CCQM-K156.1

High Polarity Analyte(s) in aqueous media: Determination of L-PFOA and L-PFOS in Ground Water

Key Comparison
Track C

Final Report
May 2023

Mine Bilsel, Taner Gökçen, Burcu Binici, Hasibe Yilmaz and Alper Isleyen
TUBITAK UME, National Metrology Institute (TUBITAK UME)
Gebze/Kocaeli, 41470 Turkey

With contributions from:

Dr.Christian Piechotta
Bundesanstalt fuer Materialforschung und –pruefung (BAM)
Berlin, Germany

Dr.Anatoliy Krylov, Dr.Alena Miheeva, Dr.Marina Palagina and Dr.Unna Ushal
Mendeleyev Institute for Metrology (VNIIM)
St.Petersburg Moskovsky, Russia

Dr.Noora Perkola
Finnish Environment Institute, Laboratory Centre (SYKE)
Helsinki, Finland

Dr. Laura Quinn, Dr. Désirée Prevoo-Franzsen, Dr. Deirdre Claasen, Dr. Maria Fernandes-Whaley
National Metrology Institute of South Africa (NMISA)
South Africa

BACKGROUND

In April 2017, the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) approved the Key Comparison (KC) CCQM-K156 “Determination of L-PFOA and L-PFOS in Ground water”. Nine national metrology institutes and designated institutes participated in CCQM-K156 while seven institutes reported their results. A subsequent comparison to CCQM-K156.1 was offered to those NMIs or DIs who did not get the chance to participate in this comparison or who would like to repeat this measurement challenge. The results of TUBITAK UME in the previous CCQM-K156 agreed well with the KCRV.

SUMMARY OF CCQM-K156

CCQM-K156/P198 was coordinated by TUBITAK UME on behalf of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) Organic Analysis Working Group (OAWG). This comparison was classified as a Track C comparison. Evidence of successful participation in formal, relevant international comparison is needed to document measurement capability claims (CMCs) made by National Metrology Institutes (NMIs) and Designated Institutes (DIs).

Nine NMIs and DIs participated in the Track C Key Comparison CCQM-K156 Determination of L-PFOA and L-PFOS in ground water. Seven institutes reported their results. Participants were requested to evaluate the mass fractions, expressed in ng/kg of L-PFOA and L-PFOS in ground water. SPE was applied in the sample pretreatment and LC-MS was applied for detection. The CCQM-K156 results for the determination of L-PFOA and L-PFOS range from 2.75 ng/kg to 5.50 ng/kg with a % RSD of 19.5 % for L-PFOA and from 2.04 ng/kg to 4.45 ng/kg with a % RSD of 21.3 % for L-PFOS. The Key Comparison Reference Value (KCRV) for L-PFOA and L-PFOS was determined from the results of the NMIs/DIs participating in the key comparison that used appropriately validated methods with demonstrated metrological traceability. The KCRV was assigned using a Hierarchical Bayesian Random Effects Model (HB REM) estimator from the values reported by six of the participants. One participant result of L-PFOS and one result of L-PFOA was excluded from the KCRV for technical reasons. The KCRV was 4.9 ng/kg ± 0.4 ng/kg for L-PFOA and 3.8 ng/kg ± 0.4 ng/kg for L-PFOS. The six institutes that were included in the assignment of consensus KCRV all agreed within their standard uncertainties.

Successful participation in CCQM-K156 demonstrated the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 200 g/mol to 700 g/mol, having high polarity pKow > -2 in mass fraction range from 0.5 ng/kg to 500 ng/kg in an aqueous media.

TABLE OF CONTENTS

BACKGROUND ii
SUMMARY OF CCQM-K156 ii
LIST OF TABLES iii
LIST OF FIGURES iv
LIST OF APPENDICES iv
ACRONYMS v
SYMBOLS vi
TIMELINE 1
MEASURANDS 1
STUDY MATERIALS 2
PARTICIPANTS, INSTRUCTIONS AND SAMPLE DISTRIBUTION 10
RESULTS 11
KEY COMPARISON REFERENCE VALUE (KCRV) 17
DEGREES OF EQUIVALENCE (DoE) 19
USE OF CCQM-K156.1 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS 21
CONCLUSIONS 21
ACKNOWLEDGEMENTS 21
REFERENCES 21

LIST OF TABLES

Table 1. Timeline for CCQM-K156.1 study 1
Table 2. Information of L-PFOA and L-PFOS 1
Table 3. Anion content of the candidate ground water sample 2
Table 4. Summary of homogeneity test 4
Table 5. Short Term Stability Test Results 8
Table 6. Long Term Stability Test Results 8
Table 7. Institutions Registered for CCQM-K156.1 10
Table 8. Certified Reference Materials Used 12
Table 9. Metrological Traceability of Participants' Results 13
Table 10. Reported Results for L-PFOA 14
Table 11. Reported Results for L-PFOS 14
Table 12. Candidate KCRVs and associated standard uncertainty for L-PFOA and L-PFOS, ng/kg 17
Table 13. Degrees of Equivalence for L-PFOA 19
Table 14. Degrees of Equivalence for L-PFOS 19

LIST OF FIGURES

Figure 1. Results of between Bottle Homogeneity for L-PFOA 4
Figure 2. Results of between Bottle Homogeneity for L-PFOS 4
Figure 3. Results of within Bottle Homogeneity for L-PFOA..... 5
Figure 4. Results of within Bottle Homogeneity for L-PFOS 5
Figure 5. Short-term stability of L-PFOA over a 4-week time period..... 6
Figure 6. Short-term stability of L-PFOS over a 4-week time period 7
Figure 7. Long-Term Stability of L-PFOA over a 12-month time period 9
Figure 8. Long-Term Stability of L-PFOS over a 12-month time period..... 9
Figure 9. Reported Results for L-PFOA, ng/kg 15
Figure 10. Reported Results for L-PFOS, ng/kg..... 15
Figure 11. KCRV for L-PFOA 17
Figure 12. KCRV for L-PFOS 18
Figure 13. Degrees of Equivalence Associated with KCRV 20

LIST OF APPENDICES

Appendix A: Call for Participation A1
Appendix B: Protocol B1
Appendix C: Registration Form C1
Appendix D: Reporting Form D1
Appendix E: Core Competency Form E1
Appendix F: Summary of Participants' Analytical Information F1
Table F-1: Summary of Sample Size, Extraction, and Cleanup for CCQM-K156.1 ..... F1
Table F-2: Summary of Analytical Techniques for CCQM-K156.1 ..... F5
Table F-3: Summary of Summary of Calibrants and Standards for CCQM-K156.1 ..... F11
Table F-4: Summary of Assessment and Verification Methods for CCQM-K156.1 ..... F14
Table F-5: Additional Comments for CCQM-K156.1 ..... F16
Appendix G: Summary of Participants' Uncertainty Estimation Approaches G1
Appendix H: Participants' Results as Reported H1

ACRONYMS

CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CMC	Calibration and Measurement Capability
CRM	Certified Reference Material
CV	coefficient of variation, expressed in %: CV = $100 \cdot s/\bar{x}$
DI	Designated Institute
DoE	degrees of equivalence
LC-HRMS	liquid chromatography with high-resolution mass spectrometry detection
LC-MS	liquid chromatography with mass spectrometry detection
LC-MS/MS	liquid chromatography with tandem mass spectrometry detection
ID	isotope dilution
IDMS	isotope dilution mass spectrometry
KC	Key Comparison
KCRV	Key Comparison Reference Value
LC	liquid chromatography
MADe	median absolute deviation from the median (MAD)-based estimate of s: MADe = $1.4826 \cdot$MAD, where MAD = median($\lvert x_i$-median(x_i)$\rvert$)
MRM	multiple reaction monitoring
NMI	National Metrology Institute
NMR	nuclear magnetic resonance spectroscopy
OAWG	Organic Analysis Working Group
pKow	logarithm of the octanol-water partition coefficient
PSE	pressurized solvent extraction
qNMR	quantitative nuclear magnetic resonance spectroscopy
SPE	solid phase extraction

SYMBOLS

d_i	degree of equivalence: x_i - KCRV
$\%d_i$	percent relative degree of equivalence: $100 \cdot d_i$/KCRV
k	coverage factor: $U(x) = k \cdot u(x)$
n	number of quantity values in a series of quantity values
s	standard deviation of a series of quantity values: $s = \sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2/(n-1)}$
t_s	Student's *t*-distribution expansion factor
$u(x_i)$	standard uncertainty of quantity value x_i
$\bar{u}(x)$	pooled uncertainty: $\bar{u}(x) = \sqrt{\sum_{i=1}^{n} u^2(x_i)/n}$
$U(x)$	expanded uncertainty
$U_{95}(x)$	expanded uncertainty defined such that $x \pm U_{95}(x)$ is asserted to include the true value of the quantity with an approximate 95 % level of confidence
$U_{k=2}(x)$	expanded uncertainty defined as $U_{k=2}(x) = 2 \cdot u(x)$
x	a quantity value
x_i	the i^{th} member of a series of quantity values
$\bar{x}$	mean of a series of quantity values: $\bar{x} = \sum_{i=1}^{n} x_i/n$

Conduct of Subsequent Comparison CCQM-K156.1

TIMELINE

Table 1 lists the timeline for the CCQM-K156.1 study.

Table 1. Timeline for CCQM-K156.1 study

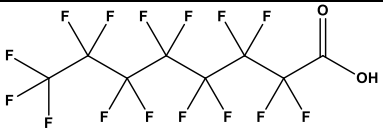
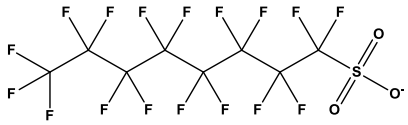
Date	Action
June 2020	Sample Preparation
July 2020	Homogeneity and Stability Testing
October 2020	Call for participation to OAWG members
November 2020	Sample Distribution
30th June 2021	Deadline for Submission of Results
October 2021	Preliminary Discussion of Results
June 2022	Draft A report distributed to OAWG
December 2022	Draft B report distributed to OAWG

MEASURANDS

The measurand was the mass fraction of linear forms for perfluoro-n-octanoic acid (L-PFOA) as acidic form and linear perfluoro-1-octane sulfonate (L-PFOS) as anion form in ground water. The indicative values are between mass fraction of 0.5 ng/kg and 20 ng/kg.

Table 2 below displays information of these compounds.

Table 2. Information of L-PFOA and L-PFOS

	L-PFOA	L-PFOS
CAS	335-67-1	45298-90-6
Molecular formula	$C_8HF_{15}O_2$	$C_8F_{17}O_3S^-$
M_W	414.07	499.12
pK_{ow}	NA	NA
Structure		

STUDY MATERIALS

The test material is ground water that was collected at 40°55'16.8" N, 29°12'43.6" E from a 250 m deep well at Kartal, Istanbul Turkey. This well supplies a significant amount of ground water that is used to fill swimming pools and for irrigation of gardens in Istanbul. The ground water was collected directly from the well into cleaned carboys and then transported to TUBITAK UME for further processing. Upon arrival at TUBITAK UME, samples were stored at -20 °C. The target mass fraction values for PFOA and PFOS was between 0.5 ng/kg and 20 ng/kg. However, based on the preliminary analysis of the collected ground water, it was concluded that in the raw water matrix, target analytes were not detected. Thus, it was decided to spike the material to achieve the target mass fraction levels for the analytes. The concentration of four anions in the raw material was also determined by ion chromatography (Dionex, ICS-3000, USA) and the results summarized in Table 3.

Table 3. Anion content of the candidate ground water sample

Anion Concentration, n=10 (mg/L)			
F^-	Cl^-	NO_3^-	SO_4^{-2}
0.164	115	1.50	214

Perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), potassium perfluoro-1-butanesulfonate (PFBS) and sodium perfluoro-1-hexanesulfonate (PFHxS) are also spiked as interferences. The homogenized water was separately dispensed into HDPE bottles to give about 154 bottles, with content of approximately 100 g each. Packing was in vacuum-sealed clear bags. Long term storage of the material at TUBITAK UME is at about 4ºC.

Each participant received five bottles of sample, which each bottle contains approximately 100 g of spiked ground water. The material was bottled considering single use. Therefore, the analysis of the water sample should be conducted using the entire contents of the bottle. The recommended minimum sample amount for analysis was at least 98 g. Measurement results were to be reported by an as-received basis.

For sample preparation, it was advised to rinse the sample bottle with extraction solvent to dissolve and collect any remaining analytes remaining on the walls of the bottle. Following this step in the provided protocol, the empty bottle was to be dried at 40 °C and reweighed with the cap, for a net mass of sample determination. All participants were required to follow the method outlined in the protocol.

Homogeneity Assessment of Study Material

A solution of analytes prepared from a mixture of linear PFAS was added to the ground water to obtain the target mass concentration of analytes. Homogenization was performed by circulating the content between two HDPE tanks for 3 h with a pressurized air driven circulation pump (DEBEM, SDB 71722, Italy). Homogenized ground water sample was dispensed into 125 mL Low Particulate Narrow-Mouth HDPE Bottles (Nalgene, Cat No: 382099-0125, USA) to give about 154 bottles, with content of approximately 100 g each. These bottles are produced in a controlled environment and certified by the producer for low particulate.

All samples were kept at the storage condition of 4ºC by TUBITAK UME. 16 bottles of samples were taken randomly, and since samples are for single use and MS_{within} is not possible, approach described in Alternative Strategies section of ISO Guide 35:2017[1] is performed on bulk material prepared from 16 samples. MS_{within} is calculated from the measurement results variance of 16 subsamples prepared from this bulk material. For each subsample 3 instrumental repetitions was done. $MS_{between}$ is calculated from the measurement results variance of 16 units selected by stratified sampling scheme covering the whole batch, while the absolute values were transformed relative to the mean.

The measurements were performed under repeatability conditions, using validated methods and according to a random sequence to clarify the possible trends in analytical sequence and filling order. The evaluation of homogeneity and calculation of uncertainty between and within bottles was performed by Equation 1 using approach described in "Alternative Strategies" section of ISO Guide 35:2017 which is recommended for single use samples.

The s_{bb} is calculated using the equation given below;

$$s_{bb}^2 = \frac{MS_{between} - MS_{within}}{n},\ n = 1 \qquad \text{Eq.1}$$

$$s_{bb} = u_{bb}$$

where,

u_{bb}: Standard uncertainty related to possible between-bottle heterogeneity
$MS_{between}$: Mean of square of variance between units

MS_{within}: Mean of square of variance within the unit n: Number of measurements within unit

The results of homogeneity is summarized in Table 4. The absolute values were transformed relative to the means and presented in Figure 1-4.

