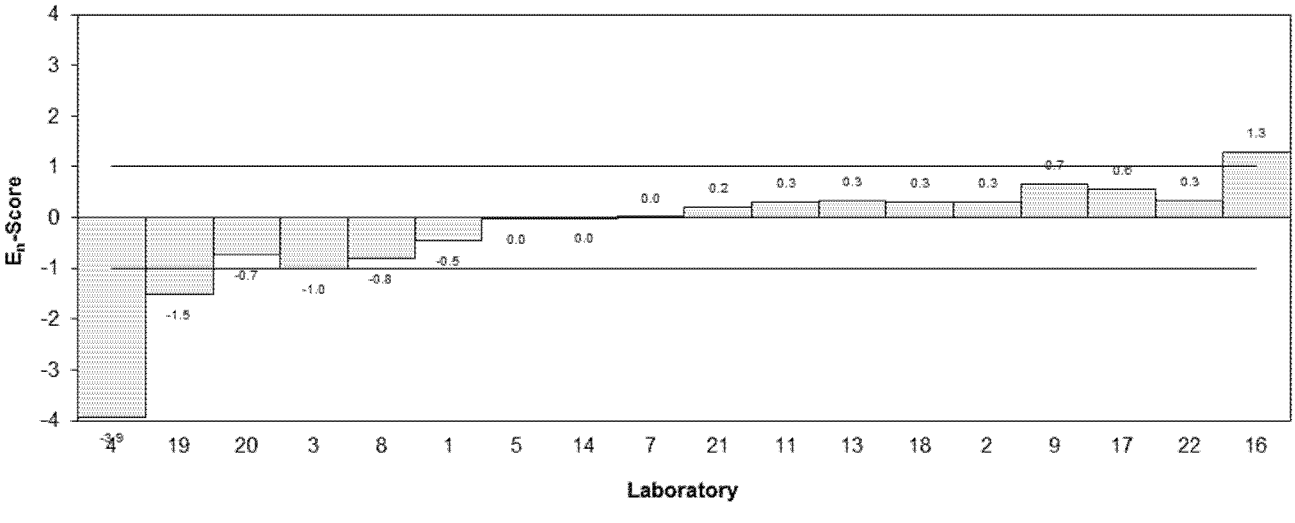


Figure 12

Table 20

Sample Details

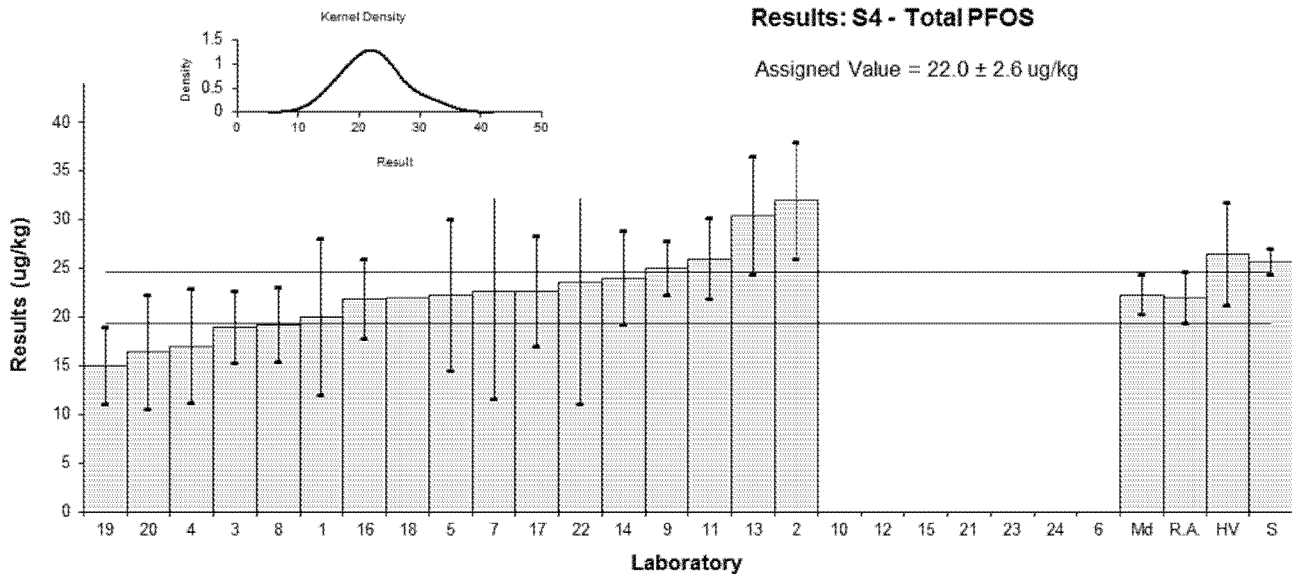
Sample No.	S4
Matrix.	Soil
Analyte.	Total PFOS
Units	ug/kg

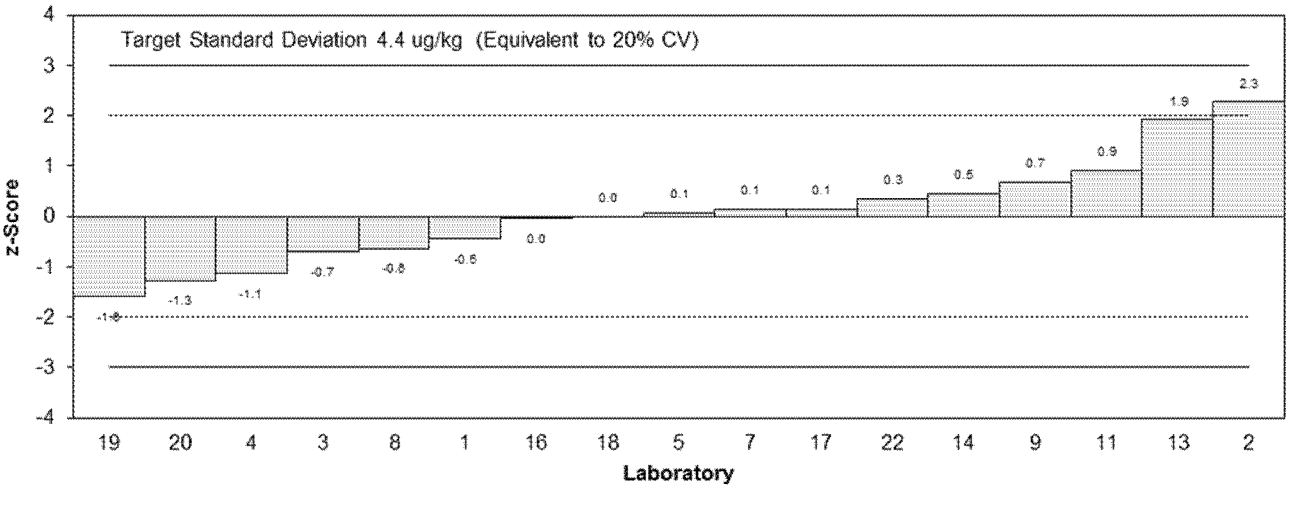
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	20	8	97	-0.45	-0.24
2	32	6.0	NR	2.27	1.53
3	18.9	3.7	94	-0.70	-0.69
4	17	5.879	70.1	-1.14	-0.78
5	22.27	7.79	103	0.06	0.03
6	NT	NT	NT		
7	22.6	11	57.5	0.14	0.05
8	19.2	3.8	89.1	-0.64	-0.61
9	25	2.8	87	0.68	0.79
10	NT	NT	NT		
11	26.0	4.2	77.7	0.91	0.81
12	NT	NT	NT		
13	30.42	6.1	103	1.91	1.27
14	24	4.8	107	0.45	0.37
15	NT	NT	NT		
16	21.8	4.09	104	-0.05	-0.04
17	22.61	5.65	93	0.14	0.10
18	22	NR	NR	0.00	0.00
19	15	4	86	-1.59	-1.47
20	16.4	5.9	NR	-1.27	-0.87
21	NT	NT	NT		
22	23.5	12.5	43	0.34	0.12
23	NT	NT	NT		
24	NT	NT	NT		

Statistics

Assigned Value	22.0	2.6
Spike	25.7	1.3
Homogeneity Value	26.5	5.3
Robust Average	22.0	2.6
Median	22.3	2.1
Mean	22.3	
N	17	
Max.	32	
Min.	15	
Robust SD	4.2	
Robust CV	19%	


Kernel Density
Density
Result
Results: S4 - Total PFOS
Assigned Value = 22.0 ± 2.6 ug/kg
Results (ug/kg)
Laboratory
19 20 4 3 8 1 16 18 5 7 17 22 14 9 11 13 2 10 12 15 21 23 24 6 Md R.A. HV S


z-Scores: S4 - Total PFOS
Target Standard Deviation 4.4 ug/kg (Equivalent to 20% CV)
z-Score
Laboratory
19 20 4 3 8 1 16 18 5 7 17 22 14 9 11 13 2

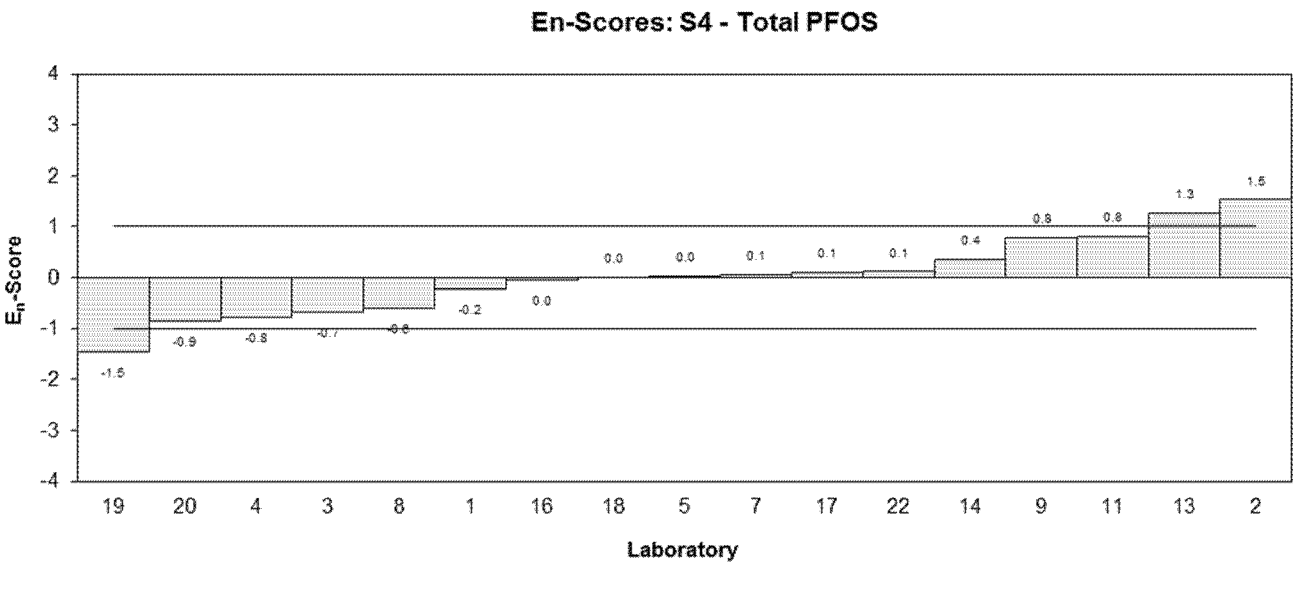

En-Scores: S4 - Total PFOS
En-Score
Laboratory
19 20 4 3 8 1 16 18 5 7 17 22 14 9 11 13 2

Figure 13

Table 21

Sample Details

Sample No.	S5
Matrix.	Water
Analyte.	Linear PFOS
Units	ug/L

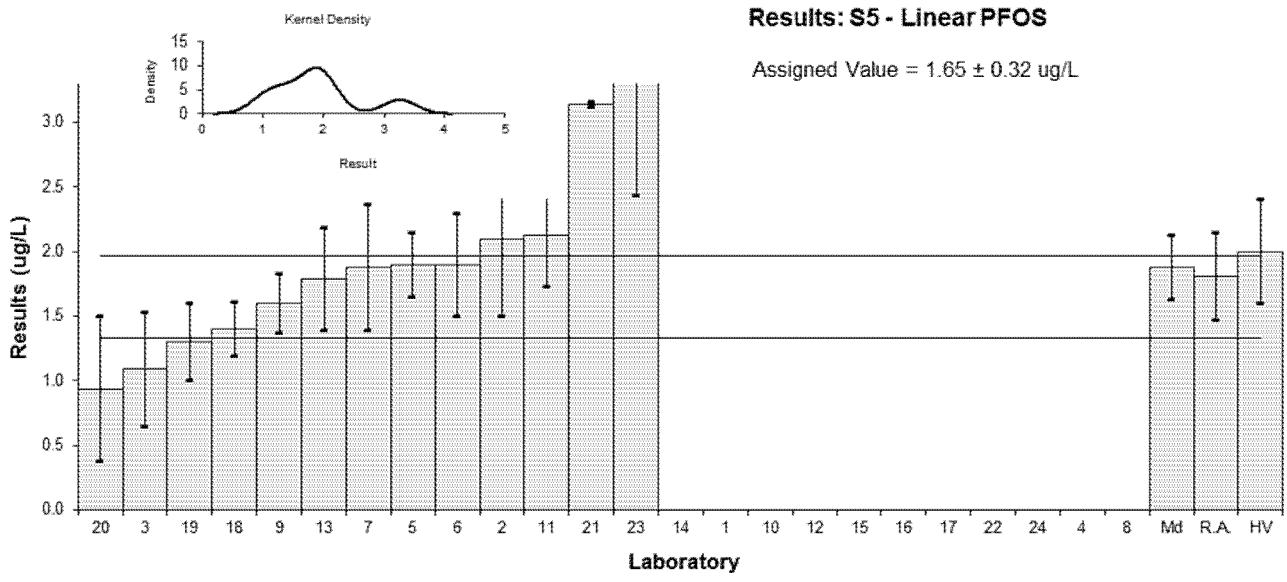
Participant Results

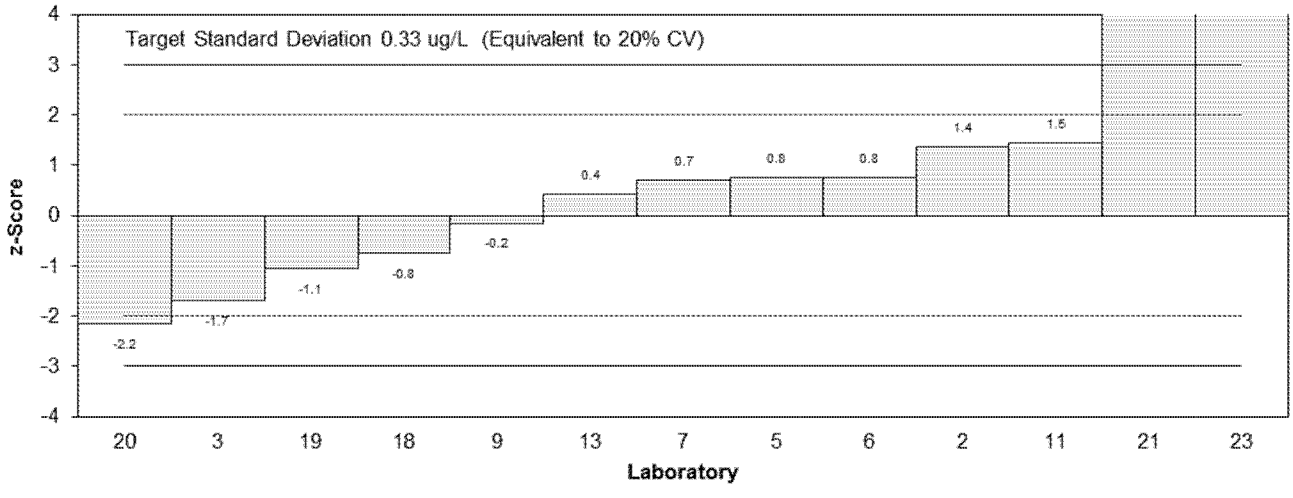
Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	NT	NT	NT		
2	2.1	0.6	NR	1.36	0.66
3	1.09	0.44	49	-1.70	-1.03
4	NT	NT	NT		
5	1.90	0.25	106	0.76	0.62
6	1.9	0.4	NR	0.76	0.49
7	1.88	0.49	52.3	0.70	0.39
8	NT	NT	NT		
9	1.6	0.23	93	-0.15	-0.13
10	NT	NT	NT		
11	2.13	0.40	70.8	1.45	0.94
12	NT	NT	NT		
13	1.79	0.4	NR	0.42	0.27
14	NR	NR	NR		
15	NT	NT	NT		
16	NT	NT	NT		
17	NT	NT	NT		
18	1.4	0.21	99	-0.76	-0.65
19	1.3	0.3	87	-1.06	-0.80
20	0.94	0.56	NR	-2.15	-1.10
21	3.14	0.016	NR	4.52	4.65
22	NT	NT	NT		
23	3.374	0.944	NR	5.22	1.73
24	NT	NT	NT		

Statistics

Assigned Value*	1.65	0.32
Spike	Not Spiked	
Homogeneity Value	2.00	0.40
Robust Average	1.81	0.34
Median	1.88	0.25
Mean	1.89	
N	13	
Max.	3.374	
Min.	0.94	
Robust SD	0.49	
Robust CV	27%	

*Robust average excluding laboratories 21 and 23.


