Improving Measurement Reliability of the PFAS TOP Assay

Final | 20 June 2019

10 YEARS & BEYOND AIGA Australasian Land & Groundwater Association

ventia

Australian Government Department of Industry, Innovation and Science National Measurement Institute

ALS

eurofins

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Acknowledgments

Ventia Utility Services Pty Ltd (Ventia), in conjunction with the National Measurement Institute (NMI), Australian Laboratory Services (ALS) and Eurofins Environment Testing Australia (Eurofins) were awarded the inaugural Australasian Land and Groundwater Association (ALGA) Research and Development Grant to complete this project.

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Steering Committee Members include:

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Ventia, NMI, ALS and Eurofins would like to thank ALGA for their foresight in creating the grant and their contribution to development of the contaminated land industry in the Australia and New Zealand region.

Ventia would also like to thank the project and steering committee members for their hard work, openness and collaborative attitude in successfully completing this much needed project.

Abbreviations

ALS	Australian Laboratory Services
6:2 FTSA	1-Octanesulfonic acid, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 8-tridecafluoro- (1H, 1H, 2H, 2H-perfluorooctane sulfonic acid)
8:2 monoPAP	Mono[2-(perfluorooctyl)ethyl] phosphate
CV	Coefficient of Variation
CRM	Certified Reference Material
ISO	International Standards Organisation
KPS	Potassium persulfate
LC	Liquid Chromatography
LOR	Limit of Reporting
MS	Mass Spectrometry
NaOH	Sodium Hydroxide
NEMP	National Environmental Management Plan (for PFAS)
NMI	National Measurement Institute (of Australia)
NT	Not Tested
PFAA	Perfluoroalkyl acids (e.g. perfluoroalkyl carboxylic acids, perfluoroalkyl carboxylates, perfluoroalkane sulfonic acids and perfluoroalkane sulfonates)
PFAS	Per- and poly-fluoroalkyl substances
PFBA	Perfluoro-n-butanoic acid
PFCA	Perfluorocarboxylic acid
PFDA	Perfluoro-n-decanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFHxA	Perfluoro-n-hexanoic acid
PFHpA	Perfluoro-n-heptanoic acid
PFNA	Perfluoro-n-nonanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFOSA or FOSA	Perfluoro-1-octanesulfonamide
PFPeA	Perfluoro-n-pentanoic acid
PFSA	Perfluorosulfonic acid
SPE	Solid Phase Extraction
TOC	Total Organic Carbon
TOF	Total Organic Fluorine
TOP	Total Oxidisable Precursor
TOPA	Total Oxidisable Precursor Assay

Executive Summary

Ventia Utility Services Pty Ltd (Ventia) – in collaboration with the National Measurement Institute (NMI), Australian Laboratory Services (ALS) and Eurofins Environment Testing Australia (Eurofins) – was awarded the inaugural Australasian Land and Groundwater Association (ALGA) Research and Development Grant to conduct an inter-laboratory assessment of the per- and poly-fluoroalkyl substances (PFAS) total oxidisable precursor (TOP) assay.

The PFAS TOP assay was first developed in 2012 as a method for identifying non-target PFAS, thereby providing a better understanding of the extent of overall PFAS contamination present within a sample.

The method for the study involved preparation of four spiked water samples by NMI and analysis of the samples by NMI, ALS and Eurofins. The four spiked water samples were:

- S1 – ultrapure water spiked with Tridol foam (40,000 x dilution) and PFOSA.
- S2 – ultrapure water spiked with spiked with 8:2 monoPAP, PFDA and PFOS.
- S3 – ultrapure water spiked with Tridol foam (40,000 x dilution), PFOSA, PFDA and PFHxS.
- S4 – diluted liquid from a worm farm (total organic carbon (TOC) content of 120 mg/L) spiked with Tridol foam (40,000 x dilution) and PFOSA, PFDA and PFHxS.

ALS and Eurofins did not know the contents of the samples, pre-analysis. All three laboratories analysed the samples pre- and post-oxidation. All laboratories based their TOP assay method on Houtz and Sedlak (2012) with modifications. In all cases, extra doses of oxidant and/or extended oxidation times were used. All laboratories reported that these modifications were required to sufficiently oxidise the samples to meet the ratio test for aqueous samples (sum of [PFAA precursors] divided by sum of [Total PFAS] <5%) recommended in the PFAS National Environmental Management Plan (NEMP) (HEPA, 2018).

Application of the TOP assay did not fully convert the precursors to PFCAs for Laboratories 1 and 3. A test for acceptability of oxidation (per the PFAS NEMP (HEPA 2018)) is presented and all results passed these criteria except for S3 for Laboratory 1.

Laboratory 2 reported 6:2 FTSA below the limit of reporting (LOR) post-oxidation indicating complete conversion of the PFAA precursor 6:2 FTSAS. Laboratory 2 diluted the sample prior to oxidation, thus reducing the organic load and perhaps improving the efficiency of the oxidation process. Sample S2, spiked with 8:2 monoPAP (a fluorotelomer precursor), showed reasonable consensus post-oxidation results for PFCAs. The data suggests the majority of 8:2 monoPAP has oxidised under the TOP assay conditions to several PFCAs, as observed in the post-TOP assay digest results.

The PFAS NEMP (HEPA 2018) defines a successful oxidation as the ratio of the sum of concentrations of PFAA precursors to the sum of total PFAS as less than 5%. Using their six times dosage of oxidant in a single incubation period (cycle), Laboratory 1 generally passed these criteria, except for a marginal exceedance for sample S3. Laboratory 2 diluted samples prior to oxidation and employed three oxidation cycles over three nights to achieve quality objectives. Laboratory 3 used six times the dosage of oxidant and two cycles for samples S1 and S2, then increased the oxidant dosage for samples S3 and S4. Applying the Houtz and Sedlak (2012) method without modification may lead to insufficient oxidation for samples with high organic content and/or high concentrations of PFAA precursors.

In addition to the results presented above, six sequential oxidant doses vs a single upfront six-times oxidant dose was investigated. There was no material difference in performance between sequential dosing and a single six-times upfront dose. One observation that is interesting to note is the increase in PFOS post-digest across the sequential doses. It is suggested that increasing the dosage may result in an elevated alkaline environment, initiating hydrolysis of PFOSA to PFOS. This observation is consistent with the PFOS results originally reported by the three labs. Both Laboratories 1 and 3, who applied higher overall dosages, reported higher PFOS concentrations. Laboratory 2, with a lower final (3x) dosage, reported lower PFOS and at a level consistent with the 3rd dose from the successive trials. The results of this trial suggest either successive small doses or a single large dose are valid approaches to achieve effective oxidation of challenging matrices. Also, high dosages may create alkaline conditions sufficient to convert precursors to PFSAs via hydrolysis rather than the expected PFCAs. Where a significant increase in PFSAs is observed from pre- to post-digest, sample dilution may be a considered approach to achieving equivalent oxidation at a lower dose and avoiding alkaline hydrolytic conditions, noting potential for the need to raise the LORs.

The results reported were used to assess the laboratories' accuracy in the measurement of PFAS before and after application of the TOP assay. The laboratories complied with the current PFAS NEMP (HEPA 2018) parameters (with some minor exceptions) however, all laboratories were required to modify the original Houtz and Sedlak (2012) approach. A consensus method is not provided here, rather, advice to laboratories on how best to develop methodology and apply to environmental samples (as presented in Section 4.1.

The results indicated that fulfilment of the PFAS NEMP (HEPA 2018) quality assurance measures require increased oxidant dosage and/or extra oxidative cycles. The advice to laboratories developing a routine TOP assay method is:

- Choose a method that will comply with the PFAS NEMP (HEPA 2018) requirements for as many sample matrices as possible. **Increased dosages and multiple cycles are recommended**.
- If samples do not comply with the PFAS NEMP (HEPA 2018) ratio test post oxidation treatment, then further oxidative treatment is required. In practice if you were performing the TOP assay on field samples, another option is to dilute the sample prior to oxidation to reduce the organic load. Dilution can result in raising the limit of reporting to an extent where the results lack analytical meaning.
- Take note of the concentrations of PFSA pre- and post-oxidation. In this study, PFOS and PFHxS were spiked into samples as monitoring compounds. For AFFF samples the PFSA should have similar concentrations pre-oxidation compared to post-oxidation (as required under PFAS NEMP (HEPA 2018) QA for equivalence of sulfonate concentrations). However, this would not be the case when dealing with, for example, fabric treatments based on acrylic polymers with perfluoroalkyl sulfonamide side branches attached. For samples like this, PFSA concentrations post-oxidation could be higher than pre-oxidation.
- Assess total PFAA after each oxidation cycle. No change in PFAA concentrations between cycles (within measurement uncertainty) is a reasonable indicator that the oxidation process is complete and that there are no significant PFAA precursors remaining.
- The maximum chain length of the oxidation products reflects the maximum possible perfluorinated chain length of the precursors. For example, assuming the sample does not contain >C8 PFAA precursors then C10 and >C10 acids should also have similar concentrations pre-oxidation versus post-oxidation.

1 Introduction

1.1 Background

The per- and poly-fluoroalkyl substances (PFAS) total oxidisable precursor (TOP) assay is an oxidative sample pre-treatment method aimed at converting perfluoroalkyl acid precursors within a sample into stable target[1] perfluoroalkyl acids (PFAAs) that can be quantified by conventional Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analytical techniques, thereby providing a better understanding of the extent of overall PFAS contamination present within a sample.

Quantifying precursors is important to better understand ongoing sources of PFAAs, including PFOS and PFOA, both at contaminated sites and in the waste industry (sewage treatment effluents, biosolids, landfill leachates). Without the ability to reliably quantify precursors, uncertainties in the long-term potential for PFAA formation hinders effective regulation, management and remediation. Currently the TOP assay, although useful as a semi-quantitative tool, is not regarded as sufficiently robust by many regulators to allow quantitative consideration of precursors in environmental regulation.

Testing by commercial and Government laboratories has shown that the Houtz and Sedlak (2012) methodology for the TOP assay is challenged when applied to foam products, environmental samples with high levels of precursors or elevated levels of total organic carbon (TOC) in aqueous samples to which this report addresses. This report does not address the TOP assay for solid samples including biota. Under the standard conditions of the assay, exhaustion of the oxidant is possible unless samples are pre-diluted, or the initial oxidant dose is increased. Incomplete oxidation may significantly underestimate the post-assay PFAA concentrations when substantial concentrations of precursor compounds are present. Also important is ensuring that the pH is strongly alkaline and maintained during the oxidation within a range that promotes effective formation of hydroxyl radicals (the oxidant species) and avoids potential perfluorinated alkyl chain shortening. Shortening of the alkyl chain to <C4 will generate compounds currently outside the suite offered by laboratories and mean a portion of the PFAS mass post-oxidation is unaccounted for. Shortening of alkyl chains also has the potential to distort the PFAA profile, which may have implications for risk assessment.

The project aimed to produce robust recommendations that can be applied to the TOP assay and reported to end users to provide improved confidence in the assay. These recommendations may include an indicator of oxidation progress, pH monitoring, labelled internal standard recovery ranges and measurement of other sample parameters such as TOC. These recommendations will improve interpretation of TOP assay results and strengthen the potential for TOP assay data to be included in regulation as a quantitative (or semi-quantitative) tool.

Further, the project will reference performance criteria proposed within the HEPA (2018) PFAS National Environmental Management Plan (NEMP) and provide recommendations to the relevance of this criteria where appropriate.

1.2 Aim

The research project aimed to:

- Conduct an interlaboratory study to evaluate the laboratories' methods for the TOP assay.
- Compare and assess the participating laboratories' accuracy in the measurement of PFAS before and after application of the TOP assay.
- Develop recommendations for the assessment and application of TOP assay data.
- Develop performance criteria for national guidance documents.

1 Target PFAAs refer to the 20-30 PFAS compounds currently offered by labratories in Australia.

2 Interlaboratory Study

This study was conducted by the National Measurement Institute (NMI) North Ryde laboratory. Three laboratories participated in the study: Australian Laboratory Services (ALS), Sydney Laboratory, Eurofins Environment Testing Australia's (Eurofins) Brisbane Laboratory and NMI North Ryde Organics laboratory. The Interlaboratory Comparison Report is presented in full in **Appendix 1**.

2.1 Test Material Preparation

Four test samples were prepared in two stages. Stage 1 included samples S1 and S2 and Stage 2 included samples S3 and S4.

Sample S1 – consisted of ultrapure water spiked with Tridol foam (40,000 x dilution) and PFOSA. Expected target analytes were 6:2 FTSA (minor component of Tridol) and PFOSA, respectively. Expected post-TOP assay analytes were PFBA to PFHpA (C4-C7) and PFOA, respectively.

Spiked Analytes	Expected Post-TOP Assay Analytes
6:2 FTSA	PFBA to PFHpA
PFOSA	PFOA

Sample S2 – consisted of ultrapure water spiked with 8:2 monoPAP, PFDA and PFOS. Expected target analytes were nil, PFDA and PFOS, respectively. Expected post-TOP assay analytes were PFBA to PFNA (C4-C9), PFDA and PFOS, respectively.

Spiked Analytes	Expected Post-TOP Assay Analytes
8:2 monoPAP	PFBA to PFNA
PFDA	PFDA
PFOS	PFOS

Sample S3 – consisted of ultrapure water spiked with Tridol foam (40,000 x dilution), PFOSA, PFDA and PFHxS. Expected target analytes were 6:2 FTSA (minor component of Tridol), PFOSA, PFDA and PFHxS, respectively. Expected post-TOP assay analytes were PFBA to PFHpA (C4-C7), PFOA, PFDA and PFHxS, respectively.

Pre-TOP Assay Analytes	Expected Post-TOP Assay Analytes
6:2 FTSA	PFBA to PFHpA
PFOSA	PFOA
PFDA	PFDA
PFHxS	PFHxS

Sample S4 – consisted of diluted liquid from a worm farm (TOC content of 120 mg/L) spiked with Tridol foam (40,000 x dilution) and PFOSA, PFDA and PFHxS. Targets were 6:2 FTSA (minor component of Tridol), PFOSA, PFDA and PFHxS, respectively. Expected post-TOP assay analytes were PFBA to PFHpA (C4-C7), PFOA, PFDA and PFHxS, respectively.

Pre-TOP Assay Analytes	Expected Post-TOP Assay Analytes
6:2 FTSA	PFBA to PFHpA
PFOSA	PFOA
PFDA	PFDA
PFHxS	PFHxS

2.2 Participants' Method

Participants were asked to perform two analyses on four samples (S1, S2, S3 & S4):

1) A pre-TOP assay analysis using their routine methods for PFAS analysis.
2) A post-TOP assay analysis using their routine methods for PFAS analysis after using an oxidative sample pre-treatment method based on Houtz and Sedlak (2012) to convert PFAA precursors into target PFAAs.

A summary of participants' test methods for oxidative treatment and PFAS analysis is presented in Tables 1 and 2, respectively. The analytes targeted by all laboratories are presented in Table 3.

Table 1: Oxidative Treatment

	Houtz & Sedlak	Laboratory 1		Laboratory 2	Laboratory 3	
		S1, S3, S4	S2	All	S1, S2	S3, S4
Sample amount (mL)	125	5	5	50*	20	20
Potassium persulfate (g)	2 (60mM)	0.480	0.240	0.8	1	1
Sodium hydroxide (mL)	1.9 (150 mM)	0.456	0.228	0.76	1	1
Number of oxidation cycles	1	1	1	3	2	3
Dosage compare to H&S	1	6	3	3	6	9
pH before heating		14		13	14	
Heating time (hr)	6	6		At least 6 or overnight for each cycle	2.5 for first cycle (s) then overnight for last cycle	
Temperature (°C)	85	80 (S1),	80	85	85	
		85 (S3, S4)				
pH after heating	n/a	14		13	13	
POST oxidation pH adjust.	5-9	7		7	7	

*Sample diluted 1:10 prior to oxidation.

Table 2: Test methods for PFAS in water (pre-and post analysis)

	Laboratory 1	Laboratory 2	Laboratory 3
Sample amount (mL)	1	20	60
Extraction	Direct injection	SPE	SPE
Instrument	LCMSMS	LCMSMS	LCMSMS
Column Type	C18	C18	C18
Column Specifications	2.0mm x 50mm (1.6um)	2.1 mm X 50 mm (1.8 µm)	2.1 mm X 50 mm (1.7 µm)
Extra column for blank separation	no	no	no
Internal standard (before extraction)	24	23	26
Recovery standard (before instrument analysis)	2	0	4
Recovery correction	no	yes	yes

Table 3: Targeted Per- and Polyfluoroalkyl Substances (PFAS)

Perfluoroalkyl carboxylic acids (PFCAs)	
Perfluorobutanoic acid (PFBA)	Perfluorodecanoic acid (PFDA)
Perfluoropentanoic acid (PFPeA)	Perfluoroundecanoic acid (PFUnA)
Perfluorohexanoic acid (PFHxA)	Perfluorododecanoic acid (PFDoA)
Perfluoroheptanoic acid (PFHpA)	Perfluorotridecanoic acid (PFTrDA)
Perfluorooctanoic acid (PFOA)	Perfluorotetradecanoic acid (PFTeDA)
Perfluorononanoic acid (PFNA)	
Perfluoroalkane sulfonic acids (PFSAs)	
Perfluoropropanesulfonic acid (PFPrS)	Perfluorooctane sulfonic acid (PFOS)
Perfluorobutanesulfonic acid (PFBS)	Perfluorononanesulfonic acid (PFNS)
Perfluoropentane sulfonic acid (PFPeS)	Perfluorodecanesulfonic acid (PFDS)
Perfluorohexane sulfonic acid (PFHxS)	
Perfluoroheptane sulfonic acid (PFHpS)	
Perfluoroalkane sulfonamides (FASAs), Perfluoroalkane sulfonamido ethanols (FASEs) and N-alkyl perfluoroalkane sulfonamido ethanols (MeFASEs, EtFASEs) Perfluoroalkane sulfonamido acetic acids (FASAAs) and N-alkyl perfluoroalkane sulfonamido acetic acids (MeFASAAs, EtFASAAs)	
Perfluorooctane sulfonamide (FOSA)	2-(N-ethylperfluoro-1-octane sulfonamido)-ethanol (N-EtFOSE)
N-methylperfluoro-1-octane sulfonamide (N-MeFOSA)	N-ethyl-perfluorooctanesulfonamidoacetic acid (N-EtFOSAA)
N-ethylperfluoro-1-octanesulfonamide (N-EtFOSA)	N-methyl-perfluorooctanesulfonamidoacetic acid (N-MeFOSAA)
2-(N-methylperfluoro-1-octane sulfonamido)-ethanol (N-MeFOSE)	
Fluorotelomers n:2 Fluorotelomer sulfonic acids (n:2 FTSAs)	
1H,1H,2H,2H-Perfluorohexanesulfonic acid (4:2 FTSA)	1H,1H,2H,2H-Perfluorodecanesulfonic acid (8:2 FTSA)
1H,1H,2H,2H-Perfluorooctanesulfonic acid (6:2 FTSA)	1H, 1H, 2H, 2H-perfluorododecane sulfonic acid (10:2 FTSA)

All laboratories based their TOP assay method on Houtz and Sedlak (2012) with disparate modifications. In all cases, extra doses of oxidant and/or extended oxidation times were used. For all samples tested, Laboratory 1 used a single cycle but used six times the amount of oxidant in comparison to Houtz and Sedlak (2012).

Laboratory 2 diluted the sample prior to oxidation and employed three oxidation cycles over three nights to achieve quality objectives. Laboratory 3 used six times the dosage of oxidant and two cycles for samples S1 and& S2 then increased dosage for samples S3 and S4.

3 Results & Discussion

3.1 PFAA Precursors

NMI used a commercial supply of "Tridol" for the spiked interlaboratory samples (undisclosed source). The major ingredients are reported to be either 6:2 Fluorotelomer mercaptoalkylamido sulfonate (6:2 FTSAS) (Figure 1) or 6:2 Fluorotelomer sulfonamide alkylbetaine (6:2 FTAB) (Figure 2) (KEMI Swedish Chemicals Agency 2015). Subsequent high-resolution accurate mass experiments using LC-QToF-MS (Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry) established, by two of the laboratories, that the main ingredient contained in the Tridol used in this study was 6:2 FTSAS. Without authentic standards the identity could not be definitely confirmed.

6:2 FTSAS has previously been reported to be present in AFFFs with product names F-500, Tridol S3%, Ansulite 3% AFFF-DC-3, Niagara 1-3, and Ansul Ansulite ARC (Weiner et al. 2013). 6:2 FTAB has been reported to be present in Forafac 1157, F-500, Niagara 1-3, and Tridol S (Moe et al. 2012 and D´Agostino & Mabury 2012).

8:2 monoPAP (Figure 3) was also used as a spike representing an 8:2 fluorotelomer PFAA precursor.

Figure 1: 6:2 Fluorotelomermercaptoalkylamido sulfonate (6:2 FTSAS)

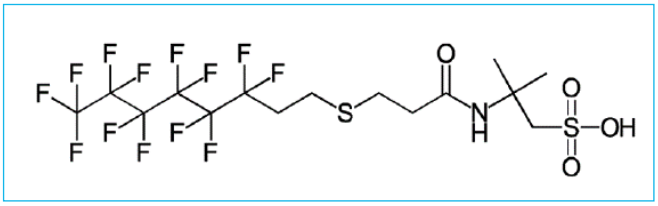


Figure 2: 6:2 Fluorotelomer sulfonamide alkylbetaine (6:2 FTAB) CAS: 34455-29-3

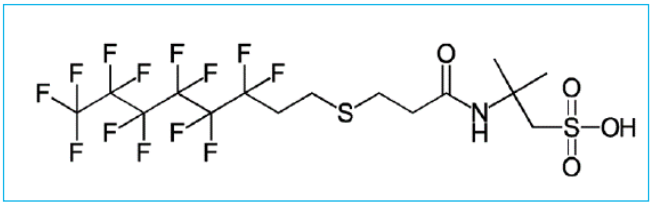
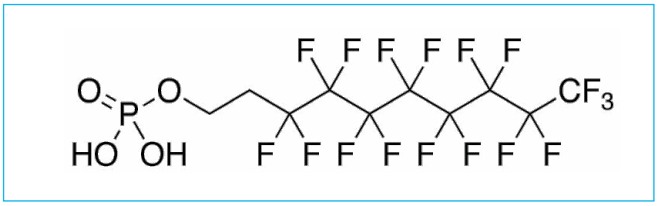


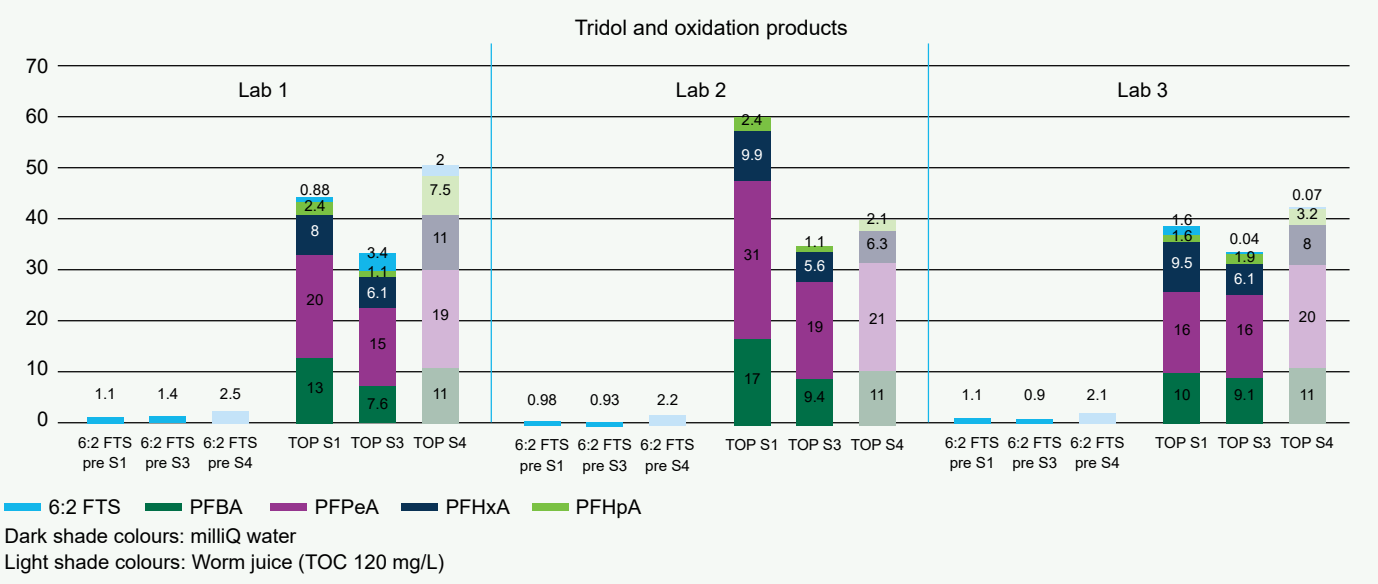
Figure 3: 8:2 monofluoroalkyl phosphate ester (8:2 monoPAP)



3.2 Laboratory Results

The results of pre- and post-TOP assay are presented diagrammatically in Figures 4 to 8.

Figure 4 – 6:2 FTS pre TOP assay and oxidation products from 150 µL Tridol / Samples S1, S3 and S4

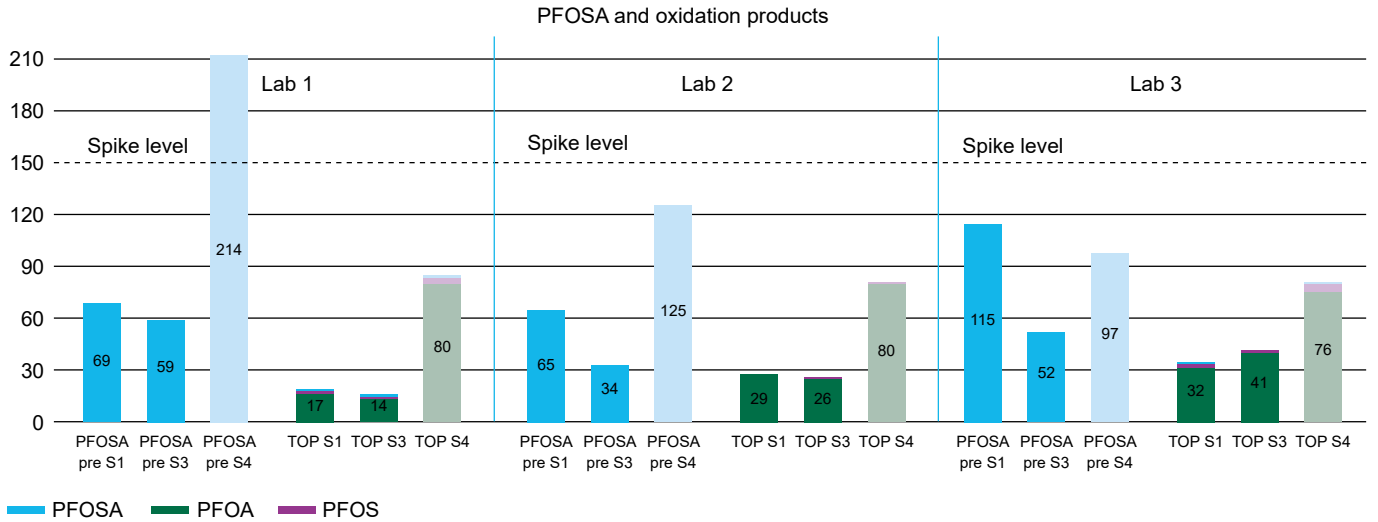


Dark shade colours: milliQ water
Light shade colours: Worm juice (TOC 120 mg/L)

Table 4: Coefficient of Variation for Samples S1, S3 and S4 (concentrations in µg/L)

	PFBA			PFPeA			PFHxA			PFHpA			6:2 FTS		
	S1	S3	S4	S1	S3	S4	S1	S3	S4	S1	S3	S4	S1	S3	S4
Lab 1	13	7.6	10.5	20	15	19	8.0	6.1	11	2.4	1.1	7.5	0.88	3.4	2.0
Lab 2	17	9.4	10.5	31	19	21	9.9	5.6	6.3	2.4	1.1	2.1	<LOR	<LOR	<LOR
Lab 3	10	9.1	10.6	16	16	20	9.5	6.1	8.0	1.6	1.9	3.2	1.6	0.042	0.065
CV%	24	11	0.6	36	11	3.7	11	4.8	27	22	34	67	n/a	n/a	n/a

Figure 5 – PFOSA pre-TOP assay and PFOA and PFOS post-TOP assay and oxidation products from 150 µg/L PFOSA spike Samples S1, S3 and S4



Dark shade colours: milliQ water
Light shade colours: Worm juice (TOC 120 mg/L)

Figure 6 – 8:2 monoPAP oxidation products Sample S2

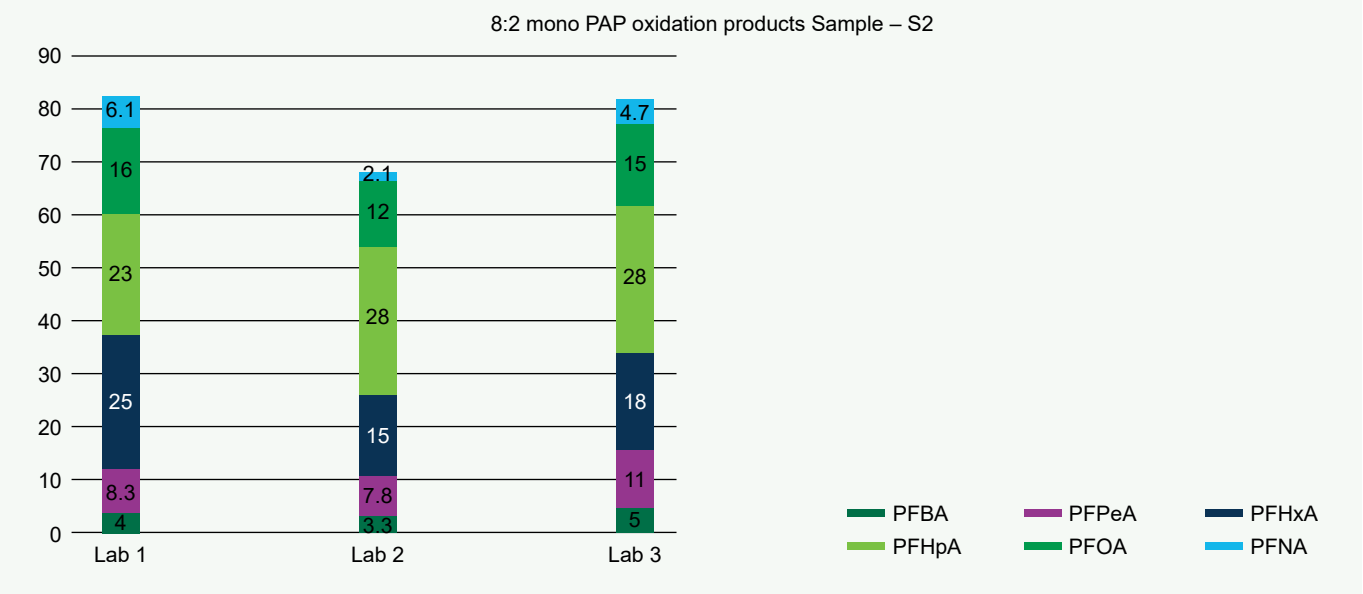


Figure 7 – PFDA results pre and post TOP assay Samples S2, S3 and S4

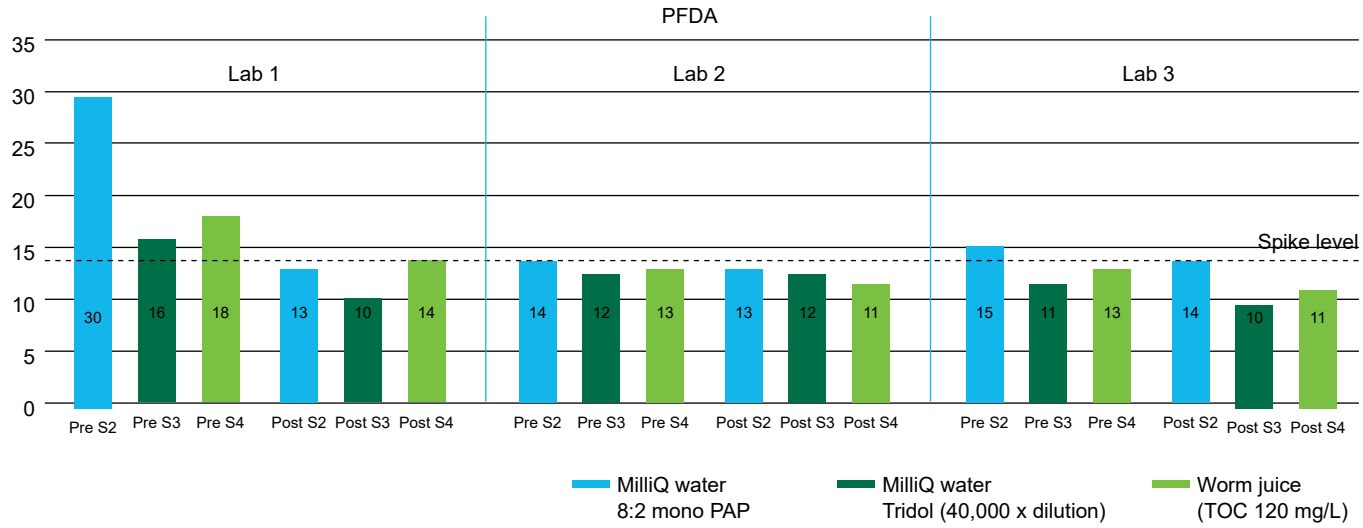


Figure 8 – PFHxS results pre and post TOP assay Samples S3 and S4

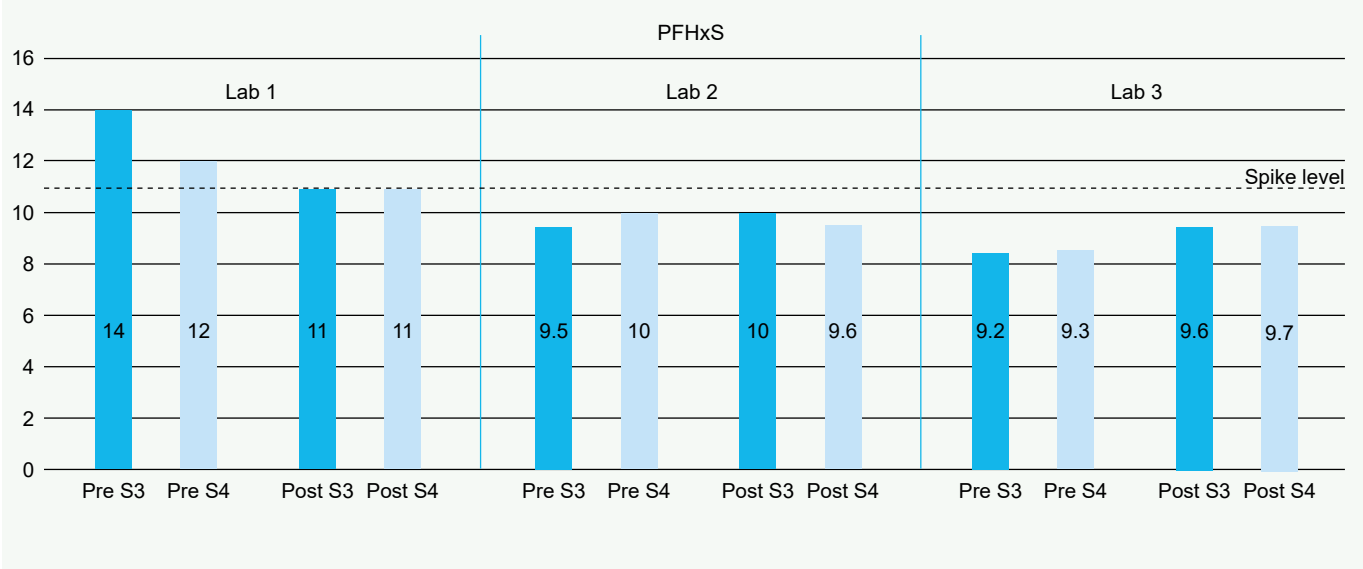


Table 5: Test for acceptability of oxidation step as per 2018 PFAS NEMP

	Sum of PFAA precursors post-oxidation µg/L			Sum of Total PFAS µg/L			Ratio (%) SumPFAA/ SumTotal PFAS µg/L			TEST* Ratio <5%		
	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3
Sample S1 POST	1.3	-	1.9	63	89	74	2.1	0	2.6	**Pass**	**Pass**	**Pass**
Sample S3 POST	4.6	-	0.08	70	83	95	6.6	0	0.1	**Fail**	**Pass**	**Pass**
Sample S4 POST	3.4	-	0.50	160	141	144	2.1	0	0.3	**Pass**	**Pass**	**Pass**

* Sum of measured PFAA precursors

** PFAS NEMP HEPA (2018) section 19.2. ND = not detected, i.e. <limit of reporting.

Raw data results and the uncertainties are presented in Appendix 1.