Table 4. Summary of homogeneity test

	L-PFOA	L-PFOS
$u_{bb,rel}(\%)$	2.30	2.37

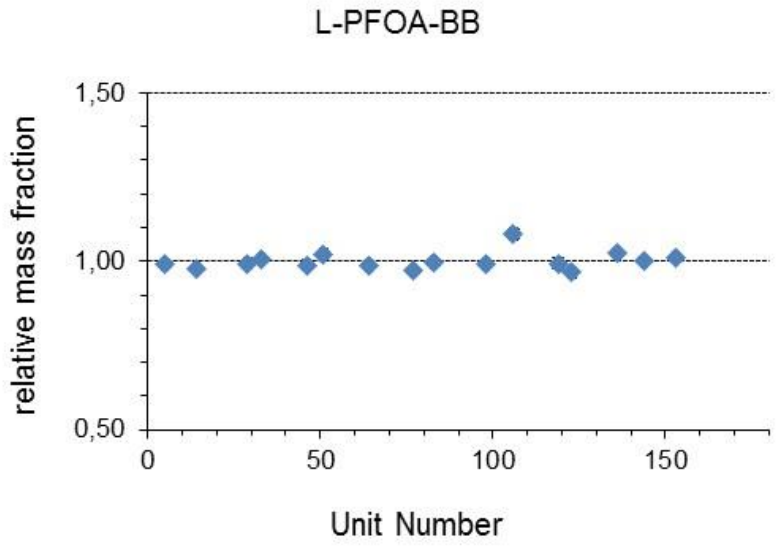


Figure 1. Results of between bottle homogeneity for L-PFOA

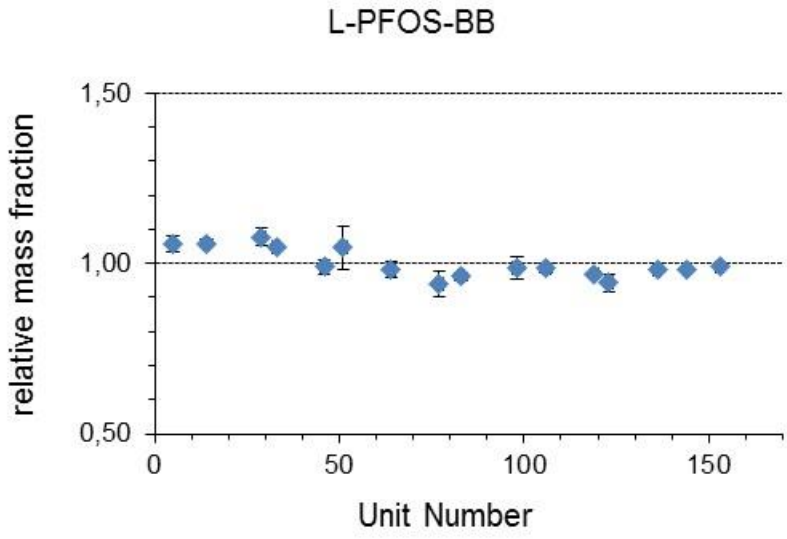


Figure 2. Results of between bottle homogeneity for L-PFOS

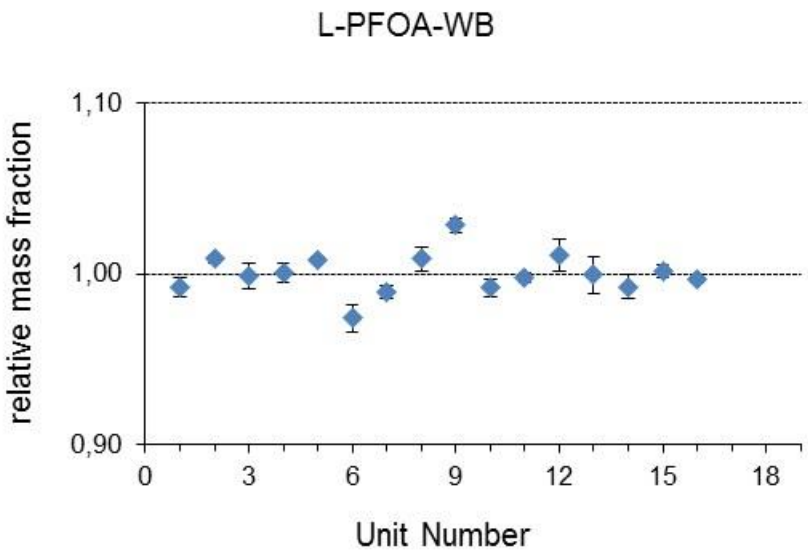


Figure 3. Results of within bottle homogeneity for L-PFOA

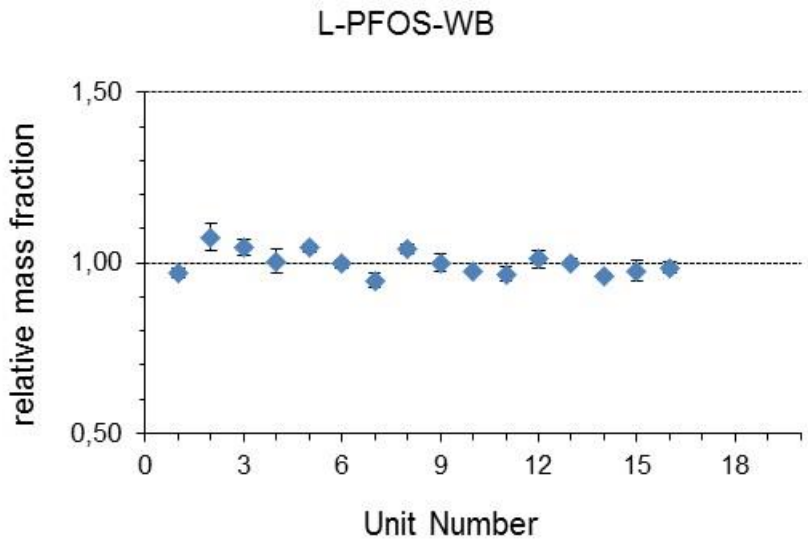


Figure 4. Results of within bottle homogeneity for L-PFOS

Stability Assessment of Study Material

Stability studies were performed with isochronous design which is cited in ISO Guide 35. For the Short Term Stability (STS) test, at temperature +60˚C and 5 time points (0, 1, 2, 3 and 4 weeks) were tested. 20 units were selected by using a stratified sampling scheme covering whole batch. 20 samples were subjected to the test temperatures for the specified time intervals.

For the Long Term Stability, the results from the previous study (CCQM-K156) were used.16 units for each laboratory (UME and SYKE) were tested at +18˚C for 0, 3, 6 and 12 months' time points.

Units were moved to +4˚C (reference temperature) after completion of the test time. All units were analysed at the same time. Samples were prepared based on the single use for each unit and were analyzed using LC-HRMS under the repeatability conditions to determine the mass fraction of L-PFOA and L-PFOS.

The data for each temperature were first examined by single Grubbs test for both 95% and 99% confidence intervals to find out outliers. Number of detected outliers are given in the Table 6 for STS and Table 7 for LTS. Since no technical reason can be found to reject these data, the outlying result were included in the calculations.

Values calculated for each time point were plotted against the time. The relationship between variables were analyzed in order to determine if any significant change exists with the testing time (regression analysis). For STS assessment, it was found that the slopes were significant for both L-PFOS and L-PFOA reported (except for 99% confidence interval). The trend graphs of short term stability are shown in Figure 5-6.

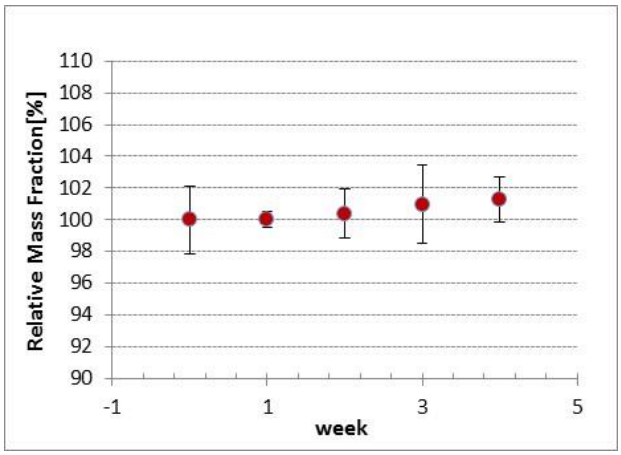


Figure 5. Short-term stability of L-PFOA over a 4-week time period.

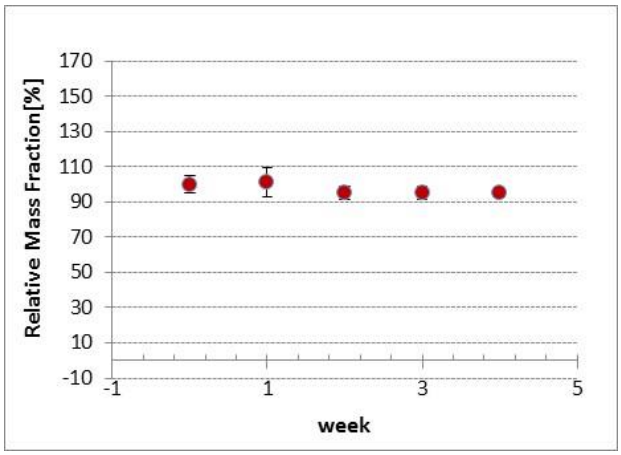


Figure 6. Short-term stability of L-PFOS over a 4-week time period.

As a consequence for the cases for which the slopes were significant, degradation is taken into account in the calculation of u_{sts} and u_{lts}. Uncertainty calculations were done using Equation 2. Maximum time for transfer is chosen as 2 weeks for STS and shelf life is chosen as 12 months for LTS.

$$u_{sts} \text{ or } u_{lts} = \frac{RSD}{\sqrt{\sum(t_i - \bar{t})^2}} \; x \; t$$

Eq.2

where,

RSD: Relative standard deviation of the slope on stability regression plot

t_i : time point for each replicate

$\bar{t}$: mean of all time points

t : maximum transfer time suggested for STS or shelf life suggested for LTS

Results are summarized in Table 5 and Table 6.

Table 5. Short Term Stability Test Results

Parameter	60 °C usts, rel for 2 weeks (%)2	Number of outliers in 95% confidence interval[1]	Number of outliers in 99% confidence interval[1]	Is there a significant trend in 95% confidence interval?	Is there a significant trend in 99% confidence interval?
		60 °C	60 °C	60 °C	60 °C
L-PFOS	2.08	1	1	Yes	Yes
L-PFOA	0.51	-	-	Yes	No

[1]Single Grubbs Test

[2] u_{sts} was calculated considering the degradation

Table 6. Long Term Stability Test Results

Parameter (Lab)	18 °C $u_{lts,rel}$ for 12 months (%)	Number of outliers in 95% confidence interval[1]	Number of outliers in 99% confidence interval[1]	Is there a significant trend in 95% confidence interval?	Is there a significant trend in 99% confidence interval?
		18 °C	18°C	18 °C	18°C
L-PFOS (TUBITAK)	1.5	1	1	No	No
L-PFOS (SYKE)	2.7	-	-	No	No
L-PFOA (TUBITAK)	1.1*	-	-	Yes	No
L-PFOA (SYKE)	2.3	-	-	No	No

*Slope is found to be significant at 95 % confidence interval, u_{lts} is calculated by taking into account the degradation (u_{lts}= slope of reg. line/√3)

The trend graphs of Long Term Stability are shown in Figures 7-8.

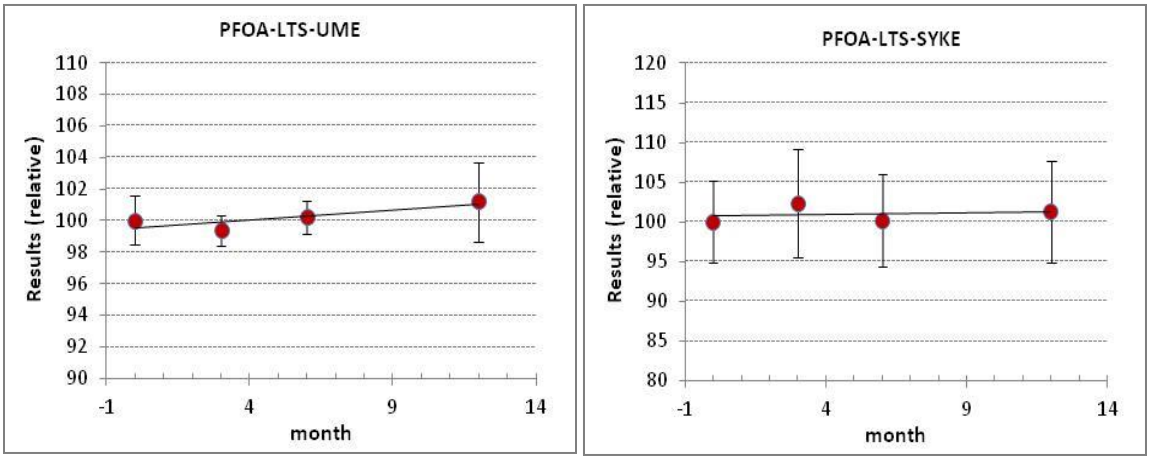


Figure 7. Long-Term Stability of L-PFOA over a 12-month time period.

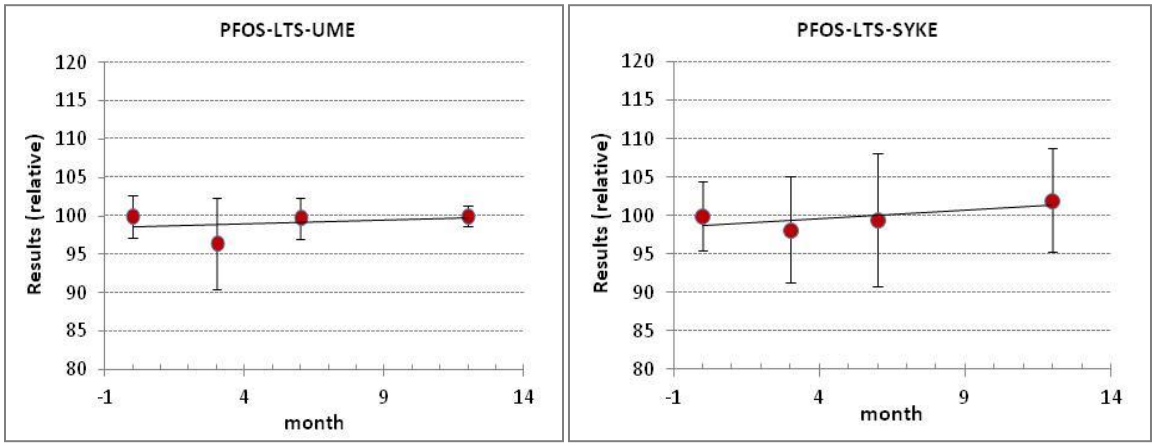


Figure 8. Long-Term Stability of L-PFOS over a 12-month time period.

