PAHs in protein matrix

EURAMET.QM-S15
PAHs in Protein Matrix

EURAMET Supplementary Comparison

Final Report
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SUMMARY

This Supplementary Comparison, EURAMET.QM-S15 " PAHs in Protein Matrix", was organized by the Federal Institute of Metrology METAS. The target analytes were four PAHs: benz[a]anthracene (BaA); benzo[a]pyrene (BaP); benzo[b]fluoranthene (BbF); and chrysene (Chr), for which maximum limits are set in the European and Swiss food legislation. The measurands were the mass fractions (µg/kg) of these PAHs in a protein-rich matrix.

Six National Metrology Institutions (NMIs) and Designated Institutes (DIs) participated in EURAMET.QM-S15. The study required solvent extraction, separation of the target analytes from interfering matrix components (clean-up), analytical/chromatographic separation and selective detection of the target analytes.

Solvent extraction (ASE, PLE, HUPsSE, QuEChERS) followed by SPE cleanup was applied by the participants in the sample pretreatment and GC-IDMS/MS, GC-HR-IDMS and LC-FLD were applied for separation and detection.

The results for the determination of the four PAHs in EURAMET.QM-S15 ranged from 0.5 µg/kg to 5.3 µg/kg for BaA, 0.6 µg/kg to 13.3 µg/kg for BaP, 0.6 µg/kg to 7.0 µg/kg for BbF and 0.5 µg/kg to 5.0 µg/kg for Chr.

Due to poor extraction efficiencies, as a result of unfavorable choice of solvent and insufficient demonstration of metrological traceability of the calibrant, only the results of two participants were included in the evaluation of the Supplementary Comparison Reference Value (SCRV). The SCRV was assigned using the weighted mean of these two participants which agreed well with gravimetrically prepared mass fractions and evidence from an ancillary study performed with external expert laboratories. Four participant results were excluded from the SCRV for the technical reasons mentioned above. The SCRV ($X \pm u(X)$) was 3.291 µg/kg ± 0.079 µg/kg for BaA, 4.28 µg/kg ± 0.10 µg/kg for BaP, 4.77 µg/kg ± 0.12 µg/kg for BbF and 2.967 µg/kg ± 0.070 µg/kg for Chr. The two institutes that were included in the assignment of consensus SCRV agreed within their standard uncertainties.

Successful participation in EURAMET.QM-S15 demonstrates the following measurement capabilities in determining the mass fractions of organic compounds, with a molecular mass between 150 g/mol and 500 g/mol, having a low polarity ($pK_{ow} < -2$), in a mass fraction range from 0.1 µg/kg to 100 µg/kg in a high protein food matrix.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS

ASE	Accelerated solvent extraction
BaA	Benz[a]anthracene
BaP	Benzo[a]pyrene
bb	Between bottles
BbF	Benzo[b]fluoranthene
BIPM	Bureau International des Poids et Mesures
CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
Chr	Chrysene
CMC	Calibration and Measurement Capability
CRM	Certified reference material
CV	Coefficient of variation, expressed in %: $CV = 100 \cdot s/\bar{x}$
DAD	Diode array detection
DI	Designated institute
DoE	Degrees of equivalence
FLD	Fluorescence detection
GC-HRMS	Gas chromatography with high resolution mass spectrometry detection
GC-HR-IDMS	Gas chromatography high resolution isotope dilution mass spectrometry
GC-MS	Gas chromatography with mass spectrometry detection
GC-MS/MS	Gas chromatography with tandem mass spectrometry detection
GC-IDMS/MS	Gas chromatography isotope dilution tandem mass spectrometry
HUPsSE	Heat-Ultrasonic-Pressure supported Solvent Extraction
ID	Isotope dilution
IDMS	Isotope dilution mass spectrometry
KC	Key Comparison
KCDB	Key comparison data base
LC-FLD	Liquid chromatography with fluorescence detection
MRM	Multiple reaction monitoring
NMI	National metrology institute
OAWG	Organic Analysis Working Group
PAHs	Polycyclic aromatic hydrocarbons
pKow	Logarithm of the octanol-water partition coefficient
PLE	Pressurized liquid extraction
PSE	Pressurized solvent extraction
QuEChERS	“Quick, Easy, Cheap, Effective, Rugged, Safe” liquid/solid extraction
RM	Reference material
SC	Supplementary comparison
SCRV	Supplementary Comparison Reference Value
SPE	Solid phase extraction
SRM	Selected reaction monitoring
wb	Within bottles

SYMBOLS

d_i	Degree of equivalence: x_i - *SCRV*
$\%d_i$	Percent relative degree of equivalence: $100 \cdot d_i/SCRV$
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

Polycyclic aromatic hydrocarbons (PAHs) are a class of low-polarity (pK_{ow} < -2) chemicals, consisting of hydrocarbons with at least two connected aromatic ring systems. Food can be contaminated by PAHs through their presence in the environment, during industrial processing or domestic food preparation (e.g. drying, heating, and grilling), and through packaging processes and materials (e.g., through contact with mineral oils). Due to their possible negative effects on health – they are associated with cancer and other diseases – the mass fractions of PAHs in foods are regulated in the European Union (EU) by the Commission Regulation (EU) 2023/915 [1] and in Switzerland by the regulation SR 817.022.15 on the maximum levels for contaminants [2]. The regulations exclusively concern benzo[a]pyrene (BaP) and the sum of benz[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fuoranthene (BbF), and chrysene (Chr) in different food products. Accurate analysis of these contaminants is therefore required.

The OAWG of CCQM have organized two previous studies concerning the determination of PAHs in food matrices. The final reports for these studies were published in 2018 (CCQM-K95.1: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea) and 2020 (CCQM-K146: Benzo[a]pyrene in Olive Oil). However, no comparison of PAHs in a protein-rich matrix has been carried out so far. Furthermore, such a comparison was not the focus of the OAWG strategy in the coming years. It was therefore decided to organize a Supplementary Comparison (SC) on RMO (EURAMET) level as a standalone study. In September 2022, the CCQM approved the Supplementary Comparison EURAMET.QM-S15 "PAHs in Protein Matrix" and it was subsequently registered in the BIPM key comparison data base (KCDB).

Extraction, separation from interfering matrix components (clean-up), chromatographic separation and quantification of organic compounds with a low polarity, such as PAHs, which are present in small amounts (mass fractions) in complex matrices, such as the protein-rich matrix investigated in this study, are core challenges for reference material producers and providers of calibration services. 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 mass fractions of the target PAHs (BaA, BaP, BbF and Chr) can be successfully evaluated after extraction and extract clean-up, including, but not limited to, gas chromatography (GC) in conjunction with isotope dilution mass spectrometry (IDMS). GC-IDMS and liquid chromatography (LC) with fluorescence detection (FLD) have been applied in previous CCQM studies (K95.1 and K146) on PAHs in food matrices and are used by commercial and official laboratories for analyzing PAHs in food. The method(s) used by participants in EURAMET.QM-S15 are intended to represent the way they deliver calibration solution services to their customers.

The following sections of this report document the timeline of EURAMET.QM-S15, the measurands, study material, participants, results, and the measurement capability claims that

participation in EURAMET.QM-S15 can support. Appendix A presents additional information based on gravimetrically prepared mass fractions and on an ancillary study with external expert food control laboratories to support the SCRV evaluation approach. The Appendices B to F reproduce the official communication materials and Appendices G to I reproduce summaries of information about the results provided by the participants.

TIMELINE

Table 1 lists the timeline for EURAMET.QM-S15.

