CCQM-K146

Low-Polarity Analyte in high fat food: Benzo[a]pyrene in Olive Oil

Key Comparison

Track A

Draft B Report

December 2019

Hongmei Li, Xiaomin Li, Qinghe Zhang, Xiaohua Lu and Shuangqing Li

National Institute of Metrology, NIM, China

Beijing, China 100029

Michael Nelson

National Institute of Standards and Technology, NIST, United States

With contributions from:

Dr. Matthias Koch

Bundesanstalt für Materialforschung und -prüfung /Department of Analytical Chemistry; Reference Materials/Division of Food Analysls (BAM), Germany

Dr Joachim Polzer, Dr Rudolf Hackenber

Federal Office of Consumer Protection and Food Safety, BVL, Germany

Md. Moniruzzaman, Dr. Mala Khan

Designated Reference Institute for Chemical Measurements (DRiCM), Bangladesh Council of Scientific & Industrial Research (BCSIR)

Dr Elias Kakoulides

EXHM/GCSL-EIM, Chemical Metrology Laboratory, Greece

Dr Kong Pak-wing, Richy; Mr Ng Chi-shing

Government Laboratory, Hong Kong Special Administrative Region (GLHK), Hong Kong, China

Dr Ting Lu, Ms Ee Mei Gui, Ms Pui Sze Cheow and Dr Tang Lin Teo

Health Sciences Authority (HSA), Chemical Metrology Laboratory, Singapore

Dr Eliane Rego, Dr Bruno Garrido, Lucas Carvalho, Rodrigo Leal, Fernando Violante

Organic Analysis Laboratory, INMETRO, Brazil

Dr. Song-Yee Baek, Sunyoung Lee, Kihwan Choi and Byungjoo Kim,

KRISS/Center for Analytical Chemistry, Rep of Korea

Ms. Milena Bucar-Miklavcic

Science and Research Center Koper, Laboratory of the Institute for Oliveculture, Slovenia

Mr. Chris Hopley

LGC, UK

Dr Jintana Nammoonnoy

Organic Analysis Laboratory, Chemical Metrology and Biometry Department, National Institute of Metrology, Thailand (NIMT)

Dr Jacolin Murray; Dr. Walter Wilson; Dr. Blaza Toman

National Institute of Standards and Technology (NIST), United States

Dr. Nobuyasu Itoh

National Metrology Institute of Japan (NMIJ), Japan

Dr. Taner Gokcen

National Metrology Institute of Turkey (UME), Turkey

Dr. Anatoliy Krylov; Dr. Alena Mikheeva

D.I. Mendeleyev Research Institute for Metrology (VNIIM), Russia

SUMMARY

The demonstration of competency and equivalence for the assessment of levels of contaminants and nutrients in primary foodstuffs is a priority within the 10-year strategy for the OAWG Track A core comparisons. The measurements are core challenges for reference material producers and providers of calibration services. This key comparison related to low polarity analytes in a high fat, low protein, low carbohydrate food matrix and Benzo[a]pyrene in edible oil was the model system selected to align with this class within the OAWG strategy. Evidence of successful participation in formal, relevant international comparisons is needed to document measurement capability claims (CMCs) made by national metrology institutes (NMIs) and designated institutes (DIs).

16 National Metrology Institutions participated in the Track A Key Comparison CCQM-K146 Low-Polarity Analyte in high fat food: Benzo[a]pyrene in Olive Oil. Participants were requested to evaluate the mass fractions, expressed in µg/kg, of Benzo[a]pyrene in the olive oil material. The KCRV was determined from the results of all NMIs/DIs participating in the key comparison that used appropriately validated methods with demonstrated metrological traceability. Different methods such as liquid-liquid extraction, GPC and SPE were applied in the sample pretreatment and HPLC-FLD, HPLC-MS/MS, and GC-MS or GC-MS/MS were applied for detection by the participants. The mass fractions for BaP were in the range of (1.78 to 3.09) µg/kg with standard uncertainties of (0.026 to 0.54) µg/kg, with corresponding relative standard uncertainties from 0.9% to 21%. Five labs withdrew their result from the statistical evaluation of the KCRV for technical reasons. One lab was excluded from the KCRV evaluation, as they did not meet the CIPM metrological traceability requirements. A Hierarchical Bayes option was selected for the KCRV value, which was determined as 2.74 µg/kg with a standard uncertainty of 0.03 µg/kg. The 10 institutes those were included in the calculation of the consensus KCRV all agreed within their standard uncertainties.

Successful participation in CCQM-K146 demonstrates the measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 0.1 µg/kg to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS

ASE	accelerated solvent extraction
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·s/$\bar{x}$
DI	designated institute
DoE	degrees of equivalence
GC-HRMS	gas chromatography with high-resolution mass spectrometry detection
GC-IT-MS	gas chromatography with ion trap mass spectrometry detection
GC-MS	gas chromatography with mass spectrometry detection
GC-MS/MS	gas chromatography with tandem mass spectrometry detection
GC-TOFMS	gas chromatography with time-of-flight mass spectrometry detection
GPC	gel permeation chromatography
HPLC-FLD	high pressure liquid chromatography with fluorescence detection
LC-MS	liquid chromatography with mass spectrometry detection
LC-MS/MS	liquid chromatography with tandem mass spectrometry detection
IDMS	isotope dilution mass spectrometry
KC	Key Comparison
KCRV	Key Comparison Reference Value
LC-SI	liquid chromatography Silica gel
MADe	median absolute deviation from the median (MAD)-based estimate of s: MADe = 1.4826·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
qNMR	quantitative nuclear magnetic resonance spectroscopy
RMP	Reference Measurement Procedure
SEC	size exclusion chromatography
SIM	selected ion monitoring
SPE	solid phase extraction
SRM	Selected reaction monitoring

SYMBOLS

d_i	degree of equivalence: x_i - KCRV
$\%d_i$	percent relative degree of equivalence: $100 \cdot d_i/\text{KCRV}$
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: $\bar{x} = \sum_{i=1}^{n} x_i/n$

INTRODUCTION

The 10-year strategy of the OAWG for Track A key comparisons includes a number of food related key comparisons highlighting the importance of the analysis of contaminants and nutrients in foodstuffs to the working group. These are core challenges for reference material producers and providers of calibration services and evidence of successful participation in formal, relevant international comparisons is needed to document measurement capability claims (CMCs) made by national metrology institutes (NMIs) and designated institutes (DIs). The area of low polarity analytes in high fat foods had not been covered by the OAWG for many years with the key comparison CCQM-K21 occurring in pp'-DDT in fish oil in 2000. This particular key comparison thus covers the measurement space described by: Molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 0.1 µg/kg to 1000 µg/kg in a in a high fat, low protein, low carbohydrate food matrix. Benzo[a]pyrene in edible oil was selected as the model system for this key comparison.

In 2017, the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) approved the Key Comparison (KC) CCQM-K146 "Low-Polarity Analyte in high fat food: Benzo[a]pyrene in Olive Oil". This comparison fits well into the OAWG strategy and the model system it covers. The olive oil is a typical matrix with higher than 60% fat, and the polarity of BaP is -6.35 in the sector of $pK_{ow} < -2$, and the mass fraction is in the sector of 1 µg/kg < w < 1 mg/kg.

BaP is one of the markers for the occurrence of PAHs in foods, for which maximum residue limits are enforced in many countries. Edible oil and fats are the main source of human PAH intake. BaP may form in edible oils by pyrolytic processes, such as incomplete combustion of organic substances. Worldwide regulatory limits of BaP in edible fats and oils are from 2.0 µg/kg to 10 µg/kg. CCQM-K146 was designed to assess participants' capabilities for (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS. The method(s) used by participants in CCQM-K146 are intended to represent the way they deliver matrix certified reference materials or testing services to their customers.

The following sections of this report document the timeline of CCQM-K146, the measurands, study material, participants, results, and the measurement capability claims that participation in CCQM-K146 can support. The Appendices reproduce the official communication materials and summaries of information about the results provided by the participants.

TIMELINE

Table 1 lists the timeline for CCQM-K146.

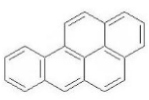
Table 1: Timeline for CCQM-K146

Date	Action
April 2016	Proposed to CCQM
October 2016	Draft protocol presented to OAWG as potential Track A Key Comparison
October 2017	OAWG authorized CCQM-K146 as a Track A Key Comparison; protocol approved
October 2017	Call for participation to OAWG members
November 2017	Study samples shipped to participants. The range in shipping times reflects delays from shipping and customs.
May 2018	Results due to coordinating laboratory
September 2018	Draft A report distributed to OAWG
April 2019	Draft B report distributed to OAWG
TBD	Final report approved by OAWG

MEASURAND

CCQM-K146 relates to "Low-Polarity Analyte in high fat food: Benzo[a]pyrene in Olive Oil". The measurand was the mass fraction of BaP in the olive oil material in μg/kg. Information on CAS number, molecular formula, molecular mass, polarity and chemical structure are shown in Table 2.

Table 2: Information on BaP

Benzo(a)pyrene	
CAS	50-32-8
Molecular formula	$C_{20}H_{12}$
M_W	252.31
pK_{ow}	-6.35
Structure	

STUDY MATERIAL

The matrix, olive oil, is a high fat and low protein, low carbohydrate matrix that falls within Sector 1 of the AOAC International food triangle. Olive oil was purchased from a local supermarket, spiked with BaP, and homogenized by vibro-mixing at room temperature for 24 hours. The indicative range for the mass fractions of the analyte was 0.1-100 μg/kg. Benzo(a)anthracene, benzo(b)fluoranthene, and chrysene were also spiked as interferences. The homogenized oil was separately dispensed into aluminum bottles under nitrogen atmosphere to give about 500 bottles, with content of 30 g each. Packing was in vacuum-sealed aluminum foil bags. Long term storage of the material at NIM was at about 25ºC.

Each participant received 2 bottles, each containing 30 g of olive oil. One sample bottle was intended for method development and the other one was to be used for determination of the final results. Samples were to be stored at room temperature. The recommended minimum sample amount for analysis was at least 0.5 g.

Homogeneity Assessment of Study Material

All samples were kept at the storage condition of 25ºC by NIM. 15 sample bottles were taken randomly, and analysis of triplicate sub-samples was carried out using an IDGC-MS/MS method while the absolute values were transformed relative to the mean. Results are shown in Figure 1.

The results of the homogeneity assessment indicated that the coefficient of variation (CV) was about 2.6% for the target BaP. One-way ANOVA with *F*-test in accordance with the requirements as stipulated in ISO Guide 35 was used to test whether there were significant between-packet differences in the concentration of the measurand (Table 3). The estimated between-packet standard deviation proved to be larger than within group standard deviation. The value of the relevant *F*-test ratios, *F*, was small, and *P*-value was larger than the usual critical 0.05 confidence level, which indicated that the inhomogeneity of the study material was insignificant.

Stability Assessment of Study Material

NIM performed long-term and short-term stability testing of BaP in the olive oil samples. Samples were stored at 50ºC for 0, 5, 8, 12, 20 and 30 days for the short-term stability with two bottles being analyzed at each time point. This study was designed to test the material stability under transportation conditions. Similarly, duplicate samples were selected randomly at the storage condition of 25ºC for testing at the 0, 1, 3, 6, 12, 16-month time points for the long-term stability study. Duplicate sub-samples were taken from each bottle and analyzed using the IDGC-MS/MS method and the absolute values were transformed relative to the mean. The trend graphs of stability are shown in Figures 2 and 3. The results of the stability assessment

indicated that the coefficient of variation (CV) was lower than 2.5% for the target BaP under both circumstances. The trend-analysis technique proposed by ISO Guide 35 was applied to assess the stability. The effect of time on the stability was evaluated using a linear approximation model by fitting linear regression lines to the data set ($Y=\beta_0-\beta_1 X$). The statistical results indicated that no significant trend at 95% confidence level was detected as the absolute values of β_1 (i.e. slope of the regression line) were smaller than the critical values of β_1 which were the uncertainty associated with the slope of the regression line for the stability times the respective Student's t-factor. Hence, the instability of the material was insignificant at the study temperature over the study period.

The stability of the study material was also evaluated through ANOVA test on the regression with results summarized in Tables 4 and 5. The obtained respective p-values for both tests (all greater than 0.05) indicated that the regressions were insignificant at 95% confidence level.

Figure 1 Homogeneity of BaP

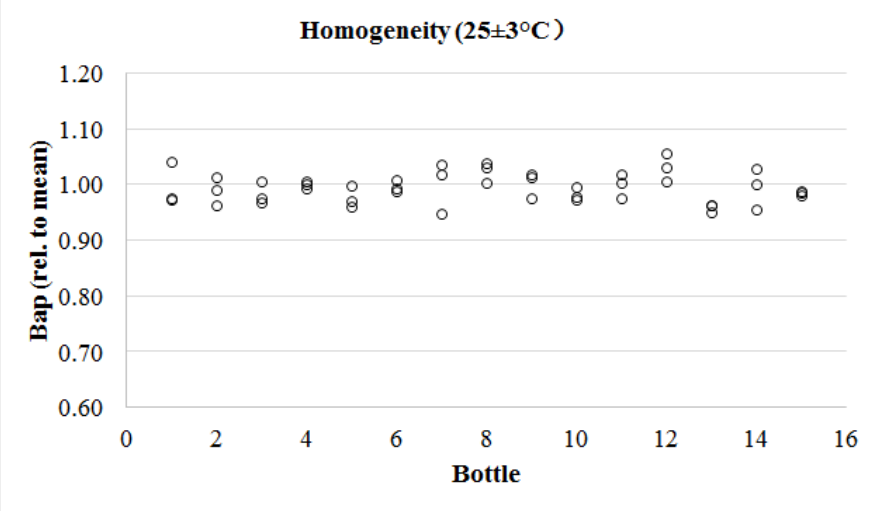


Table 3. Summary of ANOVA for homogeneity test of BaP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.013111	14	0.000937	1.628243	0.128185	2.03742
Within Groups	0.017255	30	0.000575			

Figure 2 Short-term stability of BaP

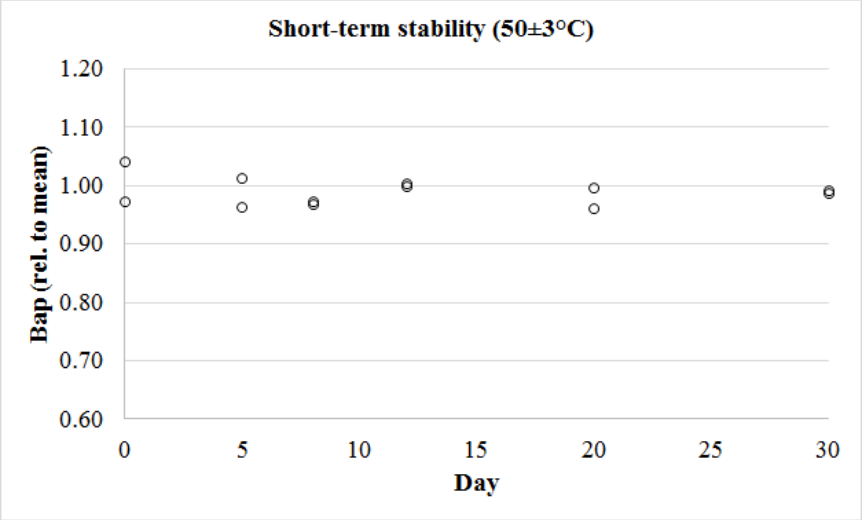


Table 4 Summary of ANOVA test for the short-term stability study of BaP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.001903	5	0.000381	0.545486	0.738671	4.387374
Within Groups	0.004186	6	0.000698			

Figure 3 Long-term stability of BaP

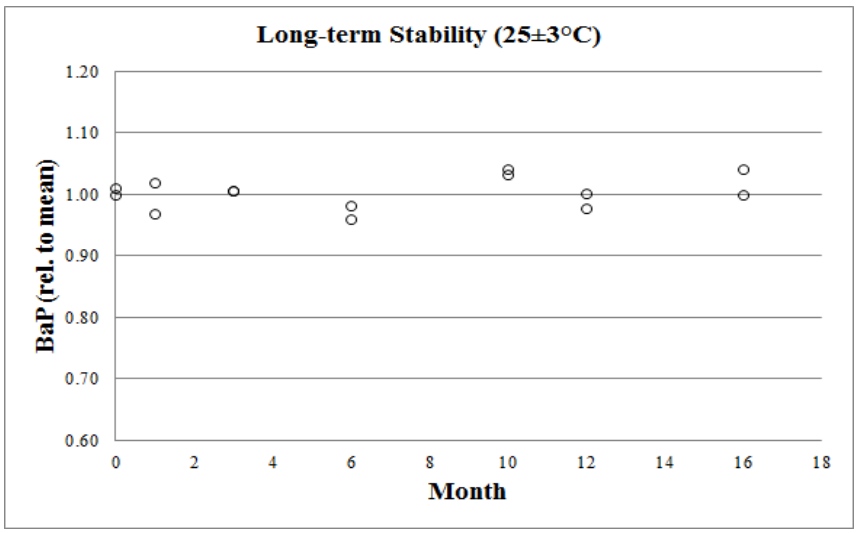


Table 5 Summary of ANOVA test for the long-term stability study of BaP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.00542	6	0.0009	2.32427	0.14719	3.86597
Within Groups	0.00272	7	0.00039			
Total	0.00814	13				

PARTICIPANTS, INSTRUCTIONS AND SAMPLE DISTRIBUTION

The call for participation was distributed in October 2017 with the intent to distribute samples in November 2017, receive results in May 2018, and discuss results in Chengdu China OAWG meeting, October 2018. See Table 1 for the study timeline. Appendix A reproduces the Call for Participation; Appendix B reproduces the study Protocol.

A total of 16 NMIs/DIs registered to participate in the measurement of BaP in the key comparison (CCQM-K146), no one registered CCQM-P185. Each participating NMI/DI was provided with two vials of comparison materials. At the time of sample dispatch, a sample receipt form was provided electronically to all participants and was to be filled in and returned to the study coordinator on receipt of the shipments. Information on participating NMIs/DIs, contacts and sample receipt details are summarized in Table 6.

Due to customs issues, samples were not received by some NMIs (BVL, INMETRO, LGC, Lab of Oliveculture), four sets of materials were delivered again in March-2018. Because of these delays, the deadline for submission of results was postponed with a final deadline of May-15-2018.

The results reporting form and core competency template were required to be completed and returned to the study coordinator before the submission deadline.

The results were to be reported in the unit of µg/kg for BaP and include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. Information on the measurement procedure (extraction, clean-up, column and conditions, quantification approach), the calibration standards, the internal standard, any quality control materials, number of replicates, the calculation of the results and the estimation of measurement uncertainty was requested.

All participants in CCQM-K146 submitted the results (Table 6).

Table 6 Information on participating NMIs/DIs, contacts and sample receipt

No.	Institute	Country	Contact Person	Sample Receipt Date	Result submission
1	Bundesanstalt für Materialforschung und –prüfung (BAM); Reference Materials/Division of Food Analysis	Germany	Dr. Matthias Koch	4-Jan-18	15-May-18
2	Federal Office of Consumer Protection and Food Safety (BVL)	Germany	Dr Joachim Polzer, Dr Rudolf Hackenber	16-Mar-18	15-May-18
3	EXHM/GCSL-EIM, Chemical metrology lab	Greece	Dr Elias Kakoulides	4-Jan-18	15-May-18
4	Government Laboratory, Hong Kong Special Administrative Region (GLHK)	Hong Kong, China	Dr Kong Pak-wing, Mr Ng Chi-shing	15-Dec-17	14-May-18
5	Health Sciences Authority (HSA), Chemical Metrology Laboratory	Singapore	Dr Tang Lin Teo, Ms Pui Sze Cheow	15-Dec-17	11-May-18
6	Organic Analysis Laboratory, INMETRO	Brazil	Dr Eliane Rego, Dr Bruno Garrido	18-Mar-17	15-May-18
7	KRISS/Center for Analytical Chemistry	Rep of Korea	Dr. Byungjoo Kim, Dr. Song-Yee Baek	18-Dec-17	15-May-18
8	Science and Research Center Koper, Laboratory of the Institute for Oliveculture	Slovenia	Ms Milena Bucar-Miklavcic	5-Mar-18	15-May-18
9	LGC	UK	Mr Chris Hopley	9-Mar-18	15-May-18
10	National Institute of Metrology (NIM), China	China	Li Xiaomin	17-Dec-17	15-May-18

11	Organic Analysis Laboratory, Chemical Metrology and Biometry Department, NIMT, Thailand	Thailand	Dr Jintana Nammoonnoy	18-Dec-17	14-May-18
12	National Institute of Standards and Technology (NIST)	United States	Dr Jacolin Murray	5-Jan-18	15-May-18
13	National Metrology Institute of Japan (NMIJ)	Japan	Dr. Nobuyasu Itoh	5-Jan-18	13-Apr-18
14	National Metrology Institute of Turkey (UME)	Turkey	Dr. Taner Gokcen	9-Jan-18	15-May-18
15	D.I. Mendeleyev Research Institute for Metrology (VNIIM)	Russia	Dr. Anatoliy Krylov Dr. Alena Mikheeva	9-Jan-18	15-May-18
16	Designated Reference Institute for Chemical Measurements (DRiCM), Bangladesh Council of Scientific & Industrial Research (BCSIR)	Bangladesh	Dr. Mala Khan	21-Dec-17	24-May-18

RESULTS

Participants were requested to report a single estimate of the mass fraction μg/kg for BaP based on measurements for one sample.