Results of this study showed that the test material can be transferred to the laboratories without applying any cooling precaution if the temperatures do not exceed 60°C for up to 2 weeks.

PARTICIPANTS, INSTRUCTIONS AND SAMPLE DISTRIBUTION

The call for participation was distributed in October 2020, with the intent to distribute samples in November 2020, receive results on 30th June 2021 and discuss results at OAWG meeting in October 2021. See Table 1 for study timeline. Appendix A reproduces the Call for Participation; Appendix B reproduces the study Protocol.

Table 7 lists the institutions that registered for CCQM-K156.1.

Table 7. Institutions Registered for CCQM-K156.1

NMI or DI	Code	Country	Contact
Bundesanstalt fuer Materialforschung und – pruefung	BAM	Germany	Dr Christian Piechotta
D.I.Mendeleyev Research Institute for Chemical Measurements	VNIIM	Russia	Dr Anatoliy Krylov, Dr Alena Miheeva, Dr Marina Palagina, Dr.Unna Ushal
Finnish Environment Institute, Laboratory Centre	SYKE	Finland	Dr Noora Perkola
National Metrology Institute of South Africa	NMISA	South Africa	Dr Laura Quinn, Dr Désirée Prevoo-Franzsen, Dr Deirdre Claasen, Dr Maria Fernandes-Whaley
National Metrology Institute of Turkey	UME	Turkey	Dr. Taner Gökçen, Mine Bilsel

The participants were informed of the date of dispatch of samples. Each participant received 5 bottles of study material. Each bottle contained approximately 100 g ground water. There was a delay in sample shipping and customs issues for VNIIM. A sample receipt form was provided electronically to all participants and was to be filled and returned to the study coordinator on receipt of the shipments. After receiving the sample, to avoid any decomposition, samples were to be kept sealed until they are used and stored at +4 ^{o}C in the original bottle, tightly capped and not exposed to intense direct light and ultraviolet radiation.

Participants were asked to report the mass fraction results (in ng/kg) for each measurand from a single bottle using their method of choice. Participants were also asked to provide information about the applied analytical procedure including the sample preparation, calibration methods and their metrological traceability. The reported mass fraction will be the overall mean from measurements of five separate units, reporting should include the values of the individual units in addition to the overall mean. Each variable contributing to the uncertainty of the result was to be

identified and quantified in order to be included in the combined standard uncertainty of the results. The results reporting form and core competency template were required to be completed and returned to the study coordinator before the submission deadline.

RESULTS

Participants were requested to report a single estimate of the mass fraction (in ng/kg) for L-PFOA and L-PFOS from a single bottle. The results range from 4.71 ng/kg to 9.36 ng/kg with a % RSD of 23.50 % for L-PFOA and from 4.62 ng/kg to 7.67 ng/kg with a % RSD of 18.58 % for L-PFOS.

In addition to the quantitative results, participants were instructed to describe their analytical methods, approach to uncertainty estimation, and complete the Core Competency table to document what which competencies were demonstrated in this study. Appendices C, D, and E reproduce the relevant report forms.

CCQM-K156.1 results were received from all the institutions that received samples. Due to the unforeseen unavailability of the laboratory staff towards the end of the study, the deadline of submission of results was postponed to July, 2021.

Calibration Materials Used by Participants

Participants established the metrological traceability of their results using certified reference materials (CRMs) with stated traceability and/or commercially available high purity materials for which they determined the purity. Table 8 lists the CRMs that were used. Table 9 lists how participants established traceability. If through their own measurements, Table 9 lists the material and its assigned purity, the method used, and how the participant demonstrated their competence in the use of the method(s).

BAM, VNIIM, SYKE, NMISA and TUBITAK UME used CRM 4056-a and CRM 4220-a from NMIJ as a calibrant material.

Table 8. Certified Reference Materials Used

CRM	Provider	Analyte	Mass Fraction[a] Delivered	In-house Purity Methods Used to Value-Assign Source Material[b]
CRM 4056-a	NMIJ	PFOA	0.959 ± 0.005 (kg/kg)	DSC, FPD, qNMR
CRM 4220-a	NMIJ	PFOS	9.93 ± 0.15 (mg/kg)	DSC, FPD, qNMR

a Stated as Value ± U_{95}(Value)
b DSC: Differential scanning calorimetry
FPD: Freezing point depression
qNMR: Quantitative nuclear magnetic resonance

Table 9. Metrological Traceability of Participants' Results

NMI/DI	Analyte	Source of Traceability	Material	Mass Fraction[a] Purity, %	Purity Techniques[b]	Evidence of Competence
BAM	PFOA PFOS	NMIJ CRM-4056-a NMIJ CRM-4220-a	N/A			
VNIIM	PFOA PFOS	NMIJ CRM-4056-a NMIJ CRM-4220-a	N/A			
SYKE	PFOA PFOS	NMIJ CRM-4056-a NMIJ CRM-4220-a	N/A			
NMISA	PFOA PFOS	NMIJ CRM-4056-a NMIJ CRM-4220-a	N/A			
TUBITAK UME	PFOA PFOS	NMIJ CRM-4056-a NMIJ CRM-4220-a	N/A			

a Stated as Value ± U_{95}(Value)
b DSC: Differential scanning calorimetry
GC-FID: Gas chromatography with flame ionization detection
HPLC-DAD: High pressure liquid chromatograph with diode-array detection
MB: Mass balance
qNMR: Quantitative nuclear magnetic resonance

Methods Used by Participants

All participants based their analyses on LC-MS/MS and HR-LC-MS. Brief descriptions of the analytical methods used by the participants, including sample preparation, analytical technique, calibrants and quantification approach are summarized in Appendix F Tables F1-5. The participants' approaches to estimating uncertainty are provided in Appendix G.

The participants used IDMS as quantification method and Solid Phase Extraction as extraction method. NMISA, BAM and SYKE used weak ion exchange cartridge Strata X-AW, VNIIM used Evolute Express Wax cartridge and TUBITAK UME used Water Oasis WAX cartridge.

Participant Results for L-PFOA and L-PFOS

The results for CCQM-K156.1 for the determination of L-PFOA and L-PFOS are detailed in Tables 10 - 11 and presented graphically in Figures 9 - 10.

Table 10. Reported Results for L-PFOA

NMI	L-PFOA ng/kg					
	x	$u(x)$	$u(x)$ %	k	$U(x)$	$U(x)$ %
VNIIM	4.71	0.37	7.86	2	0.74	15.7
BAM	8.139	0.188	2.31	2	0.376	4.62
NMISA	8.28	0.42	5.07	2	0.84	10.1
SYKE	9.04	0.60	6.64	2	1.20	13.3
TUBITAK UME	9.36	0.37	3.95	2	0.74	7.91
n	5.00					
$\bar{x}$	7.91					
s	1.86					
%CV	23.5					

n = number of results included in summary statistics; $\bar{x}$ = mean; s = standard deviation;

CV = $100 \cdot s/\bar{x}$

Table 11. Reported Results for L-PFOS

NMI	L-PFOS ng/kg					
	x	$u(x)$	$u(x)$ %	k	$U(x)$	$U(x)$ %
VNIIM	4.62	0.23	4.98	2	0.45	9.74
TUBITAK UME	7.01	0.37	5.28	2	0.75	10.7
BAM	7.362	0.071	0.96	2	0.142	1.93
SYKE	7.59	0.79	10.41	2	1.58	20.8
NMISA	7.67	0.49	6.39	2	0.98	12.8
n	5.00					
$\bar{x}$	6.85					
s	1.27					
%CV	18.6					

n = number of results included in summary statistics; $\bar{x}$ = mean; s = standard deviation;
CV = $100 \cdot s/\bar{x}$

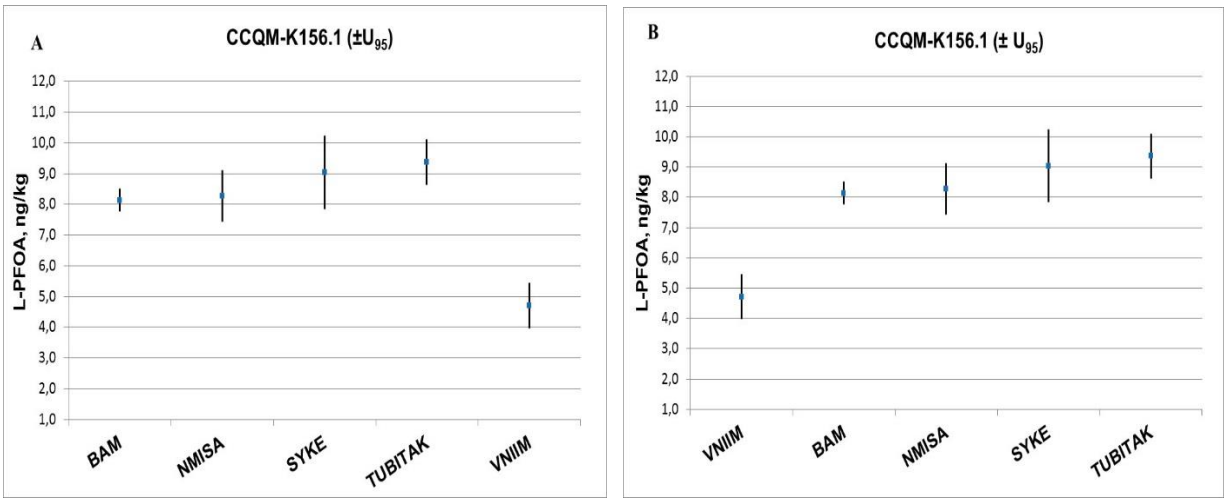


Figure 9. Reported Results for L-PFOA, ng/kg

Panels A and B display the reported results for L-PFOA; panel A displays the results sorted alphabetically by NMI acronym, panel B displays results sorted by increasing reported value. Dots represent the reported mean values, x; bars their 95 % expanded uncertainties, $U(x)$. The thin horizontal gridlines are provided for visual guidance.

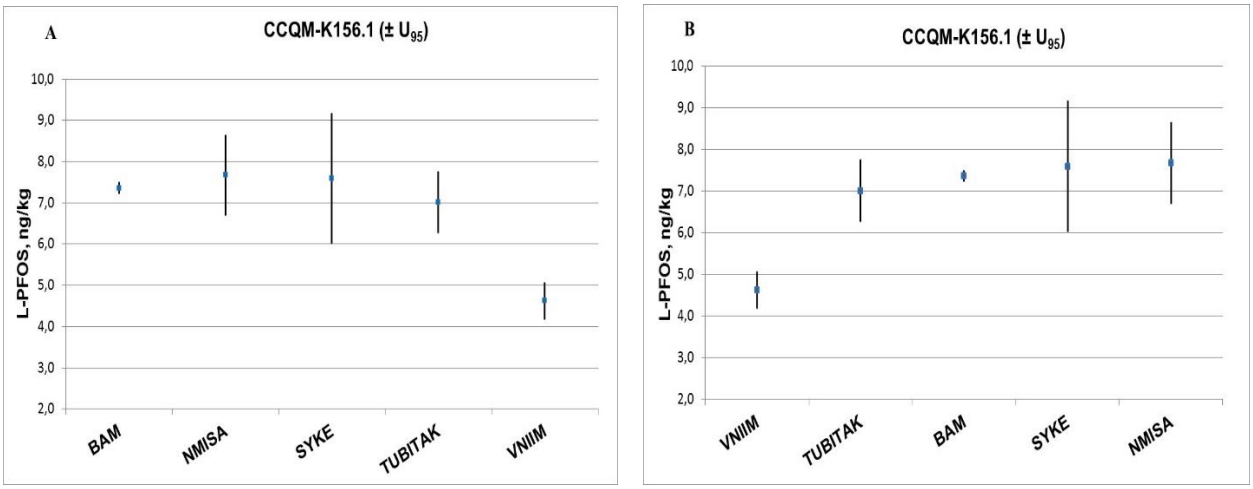


Figure 10. Reported Results for L-PFOS, ng/kg

Panels A and B display the reported results for L-PFOS; panel A displays the results sorted alphabetically by NMI acronym, panel B displays results sorted by increasing reported value. Dots represent the reported mean values, x; bars their 95 % expanded uncertainties, $U(x)$. The thin horizontal gridlines are provided for visual guidance.

Discussion of Results

The initial results were sent to participants in October 2021 and discussed at the OAWG online meeting in October 2021. BAM, VNIIM, SYKE and TUBITAK UME participated in both CCQM-K156 and CCQM-K156.1 comparisons. According to the Figures 9 and 10, the results of VNIIM for both L-PFOA and L- PFOS were low compared to the results of the remaining participants. The results range from 4.71 ng/kg to 9.36 ng/kg for L-PFOA and from 4.62 ng/kg to 7.67 ng/kg for L-PFOS.

KEY COMPARISON REFERENCE VALUE (KCRV)

The KCRV and u(KCRV) for this comparison were the results of TUBITAK UME, since TUBITAK UME was a participant in the previous CCQM-K156 and its results agreed well with the CCQM-K156 KCRV. The KCRV, standard uncertainty u(x) and expanded uncertainty U(x) are given in Table 12.

Table 12. KCRVs and associated standard uncertainties for L-PFOA and L-PFOS, ng/kg

Measurand	KCRV	u(x)	U(x)
L-PFOA	9.36	0.49	0.98
L-PFOS	7.01	0.47	0.94

The KCRVs are the results of TUBITAK UME. The uncertainty is calculated such that it also includes the dark uncertainty estimate (tau) determined in K156. The KCRV, its associated standard uncertainty and the results of the participants (with their standard uncertainties) are shown as graphs in Figures 11 - 12 for L-PFOA and L-PFOS. For L-PFOA, the result of SYKE agreed with KCRV, while the results of VNIIM, BAM and NMISA were biased low. As for L-PFOS, BAM, SYKE and NMISA results agreed with the KCRV, whereas VNIIM result was biased low.