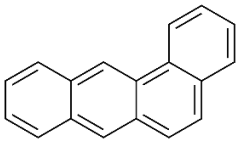
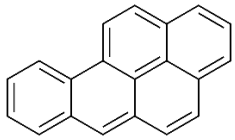
Table 1: Timeline for EURAMET.QM-S15

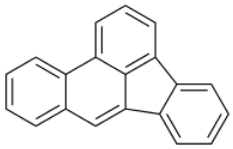
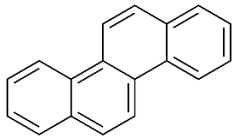
Date	Action
December 2020	Sample preparation
March to May 2022	Homogeneity and stability testing
1 February 2022	Presentation at the EURAMET SCOBA meeting
16/17 June 2022	Presentation at the CCQM OAWG meeting
September 2022	EURAMET.QM-S15 approved and registered in KCDB
September 2022	Call for participation
December 2022	Sample distribution
31 August 2023	Deadline for submission of results
November 2023	Preliminary discussion of results
April 2024	Draft A report distributed to participants
12 November 2024	Draft A report approved by participants and CCQM OAWG
17 November 2024	Draft B report approved by EURAMET TC Chair
27 November 2024	Approval for submission of Final report for publication in KCDB

MEASURANDS

EURAMET.QM-S15 relates to PAHs in a high protein food matrix. The measurands were the mass fractions of BaA, BaP, BbF and Chr in high protein powder based on the material "as received" with the assigned value expressed in µg/kg. Table 2 contains information on CAS numbers, molecular formula, molecular weight, polarity and chemical structure.

Table 2: Information of PAHs investigated in this supplementary comparison

	Benz[a]anthracene (BaA)	**Benzo[a]pyrene (BaP)**
CAS	56-55-3	50-32-8
Molecular formula	$C_{18}H_{12}$	$C_{20}H_{12}$
Molecular weight	228.3	252.3
pK_{OW}	-5.76	-6.13
Structure		

	Benzo[b]fluoranthene (BbF)	**Chrysene (Chr)**
CAS	205-99-2	218-01-9
Molecular formula	$C_{20}H_{12}$	$C_{18}H_{12}$
Molecular weight	252.3	228.3
pK_{OW}	-5.78	-5.73
Structure		

STUDY MATERIALS

The study material was a high protein powder, spiked with known mass fractions (see Appendix A) of the aforementioned PAHs. The material was produced by spiking fresh liquid protein concentrate with a solution of PAHs in acetonitrile. After mixing, the liquid protein concentrate was subjected to spray drying. A powder with about 0.8 g/g of protein was obtained. The powder was bottled into pre-cleaned glass-amber bottles, each containing 30 g of material. A more detailed description of the production of the study material is given in Appendix A.

Each participant received one bottle containing approximately 30 g of material. The recommended minimum sample amount for analysis was at least 1.0 g. Measurement results were to be reported on a "as received" basis. For each measurand, the indicative value for the mass fraction communicated to the participants was between 0.1 µg/kg and 50 µg/kg.

Homogeneity Assessment of Study Material

For the homogeneity assessment, 10 bottles covering the whole bottling range were randomly selected. Three independent test portions of each bottle were analyzed. The measurements were performed under repeatability conditions, using a validated method and according to a random sequence to prevent possible trends in analytical sequence and filling order. According to ISO Guide 35:2017 [3], the assessment of the homogeneity was carried out by a one-way analysis of variance (ANOVA). For all four PAHs, the observed *F*-values ($MS_{\text{between bottles}}/MS_{\text{within bottles}}$) were lower than the critical *F*-values, indicating that the variances of the measured values within and between the bottles do not differ significantly at a 95 % confidence level. No evidence of statistically significant inhomogeneity was therefore observed. The results of the homogeneity study and the estimated uncertainties for potential inhomogeneity (u_{bb}) are given in Table 3 and Figure 1.

Table 3: Results of the homogeneity assessment for BaA, BaP, BbF and Chr in protein matrix

	BaA	**BaP**	**BbF**	**Chr**
F_{obs}	1.099	0.147	0.222	1.051
F_{crit}	2.393	2.393	2.393	2.393
Within bottle, u_{wb} (%)	2.7	4.3	3.2	3.2
Between bottle, u_{bb} (%)	0.5	0.0	0.0	0.4
Between bottle, u^*_{bb} (%)	0.9	1.4	1.1	1.1
Uncertainty estimates for between bottle inhomogeneity, u_{bb} (%)	0.9	1.4	1.1	1.1

a) For the estimation of u_{bb} the higher value of u^*_{bb} and u_{bb} was taken. u^*_{bb} and s_{bb} were calculated according to [4].

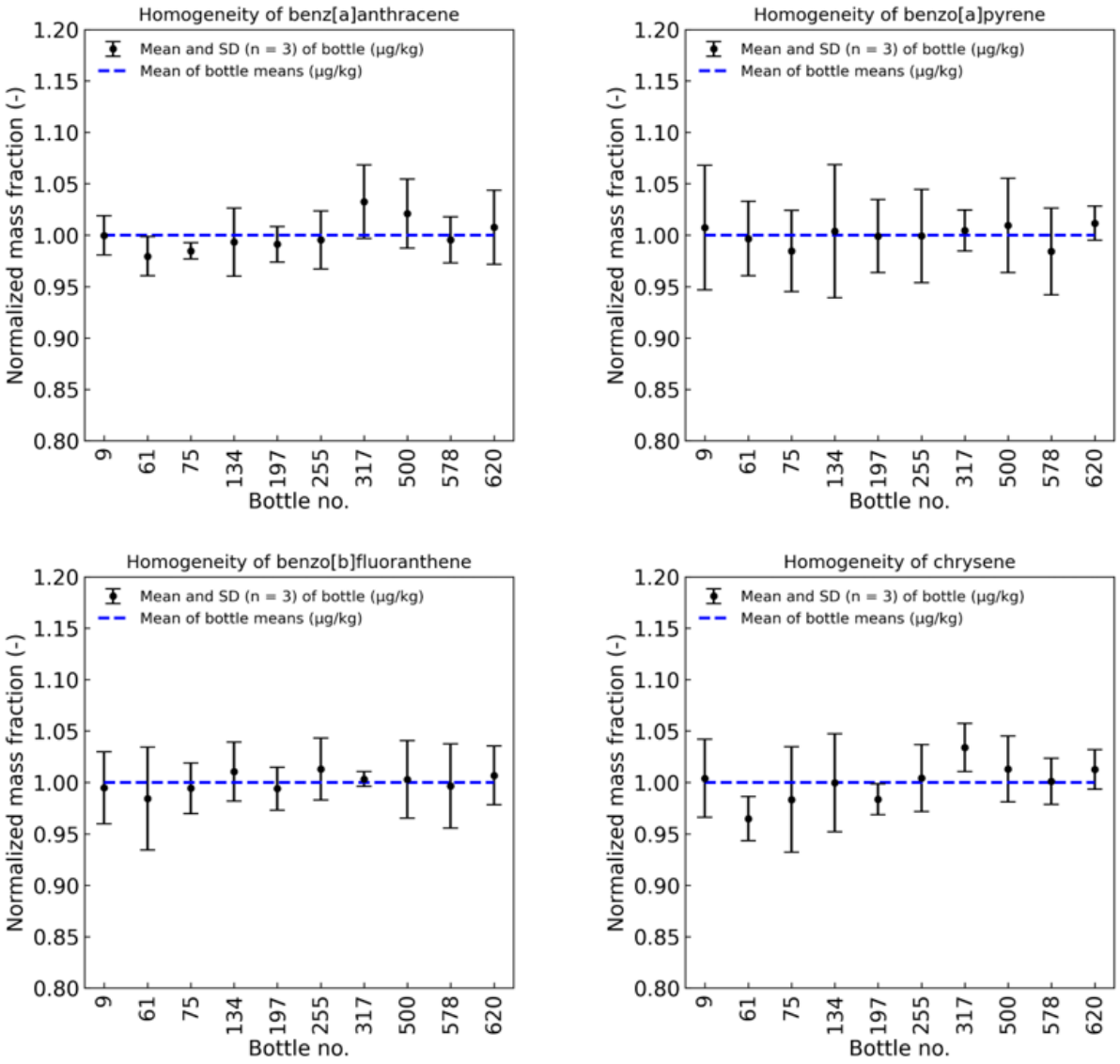


Figure 1: Homogeneity study of the four PAHs BaA, BaP, BbF and Chr.
Mean values of 10 selected bottles with their corresponding standard deviations (n = 3).