In addition to the quantitative results, participants were instructed to describe their analytical methods, approach to uncertainty estimation, and the Core Competencies they felt were demonstrated in this comparison. Appendices C, D, and E reproduce the relevant report forms.

CCQM-K146 results were received from 16 institutions.

Calibration Materials

The details of the source and purity assessment of the calibration materials used by each participant are provided in Table 7.

Table 7 Calibration materials used by participants

NMI/DI	Source(s)	Purities/Concentration and Uncertainties (95% CI)	Purity techniques	Evidence of Competence
BAM	Benzo(a)pyrene (Dr. E); NIST SRM 2260a	(98.85 ± 0.30)% (k=2)	Confirmation of identity by GC-MS (mass spectra, retention time); value assignment / purity assessment by GC-FID, confirmed with certified B(a)P standard NIST SRM 2260a	The capability is underpinned by participation in CCQM-K55 series.
BVL	NIST 1647f	(6.22 ± 0.11) mg/kg (k=2)		N/A
DRiCM	Benzo[a]pyrene (Supelco)	(99.9 ± 0.5)% (k=2)	Confirmation: FTIR (Matches: Supelco, Lib No: USER1 11) & GC- Mass Spec (Matches: NIST, Lib. No.: 99590)	N/A
EXHM	BaP (Sigma-Aldrich) NIST SRM 2260a	(194.2 ± 3.9) ng/g (k=2)	Purity checked by qNMR (971.0 mg/g ± 3.1 mg/g), solution assigned by IDMS against NIST SRM 2260a	The capability is underpinned by participation in CCQM-K131
GLHK	NIST SRM 1647f	(6.22 ± 0.11) mg/kg (k=2)		N/A
HSA	CRM (HRM-1017A) from HSA	(995.0 ± 3.5) mg/g	The certified value of benzo[a]pyrene (HRM-1017A) was determined using mass balance approach. Identity of benzo[a]pyrene was confirmed by NIST SRM1647f and SRM 2260a.	HSA's capability in the purity determination of benzo[a]pyrene with molecular mass (252.32 g/mol) and polarity (pKow: -6.35) is demonstrated

				through CCQM-K55.b (reported purity value for aldrin crossed DOE=0 line), and supported by capabilities demonstrated in the higher polarity range through CCQM-K55.c and CCQM-K55.d comparisons (reported purity values for L-valine and folic acid crossed the DOE=0 lines, respectively).
INMETRO	Benzo[a]pyrene (Supelco), lot LB52916.	(98.62 ± 0.41)% (k= 2.78)	The purity was determined in house by ^{1}H qNMR.	The capability is underpinned by participation in CCQM-K55 series.
KRISS	Benzo(a)pyrene (SUPELCO)	(98.76 ± 0.60)% (k = 2.45)	Mass-balance method and verified with qNMR.	The capability is underpinned by participating in CCQM-K55 series.
Lab of the Institute for Oliveculture	Supelco EPA 610-N PAH Kit, cat. No. 47351	(89.02 ± 1.59)% (k=2.45) provided March 2019 from LGC analysis, initially reported purity value was 98.8% without uncertainty from the Supelco certificate.	Initially, the purity value was assigned in house by qNMR, and the value did not meet the CIPM traceability requirements. After the results were discussed they asked LGC to determine the purity of the commercial material by qNMR	N/A
LGC	NIST SRM 1647f	(6.22 ± 0.11) mg/kg (k=2)		N/A
NIM	Benzo(a)pyrene GBW(E)080476	4.52 μg/mL U=2.8% (k=2)	The certified value of pure benzo[a]pyrene was determined using mass balance approach.	The capability is underpinned by participating

				in CCQM-K55 series.
NIMT	Benzo(a)pyrene (AccuStandard, Inc)	(98.86 ± 0.20)% (k = 2.45)	Mass balance method using HPLC, TGA, and KFT techniques.	The capability is underpinned by participating in CCQM-K55 series
NIST	NIST SRM 2260a	(4.71 ± 0.17) mg/kg (k=3)		The capability is underpinned by participating in CCQM-K55 series.
NMIJ	NMIJ CRM4213a	(99.2 ± 3.9) mg/kg (k=2),		The capability is underpinned by participating in CCQM-K55 series.
UME	NIST SRM 1647f	(6.22 ± 0.11) mg/kg (k=2)		N/A
VNIIM	B(α)P (Sigma Aldrich)	(99.25 ± 0.34)% (k=2)	Mass balance approach. B(α)P purity verified by comparison with NIST SRM 1647f.	The capability is underpinned by participating in CCQM-K55 series.

Table 8 Information on Internal standards

NMI/DI	Source(s)	Chemical (CP) and Isotopic Purities (IP)
BAM	Benzo(a)pyrene - ^{13}C4 (Cerilliant, CIL)	N/A
BVL	BaP D12 (LGC Standards) and ^{13}C4 BaP (Cambridge Isotope)	N/A

EXHM	Benzo[a]pyrene-D12, Chem Service	N/A
GLHK	^{13}C4-Benzo[a]pyrene	N/A
HSA	^{13}C4-Benzo[a]pyrene (Cambridge Isotope)	100 µg/mL in nonane, diluted with toluene to about 8 µg/kg.
INMETRO	Benzo[ghi]perylene	N/A
KRISS	^{13}C4-Benzo(a)pyrene Cambridge Isotope Laboratories (CIL)	N/A
Laboratory of the Institute for Oliveculture	Dibenz[a,h]anthracene	N/A
LGC	Benzo[a]pyrene D12. Sigma-Aldrich reference 451797	N/A
NIM	D12-Benzo(a)pyrene (Dr.Ehrenstorfer)	CP:99%, IP: 98.5%
NIMT	D12-Benzo(a)pyrene	N/A
NIST	BaP-D12 (NIST SRM 2270)	N/A
NMIJ	^{13}C4-benzo(a)pyrene in isooctane obtained from CIL	100 ug/mL
UME	Benzo[a]pyrene-D12 (NIST SRM 2270)	N/A
VNIIM	^{13}C-Labeled EPA 16 PAH Cocktail ES-4087 Cambridge Isotope Laboratories	5 µg/mL (^{13}C4, 99%)

BVL, GLHK, HSA, LGC, NIM, NIST, NMIJ, UME utilized existing pure or solution CRMs from other NMIs/DIs as the calibrating materials. BAM, INMETRO, KRISS, NIMT and VNIIM made value assignments of the commercial BaP materials based on qNMR

and/or mass balance approaches (Table 7), their capability is underpinned by participating in CCQM-K55 series. The standard relative uncertainties of the pure reference material range from 0.15% - 0.65%, and that of standard solutions from 0.88% - 1.97%. In the case of EXHM they calibrated the solution of the commercial material via the NIST solution SRM and thus their traceability is to the NIST material and they utilised its uncertainty in their measurement uncertainty budget for the calibrant.

Table 9 standard uncertainties of the calibrants

	Standard uncertainty of calibrants	
NMI	Pure calibrants	Solution calibrants
BAM	0.15%	/
BVL	/	0.88%
DRiCM	0.25%	
EXHM	/	1.8%
GLHK	/	0.88%
HSA	0.18%	/
INMETRO	0.15%	/
KRISS	0.25%	/
Oliveculture initially reported information*	Not provided	/
Oliveculture revised information*	0.65%	/
LGC	/	0.88%
NIM	/	1.4%
NIMT	0.10%	
NIST	/	1.27%
NMIJ	/	2.0%
UME	/	0.88%
VNIIM	0.17%	/

*The initial value used did not meet the CIPM traceability requirements. The value was revised to 0.65% after Oliveculture asked LGC to determine the purity by qNMR.

DRiCM used a Supelco material and did minimal checks on it so have not adequately assessed the purity. They also have no demonstration of any capability to carry out purity assessment, so their calibrant did not meet the CIPM traceability requirements. Laboratory of the Institute for Oliveculture also did not meet the CIPM traceability requirements from their in-house assignment of the purity of Benzo[a]pyrene by qNMR. Result from both DRiCM and Laboratory of the institute of Oliveculture were thus excluded from the KCRV calculation on this basis. After discussion in the OAWG meeting in October 2018, Laboratory of the Institute for Oliveculture carried out a series of experiments to verify the purity of Benzo[a]pyrene. They provided a detailed explanation about the bias and traceability of their method and they asked LGC to assign the value of the pure BaP by qNMR, the purity was (89.02 ± 1.59)% (k=2.45) provided in March 2019 from LGC analysis. The demonstration of their competence for their in-house measurements of purity via participation in a CCQM comparison is still lacking and thus they remained excluded from the KCRV calculation.

Internal standards used by participants are listed in Table 8, 13 used isotopically labelled internal standards from a variety of sources, 13C4-Benzo[a]pyrene and Benzo[a]pyrene D12 were the commonly used internal standards. 2 utilized structurally related compounds, 1 lab did not use an internal standard.

Methods Used by Participants

A summary of the sample intake, pre-treatment, clean-up and IS spiking and equilibration times are given in Tables 10 & 11 with full details in Appendix F-1.

BAM method A, DRiCM, EXHM, GLHK, HSA, KRISS, LGC, NIMT, NIST method 1, NMIJ etc., have applied different solvents in the extraction procedure, including acetonitrile, cyclohexane, acetone/acetonitrile, n-hexane, DMSO, iso-octane and petroleum ether. VNIIM method1 and NIM used saponification before the extraction. BAM A method, BVL, NMIJ and NIST method 2 used GPC or size exclusion chromatography method. BAM method B, Laboratory of the Institute for Oliveculture, INMETRO, NIST method 2, UME, and VNIIM method 2 did not have any extraction process, they used SPE directly for the pre-treatment. Single layer SPE cartridges that were used included Molecularly Imprinted Polymer (MIP) cartridges, Cleanert BaP3, silica-gel, C18, Al_2O_3, and LC-SI. Multi-layer SPE cartridges were the main choice for the clean-up process, including Silica gel chromatography and Molecularly

Imprinted Polymer (MIP), (Florisil/C18) SPE & NH_2 SPE, C18 SPE & Florisil SPE, EZ-POP NP (LC-Florisil® & Z-Sep/C18). The reconstitution solvents included acetonitrile, cyclohexane, n-hexane, isopropanol, toluene, dichloromethane, dichloromethane/hexane.

INMETRO, Laboratory of the Institute for Oliveculture, and NIST Method 2 used HPLC-FLD technique, GLHK used HPLC-MS/MS, and the others used GC-MS or GC-MS/MS. Details are in Appendix F-2.

DRiCM and Laboratory of the Institute for Oliveculture used external calibration methods, INMETRO used Benzo[ghi]perylene as internal standard for their calibration, and the others used IDMS methods. Details are in Appendix F-3.

The participants' approaches to estimating uncertainty are provided in Appendix G.

BAM, NIST and VNIIM use two methods for sample pretreatment and their results were combined from the two methods. NIST used the linear pool consensus approach to calculate their reported value and their uncertainty.

Table 10 Summary of sample pre-treatment

NMI/DI	Sample(g)	extraction solvent	extraction time total, temperature	repeat	others
BAM method A	4	10 mL acetonitrile	vortexing and ultrasonic extraction (15 min)	3 collecting ACN extracts, re-dissolution in 5 mL cyclohexane/ethyl acetate 1:1	GPC and re-dissolution in 1 mL toluene
BAM method B	0.5	dilution with 0.5 mL cyclohexane	vortexing of the mixture		
BVL	1	GPC	Applied GPC and reconstituted in 100 µL of toluene.		
DRiCM	1	petroleum ether	2 mL		

EXHM/GCSL-EIM	2.5	acetone/acetonitrile 40/60	3 successive extractions solvent changed into cyclohexane		
GLHK	0.5	diluted with cyclohexane	shaken by vortex mixer		
HSA	2	2.5 mL of n-hexane	vortexed for 1 min, then equilibrated at 4 °C for overnight and/or at ambient temperature (18 -25°C) for at least 1 h, then vortexed for 1 min, sonicated for 2 min and shaken on a multitube shaker for 10 min		
INMETRO	0.5	1.5 g florisil SPE	N_2 drying, 40 °C		
KRISS	5	acetone/acetonitrile (6:4=v:v)	mixer (10 min) and sonication (10 min),10 mL	twice	
Lab of the Institute for Oliveculture	0.2	SPE	N/A		
LGC	2	iso-octane	mixing (30 s) by vortexing		
NIM	0.5	1 mol/L KOH ethanol-water solution (90:10, v/v) water+ n-hexane	at 75 °C, 2H, vortex 2 min, Centrifuged 3min	twice	N_2 flow, 40 °C, reconstituted with cyclohexane
NIMT	1	3 mL of n-hexane	vortexed and sonicated for 5 min		
NIST method A	2.5	60/40 acetonitrile/acetone	10 mL 3 times		

NIST method B	2.5	Applied size exclusion chromatography	N/A		
NMIJ	5.6	10 mL DMSO, back-extraction with cyclohexane	10 min	3 times	GPC
UME	0.5	Applied SPE	N/A		
VNIIM method A	2	potassium hydroxide in hexane (30 mL）	boiling with backflow condenser for 3 hours	5 times	
VNIIM method B	2	2 mL of hexane	N/A		

Table 11 Summary of clean-up methods and internal standard spiking

NMI/DI	SPE	Recon. time, temperature	IS Spikes Before or after reconstitution	IS, Equilibration, time, temp	Final Solvent
BAM method A	N/A	N/A	before	N/A	1 mL of toluene
BAM method B	MIP cartridge	N_2 flow 40°C	before	N/A	0.2 mL of toluene
BVL	N/A	N/A	before	N/A	0.1 mL of toluene
DRiCM	Aluminium oxide	N/A	N/A	N/A	1 mL petroleum ether
EXHM/GCSL-	SPE with imprinted polymeric	concentrated	before	equilibrated for 2 days	N/A

EIM	sorbent	x 10			
GLHK	Silica gel chromatography and MIP Cartridge	N/A	N/A	N/A	N/A
HAS	Cleanert BaP3 cartridge	N/A	before	4 °C for overnight and/or at ambient temperature (18 -25°C) for at least 1 h	0.6 mL of n-hexane
INMETRO	C18 SPE	N_2 flow 40 °C	before	N/A	0.2 mL of acetonitrile
KRISS	(Florisil/C18) & (NH_2) SPE	N/A	before	N/A	toluene
Lab of the Institute for Oliveculture	LC-SI	N/A	before	N/A	1 mL of acetonitrile
LGC	SPE (Supelclean™ EZ-POP NP)	N/A	before	N/A	50 µL of acetonitrile
NIM	LC-Florisil® (upper layer) and Z-Sep/C18 (lower layer)	40 °C	before	12h	0.2 mL of isopropanol
NIMT	Cleanert BAP-3 SPE	N/A	before	N/A	0.28 mL of n-hexane
NIST method A	C18 SPE & Florisil SPE	N/A	before	N/A	0.2 mL of toluene
NIST method B	SEC semi-prep column	N/A	before	N/A	0.25 mL of dichloromethane
NMIJ	silica-gel	N/A	before	N/A	0.2 mL of toluene

UME	EZ-POP NP	N/A	before	N/A	0.25 mL of acetone
VNIIM method A	multilayer silica column	N/A	before	N/A	0.5 mL of dichloromethane/hexane
VNIIM method B	2 mL of hexane, partition on Al2O3 (4mL, equilibrated at 180 °C during 4 hours), the target fraction is dichloromethane/hexane=30/70 (50 mL)	N/A	before	N/A	0.5 mL of dichloromethane/hexane

Participant Results

The results for CCQM-K146 for the determination of BaP are detailed in Table 12 and presented graphically in Figure 4.

Table 12 Reported Results for BaP (µg/kg)

NMI/DI	x	$u(x)$	k	$U(x)$
Germany (BAM)	2.64	0.08	2	0.16
Germany (BVL)	2.61	0.22	2	0.44
Bangladesh (DRiCM)	*1.93*	*0.06*	*2*	*0.11*
Greece (EXHM/GCSL-EIM)	*1.93*	*0.17*	*2.26*	*0.38*
Hong Kong (GLHK)	2.715	0.0611	2	0.122
Singapore (HSA)	2.705	0.075	2	0.15
Brazil (INMETRO)	*1.78*	*0.173*	*2*	*0.35*
Republic of Korea (KRISS)	2.799	0.026	2.26	0.059
Slovenia (Lab of the Institute for Oliveculture)	*3.5*	*0.75*	*2*	*1.5*
Slovenia (Lab of the Institute for Oliveculture) revised*	*2.57*	*0.54*	*2*	*1.1*
UK (LGC)	2.71	0.055	2	0.11
China (NIM)	2.71	0.11	2	0.22
Thailand (NIMT)	*2.05*	*0.11*	*2.16*	*0.24*
United States (NIST)	2.73	0.16	2	0.32
Japan (NMIJ)	2.79	0.062	2.78	0.17
Turkey (UME)	*3.09*	*0.14*	*2*	*0.29*
Russia (VNIIM)	2.64	0.09	2	0.18
n	15			
$\bar{x}$	2.63			
s	0.37			
CV	14%			

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

red italic font results were excluded from the statistical calculation of the KCRV

*green represents the revised value reported by the Lab of the Institute for Oliveculture after further analysis

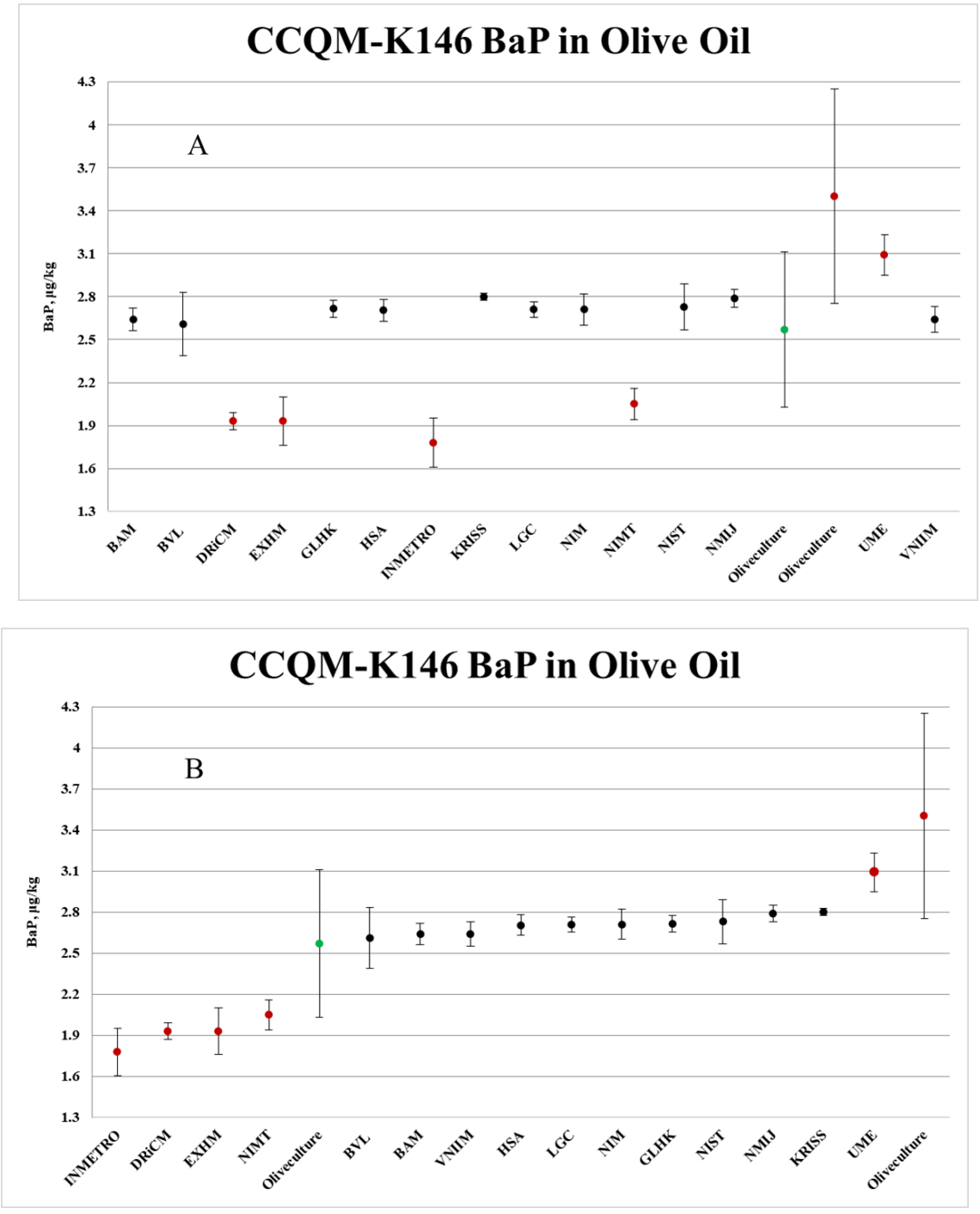


Figure 4 Dot-and-Bar Display of Reported Results for BaP, µg/kg

Panels A and B display the reported results for BaP; panel A displays the results sorted alphabetically by NMI acronym; panel B displays results sorted by increasing reported value. Dots represent the reported values, x; bars represent their standard uncertainties, $u(x)$. Solid red dots represent values excluded from statistical consideration. Solid green dot represents the revised value which was not included in the statistical consideration. The thin horizontal gridlines are provided for visual guidance.

Discussion of Results

After the discussion in the OAWG meeting in October 2018 a number of institutes carried out further investigation where their results appeared biased from the consensus.