Kernel Density
Density
Result
Results: S5 - Linear PFOS
Assigned Value = 1.65 ± 0.32 ug/L
Results (ug/L)
Laboratory
20 3 19 18 9 13 7 5 6 2 11 21 23 14 1 10 12 15 16 17 22 24 4 8 Md R.A. HV


z-Scores: S5 - Linear PFOS
Target Standard Deviation 0.33 ug/L (Equivalent to 20% CV)
z-Score
Laboratory
20 3 19 18 9 13 7 5 6 2 11 21 23

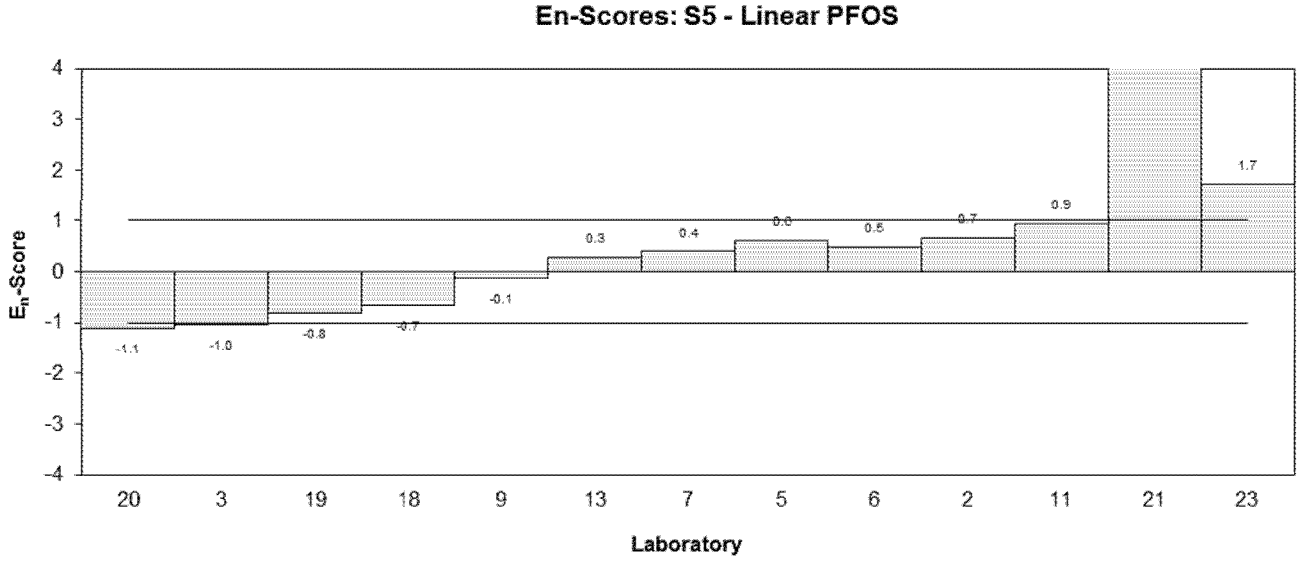

En-Scores: S5 - Linear PFOS
En-Score
Laboratory
20 3 19 18 9 13 7 5 6 2 11 21 23

Figure 14

Table 22

Sample Details

Sample No.	S5
Matrix.	Water
Analyte.	PFOA
Units	ug/L

Participant Results

Lab Code	Result	Uncertainty	Recovery
1	<0.01	NR	101
2	<0.02	NR	NR
3	0.002	0.0008	141
4	NT	NT	NT
5	0.087	0.010	94
6	5.8	1.2	NR
7	0.00214	0.00043	77.8
8	NT	NT	NT
9	0.0016	0.0001	94
10	NT	NT	NT
11	<0.01	0.01	81.2
12	NT	NT	NT
13	< 0.01	0.01	100
14	<0.1	NR	100
15	NT	NT	NT
16	<0.001	0.0002	91
17	NT	NT	NT
18	<0.001	NR	99
19	<0.01	NR	87
20	<0.05	0.018	NR
21	<0.0379	0.017	NR
22	0.00791	0.0030	43
23	0.153	0.042	NR
24	NT	NT	NT

Statistics

Assigned Value	Not Set	
Spike	Not Spiked	
Robust Average	0.011	0.011
Median	0.0079	0.0087
Mean	0.86	
N	7	
Max.	5.8	
Min.	0.0016	

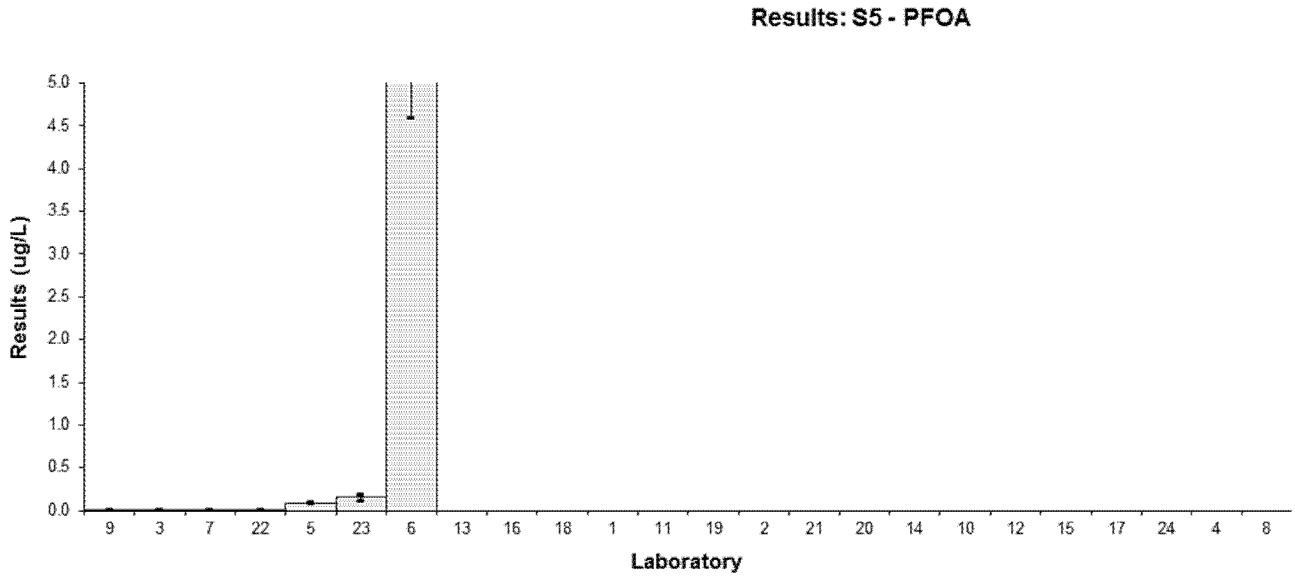

Results: S5 - PFOA
Results (ug/L)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
9 3 7 22 5 23 6 13 16 18 1 11 19 2 21 20 14 10 12 15 17 24 4 8
Laboratory

Figure 15

Table 23

Sample Details

Sample No.	S5
Matrix.	Water
Analyte.	Total PFOS
Units	ug/L

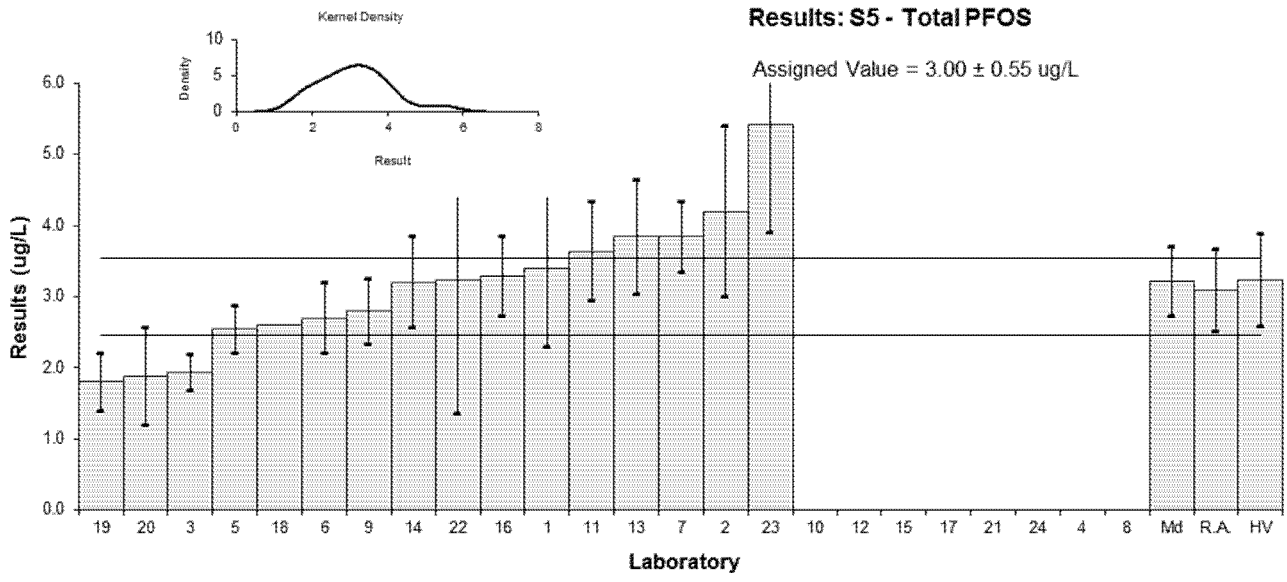
Participant Results

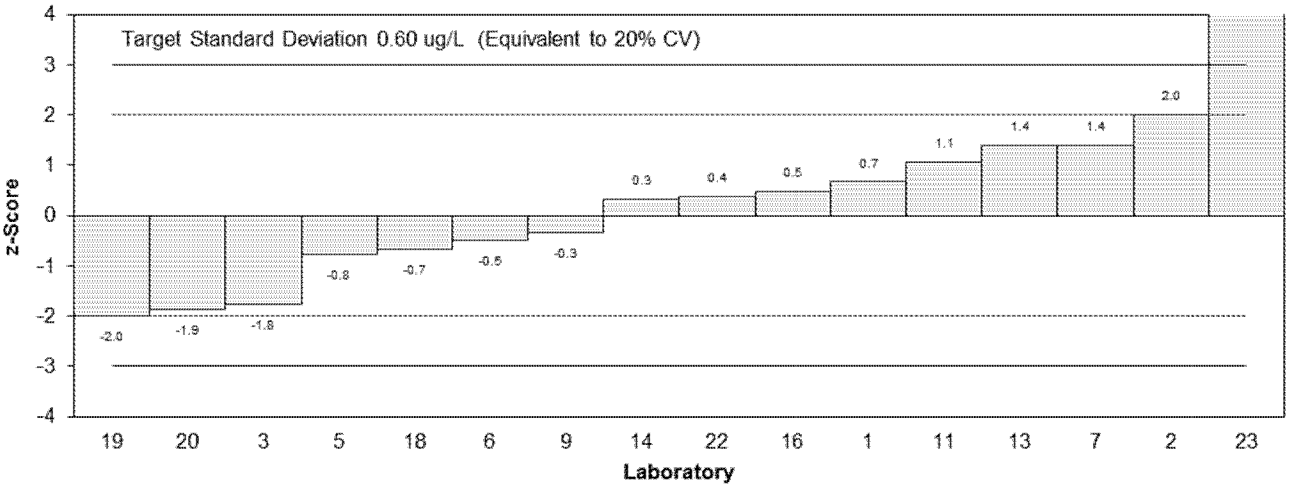
Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	3.4	1.1	72	0.67	0.33
2	4.2	1.2	NR	2.00	0.91
3	1.94	0.25	49	-1.77	-1.75
4	NT	NT	NT		
5	2.54	0.33	106	-0.77	-0.72
6	2.7	0.5	NR	-0.50	-0.40
7	3.84	0.49	52.3	1.40	1.14
8	NT	NT	NT		
9	2.8	0.46	93	-0.33	-0.28
10	NT	NT	NT		
11	3.64	0.69	70.8	1.07	0.73
12	NT	NT	NT		
13	3.84	0.8	103	1.40	0.87
14	3.2	0.64	95	0.33	0.24
15	NT	NT	NT		
16	3.290	0.5659	84	0.48	0.37
17	NT	NT	NT		
18	2.6	NR	NR	-0.67	-0.73
19	1.8	0.4	87	-2.00	-1.76
20	1.88	.68	NR	-1.87	-1.28
21	NT	NT	NT		
22	3.23	1.87	85	0.38	0.12
23	5.42	1.518	NR	4.03	1.50
24	NT	NT	NT		

Statistics

Assigned Value*	3.00	0.55
Spike	Not Spiked	
Homogeneity Value	3.24	0.65
Robust Average	3.09	0.57
Median	3.22	0.49
Mean	3.14	
N	16	
Max.	5.42	
Min.	1.8	
Robust SD	0.92	
Robust CV	30%	

*Robust average excluding laboratory 23.