Stability Assessment of Study Material

For the stability assessment, an isochronous approach was used. The investigated bottles (one for each stability point) were stored for 1.5, 3, 6 and 12 months at different temperatures: -20 °C (reference temperature), 4 °C, room temperature (ca. 20 °C) and 45 °C (up to 3 months only). After the storage time was reached for a certain stability point, the corresponding bottle was stored at the reference temperature before it was analyzed three times. For $t = 0$, data from the homogeneity study were used. According to ISO Guide 35:2017 [3] and Linsinger et al. [5], the stability was

assessed by applying a linear regression model. The slope b_1 and intercept b_0 were fit to the stability data. Using a two-tailed t-test, $t_{b1} = |b_1|/s(b_1)$, it could be shown that the slopes for all PAHs at all investigated temperatures do not differ significantly from 0 at a 95 % confidence level. Therefore, since no evidence of statistically significant instability was found at the various temperatures during the investigated storage times, b_1 was set to 0 for further calculations.

For the estimation of the uncertainties for potential long-term instability (u_{lts}) at the storage temperature of -20 °C the extrapolation model $u_{lts} = u(b_1=0) \cdot t$, with t = 24 months, was used [3,5]. The results are given in Table 4 and Figure 2. For storage temperatures at 4 °C and room temperature (ca. 20 °C) similar uncertainty estimates were obtained as for -20 °C. This indicates that the long-term stability is also given at temperatures up to room temperature.

Table 4: Results of the long-term stability assessment at a storage temperature of -20 °C.

	BaA	**BaP**	**BbF**	**Chr**
b_1 (months^{-1})	-0.001275	-0.001408	0.001560	-0.002210
b_0 (-)	1.005737	1.006334	0.992980	1.009946
$s(b_1)$ (-)	0.001948	0.002029	0.001963	0.002151
t_{b1}	0.654	0.680	0.795	1.027
t_{crit}	2.160	2.160	2.160	2.160
$s(b_1=0)$ (-)	0.001908	0.002029	0.001937	0.002156
Uncertainty estimates for long-term stability (24 months extrapolation) at -20 °C, u_{lts} (%)	4.6	4.9	4.7	5.2

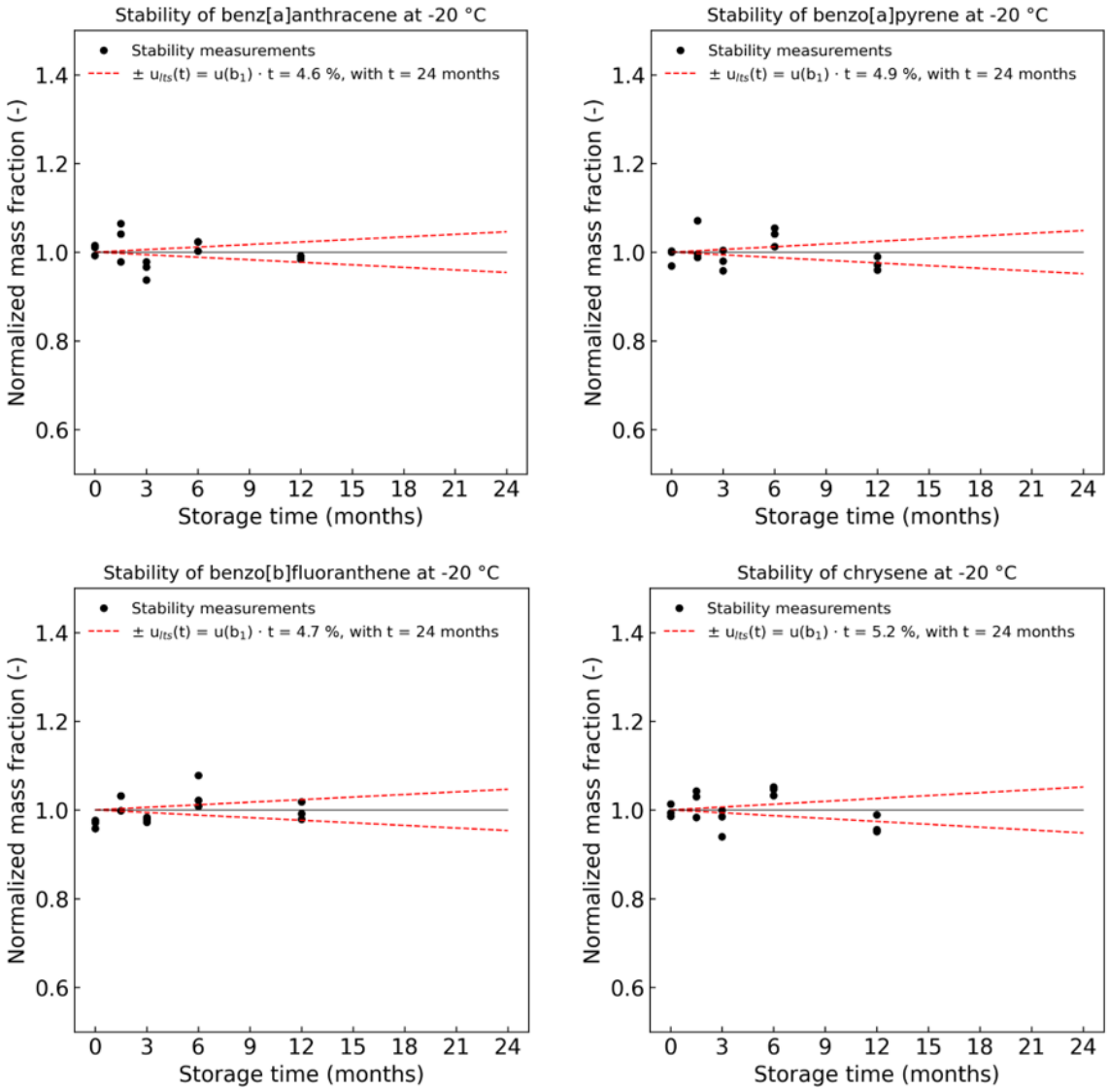


Figure 2: Long-term stability study of the four PAHs BaA, BaP, BbF and Chr

The short-term stability was evaluated at a storage temperature of 45 °C. For the estimation of the uncertainty (u_{sts}) for potential instability when the material is exposed to higher temperatures during transportation a similar extrapolation model as for the long-term stability was applied. An exposure time of 2 weeks (t = 0.5 months) was used. The results are presented in Table 5.

Table 5: Results of the short-term stability assessment at a storage temperature of 45 °C.

	BaA	BaP	BbF	Chr
b_1 (months^{-1})	-0.005666	-0.000824	0.003833	0.008454
b_0 (-)	1.008499	1.001237	0.994251	0.987319
$s(b_1)$ (-)	0.007300	0.006728	0.005405	0.005267
t_{b1}	0.776	0.123	0.709	1.605
t_{crit}	2.364	2.364	2.364	2.364
$s(b_1=0)$ (-)	0.007116	0.006300	0.005234	0.005762
Uncertainty estimates for long-term stability (24 months extrapolation) at -20 °C, u_{lts} (%)	0.36	0.32	0.27	0.29

PARTICIPANTS, INSTRUCTIONS AND SAMPLE DISTRIBUTION

The call for participation was distributed in September 2022 with the intent to distribute samples in December 2022, receive results in August 2023, and discuss first results at the online meeting on 20 November 2023. See Table 1 for study timeline. Appendix B reproduces the Call for Participation; Appendix C reproduces the study Protocol (including the updates sent to the participants). Table 6 lists the institutions that registered for EURAMET.QM-S15.