3.3 Discussion of Results

Results pre- and post-TOP assay are presented diagrammatically in Figures 4 to 8 and discussed here:

- For PFAS results post-oxidation variability within and between participants' results was observed, (Figures 4 and 5) however due to the limited amount of data and the fact that each laboratory used different methodology for oxidation and analysis, no significant trend was observed.
- Samples S1, S3 and S4 – all laboratories reported 6:2FTSA and some PFAAs pre-TOP assay which are expected impurities from the Tridol foam.
- Laboratories 1 and 3 – application of the TOP assay did not fully convert the precursors to PFCAs (Figures 4 and 5). All laboratories reported that extra doses of oxidant and/ or extended oxidation times were required to sufficiently oxidise the samples to meet the PFAS NEMP (HEPA 2018) ratio test for aqueous samples (sum of [PFAA precursors] divided by sum of [Total PFAS] <5%); results for acceptability of oxidation are presented in Table 4. All results passed these criteria except for S3 for Laboratory 1.
- PFOSA results for Samples S1 and S3 pre-oxidation compared to the spiked concentration, indicate a bias towards low results. A possible reason was the adsorption of this analyte onto the walls of the container.
- A higher result was obtained for PFOSA in the Sample S4 (high TOC liquid). It is suspected that the organic matrix kept the less polar PFAS in the solution. A similar trend was observed for 6:2 FTSA and oxidation products (Figures 4 and 5). This is further discussed in Section 3.6.
- For Samples S1, S3 & S4 – Laboratory 2 reported 6:2 FTSA below the LOR post-oxidation indicating complete conversion of the PFAA precursor. Noting that Laboratory 2 diluted the sample prior to oxidation reducing the organic load and perhaps improving the efficiency of the oxidation process. Sample S2 was spiked with 8:2 monoPAP (a fluorotelomer precursor) – see Figure 5 and Figure 6. 8:2 monoPAP is not a target compound so oxidation completion is difficult to gauge but results for the PFCAs show a reasonable consensus post-oxidation. The data suggests 8:2 monoPAP has oxidised under the TOP assay conditions to several PFCAs, as was seen in the post-TOP assay results.

- PFDA in Samples S2, S3 and S4 and PFHxS in Samples S3 and S4 were each spiked (pre-oxidation) with the same amount. These compounds are not expected to increase in concentration post-TOP assay. Results are within 72%-218% of the spiked value for PFDA and 85%-128% for PFHxS (Figures 7 and 8). PFHxS results are within acceptable analytical variability. A single PFDA result by Laboratory 1 in the pre-TOP sample was more than double the spiked value. This result is out of step with the other two lab's results.
- PFOS was spiked in Sample S2 at 10 ug/L. PFOS concentrations should not increase post-TOP assay and results confirm this premise. Results were within 95-117% of the spiked value in both pre and post-TOP assay digest samples.
- For Samples S1, S3 and S4, PFOS was not an expected oxidation product, however Laboratory 1 and 3 reported low concentrations of PFOS. As there was no reported PFOS in the pre-TOP sample it is postulated that the PFOS was formed during the oxidation (or potentially alkaline hydrolysis) of PFOSA. Laboratory 2 reported no PFOS post-TOP assay, noting the laboratory diluted the sample 1:10 prior to oxidation. This is an added variable, so no conclusion can be drawn here.

Results presented by the laboratories generally comply with the PFAS NEMP (HEPA 2018) guidelines. Section 19.2 of the PFAS NEMP (HEPA 2018) stipulates some quality measures for the TOP assay method:

1. The total PFAS concentration post-TOPA should be greater or equal to the total PFAS concentration pre-TOPA, which signifies no material losses observed in preparation steps, noting a decrease of up to 10% might be expected due to normal analytical variability.
2. The sum of PFCA post-TOPA should be equal to or greater than the sum of PFCA pre-TOPA, which signifies any precursors being converted to PFCA products.
3. The sum of PFSA post-TOPA should approximate the sum of PFSA pre-TOPA, signifying that precursors did not convert to PFSA products.
4. For a full oxidation, no PFAA precursors (e.g. 6:2 FTSA, PFOSA) are detectable post-oxidation, signifying complete oxidation.
5. For situations where near complete oxidation is acceptable, minimal PFAA precursors are detectable post oxidation signified by:
   - for aqueous samples, sum of [PFAA precursors] divided by sum of [Total PFAS] <5%.
   - noting greater leniency may be applied for samples where PFAS were detected ≤ 10 times LOR.

Table 4 shows the acceptability of the oxidation process against the criteria in the PFAS NEMP (HEPA 2018). Laboratories have generally complied with the PFAS NEMP with an exception of a marginal exceedance for Laboratory 1, for Sample S3. Clearly, all three laboratories reported ΣPFAS concentrations post-TOPA ≥ pre-TOPA and ΣPFCA post-TOPA ≥ pre-TOPA meeting these PFAS NEMP (HEPA 2018) guidelines. A limitation of the PFAS NEMP (HEPA 2018) relates to point (4). The stipulation of no PFAS precursors present post oxidation is limited to the PFAA precursors measured. This limitation and other aspects of the PFAS NEMP (HEPA 2018) are discussed in Section 4.4.

3.4 Comparison of Laboratory Methods

All laboratories based their TOP assay method on Houtz and Sedlak (2012) with modifications. In all cases, extra doses of oxidant and/or extended oxidation times were required to meet quality objectives. For all samples tested, Laboratory 1 used a single cycle but used 6 times the amount of oxidant in comparison to Houtz and Sedlak (2012). The PFAS NEMP (HEPA 2018) defines a successful oxidation as the ratio of the sum of concentrations of PFAA precursors to the sum of total PFAS as less than 5%. Using their oxidation conditions, Laboratory 1 passed these criteria except for a marginal exceedance for sample S3 (see Table 4). Laboratories 2 & 3 passed all criteria. Laboratory 2 diluted samples prior to oxidation and employed three oxidation cycles over three nights to achieve quality objectives. Laboratory 3 used 6 times the dosage of oxidant and two cycles for samples S1 & S2 then increased dosage for samples S3 & S4. All laboratories reported that these modifications were required to meet the NEMP (HEPA 2018) ratio test (sum of PFAA precursors to sum of PFAS). Applying the Houtz and Sedlak (2012) method without modification will have insufficient oxidant for samples with high organic content. It has been reported that samples with high organic content and/or high concentrations of PFAA precursors can consume all of the oxidant facilitating the need for extra dosages (Bell et al. 2019).

3.5 Oxidation Reagent Doses

All laboratories carried out additional dosage of the assay reagents (potassium persulfate and NaOH), relative to the standard Houtz and Sedlak (2012) dose, to achieve effective oxidation. A noted difference in approach was a single incubation of a larger dose versus successive incubations of a standard dose. To better understand the effectiveness of each approach, an additional trial (performed by ALS) was carried out, monitoring the progress of oxidation over successive reagent doses versus a single dose equivalent to the total dosage applied to the successive trials.

Six successive standard doses were carried out on the sample S4 with analysis carried out after each dose to monitor the progress of oxidation. A single six-times reagent dose was also carried out for comparison. All trials were carried out in duplicate. Average results are provided below.

Table 6. Successive versus single reagent dose comparison for sample S4. Results (µg/L) are averages of duplicate analyses

Dose Event	1st Dose	2nd Dose	3rd Dose	4th Dose	5th Dose	6th Dose	Single 6 x Dose
PFHxS	7.89	7.85	7.43	5.98	6.87	6.81	**7.72**
PFOS	0.03	0.03	0.23	0.94	1.58	1.59	**2.59**
PFDA	12.08	12.12	12.02	12.68	12.27	11.26	**10.23**
PFBA	0.76	1.39	5.61	10.66	7.10	7.75	**9.70**
PFPeA	0.70	1.33	10.70	18.59	14.74	14.65	**16.86**
PFHxA	0.47	0.90	3.52	7.70	10.32	8.55	**6.09**
PFHpA	0.06	0.11	2.25	3.81	2.87	2.42	**1.74**
PFOA	0.26	0.37	40.34	65.88	91.34	70.71	**103.88**
PFNA	0.08	0.08	0.10	0.15	0.12	0.11	**0.12**
Sum PFCA C4-C9	2.33	4.16	62.52	106.79	126.49	104.19	**138.39**
PFOSA	95.27	100.79	56.61	3.74	0.08	0.07	**0.58**
6:2 FTSA	17.51	16.81	16.05	7.16	0.00	0.03	**0.12**
% OXIDATION	2.0%	3.4%	46.2%	90.7%	99.9%	99.9%	**99.5%**

Dose = 80 mg KPS, 76 µL 10 N NaOH to 5 mL Sample

Light Blue = spiked positive controls, Purple = PFCA oxidation products, Green = spiked oxidation targets

Percent oxidation was calculated as the proportion of PFOSA and 6:2 FTSA relative to the sum of PFOSA, 6:2 FTSA and C4-C9 PFCAs. PFDA was excluded as this was a spiked analyte and not an expected oxidation product. Significant oxidation of the sample S4 was not apparent until the 4th successive dose, with complete oxidation at the 5th dose, plateauing at the 6th dose. There was no material difference in performance between sequential dosing and a single (6x) upfront dose (based on % oxidation of PFAA precursors pre- and post-oxidation <0.4%). One observation that is interesting to note is the increase in PFOS across the sequential doses. It is suggested that increasing dosage may result in an elevated alkaline environment, initiating hydrolysis of PFOSA to PFOS. This observation is consistent with the PFOS results originally reported by the three labs. Both Laboratories 1 and 3 who used higher overall dosages reported higher PFOS concentrations. Laboratory 2, with a lower final (3x) dosage, reported lower PFOS and at a level consistent with the 3rd dose from the successive trials.

The results of this trial suggest that either successive small doses or a single large dose are valid approaches to achieve effective oxidation of matrices presented in the NMI Interlaboratory trial. Also, such high dosages may create alkaline conditions sufficient to convert precursors to PFSAs via hydrolysis rather than the expected PFCAs. Where a significant increase in PFSAs is observed from pre- to post–TOP assay, sample dilution may be a considered approach to achieving equivalent oxidation at a lower dose and avoiding conditions of high pH, which might result in alkaline hydrolysis of precursors.

3.6 PFAS Losses to Sample Containers

PFOSA results for Samples S1 and S3 pre-TOP assay indicated a bias towards low results when compared to the spiked concentration. Additional investigation was undertaken to determine whether adsorption of target PFAS to the walls of sample containers had occurred and could account for the missing mass observed for PFOSA in the pre-TOP assay results (additional work conducted by ALS). The 6 individual containers for previously analysed samples were emptied and independently rinsed with methanol. The methanol rinsate was reduced to a known volume and analysed. Results are provided below for the sample S3 and S4 containers as well as an average for each sample (Tables 7 & 8, Figure 9 & 10). Measurable concentrations of PFHxS, PFDA, PFTeDA and particularly PFOSA were observed for all S3 and S4 container rinsates. Greater variability in concentrations were observed for the sample S3 containers compared to S4 containers. The average concentration of PFOSA in the S4 rinsate was double that of the S3 sample. This was contrary to expectations. Given the lower concentrations (relative to spike) reported by all laboratories for the S3 sample pre-TOP assay versus the S4 sample, it was expected that the S3 rinsate would be higher than the S4 rinsate to account for the greater missing mass. In fact, neither set of rinsates fully accounted for the missing mass of PFOSA. This suggests that PFOSA may have been retained elsewhere in the sample preparation process, after spiking but prior to dispatch of samples to the laboratories (e.g. sample homogenisation). This warrants further investigation. These results do not necessarily support the idea that the high organic content of the worm juice impeded adsorption, but rather that partitioning of PFOSA between adsorbed and the aqueous states may be proportional to concentration. Additionally, differences in the measured PFOSA concentrations between S3 and S4 represent differences already present in the samples as provided for testing, as opposed to greater adsorption in S3 relative to S4. Despite this, the results do support the notion of PFAS adsorption to poly-propylene containers.

Table 7. Sample S3 Container Rinsate (µg/L)

	S3-1	S3-2	S3-3	S3-4	S3-5	S3-6	S3 (Average)	Spiked	% Recovery
PFHxS	0.000	0.000	0.000	0.000	0.283	0.000	0.047	10.0	0.5%
PFDA	0.342	0.475	0.433	0.608	1.742	0.167	0.628	12.9	4.9%
PFTeDA	0.542	0.600	0.808	0.642	1.200	0.000	0.632	--	--
FOSA	6.667	8.233	7.442	11.567	23.975	2.700	10.097	150	6.7%
6-2-FTS	0.000	0.000	0.000	0.000	0.000	0.000	0.000	--	--

ND = Not Detected

Figure 9. PFAS Adsorption within Sample S3.

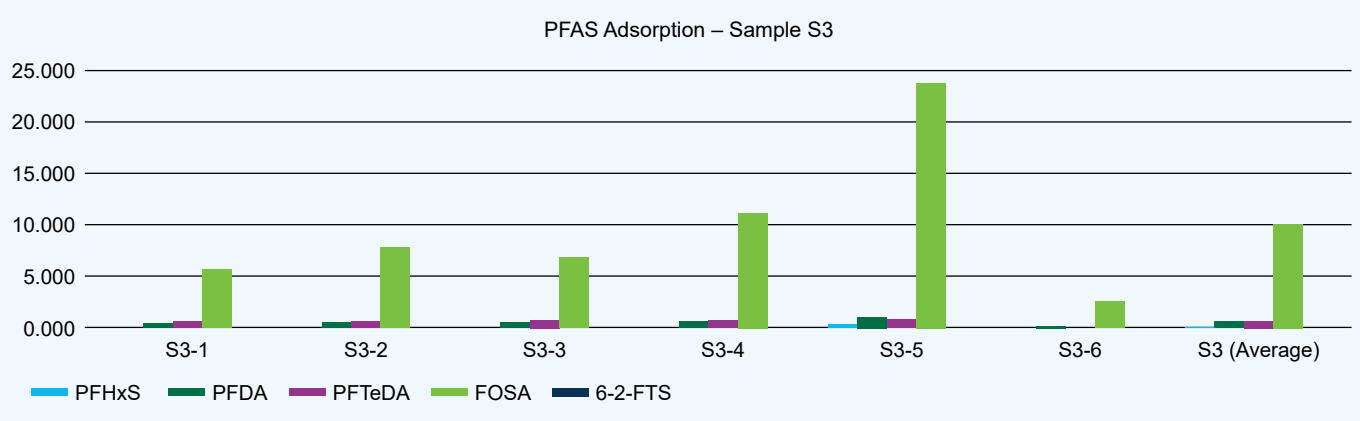
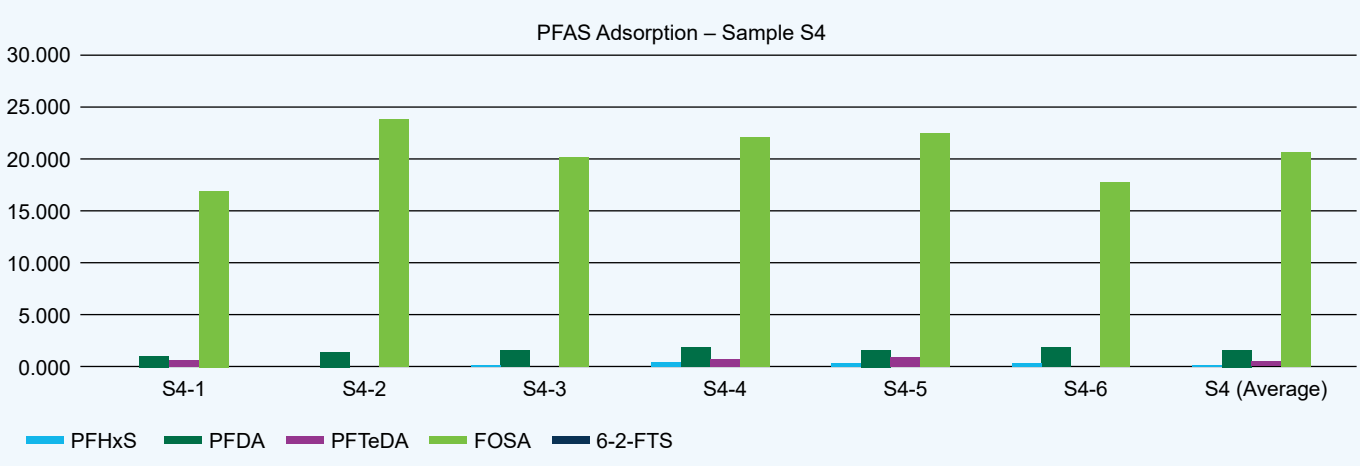


Table 8. Sample S4 Container Rinsate (µg/L)

	S4-1	S4-2	S4-3	S4-4	S4-5	S4-6	S4 (Average)	Spiked	% Recovery
PFHxS	0.092	0.158	0.267	0.367	0.258	0.408	0.258	10.0	2.6%
PFDA	1.117	1.425	1.550	1.858	1.658	1.775	1.564	12.9	12.1%
PFTeDA	0.717	0.000	0.000	0.600	1.000	0.000	0.386	--	--
FOSA	16.983	24.033	20.158	22.208	22.558	17.758	20.617	150	13.7%
6-2-FTS	0.000	0.000	0.025	0.067	0.042	0.067	0.033	--	--

ND = Not Detected

Figure 10. PFAS Adsorption within Sample S4



4 Conclusions and Recommendations

4.1 Application of TOP Assay

The results indicated that fulfilment of quality assurance measures in the PFAS NEMP (HEPA 2018) required increased oxidant dosage and/or extra oxidative cycles. The advice to laboratories developing a routine TOP assay method is:

- Choose a method that will comply with the PFAS NEMP (HEPA 2018) requirements for as many sample types as possible. **Increased dosages and multiple cycles are recommended. Additionally, adherence to strongly alkaline conditions throughout the oxidation process should be maintained.**
- If samples do not comply with the PFAS NEMP (HEPA 2018) ratio test post oxidation treatment, then further oxidative treatment is required. Another option is to dilute the sample prior to oxidation to try and reduce organic load. Note – dilution can result in raising of the LORs to an extent where the results lack analytical meaning.
- Take note of the concentrations of sulfonates pre- and post-oxidation. In this study, PFOS & PFHxS were spiked into samples as monitoring compounds. The sulfonates should have similar concentrations pre-oxidation compared to post-oxidation (as required under the PFAS NEMP (HEPA 2018) quality assurance for equivalence of sulfonate concentrations).
- Assess total PFAA after each oxidation cycle. No change in PFAA concentrations between cycles (within measurement uncertainty) is a reasonable indicator that the oxidation process is complete and that there are no significant PFAA precursors remaining.
- Assuming the sample does not contain >C8 PFAA precursors then C10 and >C10 acids should also have similar concentrations pre-oxidation versus post-oxidation.

4.2 TOP Assay Limitations

When undertaking TOP assay analysis, the following limitations need to be considered:

- The sum of the products of TOP assay expressed as fluorine is not equivalent to total (extractable or adsorbable) organic fluorine. The mass imbalance in even the most basic oxidation (e.g. 6:2-FTSA) is documented by Houtz and Sedlak (2012).
- The products of TOP assay do not necessarily represent environmental endpoints of PFAS degradation. The assay uses a strong oxidation with hydroxyl radicals that would be harsher than the expected conditions of both abiotic and biotic breakdown in the environment. Degradation can include not only oxidation but also hydrolytic processes acting on precursor compounds. For example, the metabolic endpoint of sulfonamide breakdown would be the sulfonic acid rather than a perfluorocarboxylic acid as seen in the TOP assay.
- Under the conditions of the assay, complete oxidation (i.e. destruction of fluorotelomers) will obscure some information about the origins of the contaminants. For example, where long chain perfluorocarboxylates are found to be present, post-oxidation it would not be clear whether these originated from precursors containing 8:2-FTSA or some other source.

4.3 PFAS NEMP Performance Criteria

The following provides commentary and recommended amendments to each of the quality assurance measures for the TOP assay provided in the current PFAS NEMP (HEPA 2018).

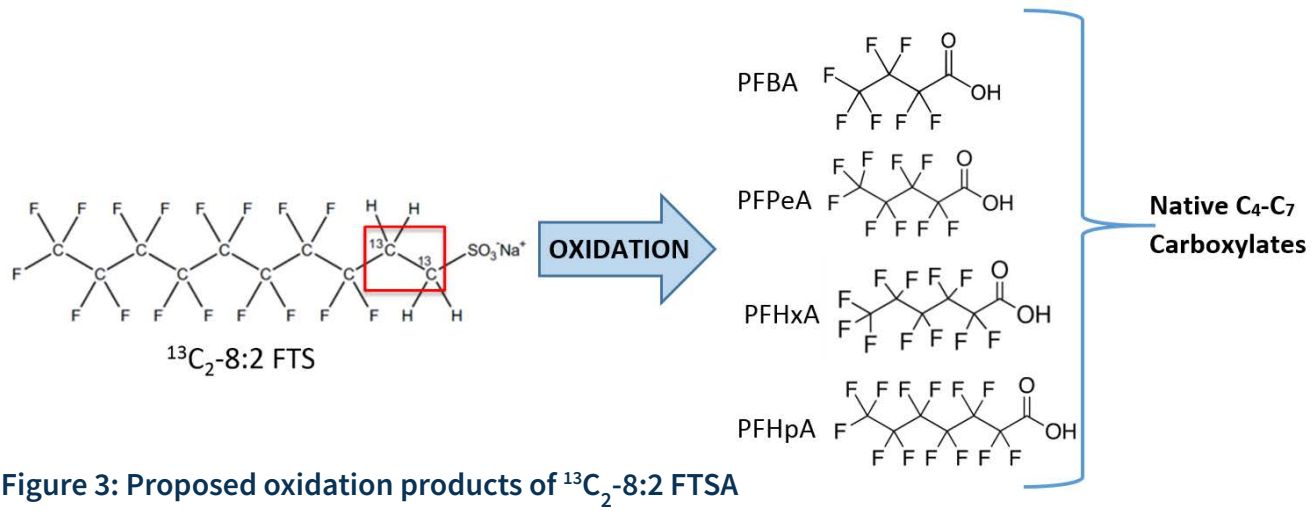


Figure 3: Proposed oxidation products of $^{13}C_2$-8:2 FTSA

If undertaking TOP Assay, that validation of the method's oxidation using detectable oxidisable precursors (e.g. labelled internal standards) is undertaken and reported, and that dilutions are also recorded and reported.

This is not straight forward in practice. Commercially available 13Cx-labelled fluorotelomers and (deuterated) sulfonamides will oxidise to unlabelled native perfluorocarboxylic acids (PFCAs) thereby positively interfering with target ions. If the appropriately 13Cx-labelled fluorotelomers were available these might then yield, upon oxidation, labelled perfluorocarboxylic acids which would interfere with either the labelled internal standards used to quantify target perfluorocarboxylic acids or the labelled surrogates used to monitor extraction efficiency.

Total PFAS concentration post-TOPA should be greater or equal to the total PFAS concentration pre-TOPA, which signifies no material losses observed in preparation steps, noting a decrease of up to 10% might be expected due to normal analytical variability

This is dependent on what pPFAA precursor compounds are present and in what proportions. The reaction pathways of oxidation dictate that in conversion to PFAA, mass can be lost. Also, conversion to PFAA with chain lengths <C4 will be unaccounted for in a standard analysis.

In the example of 6:2 FTSA, Houtz and Sedlak (2012) reported an average molar recovery from C4-C7 PFCA post assay of 73%. This represents only ~50% mass recovery of 6:2 FTSA accounted for by the C4-C7 PFCA oxidation products. Additionally, only ~50% of the fluorine in 6:2 FTSA is accounted for in the C4-C7 oxidation products. Therefore, there are circumstances where the proposed criterion may not be physically achievable. For example, if the proportion of non-target PFAA precursors to 6:2 FTSA in a sample is small, the Total PFAS post-assay may be significantly lower than Total PFAS pre-assay.

The sum of PFCA post-TOPA should be equal to or greater than the sum of PFCA pre-TOPA, which signifies any precursors being converted to PFCA products

This is a more appropriate measure than the preceding criterion, with the caveat that 'equal' is defined as within normal analytical variability.

The sum of PFSA post-TOPA should approximate the sum of PFSA pre-TOPA, signifying that precursors did not convert to PFSA products

Perfluoroalkyl sulfonic acids present in a sample are expected to remain stable under the conditions of the assay, however this criterion assumes that no PFSA will be produced from precursors, which is not necessarily the case.

This may be true for PFOS containing AFFF but this would not be the case when dealing with, for example, fabric treatments based on acrylic polymers with perfluoroalkyl sulfonamide side branches attached, which confer water and oil repellent properties. Therefore, the 'equal to or greater than' criterion specified previously for PFCA would also be applicable for PFSA.

For a full oxidation, no PFAA precursors (e.g. 6:2 FTSA, FOSA) are detectable post oxidation, signifying complete oxidation. For situations where a near complete oxidation is acceptable, minimal PFAA precursors are detectable post oxidation signified by:

- **for aqueous samples, sum of [PFAA precursors] divided by sum of [Total PFAS] <5%**
- **for soil samples, sum of [PFAA precursors] divided by sum of [Total PFAS] <10%**

(Noting greater leniency may be applied for samples where PFAS were detected ≤ 10 times LOR).

Evaluating the proportion of precursors remaining after oxidation against the sum of expected oxidation products (i.e. PFAAs) is a valuable measure of the efficacy of the assay on a per sample basis. Using sum of total PFAS could mask poor performance of the assay and is dependent on the scope of PFAS analytes reported by a particular laboratory. Amending from sum of total PFAS to sum of total PFAAs is recommended, representing a more relevant and consistent approach across laboratories.

The term sum of [PFAA precursors] also requires clarification. Laboratories only report a selection of PFAA precursors in their analytical suite. A more appropriate designation is sum of measured PFAA precursors.

The suggested change to wording is as follows:

For situations where a near complete oxidation is acceptable, minimal PFAA precursors are detectable post oxidation signified by:

- **for aqueous samples, sum of [measured PFAA precursors] divided by sum of [Total PFAAs] <5%**
- **for soil samples, sum of [measured PFAA precursors] divided by sum of [Total PFAAs] <10%**

noting greater leniency may be applied for samples where PFAS were detected ≤ 10 times LOR.

Further, an additional recommendation for consideration when a high level of analytical robustness is required:

- Inclusion of a positive control sample should be considered where a conclusive assessment of oxidation effectiveness is required. This is particularly relevant where PFAA precursors maybe oxidised to short-chain analytes not measured by the laboratory suite (e.g. many commercial laboratories in Australia only measure >C3 PFCA and >C2 PFSA).

4.4 Further studies

The members of this project recommend further work to enhance the utility of the TOP assay. Several limitations need addressing to allow the test to gain sufficient robustness (as presented in 4.3). Primarily, the TOP assay has not been assessed for fluorine mass balance in this study. Some published work has suggested that the TOP assay can result in further oxidation to non-target (<C4) acids and possibly mineralise to fluorine with therefore a significant amount of PFAA unaccounted. Further research is required to understand the chemical process and develop techniques to assess the fluorine mass imbalance of the TOP assay.

The study presented here was conducted using only three laboratories. NMI is planning to conduct a more comprehensive interlaboratory study in the near future. The use of more participant laboratories should help to produce more statistically meaningful results.

5 Disclaimer

This report has been prepared for the Australasian Land and Groundwater Association (ALGA) who commissioned part of the works as part of ALGA's Research and Development Grant program, with considerable in-kind commitment from project partners.

The report has been prepared to fulfill the objectives of the research and development project and is not intended to be a comprehensive laboratory proficiency study.

Ventia, The National Measurement Institute, or other parties involved in the study, accept no liability for use or interpretation of the report by any person or body.

6 References

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Appendix 1 – Interlaboratory Report

Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

Interlaboratory Comparison Report

PFAS TOP ASSAY

V1.3

July 2019

Revision History

Date	Issue Number	Reason for review
February 2019	1.0	Final report
February 2019	1.1	Sections 2 and 3 – small amendments.
April 2019	1.2	Appendix 1 – corrections to between labs CV (%)
July 2019	1.3	Table 1 and 2 completed

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Aim

- To evaluate the laboratories' methods for TOP assay oxidative pre-treatment
- To compare and assess laboratories' accuracy in the measurement of PFAS before and after oxidation pre-treatment

1.2 Sample Preparation

Four samples were prepared in two stages. Stage 1 included Samples S1 and S2 and Stage 2 included Samples S3 and S4.