Kernel Density
Density
Result
Results: S5 - Total PFOS
Assigned Value = 3.00 ± 0.55 ug/L
Results (ug/L)
Laboratory
19 20 3 5 18 6 9 14 22 16 1 11 13 7 2 23 10 12 15 17 21 24 4 8 Md R.A. HV


z-Scores: S5 - Total PFOS
Target Standard Deviation 0.60 ug/L (Equivalent to 20% CV)
z-Score
Laboratory
19 20 3 5 18 6 9 14 22 16 1 11 13 7 2 23

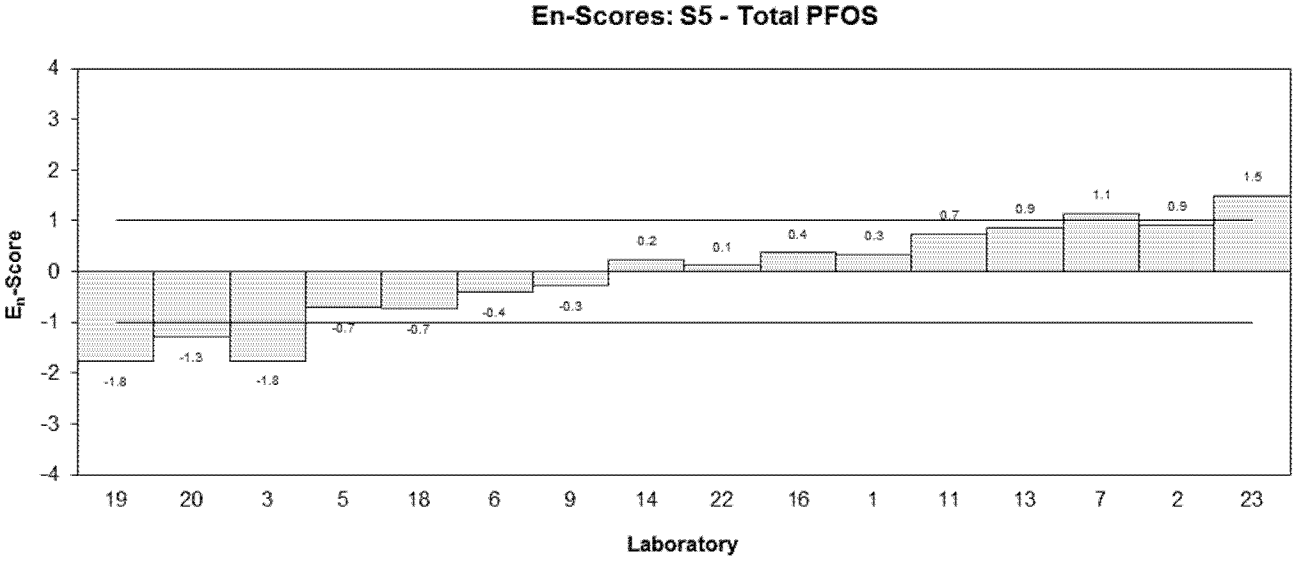

En-Scores: S5 - Total PFOS
En-Score
Laboratory
19 20 3 5 18 6 9 14 22 16 1 11 13 7 2 23

Figure 16

Table 24

Sample Details

Sample No.	S6
Matrix.	Water
Analyte.	Linear PFOS
Units	ug/L

Participant Results

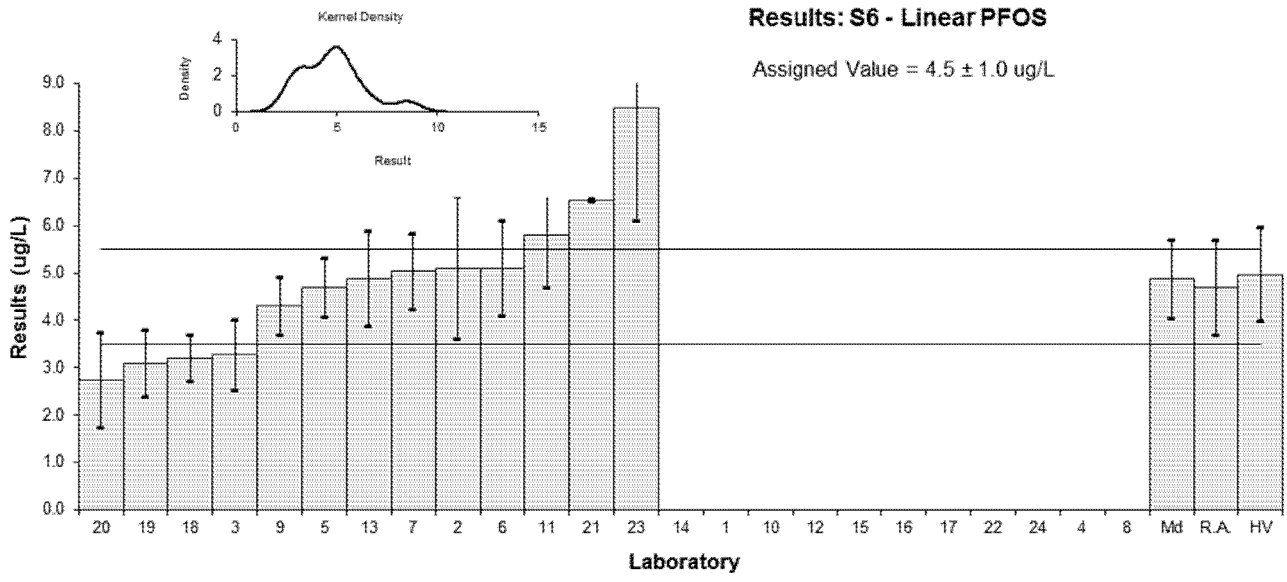
Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	NT	NT	NT		
2	5.1	1.5	NR	0.67	0.33
3	3.27	0.75	41	-1.37	-0.98
4	NT	NT	NT		
5	4.70	0.62	89	0.22	0.17
6	5.1	1.0	NR	0.67	0.42
7	5.03	0.8	79.9	0.59	0.41
8	NT	NT	NT		
9	4.3	0.6	93	-0.22	-0.17
10	NT	NT	NT		
11	5.80	1.10	72.4	1.44	0.87
12	NT	NT	NT		
13	4.87	1.0	NR	0.41	0.26
14	NR	NR	NR		
15	NT	NT	NT		
16	NT	NT	NT		
17	NT	NT	NT		
18	3.2	0.48	107	-1.44	-1.17
19	3.1	0.7	91	-1.56	-1.15
20	2.74	1	NR	-1.96	-1.24
21	6.53	0.016	NR	2.26	2.03
22	NT	NT	NT		
23	8.478	2.373	NR	4.42	1.54
24	NT	NT	NT		

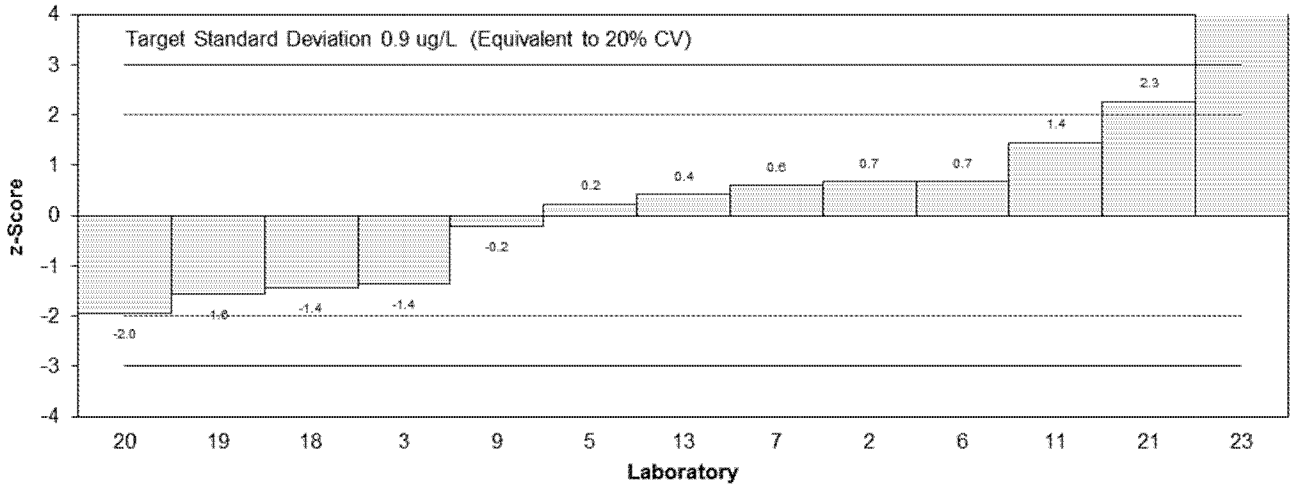
Statistics

Assigned Value*	4.5	1.0
Spike**	Not Spiked	
Homogeneity Value	4.97	0.99
Robust Average	4.7	1.0
Median	4.87	0.83
Mean	4.79	
N	13	
Max.	8.478	
Min.	2.74	
Robust SD	1.5	
Robust CV	32%	

*Robust average excluding laboratory 23.

**Sample was spiked with a technical mixture, linear PFOS value has not been certified.


Kernel Density
Density
Result
Results: S6 - Linear PFOS
Assigned Value = 4.5 ± 1.0 ug/L
Results (ug/L)
Laboratory
20 19 18 3 9 5 13 7 2 6 11 21 23 14 1 10 12 15 16 17 22 24 4 8 Md R.A. HV


z-Scores: S6 - Linear PFOS
Target Standard Deviation 0.9 ug/L (Equivalent to 20% CV)
z-Score
-2.0 -1.6 -1.4 -1.4 -0.2 0.2 0.4 0.6 0.7 0.7 1.4 2.3
Laboratory
20 19 18 3 9 5 13 7 2 6 11 21 23

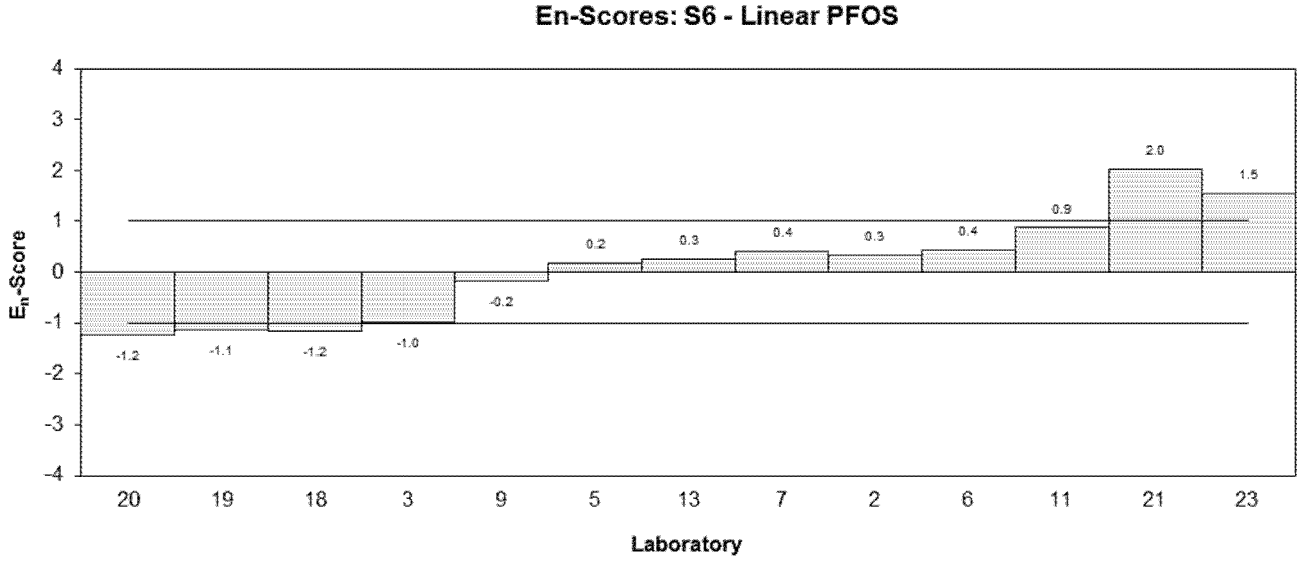

En-Scores: S6 - Linear PFOS
En-Score
-1.2 -1.1 -1.2 -1.0 -0.2 0.2 0.3 0.4 0.3 0.4 0.9 2.0 1.5
Laboratory
20 19 18 3 9 5 13 7 2 6 11 21 23

Figure 17

Table 25

Sample Details

Sample No.	S6
Matrix.	Water
Analyte.	PFOA
Units	ug/L

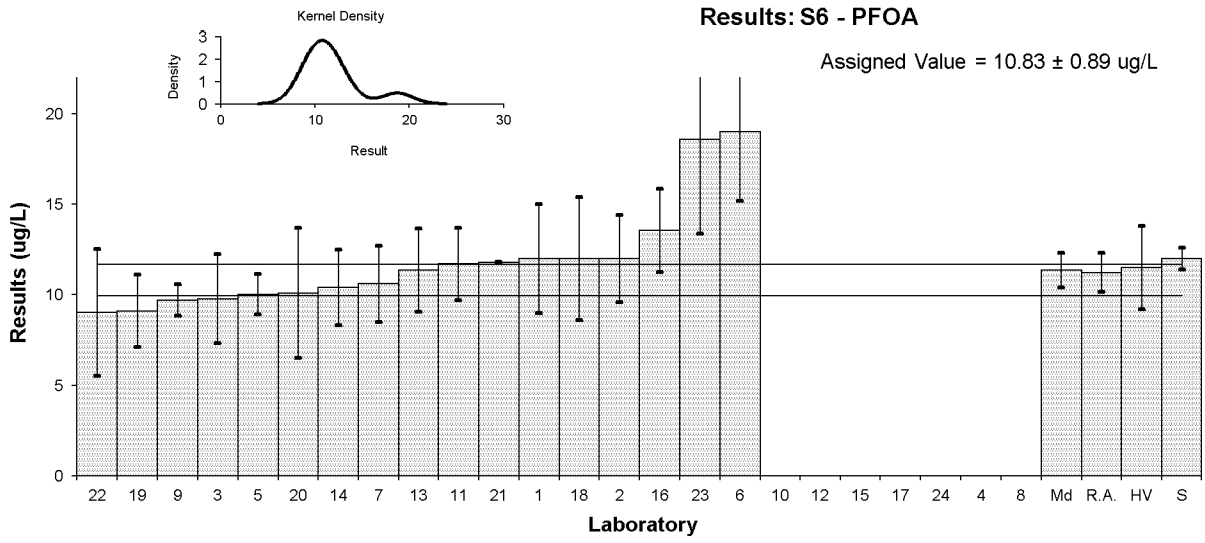
Participant Results

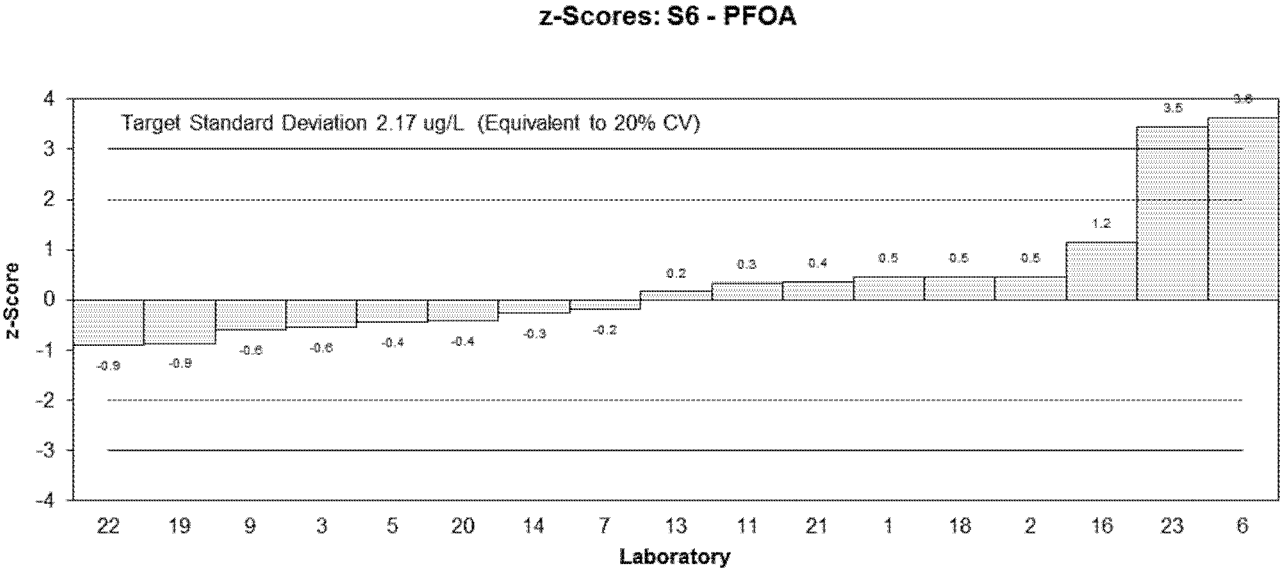
Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	12	3	131	0.54	0.37
2	12	2.4	NR	0.54	0.46
3	9.78	2.47	47	-0.48	-0.40
4	NT	NT	NT		
5	10.02	1.12	93	-0.37	-0.57
6	19	3.8	NR	3.77	2.09
7	10.6	2.1	97.4	-0.11	-0.10
8	NT	NT	NT		
9	9.7	0.87	94	-0.52	-0.91
10	NT	NT	NT		
11	11.7	2.0	103	0.40	0.40
12	NT	NT	NT		
13	11.36	2.3	112	0.24	0.22
14	10.4	2.1	100	-0.20	-0.19
15	NT	NT	NT		
16	13.54	2.302	94	1.25	1.10
17	NT	NT	NT		
18	12	3.4	95	0.54	0.33
19	9.1	2	91	-0.80	-0.79
20	10.1	3.6	NR	-0.34	-0.20
21	11.8	0.017	NR	0.45	1.09
22	9.01	3.51	62	-0.84	-0.50
23	18.598	5.207	NR	3.59	1.48
24	NT	NT	NT		

Statistics

Assigned Value*	10.83	0.89
Spike	12.00	0.60
Homogeneity Value	11.5	2.3
Robust Average	11.2	1.1
Median	11.36	0.96
Mean	11.81	
N	17	
Max.	19	
Min.	9.01	
Robust SD	1.79	
Robust CV	16%	

*Robust average excluding laboratories 6 and 23.