INMETRO found an observed difference in SPE extraction from the internal standard and the analyte, which they believed may be of relevance to their low result. Hence, INMETRO requested that their result should not be included for the KCRV calculation.

EXHM provided further information on their approach to the assessment of the calibration solution they utilised. The carried out an in-house qNMR "check" of the purity of the commercial calibrant they employed but they calibrated the solution made from that calibrant versus the NIST SRM solution. They also investigated results from solution calibration standards versus matrix-matched standards to seek further information on their low bias. They agreed to be removed for the calculation of the KCRV.

NIMT investigated interferences and also matrix effects in their extraction, they found these contributed to their biased result. They agreed to be removed from the KCRV calculation.

UME investigated the difference in calculation between a 5-point calibration and single point IDMS. A new sample was provided by NIM (A-52) for further analysis. Standards and spiking solutions were prepared freshly from new NIST CRM ampoules. The 5-point calibration curve was linear in the range of 1-50 ng/g and R^2 is 0.9996. The response factor for single point IDMS was found as 1.42. In their blank sample, a very small amount of BaP peak was observed which was equal to around 5 percent of the BaP sample peak.

Calculations were done according to both situations; by applying blank correction and without applying blank correction. Results are given at the table below:

Technique investigation of calibration curve vs. single point IDMS calibration

n=4	Blank corrected	No blank correction
5-point calibration curve	2.60 μg/kg	2.74 μg/kg
Single point IDMS	2.62 μg/kg	2.76 μg/kg

It was concluded that the problem with the previous work may be due to issues with the stock and working solution preparation. In addition, UME decided that the calculation should not apply a blank correction, as it is not the same olive sample, and the "no correction" result is more reasonable. Thus their results were excluded from the KCRV calculation.

The Oliveculture Institute (Slovenia) lacked demonstration of their capability to assign the calibrator by qNMR. Following the initial discussion of the comparison results, they carried out further work and had the purity of their calibrant certified by LGC. This lead to a large change and the purity was corrected from 98.8% to 89.02%. Their bias was further explained by their use of a correction factor, which was used because of different recoveries for BaP and their internal standard dibenzo(a,h)anthracene. They used their BaP in olive oil analysis procedure for determination of a sample from a Greek PT scheme (PROFICIENCY TEST SCHEMA 23 03 Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in water and oil sample, organized by General Chemical State Laboratory, Chemical Metrology Service, Greece). Their result was lower than the consensus in the PT scheme report, so they concluded that a correction factor was needed because of the different recovery of the analyte and IS. Following the initial discussion of the CCQM-K146 results at the OAWG meeting, they compared the results with and without the use of the correction factor and using the purity assessed by LGC. The resulting corrected value they submitted was 2.57 µg/kg. This result is displayed in green italic font in Table 12 and as a solid green dot in Figure 4. In their uncertainty evaluation, their bias estimate consists of bias of the method (7.25%) and laboratory bias from the Greek PT (19.13%), as they did not use the PT to correct the recovery. This overall approach is not how a reference measurement procedure would typically be implemented as in-house assessment of the biases should be carried out rather than any form of correction or uncertainty component being incorporated solely from an external PT result. After further investigation, they revised the uncertainty evaluation approach to the combination of purity assessment, repeatability and calibration solution preparation. The institute agreed not to be included in the KCRV calculation.

Explanation of the correction they applied is in the table below. Their result (1.86 µg/kg) from the Greek PT scheme was lower than the consensus value from the PT scheme report (2.3 µg/kg), so they concluded that a correction factor was needed. The correction factor RM_f was the ratio between the PT consensus result and their result.

$$RM_f = \frac{result_{PT\ scheme}}{result_{LAB-IZO}} = \frac{2.3}{1.86} = 1.24$$

Investigation of calibrant purity and correction factor versus results for BaP

Calibrant (B(a)P) purity determined by	certificate	qNMR Slovenia	qNMR LGC
Calibrant (B(a)P) purity	**98.8%**	**93.64%**	**89.02%**
Correction factor (RM_f)	**1.24**	**1.30**	**1.37**
'Raw' result (RR), without using correction factor	**2.85 µg/kg**	**2.70 µg/kg**	**2.57 µg/kg**
Corrected result (µg/kg) (CR; CR = RR * RM_f)	3.52 µg/kg	3.52 µg/kg	3.52 µg/kg

KEY COMPARISON REFERENCE VALUE (KCRV)

For the technical reasons discussed above, the OAWG agreed to exclude the results of EXHM, INMETRO, Oliveculture Institute, NIMT, and UME from the KCRV calculation. DRiCM did not meet the CIPM traceability requirements and was also excluded from the KCRV calculation.

Visual inspection did not indicate any reason to assume that the remaining data are not normally distributed. Visual inspection also indicated, with corroboration by the Dixon and the Grubbs statistical tests for outliers, that there are no location (X) outliers amongst the remaining dataset.

Different potential candidate KCRV values for BaP calculated from the 10 results are listed in Table 13. Neither the arithmetic mean and standard deviation of the mean, nor the robust median and median absolute deviation from the median (MAD_e) account for the participant uncertainty values, $u(X_i)$ [1]. It was preferred to utilise these as the reported uncertainty estimates of this dataset were considered by the OAWG to be generally reliable. In considering the final data set after exclusion of the six results for technical, traceability or statistical reasons, the data set was in fact very consistent. For consistent data sets with ≥ 10 results, both the Hierarchical Bayes and the DerSimmonian-Laird are considered appropriate estimators and both include the participant's uncertainties. The DSL-Mean and the Bayes summaries were calculated using the NIST Consensus Builder (NICOB) [2].

Table 13: Candidate KCRV estimators for CCQM-K146

Number of results (N) used to calculate KCRV	10
	BaP mass fraction (µg/kg)
Arithmetic Mean	2.71
Standard deviation, SD	0.06
Standard uncertainty, SD/ √ N	0.02
Median	2.71
MAD_e	0.07
standard uncertainty, 1.25 x MAD_e/ √ N	0.03
DSL-mean*	2.75
Standard uncertainty	0.02
Selected KCRV estimator	
Hierarchical Bayes*	2.74
Standard uncertainty	0.03

*Estimated using NICOB [2]

All of the estimators are in reasonable agreement and in all cases, because of the very good agreement of the dataset used to calculate the KCRV, the standard uncertainties for the KCRV estimators are smaller than most participant uncertainties.

As the data set was normally distributed and contained believable participant uncertainties, the Hierarchical Bayesian procedure implemented in the NIST Consensus Builder (NICOB) [2] was considered the most appropriate approach and implemented to estimate the KCRV and associated uncertainty. This method is based on a Gaussian random effects model:

$$X_i = \mu + \lambda_i + E_i$$

Where i indexes the participating laboratories, X_i are the lab-reported means, μ is the consensus value, λ_i are the laboratory effects distributed as Gaussian with mean 0 and variance σ_λ^2, and E_i are the lab-specific measurement errors distributed as Gaussian with mean 0 and variance $u(X_i)^2$. The parameter σ_λ^2 directly estimates the excess variance and the estimate of μ is close to the weighted mean.

The model is estimated via Markov Chain Monte Carlo (MCMC) resampling, which produces large numbers of realisations (draws) of the parameters of the random effects model. This allows the value, standard uncertainty, and 95% credible interval of a parameter to be estimated, respectively, as the arithmetic mean, standard deviation, and 95% credible interval between the 2.5^{th} percentile and 97.5^{th} percentile of a sufficiently large number (typically several tens of thousands) of draws.

The participants' data, the KCRV of the Bayes approach and their associated standard uncertainties for BaP mass fraction are plotted in Figure 5.

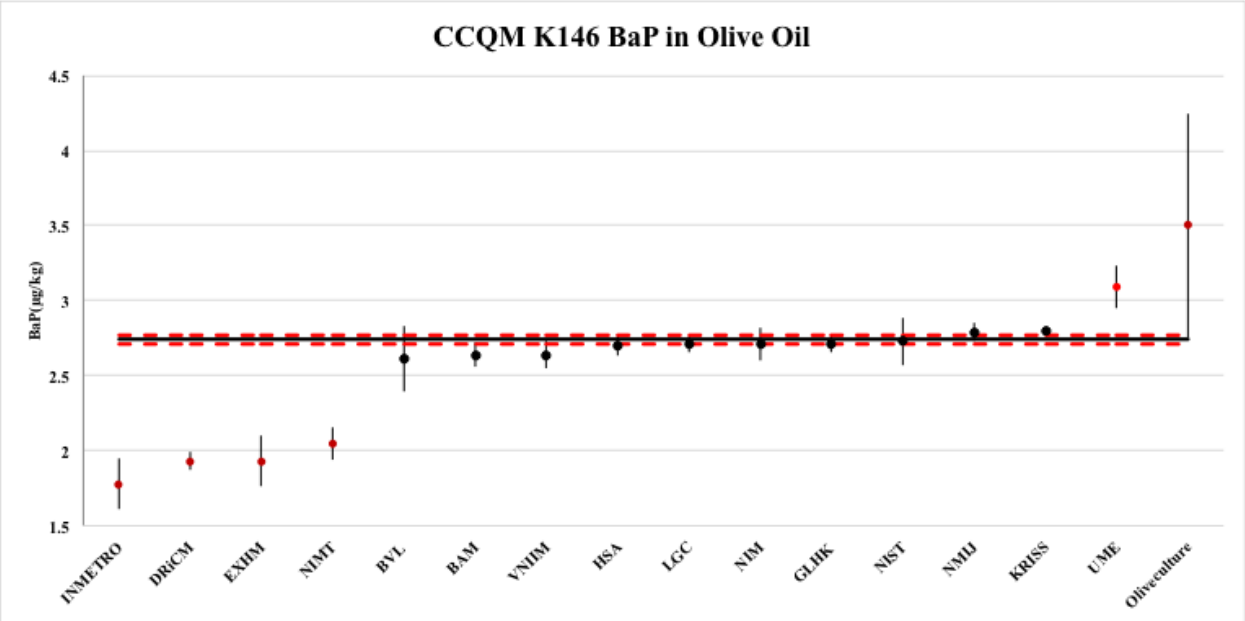


Figure 5: Reported results relative to Key Comparison Reference Value for CCQM-K146

Dots represent the reported mean values, X_i; bars their standard uncertainties, $u(X_i)$. Red dots indicate reported values that were not used to calculate the KCRV. The black horizontal line denotes the candidate KCRV. The dashed red horizontal lines denote the standard uncertainty interval of the candidate KCRV.

DEGREES OF EQUIVALENCE (DoE)

The Degrees of Equivalence (DoE) are calculated using the same hierarchical Bayesian model employed to estimate the KCRV and associated uncertainty [3].

The absolute degrees of equivalence (D_i) for the participants in CCQM-K146 are estimated as

$$D_i = X_i - X_{KCRV}$$

whereby X_i is the result reported by participant i and X_{KCRV} is the KCRV estimate. Using a Monte Carlo (MC) technique to estimate the KCRV, the respective D_i and their 95 % level of confidence expanded uncertainties, $U(D_i)$, are readily estimated along with the KCRV. This was accomplished for participants whose results were included in the KCRV calculation using the NICOB Hierarchical Bayes procedure. The distributions of the D_i were assessed as essentially symmetric, thus the $U(D_i)$ were estimated as the half- width of the interval between the 2.5th and 97.5th percentiles of the MC draws. See Appendix E for the MCMC approach used to calculate D_i for participants whose results were excluded from KCRV calculation. Table 14 and Figure 6 summarise the CCQM-K146 D_i (µg/kg) and $\%D_i$ estimates for the BaP measurand.

The percentage relative D_i, $\%D_i$, were calculated as

$$\%D_i = 100 \times \frac{D_i}{X_{KCRV}},$$

and the expanded uncertainties of the $\%D_i$, $U(\%D_i)$, were calculated as

$$U(\%D_i) = 100 \times \frac{U(D_i)}{X_{KCRV}}.$$

Table 14 Degrees of Equivalence for BaP in olive oil

NMI/DI	D_i *µg/kg*	$U(D_i)$	*%D (%)*	$U(\%D_i)$ (%)
BAM	-0.10	0.19	-3.6%	6.9%
BVL	-0.13	0.45	-4.7%	16.4%
DRiCM*	-0.81	0.13	-29.6%	4.7%
EXHM*	-0.81	0.34	-29.6%	12.4%
GLHK	-0.02	0.17	-0.7%	6.2%
HSA	-0.03	0.18	-1.1%	6.6%
INMETRO*	-0.96	0.34	-35.0%	12.4%
KRISS	0.06	0.12	2.2%	4.4%
LGC	-0.03	0.16	-1.1%	5.8%
NIM	-0.03	0.24	-1.1%	8.8%
NIMT*	-0.69	0.23	-25.2%	8.4%
NIST	-0.01	0.34	-0.4%	12.4%
NMIJ	0.05	0.16	1.8%	5.8%
Oliveculture*	0.76	1.5	27.7%	54.7%
UME*	0.35	0.29	12.8%	10.6%
VNIIM	-0.10	0.21	-3.6%	7.7%

* The measurement results reported by these institutes were not used to estimate the KCRV. The Laboratory of the Institute of Oliveculture values are based on their originally submitted data.

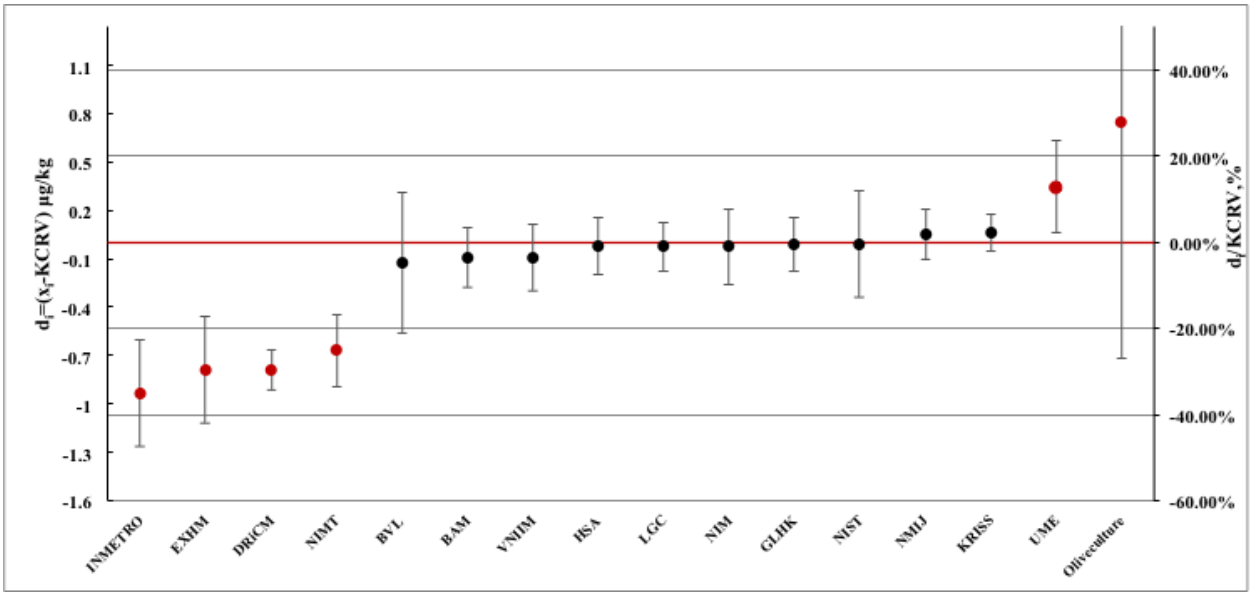


Figure 6: Degrees of Equivalence for BaP

Figure displays the DoE for BaP. All results are sorted by increasing *x*. The axis to the left edge of each panel displays the absolute DoE, *d*, in units µg/kg. The axis to the right edge of each graph displays the relative DoE, 100·*d*/KCRV. Dots represent the *d*, bars their approximate 95 % expanded uncertainties, $U_{95}(d)$. The thick red horizontal line denotes perfect agreement with the candidate KCRV. Values with red labels are excluded from values that were not used in estimating the KCRV.

USE OF CCQM-K146 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in CCQM-K146 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix.

Table 15: How Far the Light Shines

Matrix	Molecular mass (g/mol)	Analyte Polarity	Analyte Mass fraction (µg/kg)
> 60% fat* [AOAC Food Triangle Categories 1,3]	<500	pKow < -2	0.1 to 1000

This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.

Core Competency Statements and CMC support

Appendix H-1 to H-15 list the Core Competencies claimed by the participants in CCQM-K146. The information in these Tables is as provided by the participants. For any participant where their result did not agree with the KCRV and their DoE value did not cross the zero value the core competency table is flagged with a red comment at the bottom.

CONCLUSIONS

Participants in CCQM-K146 demonstrated their ability to identify and quantify low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. Six results were excluded from use in defining the consensus KCRV due to identified issues with their methodologies or traceability. All 10 results that were used for the calculation of the KCRV agreed with the value within the combined 95 % expanded uncertainties.

ACKNOWLEDGEMENTS

The study coordinators thank the participating laboratories for providing the requested information used in this study.

REFERENCES

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

2 Koepke, A., Lafarge, T., Toman, B., Possolo, A. NIST Consensus Builder User's Manual, https://consensus.nist.gov/NISTConsensusBuilder-UserManual.pdf

3 Lunn DJ, Spiegelhalter D, Thomas A, Best N (2009) Statistics in Medicine 28:3049–3082.

APPENDIX A: Call for Participation

Dear Colleagues,

Call for Participation

CCQM Key Comparison CCQM-K146 and Pilot Study CCQM-P185:

Benzo[a]pyrene in Olive Oil

You are cordially invited to participate in the CCQM Key Comparison CCQM - K146 or Pilot Study CCQM P185 on the determination of Benzo[a]pyrene in Olive Oil. The Key Comparison and Pilot Study are organized by the National Institute of Metrology, China (NIM). The objective of the comparison is to provide a platform for demonstration of a laboratory's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at levels of 0.1 to 1000 µg/kg in high fat, low protein, low carbohydrate food matrices.

The time schedule for the various stages of the Key Comparison /Pilot Study is shown as follows:

Event	Period
Preparation of sample	Oct 2015
Homogeneity testing	Nov 2015
Stability testing	From Nov 2015
Invitation of participants	October 2017

Deadline for registration	10 November 2017
Dispatch of samples	November 2017
Deadline for submission of results	20 March 2018
Distribution of preliminary report	15 April 2018
Discussion of results at the CCQM OAWG	October 2018

If you are interested to participate in the comparison, please complete the Registration Form and return it to the coordinators (E-mail: **lixm@nim.ac.cn** **&** **zhaobo@nim.ac.cn**) before 10 November 2017.

Your support and participation in this comparison are highly appreciated.

Best regards,

Dr Xiaomin LI

National Institute of Metrology, China (NIM)

APPENDIX B: Protocol

CCQM-K146/P185 Low-Polarity Analyte in high fat food: Benzo[a]pyrene in Olive Oil

Key Comparison

Track A

Coordinating Laboratory: NIM

Comparison Protocol

September, 2017

Introduction

Testing of the core competencies of laboratories that deliver measurement services of low polarity analytes and low molecule weight in high fat, low protein, low carbohydrate food material has not been covered for many years. Agreement was received in the October 2016 meeting of the OAWG in Paris to conduct the Benzo[a]pyrene (BaP) in Olive oil as the next Track A matrix comparison. This comparison fits into the 10-year strategy for the OAWG Track A comparisons which covers a range of different types of food matrices which map against the different types of capabilities needed.

BaP is a category of polycyclic aromatic hydrocarbons (PAH) that is toxic and carcinogenic to human. It also has some mutagenic properties as described by the World Health Organization, which make its presence in foods a health concern. BaP is one of the markers for the occurrence of PAHs in foods, for which maximum residue limits are enforced in many countries. Edible oil and fats are the main source of human PAH intake. BaP may form in edible oils by pyrolytic processes, such as incomplete combustion of organic substances. Worldwide regulatory limits of BaP in edible fats and oils are from 2.0 μg/kg to 10 μg/kg.

As a Track A study, it is expected that all NMIs or DIs who have, or expect to have, services related to the capabilities related to the How Far the Light Shines statement for this key comparison will participate. A parallel pilot study (CCQM-P185) will also be conducted with the same material for interested parties.

Study Material

The matrix, olive oil, is a high fat and low protein, low carbohydrate matrix that falls within Sector 1 of the AOAC International food triangle. Olive oil was purchased from a local supermarket, spiked with BaP, and homogenized by vibro-mixing at room temperature for 24 hours. The indicative range for the mass fractions of the analyte is 0.1-100 µg/kg. Benzo(a)anthracene, benzo(b)fluoranthene, and chrysene are also spiked as interferences. The homogenized oil was separately dispensed into aluminum bottles under nitrogen atmosphere to give about 500 bottles, with content of 30 g each. Packing was in vacuum-sealed aluminum foil bags. Long term storage of the material at NIM is at about 25ºC.

Measurand

The measurand of this study is benzo(a)pyrene in olive oil.

Table 1 Information of BaP

Benzo(a)pyrene	
CAS	50-32-8
Molecular formula	$C_{20}H_{12}$
M_W	252.31
pK_{ow}	-6.35
Structure	

Methods

The study will require extraction, clean-up, analytical separation, and selective detection of the analyte in olive oil. Participants are anticipated to perform measurements by isotope-dilution gas chromatography-mass spectrometry (IDGC-MS); however, other techniques such as liquid chromatography (LC) may be used.