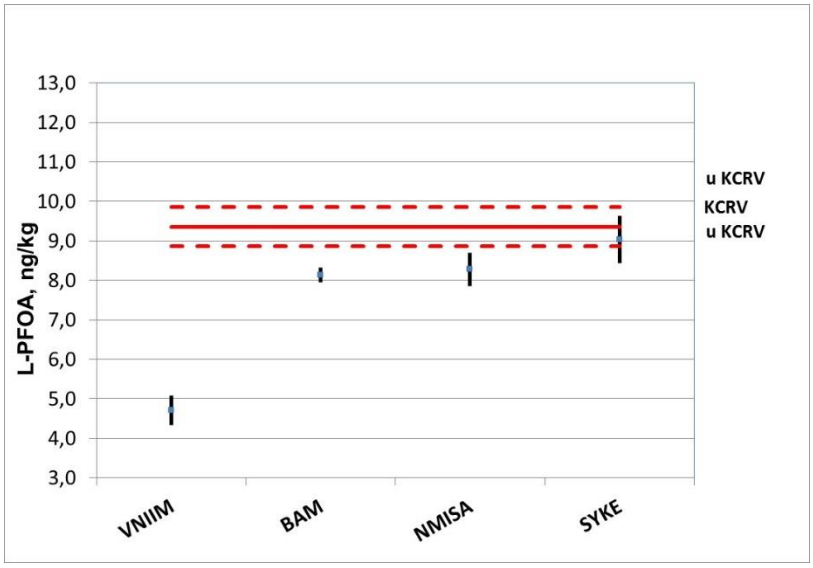


Figure 11. KCRV for L-PFOA

Figure 11 display the KCRV relative to the reported results for L-PFOA. The results are sorted by increasing reported value. Dots represent the reported mean values, *x*; bars their standard uncertainties, *u*(*x*). The red horizontal line denotes the candidate KCRV. The bracketing red lines denote the standard uncertainty of the candidate KCRV.

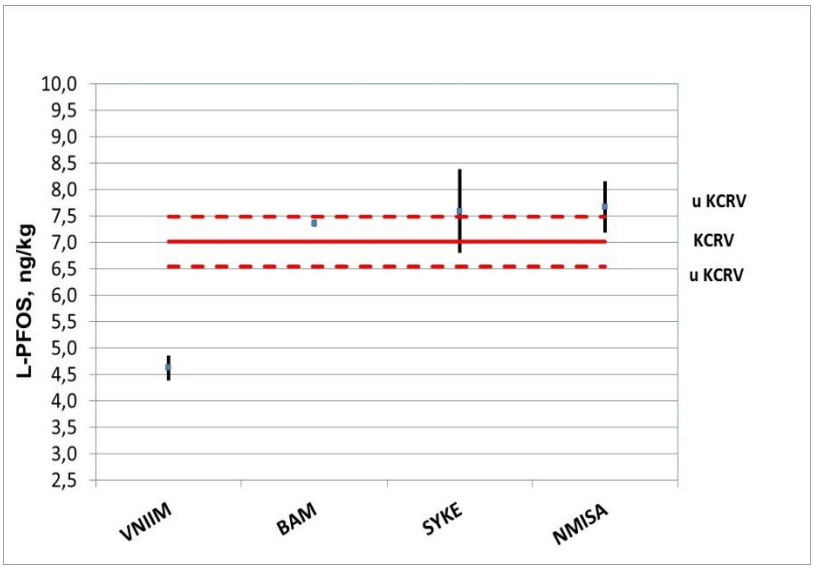


Figure 12. KCRV for L-PFOS

Figure 12 display the KCRVs relative to the reported results for L-PFOS. The results are sorted by increasing reported value. Dots represent the reported mean values, x; bars their standard uncertainties, $u(x)$. The red horizontal line denotes the candidate KCRV. The bracketing red lines denote the standard uncertainty of the candidate KCRV.

DEGREES OF EQUIVALENCE (DoE)

The absolute degrees of equivalence for the participants in CCQM-K156.1 are estimated as the signed difference between the combined value and the KCRV: $d_i = x_i$ – KCRV.

The document CCQM/13-22 Guidance note: Estimation of a consensus KCRV and associated Degrees of Equivalence, 11-Apr-2013 [2], describes the DoE estimations.

The following paragraph provides an explanation of how the uncertainty of the DoE ($U_{k=2}(d_i)$) is determined with the inclusion of covariance [2]. Since the KCRV is estimated from consensus of the results, the nominal k=2 expanded uncertainty on the d_i, $U_{k=2}(d_i)$, is estimated as twice the square root of the sum of the squares of the standard uncertainties of the two components minus twice the covariance between the x_i and the KCRV:

$$U_{k=2}(d_i) = 2\sqrt{u^2(x_i) + u^2(\text{KCRV}) - 2\text{cov}(x_i, \text{KCRV})}$$

To enable comparison with the degrees of equivalence estimates from other studies, it is convenient to express the d_i and $U_{k=2}(d_i)$ as percentages relative to the KCRV: $\%d_i = 100 \cdot d_i/\text{KCRV}$ and $U_{k=2}(\%d_i) = 100 \cdot U_{k=2}(d_i)/\text{KCRV}$.

Table 13 below lists the numeric values of d_i, $U_{95}(d_i)$, d_i, and $U_{95}(d_i)$ for all participants in CCQM-K156.1 for L-PFOA and Table 14 lists the numeric values of d_i, $U_{95}(d_i)$, d_i, and $U_{95}(d_i)$ for all participants for L-PFOS.

Table 23. Degrees of Equivalence for L-PFOA

NMI/DI	d	$U_{k=2}(d)$	$\%d$	$U_{k=2}(\%d)$
VNIIM	-4.65	1.23	-49.7	13.2
BAM	-1.22	1.05	-13.0	11.3
NMISA	-1.08	1.29	-11.5	13.8
SYKE	-0.32	1.55	-3.42	16.6

Table 14. Degrees of Equivalence for L-PFOS

NMI/DI	d	$U_{k=2}(d)$	$\%d$	$U_{k=2}(\%d)$
VNIIM	-2.39	1.05	-34.1	15.0
BAM	0.35	0.95	5.02	13.6
SYKE	0.58	1.84	8.27	26.2
NMISA	0.66	1.36	9.42	19.4

Figure 13 below graphically illustrates the presentation of both the absolute and relative DoEs for L-PFOA and L-PFOS.

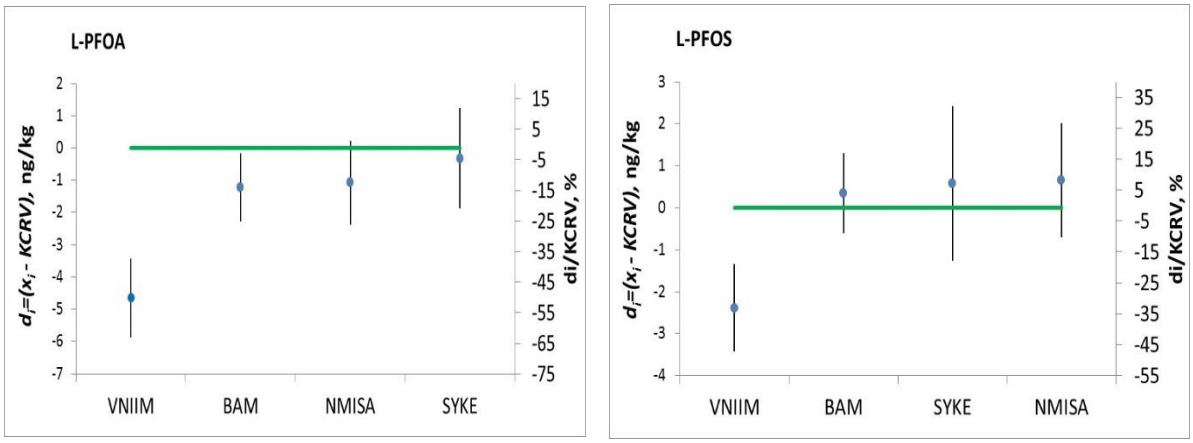


Figure 13. Degrees of Equivalence Associated with KCRV

Figure 13 display the DoE for LPFOA and L-PFOS. All results are sorted by increasing *x*. The axis to the left edge of each panel displays the absolute DoE, *d*, in units ng/kg. The axis to the right edge of each graph displays the relative DoE, 100·*d*/KCRV, as percent. Dots represent the *d*, bars their approximate 95 % expanded uncertainties, $U_{95}(d)$. The green horizontal line denotes perfect agreement with the candidate KCRV.

USE OF CCQM-K156.1 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in CCQM-K156.1 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 200 g/mol to 700 g/mol, having high polarity pKow > -2, in mass fraction range from 0.5 ng/kg to 500 ng/kg in in aqueous media.

Core Competency Statements and CMC support

Appendix E-1 to E-5 list the Core Competencies claimed by the participants in CCQM-K156.1. The information in these Tables are as provided by the participants. Details of the analytical methods used by each participant in this study are provided in Appendix F.

CONCLUSIONS

Participants in CCQM-K156.1 comparisons demonstrated their ability to determine the mass fraction expressed in ng/kg of L-PFOA and L-PFOS in Ground water. Five national metrology institutes and designated institutes participated in this Track C Key Comparison. The results of TUBITAK UME were used for the KCRV and u(KCRV) for this comparison. For L-PFOA, the result of SYKE agreed with KCRV, while the results of VNIIM, BAM and NMISA were biased low. As for L-PFOS, BAM, SYKE and NMISA results agreed with the KCRV, whereas VNIIM result was biased low.

ACKNOWLEDGEMENTS

The study coordinators would like to thank the participating laboratories for their contributions and for providing the requested information used in this study, as well as for the support of Lindsey Mackay, Katrice Lippa and the statics team Mike Nelson, Hugo Gasca Aragon and Benny Tong.

REFERENCES

[1] ISO Guide 35:2017. Reference materials–General and statistical principles for certification.

[2] CCQM/13-22 Guidance note: Estimation of a consensus KCRV and associated Degrees of Equivalence, 11-Apr-2013

APPENDIX A: Call for Participation

Dear OAWG colleagues

UME had very kindly offered to co-ordinate a follow on comparison for CCQM-K156 (PFOS and PFOA in water). This continues to be a high profile measurement area and participation in this KC will underpin a broad range of measurements in aqueous matrices so please do consider this opportunity. The draft protocol is attached. If you have any comments or would like to register could you please email Mine Bilsel on mine.bilsel@tubitak.gov.tr. The current stated timeline for measurements is October to December, however UME are very open to feedback on the working groups preferred timeline for this comparison. If you could copy me in on emails to Mine then we can all agree on the best timeframe for this comparison.

Best regards

Lindsey

APPENDIX B: Protocol

CCQM-K156.1

Determination of L-PFOA and L-PFOS in Ground Water

Key Comparison
Track C

Study Protocol
September 2020

Mine Bilsel, Taner Gökçen, Burcu Binici and Alper Işleyen
TUBITAK UME, National Metrology Institute
Gebze/Kocaeli, 41470 Turkey

INTRODUCTION

Perfluorinated alkyl substances (PFASs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been used in numerous industrial applications and products. However, because of their high stability, and resistance to biodegradation, atmospheric photooxidation, direct photolysis and hydrolysis, they have persistence in the environment. Because of their adverse effect, PFASs are identified as priority hazardous substances in the water framework directive (EU directive 2013/39/EU)[1] and production, use and import of PFOS and its salts in the EU are restricted by EU directive 2006/122/EC[2]. The annual average environmental quality standard (AA-EQS) of PFOS in inland and other surface waters is 0.65 ng/L and 0.13 ng/L, respectively. The maximum annual concentration EQS (MAC-EQS) is 36 µg/L in inland surface water and 7.2 µg/L in other surface waters. The European Union has proposed PFOA, its salts and PFOA-related substances that can be degraded to PFOA under environmental conditions, be included in the Annex A to the Stockholm Convention on Persistent Organic Pollutants (Council decision (EU) 2015/633)[3].

This Track C comparison will be used to assess the core competencies of the National Metrology Institutes/Designated Institutes (NMIs/DIs) for provision of measurement services of perfluorinated alkyl substances (PFAS) in water. This study will test the capabilities of participants for assigning the mass fraction of high-polarity analytes(pKow>-2) with molecular mass range from 200 g/mol to 700 g/mol at levels of 0.5 ng/kg to 500 ng/kg in aqueous media. These are core challenges for reference material producers and providers of calibration services. Evidence of successful participation in formal, relevant international comparisons are needed to document measurement capability claims (CMCs) made by national metrology institutes (NMIs) and designated institutes (DIs).

A subsequent comparison to CCQM-K156 will be offered to those NMIs or DIs who did not get the chance to participate in this comparison or who would like to repeat this measurement challenge.

TIMELINE

Table 1 lists the timeline for the proposed study.

Table 1: Timeline of CCQM-K156.1

Date	Action
June 2020	Sample Preparation
July 2020	Homogeneity and Stability Testing
October 2020	Call for participation to OAWG members
November 2020	Sample Distribution

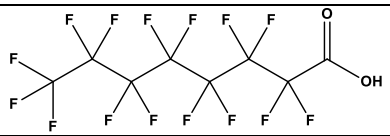
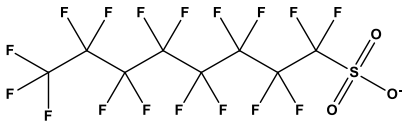
30th June 2021	Deadline for Submission of Results
October 2021	Preliminary Discussion of Results

MEASURANDS

The measurand of this study is the same as for CCQM-K156, mass fraction of linear forms for perfluoro-n-octanoic acid (L-PFOA) as acidic form and linear perfluoro-1-octane sulfonate (L-PFOS) as anion form in ground water. The indicative values are between mass fraction of 0.5 ng/kg and 20 ng/kg.

Table 2 below displays information of these compounds.

Table 2 Information of L-PFOA and L-PFOS

	L-PFOA	L-PFOS
CAS	335-67-1	45298-90-6
Molecular formula	$C_8HF_{15}O_2$	$C_8F_{17}O_3S^-$
M_W	414.07	499.12
pK_{ow}	NA	NA
Structure		

STUDY MATERIALS

The test material is ground water that was collected at 40°55'16.8" N, 29°12'43.6" E from a 250 m deep well at Kartal, Istanbul Turkey. This well supplies a significant amount of ground water that is used to fill swimming pools and for irrigation of gardens in Istanbul. The ground water was collected directly from the well into cleaned carboys and then transported to TUBITAK UME for further processing. Upon arrival at TUBITAK UME, samples were stored at -20 °C. Based on the preliminary results it was concluded that the raw material had almost none of the target analytes, thus it was decided to spike the material to reach target levels for the analytes. The approximate concentration of four anions in the raw material was also determined by ion chromatography (Dionex, ICS-3000, USA) and the results summarized in Table 3.