Kernel Density
Density
Result
Results: S6 - PFOA
Assigned Value = 10.83 ± 0.89 ug/L
Results (ug/L)
Laboratory
22 19 9 3 5 20 14 7 13 11 21 1 18 2 16 23 6 10 12 15 17 24 4 8 Md R.A. HV S


z-Scores: S6 - PFOA
Target Standard Deviation 2.17 ug/L (Equivalent to 20% CV)
z-Score
Laboratory
22 19 9 3 5 20 14 7 13 11 21 1 18 2 16 23 6

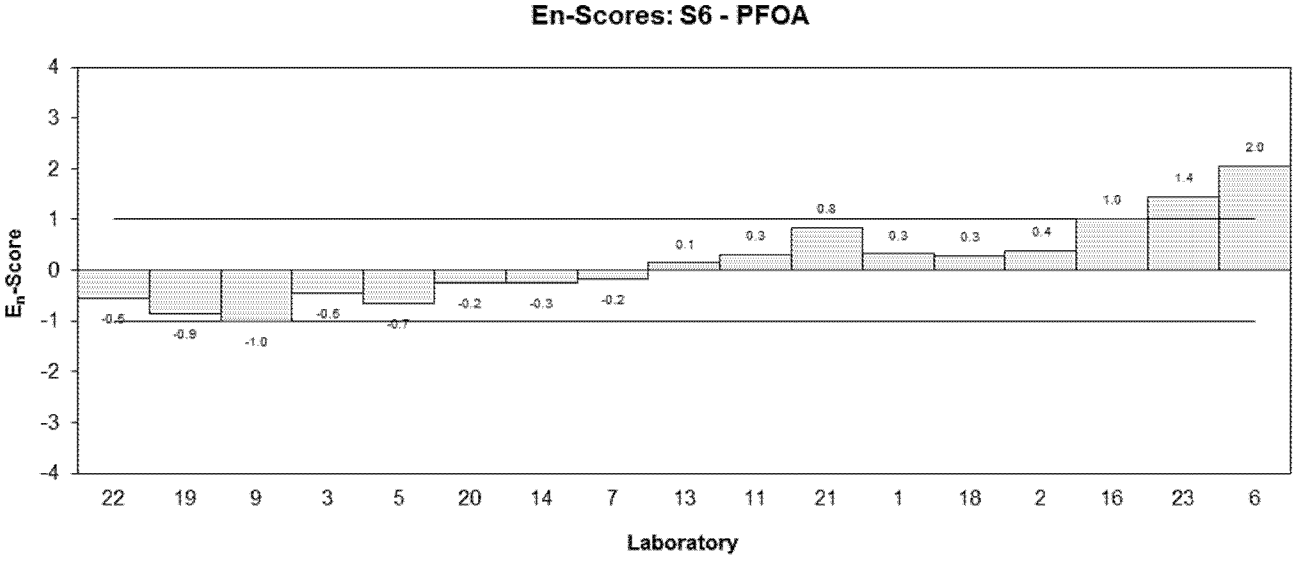

En-Scores: S6 - PFOA
En-Score
Laboratory
22 19 9 3 5 20 14 7 13 11 21 1 18 2 16 23 6

Figure 18

Table 26

Sample Details

Sample No.	S6
Matrix.	Water
Analyte.	Total PFOS
Units	ug/L

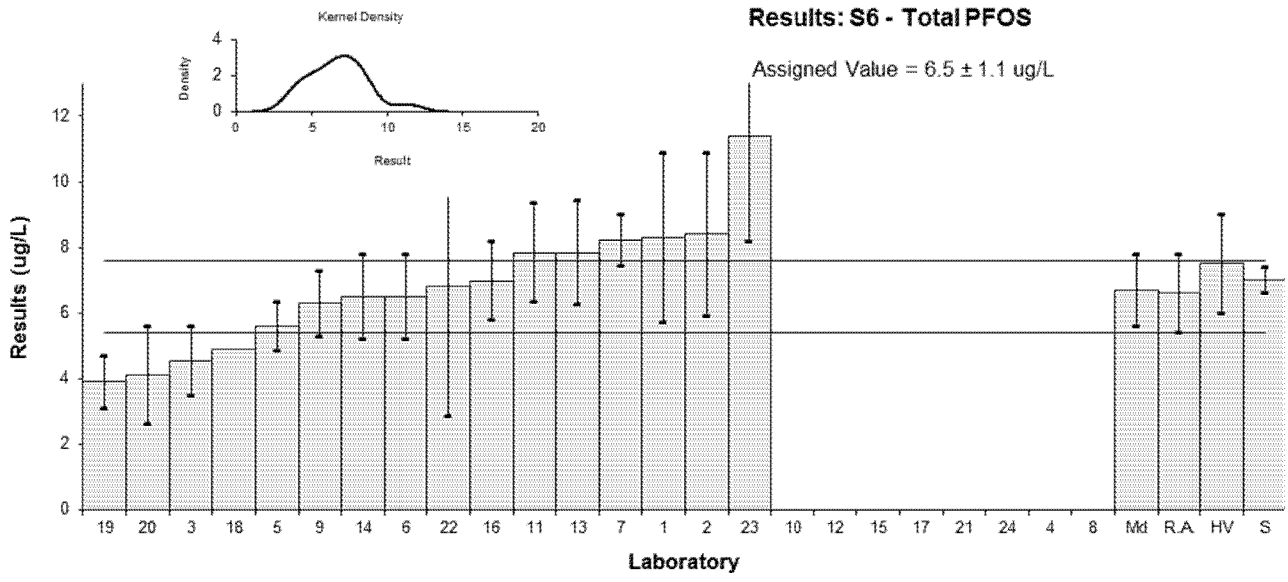
Participant Results

Lab Code	Result	Uncertainty	Recovery	z-Score	E_n-Score
1	8.3	2.6	48	1.38	0.64
2	8.4	2.5	NR	1.46	0.70
3	4.56	1.06	41	-1.49	-1.27
4	NT	NT	NT		
5	5.60	0.74	89	-0.69	-0.68
6	6.5	1.3	NR	0.00	0.00
7	8.22	0.8	79.9	1.32	1.26
8	NT	NT	NT		
9	6.3	1.0	93	-0.15	-0.13
10	NT	NT	NT		
11	7.85	1.49	72.4	1.04	0.73
12	NT	NT	NT		
13	7.85	1.6	118	1.04	0.70
14	6.5	1.3	95	0.00	0.00
15	NT	NT	NT		
16	6.979	1.200	90	0.37	0.29
17	NT	NT	NT		
18	4.9	NR	NR	-1.23	-1.45
19	3.9	0.8	91	-2.00	-1.91
20	4.12	1.5	NR	-1.83	-1.28
21	NT	NT	NT		
22	6.81	3.95	95	0.24	0.08
23	11.387	3.188	NR	3.76	1.45
24	NT	NT	NT		

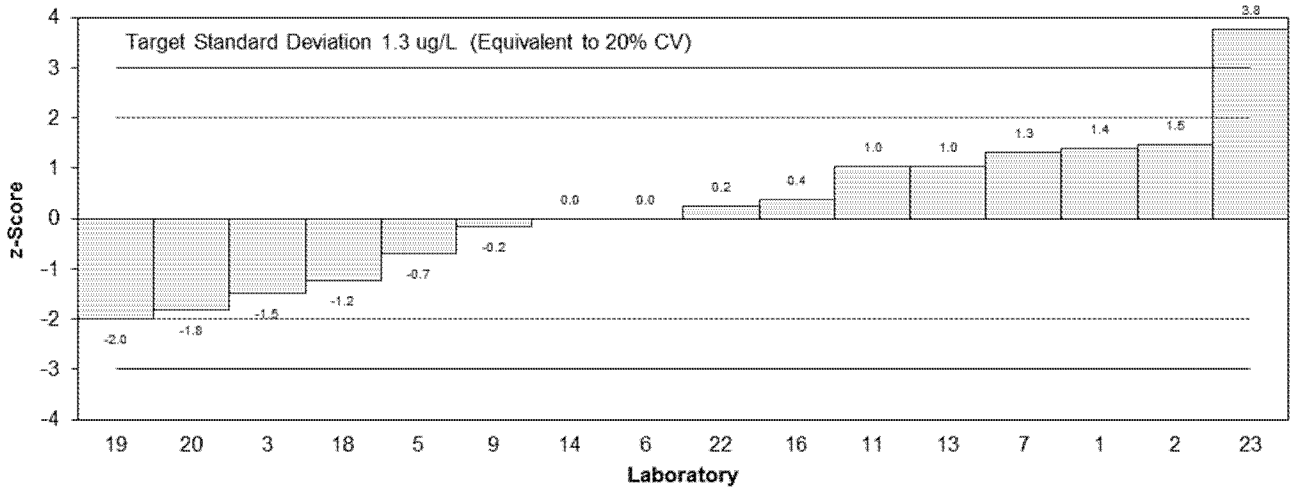
Statistics

Assigned Value*	6.5	1.1
Spike	7.00	0.40
Homogeneity Value	7.5	1.5
Robust Average	6.6	1.2
Median	6.7	1.1
Mean	6.8	
N	16	
Max.	11.387	
Min.	3.9	
Robust SD	1.90	
Robust CV	29%	

*Robust average excluding laboratory23.