Homogeneity

All samples were kept at the storage condition of 25ºC by NIM. 15 bottles of samples were taken randomly, and analysis of triplicate sub-samples was carried out using an IDGC-MS/MS method while the absolute values were transformed relative to the mean. Results are shown in Figure 1.

The results of the homogeneity assessment indicated that the coefficient of variation (CV) was about 2.6% for the target BaP. One-way ANOVA with *F*-test in accordance with the requirements as stipulated in ISO Guide 35 was used to test whether there were significant between-packet differences in the concentration of the measurand (Table 2). The estimated between-packet standard deviations proved to be larger than within group standard deviations. The value of the relevant *F*-test ratios, *F*, is small, and *P*-value is larger than the usual critical 0.05 confidence level, which indicates that the inhomogeneity of the study material was insignificant.

Figure 1 Homogeneity of BaP

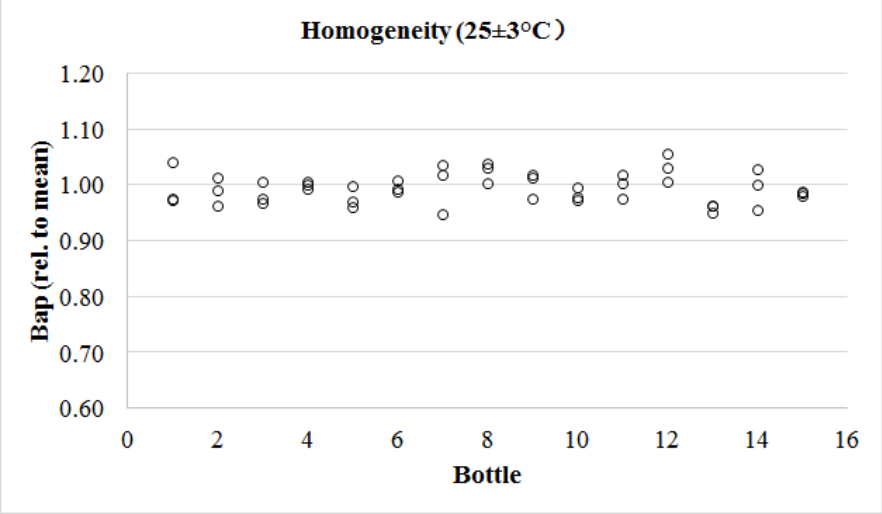


Table 2 Summary of ANOVA for homogeneity test of BaP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.013111	14	0.000937	1.628243	0.128185	2.03742

Within Groups	0.017255	30	0.000575			

Stability

NIM has performed long-term and short-term stability testing of BaP in the olive oil samples. Samples were stored at 50ºC for 0, 5, 8, 12, 20 and 30 days for the short-term stability with two bottles being analyzed at each time point. This study was designed to test the material stability under transportation conditions. Similarly, duplicate samples were selected randomly at the storage condition of 25ºC for testing at the 0, 1, 3, 6, 12, 16 months time points for the long-term stability study. Duplicate sub-samples were taken from each bottle and analyzed using the IDGC-MS/MS method and the absolute values were transformed relative to the mean. The trend graphs of stability are shown in Figure 2 and 3. The results of the stability assessment indicated that the coefficient of variation (CV) was lower than 2.5% for the target BaP under both circumstances. The trend-analysis technique proposed by ISO Guide 35 was applied to assess the stability. The effect of time on the stability was evaluated using a linear approximation model by fitting linear regression lines to the data set ($Y=\beta_0-\beta_1 X$). The statistical results indicated that no significant trend at 95% confidence level was detected as the absolute values of β_1 (i.e. slope of the regression line) were smaller than the critical values of β_1 which were the uncertainty associated with the slope of the regression line for the stability times the respective Student's t-factor. Hence, the instability of the material was insignificant at the study temperature over the study period.

The stability of the study material was also evaluated through ANOVA test on the regression with results summarized in Table 3 and 4. The obtained respective *p*-values for both tests (all greater than 0.05) indicated that the regressions were insignificant at 95% confidence level.

Figure 2 Short-term stability of BaP

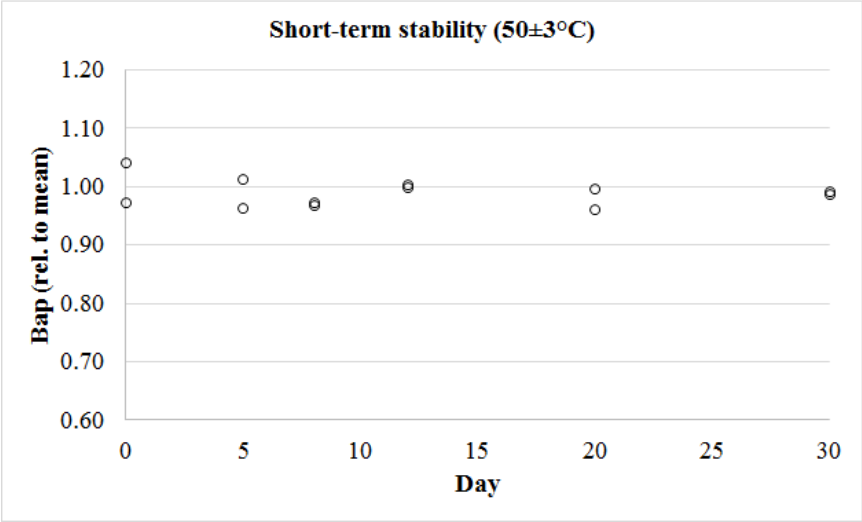


Table 3 Summary of ANOVA test for the short-term stability study of BaP

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.001903	5	0.000381	0.545486	0.738671	4.387374
Within Groups	0.004186	6	0.000698			

Figure 3 Long-term stability of BaP

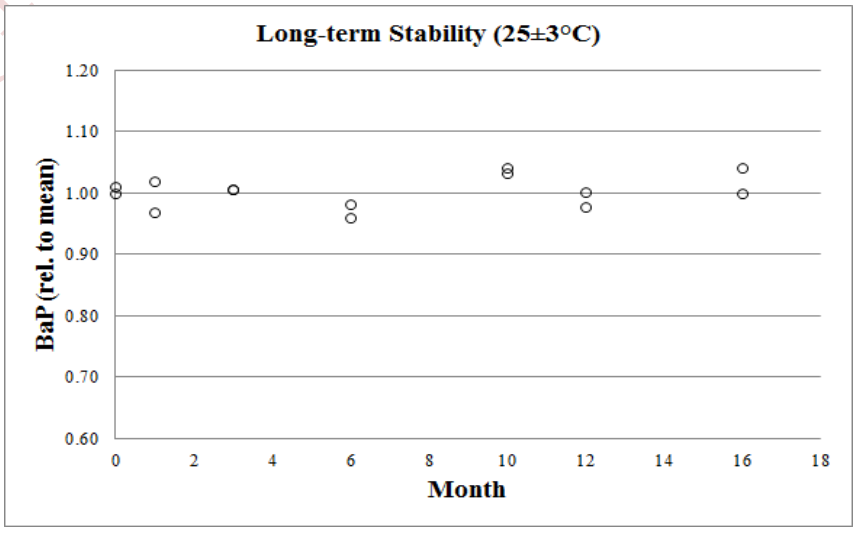


Table 4 Summary of ANOVA test for the long-term stability study of Bap

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.00542	6	0.0009	2.32427	0.14719	3.86597
Within Groups	0.00272	7	0.00039			
Total	0.00814	13				

Reference Standards Available

Solution CRMs and isotopically-labeled (deuterium or carbon-13) BaP for use as internal standard are available from a number of sources including examples indicated below (Table 5 & Table 6).

Table 5 CRMs for measurand

Supplier	Solution CRM	Certified value	Expanded uncertainty
NIM	Benzo[a]pyrene in methanol (GBW(E)080476)	4.12 ug/mL	6.7% (k=2)
NMIJ	Benzo[a]pyrene in 2,2,4-trimethylpentane (CRM 4213-a)	99.2 mg/kg	3.9 mg/kg (k=2)
NIST	Priority Pollutant PAHs in Acetonitrile （SRM 1647f）	6.22 mg/kg*	0.11g/kg* (k=2)

*Certificate currently being revised by NIST and should be available shortly.

Table 6 Reference standards for isotopically-labeled BaP

Isotopically-labeled BaP		
Cambridge Isotope Laboratories	Benzo[a]pyrene D_{12}	DLM-258
Dr. Ehrenstorfer	Benzo[a]pyrene D_{12}	97688AL
Sigma-Aldrich	Benzo[a]pyrene D_{12}	451797
Cambridge Isotope Laboratories	Benzo[a]pyrene $^{13}C_4$	CLM-2722

Study Guidelines

Each participant will receive 2 bottles, each containing 30 g of olive oil. One sample bottle is intended for method development and the other one is to be used for determination of the final results. Samples can be stored at room temperature. A minimum sample intake of 0.5 g is recommended. Participants may use their preferred laboratory procedures.

Reporting of Results

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. The results reporting form and core competency template will be provided to each participant and must be completed and returned to the study coordinator before the submission deadline.

The results should be reported in the unit of µg/kg for BaP and should include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. Information on the measurement procedure (extraction, clean-up, column and conditions, quantification approach), the calibration standards, the internal standard, any quality control materials, number of replicates, the calculation of the results and the estimation of measurement uncertainty should be included.

Evaluation of Results

All the results of the pilot and key comparison will be evaluated against the key comparison reference value (KCRV). The KCRV will be determined from the results of all NMIs/DIs participating in the key comparison that have used appropriately validated methods with

demonstrated metrological traceability. The draft A report will provide candidate estimates of the KCRV and its uncertainty for review and discussion by the OAWG.

How Far Does the Light Shine?

Successful participation in this Track A key comparison "Low-Polarity Analyte in high fat food: Benzo[a]pyrene in Olive Oil" will demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix.

This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.

Study schedule

The time schedule for the various stages of the Key Comparison /Pilot Study is shown as follows:

Table 7 Study schedule for CCQM K146/P185

Event	Period
Preparation of sample	Oct 2015
Homogeneity testing	Nov 2015
Stability testing	From Nov 2015
Invitation of participants	October 2017
Deadline for registration	10 November 2017
Dispatch of samples	November 2017
Deadline for submission of results	20 March 2018

Distribution of	preliminary report	15 April 2018
Discussion of results at the CCQM OAWG		October 2018

Contact information:

For enquiries, participants may wish to make contacts as follows:

Prof. Li Hongmei, NIM, lihm@nim.ac.cn

Dr. Li Xiaomin, NIM, lixm@nim.ac.cn

Attached: "*CCQM K146 P185 invitation letter.doc*"

"*CCQM K146 P185 sample registration form.doc*"

"*CCQM K146 P185 core competency template.doc*"

"*CCQM K146 P185 Results Report Form.xls*"

APPENDIX C: Registration Form

CCQM Key Comparison/Pilot Study

BENZO[A]PYRENE IN OLIVE OIL

CCQM-K146/CCQM-P185

REGISTRATION FORM

My institute/laboratory would like to participate in the program "Benzo[a]pyrene in Olive Oil":

□ CCQM-K146

□ CCQM-P185

INSTITUTE / LABORATORY: _____

_____

ADDRESS: _____

_____

CONTACT PERSON: **(Prof / Dr / Mr / Ms)** _____

_____

EMAIL: _____

TELEPHONE AND FAX: _____

SIGNATURE: _____

DATE: _____

Grateful if you would complete the form and email to the coordinators by 10th November 2017 at: lixm@nim.ac.cn & zhaobo@nim.ac.cn. If you do not receive an acknowledgement from us within 4 working days, please send us an email.

APPENDIX D: Reporting Form

中国计量科学研究院
National Institute of Metrology, China

CCQM Key Comparison/Pilot Study
CCQM-K146/CCQM-P185
Benzo[a]pyrene in Olive Oil
Results Report Form

Please use this excel sheet for reporting.
Please submitted this report electronically to lixm@nim.ac.cn
Please fill in all blanks and use the requested units.
Please provide any extra information in the comments section or on a separate sheet if necessary.

Part I: Participant's Information

Laboratory Name:	
Submitted by:	
Reporting Date: (dd/mm/yy)	
Programme Participated: (CCQM-K146, CCQM-P185)	

Part II: Results:

Bottle No. used for reporting	

Analyte/ Mass Fraction	Mass fraction (µg/kg)	Combined Standard uncertainty u (µg/kg)	Coverage factor (k)	Expanded uncertainty U (µg/kg)	Number of replicates (n)
Benzo (a) pyrene					

Note: Please refer to the OAWG guidance document on significant figures when reporting values

Part III: Technical Information of Methodology Used

Sample size used for analysis (g)	
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	
Analytical instrument(s) used (e.g., LC-MS/MS, GC-MS. etc) (Please specify the model)	
Chromatographic column	
The chromatographic condition(s) (e.g., GC oven temperature programme, LC Mobile phase gradient, etc)	

Method of quantification (e.g., external calibration, Internal standard calibration, IDMS, et	
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	
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
Internal standards used (Please specify the compounds, and at which stage of analysis were the internal standards added.)	

Indicate ions /MRMs measured in the mass spectrometer instrument.

Calibrant: IS:

The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

Estimation of uncertainties for each factor. 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.

Other information, observations or evidences, if any, that can further support your results.

APPENDIX E: DoE calculation

The Bayes summaries were calculated using the NIST Consensus Builder (NICOB). The D_i for participants excluded from the KCRV calculation were determined using a consistent approach based on a Hierarchical Bayesian random effects model [1] estimated via MCMC programmed in OpenBUGS [2]. The OpenBUGS script used to calculate these estimate is provided in this appendix.

Table E1 CCQM-K146 BaP in Olive Oil KCRV estimate

	value	u	dark uncertainty
Hierarchical Bayes	2.74	0.03	0.04

To enable comparison with the degrees of equivalence estimates from other studies, it is convenient to express the D_i and $U(D_i)$ as percentages relative to the KCRV: $\%D_i = 100 \cdot D_i/\text{KCRV}$ and $U(\%D_i) = 100 \cdot U(D_i)/\text{KCRV}$. Table 14 in the body of the report lists the values of D_i, $U(D_i)$, $\%D_i$ and $U(\%D_i)$ for all participants of CCQM-K146.

Example OpenBUGS code

The following Gaussian random effects model programmed in OpenBUGS was used to estimate the DoE for laboratories excluded from the KCRV calculation:

```
ModelBegin{
mu~dnorm(0,1.0E-6)
beta~dgamma(1.0E-5,1.0E-5)

#participants included in the KCRV:
for(I in 1:10){delta[i]~dnorm(mu,beta)
prec[i]<-1/(unc[i]*unc[i])
x[i]~dnorm(delta[i],prec[i])
pred[i]~dnorm(mu, prec[i])
DoE1[i]<-x[i]-pred[i]}

#participants excluded from calculation of KCRV:
for(i in 1:6){preco[i]<-1/(unco[i]*unco[i])
predo[i]~dnorm(mu, preco[i])
DoEo[i]<-xo[i]-predo[i]}
}ModelEnd
#Initial values for MCMC:
list(beta=1)

#Data; included in calculation of KCRV (mu)
x[]   unc[]
2.61  0.220
2.64  0.08
2.64  0.09
```

2.705 0.075
2.71 0.055
2.71 0.11
2.715 0.0611
2.73 0.16
2.79 0.062
2.799 0.026
END
#Data; excluded from calculation of KCRV (mu)
xo[] unco[]
1.78 0.173
1.93 0.17
2.05 0.11
3.5 0.75
3.09 0.14
1.93 0.06
END

References

[1] Toman B, Possolo A (2009) Accred Qual Assur 14:553-563.

[2] Lunn DJ, Spiegelhalter D, Thomas A, Best N (2009) Statistics in Medicine 28:3049– 3082.

APPENDIX F: Summary of Participants' Analytical Information

Table F-1: Summary of Sample Size, Extraction, and Cleanup for CCQM-K146

NMI/DI	Sample Size (g)	Pre-treatment
BAM	4	Addition of internal standard 13C4-B(a)P to weighed olive oil sample and dilution with 10 mL acetonitrile (ACN); vortexing of the mixture; ultrasonic extraction (15 min);centrifugation and separating the supernatant (ACN extract);threefold repetition of extraction/separation step and collecting ACN extracts. Evaporation of combined ACN extract and re-dissolution in 5 mL cyclohexane/ethyl acetate 1:1;separation of B(a)P by applying GPC (BioBeads SX);evaporation of GPC extract and re-dissolution in 1 mL toluene
	0.5	Addition of internal standard 13C4-B(a)P to weighed olive oil sample and dilution with 0.5 mL cyclohexane (CH); vortexing of the mixture; conditioning of MIP (molecularly imprinted polymer) cartridge (SupelMIP PAH 50mg/3ml; Supelco) with CH; loading of sample mixture onto MIP; washing of loaded MIP (3 x 1 mL CH); elution of B(a)P from MIP with ethyl acetate (3 x 1 mL).Evaporation of combined ethyl acetate extract in N2-stream (40°C) and re-dissolution in 0.2 mL toluene
BVL	1	1g of olive oil was weighed in a GPC vial. After addition of 30 µL internal standard solution (toluene) a mixture of ethylacetate: n-hexane 1:1 was added till a total volume of 6 mL was reached. The solution was homogenized using a vortexer and the vial was placed in the GPC autosampler. 5 mL of the solution was injected on the GPC column (column 25 g Bio Beads SX-3, flow 5 mL/min, solvent ethylacetate: n-hexane 1:1). The eluate fraction between 18 and 32 min was collected for further sample preparation. For this purpose the eluate was evaporated to dryness at 45°C under a gentle stream of nitrogen using a Turbovap 2 sample concentrator and reconstituted in 100 µL of toluene. After reconstitution the solution was homogenized using a vortexer and placed in the GC autosampler for measurement.
DRiCM	1	Petroleum Ether (2 ml) was used for extraction. Clean up column was prepared by using Aluminium oxide. It was first activated by introducing water followed by petroleum ether for conditioning. After the column has been conditioned sample was loaded onto column. Analyte was eluted by petroleum ether at a rate of 6 ml/min. 200 ml eluent was collected and then concentrated to 1 ml by nitrogen flow. Before injection to the instrument sample was filtered through 0.45 µm membrane filter.

EXHM	2.5	sample preconcentrated into acetone/acetonitrile 40/60 by three successive extractions solvent changed into cyclohexane. SPE with imprinted polymeric sorbent then concentrated x 10
GLHK	0.5	The sample was diluted with cyclohexane, and shaken by vortex mixer.(i) the sample was first fractionated by an activated silica gel column；(ii) the elute was pre-concentrated and was further cleaned up by Molecularly Imprinted Polymer (MIP) SPE Cartridges
HSA	2	The benzo[a]pyrene in the olive oil sample was extracted by using n-hexane as the extraction solvent. Sample blend (SB) comprising the study material and the internal standard solution was prepared gravimetrically, then vortexed for 1 min after adding 2.5 mL of n-hexane. The SB was then equilibrated at about 4ºC for overnight and/or at ambient temperature (18 -25ºC) for at least 1 h. Following the equilibration at ambient temperature, the SB was vortexed for 1 min, sonicated for 2 min and shaken on a multitube shaker for 10 min before loading into solid phase extraction (SPE) cartridges for clean-up. Solid phase extraction (SPE) was used in the clean-up step. Each SB was subjected to SPE using Cleanert BaP3 cartridge in two steps, i.e. initial clean-up and second clean-up steps. In the initial clean-up step, the cartridge was conditioned with 5 mL dichloromethane followed by 5 mL n-hexane. The blend was then loaded into the cartridge and 5 mL of n-hexane was used to wash the cartridge. Then, 10 mL of dichloromethane was used to elute the analyte. The eluent was evaporated to dryness with N2, and reconstituted with 3 mL of n-hexane before loading into another SPE cartridge for the second clean-up step. In the second clean-up step, the same procedure in the initial clean-up step was applied, except that the analyte was eluted with 6 mL of dichloromethane. The final eluent was evaporated to dryness with N2 and reconstituted with 0.6 mL of n-hexane. The extract was vortexed for 1 min and centrifuged for 5 min at 4,000 rpm before injecting the clear solution into the GC-MS.
INMETRO	0.5	0.5 g sample in 1.5 g florisil SPE. Elution: 20 mL acetonitrile. Pre-concentrated to approximately 10 mL in N2 flow, 40℃. The extract (approximately 10 mL), was passed through in 1.5 g C18 SPE. After this step, the sample was concentrated under N2 flow (40℃), until approximately 200 µL, and 20 µL of each replicate were injected 3 times.
KRISS	approximately 5 g	Liquid-Liquid Extraction:Oil samples were extracted using mixer (10 min) and sonication (10 min) with 10 mL of acetone/acetonitrile (6:4=v:v). The same procedure was repeated twice. Cleaned up by using (1) SPE dual layer (Florisil/C18) with acetonitrile as eluent and (2) SPE (NH2) with hexane/toluene as eluant.
Laboratory of the	0.2	SPE cartridge (SUPELCLEAN LC-SI, 1 g, 6 mL, cat. no. 57051) was cleaned with dichloromethane, vacuum dried and washed with hexane. Sample (0.2 g in 1 mL) and internal standard dibenz[a, h] anthracene (0.16 ng in 100 µL) were