Table 3 Anion content of the candidate ground water sample

Anion Concentration, n=10 (mg/L)			
F^-	Cl^-	NO_3^-	SO_4^{-2}
0.164	115	1.50	214

The indicative range for the mass fractions of the analyte is 0.5-500 ng/kg. Perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), potassium perfluoro-1-butanesulfonate (PFBS) and sodium perfluoro-1-hexanesulfonate (PFHxS) are also spiked as interferences. The homogenized water was separately dispensed into HDPE bottles to give about 154 bottles, with content of approximately 100 g each. Packing was in vacuum-sealed clear bags. Long term storage of the material at TUBITAK UME is at about 4ºC.

Recommended Minimum Sample Amount

The recommended minimum sample amount for analysis is 100g. The material was bottled considering single use therefore the measurement should be conducted using the entire contents of the bottle.

Dry Mass Determination (where relevant)

For sample preparation, it is adviced to rinse sample bottle with the solvent to wash away the analytes left on the walls of bottle. After this application, empty bottle should be dried at 40 °C and reweighed with the cap, so that the net mass of sample could be calculated.

Homogeneity Assessment of Study Material

All samples were kept at the storage condition of 4ºC by TUBITAK UME. 16 bottles of samples were taken randomly, and since samples are for single use and MS_{within} is not possible, approach described in Alternative Strategies section of ISO Guide 35:2017[4] is performed on bulk material prepared from 16 samples. MS_{within} is calculated from the measurement results variance of 16 subsamples prepared from this bulk material. For each subsample 3 instrumental repetitions was done. $MS_{between}$ is calculated from the measurement results variance of 16 units selected by stratified sampling scheme covering the whole batch, while the absolute values were transformed relative to the mean.

The measurements were performed under repeatability conditions, using validated methods and according to a random sequence to clarify the possible trends in analytical sequence and filling order. The evaluation of homogeneity and calculation of uncertainty between and within bottles was performed by Equation 1 using approach described in “Alternative Strategies” section of ISO Guide 35:2017 which is recommended for single use samples.

The s_{bb} is calculated using the equation given below;

$s_{bb}^2 = \frac{MS_{between} - MS_{within}}{n}$, $n = 1$ Eq.1

$s_{bb} = u_{bb}$

where,

u_{bb}: Standard uncertainty related to possible between-bottle heterogeneity
$MS_{between}$: Mean of square of variance between units

MS_{within}: Mean of square of variance within the unit n: Number of measurements within unit

The results of homogeneity is summarized in Table 4. The absolute values were transformed relative to the means and presented in Figure 1-4.

Table 4 Summary of homogeneity test

	L-PFOA	L-PFOS
$u_{bb,rel}(\%)$	2.30	2.37

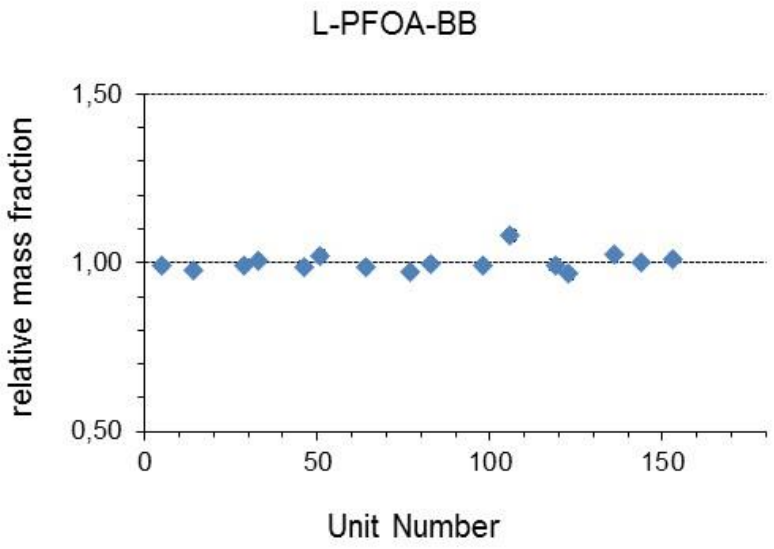


Figure 1. Between Bottle Homogeneity evaluation for L-PFOA

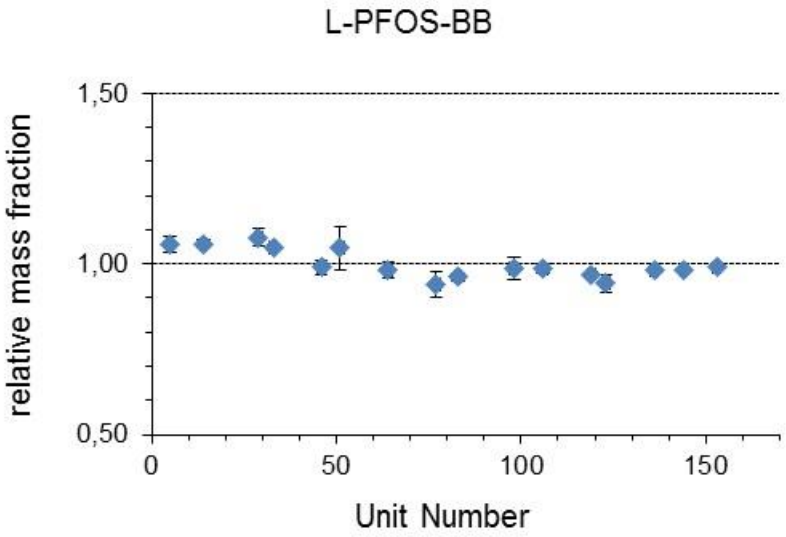


Figure 2. Between Bottle Homogeneity evaluation for L-PFOS

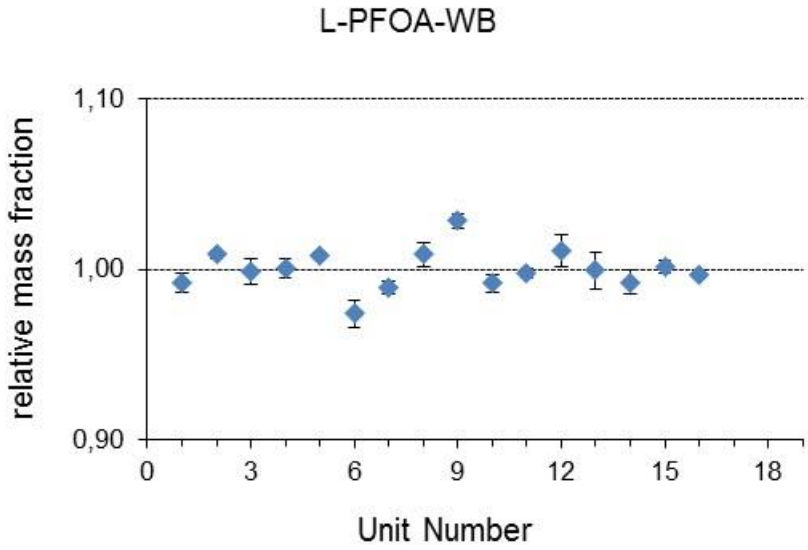


Figure 3. Within Bottle Homogeneity evaluation for L-PFOA

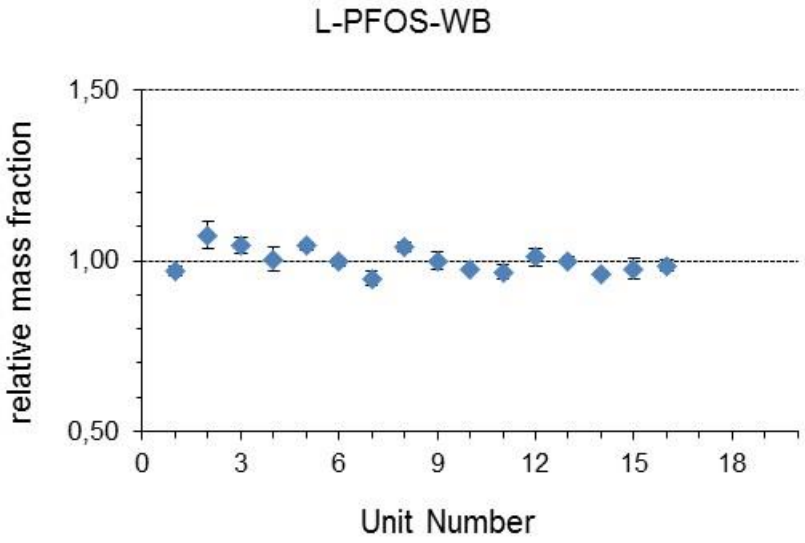


Figure 4. Within Bottle Homogeneity evaluation for L-PFOS

Stability Assessment of Study Material

Stability studies were performed with isochronous design which is cited in ISO Guide 35. For the Short Term Stability (STS) test, at temperature +60˚C and 5 time points (0, 1, 2, 3 and 4 weeks) were tested. 20 units were selected by using a stratified sampling scheme covering whole batch. 20 samples were subjected to the test temperatures for the specified time intervals.

For the Long Term Stability, the results from the previous study (CCQM-K156) were used.16 units for each laboratory (UME and SYKE) were tested at +18˚C for 0, 3, 6 and 12 months time points.

Units were moved to +4˚C (reference temperature) after completion of the test time. All units were analysed at the same time. Samples were prepared based on the single use for each unit and were analyzed using LC-HRMS under the repeatability conditions to determine the mass fraction of L-PFOA and L-PFOS.

The data for each temperature were first examined by single Grubbs test for both 95% and 99% confidence intervals to find out outliers. Number of detected outliers are given in the Table 6 for STS and Table 7 for LTS. Since no technical reason can be found to reject these data, the outlying result were included in the calculations.

Values calculated for each time point were plotted against the time. The relationship between variables were analyzed in order to determine if any significant change exists with the testing time (regression analysis). For STS assessment, it was found that the slopes were significant for both L-PFOS and L-PFOA reported (except for 99% confidence interval). The trend graphs of short term stability are shown in Figure 5-6.

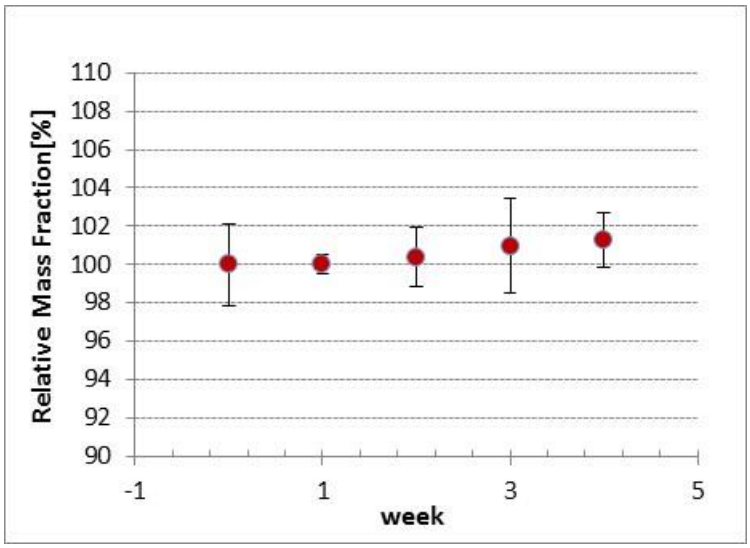


Figure 5 Short-term stability of L-PFOA at 60°C

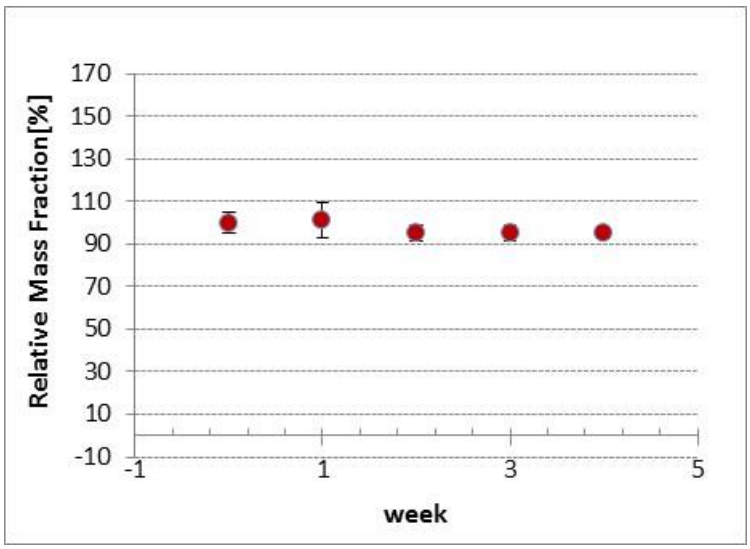


Figure 6 Short-term stability of L-PFOS at 60°C

As a consequence for the cases for which the slopes were significant, degradation is taken into account in the calculation of u_{sts} and u_{lts}. Uncertainty calculations were done using Equation 2. Maximum time for transfer is chosen as 2 weeks for STS and shelf life is chosen as 12 months for LTS.

$$u_{sts}\ or\ u_{lts} = \frac{RSD}{\sqrt{\sum(t_i-\bar{t})^2}}\ x\ t$$

Eq.2

where,

RSD: Relative standard deviation of the slope on stability regression plot

t_i : time point for each replicate

$\bar{t}$: mean of all time points

t : maximum transfer time suggested for STS or shelf life suggested for LTS

Results are summarized in Table 5 and Table 6.

Table 5 Short Term Stability Test Results

Parameter	**60 °C $u_{sts,rel}$ for 2 weeks (%)[2]**	**Number of outliers in 95% confidence interval[1]**	**Number of outliers in 99% confidence interval[1]**	**Is there a significant trend in 95% confidence interval?**	**Is there a significant trend in 99% confidence interval?**
		60 °C	**60 °C**	**60 °C**	**60 °C**
L-PFOS	2.08	1	1	Yes	Yes
L-PFOA	0.51	-	-	Yes	No

[1]Single Grubbs Test
[2] u_{sts} was calculated considering the degradation

Table 6 Long Term Stability Test Results

Parameter (Lab)	**18 °C $u_{lts,rel}$ for 12 months (%)**	**Number of outliers in 95% confidence interval[1]**	**Number of outliers in 99% confidence interval[1]**	**Is there a significant trend in 95% confidence interval?**	**Is there a significant trend in 99% confidence interval?**
		18 °C	**18°C**	**18 °C**	**18°C**
L-PFOS (TUBITAK)	1.5	1	1	No	No
L-PFOS (SYKE)	2.7	-	-	No	No
L-PFOA (TUBITAK)	1.1*	-	-	Yes	No
L-PFOA (SYKE)	2.3	-	-	No	No

*Slope is found to be significant at 95 % confidence interval, u_{lts} is calculated by taking into account the degradation (u_{lts}= slope of reg. line/√3)

The trend graphs of Long Term Stability are shown in Figures 7-8.