Sample S1 – MilliQ water spiked with Tridol foam (40,000 x dilution) and PFOSA.

Expected:

PRE TOP	POST TOP
6:2 FTS	PFBA to PFHpA
PFOSA	PFOA

Sample S2 – MilliQ water spiked with 8:2 monoPAP, PFDA and PFOS.

Expected:

PRE TOP	POST TOP
Nil	PFBA to PFNA
PFDA	PFDA
PFOS	PFOS

Sample S3 – MilliQ water spiked with Tridol foam (40,000 x dilution), PFOSA, PFDA and PFHxS.

Expected:

PRE TOP	POST TOP
6:2 FTS	PFBA to PFHpA
PFOSA	PFOA
PFDA	PFDA
PFHxS	PFHxS

Sample S4 – Diluted liquid from a worm farm (Total Organic Carbon content of 120 mg/L) spiked with Tridol foam (40,000 x dilution) and PFOSA, PFDA and PFHxS.

Expected:

PRE TOP	POST TOP
6:2 FTS	PFBA to PFHpA
PFOSA	PFOA
PFDA	PFDA
PFHxS	PFHxS

1.3 Test Material Homogeneity

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

1.4 Sample Storage and Dispatch

Prior to dispatch samples were refrigerated at 4°C.
Participants were sent 6 x 50 mL water in HDPE bottles for each sample. The samples were packed in a foam box with a cooler brick and sent by courier.
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 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.

1.5 Instructions to Participants

Participants were instructed as follows:

- Quantitatively analyse the samples using your normal test method.
- Report results in units of **µg/L** for water samples
- For each analyte in each sample report three results for pre-oxidation and three results for post-oxidation.
- 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.
- Please complete the method details as required in the Methodology sheet.
- Return the completed results sheet by e-mail proficiency@measurement.gov.au

2 PARTICIPANTS' METHOD

Participants were asked to perform two analyses on each sample:

1) A PRE TOP assay analysis using their routine methods for PFAS analysis
2) A POST TOP assay analysis using their routine methods for PFAS analysis after using an oxidative sample pre-treatment method based on Houtz and Sedlak[1] to convert non-target poly and perfluorinated PFAS (called precursors) into target perfluoroalkyl acids (PFAAs).

A brief summary of participants' test method are presented in Tables 1 and 2.

Table 1 Oxidative treatment

	Houtz & Sedlak	Laboratory 1		Laboratory 2	Laboratory 3	
		S1, S3, S4	S2	All	S1, S2	S3, S4
Sample amount (mL)	125	5	5	50*	20	20
Potassium persulfate (g)	2 (60mM)	0.480	0.240	0.8	1	1
Sodium hydroxide (mL)	1.9 (150 mM)	0.456	0.228	0.76	1	1
Number of oxidation cycles	1	1	1	3	2	3
Dosage compare to H&S	1	6	3	3	6	9
pH before heating		14		13	14	
Heating time (hr)	6	6		At least 6 or overnight for each cycle	2.5 for first cycle (s) then overnight for last cycle	
Temperature (°C)	85	80 (S1), 85 (S3, S4)	80	85	85	
pH after heating		14		13	13	
POST oxidation pH adjust.	5-9	7		neutral	5	

*Sample diluted 1:10 prior oxidation

Table 2 Test methods for PFAS in water (pre and post analysis)

	Laboratory 1	Laboratory 2	Laboratory 3
Sample amount (mL)	1	20	60
Extraction	Direct injection	SPE	SPE
Extraction solvent	Methanol		Methanol/NH4OH
Instrument	LCMSMS	LCMSMS	LCMSMS
Column Type:	C18	C18	C18
Column Specifications:	2.0mm x 50mm (1.6um)	2.1 mm X 50 mm (1.8 µm)	2.1 mm X 50 mm (1.7 µm)
Extra column for blank separation	no	no	no
Internal standard (before extraction)	24	23	26
Recovery standard (before instrument analysis)	2	0	4
Recovery correction	no	yes	yes

3 RESULTS

Results are presented in Tables 3 to 6. These results are the average of three replicate results provided by participants. All PFAA results PRE Top assay were likely impurities from Tridol foam and 8:2 monoPAP standard and were <0.20 µg/L. Raw data results and the uncertainties are presented in Appendix 1.

3.1 STAGE 1

Table 3 Sample S1- Milli-Q water

	PRE				POST			
Sample S1 Spiked analytes and level	Analyte	Concentration (µg/L)			Analyte	Concentration (µg/L)		
		Lab 1	Lab 2	Lab 3		Lab 1	Lab 2	Lab 3
Tridol 40, 000 dilution	6:2 FTS	1.1	0.98	1.1	6:2 FTS	0.88	<0.25	1.6
					PFBA	13	17	10
					PFPeA	20	31	16
					PFHxA	8.0	9.9	9.5
					PFHpA	2.4	2.4	1.6
PFOSA 150 µg/L	PFOSA	69	65*	115	PFOSA	0.38	<0.25	0.32
					PFOA	17	29	32
					PFOS	1.8	<0.25	2.7

Note: shaded cells are the expected oxidation products.
*Laboraotory 2 PFOSA result was amended on 16/08/2018. Original reported result was 9.44 ug/L.

Table 4 Sample S2– Milli-Q water

	PRE				POST			
Sample S2 Spiked analytes and level	Analyte	Concentration (µg/L)			Analyte	Concentration (µg/L)		
		Lab 1	Lab 2	Lab 3		Lab 1	Lab 2	Lab 3
8:2 monoPAP 210 ug/L					PFBA	4.0	3.3	5.0
					PFPeA	8.3	7.8	11
					PFHxA	25	15	18
					PFHpA	23	28	28
					PFOA	16	12	15
					PFNA	6.1	2.1	4.7
PFDA 13.9 µg/L	PFDA	30	14	15	PFDA	13	13	14
PFOS 10 µg/L	PFOS	10	12	9.5	PFOS	10	9.7	9.7

Note: shaded cells are the expected oxidation products.

3.2 STAGE 2

Table 5 Sample S3 – Milli-Q water

	PRE				POST			
Sample S3 Spiked analytes and level	Analyte	Concentration (µg/L)			Analyte	Concentration (µg/L)		
		Lab 1	Lab 2	Lab 3		Lab 1	Lab 2	Lab 3
Tridol 40, 000 dilution	6:2 FTS	1.4	0.93	0.90	6:2 FTS	3.4	<0.025	0.042
					PFBA	7.6	9.4	9.1
					PFPeA	15	19	16
					PFHxA	6.1	5.6	6.1
					PFHpA	1.1	1.1	1.9
PFOSA 150 µg/L	PFOSA	59	34	52	PFOSA	1.2	<0.05	0.043
					PFOA	14	26	41
					PFOS	0.82	0.17	0.94
PFDA 13.9 µg/L	PFDA	16	12	11	PFDA	10	12	10
PFHxS 10.9 µg/L	PFHxS	14	9.5	9.2	PFHxS	11	10	9.6

Note: shaded cells are the expected oxidation products.

Table 6 Sample S4 – High organic liquid from a worm farm (TOC 120 mg/L)

	PRE				POST			
Sample S4 Spiked analytes and level	Analyte	Concentration (µg/L)			Analyte	Concentration (µg/L)		
		Lab 1	Lab 2	Lab 3		Lab 1	Lab 2	Lab 3
Tridol 40, 000 dilution	6:2 FTS	2.5	2.2	2.1	6:2 FTS	2.0	<0.025	0.065
					PFBA	11	11	11
					PFPeA	19	21	20
					PFHxA	11	6.3	8.0
					PFHpA	7.5	2.1	3.2
PFOSA 150 µg/L	PFOSA	214	125	97	PFOSA	1.4	<0.05	0.43
					PFOA	80	80	76
					PFOS	2.7	0.35	4.5
PFDA 13.9 µg/L	PFDA	18	13	13	PFDA	14	11	11
PFHxS 10.9 µg/L	PFHxS	12	10	9.3	PFHxS	11	9.6	9.7

Note: shaded cells are the expected oxidation products.

The acceptability for oxidation step has been checked using the criteria in the 2018 PFAS NEMP which states that : “for situations where a near complete oxidation is acceptable, minimal PFAA precursors are detectable post oxidation signified by

- for aqueous samples, sum of [PFAA precursors] divided by sum of [Total PFAS] <5%.”[2]

Results are presented in Table 7.

Table 7 Test for acceptability of oxidation step as per 2018 PFAS NEMP

	Sum of PFAA precursors post-oxidation µg/L			Sum of Total PFAS µg/L			Ratio (%) $Sum_{PFAA}/Sum_{Total\ PFAS}$ µg/L			TEST* Ratio <5%		
	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3	Lab 1	Lab 2	Lab 3
Sample S1 POST	1.3	-	1.9	63	89	74	2.1	0	2.6	**Pass**	**Pass**	**Pass**
Sample S3 POST	4.6	-	0.08	70	83	95	6.6	0	0.1	***Fail***	**Pass**	**Pass**
Sample S4 POST	3.4	-	0.50	160	141	144	2.1	0	0.3	**Pass**	**Pass**	**Pass**

*PFAS NEMP section 19.2[2]

4 DISCUSSION

Results PRE and POST TOP assay are presented in Figures 1 to 5.

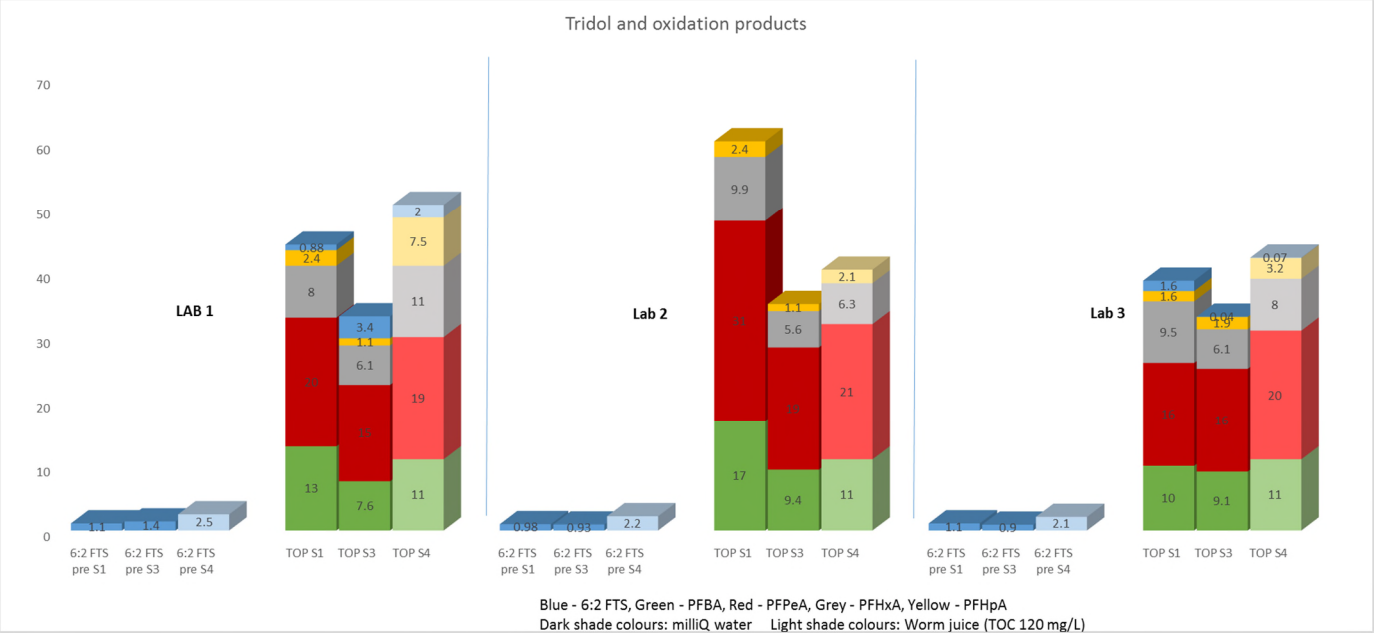

Figure 1 6:2 FTS pre TOP assay and oxidation products from 150 µL Tridol Samples S1, S3 and S4

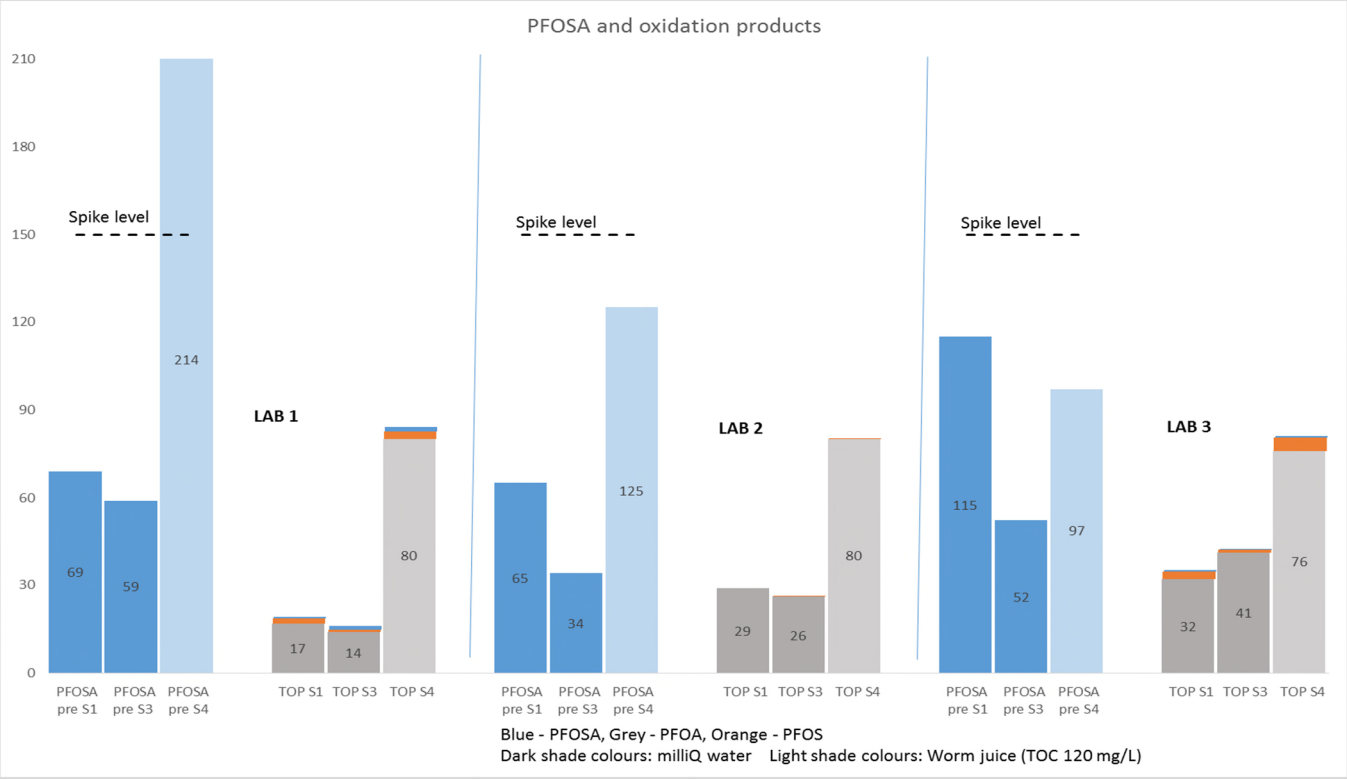

Figure 2 PFOSA pre TOP assay and oxidation products from 150 µg/L PFOSA spike Samples S1, S3 and S4

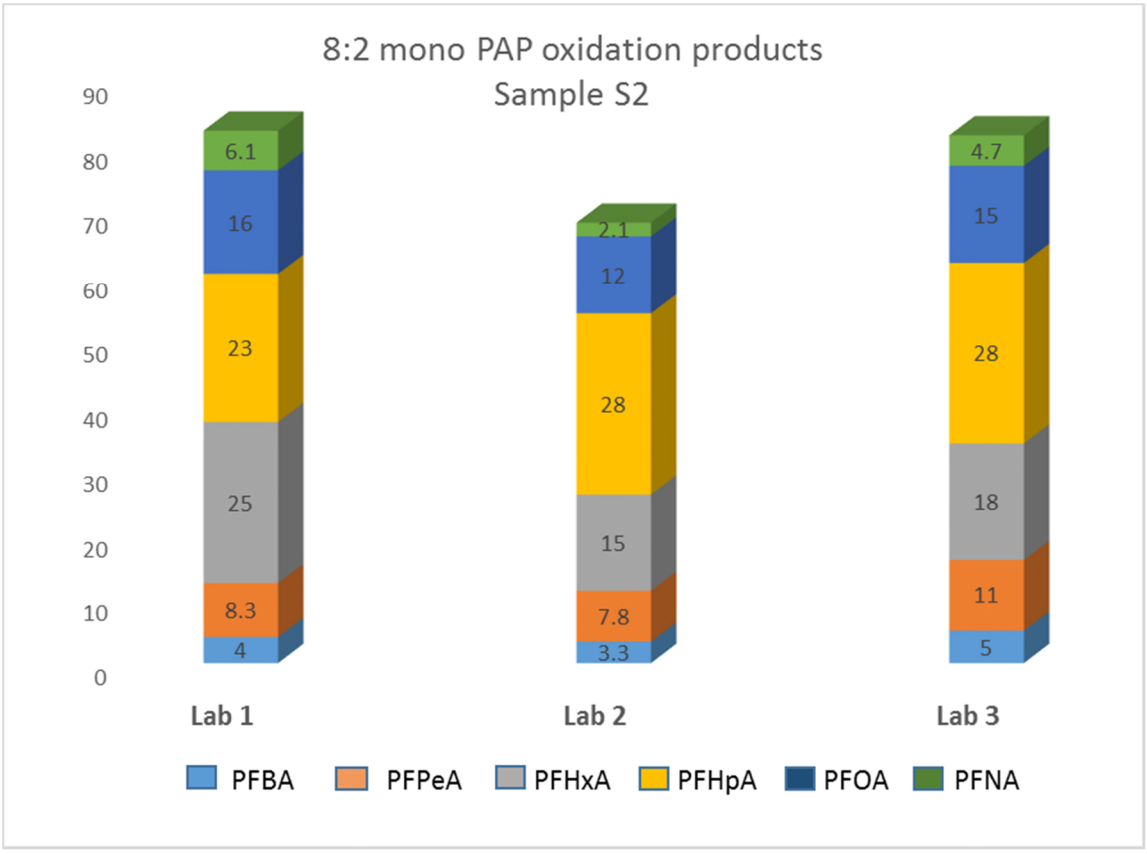

Figure 3 8:2 monoPAP oxidation products Sample S2

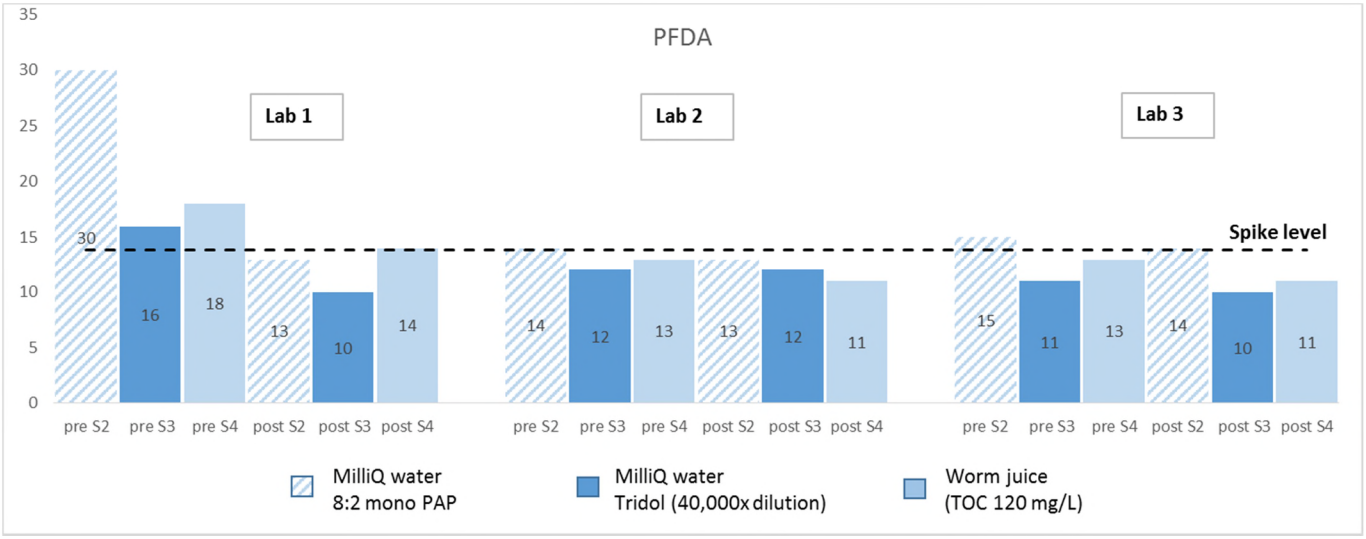

Figure 4 PFDA results pre and post TOP assay Samples S2, S3 and S4

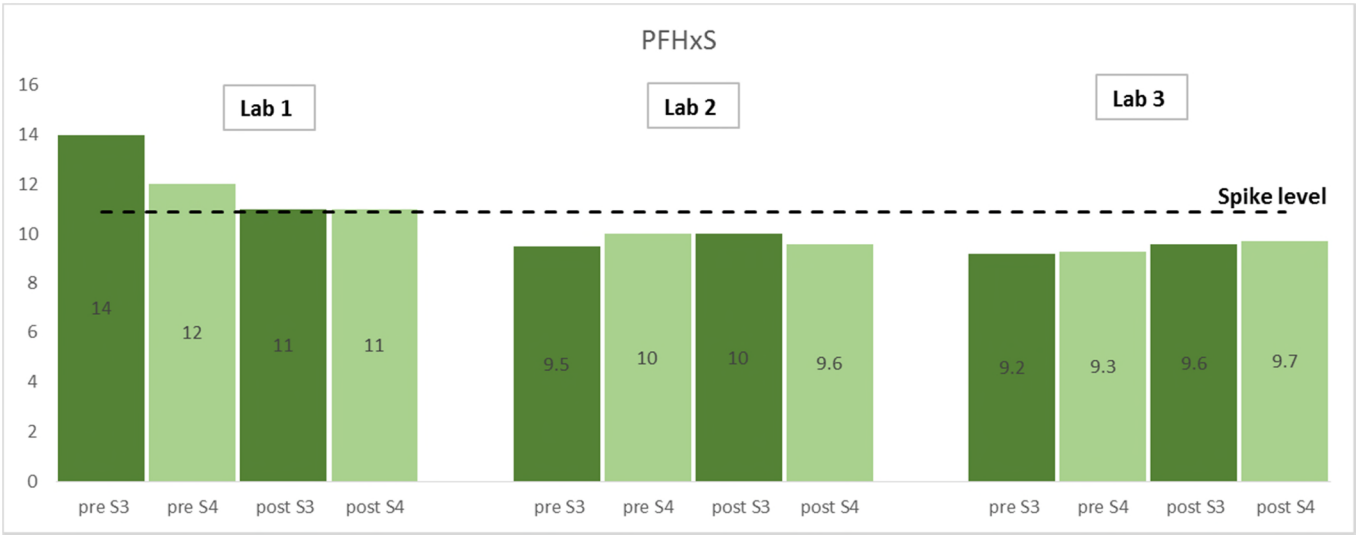

Figure 5 PFHxS results pre and post TOP assay Samples S3 and S4

1) Laboratories 1 and 3 oxidative pre-treatment did not fully convert the precursors to PFCAs (Tables 1 and 2). A test for acceptability of oxidation[2] is presented in Table 7. Laboratory 1 failed the test for Sample S3.
2) PFOSA results for Samples S1 and S3 PRE oxidation against the spiked concentration, indicate a bias towards low results. A possible reason was the adsorption of this analyte onto the walls of the container.
   A higher result was obtained for PFOSA in the Sample S4 (high organic liquid) indicating that the matrix kept the less polar PFAS in the solution. This is also valid for the oxidation product, PFOA. A similar trend was observed for 6:2 FTS and oxidation products (Figures 1 and 2).
3) For PFAS results POST oxidation pre-treatment, a high variability within and between participants' results was observed (Figures 1 and 2). Due to the limited amount of data and the fact that each laboratory used different methodology for oxidation and analysis no significant trend was observed.
4) PFDA in Samples S2, S3 and S4 and PFHxS in Samples S3 and S4 were each spiked with the same amount in the PRE and POST TOP assay samples. Laboratories results are within 72% -218% of the spiked value for PFDA and 85% - 128% for PFHxS (Figures 4 and 5)
5) PFOS was spiked in Sample S2 at 10 ug/L. Laboratories results are within 95-117% of the spiked value in both PRE and POST samples.

5 REFERENCES

[1] Houtz, F.E. & Sedlak, L.D.2012, “Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff” *Environmental Science & Technology*, 46, pp 9342-9349.

[2] PFAS National Environmental Management Plan 2018, EPA Victoria, viewed January 2019, < https://www.epa.vic.gov.au/PFAS_NMP>

APPENDIX 1 - TABLE OF RESULTS AND UNCERTAINTIES

A1.1 Results PRE and POST TOP Assay

Participant results are listed in Tables 8 to 55. Bar charts of results and uncertainties are presented in Figures 6-53.

Table 8

Sample Details

Sample No.	S1 PRE
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	6:2 FTS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	1.112	0.289		0.97	0.22		1.0	0.23
2	1.012	0.263		0.98	0.22		1.1	0.16
3	1.039	0.27		0.99	0.22		NT	NT
Mean	1.05			0.98			1.05	
Within lab CV (%)	4.9			1.0			6.7	
Between labs CV (%)	4.1							

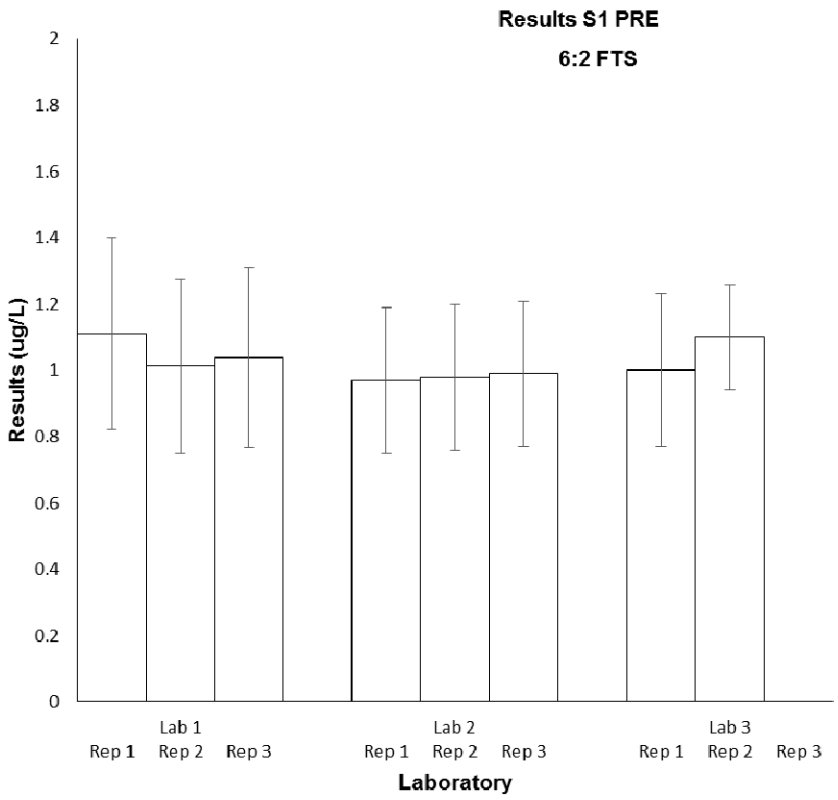

Figure 6

Table 9

Sample Details

Sample No.	S1 PRE
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFOSA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	72.79	28.388		60	12.2		110	16.5
2	69.96	27.284		69	12.5		120	18
3	65.16	25.412		65	12.4		NT	NT
Mean	69.3			64.7			115	
Within lab CV (%)	5.6			7.0			6.1	
Between labs CV (%)	34							

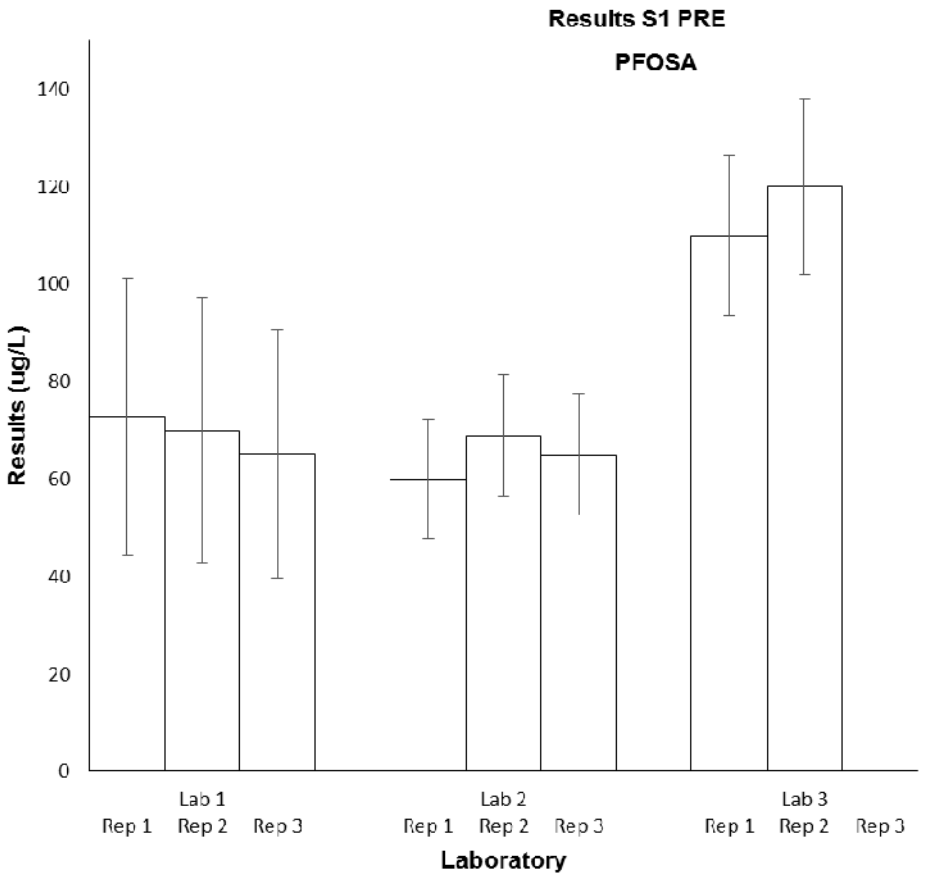

Figure 7

Table 10

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	6:2 FTS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	0.65	0.169		<0.25	0.05		2	0.3
2	0.83	0.216		<0.25	0.05		1.1	0.16
3	1.17	0.304		<0.25	0.05		NT	NT
Mean	0.88			-			1.6	
Within lab CV (%)	30			-			41	
Between labs CV (%)	39							

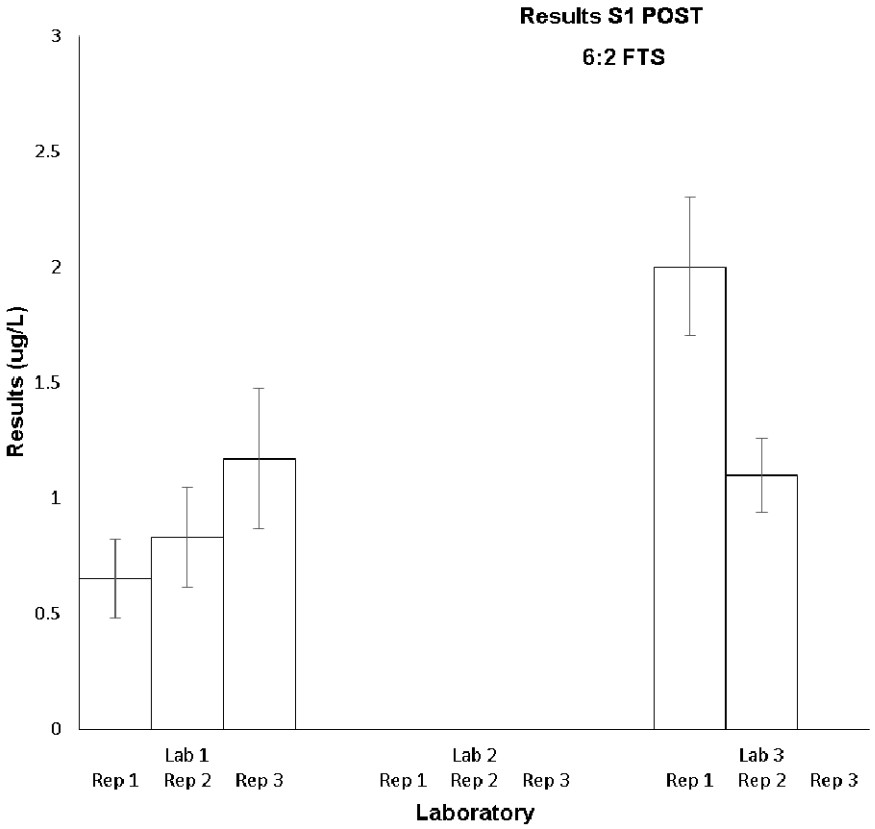

Figure 8

Table 11

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFOSA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	0.45	0.176		<0.25	0.05		0.46	0.07
2	0.37	0.144		<0.25	0.05		0.18	0.03
3	0.33	0.129		<0.25	0.05		NT	NT
Mean	0.38			-			0.32	
Within lab CV (%)	16			-			62	
Between labs CV (%)	13							