Institute for Olivecultur e		loaded and eluted with hexane/dichloromethane (70:30 v/v). The first 1 mL of eluate was discharged, next 5 mL of eluate were dried under a nitrogen stream. The residue was dissolved in 1 mL of acetonitrile and injected into the HPLC apparatus.
LGC	2	Homogenisation with analyte labelled analogue: thorough mixing (30 s) by vortexing sample with iso-octane solution of labelled analogue. SPE (Supelclean™ EZ-POP NP, Supelco PN 54341-U). 0.5 mL of sample eluted with 15 mL of acetonitrile. Collected eluent evaporated to dryness and reconstituted in 50 µL of acetonitrile.
NIM	0.5	0.5g oil+D12-PAH (equilibrate 12 hours) 5 subsamples, Mixed with 10 mL 1 mol/L KOH ethanol-water solution (90:10, v/v) (2min) and saponificated at 75 ℃ for 2 Hours, Cool down to room temperature and added 10 mL ultrapure water, then added 15 mL n-hexane vortex 2 min, Centrifuged at 20 ℃ 4000 r/min (3min), and reextracted with hexane and combined the upper phase, concentrated under a gentle nitrogen flow at 40 ℃ to near dryness and then reconstituted with cyclohexane for analysis. Cleaned up by using Supelclean™ EZ-POP NP with acetonitrile as eluent, and reconstituted with isopropanol.
NIMT	1	Oil sample was spiked with dueterated BaP (internal standard). A portion of 3 mL of n-hexane was added to the sample which was vortexed and sonicated for 5 min. Cleanert BAP-3 SPE cartridges (Agela Cleanert, 500 mg/6 mL) were used for sample clean-up. The cartridges were conditioned with 5 mL dichloromethane and 5 mL n- hexane. The samples (in 3 mL of n-hexane) were loaded onto the cartridges and washed with 12 mL n- hexane. The samples were eluted with 8 mL of dichloromethane and collected in test tubes followed by evaporated to dryness under stream of nitrogen gas in a water bath at 40 °C. The samples were then reconstituted with 0.28 mL of n-hexane and centrifuged at 10,000 rpm for 5 min prior to transferring to a GC vials for GC-MS analysis.
NIST	2.5	Liquid extraction of olive oil using 10 mL of 60/40 acetonitrile/acetone (percent volume). Sample and extraction solvent were vortexed, sonicated, and centrifuged. Top layer containing analyte was removed. Extraction was repeated with fresh extraction solvent for a total of three extractions. Method used was adapted from ISO 15753 Standard "Animal and vegetable fats and oils-Determination of polycyclic aromatic hydrocarbons." Extracts were combined and concentrated (solvent was removed, leaving residue oil). 2 mL of the acetonitrile/acetone was added to the residue oil and mixed. The extract (top layer) was loaded onto a C18 SPE cartridge. Two more extractions of the residue oil was repeated and loaded onto the SPE cartridge. PAHs were removed using 60/40 acetonitrile/acetone (percent volume) mixture. The eluant was concentrated to dryness and reconstituted in 1 mL hexanes and loaded onto Florisil SPE cartridges. The PAHs were eluted using 75/25 hexanes/dichloromethane solution (percent volume). The eluant was concentrated to 0.5 mL

		before adding 0.5 mL of toluene. This was concentrated to a final volume of 200 µL.
	2.5	After addition of an internal standard to the olive oil samples, 500 µL aliquots was injected into a size exclusion chromatography (SEC) semi-prep column (PLGel 100 µm x 100 Å, 300 x 25 mm) using dichloromethane as the mobile phase at 10 mL/min. Multiple injections per samples were separated and the fractions containing PAHs were combined. The combined fractions were concentrated to 250 µL prior to reverse phase LC analysis.
NMIJ	ca. 5.6 g	Liquid/liquid extraction (10 mL DMSO, 10 min, 3 times).Back-extraction with cyclohexane (3 times) →wash with 10% NaClaq (3 times) →concentration to 1 mL→GPC→concentration to 1 mL→SPE (silica-gel) →concentration to 0.2 mL
UME	0.5	Solid Phase Extraction with SUPELCO Supelclean EZ-POP NP (12 mL, 2.5 gram) cartridge. Clean cartridge with 10 mL acetonitrile and dry under vacuum for 15 minutes. Add 0.5 g oil sample and spike with IS solution. Elute with 15 mL acetonitrile. Extraction and clean-up in one cartridge (SUPELCO Supelclean EZ-POP NP). 15 mL acetonitrile eluate was concentrated under nitrogen stream at 40 ºC to final volume of 250 µL.
VNIIM	2	Hydrolysis reaction with potassium hydroxide (2g of Sample + 100 mL of 10% deionized water in methanol + 10 g of potassium hydroxide, boiling with backflow condenser for 3 hours). Liquid/liquid extraction in hexane (30 mL × 5 times).Washing by 700 mL of deionized water, vacuum evaporating to 2 mL, multilayer silica column (2 mL silica, 2 mL silica impregnated by KOH, 2 mL silica, 4 mL silica impregnated by H3PO4, 2 mL silica, 2 mL Na2SO4), the target fraction is dichloromethane/hexane=80/20 (50 ml), vacuum evaporating to 0.5 mL.
	2	Dissolved the Sample into 2mL of hexane, partition on Al2O3 (4mL, equilibrated at 180 °C during 4 hours), the target fraction is dichloromethane/hexane=30/70 (50 ml), vacuum evaporating to 0,5 mL.

Table F-2: Summary of Analytical Techniques for CCQM-K146

NMI/DI	Analytical Technique	Chromatographic Conditions	Ion/MRM
BAM	GC-MS	GC-MS(Agilent) 7890A with MSD 5975C inert XL HP-5MS (Agilent): 30m x 0.25mm ID x 0.25 µm Oven program: 80°C(1') → 10 K/min→ 320°C(5')Carrier gas: Helium, 1 mL/min Injection: 2 µL splitless MS: electron impact ionization 70 eV	Calibrant: Benzo(a)pyrene m/z 252 quant & 253 qual IS: 13C4-Benzo(a)pyrene m/z 256 quant & 257 qual
	GC-MS	GC-MS (Agilent) 6890N with MSD 5975B PAH-select (Agilent): 30m x 0.25 mm ID x 0.25 µm Oven program: 70°C(1') → 85 K/min→ 180°C(0') →3 K/min→ 230°C(7') →28 K/min→ 280°C(10') →14 K/min → 350°C(3') Carrier gas: Helium, 1 mL/min Injection: 5 µL large volume injection (LVI) MS: electron impact ionization 70 eV	
BVL	GC-MS, GC-HRMS	GC-MS (Thermo Trace GC Ultra coupled with Thermo DSQ II), GC-HRMS (Thermo Trace 1310 coupled with Thermo DFS) Macherey Nagel Optima 35 (30 m x 0,25 mm x 0,25 µm) and JW Select PAH (30 m x 0,25 mm x 0,25 µm) carrier gas helium 99,999%; column flow 1,0 mL/min; injection volume 2 µL, PTV 80°C (0,1 min) 14,5°C/sec 350°C (1,2 min) 14,5°C/sec 380°C (10 min) splitless time 1,2 min; oven temperature program Optima 35 80°C (2 min) 20°C/min 230°C (0 min) 2°C/min 310°C (17 min); oven temperature program JW Select PAH 80°C (2 min) 15°C/min 250°C (0 min) 1°C/min 280°C (0 min) 3°C/min 330°C (5 min); interface temperature 280 °C	Calibrant: 252 and 253 (low res); 252,0933 and 253,0967 (high res) IS: for BaP-D12 264 and 265 (low res); for 13C4-BaP 256 and 257 (low res), for BaP-D12 264,1687 and 265,1720 (high res); for 13C4-BaP 256.1075, 257.1186 (high res)
DRiCM	GC-MS	Shimadzu TQ8040. Rxi-5ms, 30 meter, 0.25mm ID, 0.25 µm df. Rate(oC/min) - Final Temperature(oC)- Hold time(min) 0 - 60.0 - 1.00, 25.00 - 275.0 - 0.00, 20.00 - 280.0 - 5.00, 10.00 - 300.0 - 5.00, Total Program Time: 21.85 min	Target Ion: 252 m/z; Reference Ion: 250 m/z

EXHM	GC-MS/MS	GC-MS/MS(Thermo GC Ultra coupled to PolarisQ) Agilent J&W DB-35 ms (30 m x 0.25 mm ID, 0.25 µm film thickness) PTV injector - 10 µL inj vol. - inj program: initial T 85 °C,split flow 100 mL/min, inj pressure 160 kPa, flow 25mL/min evaporation temp:15 °C /s to 85 °C for 0.5 min, tranfer temp: 15 min/s to 300 °C, cleaning 14.5 °C /s to 320 °C, hold 28 minHe carrier gas - 0-15 min: 1 mL/min, with 0.1 mL/min ramp to 2 mL/min - hold for 9 min oven initial T: 80°C (stable 3 min), 25 °C /min to 230, 10 °C /min to 250 °C, 3 °C /min to 310 °C, hold 5 min transfer line 280 °C	Calibrant: BaP (252 - 250/246) IS: BaP-d12 (264 - 260/258)
GLHK	LC-MS/MS	LC-MS/MS (Thermo Scientific Dionex Ultimate 3000 UPLC and AB SCIEX Triple Quad 6500) LC column: SUPELCOSIL LC-PAH (25cm x 2.1mm x 5um) Mobile phase A: Water; Mobile Phase B: Acetonitrile 40% B (0-5 min), 40-100%B (5-30 min), 100% B (30-38 min), 100-40% B (38-38.1 min), 40%B (38.1-45 min) Flowrate: 0.6 ml/min	Calibrant: 252/250 for quantification and 252/224 for qualification IS: 256/254
HSA	GC-MS	An Agilent Technologies 7890B gas chromatograph coupled to a 5977B mass spectrometer was used:(i) MS transfer line: 280 °C;(ii) Ion source: 280 °C;(iii) Quadrupole temperature: 180 °C Zebron ZB-PAH GC column, 20 m x 0.18 mm ID, 0.14 µm (i) Injection: 3 µL, 275 °C, splitless (ii) Oven: 70 °C (hold for 1 min), 70 °C/min to 180 °C (hold for 0 min), 7 °C/min to 230 °C (hold for 6 min), 40 °C/min to 280 °C (hold for 5 min), 40 °C/min to 300 °C (hold for 0 min). Post-run 300 °C for 3 min (iii) Carrier gas: Helium, 1.8 mL/min, constant flow	Benzo[a]pyrene: m/z 252.1 13C4-Benzo[a]pyrene: m/z 256.1

INMETRO	HPLC-FLD	HPLC-FLD - Thermo/Dionex Ultimate 3000 C18 250 mm x 4.6 mm size, 5 µm particle size (30 °C) The column flow was kept at 1 mL/min. Solvent A was ultrapure Water and solvent B was Acetonitrile. Gradient program was as follows. Initially solvent B was kept at 50 % until 10 min. Then it was increased to 100 % linearly until 28 min and kept until 36 min. At 36.1 min it was set back to 50 % and kept up to 40 min. Fluorescence detection was performed with excitation at 290 nm and emission at 410 nm.	not applicable
KRISS	GC/MS	GC/MS (Agilent 7890 GC/Jeol 800D-UF MS) DB-EUPAH (60m, 0.25 mm, 0.25 um) oven temp: 50°C (15 min) → 40 °C /min→ 280°C→2°C /min→320°C (10 min)	Calibrant: benzo(a)pyrene : m/z 252.0939 IS: 13C4-benzo(a)pyrene: m/z 256.1073
Lab of the Institute for Olivecultur e	HPLC-FLD	HPLC (Agilent 1100) Phenomenex Kinetex PAH 3,5 µm 150 × 4,6 mm (P/N 00F-4764-E0; S/N H17-339823) Flow: 1,2 mL/min; Gradient: A-water; B-CAN; 0' – 50% B – 8' – 100% B – 15' – 100% B – 15.5' – 50% B; eqiul. time: 3'	External calibrants
LGC	GC-MS	GC-MS (Agilent 7890A GC oven, combi PAL autosampler, Agilent 5975C MS) SGE HT8. 66 m effective length × 0.18 µm ID. 0.1 µm film thickness Injector: splitless at 260 °C. GC oven: 100 °C for 1 min. 30 °C/min to 250 °C and kept 6 min. 16 °C/min to 285 °C and kept 14.5 min. 30 °C/min to 335 and kept 7.5 min.	Calibrant: 252 Qualifiers: 250, 126 IS: 264
NIM	GC-MS/MS	GC-MS (Agilent 7890A GC oven, combi PAL autosampler, Agilent 5975C MS) Agilent DB-5 MS UI (60 m×0.25 mm×0.25 µm) Injector: splitless at 300 °C.GC oven: 70 °C for 1 min. 15 °C/min to 220 °C and kept 3min. 3°C/min to 260 °C. 30 °C/min to 300 and kept 8 min.	Benzo(a)pyrene: MRM 252->250,12D4-Benzo(a)pyrene: MRM 264->260

NIMT	GC-MS	MS conditions: MS quard 150 °C, MS source 230°C, SIM mode, m/z for Benzo(a)pyrene and D12-Benzo(a)pyrene are 250 252 253, 264 265 263;Select PAH (30 m. x 0.15 mm x 250 mm);Oven temperature: initial temperature 70 °C for 2 min→ramped rate 40 °C/min to 180 °C→ramped 7 °C/min to 230 °C for 7min→20 °C/min to 280 °C for 10 min →5 °C/min to 300 °C for 1 min. Flow rate: 2.0 ml/min, inlet temperature: 250 °C	Calibrant: Benzo(a)pyrene: 252(Q), 250, 253 IS: D12-Benzo(a)pyrene: 264 (Q), 265, 262
NIST	GC-MS	GC-MS (Agilent 6890N GC and Agilent 5973 inert MSD) using select ion mode. DB-17MS column (60 m x 0.25 mm i.d. x 0.15 µm df). Initial oven temperature was 70 °C (hold for 1 min), ramp to 150 °C at 30 °C/min, ramp to 320 °C at 2 °C/min, final temperature was held for 70 min. Flow rate of 1 mL/min.	Calibrant: Quantitation ion was 252 for BaP. Qualitative ion:250. IS: Quantitation ion: 264 for BaP-d12. Qualitative ion: 260
	LC-FLD	LC with fluorescence detection (Dionex Ultimate 3000 LC system). ZORBAX Eclipse PAH C18 (25 cm x 4.6 mm i.d., 5 µm average particle size). Mobile phase gradient: initial 50 % water and 50 % acetonitrile for 3 min, then a linear gradient to 100 % acetonitrile for 20 min, and hold at 100 % acetonitrile for 17 min. Fluorescence excitation and emission wavelengths of 404 and 440 nm were selected for 24.6 min, then change to 290 and 411 nm respectively from 24.6-40 min.	not applicable
NMIJ	GC-MS	GC-MS (Agilent Technologies 7890/5975C) DB-17MS (30 m, 0.25 mm, 0.25 um-thickness) oven temp: 50 °C (2 min-hold) → 10 °C/min → 240 °C → 1.25 °C/min → 300 °C (10 min-hold)	BaP: m/z 252.1, 250.1 13C4-BaP: m/z 256.1, 257.1
UME	GC-MS/MS	THERMO TSQ GC-Tandem Mass (triple quadrupole) THERMO TG-5MS 30 m x 0.25 mm 0.25 µm; Oven temperature:60 ºC (3 min.); with 15 ºC/min. to 160 ºC; with 5 ºC/min. to 200 ºC; with 2 ºC/min. to 240 ºC; with 5 ºC/min. to 300 ºC.	Calibrant: 252 → 250 (CE: 30V) IS: 264 → 260 (CE: 30V)
VNIIM	GC-MS/MS	Agilent GC/MS/MS 7000D HP-5MS, 30m×0.25mm×0.25µm GC oven temperature programme: 70°C (3 min) →20°C/min→ 250°C (1 min) →2°C/min→ 280°C (10 min)	B(α)P: MRM 252->250 (CE 45 eV) 13C4-B(α)P: MRM 256->254 (CE 45 eV)

Table F-3: Summary of Calibrants and Standards for CCQM-K146

NMI/DI	Type of Calibration	Method of Quantification	Calibrants	Internal Standards
BAM Method 1	6-point calibration curve	Internal standard calibration	Benzo(a)pyrene in house certified and confirmed by NIST SRM 2260a	^{13}C4-Benzo(a)pyrene
BAM Method 2	6-point calibration curve	Internal standard calibration		^{13}C4-Benzo(a)pyrene
BVL	6-point calibration curve	Internal Standard	NIST 1647f	BaP-D12 &^{13}C4-BaP
DRiCM	6-point calibration curve	External Calibration	Supelco CRM	N/A
EXHM	single-point	IDMS at exact matching with matrix-matched standard	Benzo(a)pyrene in house certified and confirmed NIST SRM 2260a	benzo[a]pyrene-d12
GLHK	Single point calibration & bracketing	IDMS	NIST SRM 1647f	^{13}C4-Benzo[a]pyrene
HSA	Single-point calibration	Exact-matching IDMS	Certified reference material (HRM-1017A)	^{13}C4-Benzo[a]pyrene
INMETRO	Five-point calibration curve. Calibration curve was prepared by two independent solutions in a blank matrix.	Internal standard calibration	Benzo(a)pyrene in house certified	Benzo[ghi]perylene
KRISS	single-point	IDMS (High resolution R=10000)	Benzo(a)pyrene in house certified	^{13}C4-Benzo[a]pyrene

Laboratory of the Institute for Oliveculture	three-point calibration curve	External calibration	/	Dibenz[(a, h] anthracen
LGC	Bracketed single point exact matching	Double exact matching IDMS	NIST SRM 1647f	Benzo[a]pyrene D12
NIM	single-point	IDMS	Benzo(a)pyrene GBW(E)080476	Benzo[a]pyrene D12
NIMT	single-point, bracketing	Exact-Matching IDMS	Benzo(a)pyrene in house certified	D12-Benzo[a]pyrene
NIST	Average response factors for both methods.	Internal standard, bracketing (IDMS). Calibrants were matrix matched (blank olive oil was spiked and this was used as calibrants). Internal standard, bracketing.	NIST SRM 2260a	BaP-d12
NMIJ	single-point	IDMS	NMIJ CRM4213a	13C4-benzo[a]pyrene
UME	Single point	IDMS	NIST SRM 1647f	Benzo[a]pyrene-d12
VNIIM	bracketing	IDMS	Benzo(a)pyrene in house certified and confirmed NIST SRM1647f	13C-Labeled EPA 16 PAH

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.

BAM

$$x_{sample} = \frac{r - i_c}{sl} \cdot \frac{m_{IS}}{m_{sample}} \cdot F_{pur}$$

xsample: mass fraction of benzo(a)pyrene in oil sample

r: area ratio native/internal standard

ic: intercept of calibration line

sl: slope of calibration line

mIS: mass of internal standard added to sample

msample: mass of oil sample

Fpur: purity of B(a)P calibation standard according to certificate

Method A

$$U = k \cdot u_c = k \cdot x_{sample} \cdot \sqrt{(u_{x,r})^2 + (u_{cal,r})^2 + (u_{pur,r})^2}$$

symbol	parameter description	value	unit
x_{sample}:	mean value of B(a)P in olive oil	2.700	µg/kg
$u_{x,r}$:	rel. standard uncertainty of measurement: SDmean/x_{sample}	0.0275	
SD_{mean}	standard deviation of the mean	0.0743	µg/kg
$u_{cal,r}$:	rel. uncertainty of calibration acc. to EURACHEM CITAC Guide	0.0351	
$u_{pur,r}$:	rel. uncertainty of B(a)P standard (purity): u_{pur}/F_{pur}	0.0015	
u_{pur}:	standard uncertainty of B(a)P standard acc. to certificate	0.0015	g/g
F_{pur}:	purity of B(a)P standard acc. to certificate	0.9885	g/g
u_c:	combined standard uncertainty	0.1205	µg/kg
k:	coverage factor	2	
U:	expanded uncertainty (95% confidence)	0.2411	µg/kg

Method B

$$U = k \cdot u_c = k \cdot x_{sample} \cdot \sqrt{(u_{x,r})^2 + (u_{cal,r})^2 + (u_{pur,r})^2}$$

symbol	parameter description	value	unit
x_{sample}:	mean value of B(a)P in olive oil	2.576	µg/kg
$u_{x,r}$:	rel. standard uncertainty of measurement: SDmean/x_{sample}	0.0256	
SD_{mean}	standard deviation of the mean	0.0658	µg/kg
$u_{cal,r}$:	rel. uncertainty of calibration acc. to EURACHEM CITAC Guide	0.0248	
$u_{pur,r}$:	rel. uncertainty of B(a)P standard (purity): u_{pur}/F_{pur}	0.0015	
u_{pur}:	standard uncertainty of B(a)P standard acc. to certificate	0.0015	g/g
F_{pur}:	purity of B(a)P standard acc. to certificate	0.9885	g/g
u_c:	combined standard uncertainty	0.0918	µg/kg
k:	coverage factor	2	
U:	expanded uncertainty (95% confidence)	0.1836	µg/kg

BVL

C(sample) = [A(analyte)*m(is)]/[A(is)*RF(mean)]*1/m(sample); C(sample): concentration of analyte in sample [ng/g]; A(analyte); A(is): chromatographic peak area of analyte; peak; m(is): mass of internal standard [ng]; RF(mean): average response factor from four calibration points; m(sample): weight of sample aliquot [g]; Response Factor = [Area(analyte)/Area(int. standard)]/[mass(int. standard)/mass(analyte)]

uncertainty contributions: weighing (sample, internal standard and calibration solution); within laboratory reproducibility (from method validation); variation of response factor (accounts for injection effects and integration bias); uncertainty of calibrant (taken from certificate NIST 1647f); additional uncertainty crosscheck NIST1647f with NIST2260a, combined uncertainty = sqrt (sum of squared standard uncertainties).