Table 6: Institutions registered for EURAMET.QM-S15

NMI or DI	Code	Country	Contact
Federal Office of Consumer Protection and Food Safety	BVL	Germany	Martin Kaminski, Isabella Zübner
National Metrology Institute of Italy	INRIM	Italy	Chiara Portesi
Institut za vode, Institute for water	IW	Bosnia and Herzegovina	Dragana Đokić Vasić
Science and Research Centre Koper, Laboratory of the Institute for Oliveculture	LAB-IZO	Slovenia	Erika Bešter
Federal Institute of Metrology	METAS	Switzerland	Simon Lobsiger
National Metrology Institute of Türkiye	TUBITAK UME	Türkiye	Mine Bilsel

Apart from INRIM, the samples were sent to the participants on 16 December 2022 under dry ice cooling. The sample for INRIM was dispatched on 28 February 2023, due to the later registration of this NMI. The samples were delivered to the participants within at least 6 days. As commented by BVL, dry ice was completely evaporated when the sample was obtained. As the stability study

showed, the samples are stable without dry ice cooling, even at elevated temperatures within the shipping time. Therefore, a significant change in the sample material due to shipping can be ruled out.

On 13 April 2023 and on 7 July 2023, METAS informed the participants that the results submission deadline was extended to 31 May 2023 and 31 August 2023, respectively, due to technical problems reported by participants.

The results had to be reported using the provided reporting excel sheet and sent by email to the study coordinator (gisela.umbricht@metas.ch) before the submission deadline. The results had to include the mass fractions of the four PAHs in protein matrix on an "as received" basis, their associated standard and expanded (95 % level of confidence) uncertainties, and the coverage factor. Participants were requested to report a single estimate of the mass fractions in µg/kg for BaA, BaP, BbF and Chr in protein matrix. The reported mass fractions should be the overall mean from replicate measurements. Reporting should include the values of the individual replicates in addition to the overall mean and an estimation of their standard and expanded measurement uncertainty. In addition to the quantitative results, participants were requested to describe their analytical methods and their approach to uncertainty estimation.

Table 7: Sample shipping and receipt and results submission dates for EURAMET.QM-S15

Code	Shipping date	Sample receipt date	Results submission
BVL	16-Dec-2022	20-Dec-2022	16-May-2023
INRIM	28-Feb-2023	N/A	30-May-2023
IW	16-Dec-2022	22-Dec-2022	30-Jun-2023
LAB-IZO	16-Dec-2022	20-Dec-2022	10-Jun-2023
METAS	N/A	N/A	1-May-2023
TUBITAK UME	16-Dec-2022	19-Dec-2022	21-Aug-2023

RESULTS

Participants were requested to report a single estimate for each of the mass fractions, in µg/kg, for BaA, BaP, BbF and Chr in the high protein food matrix. 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 study. Appendices D, E, and F reproduce the relevant report forms. EURAMET.QM-S15 results were received from all institutions that received samples.

Calibration Materials Used by Participants

Participants established the metrological traceability of their results using certified reference materials (CRMs) with stated traceability. Table 8 lists the CRMs that were used. Table 9 lists how participants established traceability. If through their own measurements, Table 9 lists the material, its assigned value, the method used, and how the participant had demonstrated their competence in the use of the method(s).

Four participants used the SRM 1647f from NIST as the primary calibrant to establish metrological traceability. IW and INRIM used calibrants consisting of PAH mixtures in acetonitrile from non-NMI sources. While IW used a RM from Dr. Ehrensdorfer, INRIM used a CRM from Merck (Supelco). Both laboratories did not perform a re-value assignment against a CRM from an NMI and, at the time of this comparison, had not demonstrated their capabilities in assigning a value to these commercial materials through past participations in solution comparisons. Because they did not meet the requirements defined by the CIPM MRA [6] and described in section 7.7 of the OAWG Practices and Guidelines document [7], the values from IW and INRIM were not included in the SCRV calculation.

Table 8: Certified reference materials used

CRM	Provider	Analyte	Mass fraction[a] delivered, µg/g	Mass fraction[a] source material, %	In-house purity methods used to value-assign source material
SRM 1647f	NIST	BaA BaP BbF Chr	5.16 ± 0.07 6.22 ± 0.11 5.29 ± 0.06 4.67 ± 0.08	N/A	N/A

a Stated as Value ± U_{95}(Value)

Table 9: Metrological traceability of participants' results

NMI/DI	Analyte	Source of traceability	Material	Mass Fraction or mass concentration[a]	Measurement techniques[b]	Evidence of competence
BVL	BaA BaP BbF Chr	SRM 1647f	N/A			
INRIM	BaA BaP BbF Chr	CRM from commercial supplier (not NMI)	CRM47940, Lot LRAD2034 (Merck, Supelco)	(9.99 ± 0.12) µg/mL (10.00 ± 0.11) µg/mL (9.99 ± 0.12) µg/mL (10.00 ± 0.14) µg/mL	Re-value assignment against CRM from NMI not performed	No evidence of competence provided
IW	BaA BaP BbF Chr	RM from commercial supplier (not NMI)	PAH-Mix 9 100 µg/mL in Acetonitrile, DRE-XA20950009AL, Lot G1126284AL, (Dr. Ehrensdorfer)	(100.01 ± 3.09) µg/mL (100.00 ± 2.06) µg/mL (100.00 ± 2.03) µg/mL (100.00 ± 3.10) µg/mL	Re-value assignment against CRM from NMI not performed	No evidence of competence provided
LAB-IZO	BaA BaP BbF Chr	SRM 1647f	N/A			
METAS	BaA BaP BbF Chr	SRM 1647f	N/A			
TUBITAK UME	BaA BaP BbF Chr	SRM 1647f	N/A			

a Stated as Value ± U_{95}(Value)
b Measurement techniques to assign or verify mass fractions of RMs/CRMs provided by commercial suppliers

Methods Used by Participants

The participants extraction (including the solvents used) and the measurement techniques are summarized in Table 10. A complete description of the analytical methods used by the participants, including sample preparation, analytical technique, and quantification approach is summarized in Appendix G. The participants' approaches to estimating uncertainty are provided in Appendix H.

Table 10: Extraction and measurement techniques used by the participants

NMI/DI	Analytical instrumentation	Method of quantification and type of calibration	IDMS	Extraction technique	Extraction solvent(s)
BVL	GC-HR-IDMS	Multi-point-external calibration with internal standard	Yes	HUPsSE	methanol : tert-butylmethylether (1:1)
INRIM	HPLC-FLD	6-point external calibration	No, benzo[b]-chrysene used as ISTD	QuEChERS	acetonitrile
IW	HPLC-DAD/FLD	Multi-point external calibration	No	QuEChERS	acetonitrile
LAB-IZO	HPLC-FLD	4-point external calibration	No, benzo[b]-chrysene used as ISTD	Ultrasonic bath	n-hexane
METAS	GC-IDMS/MS	Linear regression IDMS (6-point)	Yes	ASE	methanol : tert-butylmethylether (1:1)
TUBITAKUME	GC-IDMS/MS	IDMS (8-point)	Yes	PSE	n-hexane

Participant Results for BaA, BaP, BbF and Chr

The results for EURAMET.QM-S15 for the determination of BaA, BaP, BbF and Chr are detailed in Table 11 to Table 14 and presented graphically in Figure 3.

As described above, the results of IW and INRIM were excluded from SCRV calculations because of not providing evidence for metrological traceability of their calibrants. The results of these two institutes are therefore displayed in red (framed in red in illustrations) in this report.

The results of LAB-IZO and TUBITAK UME are displayed in blue (framed in blue in illustrations) in this report. As discussed below the low values reported by these two institutes can be attributed to low extraction efficiencies caused by the solvent(s) used.

For the statistics reported in Table 11 to Table 14, all values were considered. However, for the SCRV calculation only the values reported by BVL and METAS were used.