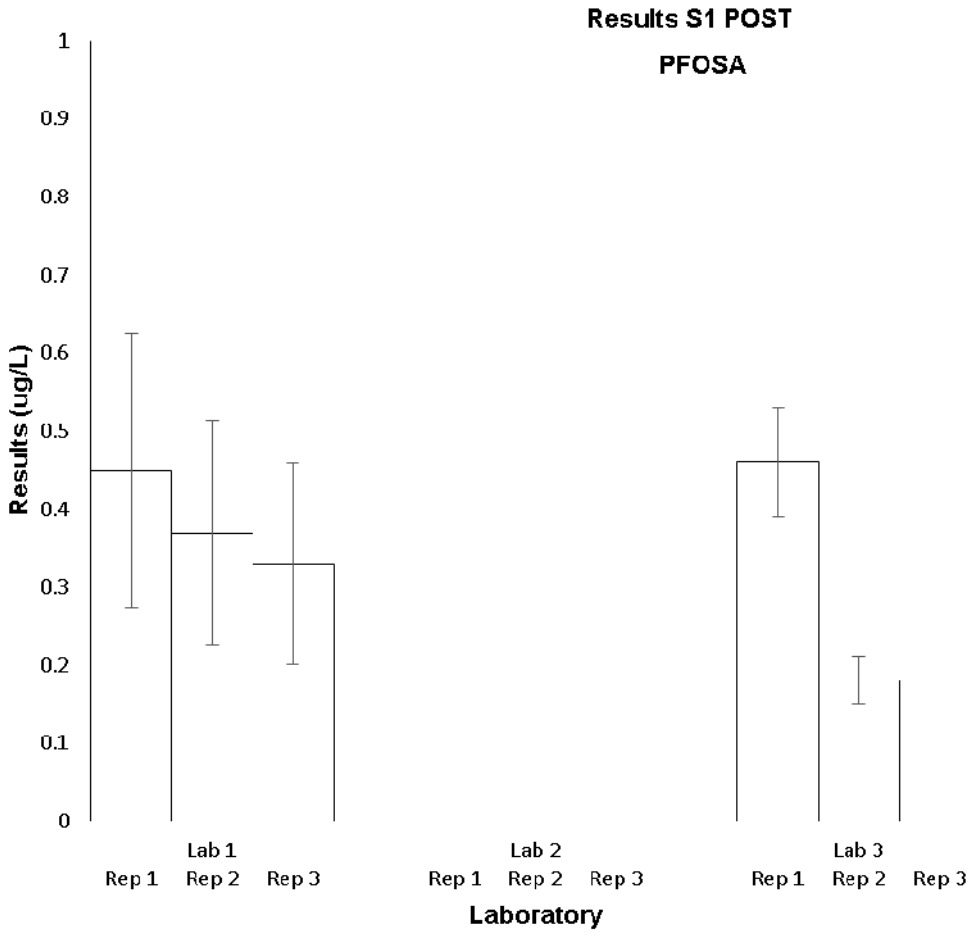

Figure 9

Table 12

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFBA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	11.72	2.953		14.7	2.6		9.8	1.5
2	13.14	3.311		18.3	3.2		11	1.6
3	12.55	3.163		16.9	3		NT	NT
Mean	12.5			16.6			10.4	
Within lab CV (%)	5.7			10.9			8.2	
Between labs CV (%)	24							

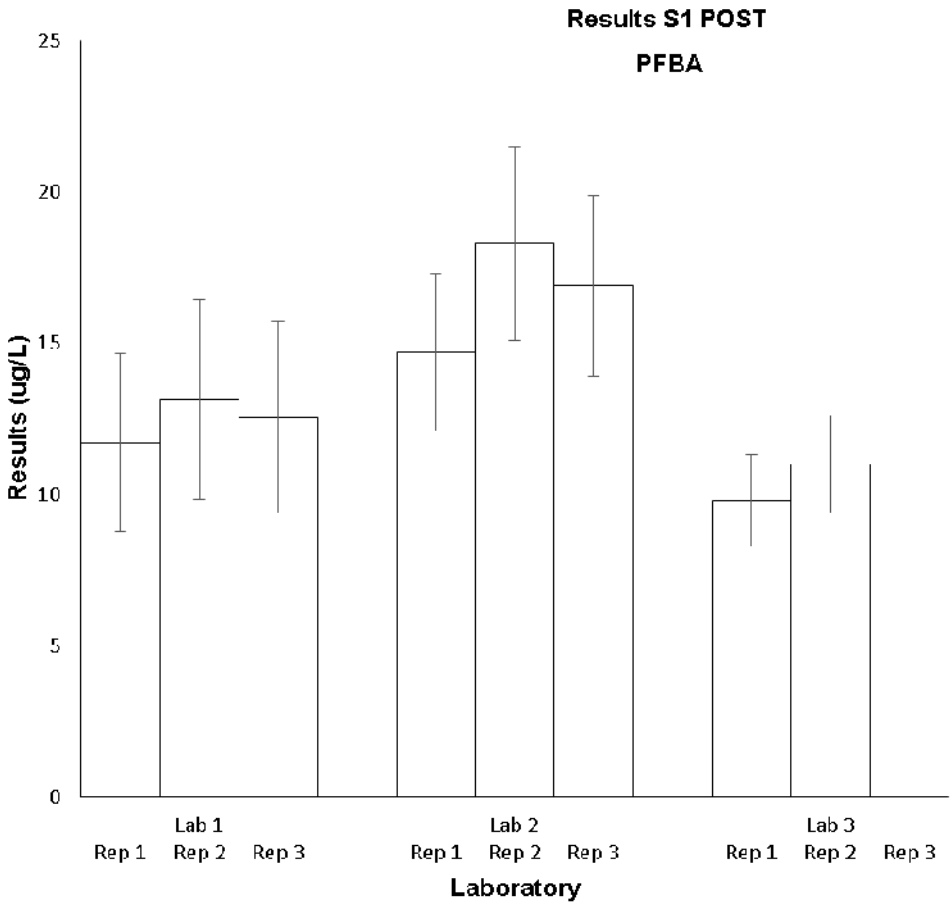

Figure 10

Table 13

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFPeA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	19.38	4.651		29.9	7.7		14	2.1
2	21.16	5.078		30.2	7.8		17	2.6
3	19.46	4.67		32.5	8.4		NT	NT
Mean	20.00			30.9			16	
Within lab CV (%)	5.0			4.6			13.7	
Between labs CV (%)	36							

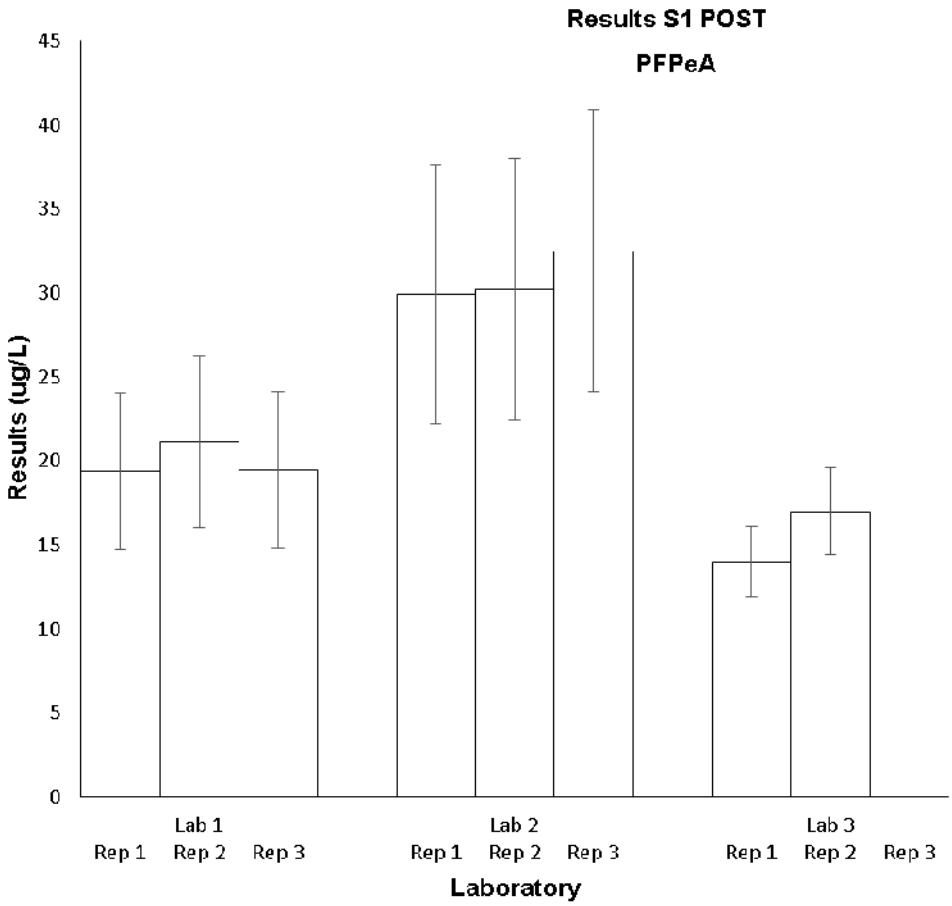

Figure 11

Table 14

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFHxA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	8.12	2.387		10.7	2.7		9	1.3
2	8.02	2.35		10.1	2.5		10	1.5
3	7.71	2.267		8.9	2.2		NT	NT
Mean	7.95			9.9			9.5	
Within lab CV (%)	2.7			9.3			7.4	
Between labs CV (%)	11							

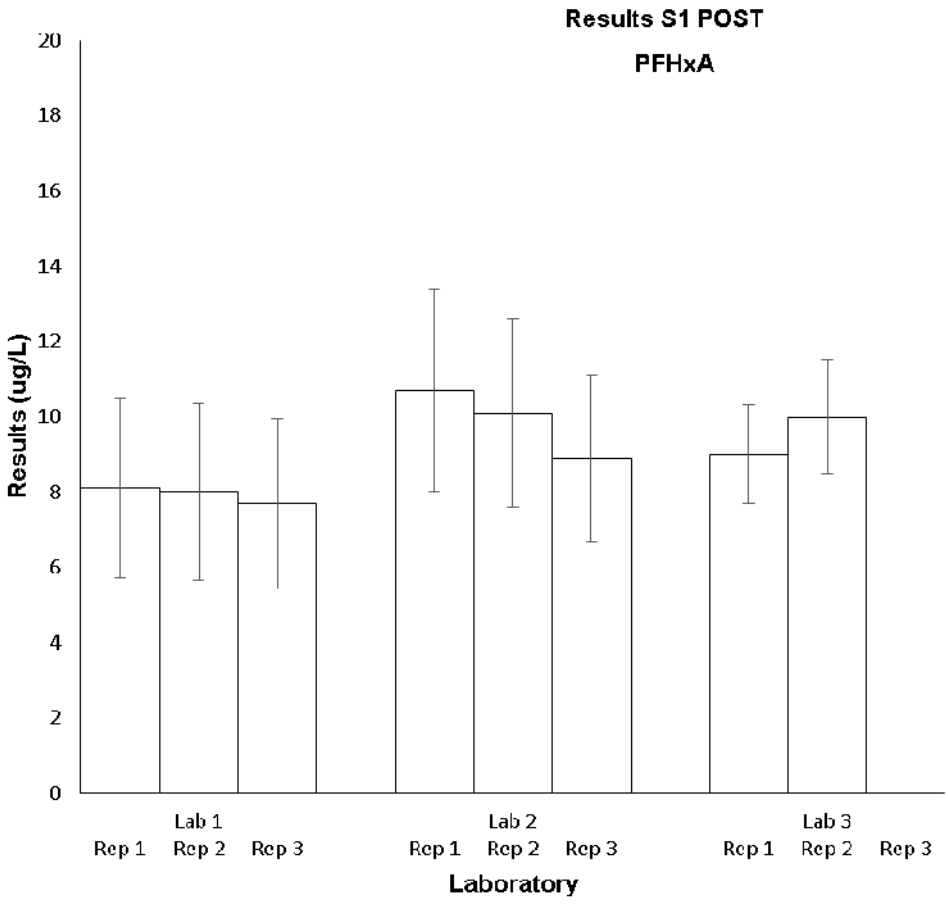

Figure 12

Table 15

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFHpA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	2.49	0.475		2.6	0.6		1.4	0.2
2	2.46	0.469		2.1	0.5		1.8	0.27
3	2.35	0.448		2.5	0.6		NT	NT
Mean	2.43			2.4			1.6	
Within lab CV (%)	3.0			11.0			17.7	
Between labs CV (%)	22							

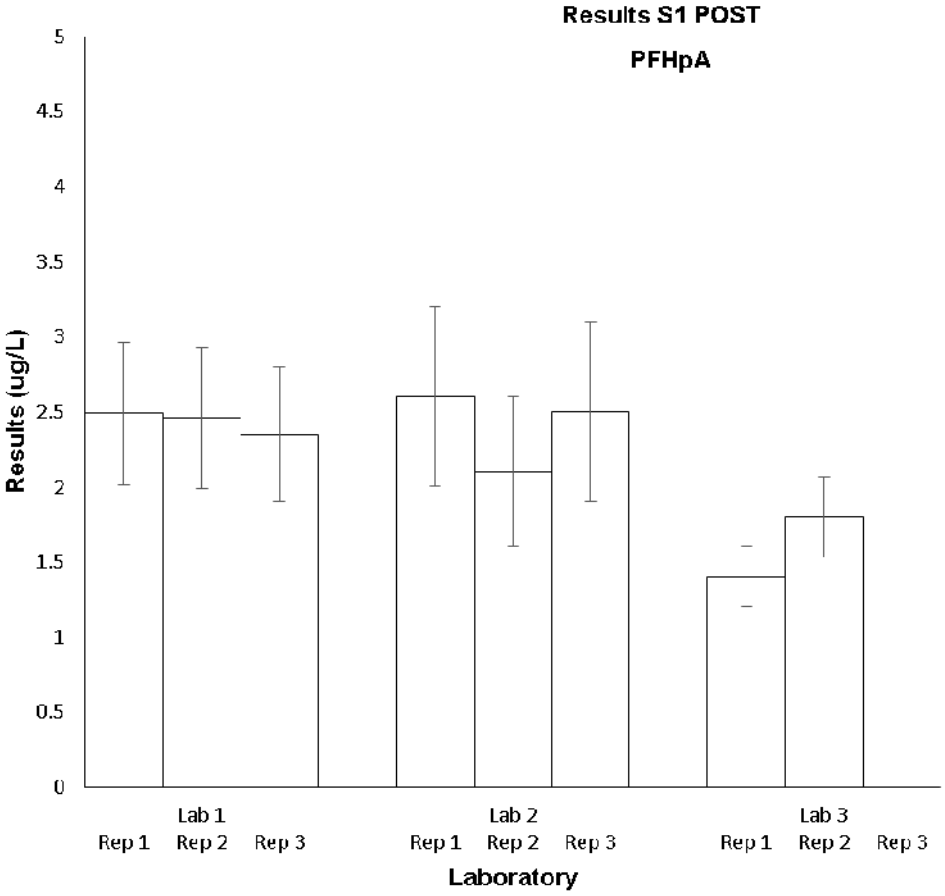

Figure 13

Table 16

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFOA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	15.89	4.736		26.3	4.9		26	3.9
2	19.15	5.707		21.1	4		37	5.6
3	15.7	4.679		38.6	7.3		NT	NT
Mean	16.9			28.7			32	
Within lab CV (%)	11			31			25	
Between labs CV (%)	30							

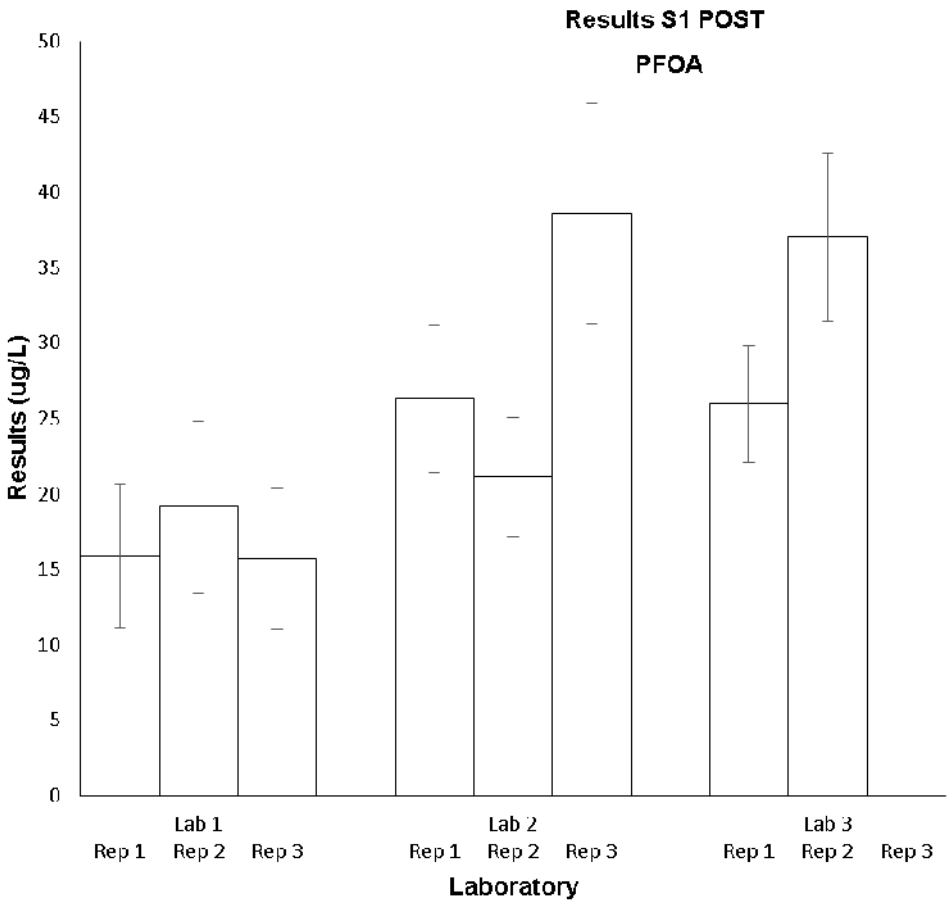

Figure 14

Table 17

Sample Details

Sample No.	S1 POST
Matrix.	MilliQ water, Tridol and PFOSA
Analyte.	PFOS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	1.8	0.382		<0.25	0.05		2.1	0.3
2	1.6	0.339		<0.25	0.05		3.3	0.5
3	2.1	0.445		<0.25	0.05		NT	NT
Mean	1.8			-			2.7	
Within lab CV (%)	14			-			31	
Between labs CV (%)	27							

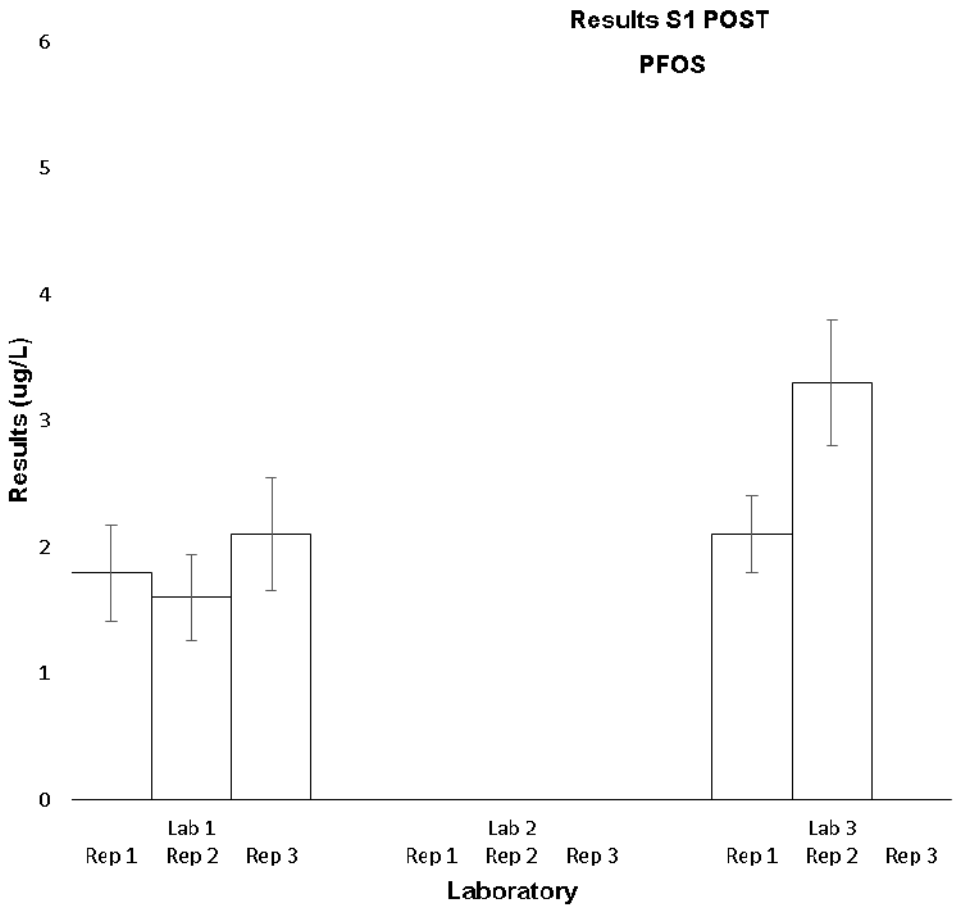

Figure 15

Table 18

Sample Details

Sample No.	S2 PRE
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFDA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	31.82	7		13.7	2.9		14	2.1
2	27.16	6.627		14.4	3.1		15	2.3
3	31.84	7.769		14.3	3.1		15	2.3
Mean	30.27			14.1			14.7	
Within lab CV (%)	8.9			2.7			3.9	
Between labs CV (%)	47							

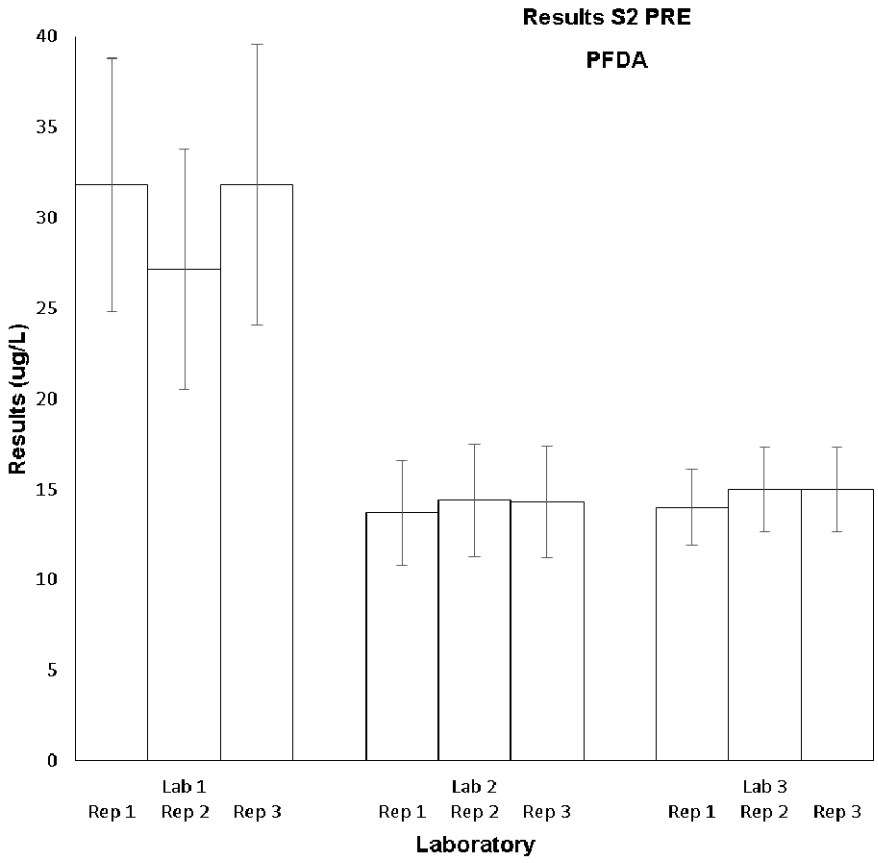

Figure 16

Table 19

Sample Details

Sample No.	S2 PRE
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFOS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	10.13	2.148		11.3	2.4		9.3	1.4
2	9.55	2.025		11.4	2.4		9.7	1.5
3	10.6	2.247		12.3	2.4		9.4	1.4
Mean	10.09			11.7			9.5	
Within lab CV (%)	5.2			4.7			2.2	
Between labs CV (%)	11							

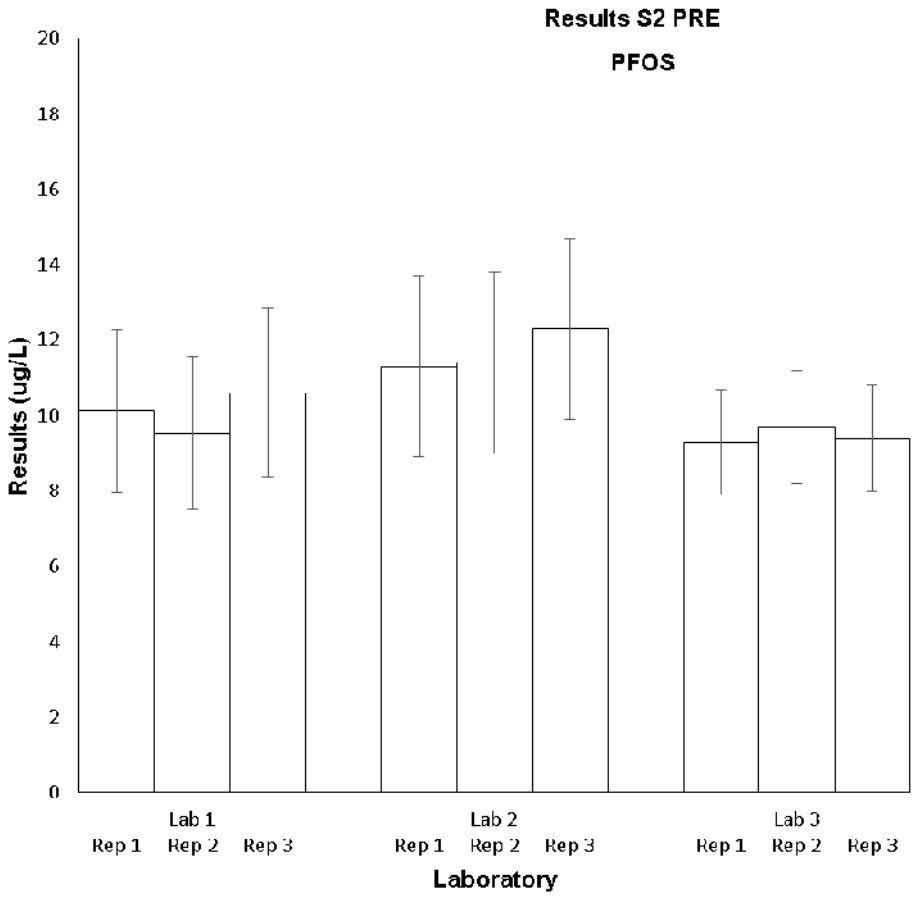

Figure 17

Table 20

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFBA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	4.247	1.07		3.1	0.5		5.3	0.8
2	3.799	0.957		3.1	0.5		5	0.75
3	3.967	1		3.7	0.7		4.6	0.7
Mean	4.00			3.3			5.0	
Within lab CV (%)	5.7			11			7.1	
Between labs CV (%)	20							

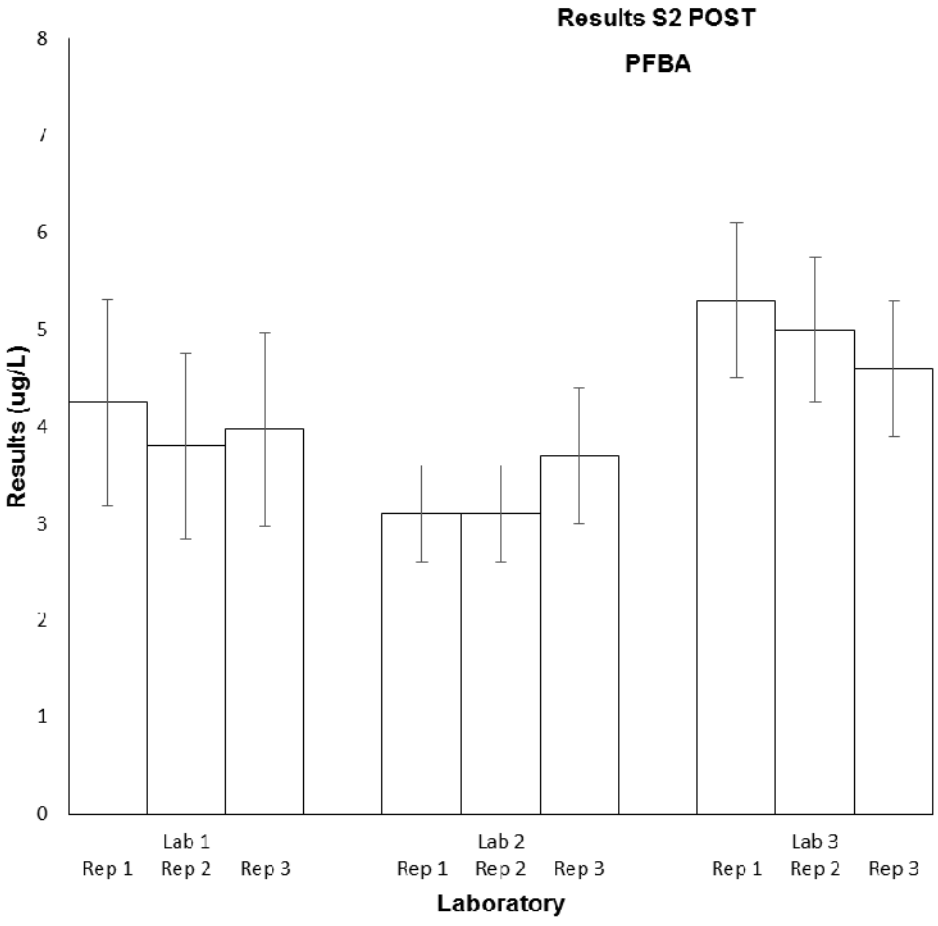

Figure 18

Table 21

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFPeA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	8.826	2.118		7.2	1.9		11	1.7
2	7.755	1.861		7.5	1.9		10	1.5
3	8.289	1.989		8.6	2.2		11	1.7
Mean	8.29			7.8			10.7	
Within lab CV (%)	6.5			9.5			5.4	
Between labs CV (%)	17							