Weighing processes		
weighing of sample	0.01	1000 +/- 0,1 mg
addition of internal standard solutic	0.38	weight of sample taken 26 mg +/- 0,1 mg
Calibration		
Uncertainty of calibrant	0.88	NIST 1647f, see certificate
weighing NIST 1647f	0.06	weight of sample taken 155 mg +/- 0,1 mg
crosscheck NIST SRMs	3.030	difference between response factors NIST 2260a and NIST 1647f
variation response factor	5.100	accounts for injection and integration bias

mean RF	std. dev.	CV [%]	analyte	analysis number	
1.00642	0.047	4.63	(BaP)	AS180057	D12
0.896229	0.046786	5.22	(BaP)	AS180057	13C4
0.913881	0.055092	6.03	(BaP)	AS180067	D12
0.838537	0.036689	4.38	(BaP)	AS180067	13C4

mean	5.1

Method		
within lab. reproducibility	6	taken from validation
combined standard uncertainty [%]	8.49	%
expanded uncertainty [%]		
k=2	17.0	%

DRiCM

From Calibration Curve mass fraction was calculated. Straight line equation for calibration curve was y= 41617.73 x + 31438.4 with regression coefficient of 0.999.

i) Fractional Uncertainty Method were used to combine uncertainty with different units. Fractional uncertainty= δx/lxl. ii) Combined Standard Uncertainy = Square root of the summation of the square of standard uncertainty of each uncertainty sources. Uncertainty Budget (Source - Mean Value- Expanded Uncertainty - Fractional Uncertainty(%) - Divisor - Standard Uncertainty)

Repeatability (µg/Kg) - 1.93 - 0 - 2.87 - 0 - 2.87

Pipette (mL) - 1 - 0.01 - 1 - 2 - 0.5

Balance (gm) - 1 - 0.0003 - 0.03 - 2 - 0.02

Reference Standard (%) - 99.9 - 0.5 - 0.5 - 2 - 0.25

EXHM

The measurement equation is:

$$w_{M,S} = w_{M,C} \frac{100}{Rechh} \times \frac{m_{is,S}}{m_{M,S}} \times \frac{m_{M,C}}{m_{is,C}} \times \frac{R_S}{R_B}$$

where $w_{M,S}$ = mass fraction of the analyte (B[a]P) in the sample, (µg/kg)

$w_{M,C}$ = mass fraction of the analyte (B[a]P) in the calibration solution, (µg/kg)

Rec = recovery (%), assessed against other independent methods

$m_{is,S}$ = mass of internal standard solution added to sample blend, (g)

$m_{M,S}$ = mass of test material in sample blend, (g)

$m_{M,C}$ = mass of the analyte (B[a]P) solution added to calibration blend, (g)

$m_{is,C}$ = mass of internal standard solution added to calibration blend, (g)

R_S = measured peak area ratio of the selected ions in the sample blend

R_C = measured peak area ratio of the selected ions in the calibration blend

The equation used to estimate standard uncertainty is:

$$u(w_{BS}) = \sqrt{\left({s_R}/{\sqrt{n}}\right)^2 + \sum\left(C_j u(m_i)\right)^2 + \sum\left(C_j u(R_i)\right)^2 + \left(C_j u(w_{MC})\right)^2 + + \left(C_j u(R)\right)^2}$$

where s_R is the standard deviation under reproducibility conditions, n the number of determinations and C_j the sensitivity coefficients associated with each uncertainty component. The uncertainty of the peak area ratios was considered to have been included in the estimation of method precision.

Uncertainty estimation was carried out according to JCGM 100: 2008. The standard uncertainties were combined as the sum of the squares of the product of the sensitivity coefficient (obtained by partial differentiation of the measurement equation) and standard uncertainty to give the square of the combined uncertainty. The square root of this value was multiplied by a coverage factor (95% confidence interval) from the t-distribution at the total effective degrees of freedom obtained from the Welch-Satterthwaite equation to give the expanded uncertainty.

Benzo[a]pyrene

uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	1,93	1,000	0,16	0,0840	0,1620	0,0263
mass fraction of B[a]P in the calibration solution, (*µg/kg*)	194,20	0,010	1,95	0,0180	0,0194	0,0004
recovery (%)	100,00	-0,019	2,347	0,0235	-0,0453	0,0020
mass of B[a]P-d_{12} solution added to sample blend, (*g*)	0,07568	25,488	0,00007	0,0009	0,0018	0,0000
mass of test material in sample blend, (*g*)	10,00000	-0,193	0,00032	0,0000	-0,0001	0,0000
mass of B[a]P solution added to calibration blend, (*g*)	0,09547	20,205	0,00003	0,0003	0,0006	0,0000
mass of B[a]P-d_{12} solution added to calibration blend, (*g*)	0,07575	-25,465	0,00003	0,0004	-0,0008	0,0000
measured peak area ratio of the selected ions in the sample blend	1,762	1,095	considered to be included in the			
measured peak area ratio of the selected ions in the calibration blend	1,692	-1,140	estimation of method precision			

result (ng/g)	1,93
combined standard uncertainty (ng/g)	0,17
relative standard uncertainty (%)	8,78
effective degrees of freedom	9,5
coverage factor	2,26
expanded uncertainty (ng/g)	0,38

GLHK

$$C_x = C_z \times \frac{M_y}{M_x} \times \frac{M_z}{M_{yc}} \times \frac{R_b}{R_{BC}}$$

Cz = mass fraction of the analyte solution used to prepare the calibration blend

My = mass of standard standard added to the sample blend (g)

Mx = mass of sample (g)

Mzc = mass of standard solution added to the calibration blend (g)

Myc = mass of internal standard added to the calibration blend (g)

RB = isotope amount ratio in sample blend

RBC = isotope amount ratio in calibration blend

BAPY	Description	**Value *x***	**Standard uncertainty $u(x_i)$**	**Relative standard uncertainty $u(x_i)/x_i$**

c_{Z0} (ug/g)	Mass fraction of stock solution	6.22	0.055	0.00884
c_{z1}(g)	Weight of stock solution	0.18812	0.0001	0.00053
c_{z2} (g)	Weight of solvent for dilution	2.70461	0.0001	0.00004
c_{z3} (g)	Weight of intermediate solution	0.25125	0.0001	0.00040
c_{z4} (g)	Weight of solvent for dilution	7.42459	0.0001	0.00001
m_y (g)	Weight of internal standard in sample blend	0.12559	0.0001	0.00080
m_x (g)	Weight of sample	0.54392	0.0001	0.00018
m_{yc} (g)	Weight of internal standard in calibration blend	0.45123	0.0001	0.00022
m_z (g)	Weight of standard added to calibration blend	0.36201	0.0001	0.00028
R_b	Isotope ratio of sample	0.97597	0.0078	0.00796
R_{bc}	Isotope ratio ofcalibration blend	0.97633	0.0077	0.00785
R	Run to run variability	1.000	0.0094	0.00941
R_{bc}	Accuracy of CRM	1.000	0.0109	0.01091
R	Spike recovery	1.000	0.0097	0.00973
$c_{x,i}$ (ug/g)			0.0587	0.02251

HSA

$$C_X = C_Z \cdot \frac{m_Y \cdot m_{Zc}}{m_X \cdot m_{Yc}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$$

Factors	Details of the factors	How the values were determined

C_Z =	mass fraction of benzo[a]pyrene in the calibration standard solution used to prepare the calibration blend	Gravimetric values of serial dilution of the calibration solution and the purity value of benzo[a]pyrene calibrant
m_Y =	mass of internal standard solution added to the sample blend	Weighing
m_{Yc} =	mass of internal standard solution added to the calibration blend	Weighing
m_{Zc} =	mass of standard solution added to the calibration blend	Weighing
m_X =	mass of study material in the sample blend	Weighing
R_X =	observed isotope abundance ratio in the study material	Peak area ratio of 252.1/256.1 in the study material
R_Y =	observed isotope abundance ratio in the internal standard	Peak area ratio of 252.1/256.1 in the internal standard solution
R_Z =	observed isotope abundance ratio in the calibration standard	Peak area ratio of 252.1/256.1 in the calibration standard solution
R_B =	observed isotope abundance ratio in the sample blend	Peak area ratio of 252.1/256.1 in the sample blend
R_{Bc} =	observed isotope abundance ratio in the calibration blend	Peak area ratio of 252.1/256.1 in the calibration blend

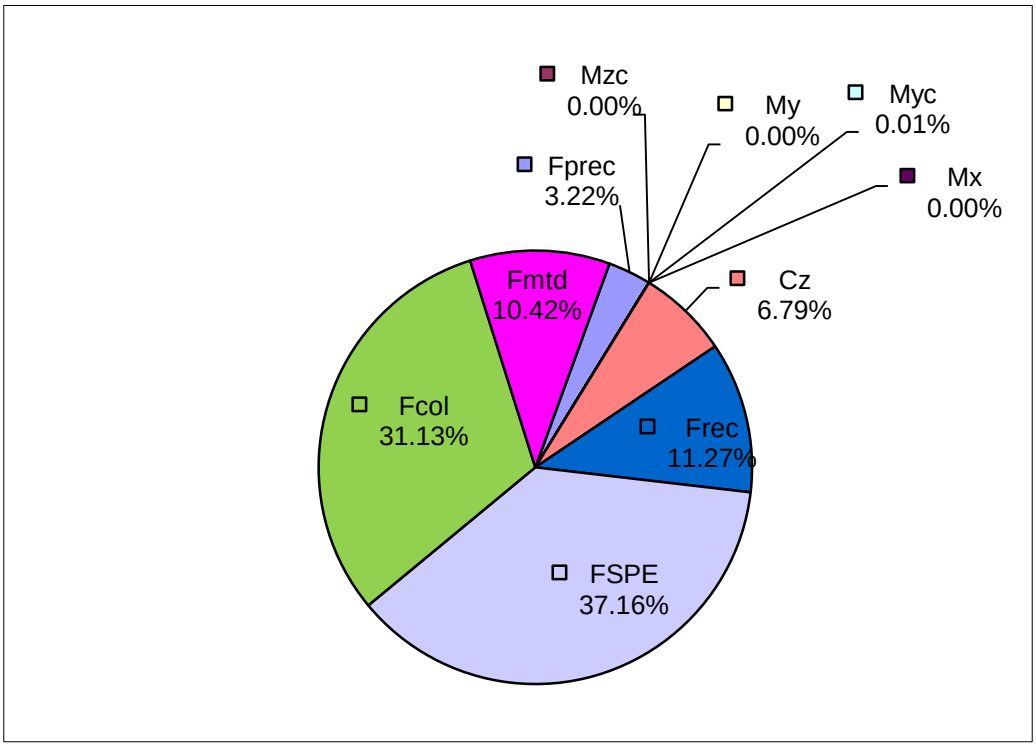
$$C_X = F_{prec} \cdot F_{col} \cdot F_{SPE} \cdot F_{mtd} \cdot F_{rec} \cdot C_Z \cdot \frac{m_Y \cdot m_{Zc}}{m_X \cdot m_{Yc}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$$

where

additional factors (F) contributing to biases in the result value of benzo[a]pyrene were included, with an uncertainty associated to each factor.

F_{prec} =	Factor representing method precision
F_{col} =	Factor representing any bias in the result due to choice of GC column
F_{SPE} =	Factor representing any bias in the result due to choice of SPE cartridge for clean-up
F_{mtd} =	Factor representing any bias in the result due to the choice of calibration method, i.e. exact matching IDMS vs linear regression IDMS
F_{rec} =	Factor representing method recovery

The full uncertainty budget for the determination of benzo[a]pyrene is given in the Table below:



Parameter	x_i	u_{xi}	u_{xi}/x_i	Contribution	Sources of uncertainty
F_{prec}	1	0.00497	0.4971%	3.224%	Standard deviation of the mean of six independent determinations on the study material.
F_{col}	1	0.01545	1.5446%	31.133%	Comparison of results obtained using Zebron ZB-PAH column and Restek Rxi-PAH column on the same subsamples.
F_{SPE}	1	0.01688	1.6876%	37.162%	Comparison of results obtained using Cleanert BaP3 SPE cartridge and SupelMIP SPE cartridge.
F_{mtd}	1	0.00893	0.8934%	10.416%	Comparison of results obtained using exact matching IDMS and linear regression IDMS on the same subsamples.
F_{rec}	1	0.00929	0.9292%	11.267%	Method recovery using in-house

					spiked olive oil as quality control.
C_Z	0.002996	0.0000216	0.7212%	6.788%	• Uncertainty in the purity value of benzo[a]pyrene certified reference material (HRM-1017A). • Uncertainty in weighing based on balance calibration certificate. • Bias in the preparation of calibration blends. • Bias in results determined using the solution calibration blend (in toluene) vs the matrix-matched calibration blend.
m_Y	0.7086	0.0000778	0.0110%	0.002%	Uncertainty in weighing based on balance calibration certificate.
m_{Yc}	0.3316	0.0000778	0.0235%	0.007%	
m_{Zc}	0.8638	0.0000778	0.0090%	0.001%	
m_X	1.9588	0.0000778	0.0040%	0.0002%	
R_X, R_Y, R_Z	Negligible				
R_B, R_{Bc}	Uncertainty included in method precision				

INMETRO

$$w_a = c_0 * \frac{m_{IS} * P_a}{m_{sol}}$$

$$c_0 = \left(\frac{A - b_0}{b_1}\right)$$

wa = Mass fraction of the analyte (measurand)

A = Area of the analyte in the sample

b0 = Linear coefficient of the calibration curve

msol = Final mass of the injected dilute solution

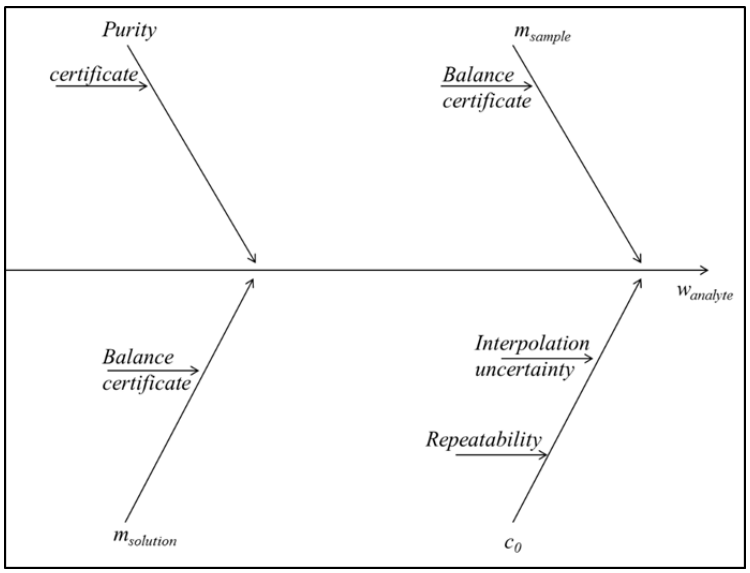
b1 = Angular coefficient of the calibration curve

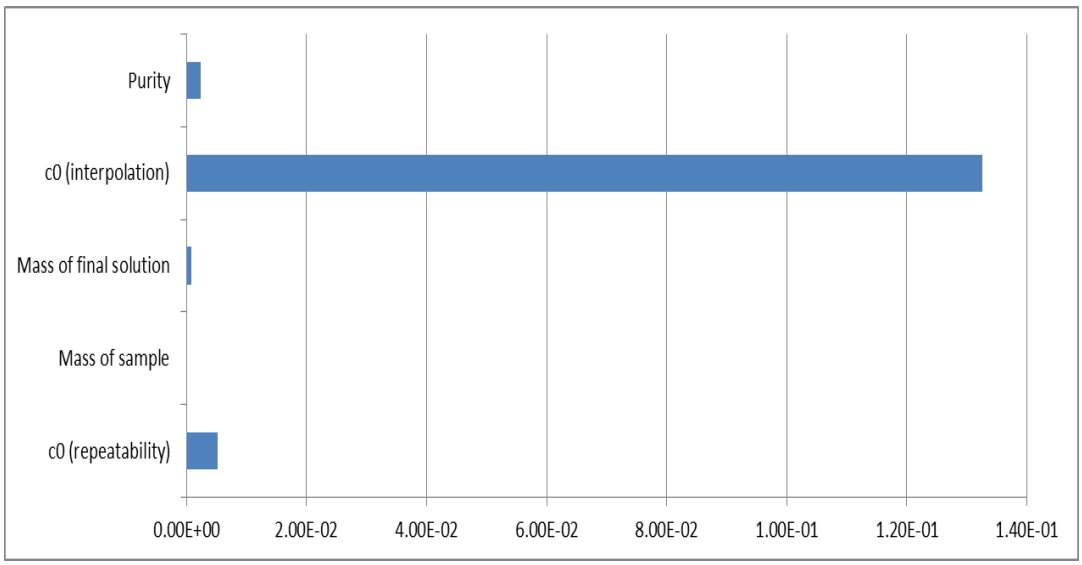
mIS = Weighed mass of IS solution

Pa = Purity of the standard used in the calibration curve

c0 = mass fraction in the injected dilute solution

Ishikawa diagram





Uncertainty sources	Value	Type	Distribution	Standard uncertainty	Sensitivity coeff.	Uncertainty comp.
c0 (repeatability)	0.0101	A	Normal	3.21E-05	1.61E+02	5.18E-03
Mass of sample	0.50996	B	Normal	4.50E-05	-3.20E+00	1.44E-04
Mass of final solution	0.08338	B	Normal	4.50E-05	1.96E+01	8.82E-04
Angular coefficient (b1)	83.16420021	A	Normal	-	-	
Linear coefficient (b0)	0.019635619	A	Normal	-	-	
c0 (interpolation)	0.0101	A	Normal	8.22E-04	1.61E+02	1.33E-01
Purity	986.2	B	Normal	1.47E+00	1.66E-03	2.44E-03
					Combined uncertainty	0.132736421

KRISS

$$C_{\text{sample}} = f \bullet \frac{M_{\text{is-sol,spiked}} \cdot AR_{\text{sample}} \cdot M_{\text{s-sol,std. mix.}} \cdot C_{\text{s-sol}}}{M_{\text{sample}} \cdot AR_{\text{std. mix.}} \cdot M_{\text{is-sol,std.mix.}}}$$

f: dry-mass correction factor; it is not applied in this experiment.

C_{sample}: is the concentration of analytes in the sample;

$C_{s\text{-}sol}$: is the concentration of the analytes standard solution;

M_{sample}: is the mass of the sample taken for analysis;

$M_{is\text{-}sol,\ spiked}$: is the mass of the isotope standard solution added to the sample aliquot;

$M_{is\text{-}sol,\ std.\ mix.}$: is the mass of the isotope standard solution added to the isotope ratio standard solution;

$M_{s\text{-}sol,\ std.\ mix.}$: is the mass of the standard solution added to the isotope ratio standard solution;

AR_{sample}: is the area ratio of analyte/isotope for sample extract, observed by GC/MS;

$AR_{std.\ mix.}$: is the area ratio of analyte/isotope for the isotope ratio standard solution, observed by GC/MS.

$$u(C_{\text{mean}}) = \sqrt{u^2_{\text{s.p.,systematic}} + s^2}$$

Systematic	U,sys (rel%)	DOF
Uncertainty of purity of primary standard	0.25%	6
Uncertainty of gravimetric preparation for standard solution	0.47%	3
Uncertainty of gravimetric mixing for calibration isotope standard mixtures.	0.35%	4
Area ration of BaP/13C-BaP for the calibration standard mixture, observed by GC/MS	0.31%	2
SUM	0.71%	9

Laboratory of the Institute for Oliveculture

Mass fraction of B[a]P

$$Mass\ \ fraction\ \ B[a]P(\mu g/kg) = \frac{X_{HPLC} \times V_s \times 1000 \times Rec \times RM_f}{V_i \times m_{sample}}$$

Where:

X_{HPLC} amount of HPLC-FLD determined B[a]P according to 3-point calibration using the linear formula X_{HPLC} (ng) = (y-b)/m [y = peak area in arbitrary units; b = 0.108406; m = 4944.19032; r2 = 0.99993; origin connected, equal weighing]

V_s volume of solution before the injection in HPLC (1000 µL)

Rec Recovery = (amount of IS added to the sample prior to the extraction (ng))/(amount of IS found (ng))

RM_f Factor correcting for the difference between RM assigned value and the found value (in our case = 2.3/1.86)

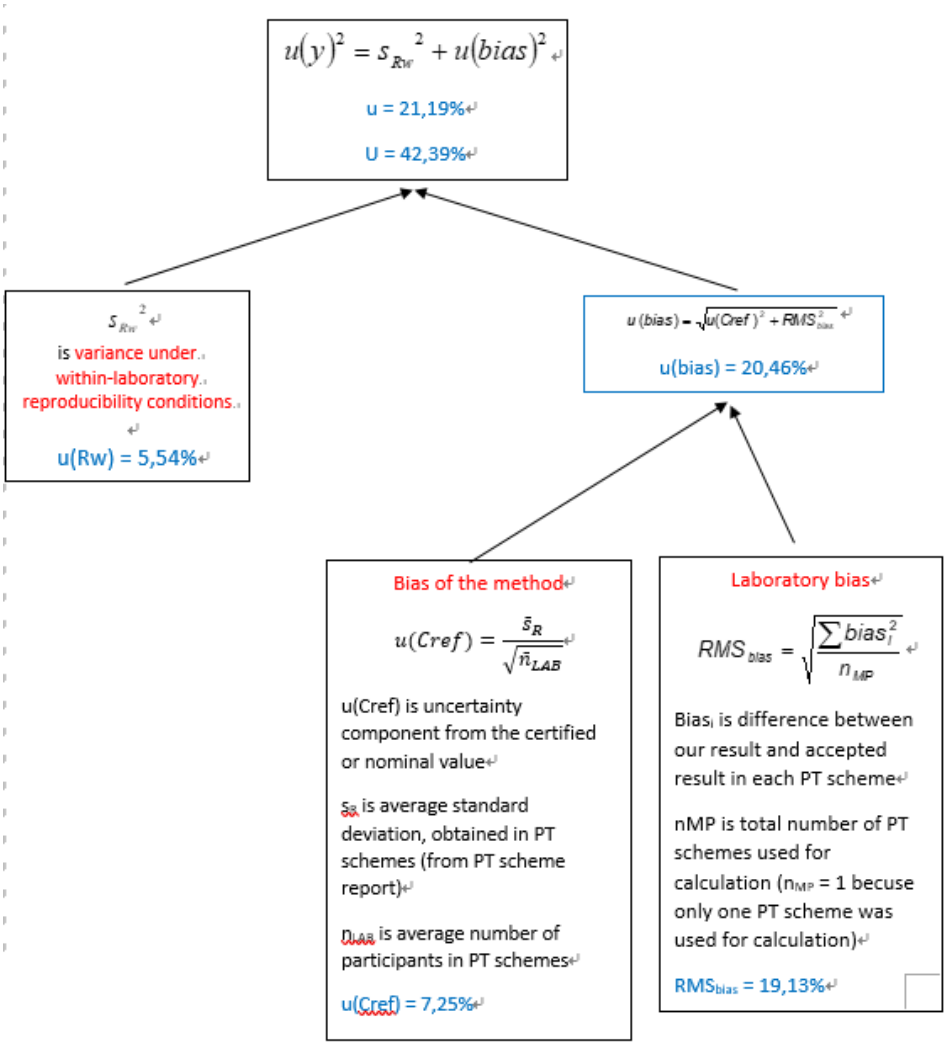
V_i volume of injection (30 µL)

m_{sample} mass of sample (ca. 200 mg)

The overall uncertainty was estimated according to document SANTE/11813/2017, Appendix C (page 31), 2nd approach (equation 2). The first (relative) contribution (u' RSDwR) was 5.5 %, and the second contribution (u' bias) was 20.5 %. The second contribution (u' bias) was evaluated analysing the RM material and comparing our results with RM data from PT (PROFICIENCY TEST SCHEMA 23 03 Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in water and oil sample, organized by General Chemical State Laboratory, Chemical Metrology Service, Greece). The overall relative uncertainty was 21.2 % and the expanded uncertainty, using coverage factor 2, was 42.4 %.