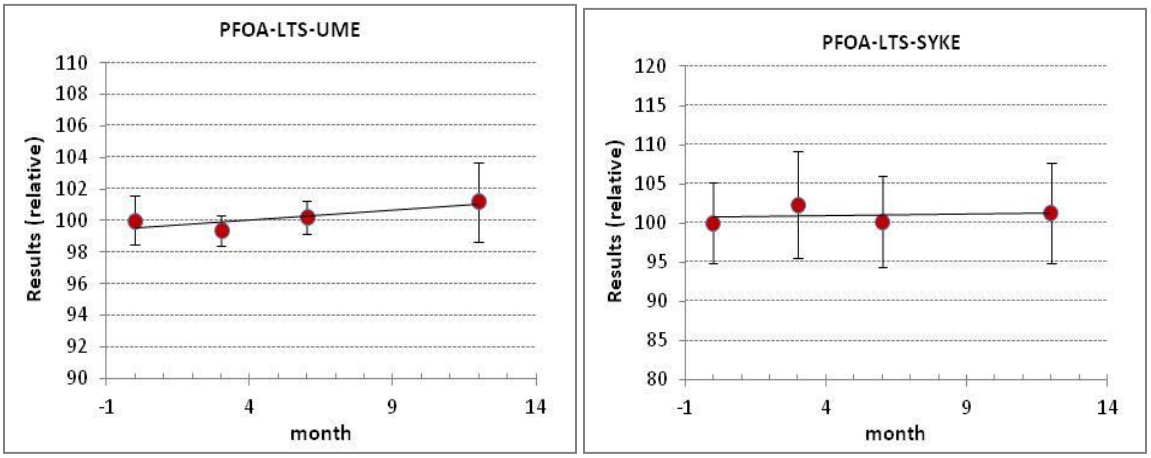


Figure 7 Long-Term Stability of L-PFOA

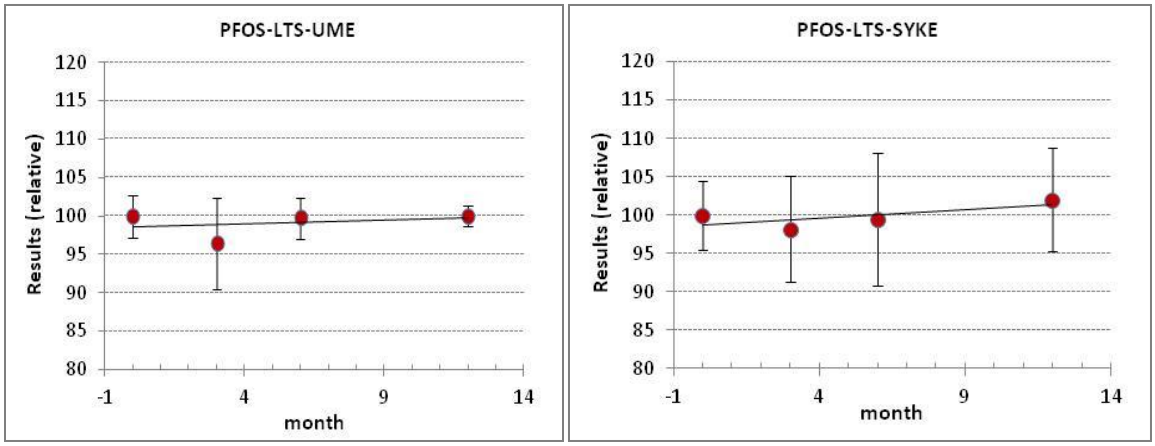


Figure 8 Long-Term Stability of L-PFOS

Results of this study showed that the test material can be transferred to the laboratories without applying any cooling precaution if the temperatures do not exceed 60°C for up to 2 weeks.

INSTRUCTIONS AND SAMPLE DISTRIBUTION

Each participant will receive 5 bottles, each containing approximately 100 g of ground water. Samples will be shipped at room temperature and should be stored at 4°C until analysis. At the time of sample dispatch, a sample receipt form will be provided electronically to all participants and must be filled in and returned to the study coordinator on receipt of the shipments. If there is any damage to the sample, TUBITAK UME will send a substitute sample on request. After receiving the sample, to avoid any decomposition, samples should be kept sealed until they are used. They should be stored at +4°C in the original bottle, tightly capped and not exposed to intense direct light and ultraviolet radiation. The Samples should be equilibrated to room temperature before the analysis. The measurement should be carried out immediately after opening the samples.

RESULTS

Participants will be requested to report a single estimate of the mass fraction in ng/kg for L-PFOA and L-PFOS in ground water. The reported mass fraction will be the overall mean from measurements of five separate units, reporting should include the values of the individual units in addition to the overall mean. All results must be linked to the TUBITAK UME sample identification number (unit number). The completed table should be submitted together with the measurement result. Please complete and submit Report Form and the Core Competency Table to

TUBITAK UME (E-mail: taner.gokcen@tubitak.gov.tr and mine.bilsel@tubitak.gov.tr) by e-mail before the scheduled deadline.

In addition to the quantitative results, participants will be instructed to describe their analytical methods, approach to uncertainty estimation, and the Core Competencies they felt were demonstrated in this study.

Available Calibration Materials

Participants may establish the metrological traceability of their results using certified reference materials (CRMs) with stated traceability and/or commercially available high purity materials for which they determined the purity. Table 7 lists the CRMs that are available for use for this study.

Table 7 : Certified Reference Materials Available for Use

CRM	Provider	Analyte	Mass Fraction[a] Delivered, (kg/g)	Mass Fraction[a] Source Material, %	In-house Purity Methods Used to Value-Assign Source Material[b]
CRM 4056-a	NMIJ	PFOA	0.959 ± 0.005 (kg/g)	-	-
CRM 4220-a	NMIJ	PFOS	9.93 ± 0.15 (mg/kg)	-	-

a Stated as Value ± U_{95}(Value)

Stable isotope internal standards for both PFOA and PFOS are available.

USE OF CCQM-K156.1 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in CCQM-K156.1 will demonstrate the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 200 g/mol to 700 g/mol, having high polarity pKow > -2, in mass fraction range from 0.5 ng/kg to 500 ng/kg in aqueous media.

Core Competency Statements and CMC support

The Core Competencies are given in Appendix B.

REFERENCES

[1] Directive 2013/39/EC amending for the 30th time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates).

[2] Directive 2006/122/EC amending for the 30th time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates).

[3] Council Decision (EU) 2015/633 of 20 April 2015 on the submission, on behalf of the European Union, of a proposal for the listing of additional chemicals in Annex A to the Stockholm Convention on Persistent Organic Pollutants.

[4] ISO Guide 35:2006. Reference materials–General and statistical principles for certification.

APPENDIX C: Registration Form

CCQM Key Comparison

L-PFOA and L-PFOS in Ground Water
CCQM-K156.1

REGISTRATION FORM

Participation to:

☐ CCQM-K156.1

ORGANISATION / DEPARTMENT / LABORATORY

FULL ADDRESS

CONTACT PERSON

Additional Information to assist with shipping: (e.g. permit requirements if any)

TELEPHONE

FAX

E-MAIL

DATE

Please complete the form and send it back to taner.gokcen@tubitak.gov.tr and mine.bilsel@tubitak.gov.tr before 13.11.2020.

APPENDIX D: Reporting Form

TÜBİTAK UME

Determination of L-PFOA and L-PFOS in Ground Water

CCQM-K156.1

RESULT REPORTING FORM

Please use this excel sheet for reporting.

Report should be send to taner.gokcen@tubitak.gov.tr and mine.bilsel@tubitak.gov.tr electronically by 30th June 2021.

Please fill in all blanks and use the requested units.

Additional information can be given in remarks section or a separate sheet if necessary.

Participant Information:

Name of Institute:	
Postal Address:	
Name of Contact Person:	
Telephone / Fax::	
E-mail Address:	
Date of Reporting:	…./…./2021

Results:

Analytes	Sample Name/ Unit No	Mass Fraction (ng/kg)	Mean Value (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
L-PFOA*						
L-PFOS*						
Remarks						

Please provide results for at least 3 samples

Measurement Equation and Uncertainty Budget

Please give the measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations.

Please describe individual uncertainty contributions and estimation of uncertainties for each factor. Please give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Additional information, observations or comments

Analytes	Sample Name/ Unit No	Mass Fraction (ng/kg)	Mean Value (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
L-PFOA*						
L-PFOS*						
Remarks						

*lease provide results for at least 3 samples

Measurement Equation and Uncertainty Budget

Please give the measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations.

Please describe individual uncertainty contributions and estimation of uncertainties for each factor. Please give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Additional information, observations or comments

Technical Information about the analytical procedure

Sample preparation:

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Sample size used for analysis :					
Please describe how the mass of the total sample in the bottle is determined					
Sample pre-treatment (if applicable) (eg blend preparation, equilibration time)					
Extraction method: (Please briefly describe the extraction procedures, e.g. Liquid/Solid extraction, Soxhlet, etc., solvents, volumes, time, temperature etc.)					
Post extraction clean-up method and the transformation procedures, if any: (e.g., SPE, GPC, etc) (Please briefly describe the clean-up and transformation procedures including any dilutions or concentration steps prior to analysis)					
Derivatization, if any:					
Remarks:					

Instrumental Analysis:

Analytical instrument(s) used: (e.g. LC-MS/MS, GC-MS, etc. Please specify the model)	
Chromatographic column(s):	
The chromatographic condition(s): (e.g. GC oven temperature programme, LC Mobile phase gradient, etc)	
Indicate ions /MRMs measured in the mass spectrometer instrument:	Calibrants: IS:
Others:	

Calibration and Quantification:	L-PFOA	L-PFOS
Method of quantification: (e.g. external calibration, Internal standard calibration, IDMS, etc)		
Type of calibration: (e.g. single-point, bracketing, three-point calibration curve, etc.)		
Native calibration standards: (source, confirmation of identity, value assignment, uncertainty and traceability)		
Batch number of calibrants or reference materials:		
Reference material used for calibration is in compliance with the requirements for Traceability in CIPM MRA (Document No.: CIPM 2009-24; Latest update: Revised 13 Oct. 2009):	Yes/ No	Yes/ No
Internal standards used: Please specify the compounds, and at which stage of analysis were the internal standards added		
Information about quality control sample(s):		
Remarks:		

NA can be used for not applicable areas

Remarks: Please write your comments regarding issues such interferences, accuracy, small changes during the measurement.

Date:

APPENDIX E: Core Competency Tables

CCQM OAWG: Competency Template for Analyte(s) in Matrix

CCQM-K156.1	***BAM***	**Determination of L-PFOA and L-PFOS in Ground Water**
Scope of Measurement: This Key Comparison will demonstrate the capabilities of participants for assigning mass concentration of analytes with molecular mass range from 200 to 700 g/mol in aqueous matrices at the 0.5-500 ng/kg mass concentration levels.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	x	NMIJ CRM 4056-a NMIJ CRM 4220-a
Identity verification of analyte(s) in calibration material.#	**X**	Retention time and MRMs
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	**N/A**	
For calibrants which are a calibration solution: Value-assignment method(s).#	**N/A**	
Sample Analysis Competencies		
Identification of analyte(s) in sample	x	Retention time and MRMs
Extraction of analyte(s) of interest from matrix	**x**	Solid phase extraction – weak anion exchange. SPE: 3 mL Strata X-AW 33 µm, 60 mg cartidges
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	**x**	Solid phase extraction – weak anion exchange. SPE: 3 mL Strata X-AW 33 µm, 60 mg cartidges
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	**N/A**	
Analytical system	**x**	Agilent 1260 (Binary pump, autosampler, thermostatted column compartment, 1200 diode array detector) with AB Sciex TSQ 6500 mass spectrometer in ESI negative mode
Calibration approach for value-assignment of analyte(s) in matrix	**x**	a) Isotope dilution calibration b) Five-point calibration curve (2.5 - 12.5 ng/L)
Verification method(s) for value-assignment of analyte(s) in sample (if used)	**x**	Determination of recovery rate of spiked water samples
Other	**N/A**	

The result reported by BAM for L-PFOA was not consistent with the KCRV at the 95% confidence interval and the DoE did not cross zero.

CCQM OAWG: Competency Template for Analyte(s) in Matrix

CCQM-K156.1	***VNIIM***	**Determination of L-PFOA and L-PFOS in Ground Water**
Scope of Measurement: This Key Comparison will demonstrate the capabilities of participants for assigning mass concentration of analytes with molecular mass range from 200 to 700 g/mol in aqueous matrices at the 0.5-500 ng/kg mass concentration levels.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	√	**NMIJ CRM 4056-a** **NMIJ CRM 4220-a**
Identity verification of analyte(s) in calibration material.#	N/A	***Indicate method(s) you used to identify analyte(s)***
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	***Indicate how you established analyte mass fraction/purity (i.e., mass balance (list techniques used), qNMR, other)***
For calibrants which are a calibration solution: Value-assignment method(s).#	√	**In accordance with Certificate of Analysis NMIJ CRM 4056-a and NMIJ CRM 4220-a**
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	Retention time, MRMs
Extraction of analyte(s) of interest from matrix	√	SPE (with EVOLUTE EXPRESS WAX Cartridge 150 mg , 6 ml) was used for extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	N/A	SPE (with EVOLUTE EXPRESS WAX Cartridge 150 mg , 6 ml) was used for cleanup
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	*Indicate chemical transformation method(s), if any, (i.e., hydrolysis, derivatization, other)*
Analytical system	√	LC-MS/MS Agilent Technologies Triple Quad 1200/6460
Calibration approach for value-assignment of analyte(s) in matrix	√	a) IDMS b) single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	√	Method of standard addition - the PFCs were added directly to the aliquot of pure water
Other	N/A	*Indicate any other competencies demonstrated.*

The result reported by VNIIM for L-PFOA and L-PFOS was not consistent with the KCRV at the 95% confidence interval and the DoE did not cross zero.