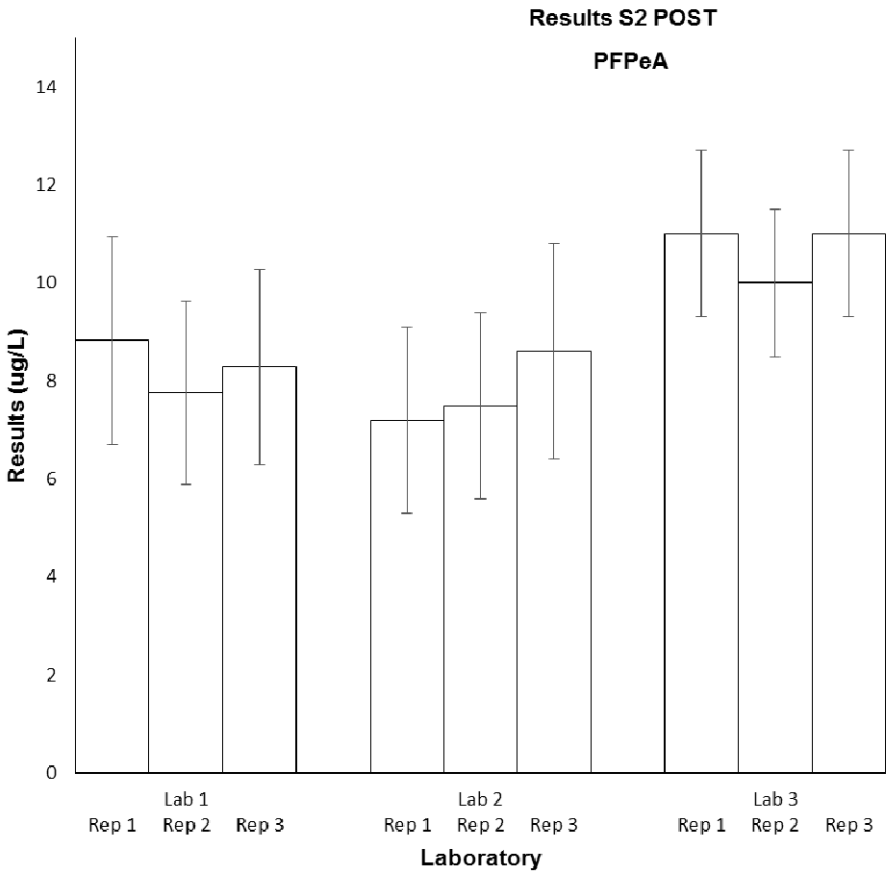

Figure 19

Table 22

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFHxA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	29.3	8.614		14.5	3.6		18	2.7
2	21.23	6.242		13.5	3.4		18	2.7
3	23.02	6.768		15.5	3.9		17	2.7
Mean	24.5			14.5			17.7	
Within lab CV (%)	17			6.9			3.3	
Between labs CV (%)	27							

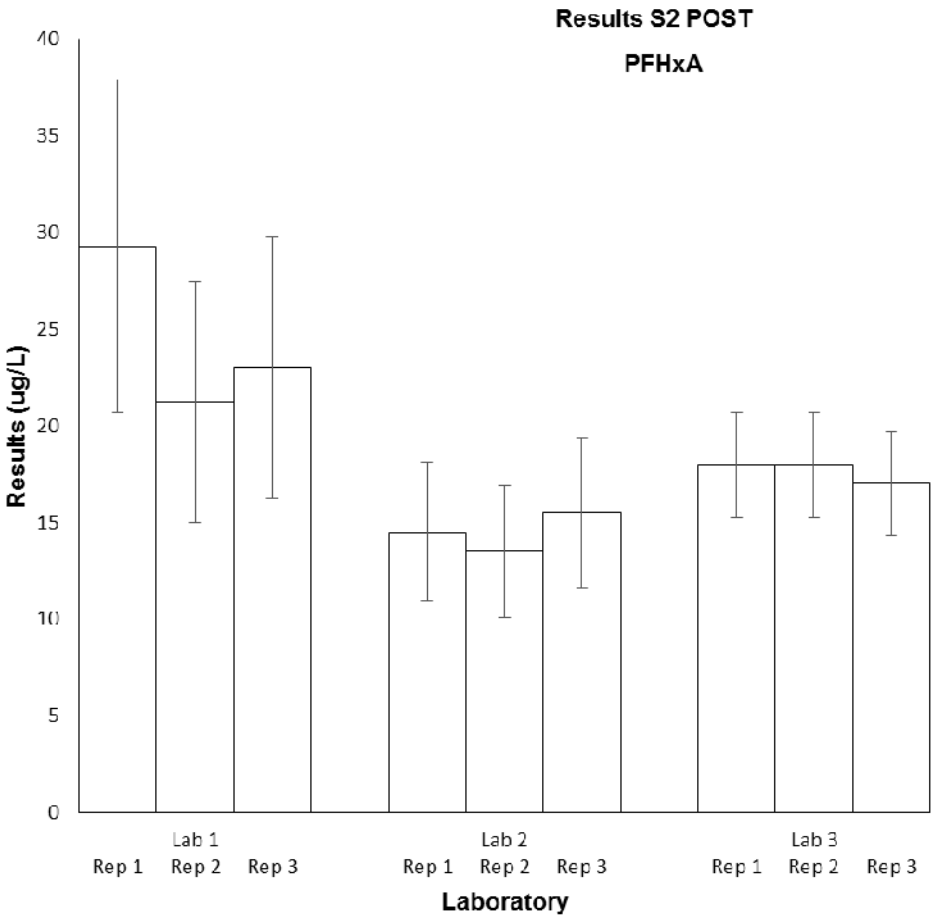

Figure 20

Table 23

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFHpA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	24.13	4.6		27.1	6.6		28	4.2
2	20.68	3.942		27.4	6.7		28	4.2
3	24.12	4.598		28.11	6.9		28	4.2
Mean	22.98			27.5			28	
Within lab CV (%)	9			1.9			0.0	
Between labs CV (%)	11							

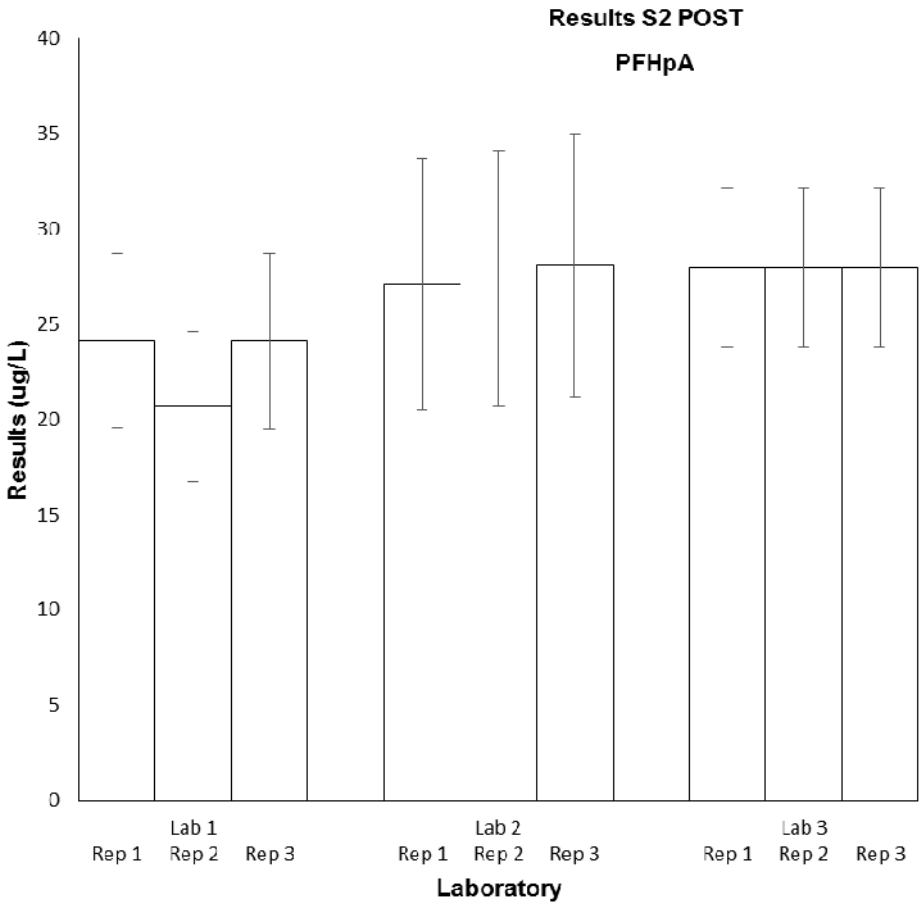

Figure 21

Table 24

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFOA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	15.68	4.673		11.9	2.2		14	2.1
2	15.57	4.64		10.5	2		15	2.2
3	16.44	4.899		12	2.3		15	2.2
Mean	15.90			11.5			14.7	
Within lab CV (%)	3.0			7.3			3.9	
Between labs CV (%)	16							

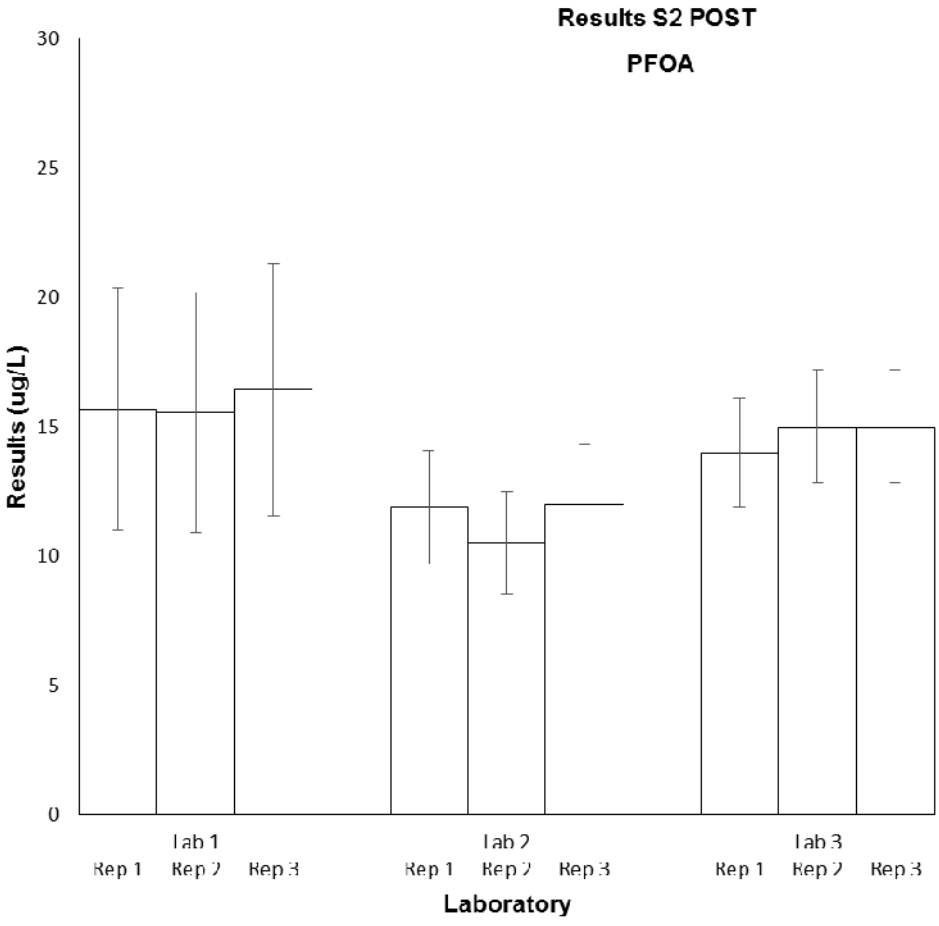

Figure 22

Table 25

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFNA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	6.105	1.392		2.1	0.4		4.6	0.7
2	5.949	1.357		1.9	0.3		4.7	0.7
3	6.166	1.406		2.2	0.4		4.7	0.7
Mean	6.073			2.1			4.7	
Within lab CV (%)	1.8			7.4			1.2	
Between labs CV (%)	48							

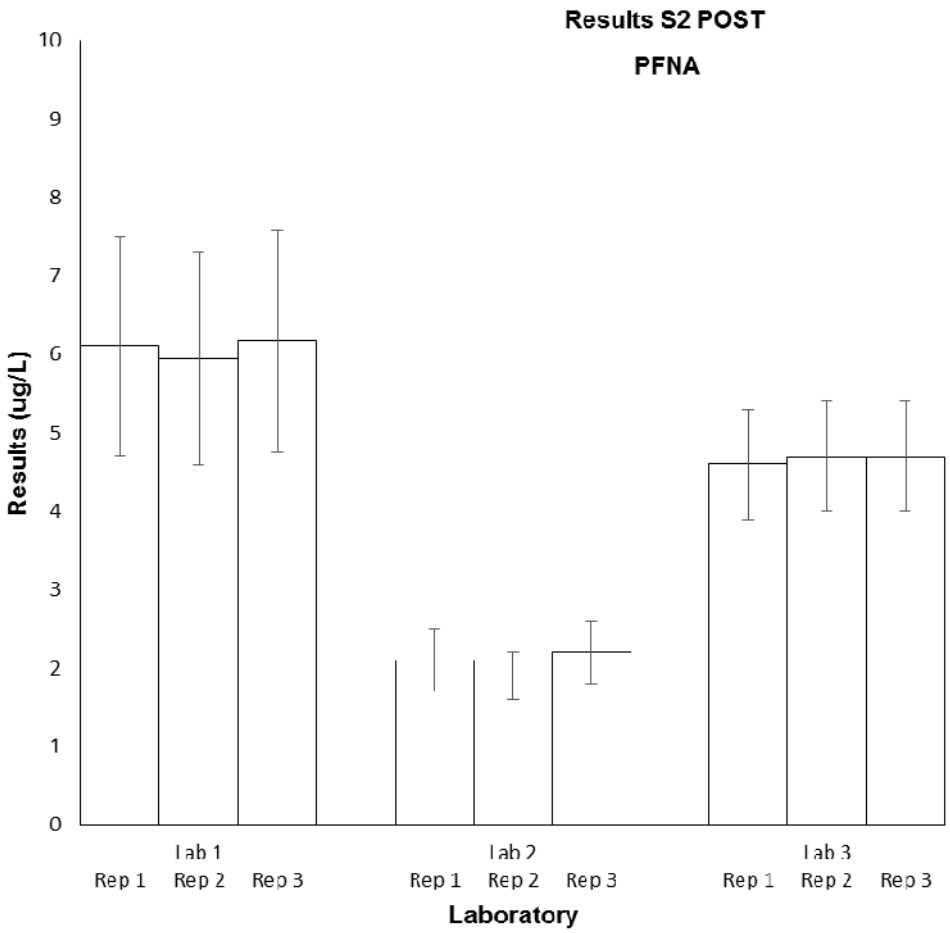

Figure 23

Table 26

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFDA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	12.64	3.085		13.1	2.8		13	1.9
2	12.14	2.963		11.4	2.4		15	2.3
3	13.21	3.224		12.9	2.7		14	2.2
Mean	12.66			12.5			14	
Within lab CV (%)	4.2			7.5			7.1	
Between labs CV (%)	6.0							

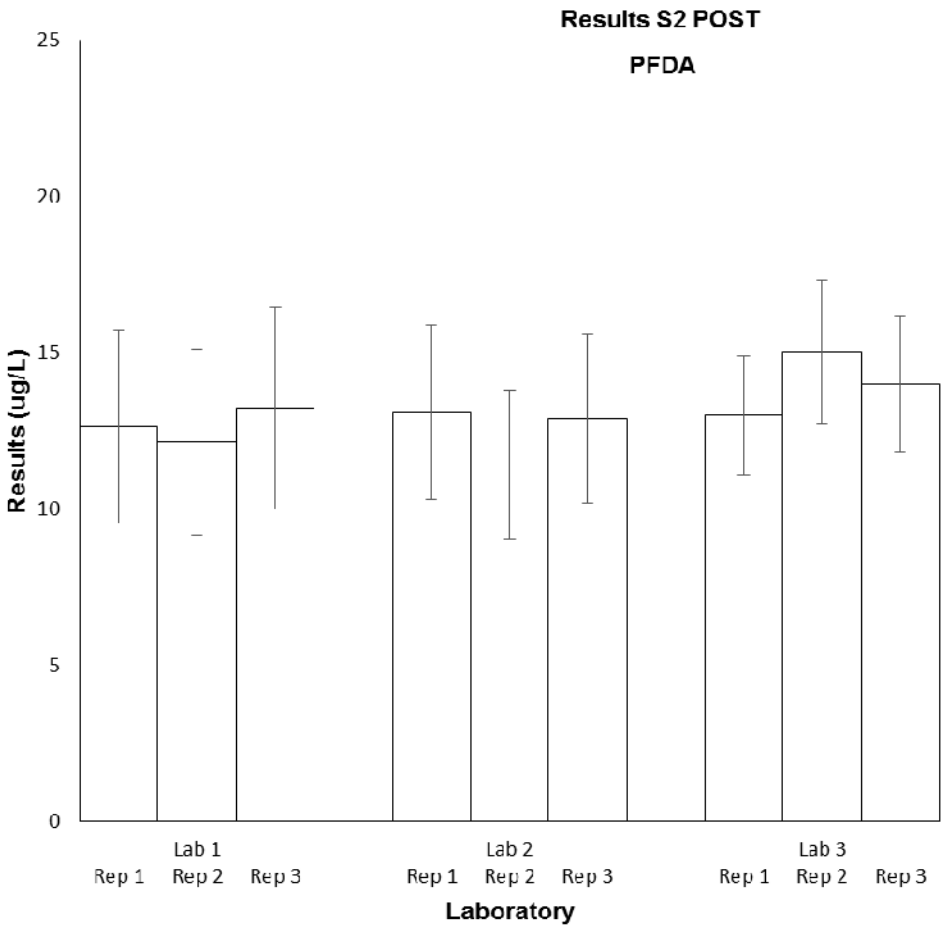

Figure 24

Table 27

Sample Details

Sample No.	S2 POST
Matrix.	MilliQ water, 8:2 monoPAP, PFDA and PFOS
Analyte.	PFOS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	10.62	2.251		9.5	2		9.2	1.4
2	10.37	2.198		9.8	2.1		10	1.5
3	10.25	2.173		9.8	2.1		10	1.5
Mean	10.41			9.7			9.7	
Within lab CV (%)	1.8			1.8			4.7	
Between labs CV (%)	4.0							

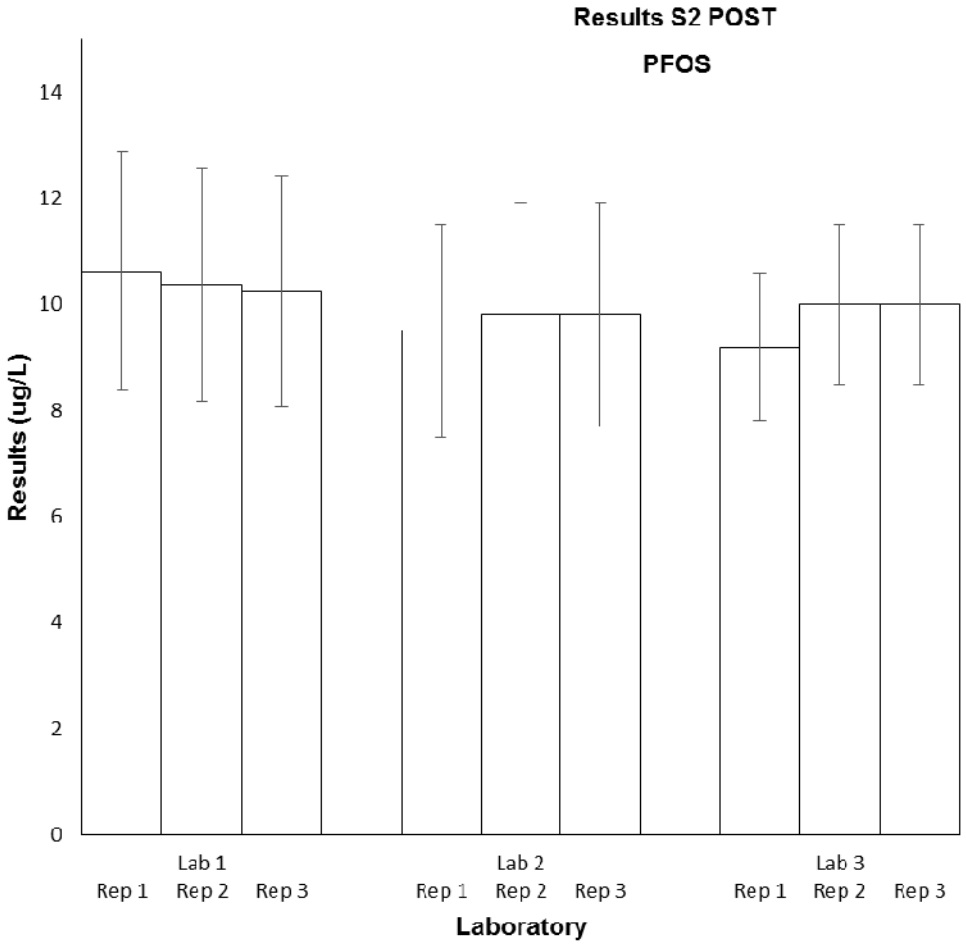

Figure 25

Table 28

Sample Details

Sample No.	S3 PRE
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	6:2 FTS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	1.416	0.6		0.84	0.2		0.87	0.12
2	1.385	0.587		1.1	0.262		0.87	0.12
3	1.276	0.541		0.86	0.204		0.97	0.14
Mean	1.359			0.93			0.90	
Within lab CV (%)	5.4			16			6.4	
Between labs CV (%)	24							

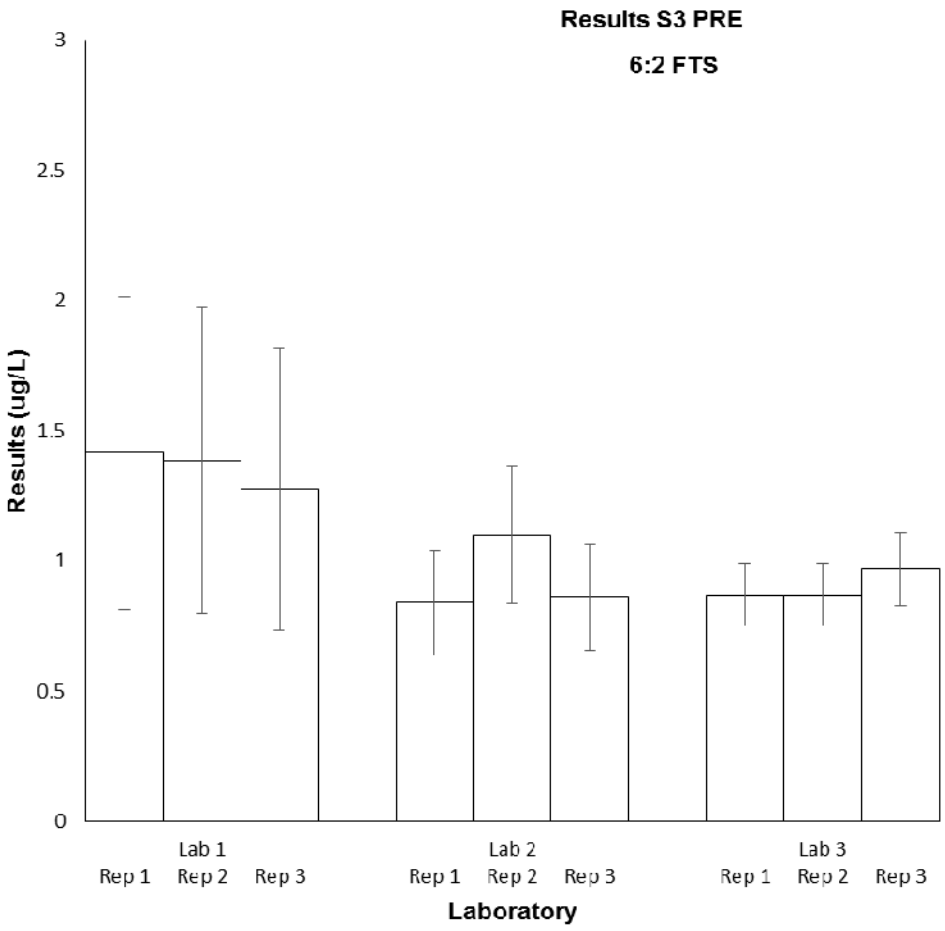

Figure 26

Table 29

Sample Details

Sample No.	S3 PRE
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFOSA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	57.087	5.732		33	7.9		48.8	6.8
2	66.048	6.631		30	7.2		53.3	7.5
3	54.53	5.475		39	9.4		52.6	7.4
Mean	59.22			34			51.6	
Within lab CV (%)	10			13			4.7	
Between labs CV (%)	27							

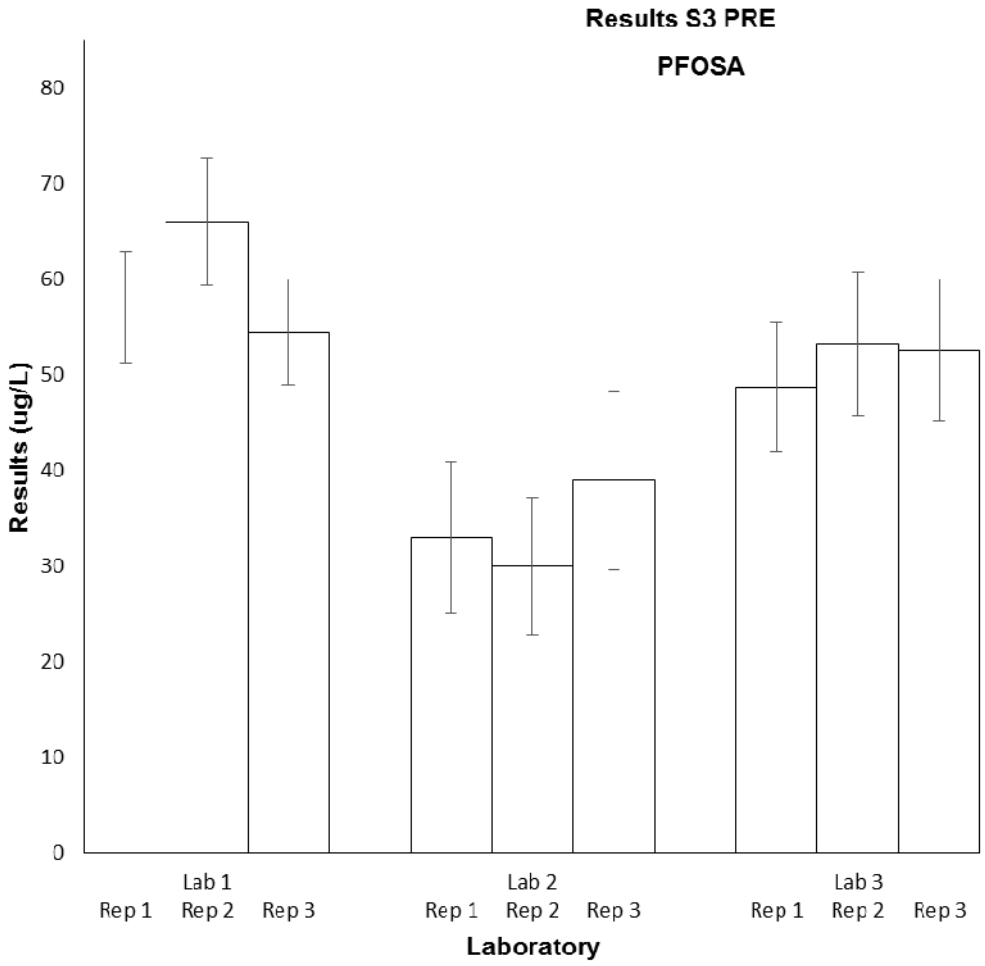

Figure 27

Table 30

Sample Details

Sample No.	S3 PRE
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFDA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	13.918	5.929		12	2.97		11.1	1.55
2	15.181	6.467		11	2.64		10.9	1.53
3	17.49	7.451		12	2.97		10.2	1.43
Mean	15.53			11.7			10.7	
Within lab CV (%)	12			4.9			4.4	
Between labs CV (%)	20							

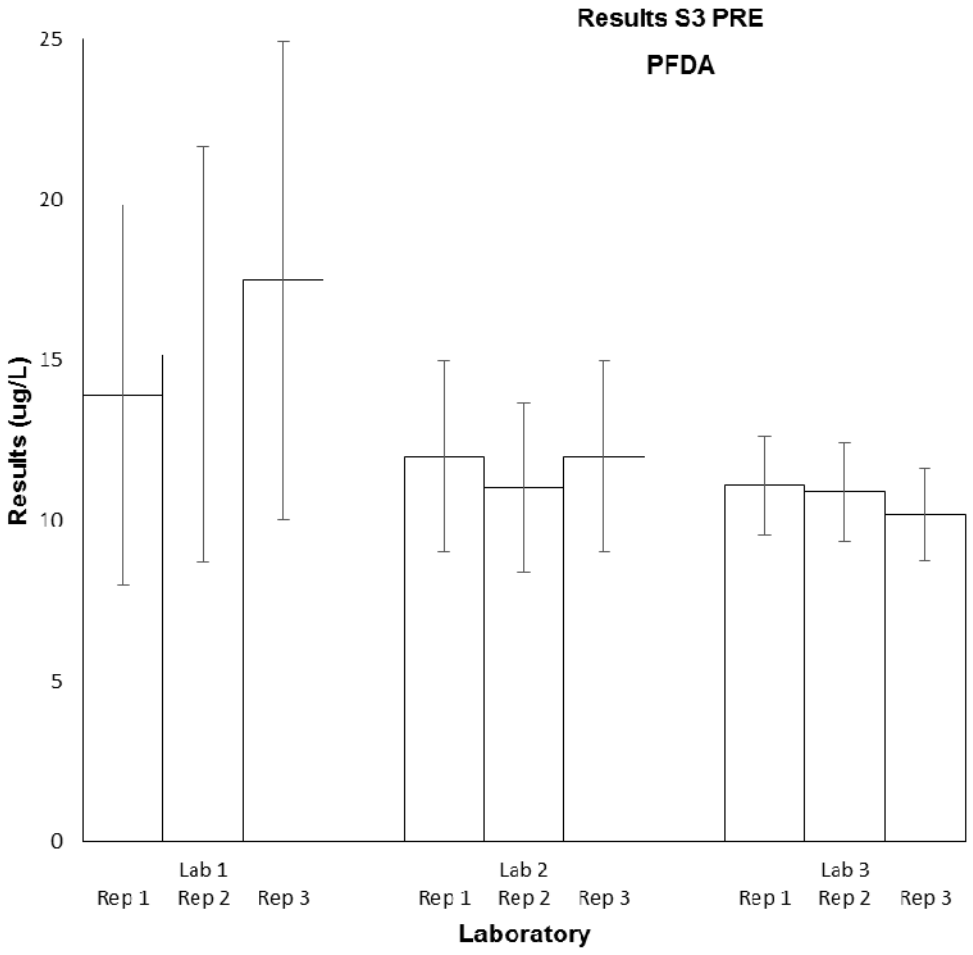

Figure 28

Table 31

Sample Details

Sample No.	S3 PRE
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFHxS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	14.556	6.026		9.5	2.61		9.01	1.3
2	14.398	5.961		10	2.78		8.95	1.3
3	14.115	5.844		8.9	2.47		9.75	1.4
Mean	14.356			9.5			9.24	
Within lab CV (%)	1.6			5.8			4.8	
Between labs CV (%)	26							

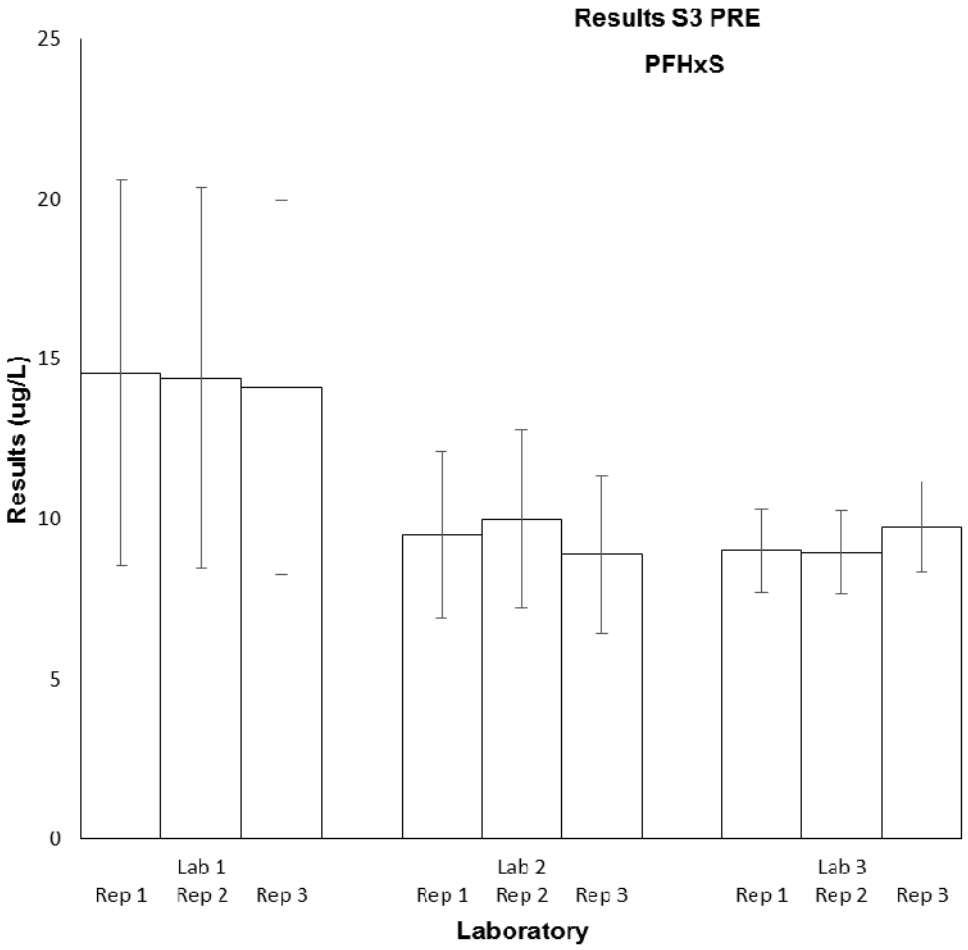

Figure 29

Table 32

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	6:2 FTS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	2.943	1.419		<0.025	0.6		0.032	0.004
2	3.36	1.62		<0.025	0.006		0.04	0.006
3	3.823	1.843		<0.025	0.006		0.054	0.008
Mean	3.38			-			0.042	
Within lab CV (%)	13			-			27	
Between labs CV (%)	138							