z-Scores: S6 - Total PFOS



En-Scores: S6 - Total PFOS

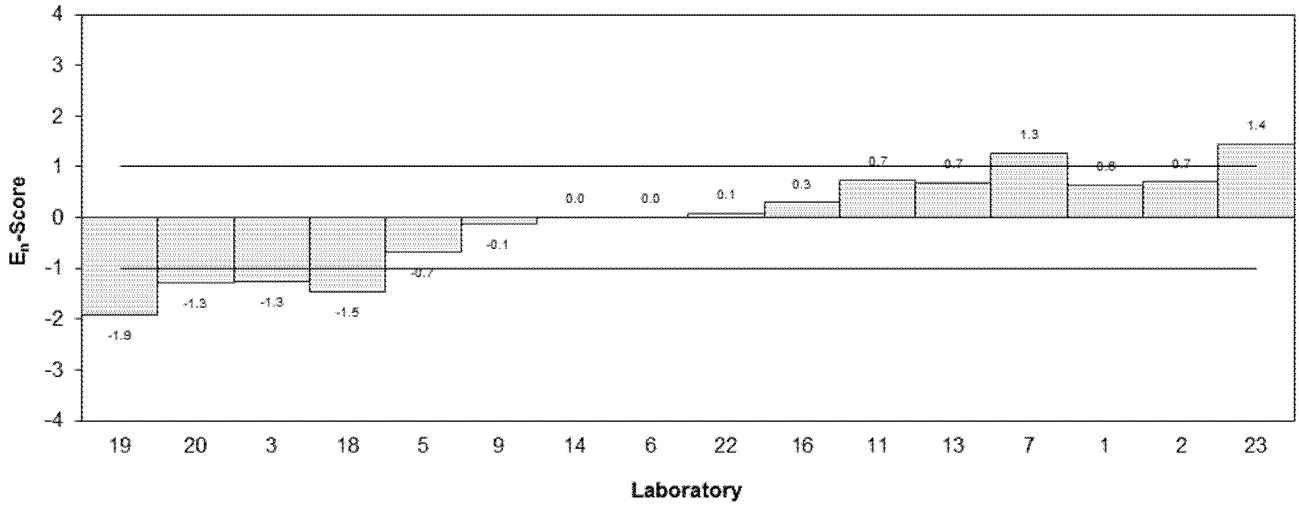


Figure 19

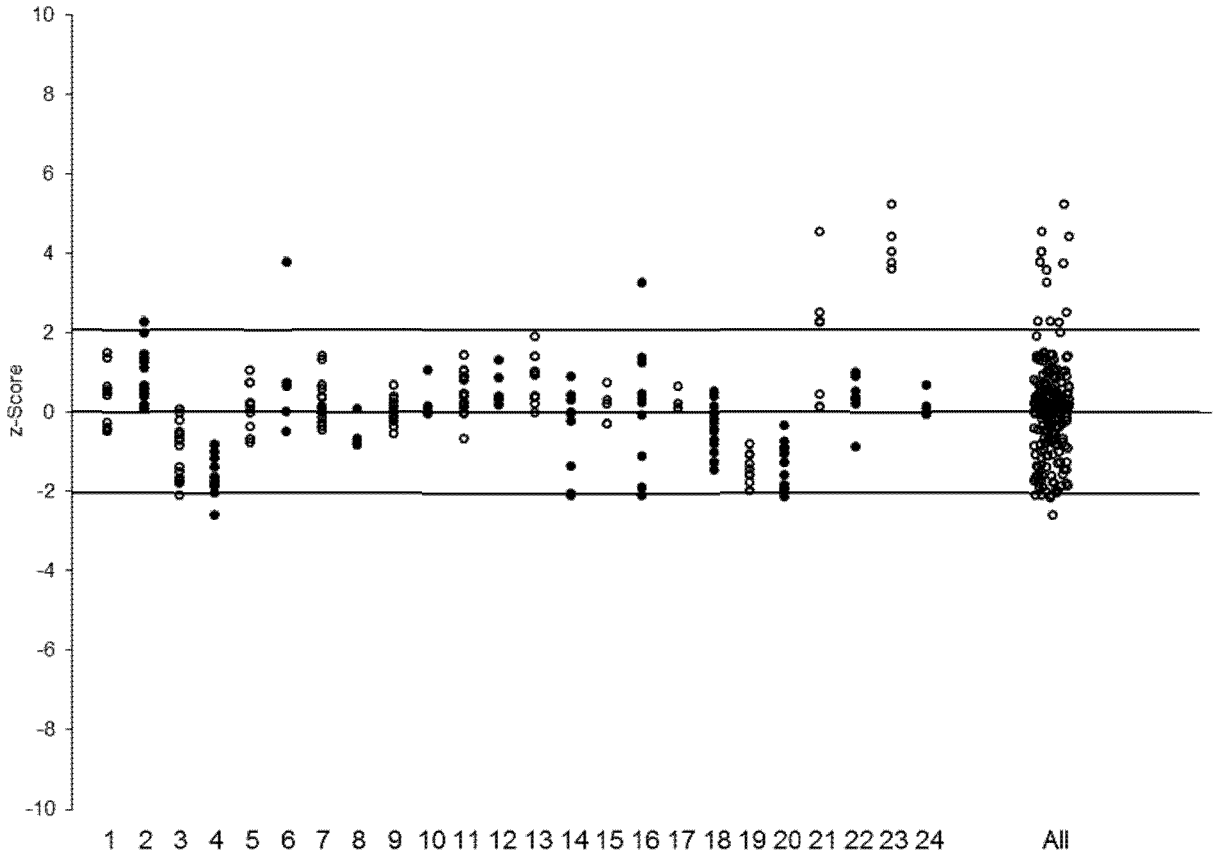


Figure 20 z-Score Dispersal by Laboratory

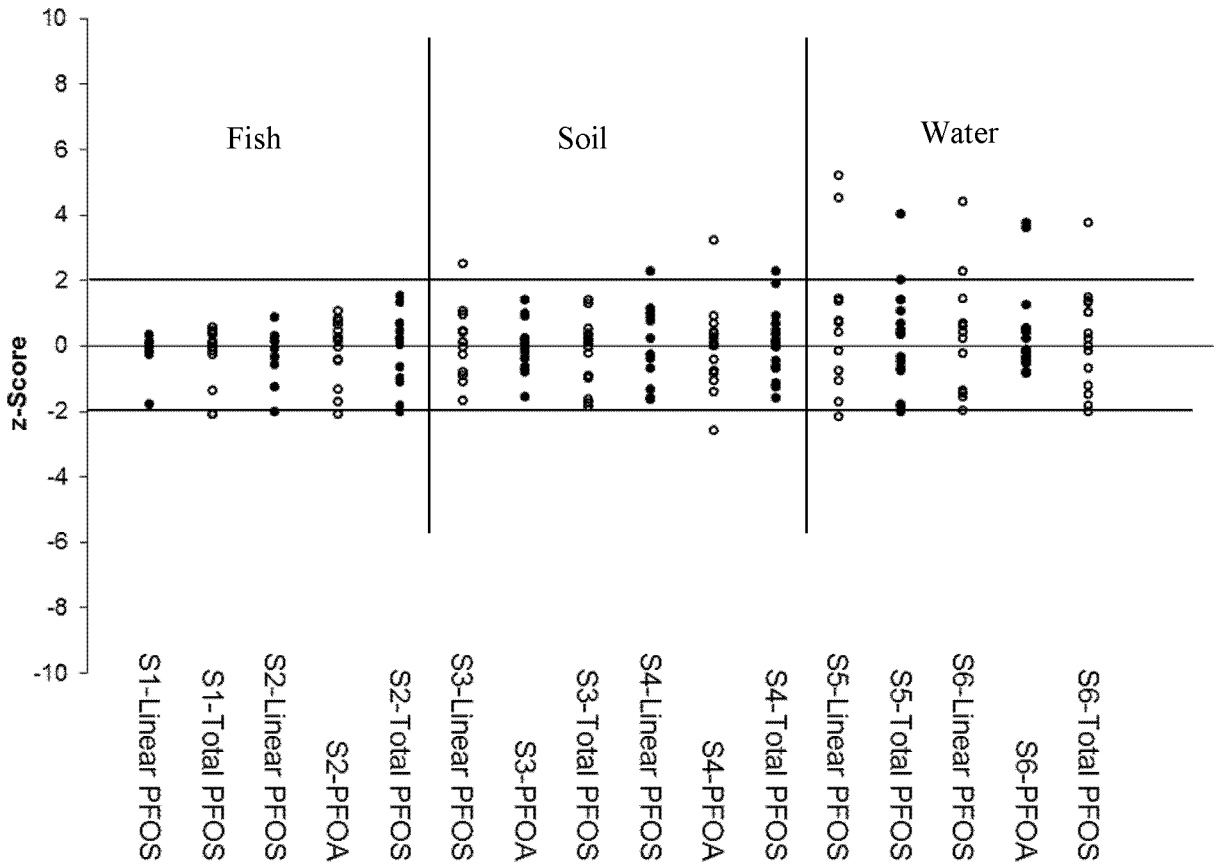


Figure 21 z-Score Dispersal by Analyte

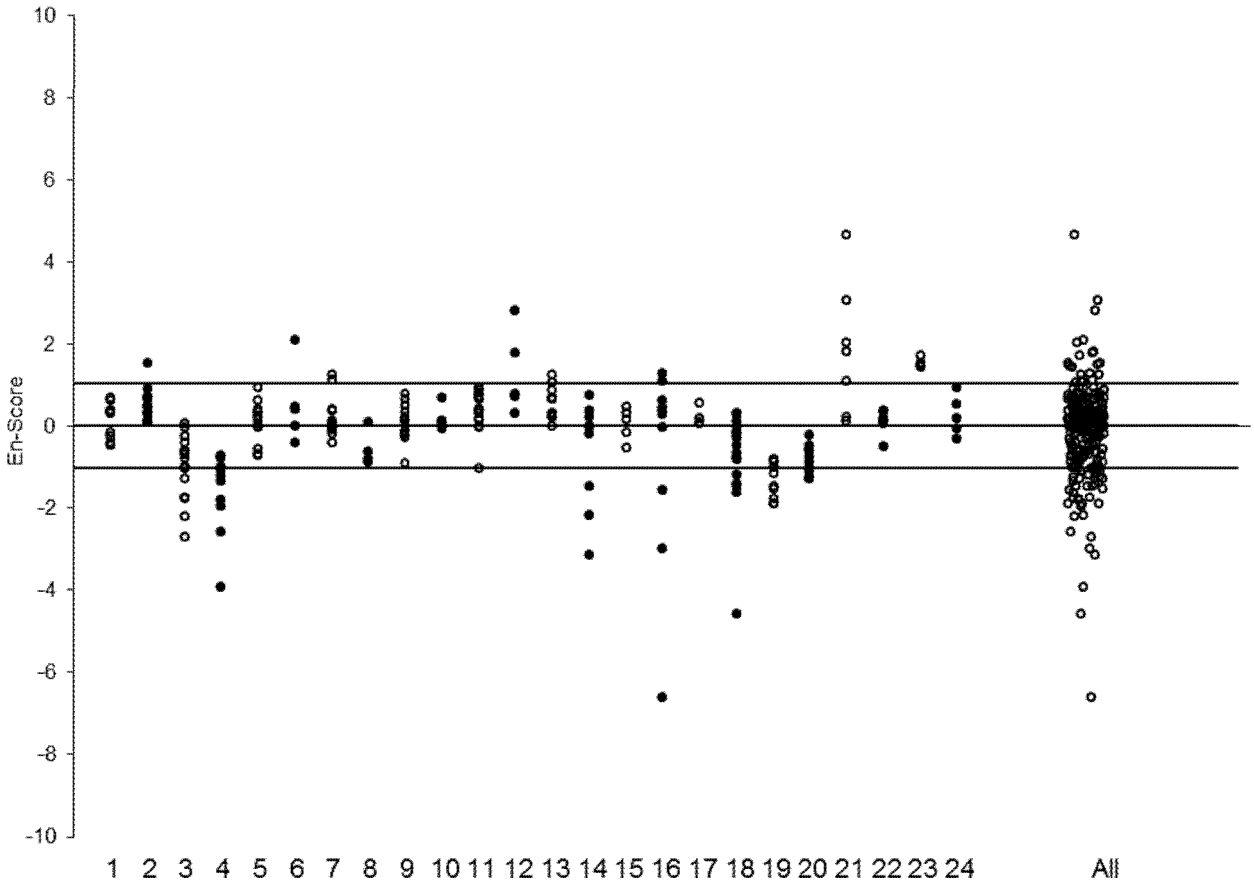


Figure 22 E_n-Score Dispersal by Laboratory

6 DISCUSSION OF RESULTS

6.1 Assigned Value

The robust average of participants' results was used as the assigned values for all samples. The robust averages and associated expanded uncertainties, were calculated using the procedure described in 'ISO13528:2015(E), Statistical methods for use in proficiency testing by interlaboratory comparisons'.[8] The calculation of the expanded uncertainty for the robust average of PFOA in Sample S6 is presented in Appendix 3.

Results less than 50% and greater than 150% of the robust average were removed before calculation of the assigned value.[3,4]

Assigned values for spiked Samples S2, S4 and S6 were within the range 72 – 92% of the spiked concentration for that analyte (Table 27).

No assigned values were calculated for PFOA in Samples S1 and S5, the levels in the incurred samples were very low and only a few laboratories were able to report numeric results.

Traceability: The consensus of participants' results is not traceable to any external reference, so although expressed in SI units, metrological traceability has not been established.

Table 27 Comparison of Assigned Value and Spiked Concentration.

Sample	Matrix	Analyte	Units	Spiked Concentration	Assigned Value	Assigned/ Spike (%)
S2	Fish	PFOA	µg/kg	69.9	50.5	72
S2	Fish	Total PFOS	µg/kg	64.9	53.7	83
S4	Soil	PFOA	µg/kg	14.8	12.0	81
S4	Soil	Total PFOS	µg/kg	25.7	22.0	86
S6	Water	PFOA	µg/L	12.0	10.83	90
S6	Water	Total PFOS	µg/L	7.0	6.5	93

6.2 Measurement Uncertainty Reported by Participants

Participants were asked to report an estimate of the expanded uncertainty associated with their results and the basis of this uncertainty estimate (Table 4).

It is a requirement of the ISO Standard 17025 that laboratories have procedures to estimate the uncertainty of chemical measurements and to report this uncertainty in specific circumstances, including: 'when the client's instruction so requires.'

Two hundred and thirty of two hundred and forty-two results (95%) were reported with an associated estimate of expanded measurement uncertainty. Laboratories **16** and **18** reported expanded measurement uncertainties only for some analytes.

The magnitude of the reported expanded uncertainties was within the range 0% to 72% of the reported value. Twenty-one were less than 10% relative, which the study coordinator believes is unrealistically small for a routine PFOS/PFOA measurement.

Results returning a satisfactory z-score but an unsatisfactory E_n-score may have underestimated the uncertainty.

Some participants attached an estimate of the expanded measurement uncertainty to a result reported as less than their limit of reporting.

In some cases the results were reported with an inappropriate number of significant figures. The recommended format is to write uncertainty to no more than two significant figures and then to write the result with the corresponding number of decimal places (for example instead of 210 ± 39.354 µg/kg better report 210 ± 39 µg/kg)[7].

6.3 z-Score

A target standard deviation equivalent to 20% coefficient of variation (CV) was used to calculate z-scores. The between laboratory coefficient of variation predicted by the modified Horwitz equation[9] and the between laboratories CV are presented for comparison in Table 28.

Table 28 Target standard deviation, between laboratories CV and modified Horwitz values

Sample	Analyte	Assigned value	Unit	Target SD (as CV, %)	Modified Horwitz CV (%)	Between laboratories' CV (%)
S1	Linear PFOS	18.62	µg/kg	20	22	2.7
S1	Total PFOS	20.6	µg/kg	20	22	9.2
S2	Linear PFOS	38.6	µg/kg	20	22	7.3
S2	PFOA	50.5	µg/kg	20	22	17
S2	Total PFOS	53.7	µg/kg	20	22	21
S3	Linear PFOS	179	µg/kg	20	22	22
S3	PFOA	5.83	µg/kg	20	22	9.6
S3	Total PFOS	262	µg/kg	20	20	11
S4	Linear PFOS	16.3	µg/kg	20	22	27
S4	PFOA	12.0	µg/kg	20	22	14
S4	Total PFOS	22.0	µg/kg	20	22	19
S5	Linear PFOS	1.65	µg/L	20	22	27
S5	Total PFOS	3.00	µg/L	20	22	30
S6	Linear PFOS	4.5	µg/L	20	22	32
S6	PFOA	10.83	µg/L	20	22	16
S6	Total PFOS	6.5	µg/L	20	22	29

The dispersal of participants' z-scores is graphically presented in Figure 20 and by analyte in Figure 21.

Of the two hundred and thirty-two results for which z-scores were calculated, 213 (92%) returned a satisfactory z-score of $|z| \leq 2$.

Nine laboratories analysed all three matrices. Laboratories **1**, **7**, **9**, **11** and **18** returned satisfactory z-scores for all analytes tested for which z-scores were calculated.

Seven laboratories analysed two matrices. Laboratories **5**, **13**, **22** and **19** returned satisfactory z-scores for all analytes tested for which z-scores were calculated.

Eight laboratories analysed only one matrix. Laboratories **8**, **10, 12**, **15**, **17** and **24** returned satisfactory z-scores for all analytes tested for which z-scores were calculated.

Laboratory **23** enrolled for water matrix only. This laboratory returned $|z| > 3$ for all test samples demonstrating an unsatisfactory performance.

Scatter plots of z-scores for all analytes are presented in Figures 23-29. Scores are predominantly plotted in quadrants I and III, indicating laboratory bias is the major contributor to the variability of results.