Re-evaluated uncertainty in March 2019

There are two contributions to measurement uncertainty: variance under within-laboratory reproducibility conditions and estimated variance of method bias and laboratory bias. Details on calculation are given on next page. Individual contributions to total MU are written in blue.



LGC

$$W_{X_i} = \frac{1}{m_X} \times (m_Z \times W_Z) \times \frac{m_{Y,SB}}{m_{Y,CB}} \times \frac{R_{SB_i}}{R_{CB_i}}$$

— W_Xi is the mass fraction of the analyte in the sample calculated for injection i,

— m_X is the mass of the sample weighed,

— m_Z is the mass of the analyte solution added to the calibration blend,

— W_Z is the mass fraction of the analyte in the solution added to the calibration blend

— m_(Y,CB) is the mass of the labelled analogue solution added to the calibration blend,

— m_(Y,SB) is the mass of the labelled analogue solution added to the sample blend,

— R_SBi is the height ratio of each of the individual sample injection i.

— R_CBi is the average height ratio of the 2 bracketing calibration blends of injection i.

The mass fraction of each individual sample was calculated as the average of the 5 calculated mass fractions of the individual injections:

Uncertainty of each individual result was estimated as:

$$u_{W_X} = W_{\mathrm{X}} \times \sqrt{\left(\frac{u_{m_{\mathrm{X}}}}{m_{\mathrm{X}}}\right)^2 + \left(\frac{u_{m_{\mathrm{Z}}}}{m_{\mathrm{Z}}}\right)^2 + \left(\frac{u_{W_{\mathrm{Z}}}}{W_{\mathrm{Z}}}\right)^2 + \left(\frac{u_{m_{\mathrm{Y,\,SB}}}}{m_{\mathrm{Y,\,SB}}}\right)^2 + \left(\frac{u_{m_{\mathrm{Y,CB}}}}{m_{\mathrm{Y,\,CB}}}\right)^2 + \left(\frac{u_{\left(\frac{R_{\mathrm{SB}}}{R_{\mathrm{CB}}}\right)}}{\frac{R_{\mathrm{SB}}}{R_{\mathrm{CB}}}}\right)^2}$$

Where:

— $u_{(m\_X)}$ is the uncertainty of the mass of sample used, estimated from the balance certificate.

— $u_{(m\_Z)}$ is the uncertainty of the mass of analyte solution added to the calibration blend, estimated from the balance certificate.

— $u_{(W\_Z)}$ is the uncertainty of the mass fraction of the calibration solution, estimated by the combination of the uncertainty of the masses of the individual components, and these from the balance certificate.

— $u_{(m\_Y,SB\ )}$ is the uncertainty of the mass of the analyte labelled analogue solution added to the sample blend, estimated from the balance certificate.

— $u_{(m\_Y,CB)}$ is the uncertainty of the mass of the analyte labelled analogue solution added to the calibration blend, estimated from the balance certificate.

— $u_{(R\_SB\ /\ R\_CB)}$ is the standard deviation of 5 bracketed response ratios.

— (R_{SB} / R_{CB}) is average of the 5 bracketed response ratios.

Total combined uncertainty was estimated by averaging the individual combined standard uncertainties.

Next table shows the uncertainty budget for the 6 individual replicates:

		R_SB/R_CB	m_X	m_Y,SB	m_Y,CB	m_Z	w_Z
Rep 1	Variance	3.34E-04	9.79E-09	6.49E-07	6.49E-07	6.11E-07	7.82E-05
	Budget (%)	81	0.0024	0.16	0.16	0.15	19
Rep 2	Variance	1.15E-04	9.81E-09	6.51E-07	6.45E-07	6.12E-07	7.82E-05
	Budget (%)	59	0.005	0.33	0.33	0.31	40
Rep 3	Variance	1.05E-04	9.80E-09	6.45E-07	6.45E-07	6.12E-07	7.82E-05
	Budget (%)	57	0.005	0.35	0.35	0.33	42
Rep 4	Variance	3.35E-04	9.71E-09	5.87E-07	5.88E-07	6.12E-07	7.82E-05
	Budget (%)	81	0.0023	0.14	0.14	0.15	19
Rep 5	Variance	4.35E-04	9.78E-09	5.94E-07	5.95E-07	6.21E-07	7.82E-05
	Budget (%)	84	0.0019	0.12	0.12	0.12	15
Rep 6	Variance	7.95E-04	9.68E-09	5.88E-07	5.95E-07	6.21E-07	7.82E-05
	Budget (%)	91	0.0011	0.07	0.07	0.07	8.9

NIM

The mass fraction (µg/kg) of analytes (C_x) in the sample was calculated as follows:

The expanded measurement equation given was used to calculate the mass fraction of the measurand. The additional factors (F) in the expanded measurement equation represent aspects of the measurement procedure that may influence the measured mass fraction value. They are given a value of 1 but they add an uncertainty component to the uncertainty budget.

Expanded measurement equation:

$C_x = F_P \times (M_{IS} \times M_{AC} \times R_b)/(M_S \times M_{IC} \times R_{bc})$

Where :

Cx is the mass fraction of analytes in the sample (ng/g);

F_P is the method precision factor

M_{IS} is mass of internal standard (isotopologue) added to the sample blend (g)

M_{AC} is mass of analyte added to the calibration blend (g)

R_b is peak area ratio of analyte /isotopologue in sample blend

M_S is mass of sample (g)

M_{IC} is mass of internal standard(isotopologue) added to the calibration blend (g)

R_{bc} is peak area ratio of analyte /isotopologue in calibration blend

Source of uncertainty	Value	u(x)	u(x)/(x)
M_{IS} (g)	0.08	0.00019	0.24%
M_S (g)	0.5	0.00019	0.04%
M_{IC} (g)	0.2	0.00019	0.10%
M_{AC}(g)	0.2	0.0028	1.40%
Method Precision, F_P			3.80%
Coverage factor, *k*			2
Relative combined standard uncertainty (u_c)			4.06%
Relative expanded uncertainty (*Uc*)			8.1%
Mass Fraction (µg/kg)			2.71
Expanded uncertainty, *U* (µg/kg)			0.22

NIMT

$$w_x = F_P.F_E.w_z \cdot \frac{m_y \cdot m_{zc}}{m_x \cdot m_{yc}} \cdot \frac{R'b}{R'bc}$$

wx = mass fraction of benzo(a)pyrene (ng/g) in olive oil

wz = mass fraction of benzo(a)pyrene (ng/g) in the calibration solution used to prepare the calibration blend

my = mass of internal standard solution added to the sample blend

myc = mass of internal standard solution added to the calibration blend

mzc= mass of calibration standard added to calibration blend

mx = mass of sample used

FE = extraction efficiency factor, given a value of 1

FP = method precision factor

R'b and R'bc= the isotope amount ratios of the analyte and the internal standard in the sample and calibration blends, respectively

$$\frac{u(x)}{x} = \sqrt{\left(\frac{u(m_y)}{m_y}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(F_E)}{F_E}\right)^2 + \left(\frac{u(F_P)}{F_P}\right)^2 + \left(\frac{u(w_{zc})}{w_{zc}}\right)^2 + \left(\frac{u(m_{ZC})}{m_{ZC}}\right)^2 + \left(\frac{u(m_{yC})}{m_{yC}}\right)^2}$$

$u(w_{z,c})$ is the standard uncertainty of the mass fraction of analyte in the calibration solution used to prepare the calibration blend.

(m_y), $u(m_{y,c})$, $u(m_x)$ and $u(m_{z,c})$ are standard uncertainties of the masses.

u (F_P) is the standard uncertainty of the precision factor

u(F_E) is the standard uncertainty of the extraction efficiency factor

Note: For the uncertainty contributing to the R'b and R'b,C ,the precision in measuring the isotope amount ratios of the analyte and the internal standard in the sample and calibration blends was assumed to be incorporated in the overall method precision. The effect of any biases on these ratios was assumed to be negligible as any systematic biases should cancel out since the calibration blends and sample blends were exact-matched for analyte concentration and isotope ratio. Other biases that may arise from interferences, extractions are captured in other factors.

Uncertainty Analysis Results

wx=	**2.052**	ng/g
u(x) =	0.111	ng/g
u(x)/x =	5.39%	
Veff(total) =	13.380	

k=	2.16	(@ 95% level)
U(x) =	**0.239**	
%U(x) =	**11.65%**	

Combination of Uncertainties

Factor	**Value** **x**	**Uncertainty** **u(x)**	**Standard uncertainty** **u(x)/(x)**
Measurement equation factors			
Method Precision, F_P	1.0000	0.04486	4.486%
m_{zc}	0.04035	0.000080	0.1973%
m_y	0.04140	0.000080	0.1923%
m_{yc}	0.04099	0.000080	0.1942%
m_x	0.98860	0.000080	0.0081%
w_z	51.1204	1.126682	2.2040%
Additional Factors			
Extraction effect, F_E	1.000	0.0200	2.000%

NIST

For calibration solutions: RF= (area PAH in calibrant/area labeled PAH in calibrant)*(mass of labeled PAH in calibrant/mass of PAH in calibrant)

For olive oil samples: mass fraction of PAH = [(area PAH in sample/area labeled PAH in sample)*(mass of labeled PAH in sample/RF)]/mass of olive oil sample used.

The above equations were used to obtain the mass fraction of BaP in the olive oil for both methods. The results from the two methods were combined using Linear Pool method using the NIST Consensus Builder [1].

References:

[1] Koepke, A, Lafarge, T, Toman, B, Possolo, A NIST Consensus Builder- User's Manual (Gaithersburg, MD: National Institute of Standards and Technology) consensus.nist.gov, 2017.

Uncertainty quantification was performed using the Observation Equation approach [2,3] evaluated using Bayesian Markov Chain Monte Carlo [4]. Individual uncertainty components were:

Uncertainty source Standard uncertainty

Balance: 0.0000625g

BaP mass fraction of SRM 2260a (Method 1 and 2): 0.06 µg/g (1.27 %)

Internal standard solution, BaP-d12 mass fraction (Method 1): 0.0007 µg/g (1.77 %)

Internal standard solution, BaP-d12 mass fraction (Method 2): 0.0134 µg/g (1.52 %)

Response factor consensus for method 1: 0.03 (2.75 %)

Response factor consensus for method 2: 0.02 (2.16 %)

In addition to these uncertainty components, the Observation Equation approach also accounted for uncertainty due to repeatability within each method.

Results from each of the methods:

Method 1 (µg/kg): 2.81 with standard error 0.08 (2.85 %)

Method 2 (µg/kg): 2.56 with standard error 0.12 (4.69 %)

The results from the two methods were combined using the Linear Pool method of the NIST Consensus Builder [1] to obtain: 2.73 µg/kg with standard uncertainty of 0.16 µg/kg and expanded uncertainty of 0.32 µg/kg. This final uncertainty accounted for all of the sources listed above (within method), as well as the between method variability of Methods 1 and 2.

References:

[1] Koepke, A, Lafarge, T, Toman, B, Possolo, A NIST Consensus Builder- User's Manual (Gaithersburg, MD: National Institute of Standards and Technology) consensus.nist.gov, 2017.

[2] Possolo A, Toman B (2007) Metrologia 44:464–475.

[3] Toman B, Nelson M, Bedner M (2017) Metrologia 54, S16 – S28

[4] Gelman A, Carlin J B, Stern H S and Rubin D B (2003) Bayesian Data Analysis 2nd edn (London/Boca Raton, FL: Chapman and Hall/CRC)

NMIJ

Canal = {[(Fprep(sample) * Rsample - Rblank - Rsurrogate)/(Rcalib - Rsurrogate)] * Fcalib * Mcalib * Ccalib * Fsur(sample) * Fdilut * Msur(sample)} / (Msample * Msur(calib))

Canal is a concentration of BaP in the sample (mg/kg),

Fprep(sample) is a factor for reproducibility of sample preparation,

Type A uncertainty (repeatability of result, n=5)

Rsample is a ratio of peak area of BaP/13C-BaP observed for the sample solution,

Type A uncertainty (repeatability of measurement, n=5)

Rblank is a ratio of peak area of BaP/13C-BaP observed for the blank solution,

Type A uncertainty (repeatability of measurement, n=5)

Rsurrogate is a ratio of peak area of BaP/13C-BaP observed for the surrogate solution,

Type A uncertainty (repeatability of measurement, n=5)

Rcalib is a ratio of peak area of BaP/13C-BaP observed for the calibration solution,

Type A uncertainty (repeatability of measurement, n=5)

Fcalib is a factor for reproducibility of preparation of the calibration solution,

Type A uncertainty (from ANOVA, n=3)

Mcalib is mass of the standard solution of BaP taken for preparation of the calibration solution,

Type A uncertainty (reproducibility of weighing, n=10) and type B uncertainty (linearity of weighing, certificate of calibration) were combined.

Ccalib is concentration of BaP in the calibration solution, (mg/kg)

u(Ccalib) is uncertainty of concentration in the calibration solution prepared from NMIJ CRM BaP

Fsur(sample) is a factor for reproducibility of addition of the surrogate solution,

Type A uncertainty (reproducibility of weighing, n=10) and type B uncertainty (linearity of weighing, certificate of calibration) were combined.

Fdilut is a factor for reproducibility of preparation of the surrogate solution,

Type A uncertainty (reproducibility of weighing, n=10) and type B uncertainty (linearity of weighing, certificate of calibration) were combined.

Msur(sample) is mass of the surrogates solution added to the sample, (mg)

Type B uncertainties (linearity of weighing, from certificate of calibration) were combined.

Msample is mass of the CCQM sample taken for sample preparation, (mg)

Type B uncertainties (linearity of weighing, certificate of calibration) were combined.

Msur(calib) is mass of the surrogate solution taken for preparation of the calibration solution, (mg)

Type A uncertainty (reproducibility of weighing, n=10) and type B uncertainty (linearity of weighing, certificate of calibration) were combined.

	Value, xi	Uncertainty, u(xi)	degree of freedom	type of uncertainty
Fprep(sample)	1.0000	0.0018	4	A
Rsample	0.9177	0.0021	4	A
Rblank	0.0061	0.0015	4	A
Rsurrogate	0.0532	0.0008	4	A
Rcalib	0.7857	0.0008	4	A
Fcalib	1.0000	0	2	A
Mcalib (mg)	403.65	2.22	large	A + B
Ccalib (mg/kg)	1.020	0.021	large	A + B
Fsur(sample)	1.0000	0.0008	large	A + B
Fdilut	1.0000	0.0055	large	A + B
Msur(sample) (mg)	12.997	0.002	large	B
Msample (mg)	5589.85	0.06	large	B
Msur(calib) (mg)	391.98	0.06	large	A + B
	Concentration (ug/kg)	combined uncertainty (ug/kg)	k	expanded uncertainty (ug/kg)
BaP	2.79	0.062	2.78	0.17

UME

$$Conc._{Native} = \frac{\left(\frac{Area_{Native}}{Area_{IS}}\right) x \left((IS_{Conc.}) x (IS_{spiked})\right)}{\frac{RF}{Sample\ Amount}}$$

$$RF = \frac{\frac{Area_{Native}}{Area_{IS}}}{\frac{Conc._{Native}}{Conc._{IS}}}$$

Uncertainty Budget of Benzo[a]pyrene				
Parameters	Unit	Value (X)	u(x)	u(x)/X
Mass of sample intake	g	0.5	1.63144E-05	3.26E-05
Spiking Labelled stock solution	g	0.075	0.00000004	5.15E-07
Native stock solution	µg/kg	100	0.00088426	8.84E-06
Calibration		1.440	0.025	1.72E-02
Recovery		0.986	0.040	4.06E-02
Repeatability	µg/kg	3.09	0.049	1.60E-02
Relative Standard Measurement Uncertainty				0.047
Result (µg/kg)		3.09		
Combined Standard Measurement Uncertainty			0.14	
Expanded Uncertainty (k=2)			0.29	
Relative Mesurement Uncertainty (%)			9.38	

COMBINED STANDARD MEASUREMENT UNCERTAINTY

$$\frac{u_c(Analyte)}{c_{Analyte}} = \sqrt{\left(\frac{u(m_{SI})}{m_{SI}}\right)^2 + \left(\frac{u(m_{SLS})}{c_{SLS}}\right)^2 + +\left(\frac{u(C_{NSS})}{C_{NSS}}\right)^2 + \left(\frac{u(RF)}{RF}\right)^2 + \left(\frac{u(R_m)}{R_m}\right)^2 + \left(\frac{u(r)}{r}\right)^2}$$

Uncertainty Sources :
1-Mass of sample intake
2-Spiking of labelled stock solution
3-Native stock solution
4-Calibration
5-Recovery
6-Repeatability

$$w_{an} = \frac{\left(\frac{A_{an_s} \times m_{IS_s}}{A_{IS_s}} - \frac{A_{an_{bl}} \times m_{IS_{bl}}}{A_{IS_{bl}}}\right)}{RF \times m_{sample}}$$

$$RF = \frac{A_{an} \times m_{IS}}{A_{IS} \times m_{an}}$$

---

w_{an} – the mass fraction of the Benzo(a)pyrene in the Sample, mkg/kg;

A_{an} – the area of the Benzo(a)pyrene in Sample(s)/blank(bl);

A_{IS} – the area of the Internal Standard in the Sample(s) or Blank(bl);

m_{IS} – the mass of Internal Standard added into the Sample(s) or Blank(bl) before Sample preparation, mkg;

m_{sample} – the mass of the Sample, kg;

RF – the Response Factor.