Table 11: Reported Results for benz[a]anthracene (BaA)

	benz[a]anthracene, µg/kg					
NMI/DI	***x***	***u*(*x*)**	***u*(*x*) %**	***k***	***U*(*x*)**	***U*(*x*) %**
BVL	3.20	0.19	5.9	2.00	0.39	12.2
INRIM	3.63	0.21	5.8	2	0.42	11.6
IW	5.333	0.575	10.8	2	1.149	21.5
LAB-IZO	0.619	0.055	8.9	2	0.109	17.6
METAS	3.31	0.086	2.6	2	0.18	5.4
TUBITAK UME	0.51	0.05	9.8	2	0.10	19.6
n	6					
$\bar{x}$	2.77					
s	1.87					
CV	67.6					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = 100·*s*/$\bar{x}$, *u*(*x*) % and *U*(*x*) % were calculated based on the reported values of the participants.

Table 12: Reported Results for benzo[a]pyrene (BaP)

	benzo[a]pyrene, µg/kg					
NMI/DI	***x***	***u*(*x*)**	***u*(*x*) %**	***k***	***U*(*x*)**	***U*(*x*) %**
BVL	4.20	0.18	4.3	2.00	0.36	8.6
INRIM	5.27	0.21	4.0	2	0.42	8.0
IW	13.333	1.438	10.8	2	2.875	21.6
LAB-IZO	0.568	0.051	9.0	2	0.102	18.0
METAS	4.31	0.114	2.6	2	0.23	5.3
TUBITAK UME	0.58	0.04	6.9	2	0.09	15.5
n	6					
$\bar{x}$	4.71					
s	4.68					
CV	99.3					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = 100·*s*/$\bar{x}$, *u*(*x*) % and *U*(*x*) % were calculated based on the reported values of the participants.

Table 13: Reported Results for benzo[b]fluoranthene (BbF)

	benzo[a]pyrene, µg/kg					
NMI/DI	***x***	***u*(*x*)**	***u*(*x*) %**	***k***	***U*(*x*)**	***U*(*x*) %**
BVL	4.60	0.32	7.0	2.00	0.65	14.1
INRIM	5.19	0.16	3.1	2	0.32	6.2
IW	7.000	0.755	10.8	2	1.510	21.6
LAB-IZO	0.600	0.068	11.3	2	0.137	22.8
METAS	4.79	0.124	2.6	2	0.25	5.2
TUBITAK UME	0.78	0.04	5.1	2	0.09	11.5
n	6					
$\bar{x}$	3.83					
s	2.57					
CV	67.3					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = 100·$s/\bar{x}$, *u*(*x*) % and *U*(*x*) % were calculated based on the reported values of the participants.

Table 14: Reported Results for chrysene (Chr)

	benzo[a]pyrene, µg/kg					
NMI/DI	***x***	***u*(*x*)**	***u*(*x*) %**	***k***	***U*(*x*)**	***U*(*x*) %**
BVL	2.92	0.15	5.1	2.00	0.30	10.3
INRIM	3.16	0.20	6.3	2	0.40	12.7
IW	5.000	0.539	10.8	2	1.078	21.6
LAB-IZO	0.942	0.087	9.2	2	0.174	18.5
METAS	2.98	0.079	2.7	2	0.16	5.4
TUBITAK UME	0.46	0.04	8.7	2	0.08	17.4
n	6					
$\bar{x}$	2.58					
s	1.65					
CV	64.1					

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = 100·$s/\bar{x}$, *u*(*x*) % and *U*(*x*) % were calculated based on the reported values of the participants.

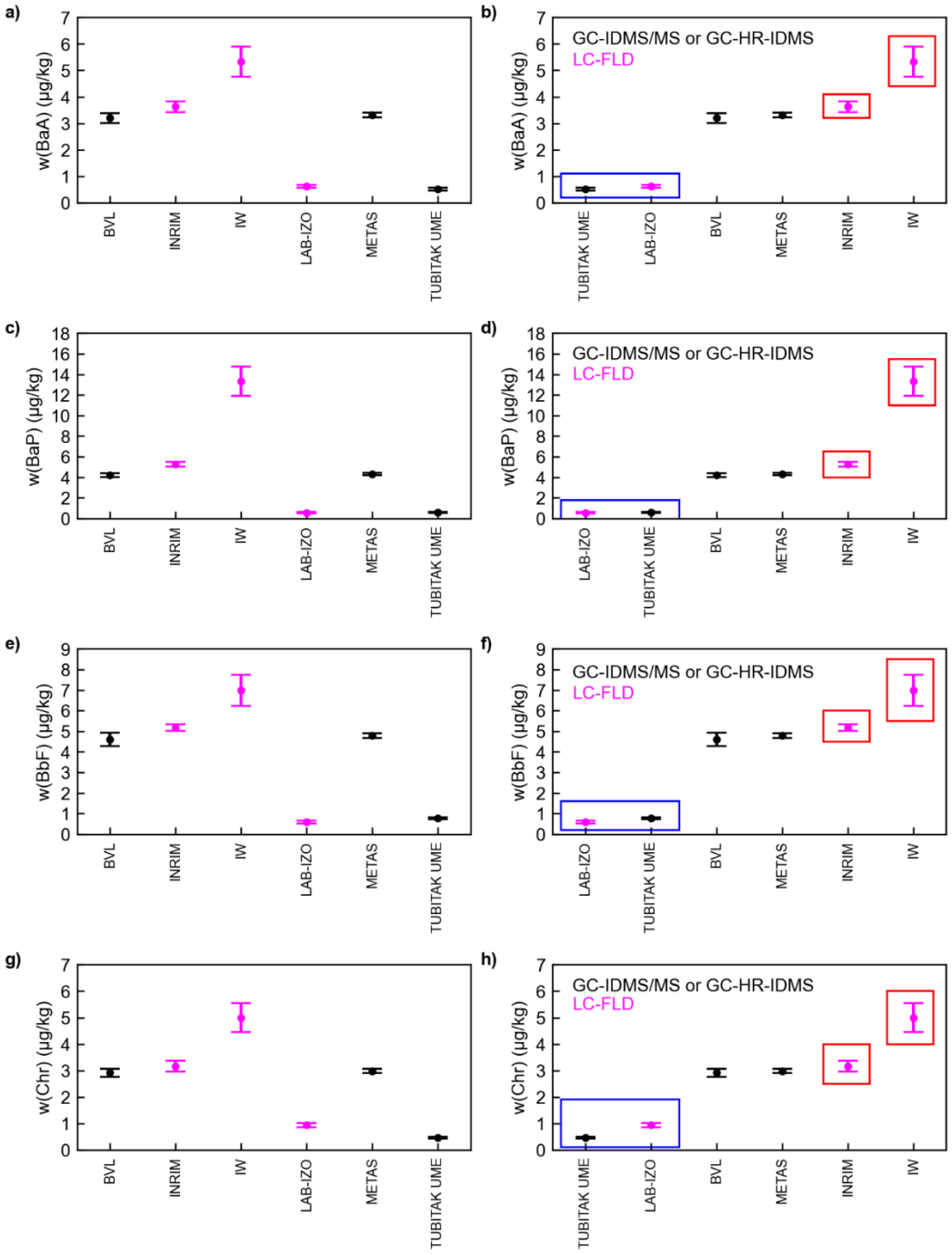


Figure 3: Illustrated reported results for BaA, BaP, BbF and Chr.
Panels a) and b) display the reported results for BaA, panels c) and d) for BaP, panels e) and f) for BbF and panels g) and h) for Chr. Panels a), c), e) and g) display the results sorted alphabetically by NMI acronym, panels b), d), f) and h) display results sorted by increasing reported value. Dots represent the reported mean values, x; bars their standard uncertainties, $u(x)$.