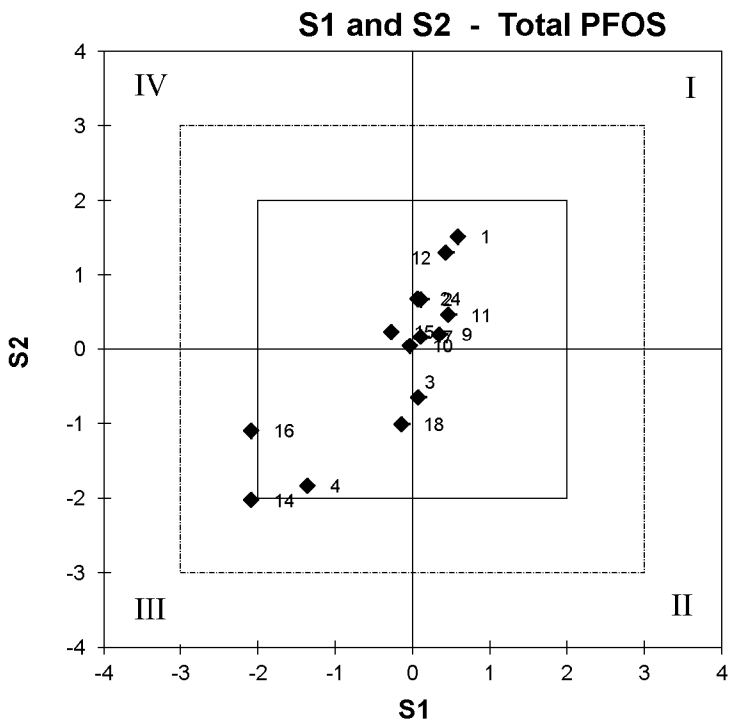


Figure 23 Fish z-score scatter plot Total PFOS

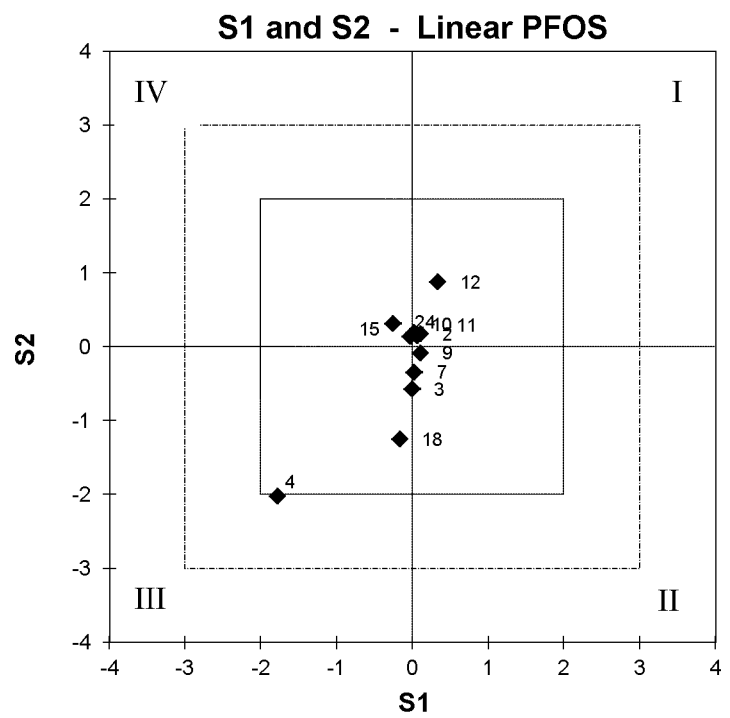


Figure 24 Fish z-score scatter plot Linear PFOS

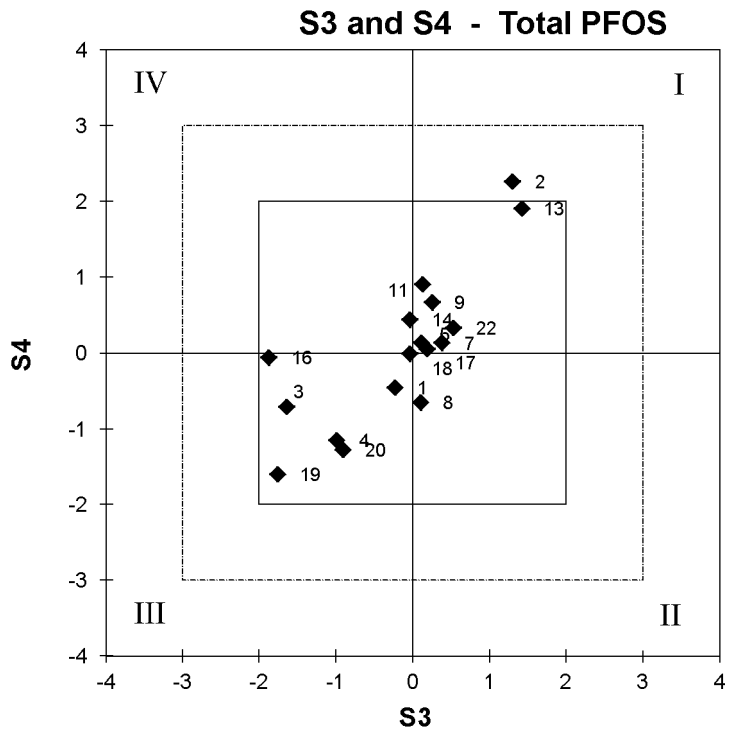


Figure 25 Soil z-score scatter plot Total PFOS

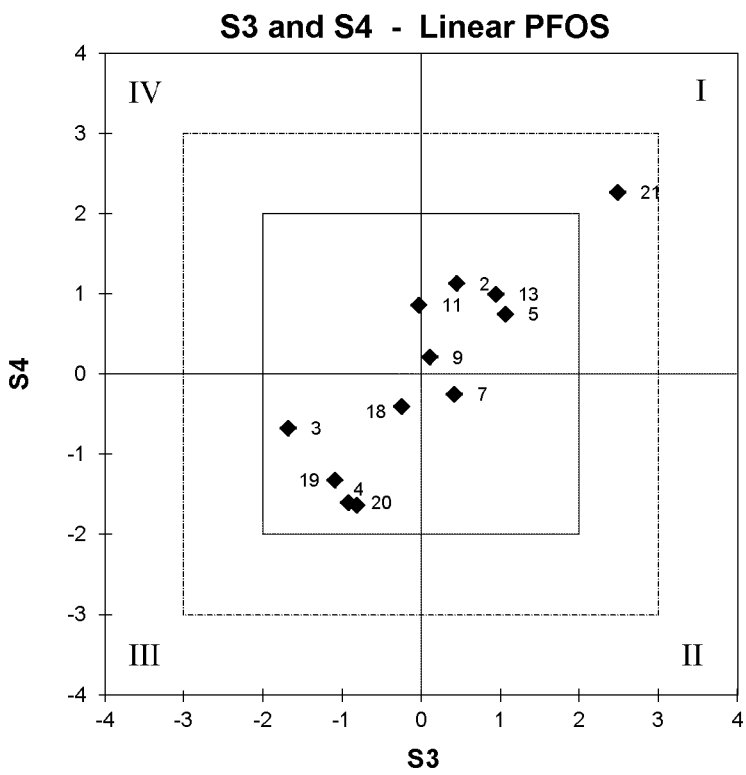


Figure 26 Soil z-score scatter plot Linear PFOS

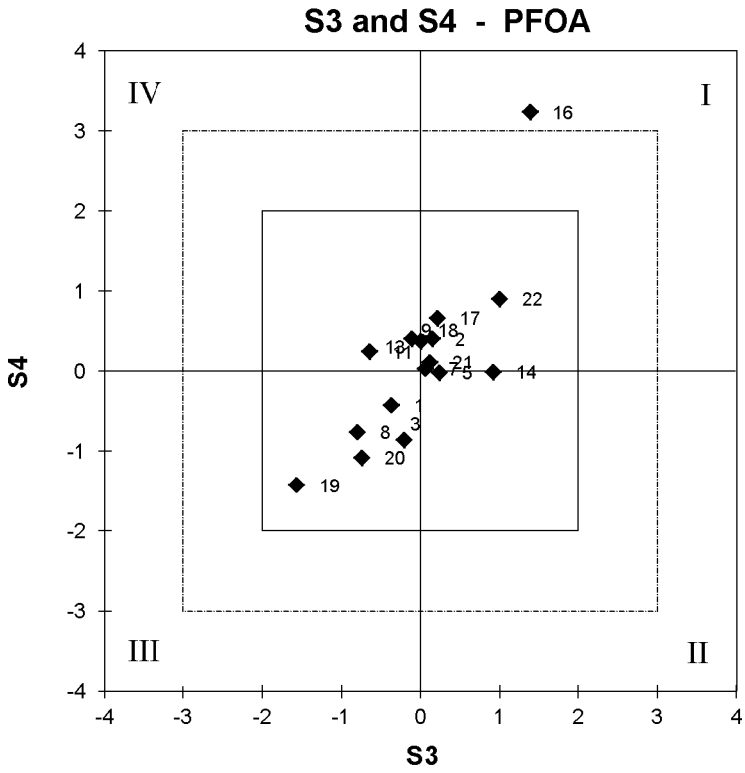
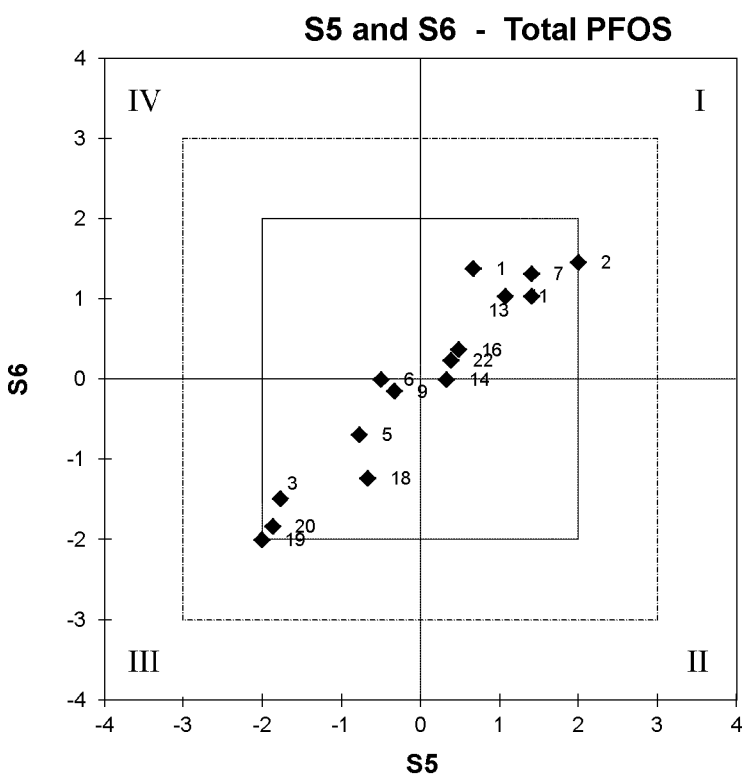
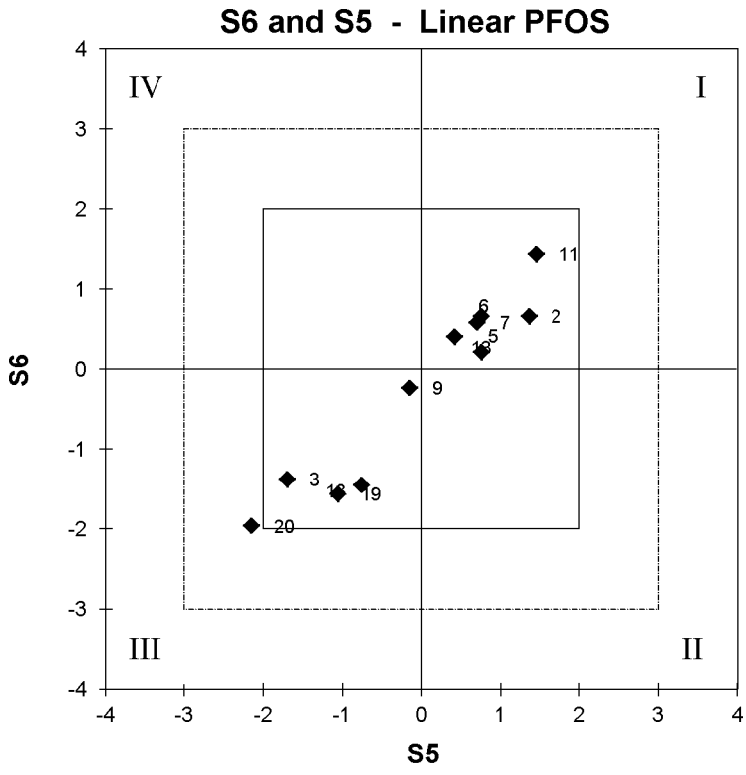


Figure 27 Soil z-score scatter plot PFOA



Laboratory 23 is off-scale.

Figure 28 Water z-score scatter plot Total PFOS



Laboratories 21 and 23 are off-scale.

Figure 29 Water z-score scatter plot Linear PFOS

6.4 E_n-Score

Where a laboratory did not report an uncertainty estimate an uncertainty of zero (0) was used to calculate the E_n-score.

Of two hundred and thirty-two E_n-scores, 174 (75%) were satisfactory with $|E_n| \leq 1$.

Laboratories **1** and **9** that analysed three matrices returned satisfactory E_n-score for all analytes tested for which E_n-scores were calculated.

Laboratory **5** that analysed two matrices and laboratories **8**, **10**, **15**, **17**, **22** and **24** that analysed one matrix returned satisfactory E_n-score for all analytes for which E_n-scores were calculated.

Laboratory **23** analysed one matrix, water, and returned $|E_n| > 1$ for all test samples. This indicates that the reported results did not agree with the assigned value and that the uncertainties associated with the results have been understated.

The dispersal of participants' E_n-scores is presented in Figure 22.

6.5 Participants' Methods

Participants were requested to analyse the samples using their normal test method and to report a single result as they would normally report to a client. Results reported in this way reflect the true variability of results reported to clients. The method descriptions provided by participants are presented in Tables 1 to 6. The study coordinator thanks all laboratories that completed the method questionnaire. A summary is presented below.

Fish samples

Pre-treatment: freezing (1), enzyme treatment (2) and alkalinization (2).
Extraction technique: sonication (4), liquid-liquid (1), solid-liquid (1), ion pairing (3) and mechanical agitation (1).

Clean-up: weak anion (1), SPE (4), filtration (1) active carbon (1) and C18, Carbon (1).
Extraction solvent: acetonitrile (6), methanol (4) and MTBE (4).
Calibration standard for PFOS analysis: mixed (8) and linear (6).

Soil samples

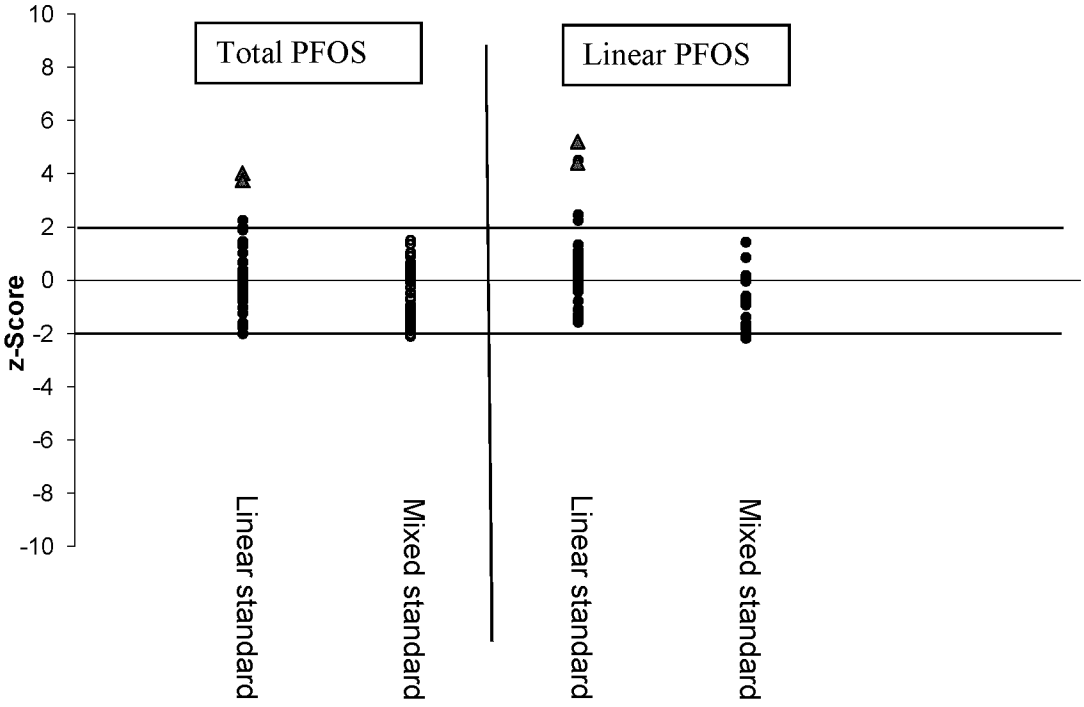
Pre-treatment: cooling (1), acidification (1), digestion with base (1) and pH adjustment (1).
Extraction technique: sonication (7), tumble (1), mechanical agitation (1) and leaching (1).
Clean-up: DSPE (Carbon) (4), SPE (1) and filtration (3).
Extraction solvent: methanol (14), acetonitrile (1), acetonitrile/methanol (2) and MTBE (1).
Calibration standard for PFOS analysis: mixed (9) and linear (9).

Water samples

Pre-treatment: Trizma buffer (1).
Extraction technique: direct injection (6), filtration (1), SPE (11) and dispersive liquid-liquid microextraction (1).
Extraction solvents: methanol (7), ammonia/methanol (3) and octanol (1).
Calibration standard for PFOS analysis: mixed (7) and linear (10).

For all matrices the analytical detection method of choice was LC-MSMS.

Laboratories used different extraction and sample preparation methods. Due to the limited amount of data and the variety of analytical methods used no significant trend with extraction and sample preparation was identified. Participants used either mixed or linear standards to quantify total and linear PFOS. A plot of z-scores for total and linear PFOS vs mixed or linear calibration standards is presented in Figure 30.



▲Laboratory 23 participated only in water samples and performance was unsatisfactory for all z-scores.

Figure 30 Linear vs mixed calibration standards for total and linear PFOS measurements

6.6 Effects of Sample Matrix

Samples S1 and S2 were fish, Samples S3 and S4 were soil and Samples S5 and S6 were water. Water samples received the least number of percent satisfactory z-scores.

Table 29 Satisfactory z-scores for each matrix

Sample		Expected number of z-scores	Actual number of z-scores (% of expected no of z-scores)	Satisfactory	% satisfactory
S1 Fish	Incurred	34	25 (74%)	23	92
S2 Fish	Spiked	51	39 (76%)	37	95
S3 Soil	Incurred	54	46 (85%)	45	98
S4 Soil	Spiked	54	47 (87%)	43	91
S5 Water	Incurred	34	29 (85%)	25	86
S6 Water	Spiked	51	46 (90%)	41	89

7 REFERENCES

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[2] NMI Chemical Proficiency Testing Study Protocol.
http://www.measurement.gov.au → products and services → chemical proficiency testing → details of our program → Study Protocol

[3] NMI Chemical Proficiency Testing Statistical Manual.
http://www.measurement.gov.au → products and services → chemical proficiency testing → details of our program → Statistical Manual

[4] Thompson, M. Ellison S. L. R and Wood, R., The international harmonized protocol for proficiency testing of (chemical) analytical laboratories, Pure Appl. Chem. 78, 145-196, 2006.

[5] National Measurement Institute AQA 15-03 PFOS/PFOA in Water and Soil,
http://www.measurement.gov.au/Publications/ProficiencyStudyReports/Pages/default.aspx

[6] ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories, ISO, Geneva.