A_{an} – the area for the Benzo(a)pyrene in the Calibration solution;

A_{IS} – the area for the Internal Standard in the Calibration solution;

m_{an} – the mass of Benzo(a)pyrene into the Calibration solution, mkg

m_{IS} – the mass of Internal Standard into the Calibration solution, mkg;

$\frac{u_{(x)}}{x} = \sqrt{\left(\frac{u_{(RF)}}{RF}\right)^2 + \left(\frac{u_{(w_{bl})}}{w_{bl}}\right)^2 + \left(\frac{u_{(w_s)}}{w_s}\right)^2}$	$u_{(RF)}$	– the standard uncertainty of the Calibration (response factor- RF);
	$u_{(w_{bl})}$	– the standard uncertainty of the mass fraction of analyte in the Blank;
	$u_{(w_s)}$	– the standard uncertainty of the mass fraction of analyte in the Sample
$\frac{u_{(RF)}}{RF} = \sqrt{\left(\frac{u_{(m_{IS})}}{m_{IS}}\right)^2 + \sum\left(\frac{u_{(m_{nat})}}{m_{nat}}\right)^2 + \left(\frac{u_{(w_{pur})}}{w_{pur}}\right)^2 + \sum\left(\frac{u_{(m_{cal})}}{m_{cal}}\right)^2 + \left(\frac{u_{(RF_{av})}}{RF_{av}}\right)^2}$	$u_{(m_{IS})}$	– the standard uncertainty of the mass (preparation of the Internal Standard solution)
	$u_{(m_{nat})}$	– the standard uncertainty of the masses (preparation of the Native Stock solution)
	$u_{(w_{pur})}$	– the standard uncertainty of the purity of Benzo(a)pyrene
	$u_{(m_{cal})}$	– the standard uncertainty of the masses (preparation of the Calibration solution)
	$u_{(RF_{av})}$	– the standard uncertainty of calibration (Standard Deviation of RF)
$\frac{u_{(w_{bl})}}{w_{bl}} = \sqrt{\left(\frac{u_{(m_{IS_{bl}})}}{m_{IS_{bl}}}\right)^2 + \left(\frac{u_{(w_{av_{bl}})}}{w_{av_{bl}}}\right)^2}$	$u_{(m_{IS_{bl}})}$	– the standard uncertainty of the mass (addition the Internal Standard into the Blank)
	$u_{(w_{av_{bl}})}$	– the standard uncertainty of mass fraction of Benzo(a)pyrene in Blank (Standard Deviation of results)
$\frac{u_{(w_s)}}{w_s} = \sqrt{\left(\frac{u_{(m_{IS_s})}}{m_{IS_s}}\right)^2 + \left(\frac{u_{(m_s)}}{m_s}\right)^2 + \left(\frac{u_{(w_{av_s})}}{w_{av_s}}\right)^2}$	$u_{(m_{IS_s})}$	– the standard uncertainty of the mass (addition the Internal Standard solution into the Sample)
	$u_{(m_s)}$	– the standard uncertainty of the mass of the Sample (weighing the Sample)
	$u_{(w_{av_s})}$	– the standard uncertainty of mass fraction of Benzo(a)pyrene in Sample (Standard Deviation of results)

Source of uncertainty	u, %
$u_{(m_{IS})}$	1.2
$u_{(m_{nat})}$	2.1
$u_{(w_{pur})}$	0.17
$u_{(m_{cal})}$	0.63
u_{cal}	0.33
$u_{(m_{IS_{bl}})}$	0.81
$u_{(w_{avr_{bl}})}$	1.6
$u_{(m_{IS_s})}$	0.81
$u_{(m_s)}$	0.09
$u_{(w_{avr_s})}$	0.6
Relative Standard Uncertainty	**3.3**
Relative expanded uncertainty	**6.6**

APPENDIX H: Core Competencies

Table H-1: Core Competencies Demonstrated in CCQM-K146 by BAM

CCQM-K146	*BAM*	Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		'Highly-pure' substance Benzo(a)pyrene (LGC/Dr. Ehrenstorfer); Lot-no. G147406; ISO Guide 34 Reference Material
Identity verification of analyte in calibration material. #	✓	GC-MS, mass spectra and retention time
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	Value-Assignment / Purity Assessment by GC-FID; confirmed with certified B(a)P standard NIST SRM 2260a
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	GC-MS, mass spectra and retention time
Extraction of analyte(s) of interest from matrix	✓	Method A: Ultrasonic extraction with acetonitrile (total of 4 repetitions) Method B: SPE by using molecularly imprinted polymer (MIP) cartridge
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Method A: Gel permeation chromatography (GPC) Method B: After extraction MIP also used for clean-up (washing with cyclohexane)

Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) internal standard $^{13}C_4$-B(a)P b) 6-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

Table H-2: Core Competencies Demonstrated in CCQM-K146 by **BVL**

CCQM-K146	***BVL***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate measurement capabilities including: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS. The study will test the capabilities of participants for assigning the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/ mol at levels of 1 to 50 µg/kg in a high fat, low protein, low carbohydrate food matrix.		
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?		*Calibration solution PAH in acetonitrile, NIST standard reference material 1647f*
Identity verification of analyte in calibration material. #	✓	*Chromatographic retention time, mass ratio*
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).#	✓	*NIST standard reference material 2260a for crosscheck*
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	*Chromatographic retention time, mass ratio*
Extraction of analyte(s) of interest from matrix	✓	*No extraction needed*
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	*GPC*
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	*GC-HRMS (R=10.000) and GC-MS*
Calibration approach for value-assignment of analyte(s) in matrix	✓	*a) isotopic dilution mass spectrometry*

		b) 6-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

Table H-3: Core Competencies Demonstrated in CCQM-K146 by **EXHM/GCSL-EIM**

CCQM-K146	***EXHM***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		NIST SRM 2260a
Identity verification of analyte in calibration material. #	✓	GC-IT-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	in-house calibrant prepared by Sigma Aldrich TraceCert BaP – 1 H NMR used to identify structure 971.0 mg/g ± 3.1 mg/g by qNMR to NMIJ CRM 4601-a
For calibrants which are a calibration solution: Value-assignment method(s).#	✓	194.2 ng/g ± 3.9 ng/g by IDMS against SRM 2260a
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	mass spectrometry
Extraction of analyte(s) of interest from matrix	✓	preconcentration and extraction of PAHs with acetone/acetonitrile
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	change of solvent into cyclohexane SPE using molecularly imprinted polymers
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	n/a	<none>
Analytical system	✓	GC-IT-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS at exact matching, single point

Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	spiking the sample with b-chrysene, homogenization, extraction of PAHs with acetone/acetonitrile, clean-up using C18 and fluorisil cardridges, measurement using LC-FLD
Other		

The result for EXHM was not consistent with the KCRV at the 95% confidence intervals and the DoE does not cross zero. EXHM have attributed this inconsistency to the preparation of the matrix matched standard

Table H-4: Core Competencies Demonstrated in CCQM-K146 by **GLHK**

CCQM-K146	***GLHK***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pK_{ow} < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		Calibration solution from NIST (NIST SRM 1647f)
Identity verification of analyte in calibration material. #	NA	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	NA	
For calibrants which are a calibration solution: Value-assignment method(s).#	NA	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	LC-MS/MS, identification by a) chromatographic retention time and b) MRM ratio
Extraction of analyte(s) of interest from matrix	✓	Dilution
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Silica gel chromatography and Molecularly Imprinted Polymer (MIP) Cartridge
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	NA	
Analytical system	✓	LC-MS/MS Thermo Scientific Dionex Ultimate 3000 UPLC and AB

		SCIEX Triple Quad 6500
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS (Single point calibration and bracketing)
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	The sample was also verified by GC-MS/MS technique. Besides, the calibration approach was verified by using gravimetric standard addition method.
Other	NA	

Table H-5: Core Competencies Demonstrated in CCQM-K146 by **HSA**

CCQM-K146	***HSA***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?	✓	High purity certified reference material (CRM) of benzo[a]pyrene (HRM-1017A) from Health Sciences Authority with a purity value of (995.0 ± 3.5) mg/g was used as the calibrant.
Identity verification of analyte in calibration material. #	✓	The identity of benzo[a]pyrene was verified by comparing its mass spectrum with the certified reference materials SRM 1647f Priority Pollutant Polycyclic Aromatic Hydrocarbons in Acetonitrile and SRM 2260a Aromatic Hydrocarbons in Toluene from NIST, based on retention time and m/z ratio on the GC-MS.
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	The certified value of benzo[a]pyrene (HRM-1017A) was determined using mass balance approach. The capability is underpinned by HSA's participation in CCQM-K55b, c and d.
For calibrants which are a calibration solution: Value-assignment method(s).#	NA	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and m/z ratio based on SIM mode with one ion on GC-MS.
Extraction of analyte(s) of interest from matrix	✓	The benzo[a]pyrene in the olive oil sample was extracted by using n-hexane as the extraction solvent. Sample blend (SB) comprising the study material and the internal standard solution was prepared gravimetrically, then vortexed for 1 min after adding 2.5 mL of n-hexane. The SB was then equilibrated at about 4 °C for overnight and/or at ambient temperature (18 - 25 °C) for at least 1

		h. Following the equilibration at ambient temperature, the SB was vortexed for 1 min, sonicated for 2 min and shaken on a multi-tube shaker for 10 min before loading into solid phase extraction (SPE) cartridges for clean-up.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Solid phase extraction (SPE) was used in the clean-up step. Each SB was subjected to SPE using Cleanert BaP3 cartridge in two steps, i.e. initial clean-up and second clean-up steps. In the initial clean-up step, the cartridge was conditioned with 5 mL dichloromethane followed by 5 mL n-hexane. The blend was then loaded into the cartridge and 5 mL of n-hexane was used to wash the cartridge. Then, 10 mL of dichloromethane was used to elute the analyte. The eluent was evaporated to dryness with N_2, and reconstituted with 3 mL of n-hexane before loading into another SPE cartridge for the second clean-up step. In the second clean-up step, the same procedure in the initial clean-up step was applied, except that the analyte was eluted with 6 mL of dichloromethane. The final eluent was evaporated to dryness with N_2 and reconstituted with 0.6 mL of n-hexane. The extract was vortexed for 1 min and centrifuged for 5 min at 4,000 rpm before injecting the clear solution into the GC-MS.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	NA	
Analytical system	✓	Agilent 7890B/ 5977B GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	A single-point calibration, using exact-matching IDMS method.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Commercial cold-pressed olive oil (pre-screened) containing negligible amount of benzo[a]pyrene was spiked gravimetrically with SRM 1647f. The spiked material was analysed in parallel with the SBs for quality control (QC). Each QC was subjected to the same extraction and clean-up as the study sample. The recovery results ranged between 100.9% and 102.0% were used to estimate the uncertainty of method recovery (F_{rec}).
Other	NA	

Table H-6: Core Competencies Demonstrated in CCQM-K146 by **INMETRO**

CCQM-K146	***INMETRO***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		*Pure material with purity assessed in house by qNMR .* - *Benzo[a]pyrene: Supelco, purity mass fraction Purity: 98.62% ± 0.41 % (k= 2.78; 95%)*
Identity verification of analyte in calibration material. #	✓	*GC-MS , NMR*
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	*Analyte purity mass fraction was established by qNMR and cross-checked by mass balance*
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	<Specify>
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	*Retention time*
Extraction of analyte(s) of interest from matrix	✓	*SPE (Florisil)*
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	*SPE (C18)*
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	*LC-FLD*
Calibration approach for value-assignment of analyte(s) in matrix	✓	*Matrix calibration curve: five-point calibration curve, using Benzo[ghi]perylene as internal standard*

Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	*a) LC-FLD, Standard addition to sample using five-point calibration curve with Benzo[ghi]perylene as internal standard* *b) GC-IDMS, single-point calibration using isotopically labelled analog compound as internal standard*
Other		

The result for INMETRO was not consistent with the KCRV at the 95% confidence interval and the DoE does not cross zero. The low result may be attributed to SPE poor performance. Only a limited number of SPE cartridges were available, so the procedure could not be extensively tested. HPLC-FLD was used instead of IDMS due to problems with the GCMS system at the time of the comparison. Method recovery (97 %) was determined with a spiked sample (as no matrix CRM was available) and this might have not reflected the behavior of the real sample. Because FLD detection was employed, a labelled IS could not be used and this might have exacerbated the problem of extraction recovery.

Table H-7: Core Competencies Demonstrated in CCQM-K146 by **KRISS**

CCQM-K146	***KRISS***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		Neat commercial calibrant for benzo(a)pyrene was from SUPELCO. Purity was assayed by KRISS with mass-balance method and verified with qNMR.
Identity verification of analyte in calibration material. #	✓	ID-GC/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	The purity of the primary materials was determined following protocols maintained in KRISS. GC-FID used for the analysis of structurally related impurities, Karl-Fischer Coulometry for water content, thermogravimetric analysis for non-volatile impurities, headspace-GC/MS for residual solvents. As a result, the purity of benzo(a)pyrene was 98.76 % ± 0.60 % (k = 2.45)
For calibrants which are a calibration solution: Value-assignment method(s).#	✓	Calibration solutions were gravimetrically prepared in KRISS and verified by cross-checking of multiple calibration solutions. Secondary confirmation by comparison with NIST SRM 1647f
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	GC retention time, mass spec ion ratios, comparison of GC/MS measurement results by low and high resolution SIM.
Extraction of analyte(s) of interest from matrix	✓	Liquid-Liquid Extraction. Oil samples were extracted using mixer (10 min) and sonication (10 min) with 10 mL of acetone/acetonitrile (6:4=v:v). The same procedure was repeated twice.

Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Cleaned up by using (1) SPE dual layer (Florisil/C18) with acetonitrile as eluent and (2) SPE (NH2) with hexane/toluene as eluent.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)		N/A
Analytical system	✓	GC/MS, resolution = 10000 (HR)
Calibration approach for value-assignment of analyte(s) in matrix	✓	Gravimetrically prepared calibration solution was used as a calibrant. For ID-GC/MS analysis, calibration bland was prepared by gravimetrically mixing the calibration solution and the internal standard solution ($^{13}C_4$-BaP). IDMS with exact matching single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Gravimetrically fortified blank olive oil was used for the verification of analytical method. NIST SRM 1647f analyzed in parallel with each sample for confirmation. As the confirmatory method, we also applied different GC/MS analysis condition to pretreated samples, such as low-resolution (R=1000) and MRM (m/z 252 -> 250 for BaP, m/z 256->254 for 13C4-BaP), which were good agreement with the primary method (GC/MS: R =10000)
Other		

Table H-8: Core Competencies Demonstrated in CCQM-K146 by **Laboratory of the Institute for Oliveculture -1 2018.05.15**

CCQM-K146	Laboratory of the Institute for Olivecult-u re	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		Pure materials from Supelco
Identity verification of analyte in calibration material. #	X	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	X	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time
Extraction of analyte(s) of interest from matrix	X	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	X	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	HPLC with FLD
Calibration approach for value-assignment	✓	3-point calibration curve, including recovery based on

of analyte(s) in matrix		internal standard
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

The result for the Oliveculture Institute was not consistent with the KCRV at the 95% confidence interval and the DoE does not cross zero. The expected reason is the purity assignment of their calibrant and the correction factor that was applied.

Table H-9: Core Competencies Demonstrated in CCQM-K146 by **LGC**

CCQM-K146	**LGC**	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		CRM from NIST (PAHs in solution). Ref. SRM 1647f
Identity verification of analyte in calibration material. #	NA	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	NA	
For calibrants which are a calibration solution: Value-assignment method(s).#	NA	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	2 ions in SIM mode, retention time and peak shape.
Extraction of analyte(s) of interest from matrix	NA	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE (Supelclean™ EZ-POP NP, Supelco PN 54341-U). 0.5 mL of sample eluted with 15 mL of acetonitrile.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	NA	
Analytical system	✓	GC-MS
Calibration approach for value-assignment of	✓	Bracketed single point double exact matching IDMS.

analyte(s) in matrix		Internal standard: BaP-D12.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	FAPAS T0669QC run with each batch. Value assigned in-house. Value confirmed using a different IS: BaP-4C13.
Other		

Table H-10: Core Competencies Demonstrated in CCQM-K146 by **NIM**

CCQM-K146	***NIM***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		CRM from GBW(E)080476
Identity verification of analyte in calibration material. #	✓	Benzo[a]pyrene identity confirmed using mass spectra.
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).#	✓	Used benzo[a]pyrene certified mass fraction, uncertainties, and SI traceability as listed on Certificate of Analysis of GBW(E)080476
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention Time, MRM ions
Extraction of analyte(s) of interest from matrix	✓	Saponicated with potassium hydroxide Liquid/liquid extraction by hexane twice
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE (Supelclean™ EZ-POP NP)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	15 mL acetonitrile eluate from SPE was concentrated under nitrogen stream, and reconstituted by isopropanol to 200 µL prior to GC-MS/MS analysis.
Analytical system	✓	Agilent GC-MS/MS
Calibration approach for value-assignment	✓	Single point exact matching IDMS. Internal standard:

of analyte(s) in matrix		BaP-D12.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Used blank spiked olive oil sample for the verification of analytical method. NIST SRM 1647f analyzed in parallel for confirmation.
Other	N/A	

Table H-11: Core Competencies Demonstrated in CCQM-K146 by **NIMT**

CCQM-K146	***NIMT***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		Benzo(a)pyrene was obtained from AccuStandard, Inc. (USA)
Identity verification of analyte in calibration material. #	✓	*GC-MS*
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	*Purity of Benzo(a)pyrene was assessed by NIMT using mass balance method (normalized HPLC, TGA, KFT techniques*)
For calibrants which are a calibration solution: Value-assignment method(s).#	✓	*Gravimetric*
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	*The analyte in the sample was identified against Benzo(a)pyrene standards by comparing their retention times and m/z of GC-MS.*
Extraction of analyte(s) of interest from matrix	N/A	<Specify>
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Cleanert BAP-3 SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	<Specify>
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS

		b) exact-matching
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	<Specify>
Other	N/A	

The result for NIMT was not consistent with the KCRV at the 95% confidence interval and the DoE does not cross zero. NIMT have not identified a specific reason for this deviation.

Table H-12: Core Competencies Demonstrated in CCQM-K146 by **NIST**

CCQM-K146	***NIST***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
Competency	**✓, ✗, 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?		Used NIST SRM 2260a Aromatic Hydrocarbons in Toluene as a calibration solution.
Identity verification of analyte in calibration material. #	✓	Method 1: Benzo[*a*]pyrene identity confirmed using retention time and mass spectra. Method 2: Benzo[*a*]pyrene identity confirmed using retention time and fluorescence spectra compared to authentic neat benzo[*a*]pyrene standard.
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).#	✓	Used benzo[*a*]pyrene certified mass fraction, uncertainties, and SI traceability as listed on Certificate of Analysis of NIST SRM 2260a.
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Method 1: Benzo[*a*]pyrene identity confirmed in samples using retention and MS (SIM mode with presence of quantitation ion and confirmation ion). Method 2: Benzo[*a*]pyrene identity confirmed in samples using retention time, LC-Fluorescence.
Extraction of analyte(s) of interest from matrix	✓	Used liquid/liquid extraction for Method 1.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Used solid phase extraction (SPE) for Method 1 and size exclusion chromatography (SEC) for Method 2.
Transformation - conversion of analyte(s)	N/A	

of interest to detectable/measurable form (if used)		
Analytical system	✓	GC-MS and LC-Fluorescence
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) Used internal standard mode using benzo[*a*]pyrene-d_{12} for benzo[*a*]pyrene. The internal standard concentrations were similar to those of the analyte. b) Used an average response factor for calibrants that bracketed the concentrations of the analyte.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Used in house spiked olive oil sample for both methods and used NIST SRM 2779 Gulf of Mexico Crude Oil as an additional control for Method 1.
Other	N/A	

Table H-13: Core Competencies Demonstrated in CCQM-K146 by **NMIJ**

CCQM-K146	***NMIJ***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		Calibration solution: NMIJ CRM 4213a (Benzo[*a*]pyrene in 2,2,4-trimethylpentane)
Identity verification of analyte 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 its mass spectrum with GC/MS
Extraction of analyte(s) of interest from matrix	✓	Liquid/liquid extraction with DMSO
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	GPC and SPE (silica-gel)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC/MS under SIM mode
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS with $^{13}C_4$-BaP and single-point calibration

Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Verified with BaP fortified toluene at comparable level
Other		

Table H-14: Core Competencies Demonstrated in CCQM-K146 by **UME**

CCQM-K146	*UME*	Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		Calibration Solution, NIST SRM 1647f
Identity verification of analyte in calibration material. #	√	GC-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).#	√	Certificate
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	GC-MS/MS
Extraction of analyte(s) of interest from matrix	√	SPE (SUPELCO Supelclean EZ-POP NP)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	SPE (SUPELCO Supelclean EZ-POP NP)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	√	15 mL acetonitrile eluate from SPE was concentrated under nitrogen stream to 250 µL prior to GC-MS/MS analysis.
Analytical system	√	Thermo TSQ GC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	√	Single point IDMS, IS solution from NIST SRM 2270
Verification method(s) for value-assignment	**N/A**	

of analyte(s) in sample (if used)		
Other		

The result for UME was not consistent with the KCRV at the 95% confidence interval and the DoE does not cross zero. The expected reason may be due to stock and working sample preparation.

Table H-15: Core Competencies Demonstrated in CCQM-K146 by **VNIIM**

CCQM-K146	***VNIIM***	**Low-Polarity Analyte in high fat food - Benzo[a]pyrene in Olive Oil**
Scope of Measurement: Participation in this study would provide the opportunity to demonstrate participant's capabilities in determining the low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 0.1 to 1000 µg/kg in a high fat, low protein, low carbohydrate food matrix. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as GC/MS, GC-HRMS, HPLC-FLD or LC-MS.		
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?		Highly-pure substance B(α)P
Identity verification of analyte in calibration material. #	✓	GC/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	B(α)P (99.25 ± 0.34) % In-house evaluation of B(α)P purity by using mass balance approach Structurally related organics: HPLC-DAD, GC-MS Moisture: Mettler Toledo Karl Fisher Coulometer VOC: Headspace GC/MS and Termodesorbtion GC/MS Total non-volatiles: ICP/MS/MS
For calibrants which are a calibration solution: Value-assignment method(s).#		<Specify>
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention Time, MRM ions
Extraction of analyte(s) of interest from matrix	✓	Hydrolysis reaction with potassium hydroxide Liquid/liquid extraction in hexane
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Washing by deionized water Multilayer silica column

Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)		No
Analytical system	✓	GC/MS/MS Agilent 7000D
Calibration approach for value-assignment of analyte(s) in matrix	✓	Bracketing>
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Second (alternative) clean-up procedure (partition on Al_2O_3) Measuring by using SRM NIST 1647f
Other		