Discussion of Results

The results can mainly be divided into three subgroups (1) to (3) with increasing PAH mass fractions:

(1) TUBITAK UME and LAB-IZO, mass fractions close to or below 1 µg/kg
(2) BVL, METAS and INRIM, mass fractions in the range of 3 µg/kg to 5 µg/kg
(3) IW, mass fractions in the range of 5 µg/kg to 13 µg/kg

Influence of the extraction

The extraction is one of the most important steps of the analytical process. The use of internal standards (ISTDs) can also lead to erroneous results if the equilibrium between ISTD in the extraction solvent and the matrix is not fully achieved. All except IW used an internal standard (see a more detailed discussion on IDMS below) and added it before extraction. All participants used either ASE/PSE, HUPsSE, QuEChERS or treatment in an ultrasonic bath to equilibrate the extraction mixture in combination with various extraction solvents.

METAS, as the pilot in EURAMET.QM-S15, investigated and discussed the influence of the solvent and the different techniques on the extraction efficiency of the four PAHs BaA, BaP, BbF and Chr in the same protein-rich matrix as used in this supplementary comparison in a separate research article [8]. As illustrated in Figure 4, only about 10 % of the content of BaA, BaP, BbF or Chr were extracted when nonpolar solvents like n-hexane were used. The reason for the low values reported by subgroup (1) can therefore be attributed to a low extraction efficiency of the solvent(s) used. Both participants used n-hexane as extraction solvent. Based on these findings, values reported from subgroup (1) (TUBITAK UME and LAB-IZO) were excluded from SCRV calculation.

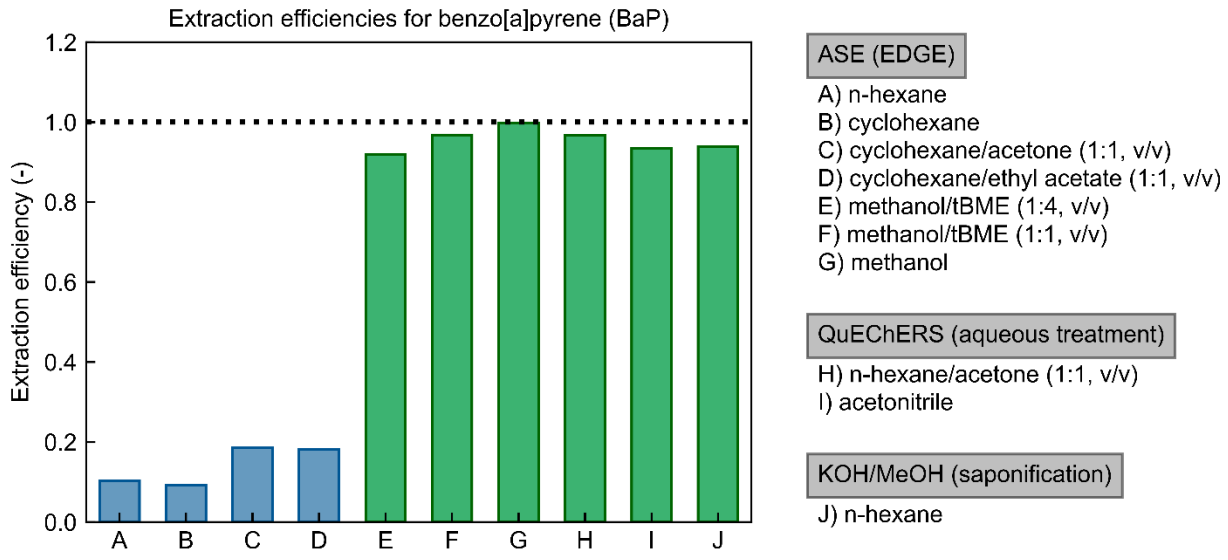


Figure 4: Solvent extraction efficiencies for BaP.
The Figure was adapted from [8]. For BaA, BbF and Chr similar extraction efficiencies were obtained, see [8]

The three institutes in subgroup (2) (BVL, METAS and INRIM) either used polar protic solvents like methanol or treated the sample with water before QuEChERS extraction with acetonitrile. As illustrated in Figure 4 these selections of solvents or extraction techniques can be regarded as suitable to extract the main content of the PAHs out of this matrix type.

IW in subgroup (3) generally obtained higher mass fractions than all the other participants. In the case of BaP, for example, IW reported a mass fraction close to 13 µg/kg, which is about a factor of 3 higher than the values reported by subgroup (2). IW used a QuEChERS type extraction with acetonitrile and a buffer salt mixture, which does not actually contradict the study on the extraction efficiency. The reason for the significantly higher mass fractions may be that IW did not use an ISTD at all.

Influence of the clean-up

The determination of low levels of PAHs in food samples usually requires a thorough clean-up by SPE or GPC, especially when GC-MS is applied with the purified extracts. All except INRIM used SPE (one step or two steps) for clean-up. The procedure used by INRIM consisted of centrifugation of the crude extract followed by subsequent processing (solvent change to isopropanol) of the supernatant.

Influence of the chromatographic method and detection technique

Three participants used GC-IDMS (BVL: GC-HR-IDMS; METAS and TUBITAK UME: GC-IDMS/MS) for chromatographic separation and detection. The other three participants used LC-FLD (LAB-IZO and INRIM) or LC-DAD/FLD (IW). The usage of both techniques is common for the analysis of PAHs in food samples. This is also shown by an ancillary study with external expert laboratories where one half of the participants used GC-IDMS and the other half LC-FLD (see Appendix A). In this ancillary study, no patterns were evident that would suggest that significantly different results were obtained with one technique than the other.

Influence of the quantification method and type of calibration

BVL, METAS and TUBITAK UME used IDMS for quantification, which is accepted to be a method that had been demonstrated to provide full metrological traceability. Because for FLD isotope dilution is not applicable, the participants using FLD as detection technique applied a method that had not been demonstrated to provide full metrological traceability. All participants used an external multi-point calibration by applying a linear regression model. Using various approaches, all participants except INRIM considered uncertainty components for the linear regression model.

Influence of other methodological approaches

Review of the approaches presented by the participants for estimating the measurement uncertainties shows that, except for INRIM, gravimetric approaches were used for all steps crucial for calculating the result. INRIM appears to have performed some of the operations in the laboratory on a volumetric basis, which could contribute to greater uncertainties.

SUPPLEMENTARY COMPARISON REFERENCE VALUE (SCRV)

As discussed above, only the values from BVL and METAS remained for the calculation of the SCRV. The reported BaA, BaP, BbF and Chr values from BVL and METAS agree within their stated uncertainties. Therefore, the uncertainty-weighted mean was selected as SCRV estimator, as described in [9,10].

Table 15 lists the SCRV, X, and standard uncertainties, $u(X)$, using the relevant equations in [9,10] for the uncertainty-weighted mean.

Table 15: SCRV Values for BaA, BaP, BbF and Chr.

		BaA, µg/kg		BaP, µg/kg	
Estimator	u?[a]	X	$u(X)$	X	$u(X)$
Uncertainty-weighted mean[b]	Yes	3.291	0.079	4.28	0.10

		BbF, µg/kg		Chr, µg/kg	
Estimator	u?[a]	X	$u(X)$	X	$u(X)$
Uncertainty-weighted mean[b]	Yes	4.77	0.12	2.967	0.070

a) Does the estimator utilize the information in the reported uncertainties?
b) Estimated using equations in [9,10]

Figure 5 and Figure 6 below display the application of the SCRVs to the reported data. The exclusion of two-thirds of the participants' results led to a small data basis for SCRV calculation. In order to support the selected approach, additional information on gravimetrically prepared mass fractions and on an ancillary interlaboratory comparison study with external expert food control laboratories is presented in Appendix A.