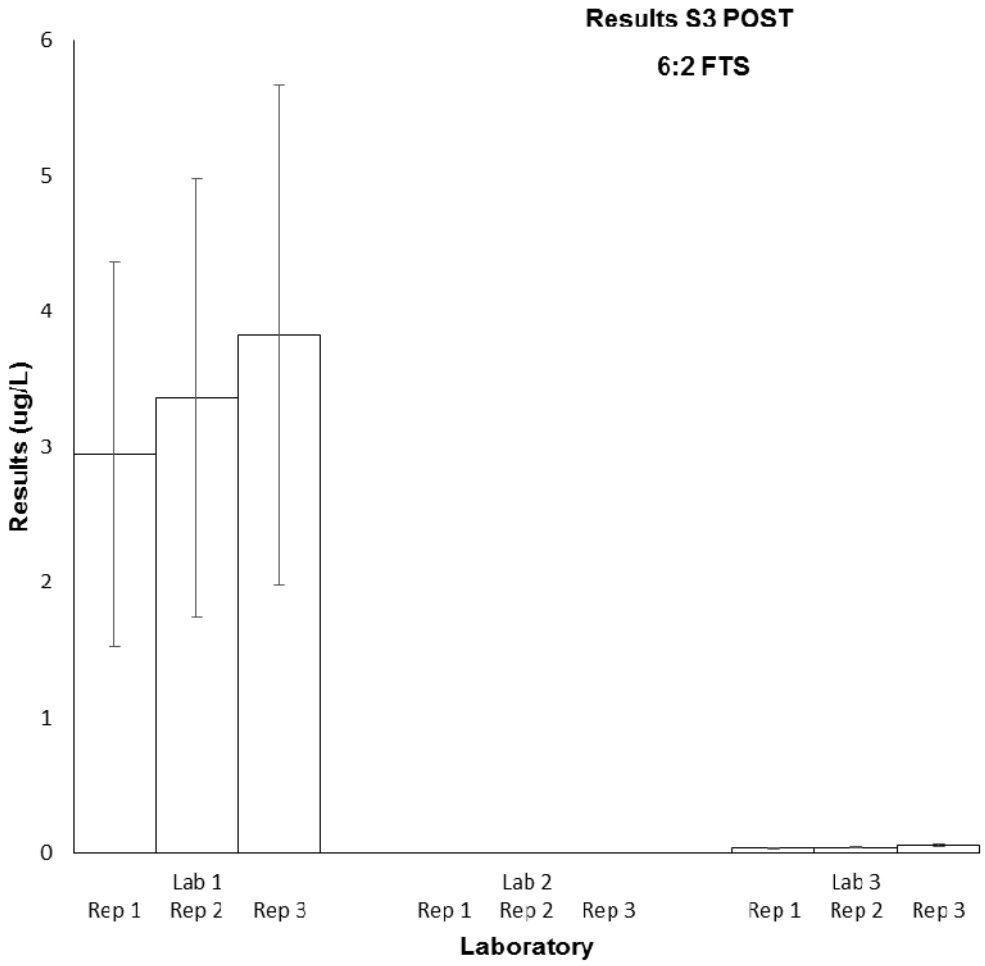

Figure 30

Table 33

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFBA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	8.156	6.215		9	2.1		9.16	1.3
2	7.363	5.611		10	2.4		8.63	1.2
3	7.393	5.633		9.2	2.2		9.37	1.3
Mean	7.637			9.4			9.05	
Within lab CV (%)	5.9			5.6			4.2	
Between labs CV (%)	11							

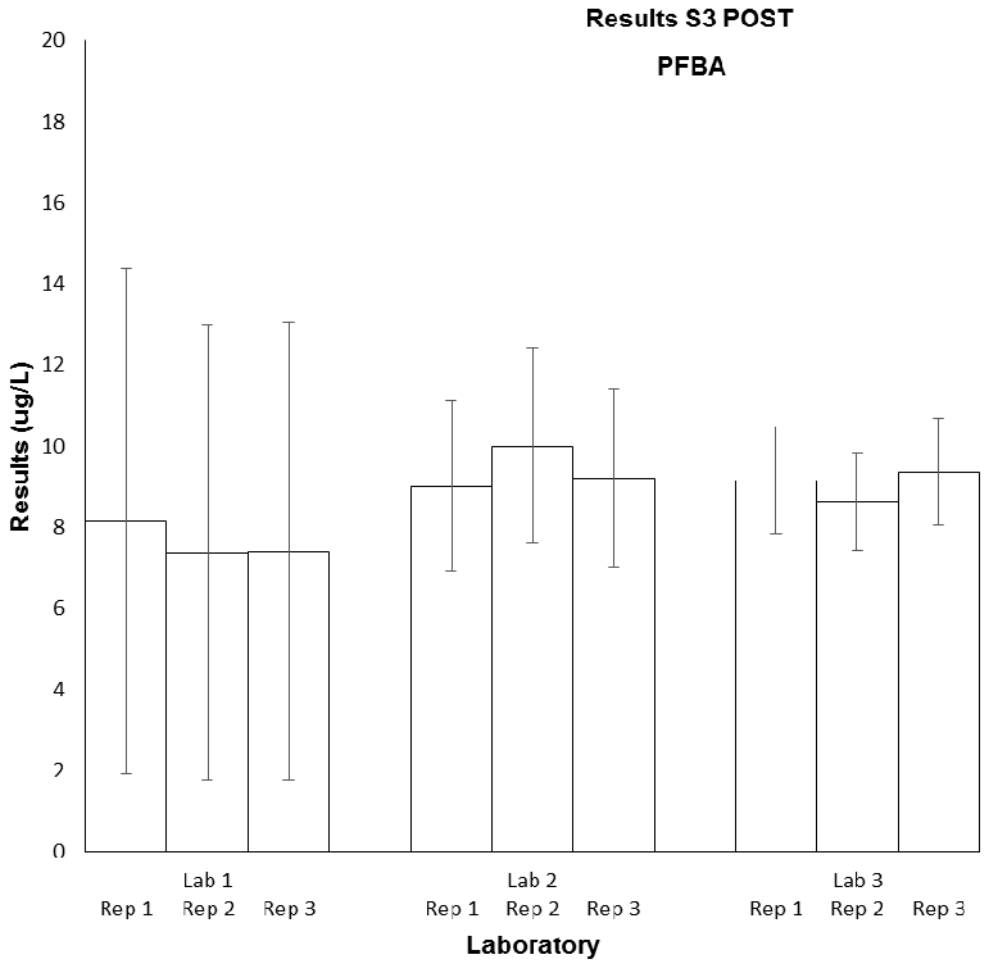

Figure 31

Table 34

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFPeA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	14.71	7.149		19	4.6		15.9	2.2
2	15.585	7.574		20	4.8		15.3	2.1
3	15.242	7.408		17	4.1		17.3	2.4
Mean	15.18			18.7			16.2	
Within lab CV (%)	2.9			8.2			6.3	
Between labs CV (%)	11							

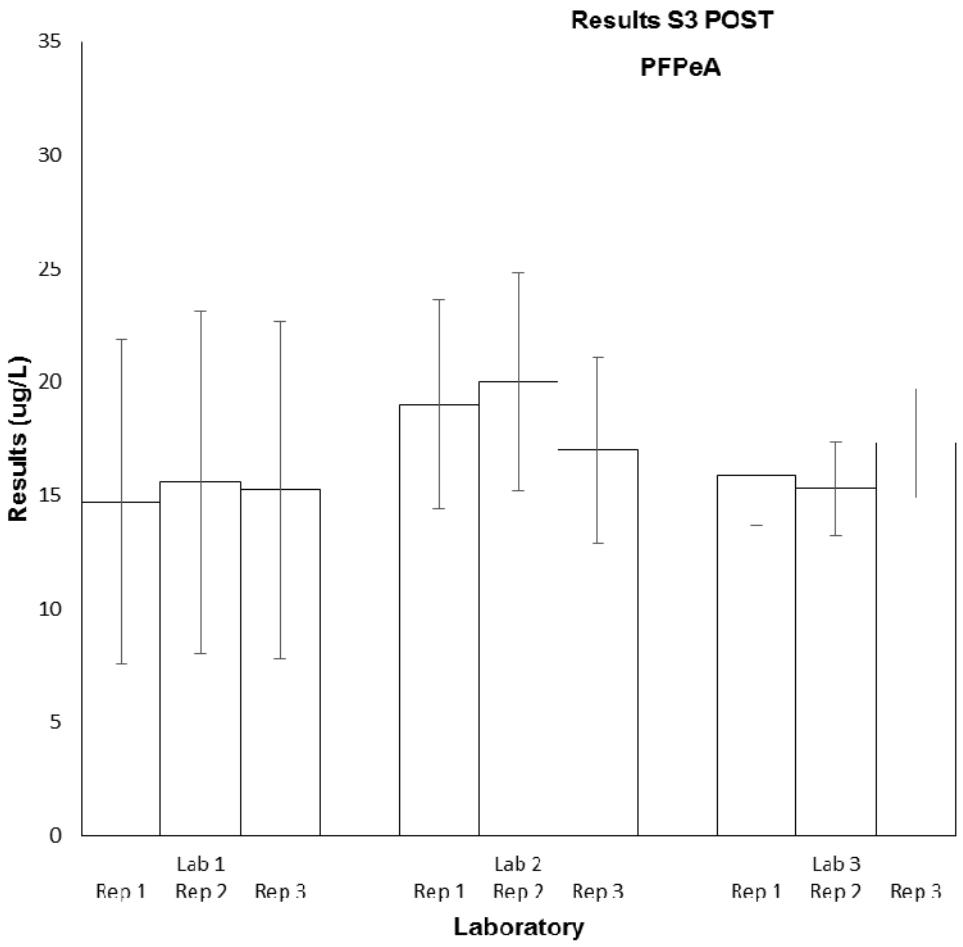

Figure 32

Table 35

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFHxA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	5.973	3.225		4.6	1.1		5.95	0.8
2	5.893	3.182		5.9	1.4		5.73	0.8
3	6.46	3.488		6.3	1.5		6.52	0.9
Mean	6.11			5.6			6.07	
Within lab CV (%)	5.0			16			6.7	
Between labs CV (%)	4.8							

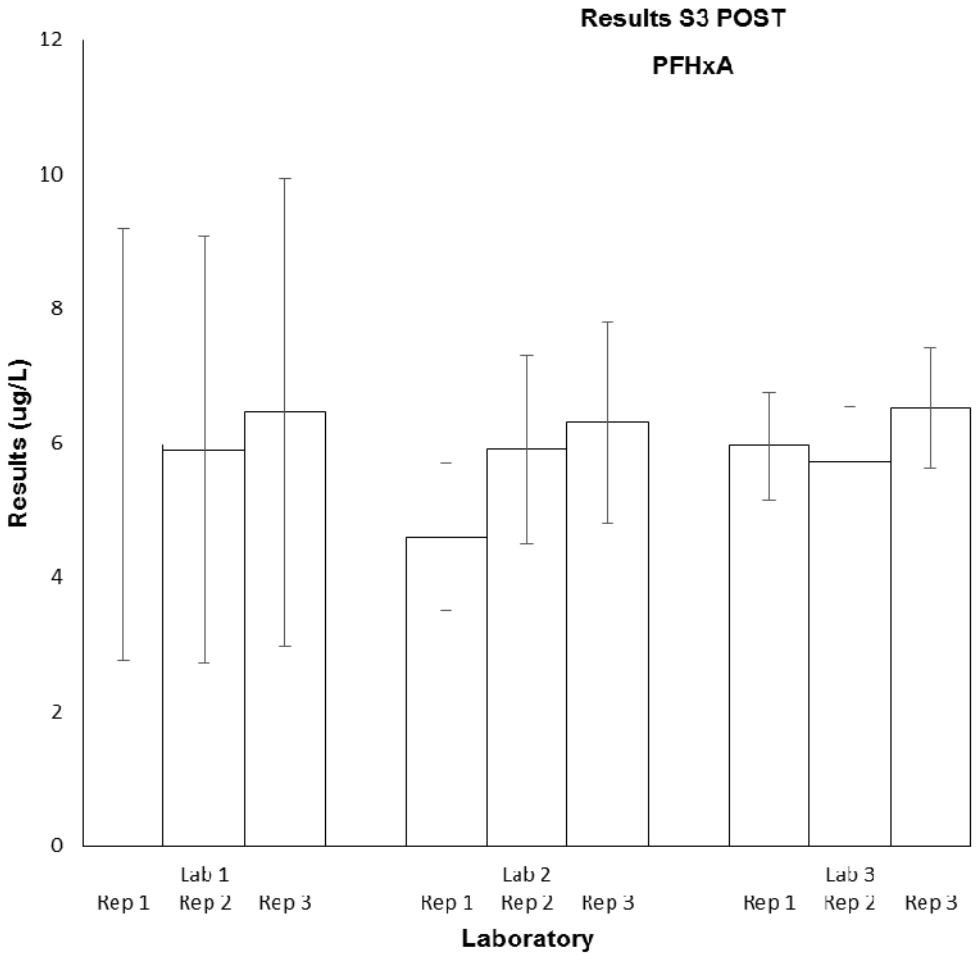

Figure 33

Table 36

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFHpA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	1.078	0.677		1	0.25		1.87	0.26
2	1.068	0.671		1.1	0.27		2.11	0.29
3	1.166	0.732		1.1	0.27		1.67	0.23
Mean	1.104			1.1			1.88	
Within lab CV (%)	4.9			5.4			12	
Between labs CV (%)	34							

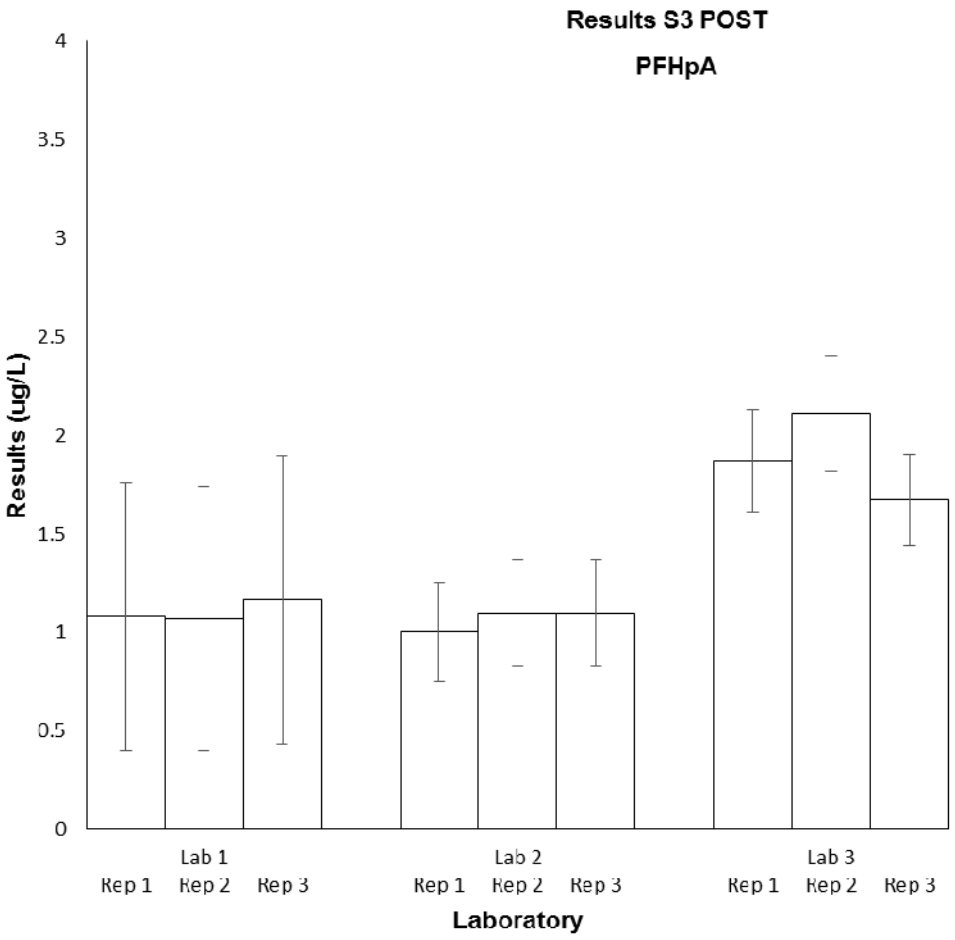

Figure 34

Table 37

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFOSA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	0.679	0.068		<0.05	0.6		0.029	0.004
2	1.37	0.138		<0.05	0.012		0.057	0.008
3	1.557	0.156		<0.05	0.012		0.042	0.006
Mean	1.20			-			0.043	
Within lab CV (%)	38			-			33	
Between labs CV (%)	132							

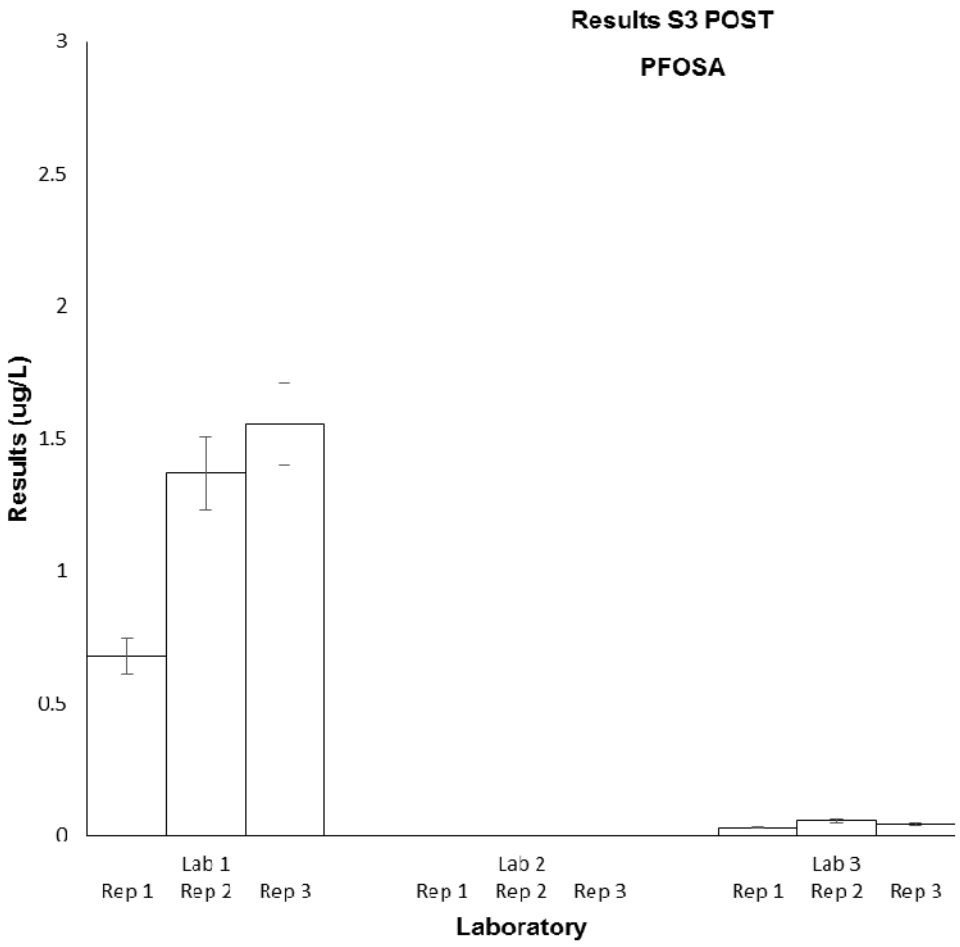

Figure 35

Table 38

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFOA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	13.81	8.88		25	6		40.9	5.7
2	14.01	9.008		23	5.8		41.3	5.8
3	14.17	9.111		29	6.9		39.6	5.6
Mean	14.00			26			40.6	
Within lab CV (%)	1.3			12			2.2	
Between labs CV (%)	50							

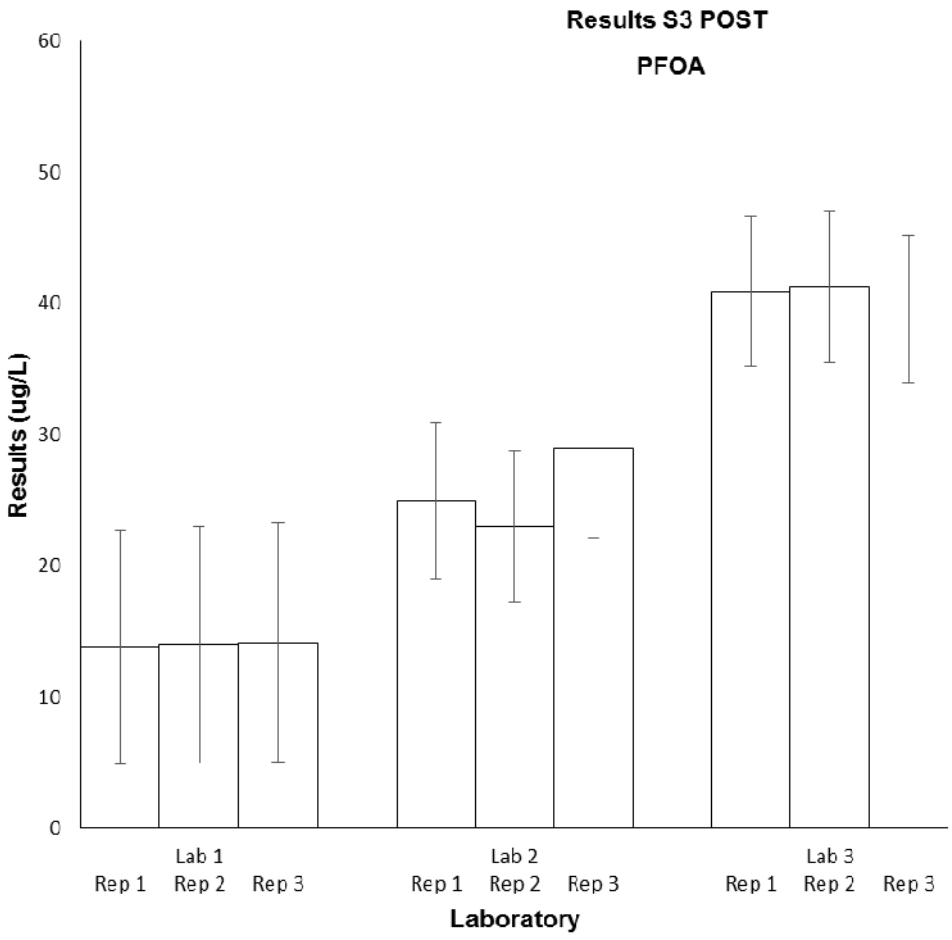

Figure 36

Table 39

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFOS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	0.691	0.285		0.18	0.05		0.828	0.124
2	0.89	0.367		0.15	0.04		1.009	0.151
3	0.865	0.356		0.18	0.05		0.989	0.148
Mean	0.815			0.17			0.942	
Within lab CV (%)	13			10			11	
Between labs CV (%)	64							

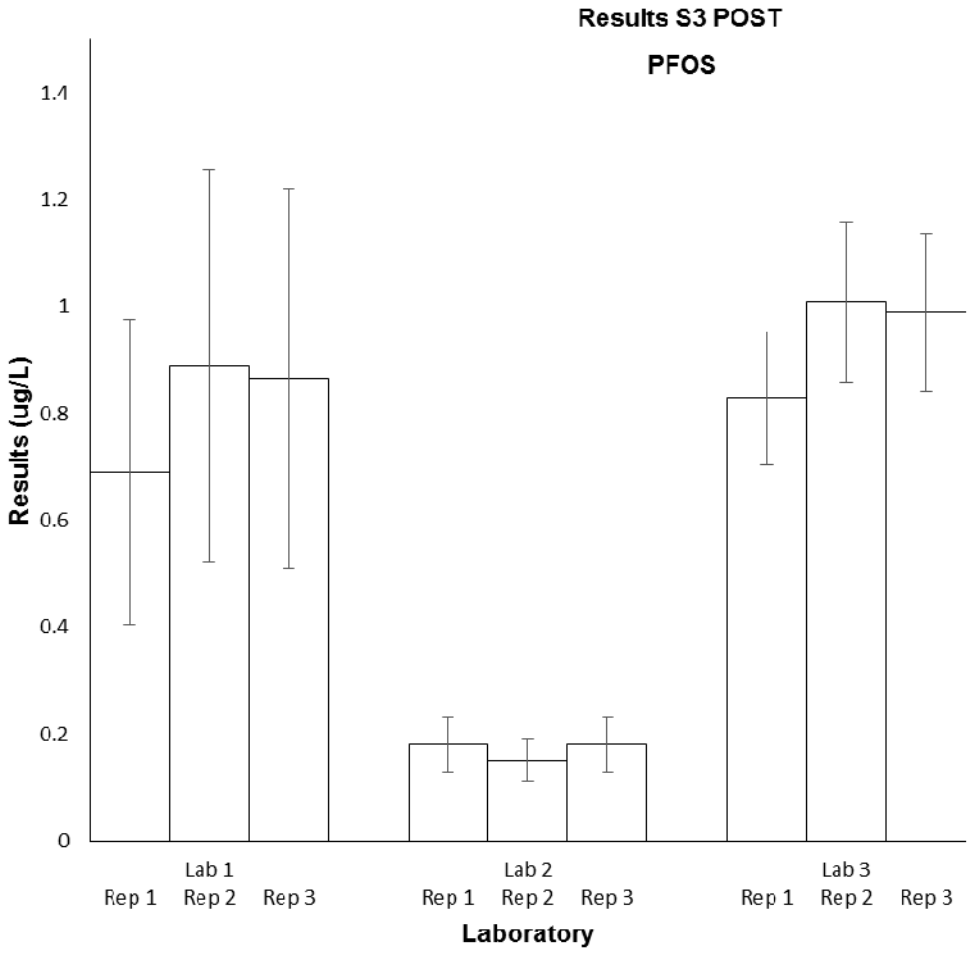

Figure 37

Table 40

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFDA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	8.38	6.369		12	2.9		10.1	1.4
2	10.86	8.254		9.5	2.4		9.89	1.4
3	11.02	8.375		14	3.4		9.91	1.4
Mean	10.09			11.8			9.97	
Within lab CV (%)	15			19			1.2	
Between labs CV (%)	9.8							

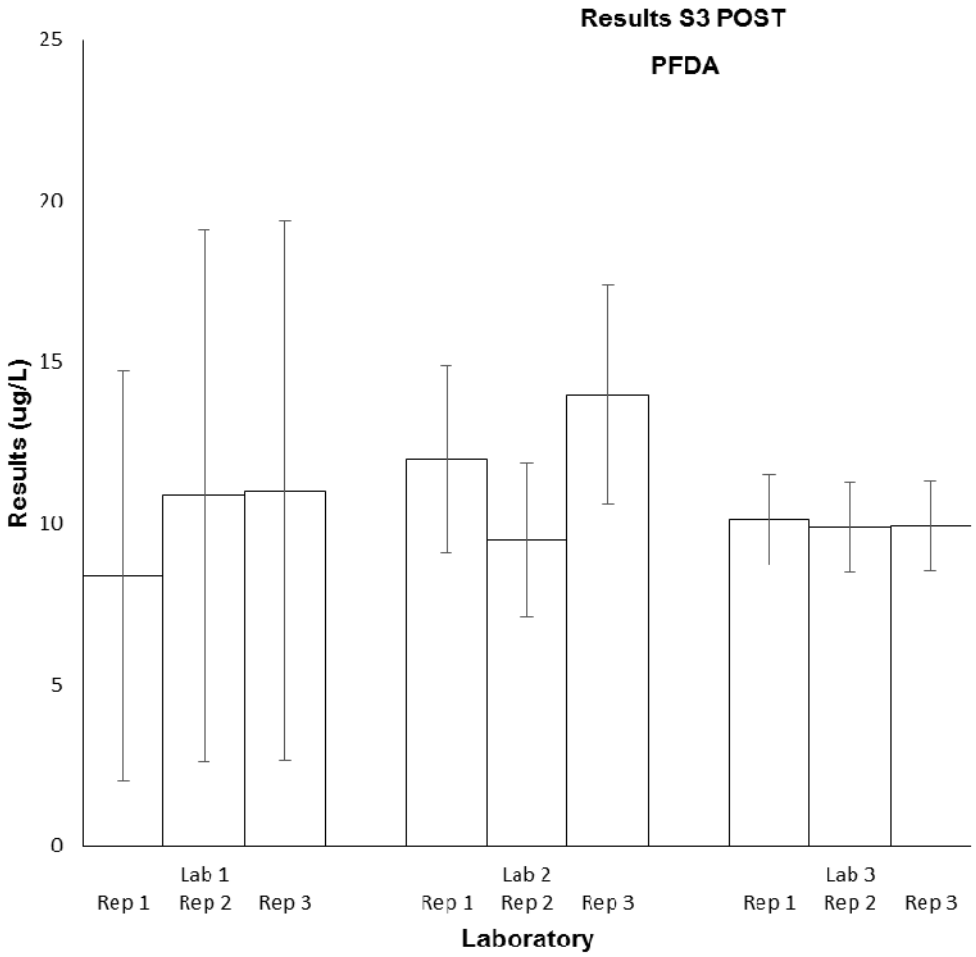

Figure 38

Table 41

Sample Details

Sample No.	S3 POST
Matrix.	MilliQ water, Tridol, PFDA and PFHxS
Analyte.	PFHxS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	10.757	5.422		10	2.8		9.37	1.3
2	11.39	5.741		11	3.1		9.81	1.4
3	11.52	5.806		9.4	2.7		9.55	1.3
Mean	11.22			10.1			9.58	
Within lab CV (%)	3.6			8.0			2.3	
Between labs CV (%)	8.1							

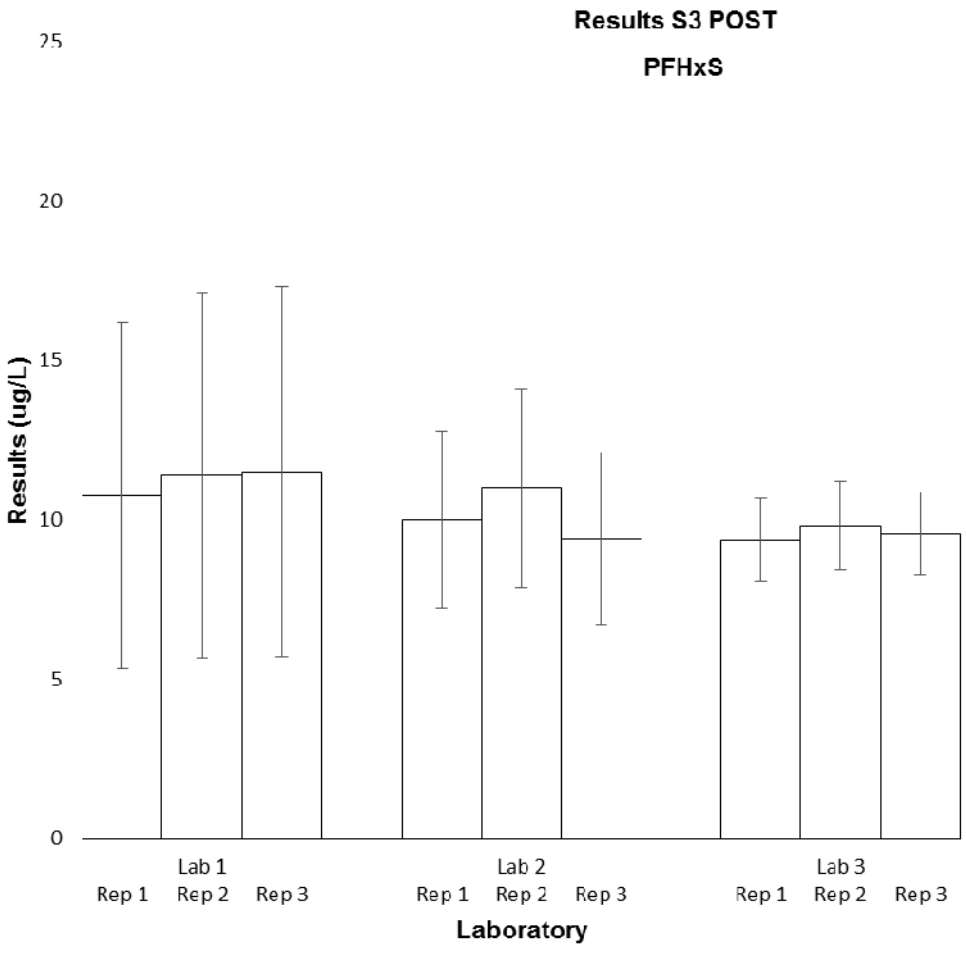

Figure 39

Table 42

Sample Details

Sample No.	S4 PRE
Matrix.	Worm juice, Tridol, PFDA and PFHxS
Analyte.	6:2 FTS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	2.532	1.074		2.5	0.6		2.06	0.29
2	2.614	1.108		2.4	0.58		2.12	0.29
3	2.339	0.992		1.7	0.41		1.97	0.27
Mean	2.495			2.2			2.05	
Within lab CV (%)	5.7			20			3.7	
Between labs CV (%)	10							