CCQM OAWG: Competency Template for Analyte(s) in Matrix

CCQM-K156.1	***SYKE***	**Determination of L-PFOA and L-PFOS in Ground Water**
Scope of Measurement: This Key Comparison will demonstrate the capabilities of participants for assigning mass concentration of analytes with molecular mass range from 200 to 700 g/mol in aqueous matrices at the 0.5-500 ng/kg mass concentration levels.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	x	L-PFOS: NMIJ CRM 4220-a L-PFOA: NMIJ CRM 4056-a
Identity verification of analyte(s) in calibration material.#	x	Retention time, specific ions at mass spectrometer (MRM)
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	x	By weighing and preparing dilutions with analytical scale (U +/- 0.0006-0.04%) at controlled temperature.
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	x	Retention time, specific ions at mass spectrometer (MRM)
Extraction of analyte(s) of interest from matrix	x	Solid-phase extraction (weak anion exchange)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	x	Solid-phase extraction (weak anion exchange)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	x	UHPLC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	x	a) IDMS b) 8-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	x	CRM IRMM-428-4
Other	N/A	

CCQM OAWG: Competency Template for Analyte(s) in Matrix

CCQM-K156.1	*NMISA*	**Determination of L-PFOA and L-PFOS in Ground Water**
Scope of Measurement: This Key Comparison will demonstrate the capabilities of participants for assigning mass concentration of analytes with molecular mass range from 200 to 700 g/mol in aqueous matrices at the 0.5-500 ng/kg mass concentration levels.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	*X*	*PFOA – Highly pure material - CRM procured from National Metrology Institute of Japan (NMIJ CRM 4056-a) PFOS – Calibration solution - CRM procured from National metrology Institute of Japan (NMIJ CRM 4220-a)*
Identity verification of analyte(s) in calibration material.	*X*	*Verification of NMIJ CRM against Wellington commercial standards and through mass spectral ion ratios from MRMs and retention times using Liquid Chromatography – tandem mass spectrometry (LC-MS/MS)*
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	*X*	*Retention time and mass spec ion ratios, verified against NMIJ standard*
Extraction of analyte(s) of interest from matrix	*X*	*Agitation at increased temperature and ultrasonic extraction*
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	*X*	*SPE: Weak anion exchange*
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	X	*ESI LC-MS/MS (SCIEX QTRAP® 6500+ System)*
Calibration approach for value-assignment of analyte(s) in matrix	X	*a) IDMS* *b) Bracketing double IDMS*
Verification method(s) for value-assignment of analyte(s) in sample (if used)	X	*Verification against NMIJ CRM and carbon-labeled isotopes; through mass spectral ion ratios from MRMs and retention times using Liquid Chromatography – tandem mass spectrometry (LC-MS/MS)*
Other	N/A	

CCQM OAWG: Competency Template for Analyte(s) in Matrix

CCQM-K156.1	***TUBITAKUME***	**Determination of L-PFOA and L-PFOS in Ground Water**
Scope of Measurement: This Key Comparison will demonstrate the capabilities of participants for assigning mass concentration of analytes with molecular mass range from 200 to 700 g/mol in aqueous matrices at the 0.5-500 ng/kg mass concentration levels.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	√	L-PFOA, NMIJ 4056-a L-PFOS, NMIJ 4220-a
Identity verification of analyte(s) in calibration material.#	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	Retention time and MRMs
Extraction of analyte(s) of interest from matrix	√	Solid phase extraction (SPE)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	Solid phase extraction (SPE)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	√	HR-LC-MS (Thermo Orbitrap Q Exactive)
Calibration approach for value-assignment of analyte(s) in matrix	√	IDMS, 5-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other		

APPENDIX F: Summary of Participants' Analytical Information

The following Tables summarize the detailed information about the analytical procedures each participant provided in their "Analytical Information" worksheets. The presentation of the information in many entries has been consolidated and standardized.

The participant's measurement uncertainty statements are provided verbatim in Appendix G.

Table F-1: Summary of Sample Size, Extraction, and Cleanup for CCQM-K156.1

Institute	Pre-treatment	Extraction Method	Sample Size (g)	Clean-up
BAM	No sample pre-treatment was applied	SPE: 3 mL Strata X-AW 33 µm, 60 mg cartidges. Conditioning: 2 mL 0.1% NH3 in MeOH, 2 mL MeOH, 2 mL H2O. Load sample with 1.25 mL/min. Wash steps: 2 mL H2O, 2 mL acetone : ACN : formic acid (50:50:1, v/v/v), 2 mL MeOH. Elution: 2 mL 0.1% NH3 in MeOH.	whole sample volume	The extract from the SPE is evaporated with N2 at 40°C to dryness for 30 min. The residue is reconstituted in 1 mL MeOH/H2O (50:50, v/v) and is ready for analysis

Institute	Pre-treatment	Extraction Method	Sample Size (g)	Clean-up
NMISA	The internal standard was added to the sample and allowed to equilibrate for 48 hours on a orbital shaker at 40°C.	The samples were placed in an ultrasonic bath for 20 min. Thereafter the samples were placed on an orbital shaker for 20 min. The sample was acidified using acetic acid and then placed on the shaker for a further 40 min.	100.06, 100.08, 100.05	The samples were extracted using weak anion exchange ion a Strata-X-AW 33µm polymeric weak anion 500 mg/ 6mL cartridge. The cartridge was washed with 0.06 M ammonia in methanol and conditioned with 0.07 M acetic acid in water; the full sample is loaded and the cartridge rinsed with 0.07 M acetic acid in water followed by 0.07 M acetic acid in methanol. The SPE cartridge was dried for 10 min under vacuum. Once the bed was completely dried the SPE was eluted with 0.06 M ammonia in methanol. The eluent was reduced to dryness and the sample was reconstituted in 1:1 methanol and water.

Institute	Pre-treatment	Extraction Method	Sample Size (g)	Clean-up
VNIIM	Sample was equilibrated at room temperature for 30 minutes before any kind of handling	PFCs were concentrated on the cartridge EVOLUTE EXPRESS WAX Cartridge 150 mg 6 ml Conditioning: - 8 ml of 0.1% ammonium solution in methanol - 8 ml of methanol - 8 ml of water - drying by air. The Sample (with IS) was passed through the cartridge at a rate of 1-2 drops per second. Bottle was rinsed with 10 ml of deionized water which was transferred to the cartridge as well. Then cartridge was dried by air. Extraction: - 4 ml of 0.1% ammonia solution in methanol Extract was collected into polypropylene vial and evaporated until dry by nitrogen stream and reconstructed with 200 µl of mix (water :methanol)=(1:1).	99987.65 mg, 100019,24 mg, 100065.23 mg, 100046.17 mg, 100035.27 mg	N/A
SYKE	Spiking of internal standards and pH adjustment with formic acid	Solid-phase extraction with weak anion-exchange cartridges. Elution with 6 ml of basic methanol.	0.10007 kg, 0.10006 kg, 0.10007 kg, 0.10004 kg, 0.10006 kg	N/A

Institute	Pre-treatment	Extraction Method	Sample Size (g)	Clean-up
TUBITAKUME	IS spiked and bottle shaken by hand for 10 minutes and then waited for one hour at room temperature for equilibration.	Extraction method from ISO 25101:2009 followed by using Waters OASIS Wax 150 mg 6 mL cartridge. Cartridge conditioned by 4 mL of ammonia/methanol solution, 4 mL of methanol and lastly 4 mL of water. Whole water sample eluted immediately after conditioning. Cartridge dried under vacuum. Washed with 4 mL acetate buffer solution. Bottle washed with 4 mL ammonia/methanol solution. Cartridge eluted first with 4 mL methanol and then with ammonia/methanol solution used for bottle wash.	97.861g, 97.935g, 98.038g, 97.777g, 98.287g	Concentrated under gentle stream of nitrogen at 40 °C till 200 µL.

Table F-2: Summary of Analytical Techniques for CCQM-K156.1

<table>
<tr><th>Institute</th><th>Analytical Technique</th><th>Chromatographic Column</th><th>Chromatographic and Mass Spectrometry Conditions</th><th>ion/MRM monitored</th></tr>
<tr><td>BAM</td><td>Agilent 1260 (Binary pump, autosampler, thermostatted column compartment, 1200 diode array detector) with AB Sciex TSQ 6500 mass spectrometer in ESI negative mode</td><td>Phenomenex Luna Omega 3 µm PS C18 100 Ä, LC Column 100 x 2.1 mm</td><td>A: 10 mmol NH4Ac in H2O, B: MeOH. Flow rate: 0.35 mL/min, column temperature 35°C, and 50µL injection volume. LC gradient: 75% B (2 min), 75%-25% B (2.00-4.00 min), 25%-5% B (4.00-20.00 min), 5% B (4 min), 5%-75% B (24.00-24.50 min). All reagents and solvents have LC-MS grade.</td><td>Calibrants: L-PFOS: 499.0 to 80.0 m/z, 499.0 to 99.0 m/z. L-PFOA 413.0 to 369.0 m/z, 413.0 to 169.0 m/z. Quantifier ion L-PFOS 99.0 m/z and L-PFOA 169.0 m/z
IS: PFOS (Perfluoro-1-[1,2,3,4-13C4] octanesulfonate): 503.0 to 80.0 m/z. PFOA (Perfluoro-n-[1,2,3,4-13C4] octanoic acid): 417.0 to 372.0 m/z</td></tr>
<tr><td>NMISA</td><td>LC-MS/MS - SCIEX QTRAP 6500+ System</td><td>FluoroSep RP-Octyl FO (5 µm x 150 mm x 2.0 mm i.d.)</td><td>Solvent A (5 MM Ammonium Formate; pH 6.2); Solvent B (Methanol)
Column temperature = 30°C
<table><tr><th>Time</th><th>Flow (ml/min)</th><th>Solvent A</th><th>Solvent B</th></tr><tr><td>Initial</td><td>0.4</td><td>60</td><td>40</td></tr><tr><td>1</td><td>0.4</td><td>60</td><td>40</td></tr><tr><td>25</td><td>0.4</td><td>10</td><td>90</td></tr><tr><td>30</td><td>0.4</td><td>10</td><td>90</td></tr><tr><td>35</td><td>0.4</td><td>8</td><td>92</td></tr><tr><td>40</td><td>0.4</td><td>0</td><td>100</td></tr><tr><td>40.1</td><td>0.4</td><td>60</td><td>40</td></tr><tr><td>50</td><td>0.4</td><td>60</td><td>40</td></tr></table></td><td>Calibrants:
PFOS: 499>80; 499>99;
PFOA: 413>369; 413>169

IS:
M8PFOS: 507>80; 421>172
M8PFOA: 421>376; 507>99</td></tr>
</table>

<table>
<tr><th>Institute</th><th>Analytical Technique</th><th>Chromatographic Column</th><th>Chromatographic and Mass Spectrometry Conditions</th><th>ion/MRM monitored</th></tr>
<tr>
<td>VNIIM</td>
<td>LC-MS/MS
Agilent Technologies
Triple Quad
LC/MS 6460</td>
<td>Kromasil C18 5µm 100Å 150mm X 4.6mm (Nouryon)</td>
<td>Eluent A: Ammonium acetate + Acetic acid buffer (2mM);
Eluent B: Acetonitrile;
Flow: 0,7 ml/min;
Injection volume: 10µl
<table>
<tr><th>Time</th><th>A, %</th><th>B, %</th></tr>
<tr><td>0</td><td>47</td><td>53</td></tr>
<tr><td>1,9</td><td>47</td><td>53</td></tr>
<tr><td>2,0</td><td>25</td><td>75</td></tr>
<tr><td>4,8</td><td>25</td><td>75</td></tr>
<tr><td>4,9</td><td>47</td><td>53</td></tr>
</table>
</td>
<td>
<table>
<tr><td colspan="2">MRMs (negative polarity):</td></tr>
<tr><td colspan="2">Analyts</td></tr>
<tr><td>PFOS</td><td>499→99</td></tr>
<tr><td>PFOA</td><td>413→369</td></tr>
<tr><td colspan="2">IS</td></tr>
<tr><td>For IS (PFOS 8C13)</td><td>507→99</td></tr>
<tr><td>For IS (PFOA 8C13)</td><td>421→372</td></tr>
</table>
</td>
</tr>
<tr>
<td>SYKE</td>
<td>LC-MS/MS;
Waters Acquity UPLC and Xevo TQ MS</td>
<td>Waters Acquity BEH C18</td>
<td>LC mobile phases: A: 2 mM ammonium acetate in water, B: 2 mM ammonium acetate in methanol
Gradient from 70% of A to 0% of A</td>
<td>Calibrants:
PFOA: 413 > 369
PFOS: 499 > 99 and 499 > 80
IS:
13C4 PFOA: 417 > 372
13C4 PFOS: 503 > 99 and 503 > 80</td>
</tr>
</table>

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
TUBITAKUME	HR-LC-MS (THERMO Orbitrap Q Exactive)	Luna 5u C18(2) 100 A 150 x 3 mm 5 µm (before injection) Luna 5u PFP(2) 100 A 150 x 2 mm	Mobile phase A: 20 mM NH4Ac (10% MeOH); Mobile Phase B: MeOH Start with 30% B gradient to 85% in 18 min (hold 2 min), return to 30% B in 1 min (hold 9 min). Flow: 0.2 ml/min Injection: 25 µl Column temperature: 45 °C MS Parameters: Scan range: 60.0 to 750.0 m/z Resolution: 70000 Polarity: negative AGC target: 3e6 Sheath gas flow rate: 35, Aux : 5, Sweep: 0 Spray voltage: 2.50 kV Capillary: 300 °C Aux gas heater temp: 250 °C	Calibrants: PFOA: 412.966 PFOS: 498.931 IS: PFOA-13C4: 416.981 PFOS-13C4: 502.944

Table F-3: Summary of Calibrants and Standards for CCQM-K156.1

Institute	Type of Calibration	Calibrants	Internal Standards
BAM	Internal standard calibration- IDMS Five-point calibration curve (2.5 - 12.5 ng/L)	NMIJ CRM 4056-a Perfluorooctanoic acid NMIJ CRM 4220-a Potassium Perfluorooctanesulfonate in methanol	Wellington 13C-labeled PFOA (Perfluoro-n-[1,2,3,4-13C4]octanoic acid), 50µg/mL (in MeOH). Internal standard is directly added to the aqueous sample Wellington 13C-labeled PFOS (Perfluoro-1-[1,2,3,4-13C4] octanesulfonate), 50µg/mL (in MeOH). Internal standard is directly added to the aqueous sample
NMISA	Double – IDMS Bracketing	CRM procured from National metrology Institute of Japan (NMIJ CRM 4056-a) CRM procured from National metrology Institute of Japan (NMIJ CRM 4220-a)	M8PFOA - added at the beginning of the extraction procedure M8PFOS- added at the beginning of the extraction procedure
VNIIM	IDMS Single-point	NMIJ CRM 4056-a (0.959±0.005) kg/kg NMIJ CRM 4220-a (9.93±0.15) mg/kg	CIL PFOA (13C8, 99%), Lot#:SEAD-010 50±0,5 (µg/mL) CIL PFOS (13C8, 99%), Lot#:SEAD-013 50±0,5 (µg/mL)
SYKE	IDMS Eight-point calibration curve	Wellington PFC-MXB NMIJ CRM 4056-a Wellington PFC-MXB NMIJ CRM 4220-a	13C4-PFOA; added in the beginning of the analysis (before pH adjustment and extraction) 13C4-PFOS; added in the beginning of the analysis (before pH adjustment and extraction)