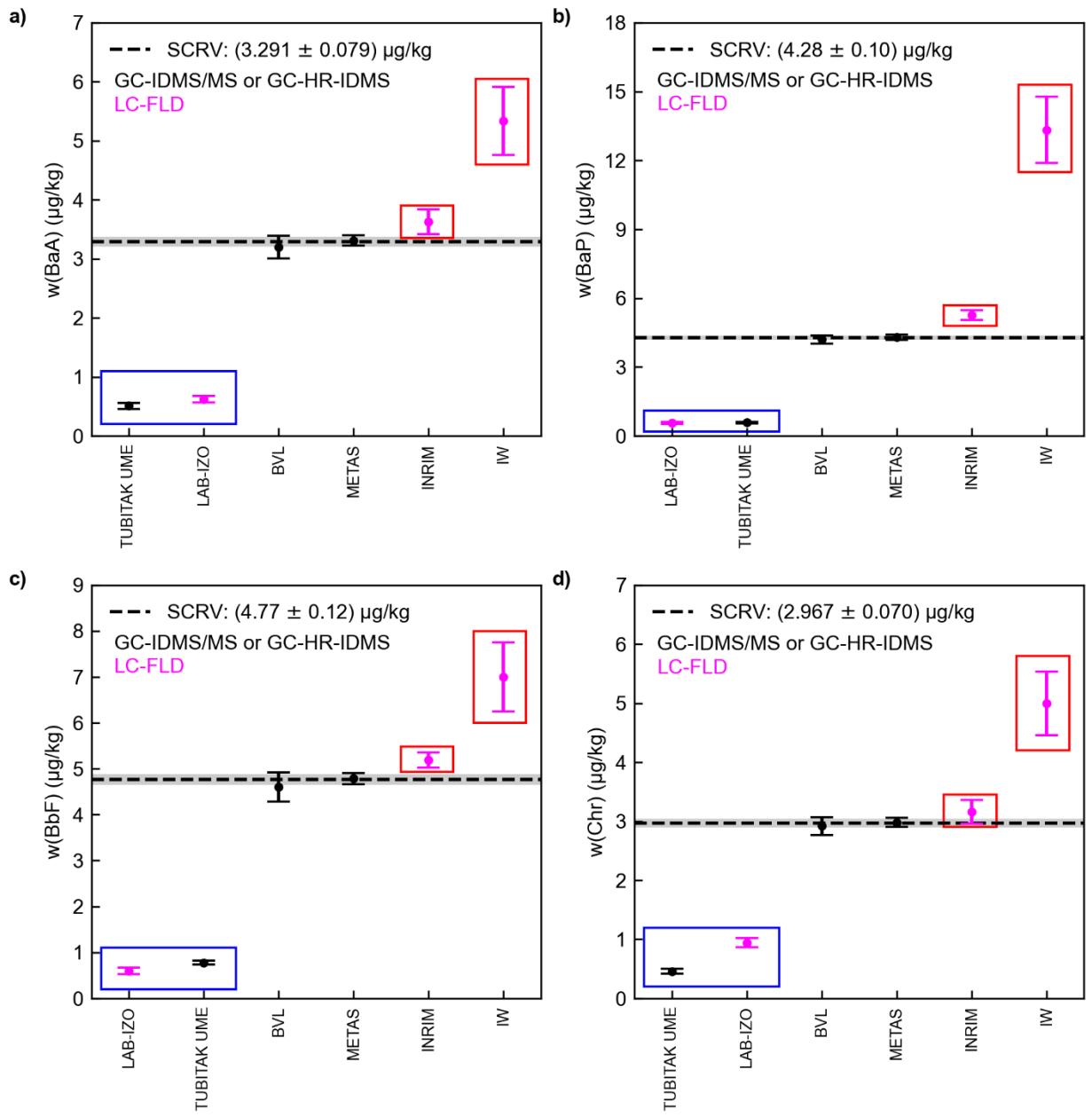


Figure 5: Supplementary Comparison Reference Values (SCRVs).
Panels a) to d) display the uncertainty-weighted mean SCRVs relative to the reported results for BaA, BaP, BbF and Chr. In all panels, the results are sorted by increasing reported value. Dots represent the reported mean values, x; bars their standard uncertainties, $u(x)$. The black horizontal line denotes the SCRV. The grey band denotes the standard uncertainty of the SCRV.

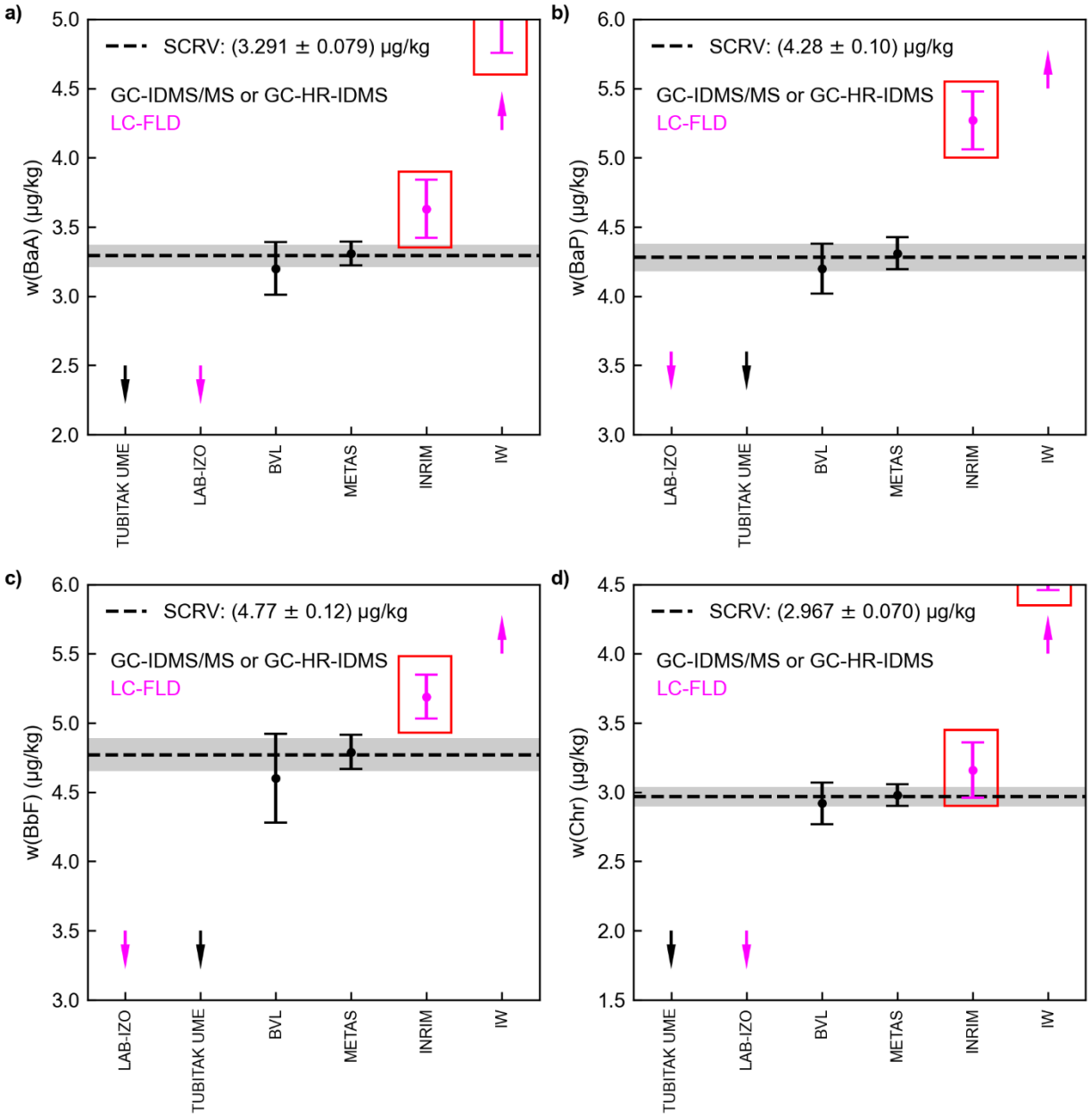


Figure 6: Enlarged plots of the Supplementary Comparison Reference Values (SCRVs).
Panels a) to d) display the uncertainty-weighted mean SCRVs relative to the reported results for BaA, BaP, BbF and Chr. In all panels, the results are sorted by increasing reported value. Dots represent the reported mean values, x; bars their standard uncertainties, $u(x)$. The black horizontal line denotes the SCRV. The grey band denotes the standard uncertainty of the SCRV.