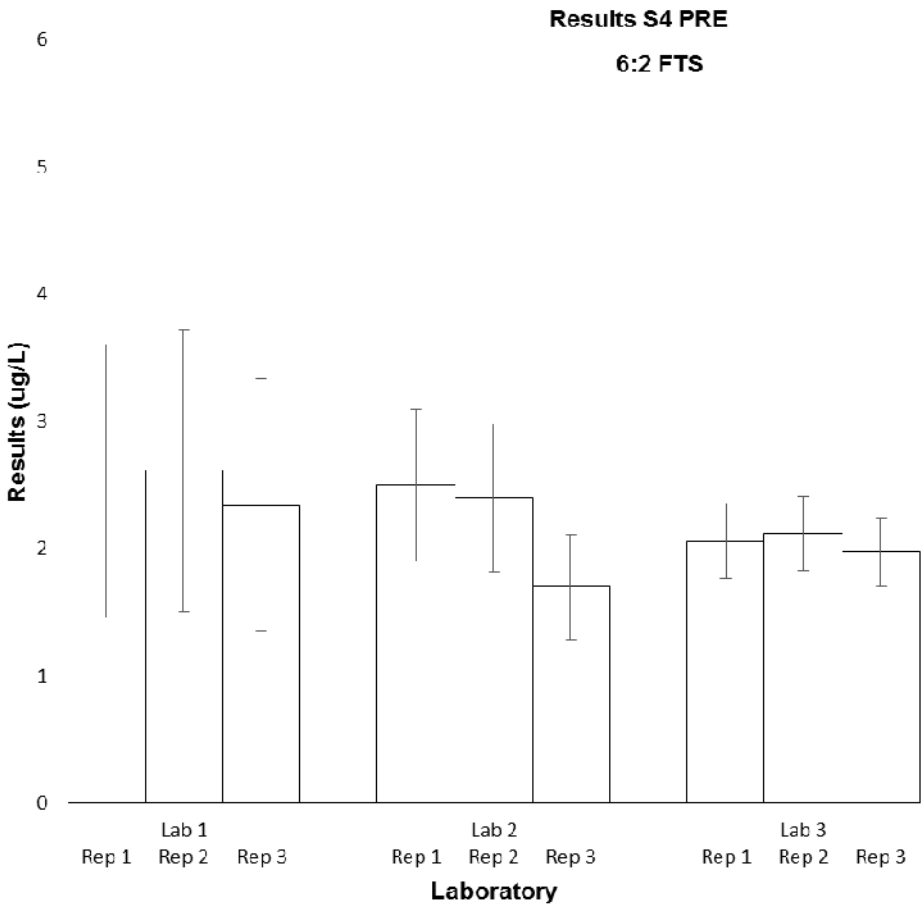

Figure 40

Table 43

Sample Details

Sample No.	S4 PRE
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFOSA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	219.3	22.018		130	31		92.2	12.9
2	235.5	23.644		107	26		100	14
3	186.2	18.694		137	33		98.3	13.7
Mean	213.7			125			96.8	
Within lab CV (%)	12			13			4.2	
Between labs CV (%)	42							

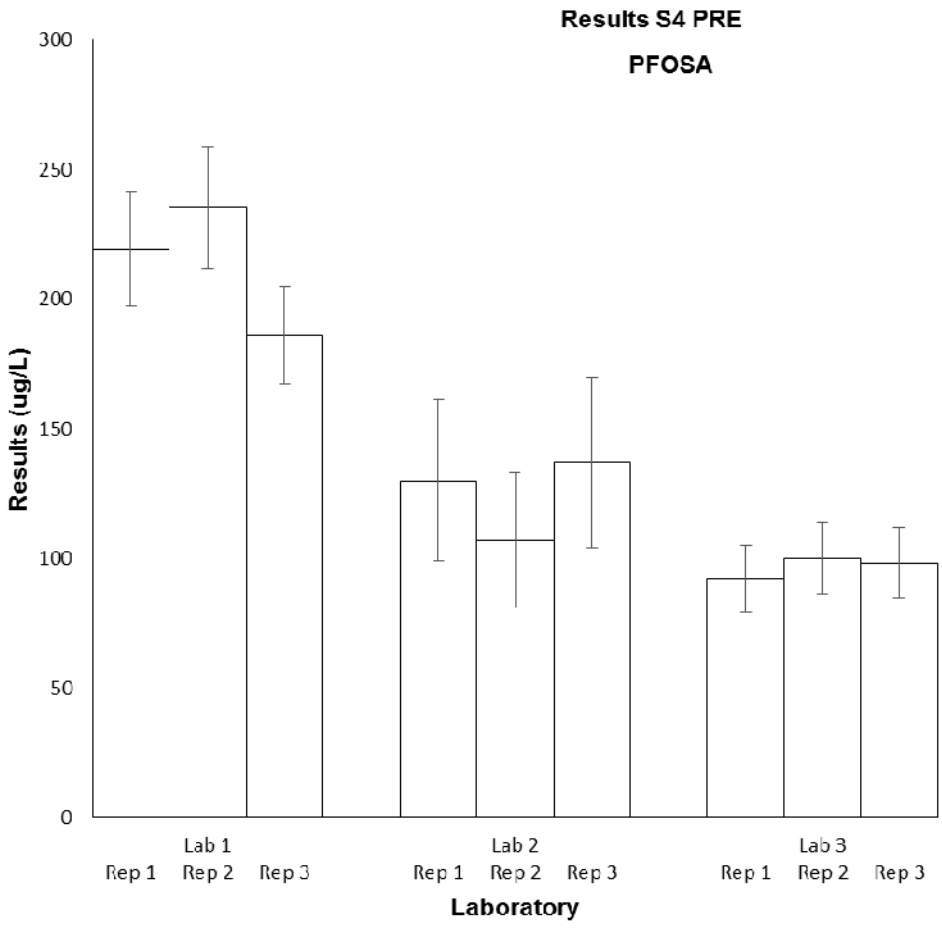

Figure 41

Table 44

Sample Details

Sample No.	S4 PRE
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFDA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	18.317	7.803		13	3.2		12.1	1.7
2	15.717	6.695		12	3		13.4	1.9
3	19.775	8.424		13	3.2		14	1.9
Mean	17.936			12.7			13.2	
Within lab CV (%)	11			4.6			7.4	
Between labs CV (%)	20							

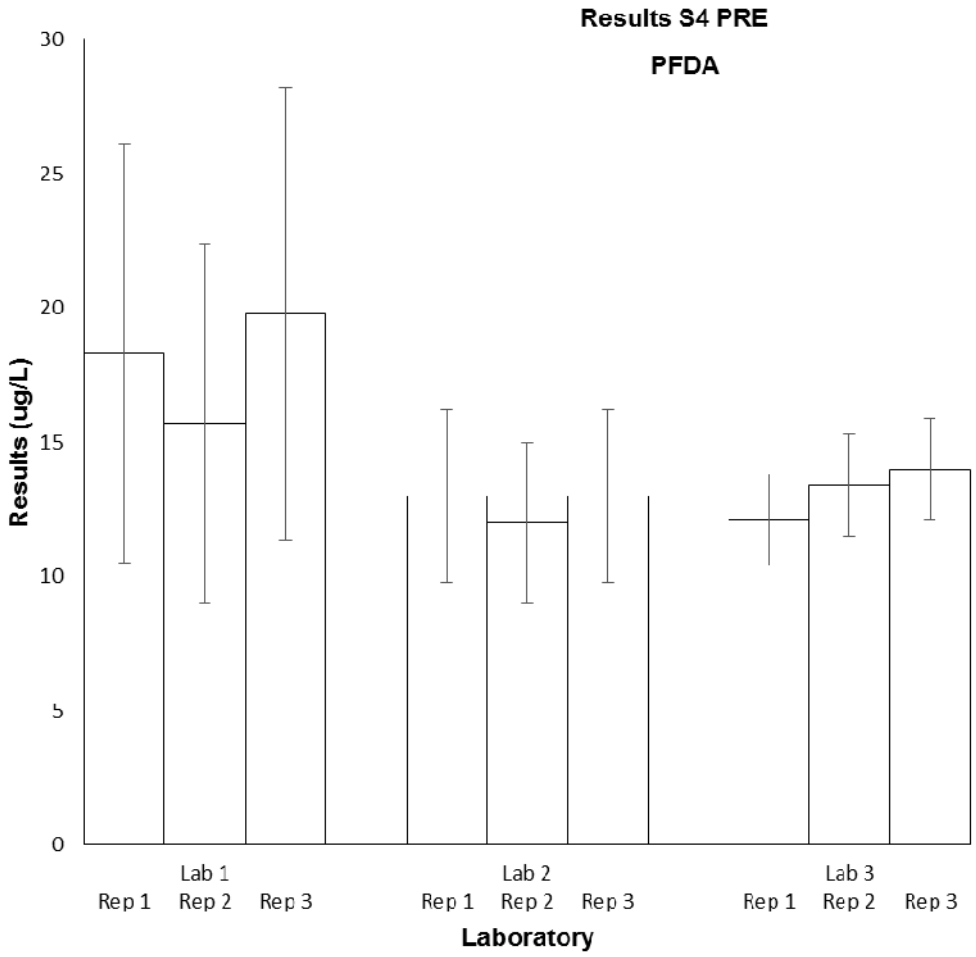

Figure 42

Table 45

Sample Details

Sample No.	S4 PRE
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFHxS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	12.01	4.972		9.9	2.7		9.4	1.32
2	12.56	5.2		12	3.3		9.57	1.32
3	12.28	5.084		9.3	2.6		8.96	1.25
Mean	12.28			10.4			9.31	
Within lab CV (%)	2.2			14			3.4	
Between labs CV (%)	14							

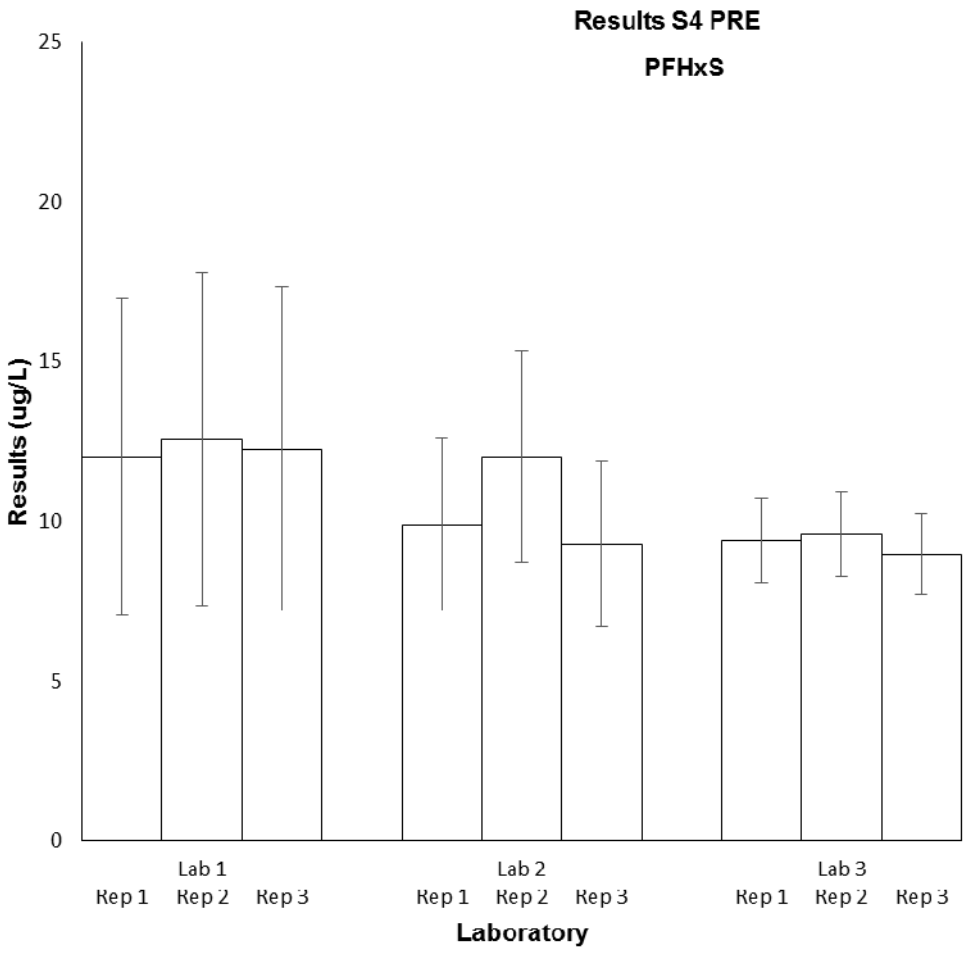

Figure 43

Table 46

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	6:2 FTS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	1.99	0.959		<0.025	0.6		0.071	0.0099
2	1.83	0.882		<0.025	0.006		0.043	0.006
3	2.3	1.109		<0.025	0.006		0.081	0.011
Mean	2.04			-			0.065	
Within lab CV (%)	12			-			30	
Between labs CV (%)	133							

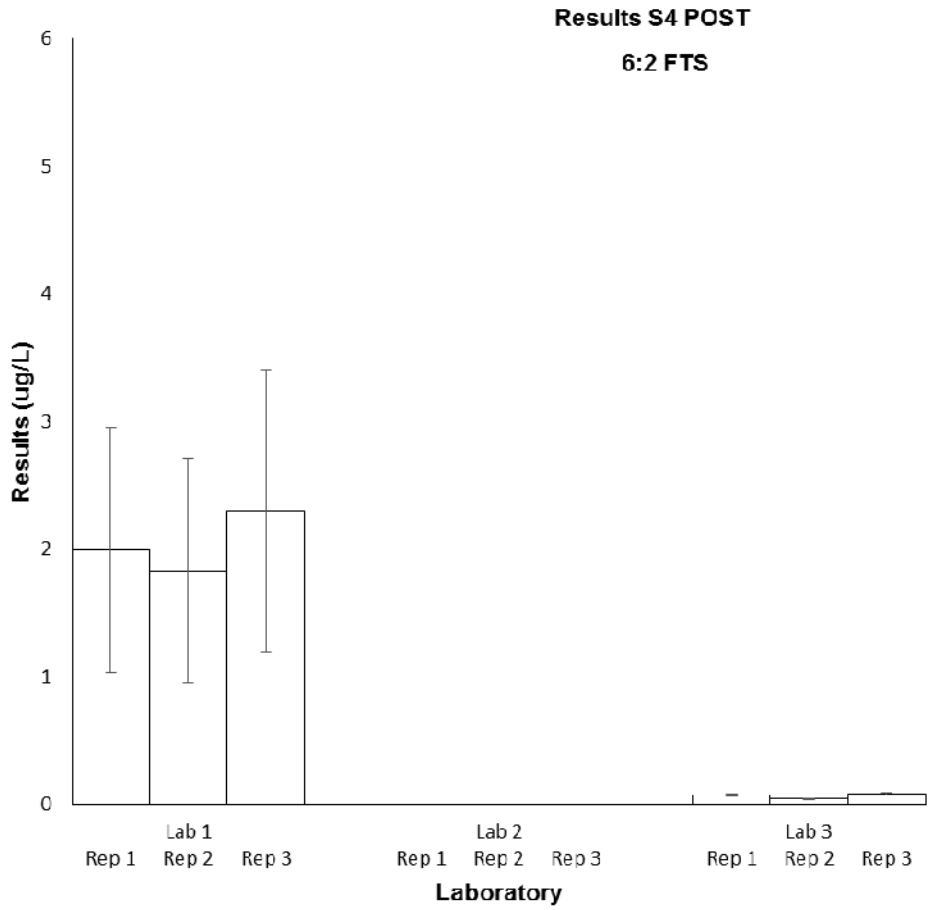

Figure 44

Table 47

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFBA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	11.39	8.679		9.5	2.7		11.9	1.66
2	9.359	7.132		11	2.75		10.3	1.44
3	10.86	8.275		11	2.75		9.67	1.35
Mean	10.54			10.5			10.6	
Within lab CV (%)	10			8.2			11	
Between labs CV (%)	0.6							

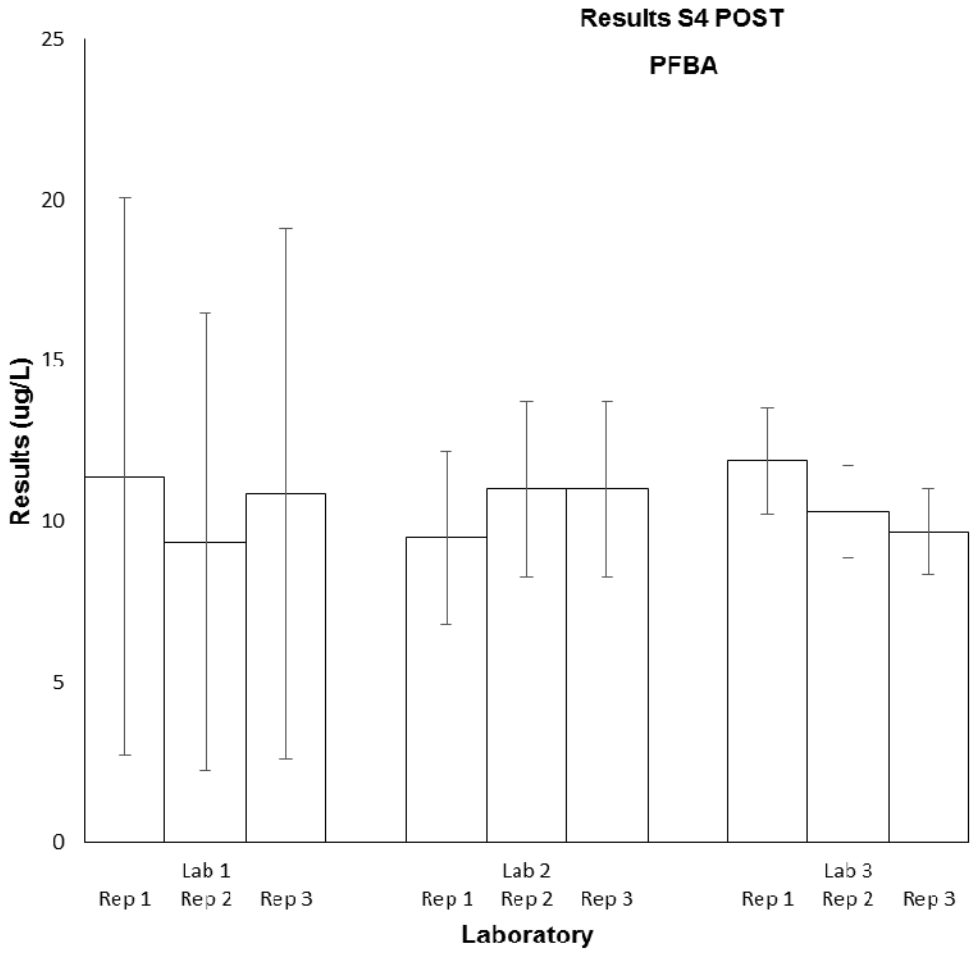

Figure 45

Table 48

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFPeA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	19.3	9.38		19	4.8		22.3	3.1
2	19.54	9.496		19	4.75		19.8	2.8
3	18.9	9.185		24	6		18.8	2.6
Mean	19.25			21			20.3	
Within lab CV (%)	1.7			14			8.9	
Between labs CV (%)	3.7							

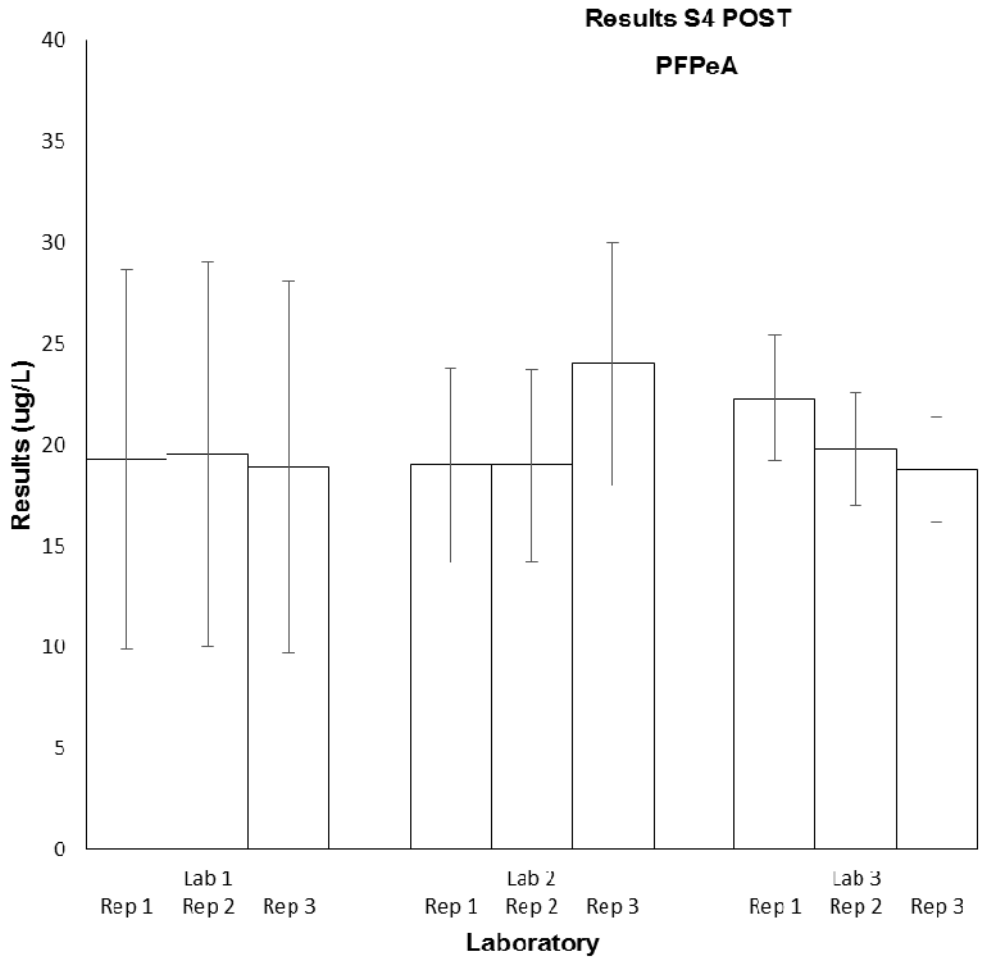

Figure 46

Table 49

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFHxA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	10.16	5.486		5.5	1.5		8.93	1.25
2	10.85	5.859		6.7	1.6		7.4	1.04
3	11.2	6.048		6.7	1.6		7.56	1.06
Mean	10.74			6.3			7.96	
Within lab CV (%)	4.9			11			11	
Between labs CV (%)	27							

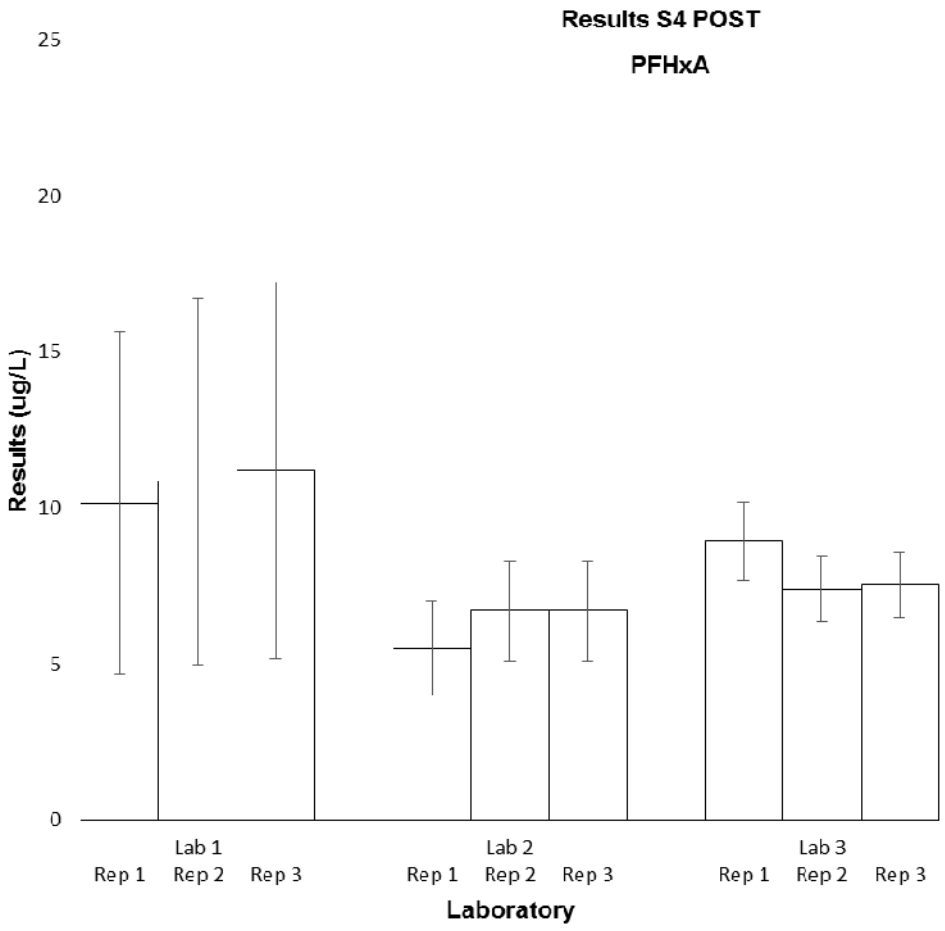

Figure 47

Table 50

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFHpA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	7.15	4.49		2.2	0.5		3.26	0.46
2	7.81	4.905		1.9	0.46		3.28	0.46
3	7.67	4.817		2.2	0.53		3.13	0.44
Mean	7.54			2.1			3.22	
Within lab CV (%)	4.6			8			2.5	
Between labs CV (%)	67							

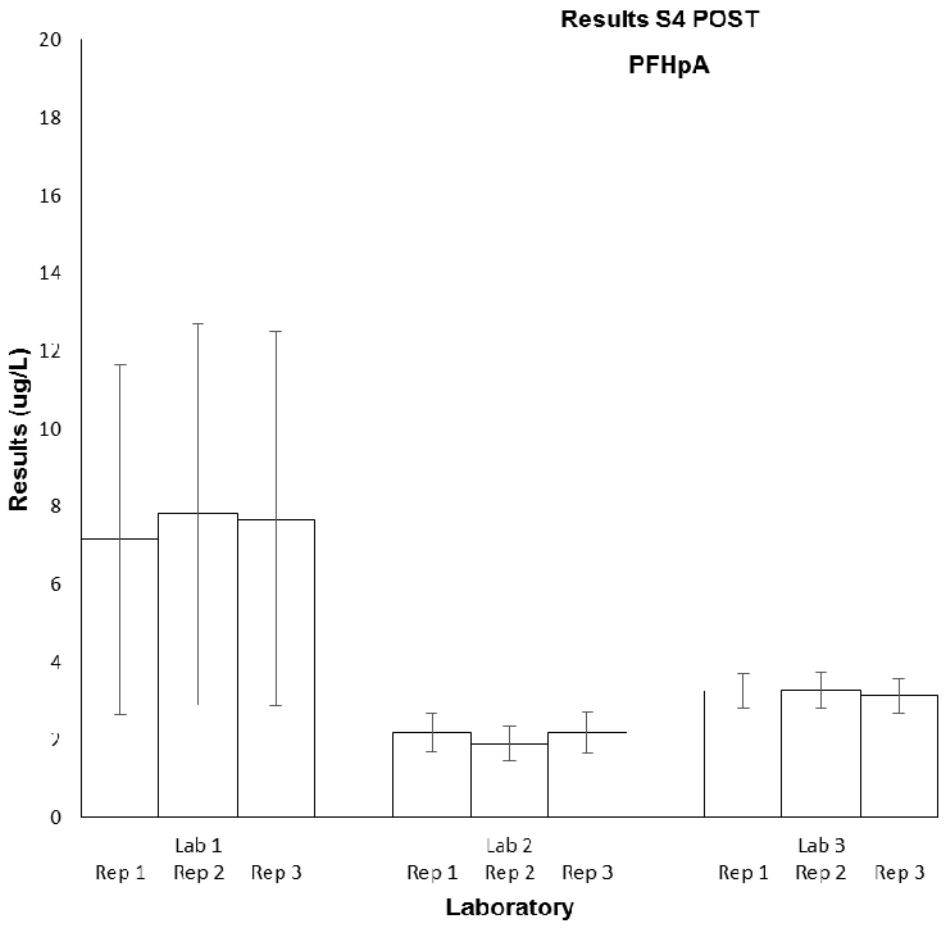

Figure 48

Table 51

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFOSA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	2.18	0.219		<0.05	0.6		0.43	0.06
2	0.8	0.08		<0.05	0.012		0.35	0.049
3	1.3	0.131		<0.05	0.012		0.5	0.07
Mean	1.43			-			0.43	
Within lab CV (%)	49			-			18	
Between labs CV (%)	76							

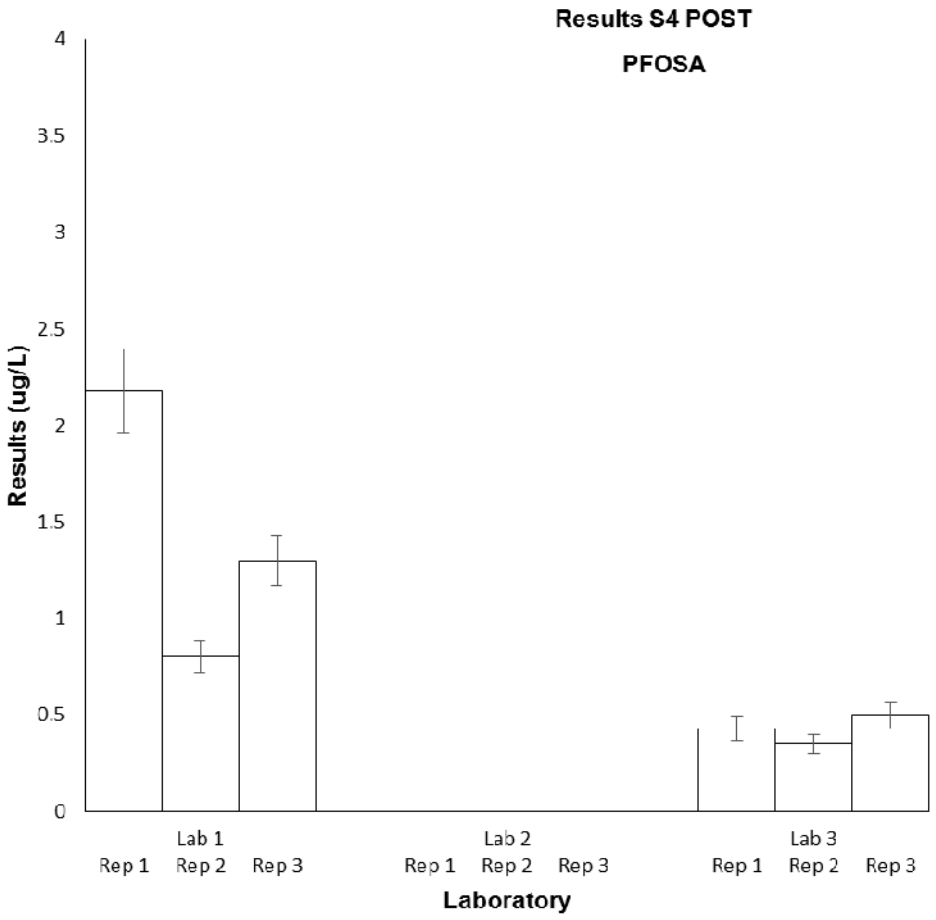

Figure 49

Table 52

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFOA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	71.89	46.225		83	21		73.3	10.3
2	86.57	55.665		63	16		77.3	10.8
3	81.82	52.61		94	23.9		75.9	10.6
Mean	80.09			80			75.5	
Within lab CV (%)	9.4			20			2.7	
Between labs CV (%)	3.3							