Institute	Type of Calibration	Calibrants	Internal Standards
TUBITAKUME	IDMS 5 point calibration	NMIJ, NMIJ-4056a, 0.959 ± 0.005 kg/kg, SI traceable	Wellington MPFAC-MXA mix solution used, added before extraction
		NMIJ, NMIJ-4220a, 9.93 ± 0.15 mg/kg (potassium salt value), SI traceable	Wellington MPFAC-MXA mix solution used, added before extraction

Table F-4 Assessment and Verification Methods for CCQM-K156.1

Institute	Purity Assessment	Result Verification
BAM	N/A	Determination of recovery rate of spiked water samples
NIMSA	N/A	Verification against NMIJ CRM and carbon-labeled isotopes; through mass spectral ion ratios from MRMs and retention times using Liquid Chromatography – tandem mass spectrometry (LC-MS/MS)
VNIIM	N/A	Method of standard addition - the PFCs were added directly to the aliquot of pure water
SYKE	N/A	CRM IRMM-428-4
TUBITAKUME	N/A	

Table F-5: Additional Comments for CCQM-K156.1

Institute	Additional Comments
BAM	N/A
NMISA	Three samples from bracketing experiment were used for reporting There was PFOA detected as a background contaminant during the extraction procedure. The contribution was stable and could be accounted for. No background contamination was observed for PFOS.
VNIIM	Samples No. 0019 and 0102 were used for the preliminary experiments Polypropylene laboratory glassware was used at all stages of Sample preparation and analysis (no glass, Teflon, metal etc.) Quality control samples were prepared by adding the both analytes to 100 ml of deionized water at levels about Samples contained. The differences between added and measured values were not more then 7 %. This is less than U of measurements and due to that this contribution was not incorporated into U. PFOA was found into the blank Samples and it was accounted in the Uncertainty budget.
SYKE	N/A
TUBITAKUME	Wellington mixed mass labelled PFACs and PFASs (MPFAC-MXA) used as internal standard. PFOA: 13C4, PFOS: 13C4. For PFOS native solution is potassium salt and IS solution is sodium salt, mass corrections applied for anion conversion. Eventhough a column connected prior to injection port for delay of interferences from the system a small peak observed for PFOA, a blank correction applied for PFOA results.

APPENDIX G: Summary of Participants' Uncertainty Estimation Approaches

The following are text excerpts and/or pictures of the uncertainty-related information provided by the participants in the reporting form. Information is grouped by participant and presented in alphabetized acronym order.

Uncertainty Information from BAM

CCQM K156.1 L-PFOS in water

uncertainty budget

measurement equation: $x_{sample} = \frac{r - i_c}{sl} \cdot \frac{m_{is}}{m_{sample}} \cdot F_{ex} \cdot F_{purity}$ all F = 1

symbol	parameter description	uncertainty estimate	u_rel L-PFOS
r	area ratio native/internal std	residual scatter of calibration	0,003
i_c	intercept of calibration line	estimate combining u(ic), u(sl), and covar	0,009
sl	slope of calibration line		
m_is	mass of internal standard added to sample	SD of replicate weighings, converted to u rel	0,003
m_sample	mass sample	SD of replicate weighings of mass standard, converted to u rel	0,001
F_ex	extraction variability	repeatability SD of replicate determinations, converted to u_rel	0,033
F_purity	purity correction factor	certificate uncertainty NMIJ CRM 4220-a	0,008
x_sample	**result**		**0,035**

expansion factor k = 2

expanded relative uncertainty U:	0,071
expanded uncertainty U ng/kg:	0,142
standard uncertainty ng/kg	0,071

CCQM K156.1 L-PFOA in water

uncertainty budget

measurement equation: $x_{sample} = \frac{r - i_c}{sl} \cdot \frac{m_{is}}{m_{sample}} \cdot F_{ex} \cdot F_{purity}$ all F = 1

symbol	parameter description	uncertainty estimate	u_rel L-PFOA
r	area ratio native/internal std	residual scatter of calibration	0,012
i_c	intercept of calibration line	estimate combining u(ic), u(sl), and covar	0,084
sl	slope of calibration line		
m_is	mass of internal standard added to sample	SD of replicate weighings, converted to u rel	0,003
m_sample	mass sample	SD of replicate weighings of mass standard, converted to u rel	0,001
F_ex	extraction variability	repeatability SD of replicate determinations, converted to u_rel	0,041
F_purity	purity correction factor	certified uncertainty NMIJ CRM 4056-a Perfluorooctanoic acid	0,003
x_sample	**result**		**0,094**

expansion factor k = 2

expanded relative uncertainty U:	0,188
expanded uncertainty U ng/kg:	0,376
standard uncertainty ng/kg	0,188

Uncertainty Information from NMISA

$$W_x = W_z \times \frac{m_z}{m_{yc}} \times \frac{m_y}{m_x} \times \frac{R'_B}{R'_{BC}}$$

W_x = The final concentration of the native substance in the sample solution

W_z = The concentration of the native CRM calbration blend added to the calibration solution

m_z = The weight of the calibration blend added to the calibration solution

m_{yc} = The weight of the isotope added to the calibration solution

m_y = The weight of the isotope added to the sample

m_x = The weight of the sample

R'_B = Peak area of native in the sample divided by the peak area of the isotope in the sample

R'_{Bc} = Peak area of native in the calibration solution divided by the peak area of the isotope in the calibration

PFOA - Uncertainty		x	u	u/x	u/x2	
Wz	[native] solution added to calibration blend (ug/ g)	4.95539	1.718E-02	3.467E-03	1.202E-05	
mz	weight native solution added to calibration blend (g)	0.17305	1.000E-04	5.779E-04	3.339E-07	
my	weight of Isotope solution added to sample (g)	0.16859	1.000E-04	5.932E-04	3.518E-07	
myc	weight of Isotope solution added to calibration blend (g)	0.16844	1.000E-04	5.937E-04	3.525E-07	
mx	Mass of sample analysed	100.063	1.000E-04	9.994E-07	9.987E-13	
RB	ratio of peaks areas of native/ labelled in the samples	0.90705	2.174E-02	2.397E-02	5.744E-04	
RBC	ratio of peaks areas of RM native/ labelled in the calibration blend	0.93044	1.931E-02	2.075E-02	4.306E-04	
Branched interferences	Addition of +/-6.7 % uncertainty for branched co-elutions	1	3.868E-02	3.868E-02	1.496E-03	
Precision	Repeat measurements	8.32127	8.567E-02	1.030E-02	1.060E-04	
					0.00262	
					0.42	u
					0.84	U (k=2)
					10.15	Rel U

PFOS - Uncertainty		x	u	u/x	u/x2	
Wz	CRM uncertainty	9.93	7.500E-02	7.553E-03	5.705E-05	
mz	Weight of calibration blend (g)	0.1161675	1.394E-04	1.200E-03	1.440E-06	
my	Weight of Isotope added to sample (g) - ESDM of 3 spikes	0.105966667	4.421E-04	4.172E-03	1.741E-05	
myc	Weight of Isotope added to calibration blend (g) -ESDM of 3 spikes	0.107055	1.965E-04	1.836E-03	3.369E-06	
mx	Weight of sample (g) - ESDM of the 3 sample weights used	100.0628933	9.582E-03	9.576E-05	9.170E-09	
R'B/R'BC	Ratio of peaks areas of native/ labelled in the samples - STD DEV	0.993899569	4.717E-02	4.746E-02	2.252E-03	
Precision	Stdev of bracket calcs	0.007665387	1.324E-04	1.727E-02	2.982E-04	
Branched interferences	Addition of +/-6.7% uncertainty for branched co-elutions	1	3.868E-02	3.868E-02	1.496E-03	
					0.00413	
					0.00049	u
					0.00098	U
					12.79	%Rel U

$$\omega_{PFC} = \frac{m_{PFC}}{m_{sample}} = \frac{\frac{S_{PFC} * m_{IS}}{S_{IS} * RF}}{m_{sample}}$$

ω_{PFC} - mass fraction of PFOA or PFOS (ng/kg)
m_{PFC} - mass of PFOA or PFOS (ng)
m_{sample} - mass of the Sample (kg)
S_{PFC} - PFOA or PFOS peak area (conventional units)
S_{IS} - Internal Standard peak area (conventional units)
m_{IS} - mass of Internal Standard (ng)
RF - average Response Factor

$$u_{PFC} = \sqrt{u_A^2 + u_B^2} = \sqrt{S_1^2 + S_2^2 + u_{m\ sample}^2 + u_{m\ IS}^2 + u_{calibration\ solution}^2 + u_{calibrant}^2 + u_{RF}^2 + u_{blank}^2}$$

u_{PFC} - Relative combined standard Uncertainty of the PFOA/PFOS measurement (%)
u_A - Uncertainty type A (%)
u_B - Uncertainty type B (%)
S_1 - RSD of results of three Samples (%)
S_2 - maximum RSD of results of one Sample and three inputs (%)
$u_{m\ sample}$ - Uncertainty of Sample weighing (%)
$u_{m\ IS}$ - Uncertainty of IS adding (%)
$u_{calibration\ solution}$ - Uncertainty of calibration solutions preparing (%)
$u_{calibrant}$ - Uncertainty of calibrant (%)
u_{RF} - RSD of Response Factor (%)
u_{blank} - Uncertainty of blank (%)

Contribution to Uncertainty	Type	PFOA	PFOS
S1	A	2,39	2,42
S2	A	1,86	0,9
$u_{m\ sample}$	A+B	0,004	0,004
$u_{m\ IS}$	A+B	0,18	0,25
$u_{calibration\ solution}$	A+B	0,99	0,69
$u_{calibrant}$	B	0,52	1,51
u_{RF}	A+B	5,24	3,83
u_{blank}	B	4,82	-
Combined Standard Uncertainty (u_{PFC}), %		7,82	4,91
Coverage factor (k)		2	2
Expanded Uncertainty (U), %		15,64	9,83

Uncertainty Information from SYKE

External calibration: analyte concentration = analyte peak area *(ISTD conc / ISTD area)

Mass fraction in sample: Analyte peak area * (ISTD conc / ISTD area) / sample weight

Adding of ISTD: volume 100 µl; uncertainty of the calibrated automatic pipette = +/- 1.4 µl = +/- 1.4%

Weighing of the sample bottle: uncertainty +/- 0.01 g = +/- 0.01%

Weighing of calibration standards: uncertainty +/- 0.0006-0.04% = max 0.04%

RSD of LC-MS (5 replicate injections of the sample extracts): PFOS max 5%; PFOA max 3%

Combined standard uncertainty: PFOS 10.4%; PFOA 6.6%

Expanded uncertainty: PFOS 21%; PFOA 13%

Uncertainty Information from TUBITAK UME

$$C_X = \frac{A_x x n_{ISx}}{A_{isx} x RF x M_{sample}}$$

C_X	: Concentration of analyte in unknown sample (ng/kg)
A_X	: Peak area of analyte in unknown sample
A_{ISX}	: Peak area of labelled analyte
n_{ISX}	: Total amount of added internal standard (ng)
M_{sample}	: Sample mass (g)
RF	: Response Factor

$$\frac{U_c(A)}{C_A} = \sqrt{(\frac{u(W_{SM})}{W_{SM}})^2 + (\frac{u(W_{IS})}{W_{IS}})^2 + (\frac{u(C_{NS})}{C_{NS}})^2 + (\frac{u(c_0)}{c_0})^2 + u(R_m)^2 + u(r)^2}$$

Uncertainty Budget of L-PFOA

Parameters	**Unit**	**Value (X)**	**u(x)**	**u(x)/X**
Mass of starting material	g	97.98	5.33E-05	5.44E-07
Spiking Labelled stock solution	g	0.37	1.95E-07	5.33E-07
Native stock solution		86.83	2.44E-01	2.81E-03
Calibration		4.72	1.30E-02	2.75E-03
Recovery		1.00	3.92E-02	3.90E-02
Repeatability		4.72	3.05E-02	6.47E-03

Relative Standard Measurement Uncertainty			3.98E-02
Result (ng/kg)	9.36		
Combined Standard Measurement Uncertainty		0.37	
Expanded Uncertainty (k=2)		0.74	
Relative Mesurement Uncertainty (%)		7.95	

Uncertainty Budget of L-PFOS

Parameters	Unit	Value (X)	u(x)	u(x)/X
Mass of starting material	g	97.98	5.33E-05	5.44E-07
Spiking Labelled stock solution	g	0.37	1.95E-07	5.33E-07
Native stock solution		91.74	6.93E-01	7.55E-03
Calibration		3.68	8.12E-03	2.21E-03
Recovery		0.94	4.89E-02	5.19E-02
Repeatability		3.68	3.53E-02	9.59E-03

Relative Standard Measurement Uncertainty			5.34E-02
Result (ng/kg)	7.01		
Combined Standard Measurement Uncertainty		0.37	
Expanded Uncertainty (k=2)		0.75	
Relative Mesurement Uncertainty (%)		10.67	

APPENDIX H: Participants' Quantitative Results as Reported

The following are text excerpts and/or pictures of the quantitative results as provided by the participants in the reporting form. Information is grouped by participant and presented in alphabetized acronym order.

Quantitative Results from BAM

Measurand	Mass Fraction (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
L-PFOA	8.139	0.188	2	0.376
L-PFOS	7.362	0.071	2	0.142

Quantitative Results from NMISA

Measurand	Mass Fraction (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
L-PFOA	8.28	0.42	2.0	0.84
L-PFOS	7.67	0.49	2.0	0.98

Quantitative Results from VNIIM

Measurand	Mass Fraction (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
L-PFOA	4.71	0.37	2	0.74
L-PFOS	4.62	0.23	2	0.45

Quantitative Results from SYKE

Measurand	Mass Fraction (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
L-PFOA	9.04	0.60	2	1.20
L-PFOS	7.59	0.79	2	1.58

Quantitative Results from TUBITAK UME

Measurand	Mass Fraction (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
L-PFOA	9.36	0.37	2	0.74
L-PFOS	7.01	0.37	2	0.75