[7] Eurachem/CITAC Guide Quantifying Uncertainty in Analytical Measurement, Third edition, (2012), http://www.eurachem.org/images/stories/Guides/pdf/QUAM2012-P1.pdf (Accessed July 2016)

[8] ISO13528:2015(E), Statistical methods for use in proficiency testing by interlaboratory comparisons, ISO, Geneva.

[9] Thompson, M., Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386, 2000.

[10] Thompson, M. and Fearn, T., A new test for sufficient homogeneity, Analyst, 126, 1414-1417, 2001.

APPENDIX 1 – PARTICIPATING LABORATORIES

ACS Laboratories (Australia), VIC	Advanced Analytical Australia, NSW
ALS Czech Republic, s.r.o., Czech Republic	ALS Laboratory Group Ontario, CANADA
AsureQuality Limited, NEW ZEALAND	Australian Laboratory Services, NSW
AXYS Analytical Services Ltd, CANADA	Korea University, SOUTH KOREA
CHEMCENTRE WA	National Centre Food Safety Risk Assessment, CHINA
Eulji University, KOREA	Eco-Research, ITALY
Envirolab Services NSW	Eurofins mgt, QLD
Eurofins GFA Lab Service GERMANY	Health Canada, Ottawa, CANADA
Government Laboratory HONG KONG	NRCET ENTOX, Queensland University, QLD
Office of Environment and Heritage, Department of Premier and Cabinet Environmental Protection Science, NSW	SGS Belgium Division IAC, BELGIUM
SGS Leeder Consulting VIC	TestAmerica Denver, USA
TestAmerica Sacramento California, USA	University of A Coruna (UDC), SPAIN

APPENDIX 2 - SAMPLE PREPARATION, HOMOGENEITY TESTING AND STABILITY CHECK

A2.1 Sample Preparation

Analytical standards used for spiking Samples S2, S4 and S6 were purchased from Sigma Aldrich. On the analytical report provided with the standards, PFOA (product 33824, batch S ZBD308XV) has a stated purity of 99.4% and PFOS (product 33829, batch SZBE107XV) of 98%. No value was supplied for linear PFOS.

Sample S1: Dusky flathead (Platycephalus fuscus) from a contaminated site were composited.

Sample S2: Yellow bream (Acanthopagrus australis) and sand whiting (Sillago ciliate) from a control site were composited. A portion of the composited fish was sub-sampled and placed in the -80°C freezer for use as Sample S2 unspiked. Approximately 955.6 g of fish composite was placed on a tray and sprayed with a mist of the spiking solution using a commercial pump. This was repeated until all spiking solution was used. The fish was thoroughly mixed.

Both Samples S1 and S2 were placed on stainless steel trays as patties of no more than six centimeters square and frozen overnight in -80°C freezer. The patties of fish were embrittled by immersion in liquid nitrogen and then passed through a Retsch SM2000 mill that was cooled with liquid nitrogen and dry ice. After grinding, samples were placed in a refrigerator in a covered Pyrex dish. The next day after all the carbon dioxide sublimed, 5 to 8 g portions of fish were packed into labelled 50 mL Greiner tubes.

Sample S3: Soil obtained from a contaminated site and uncontaminated dried soil were sieved through 212 µm sieve. 903 g of contaminated soil and 310 g of uncontaminated soil were mixed for 90 min using a V-mixer. The mixture was divided into 20 g, packed into labelled 50 mL Greiner tubes.

Sample S4: 1181 g of dried uncontaminated soil sieved through 355 µm sieve was placed in a stainless steel pot. A slurry was produced by adding acetone. The slurry was spiked with solutions of PFOA and PFOS prepared in methanol. The slurry was stirred using an IKA stirrer, left to evaporate overnight and purged with nitrogen for further drying. The soil was then mixed, dispensed into 20 g portions and packed into labelled 50 mL Greiner tubes..

Sample S5: Contaminated water was filtered through an Advantec Toyo GB 140 glass fibre filter. 305 g of filtered contaminated water was diluted with Milli-Q water to a final volume of 4578 g. Water was placed in a stainless steel pot, mixed with an IKA stirrer and dispensed into labelled 65 mL HDPE containers.

Sample S6: 7167 g of autoclaved tap water was spiked with standard solutions of PFOA and PFOS prepared in methanol. The spiked water was stirred using an IKA stirrer and dispensed into labelled 65 mL HDPE containers.

Fish samples were stored in -80°C freezer and the soil and the water samples in a cool room at 4^0C prior to dispatch to participants.

A2.2 Sample Analysis and Homogeneity Testing

Homogeneity testing was based on that described in the International Protocol.[4] Seven bottles were selected at random from each set of samples and analytes measured. The measurements were made under repeatability conditions in random order.

A2.2.1 Fish

Fish samples were prepared at NMI North Ryde (see A2.1) and analysed by National Research Centre for Environmental Toxicology (Entox) from University of Queensland.

Fish Samples S1 and S2 were analysed in duplicate by weighing 1 g of sample and spiking with 20 µl (0.2 ppm) ^{13}C-labelled PFAS solution mixture. The samples were digested with 200 mM NaOH/MeOH and subsequently extracted twice by ultra-sonication in acetonitrile and acidified with 4M HCl/MeOH. The supernatants were combined and reduced to 2 ml, then liquid-liquid extracted three times with n-hexane. The extract was reduced to 250 µl and reconstituted to 1 ml using methanol, then loaded onto a 100 mg BondElut carbon cartridge (Agilent Technologies) and eluted with methanol. The final extract was reduced to 400 µl and reconstituted with 5 mM ammonium acetate in water to 1 ml, then spiked with 20 µl (0.2 ppm) $^{13}C_8$-PFOS and $^{13}C_8$-PFOA solution mixture.

Instrument analysis was performed using high performance liquid chromatography (HPLC, Shimadzu Corp., Kyoto Japan) coupled to a tandem mass spectrometer (QTrap 5500 AB-Sciex, Concord, Ontario, Ca) operating in negative electrospray ionisation mode and using scheduled multiple reaction monitoring (SMRM). For separation a volume of 5 µl was injected onto a Gemini NX C18 column (50 x 2 mm, 3 µm, 110 Å, Phenomenex, Lane Cove, Australia) held at a constant temperature of 50 °C. PFASs were separated by gradient elution on the HPLC using mobile phase 10 % (A) and 90 % (B) methanol, respectively, with 5mM ammonium acetate. Identification and confirmation of peaks was done using retention times and comparing the ratios of MRM transition intensity between the samples and the calibration standards in the same batch of analysis. Quantification was conducted using ^{13}C-labelled PFASs. Calibration standards were made up in 1000 µl (400 µL methanol/600 µL using 5 ml ammonium acetate in water). The concentration range of the eight prepared calibration standards was 0.1– 100 ppb (0.1; 0.4; 1; 4; 10; 20; 40;100). Calibration standards were injected three times in each batch of samples. Quality control standards, including duplicate samples, native spikes and deionized water procedural blanks and instrument blanks were added to each batch and treated in the same way as real samples. All values reported are corrected for recovery of the surrogate standards.

Results of the fish homogeneity testing are presented in tables 30-34. For each sample, the mean of the 14 measurements were used as the homogeneity value. All samples were found to be sufficiently homogeneous for use in this PT study.

Table 30 Sample S1, Linear PFOS homogeneity testing results

Bottle Fill Number	Linear PFOS µg/kg	
	Replicate 1	Replicate 2
1	18.0	16.5
16	18.7	17.5
17	18.6	18.1
30	20.1	19.0
32	19.3	18.2
39	19.4	18.7
43	19.8	18.9
Mean	18.6	
CV	5.0%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.29	0.73	**Pass**
S_{an}/σ	0.20	0.5	**Pass**
s^2_{sam}	0.363	3.41	**Pass**

Table 31 Sample S1, Total PFOS homogeneity testing results

Bottle Fill Number	Total PFOS µg/kg	
	Replicate 1	Replicate 2
1	19.7	18.0
16	20.4	19.2
17	20.3	19.9
30	20.8	21.8
32	19.8	21.0
39	21.2	20.5
43	21.6	20.7
Mean	20.4	
CV	4.9%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.35	0.73	**Pass**
S_{an}/σ	0.19	0.5	**Pass**
s^2_{sam}	0.441	3.97	**Pass**

Table 32 Sample S2, Linear PFOS homogeneity testing results

Bottle Fill Number	Linear PFOS µg/kg	
	Replicate 1	Replicate 2
3	42.7	39.0
23	44.4	40.0
39	43.0	46.9
42	43.0	40.1
44	44.2	37.1
45	40.5	39.5
48	44.1	41.3
Mean	41.8	
CV	6.3%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.43	0.73	**Pass**
S_{an}/σ	0.34	0.5	**Pass**
s^2_{sam}	0.000	25.09	**Pass**

Table 33 Sample S2, Total PFOS homogeneity testing results

Bottle Fill Number	Total/Linear PFOS µg/L	
	Replicate 1	Replicate 2
3	55.5	59.6
23	60.5	54.8
39	60.1	63.6
42	57.6	55.4
44	50.5	61.0
45	54.2	56.2
48	62.2	57.3
Mean	57.7	
CV	6.2%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.54	0.73	**Pass**
S_{an}/σ	0.33	0.5	**Pass**
s^2_{sam}	0.000	46.14	**Pass**

Table 34 Sample S2, PFOA homogeneity testing results

Bottle Fill Number	PFOA µg/kg	
	Replicate 1	Replicate 2
3	48.5	49.8
23	50.1	49.4
39	51.1	55.2
42	55.2	49.6
44	50.7	54.7
45	48.7	50.9
48	53.7	57.2
Mean	51.8	
CV	5.5%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.38	0.73	**Pass**
S_{an}/σ	0.24	0.5	**Pass**
s^2_{sam}	2.276	28.79	**Pass**

Soil and water samples were prepared (see A2.1) and analysed at NMI North Ryde. A brief description of analysis method is presented below.

A2.2.2 Soil and Water

Soil samples S3 and S4 were prepared in duplicate by accurately weighing 1 g of sample and spiking with 60 µL of labelled surrogate standard in methanol. The samples were extracted by overnight tumbling in alkaline methanol (0.01 N potassium hydroxide), then centrifuged and a portion was filtered and evaporated. The filtered extract was reconstituted to 600 µL in mobile phase and spiked with 10 µL labelled recovery standard in methanol.

Water samples S5 and S6 were prepared by accurately weighing the entire content of the sample bottles (~50 mL) then spiking with 30 µL of labelled surrogate standard in methanol. The samples were pre-treated with 1N acetic acid then extracted by solid phase extraction (Waters Oasis HLB, 6 mL, 500 mg, 60 µm particle size) under vacuum and eluted using methanol. After evaporation under nitrogen, the extract was reconstituted to 600 µL in mobile phase and spiked with 10 µL labelled recovery standard in methanol. All chemicals are analytical reagents or LCMS grade solvents.

Instrument analysis was performed using an Agilent 1100 High Performance Liquid Chromatography (HPLC) coupled with an ABSciex 4000 Qtrap Mass spectrometer, operating in multiple reaction monitoring mode. 5 µL of extract was injected onto a Waters XBridge BEH column (1 mm x 150 mm x 3.5 µm, 130 Å) with a mobile phase gradient consisting of water:methanol (2 mM ammonium acetate). Two mass transitions were monitored for each target analyte and labelled surrogate, and abundance ratios checked. The instrument mass accuracy is calibrated annually during preventative maintenance, and the six point calibration curve established for each analytical batch. A solvent batch blank is extracted and analysed with each batch, and sample results must be at least three times the level of any analyte detected in the batch blank to be reported. Quantification is based on the use of the labelled surrogates using relative retention factors from the multipoint calibration, and is corrected for surrogate recoveries. The analysis is based on USEPA 537 and used calibration, surrogate and recovery standards supplied by Wellington Laboratories, Canada.

Chromatographic separation of the main four PFOS isomers was performed by optimising column and mobile phase conditions. Total PFOS was determined by integrating all peaks that eluted from the LC column within the retention time window established during analysis of a technical PFOS mixture standard (Wellington Laboratories). Linear PFOS was determined by integrating the peak that co eluted with the linear labelled surrogate PFOS.

Both total and linear PFOS were quantitated using linear PFOS calibration standards.

Results of the soil homogeneity testing are presented in tables 35-40. For soil samples, the mean of the 14 measurements were used as the NMI homogeneity value. All soil samples were found to be sufficiently homogeneous for use in this PT study.

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Table 35 Sample S3, Linear PFOS homogeneity testing results

Bottle Fill Number	Linear PFOS µg/kg	
	Replicate 1	Replicate 2
1	186	190
16	214	193
17	201	176
30	195	187
32	192	186
39	218	182
43	196	194
Mean	194	
CV	5.9%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.52	0.73	**Pass**
S_{an}/σ	0.34	0.5	**Pass**
s^2_{sam}	0.000	537.13	**Pass**

Table 36 Sample S3, Total PFOS homogeneity testing results

Bottle Fill Number	Total PFOS µg/kg	
	Replicate 1	Replicate 2
1	258	261
16	297	268
17	279	252
30	272	269
32	266	257
39	298	256
43	275	270
Mean	270	
CV	5.2%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.51	0.73	**Pass**
S_{an}/σ	0.29	0.5	**Pass**
s^2_{sam}	0.000	904.12	**Pass**

Table 37 Sample S3, PFOA homogeneity testing results

Bottle Fill Number	PFOA µg/kg	
	Replicate 1	Replicate 2
1	5.8	5.3
16	6.3	6.0
17	5.9	5.8
30	6.2	6.1
32	5.6	5.6
39	6.2	5.8
43	6.2	6.6
Mean	5.9	
CV	5.6%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.29	0.73	**Pass**
S_{an}/σ	0.19	0.5	**Pass**
s^2_{sam}	0.063	0.34	**Pass**

Table 38 Sample S4, Linear PFOS homogeneity testing results

Bottle Fill Number	Linear PFOS µg/kg	
	Replicate 1	Replicate 2
3	21.6	21.8
15	21.6	22.8
21*	27.4	20.8
27	23.8	23.8
29	23.2	22.1
35	23.9	22.1
38	23.4	22.1
Mean	22.7	
CV	4.0%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.43	0.78	**Pass**
S_{an}/σ	0.18	0.5	**Pass**
s^2_{sam}	0.193	5.18	**Pass**

*Results on bottle fill 21 were not included in the test for homogeneity being identified as an analytical outliers (test Sa/σ) due to the difference between replicates.[10]

Table 39 Sample S4, Total PFOS homogeneity testing results

Bottle Fill Number	Total PFOS µg/kg	
	Replicate 1	Replicate 2
3	25.3	25.6
15	25.9	26.5
21*	31.8	24.8
27	27.3	27.7
29	27.3	25.8
35	27.4	25.8
38	27.3	25.7
Mean	26.5	
CV	3.3%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.32	0.78	**Pass**
S_{an}/σ	0.15	0.5	**Pass**
s^2_{sam}	0.107	6.71	**Pass**

*Results on bottle fill 21 were not included in the test for homogeneity being identified as an analytical outliers (test Sa/σ) due to the difference between replicates.[10]

Table 40 Sample S4, PFOA homogeneity testing results

Bottle Fill Number	PFOA µg/kg	
	Replicate 1	Replicate 2
3	15.2	15.4
15	13.7	14.7
21*	17.2	12.8
27	15.0	13.8
29	16.3	15.6
35	16.5	16.6
38	15.7	14.3
Mean	15.2	
CV	6.4%	

Thompson and Fearn Homogeneity Tests

Test	Value	Critical	Result
Cochran	0.40	0.78	**Pass**
S_{an}/σ	0.21	0.5	**Pass**
s^2_{sam}	0.608	2.81	**Pass**

*Results on bottle fill 21 were not included in the test for homogeneity being identified as an analytical outliers (test Sa/σ) due to the difference between replicates.[10]

Results of the water homogeneity testing are presented in tables 41-45. Since the entire water sample was used in each analysis, it was not possible to apply analysis of variance (ANOVA) to determine if samples were sufficiently homogeneous. When it is not possible to conduct replicate measurements, the standard deviation of the results (sd) will be compared with the target standard deviation of the PT (σ) calculated as described in section 4.4. The proficiency test samples may be considered sufficiently homogeneous if : $sd \leq 0.3\ \sigma$.[8]

For water samples, the mean of the 10 measurements were used as the NMI homogeneity value. All soil samples were found to be sufficiently homogeneous for use in this PT study.