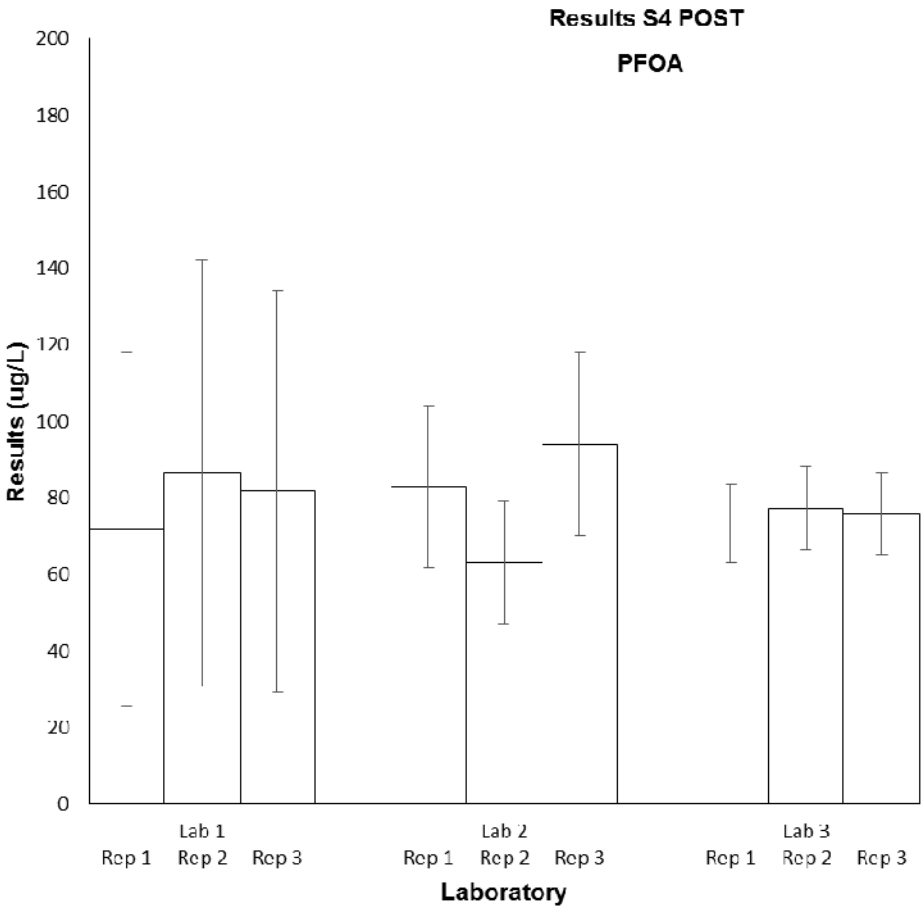

Figure 50

Table 53

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFOS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	2.66	1.096		0.4	0.11		3.82	0.12
2	2.12	0.873		0.29	0.08		4.02	0.6
3	3.41	1.405		0.35	0.09		5.75	0.86
Mean	2.73			0.35			4.53	
Within lab CV (%)	24			16			23	
Between labs CV (%)	83							

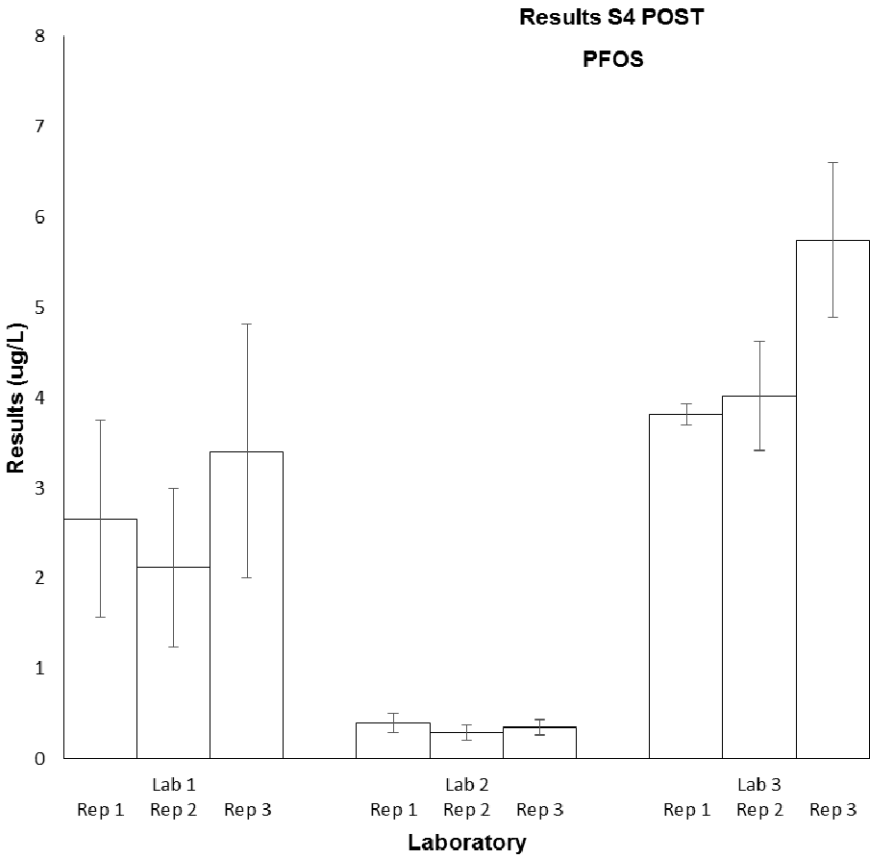

Figure 51

Table 54

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFDA
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	12.25	9.31		12	3		11.4	1.59
2	14.41	10.952		8.8	2.2		10.3	1.44
3	14.35	10.906		13	3.3		9.74	1.36
Mean	13.67			11.3			10.5	
Within lab CV (%)	9.0			19			8.1	
Between labs CV (%)	14							

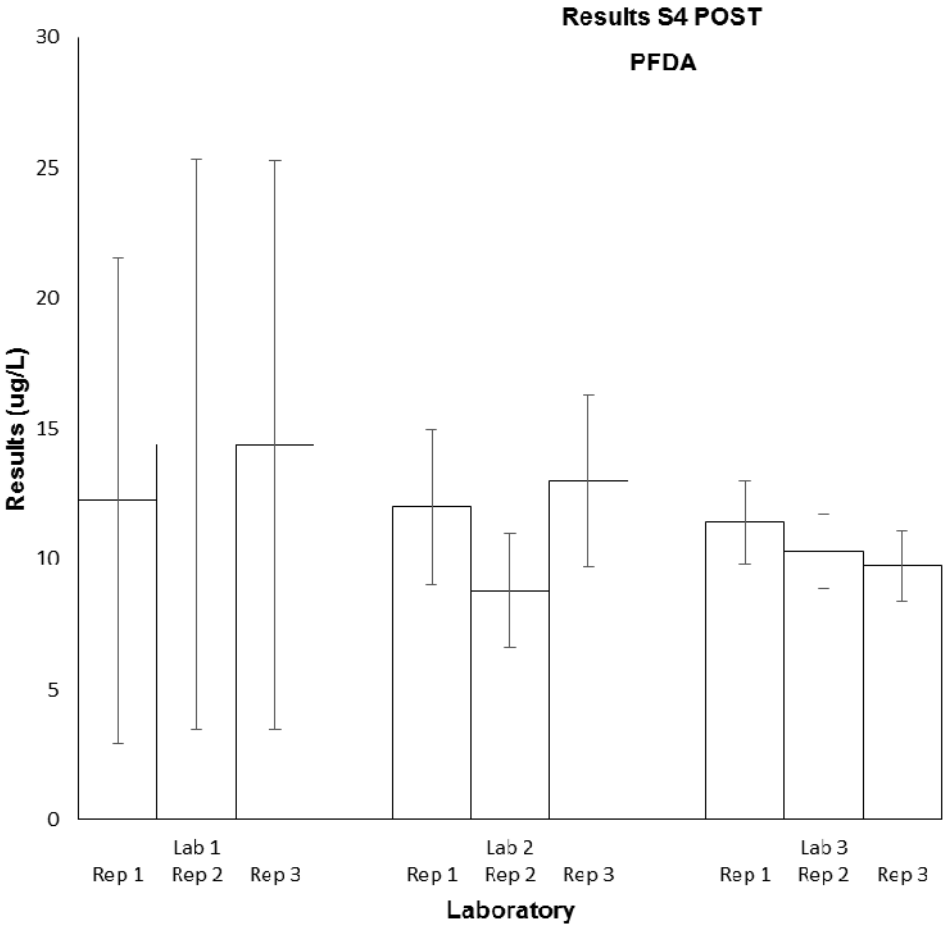

Figure 52

Table 55

Sample Details

Sample No.	S4 POST
Matrix.	Worm Juice, Tridol, PFDA and PFHxS
Analyte.	PFHxS
Units	ug/L

Participants' Results

Replicates	Lab 1			Lab 2			Lab 3	
	Result	Uncertainty		Result	Uncertainty		Result	Uncertainty
1	9.71	4.894		9.5	2.7		10.4	1.45
2	12.42	6.26		11	3.1		9.52	1.33
3	12.13	6.114		8.2	2.3		9.1	1.27
Mean	11.42			9.6			9.66	
Within lab CV (%)	13			15			6.9	
Between labs CV (%)	10							

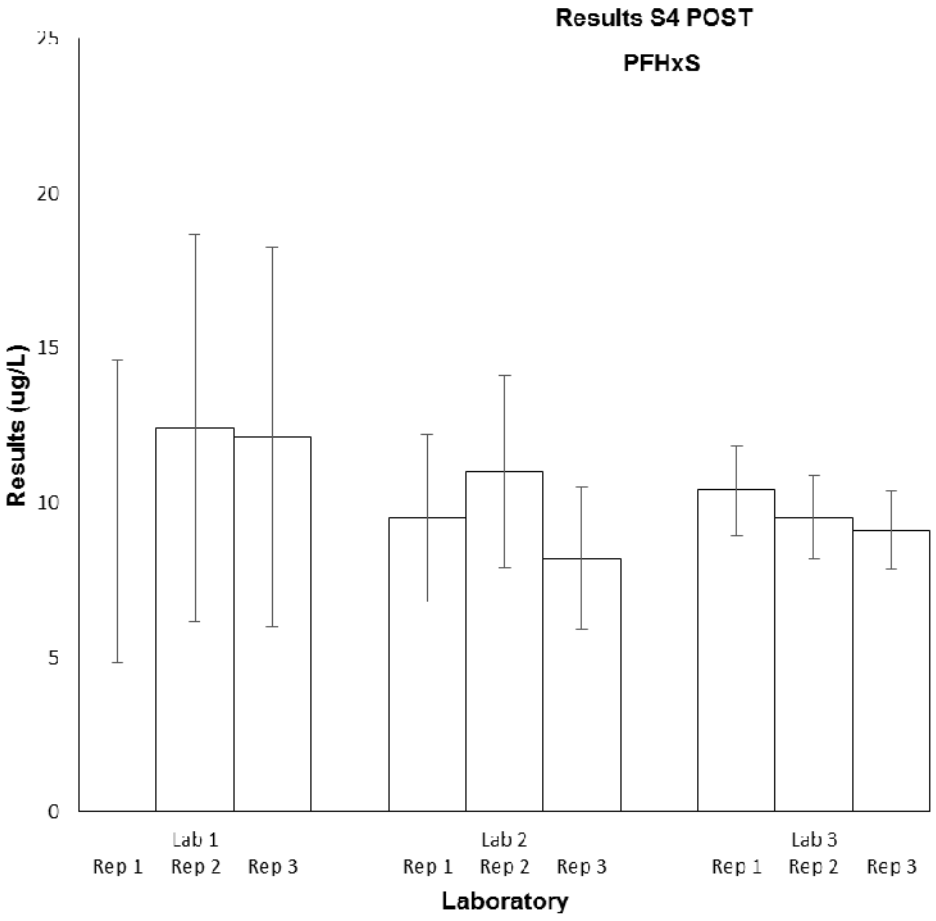

Figure 53

A2.1 PRE TOP ASSAY Perfluoroalkyl Carboxylic Acids (PFCAs) Incurred

PFCA's found in the PRE TOP assay samples are likely impurities in the Tridol foam and 8:2 monoPAP. Results are presented below.

Laboratory **1** PRE

Sample **S1**

	R1	U1	R2	U2	R3	U3	
PFBA	<0.1	0.025	<0.1	0.025	<0.1	0.025	µg/L
PFPeA	0.021	0.005	0.023	0.006	0.024	0.006	µg/L
PFHxA	0.055	0.016	0.057	0.017	0.059	0.017	µg/L
PFHpA	<0.02	0.005	<0.02	0.005	<0.02	0.005	µg/L
PFOA	0.036	0.011	0.037	0.012	0.042	0.013	µg/L
PFNA	<0.02	0.005	<0.02	0.005	<0.02	0.005	µg/L

Sample **S2**

	R1	U1	R2	U2	R3	U3	
PFBA	<0.1	0.025	<0.1	0.025	<0.1	0.025	µg/L
PFPeA	<0.02	0.005	<0.02	0.005	<0.02	0.005	µg/L
PFHxA	<0.02	0.006	<0.02	0.006	<0.02	0.006	µg/L
PFHpA	<0.02	0.005	<0.02	0.005	<0.02	0.005	µg/L
PFOA	<0.01	0.004	<0.01	0.004	<0.01	0.004	µg/L
PFNA	0.159	0.037	0.167	0.038	0.142	0.033	µg/L

Sample **S3**

	R1	U1	R2	U2	R3	U3	
PFBA	<0.1	0.027	<0.1	0.027	<0.1	0.027	µg/L
PFPeA	0.03	0.007	0.035	0.008	0.027	0.006	µg/L
PFHxA	0.034	0.009	0.035	0.009	0.026	0.007	µg/L
PFHpA	0.017	0.007	0.018	0.008	0.016	0.007	µg/L
PFOA	0.077	0.018	0.083	0.020	0.071	0.017	µg/L
PFNA	0.091	0.008	0.098	0.008	0.095	0.008	µg/L

Sample **S4**

	R1	U1	R2	U2	R3	U3	
PFBA	<0.1	0.027	<0.1	0.027	<0.1	0.027	µg/L
PFPeA	<0.02	0.004	<0.02	0.004	0.036	0.008	µg/L
PFHxA	0.11	0.028	0.105	0.027	0.091	0.023	µg/L
PFHpA	0.025	0.011	0.03	0.013	0.026	0.011	µg/L
PFOA	0.073	0.017	0.088	0.021	0.08	0.019	µg/L
PFNA	0.093	0.008	0.108	0.009	0.097	0.008	µg/L

Laboratory **2** PRE

Sample **S1**

	R1	U1	R2	U2	R3	U3	
PFBA	0.02	0.004	0.02	0.004	0.02	0.004	µg/L
PFPeA	0.015	0.004	0.015	0.004	0.014	0.004	µg/L
PFHxA	0.019	0.005	0.019	0.005	0.019	0.005	µg/L
PFHpA	0.011	0.003	0.011	0.003	0.01	0.003	µg/L
PFOA	0.038	0.007	0.04	0.007	0.04	0.007	µg/L
PFNA	<0.01	0.002	<0.01	0.002	<0.01	0.002	µg/L

Sample **S2**

	R1	U1	R2	U2	R3	U3	
PFBA	0.03	0.005	0.03	0.005	0.03	0.005	µg/L
PFPeA	0.01	0.003	0.01	0.003	0.01	0.003	µg/L
PFHxA	<0.01	0.002	<0.01	0.002	<0.01	0.002	µg/L
PFHpA	<0.01	0.002	<0.01	0.002	<0.01	0.002	µg/L
PFOA	0.02	0.004	0.02	0.004	0.02	0.004	µg/L
PFNA	0.07	0.01	0.07	0.01	0.07	0.01	µg/L

Sample **S3**

	R1	U1	R2	U2	R3	U3	
PFBA	< 0.05	0.012	0.05	0.012	<0.05	0.012	µg/L
PFPeA	0.02	0.005	0.02	0.005	0.01	0.003	µg/L
PFHxA	0.02	0.005	0.02	0.005	0.02	0.002	µg/L
PFHpA	0.01	0.002	0.01	0.002	0.01	0.002	µg/L
PFOA	0.05	0.012	0.06	0.014	0.05	0.012	µg/L
PFNA	0.07	0.017	0.07	0.017	0.06	0.014	µg/L

Sample **S4**

	R1	U1	R2	U2	R3	U3	
PFBA	0.05	0.012	0.06	0.018	<0.05	0.012	µg/L
PFPeA	0.02	0.005	0.03	0.009	0.01	0.003	µg/L
PFHxA	0.04	0.010	0.06	0.018	0.04	0.01	µg/L
PFHpA	0.01	0.002	0.01	0.002	0.01	0.002	µg/L
PFOA	0.05	0.012	0.05	0.012	0.06	0.013	µg/L
PFNA	0.07	0.017	0.06	0.018	0.06	0.018	µg/L

Laboratory **3** PRE

Sample **S1**

	R1	U1	R2	U2	R3	U3	
PFBA	<0.01		<0.01				µg/L
PFPeA	<0.01		<0.01				µg/L
PFHxA	0.028	0.0014	0.023	0.003			µg/L
PFHpA	0.019	0.0028	0.014	0.002			µg/L
PFOA	0.042	0.0063	0.042	0.0063			µg/L
PFNA	<0.01		<0.01				µg/L

Sample **S2**

	R1	U1	R2	U2	R3	U3	
PFBA	<0.05		<0.05		<0.05		µg/L
PFPeA	<0.01		<0.01		<0.01		µg/L
PFHxA	<0.01		<0.01		<0.01		µg/L
PFHpA	<0.01		<0.01		<0.01		µg/L
PFOA	<0.01		<0.01		<0.01		µg/L
PFNA	0.061	0.009	0.061	0.009	0.062	0.009	µg/L

Sample **S3**

	R1	U1	R2	U2	R3	U3	
PFBA	0.053	0.007	0.054	0.007	0.055	0.007	µg/L
PFPeA	0.022	0.003	0.022	0.003	0.022	0.003	µg/L
PFHxA	0.021	0.003	0.02	0.003	0.022	0.003	µg/L
PFHpA	0.012	0.002	0.013	0.002	0.013	0.002	µg/L
PFOA	0.057	0.008	0.058	0.008	0.059	0.008	µg/L
PFNA	0.076	0.011	0.074	0.011	0.073	0.011	µg/L

Sample **S4**

	R1	U1	R2	U2	R3	U3	
PFBA	0.094	0.013	0.097	0.013	0.096	0.013	µg/L
PFPeA	0.035	0.0049	0.033	0.0046	0.034	0.0047	µg/L
PFHxA	0.049	0.0068	0.045	0.0063	0.046	0.0064	µg/L
PFHpA	0.016	0.0022	0.016	0.0022	0.016	0.0022	µg/L
PFOA	0.073	0.01	0.075	0.011	0.071	0.01	µg/L
PFNA	0.080	0.011	0.076	0.011	0.082	0.012	µg/L

APPENDIX 2 - SAMPLE PREPARATION AND HOMOGENEITY TESTING

A2.1 Sample Preparation

Five analytical standards used for spiking samples in this study were purchased from HPC Standards GmbH, Toronto Research Chemicals and Sigma-Aldrich. On the analytical reports provided with the standards, all analytes have a stated purity of >95%. Tridol foam was obtained from a commercial supplier.

Sample **S1**: 6000.8 g of Milli-Q water was spiked with 150 µL Tridol foam and 150 µg/L PFOSA. The spiked water was stirred using an IKA overhead stirrer and dispensed into labelled 65 mL HDPE containers.

Sample **S2**: 6000.5 g of Milli-Q water was spiked with 214 µg/L 8:2 monoPAP, 13.9 µg/L PFDA and 10 µg/L PFOS. The spiked water was stirred using an IKA overhead stirrer and dispensed into labelled 65 mL HDPE containers.

Sample **S3**: 6006.2 g of Milli-Q water was spiked with 150 µL Tridol foam, 150 µg/L PFOSA, 13.9 µg/L PFDA and 10.9 µg/L PFHxS. The spiked water was stirred using an IKA overhead stirrer and dispensed into labelled 65 mL HDPE containers.

Sample **S4**: Liquid from a worm farm was filtered through an ADVANTEC Glass Fibre filter (GB 140) 150 mm. Sample was analysed by the Inorganics Section at NMI North Ryde for Total Organic Carbon (TOC) and found to contain 400 mg/L. 1822.5 g of filtered worm juice was mixed with 4207.1 g of MilliQ-water and spiked with 150 µL Tridol foam, 150 µg/L PFOSA, 13.9 µg/L PFDA and 10.9 µg/L PFHxS. The spiked diluted worm juice was stirred using an IKA stirrer and dispensed into labelled 65 mL HDPE containers.

All samples were stored at 4°C prior to dispatch to participants.

A2.2 Homogeneity Testing

Water samples were prepared (see A2.1) and analysed at NMI North Ryde. A brief description of analysis method is presented below. The measurements were made under repeatability conditions in random order.

PRE TOP assay samples were prepared by accurately weighing the entire content of the sample bottles (~60 mL) then spiking with 25 µL of labelled surrogate standard in methanol. Samples were pre-treated with 1N acetic acid then extracted by solid phase extraction (Strata XL-AW, 6 cc/ 500 mg, 100 µm particle size) under vacuum and eluted using ammonia/ methanol. After evaporation under nitrogen, the extract was reconstituted to 1 mL in ammonia/ methanol solution and spiked with 50 µL labelled Recovery Standard in methanol.

Instrument analysis was performed using a Ultra High Performance Liquid Chromatograph/ mass spectrometer (UPLC) Waters Xevo TQS, operating in multiple reaction monitoring mode. 2 µL of extract was injected onto a Waters Aquity BEH C18 column (1.7 um, 2.1 x 50 mm) with a mobile phase gradient consisting of water:methanol (2 mM ammonium acetate).

Two mass transitions were monitored for each target analyte (exception for PFBA and PFPeA with one transition) and labelled surrogate, and abundance ratios checked.

The instrument mass accuracy is calibrated annually during preventative maintenance, and the eight 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 method and used calibration, surrogate and recovery standards supplied by Wellington Laboratories, Canada.

POST TOP assay Samples S1 and S2 were prepared by accurately weighing aliquots (~20 mL) taken from the samples provided. Each aliquot was oxidised in two stages:

- 1st stage: 1 g of potassium persulfate and 1 mL of 10N sodium hydroxide solution added to the samples for pH >13 and kept at 85 °C for 2.5 hours.

- 2nd stage: 1 g of potassium persulfate and 1 mL of 10N sodium hydroxide solution added to the samples for pH >13 and kept at 85 °C overnight.

POST TOP assay Samples S3 and S4 were prepared by accurately weighing aliquots (~20 mL) taken from the samples provided. Each aliquot was oxidised in three stages:

-1st and 2nd stage: 1 g of potassium persulfate and 1 mL of 10N sodium hydroxide solution added to the samples for pH > 13 and kept at 85 °C for 2.5 hours.

- 3rd stage: 1 g of potassium persulfate and 1 mL of 10N sodium hydroxide solution added to the samples for pH > 13 and kept at 85 °C overnight.

After the oxidation step, all samples were acidified with HCl to pH=4 and extracted as per PRE TOP assay method above.

Stage 1

Twenty bottles were selected at random. Ten bottles were tested PRE oxidation and ten bottles POST oxidation. All samples were judged to be sufficiently homogeneous for use in this study.

The results of the homogeneity testing for Samples S1 and S2 are presented in tables 56-59.

Table 56 Homogeneity testing S1 PRE

Bottle fill number	6:2 FTS (µg/L)	PFOSA (µg/L)
3	1.09	55.0
29	1.06	78.1
31	1.15	63.6
41	1.09	74.9
43	1.07	85.7
58	1.12	108
61	1.14	81.5
69	1.11	85.9
73	1.01	81.2
90	1.06	91.6
Mean	1.09	80.5
CV	3.9%	18%

Table 57 Homogeneity testing S1 POST

Bottle fill number	6:2 FTS (µg/L)	PFBA (µg/L)	PFPeA (µg/L)	PFHxA (µg/L)	PFHpA (µg/L)	PFOSA (µg/L)	PFOA (µg/L)	PFOS (µg/L)
11	1.03	9.41	15.3	7.81	1.26	0.145	23.4	2.02
14	0.850	8.56	13.7	6.26	1.15	0.122	17.9	1.71
19	0.887	9.02	13.9	7.27	1.27	0.164	20.7	1.94
26	0.534	9.78	15.9	7.68	1.26	0.064	24.1	1.31
50	0.408	8.69	14.0	6.28	1.20	0.080	29.7	1.87
63	0.703	9.63	15.6	7.59	1.35	0.189	32.2	2.67
66	0.969	9.72	15.7	7.50	1.41	0.217	30.7	3.47
76*	-	-	-	-	-	-	-	-
84	0.638	11.6	18.6	8.41	1.52	0.120	38.7	2.68
85	0.311	10.6	17.4	7.72	1.28	0.057	34.0	1.61
Mean	0.703	9.66	15.5	7.39	1.30	0.128	27.9	2.14
CV	36	9.7	11	9.5	8.6	43	24	31

Bottle fill 76 not analysed due to inadvertent sample loss during oxidation.

Table 58 Homogeneity testing S2 PRE

Bottle fill number	PFDA (µg/L)	PFOS (µg/L)
13	12.8	8.25
15	11.9	7.57
25	12.3	7.99
42	14.0	8.63
45	12.8	8.23
60	12.2	8.03
68	13.3	8.73
70	13.1	8.05
75	14.1	8.24
94	12.4	7.98
Mean	12.9	8.17
CV	5.8%	4.1%

Table 59 Homogeneity testing S2 POST

Bottle fill number	PFBA (µg/L)	PFPeA (µg/L)	PFHxA (µg/L)	PFHpA (µg/L)	PFOA (µg/L)	PFNA (µg/L)	PFDA (µg/L)	PFOS (µg/L)
6	3.78	8.21	13.3	22.6	10.5	3.32	11.3	7.42
21	3.93	8.17	13.5	22.8	11.9	3.94	11.8	7.45
28	3.96	8.46	14.1	24.4	12.0	3.71	11.8	7.35
30	4.08	8.48	13.8	24.3	12.2	3.86	12.1	8.13
37	4.18	9.09	15.7	26.8	12.6	3.99	12.6	7.99
52	3.80	8.13	13.3	23.3	12.2	3.97	11.4	7.65
59	4.44	9.29	15.7	27.3	13.6	4.48	12.5	8.22
69	3.75	7.93	12.9	23.0	11.8	3.74	11.1	7.10
81	3.64	7.85	12.9	21.5	10.4	3.44	10.4	6.59
86	4.27	8.87	15.0	24.6	12.3	3.94	12.4	7.92
Mean	3.98	8.45	14.0	24.0	11.9	3.84	11.7	7.58
CV	6.4	5.8	7.8	7.6	7.9	8.4	5.9	6.7

Stage 2

Fourteen bottles were selected at random. Seven bottles were tested PRE oxidation and seven bottles POST oxidation. All samples were found to be sufficiently homogeneous for use in this study.

The results of the homogeneity testing are presented in Tables 60-63.

Table 60 Homogeneity testing S3 PRE

Bottle fill number	6:2 FTS (µg/L)	PFOSA (µg/L)	PFDA (µg/L)	PFHxS (µg/L)
28	0.953	54.3	11.9	9.85
38	0.816	45.8	9.96	8.57
49	0.864	49.5	10.1	8.95
53	0.789	50.3	9.71	8.53
65	0.954	59.1	11.5	10.3
70	0.918	54.9	11.2	8.94
71	0.771	50.3	9.83	8.70
28	0.953	54.3	11.9	9.85
Mean	0.866	52.0	10.6	9.12
CV	8.9	8.4	8.5	7.5

Table 61 Homogeneity testing S3 POST

Bottle fill number	6:2 FTS (µg/L)	PFBA (µg/L)	PFPeA (µg/L)	PFHxA (µg/L)	PFHpA (µg/L)	PFOSA (µg/L)	PFOA (µg/L)	PFOS (µg/L)	PFDA (µg/L)	PFHxS (µg/L)
8	0.028	7.91	13.9	4.45	1.64	0.021	30.0	0.503	9.79	9.38
19	0.052	7.51	14.1	6.02	1.28	0.030	31.7	0.811	8.22	8.27
24	0.042	8.39	15.4	4.73	1.51	0.057	41.7	0.412	9.72	9.68
54	0.038	7.82	15.6	4.49	2.05	0.161	31.0	1.009	9.88	9.32
58	0.032	8.15	16.3	5.36	2.50	0.129	33.2	0.837	11.3	10.3
72	0.042	10.1	18.6	7.38	1.70	0.027	34.9	0.844	9.89	10.2
83	0.025	8.70	17.7	6.24	1.76	0.038	38.2	0.967	9.73	9.38
Mean	0.037	8.37	15.9	5.52	1.78	0.066	34.4	0.769	9.79	9.50
CV	25	10	11	20	22	85	12	29	9.1	7.1

Table 62 Homogeneity testing S4 PRE

Bottle fill number	6:2 FTS (µg/L)	PFOSA (µg/L)	PFDA (µg/L)	PFHxS (µg/L)
20	1.86	97.6	11.4	8.40
32	1.56	81.3	10.0	7.20
44	1.82	100	11.7	8.60
57	2.39	127	16.3	11.4
72	1.83	89.6	10.1	7.94
75	2.51	117	14.4	10.2
80	1.99	86.3	10.3	7.97
20	1.86	97.6	11.4	8.40
Mean	1.99	99.8	12.0	8.82
CV	17	16	20	16

Table 63 Homogeneity testing S4 POST

Bottle fill number	6:2 FTS (µg/L)	PFBA (µg/L)	PFPeA (µg/L)	PFHxA (µg/L)	PFHpA (µg/L)	PFOSA (µg/L)	PFOA (µg/L)	PFOS (µg/L)	PFDA (µg/L)	PFHxS (µg/L)
17	0.056	9.79	18.8	7.27	2.98	0.580	75.7	4.93	10.3	9.18
25*	0.038	7.01	13.6	5.61	2.25	0.284	52.2	3.41	8.56	6.26
26	0.105	9.94	19.6	7.93	3.04	0.506	75.8	4.66	10.0	9.05
43	0.080	10.8	20.0	7.81	3.14	0.411	80.2	4.44	9.31	9.78
64	0.053	10.0	19.2	8.12	3.04	0.363	70.1	4.37	9.73	9.38
83	0.047	10.5	20.7	8.28	3.50	0.300	80.6	4.01	10.5	10.30
89	0.066	10.3	20.8	8.41	3.76	0.407	81.3	5.27	10.9	9.54
Mean	0.068	10.2	19.9	7.97	3.24	0.428	77.3	4.61	10.1	9.54
CV	32	3.7	4.1	5.1	10	24	5.5	9.6	5.6	4.8

* Results for bottle fill 25 are considered outliers and were not included in the test for homogeneity.

APPENDIX 3 - ACRONYMS AND ABBREVIATIONS

6:2 FTS	1H, 1H, 2H, 2H-perfluorooctane sulfonate
8:2 monoPAP	Mono[2-(perfluorooctyl)ethyl] phosphate
CV	Coefficient of Variation
CRM	Certified Reference Material
PFOSA	Perfluoro-1-octanesulfonamide
ISO	International Standards Organisation
LC	Liquid Chromatography
MS	Mass Spectrometry
NMI	National Measurement Institute (of Australia)
NT	Not Tested
PFAS	Per- and poly fluorinated alkyl substances
PFBA	Perfluoro-n-butanoic acid
PFDA	Perfluoro-n-decanoic acid
PFHxS	Potassium perfluorohexanesulfonate
PFHxA	Perfluoro-n-hexanoic acid
PFHpA	Perfluoro-n-heptanoic acid
PFNA	Perfluoro-n-nonanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFOSA	Perfluoro-1-octanesulfonamide
PFPeA	Perfluoro-n-pentanoic acid
SPE	Solid Phase Extraction
TOP	Total oxidisable precursor

END OF REPORT

10 YEARS & BEYOND AIGA Australasian Land & Groundwater Association

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