Table 41 Sample S5, Linear PFOS homogeneity testing results

Bottle Fill Number	Linear PFOS
	µg/L
2	1.99
3	2.05
5	1.93
15	2.19
27	2.01
30	2.07
31	2.06
58	2.05
63	1.87
67	1.81
Mean	**2.00**
sd	**0.10**
Target σ	**0.33**

Test	Value	Critical	Result
$sd/\sigma \leq 0.3$	0.30	0.3	**Pass**

Table 42 Sample S5, Total PFOS homogeneity testing results

Bottle Fill Number	Total PFOS
	µg/L
2	1.99
3	2.05
5	1.93
15	2.19
27	2.01
30	2.07
31	2.06
58	2.05
63	1.87
67	1.81
Mean	**3.24**
sd	**0.14**
Target σ	**0.60**

Test	Value	Critical	Result
$sd/\sigma \leq 0.3$	0.23	0.3	**Pass**

Table 43 Sample S6, Linear PFOS homogeneity testing results

Bottle Fill Number	Linear PFOS
	µg/L
6	5.15
17	4.95
21	5.24
31	5.02
38	4.95
55	4.70
62	4.96
64	4.95
77	5.05
96	4.78
Mean	**4.97**
sd	**0.16**
Target σ	**0.90**

Test	Value	Critical	Result
$sd/\sigma \leq 0.3$	0.18	0.3	**Pass**

Table 44 Sample S6, Total PFOS homogeneity testing results

Bottle Fill Number	Total PFOS
	µg/L
6	7.75
17	7.61
21	7.67
31	7.50
38	7.70
55	7.22
62	7.44
64	7.62
77	7.59
96	7.27
Mean	**7.54**
sd	**0.18**
Target σ	**1.3**

Test	Value	Critical	Result
$sd/\sigma \leq 0.3$	0.13	0.3	**Pass**

Table 45 Sample S6, PFOA homogeneity testing results

Bottle Fill Number	PFOA
	µg/L
6	11.6
17	11.3
21	12.0
31	11.4
38	11.5
55	11.0
62	11.5
64	11.6
77	11.9
96	11.3
Mean	**11.5**
sd	**0.29**
Target σ	**2.17**

Test	Value	Critical	Result
$sd/\sigma \leq 0.3$	0.13	0.3	**Pass**

A2.3 Stability Testing for Fish Samples S1 and S2

For stability testing fish Samples S1 and S2 were analysed at NMI North Ryde. Samples were prepared in duplicate by accurately weighing 1 g of the sample then spiking with 60 µL of labelled surrogate standard in methanol. The samples were extracted by overnight tumbling in alkaline methanol (0.01 N potassium hydroxide), then centrifuged and a portion was diluted in milliQ water and extracted by solid phase extraction (Waters Oasis HLB, 6 mL, 500 mg, 60 µm particle size) under vacuum and eluted using methanol. After evaporation under nitrogen, the extract was reconstituted to 600 µL in mobile phase and spiked with 10 µL labelled recovery standard in methanol. All chemicals are analytical reagents or LCMS grade solvents. Instrument analysis was performed as described in section A2.2.2.

A stability study of the fish samples was carried out. A factor considered to affect the stability of fish samples was storage condition during transportation.

Storage condition during transportation was considered at room temperature and assessed by comparing the average of measurements performed on two jars of each samples starting 17/06/2016 (sample dispatch) for one month to 18/07 2016. Results were in good agreement with each other, with the spiked level and the assigned value (Figures 31 and 32).

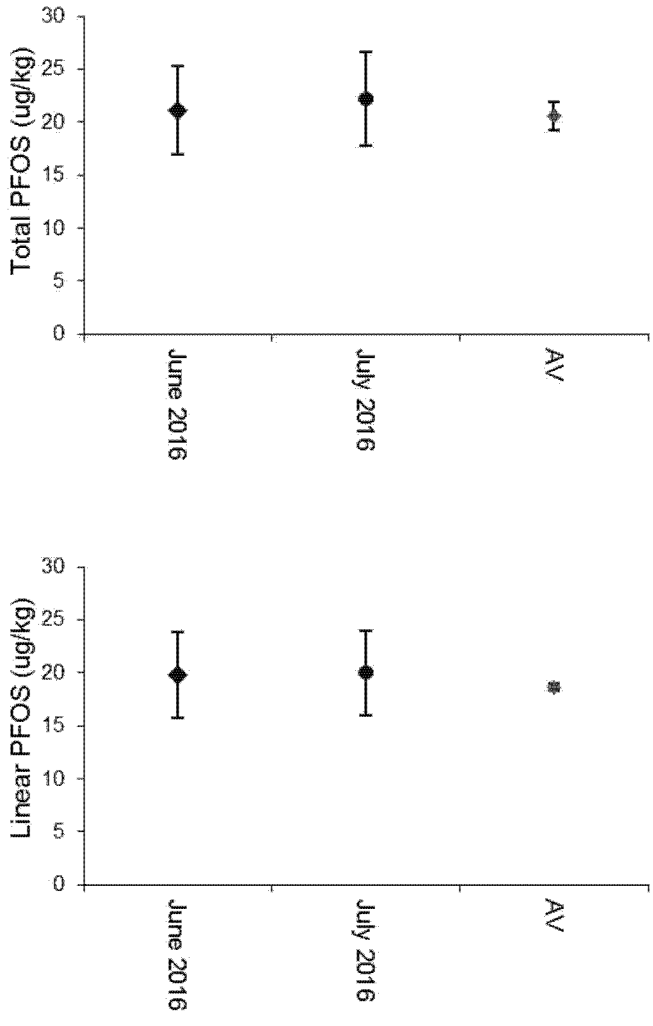

Figure 31 Total and linear PFOS stability results and assigned value (AV) in incurred fish Sample S1 (room temperature)

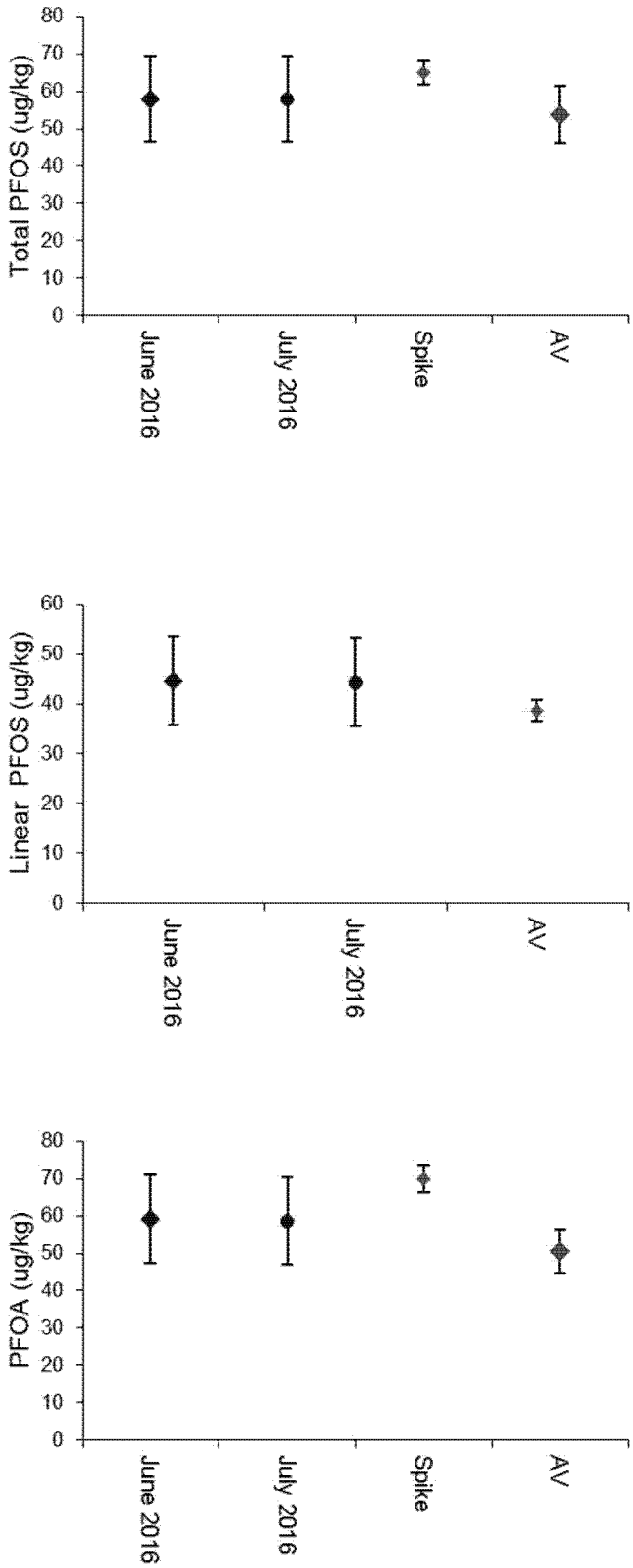

Figure 32 Stability results, spike level and assigned value (AV) in fish Sample S2 (room temperature)

To confirm the stability during transportation and over time, results returned by participants were combined with the reported participant date of analysis and subjected to a trend analysis. No trend was evident providing further evidence of samples stability. An example for Total PFOS and PFOA in Sample S2 are presented in Figure 33.

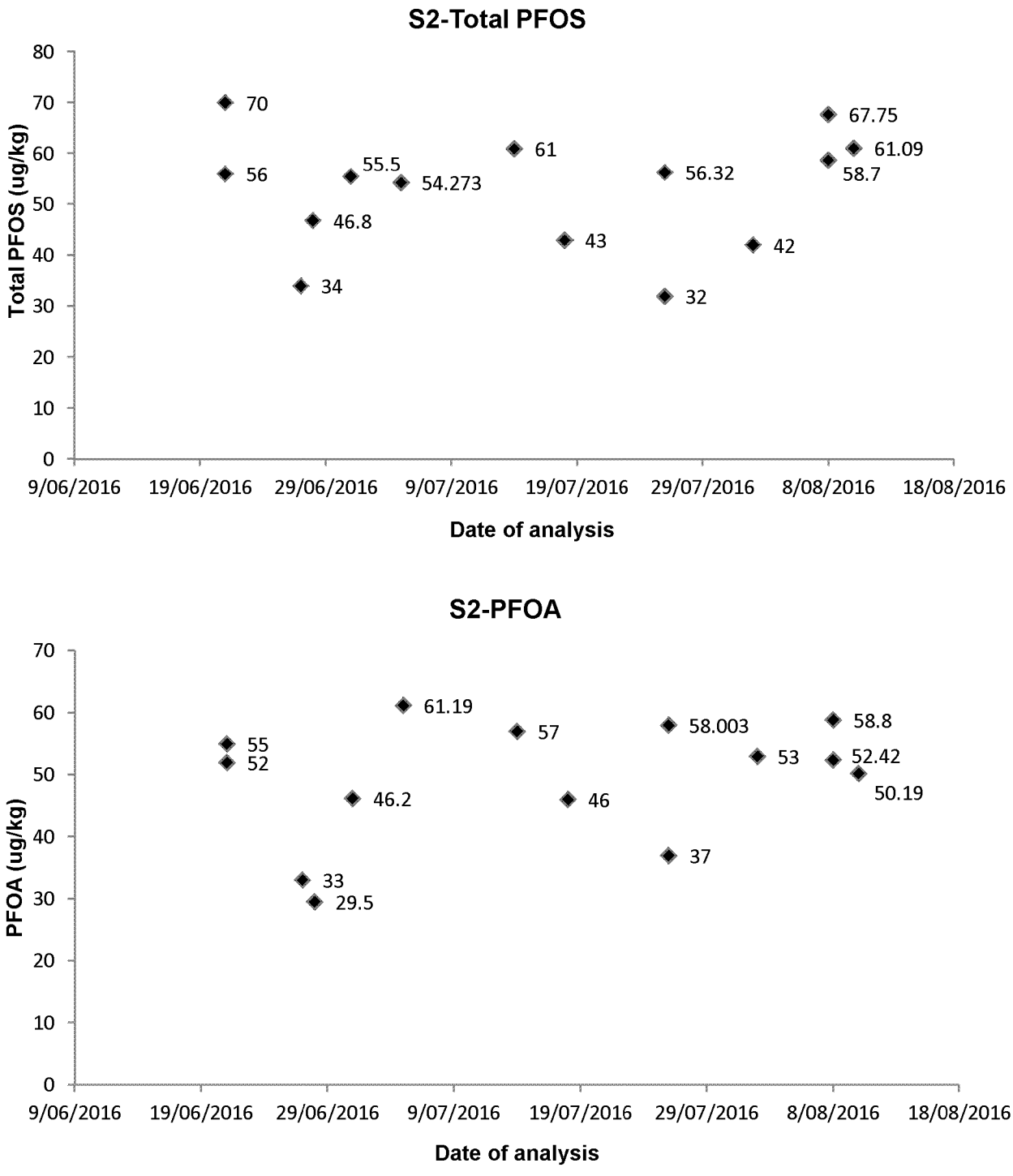

Figure 33 Participant results and date of sample analysis trend for Total PFOS and PFOA in fish Sample S2

APPENDIX 3 - ROBUST AVERAGE AND ASSOCIATED UNCERTAINTY

The robust average was calculated using the procedure described in 'ISO13258:2015(E), Statistical methods for use in proficiency testing by interlaboratory comparisons – Annex C'[7] the uncertainty was estimated as:

$$u_{rob\ av} = 1.25 * S_{rob\ av} / \sqrt{p} \qquad \text{Equation 4}$$

where:

$u_{rob\ av}$	robust average standard uncertainty
$S_{rob\ av}$	robust average standard deviation
p	number of results

The expanded uncertainty ($U_{rob\ av}$) is the standard uncertainty multiplied by a coverage factor of 2 at approximately 95% confidence level.

The robust average of all seventeen results was calculated (Table 46).

Table 46 Robust average for all PFOA results in Sample S6

Lab. Code	PFOA (µg/kg)
1	12
2	12
3	9.78
4	NT
5	10.02
6	*19*
7	10.6
8	NT
9	9.7
10	NT
11	11.7
12	NT
13	11.36
14	10.4
15	NT
16	13.54
17	NT
18	12
19	9.1
20	10.1
21	11.8
22	9.01
23	*18.598*
24	NT
Robust average	11.23

Result from laboratories **6** and **23** was removed as this is outside of a range ± 50% of the robust average. The robust average and associated uncertainty of the remaining sixteen results were then used as the assigned value for this analyte (Table 47).

Table 47 Uncertainty estimate for PFOA in Sample S6 after removing outlier

No. results (p)	15
Robust Average	10.83 µg/L
$S_{rob\ av}$	1.37 µg/L
$u_{rob\ av}$	0.443 µg/L
k	2
$U_{rob\ av}$	0.89 µg/L

The assigned value for PFOA in Sample S6 is 10.83 ± 0.89 µg/L.

APPENDIX 4 - ACRONYMS AND ABBREVIATIONS

CV	Coefficient of Variation
EPA	Environment Protection Authority
ISO	International Standards Organisation
LC	Liquid Chromatography
Max	Maximum value in a set of results
Md	Median
Min	Minimum value in a set of results
MS	Mass Spectrometry
NMI	National Measurement Institute (of Australia)
NR	Not Reported
NT	Not Tested
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PT	Proficiency Test
Robust CV	Robust Coefficient of Variation
Robust SD	Robust Standard Deviation
S	Spiked or formulated concentration of a PT sample
SPE	Solid Phase Extraction
Target SD	Target Standard Deviation
σ	Target standard deviation

s