DEGREES OF EQUIVALENCE (DoE)

The estimation of the consensus SCRV and the associated degrees of equivalences was based on the CCQM/13-22 guidance [9]. The absolute degrees of equivalence (DoE) for the participants in EURAMET.QM-S15 were estimated as the signed difference between the combined value and the SCRV:

$$d_i = x_i - \mathrm{SCRV}$$

The uncertainty of the DoE ($U_{k=2}(d_i)$) was determined with the inclusion of covariance [9]]. Since the SCRV was estimated from consensus of all results, the nominal $k = 2$ expanded uncertainty on the d_i, $U_{k=2}(d_i)$, was estimated as twice the square root of the sum of the squares of the standard uncertainties of the two components minus twice the covariance between the x_i and the SCRV:

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

To enable comparison with the degrees of equivalence estimates from other studies, the d_i and $U_{k=2}(d_i)$ were expressed as percentages relative to the SCRV:

$$\%d_i = \frac{100 \cdot d_i}{\mathrm{SCRV}};\ U_{k=2}(\%d_i) = \frac{100 \cdot U_{k=2}(d_i)}{\mathrm{SCRV}}$$

Table 16 below lists the numeric values of d_i, $U_{95}(d_i)$, d_i, and $U_{95}(d_i)$ for all participants in EURAMET.QM-S15 for BaA, BaP, BbF and Chr.

Table 16: Degrees of Equivalence for BaA, BaP, BbF and Chr

	BaA, µg/kg				BaP, µg/kg			
NMI/DI	*D*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$
BVL	-0.09	0.35	-2.8	10.5	-0.08	0.30	-1.8	7.1
INRIM	0.34	0.45	10.3	13.6	0.99	0.46	23.2	10.8
IW	2.04	1.16	62.0	35.3	9.05	2.88	211.6	67.4
LAB-IZO	-2.67	0.19	-81.2	5.8	-3.71	0.22	-86.7	5.1
METAS	0.02	0.07	0.6	2.2	0.03	0.12	0.7	2.9
TUBITAK UME	-2.78	0.19	-84.5	5.7	-3.70	0.21	-86.4	4.9

	BbF, µg/kg				Chr, µg/kg			
NMI	*D*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$
BVL	-0.17	0.60	-3.5	12.5	-0.05	0.27	-1.6	8.9
INRIM	0.42	0.39	8.9	8.3	0.19	0.42	6.5	14.3
IW	2.23	1.53	46.9	32.1	2.03	1.09	68.5	36.6
LAB-IZO	-4.17	0.27	-87.4	5.6	-2.02	0.22	-68.2	7.5
METAS	0.02	0.09	0.5	1.9	0.01	0.07	0.4	2.5
TUBITAK UME	-3.99	0.24	-83.6	5.1	-2.51	0.16	-84.5	5.4

Figure 7 and Figure 8 below graphically illustrates both the absolute and relative DoEs for the four measurands using the uncertainty-weighted mean SCRV.

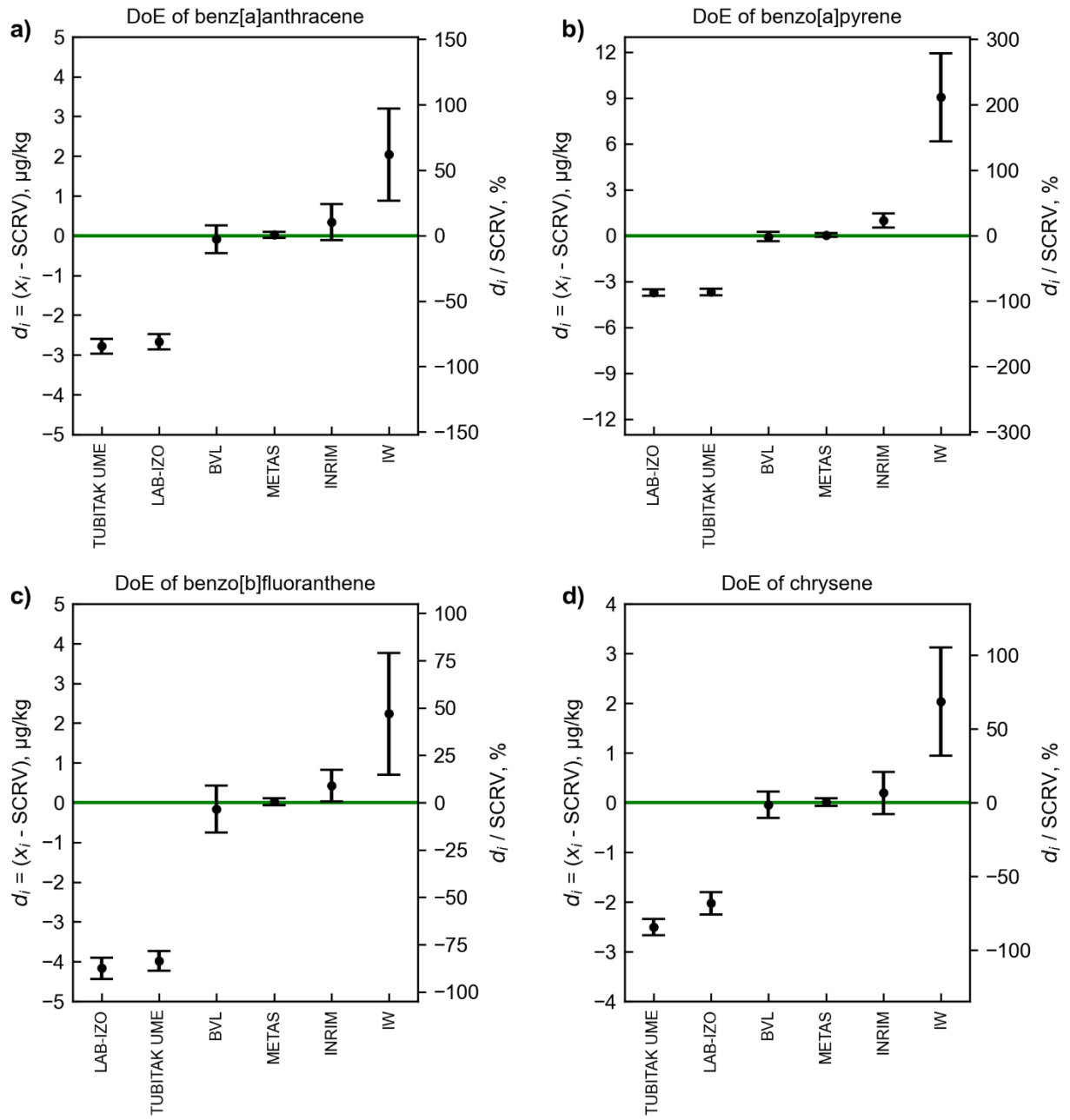


Figure 7: Degrees of Equivalence (DoEs) associated with the SCRVs.
Panels a) to d) display the DoEs for the uncertainty-weighted mean SCRVs for BaA, BaP, BbF and Chr. All results are sorted by increasing d. The axis to the left edge of each panel displays the absolute DoE, d, in µg/kg. The axis to the right edge of each panel displays the relative DoE, $100 \cdot d/\mathrm{SCRV}$, as percent. Dots represent the d, bars their approximate 95 % expanded uncertainties, $U_{95}(d)$. The thick green horizontal line denotes perfect agreement with the SCRV.

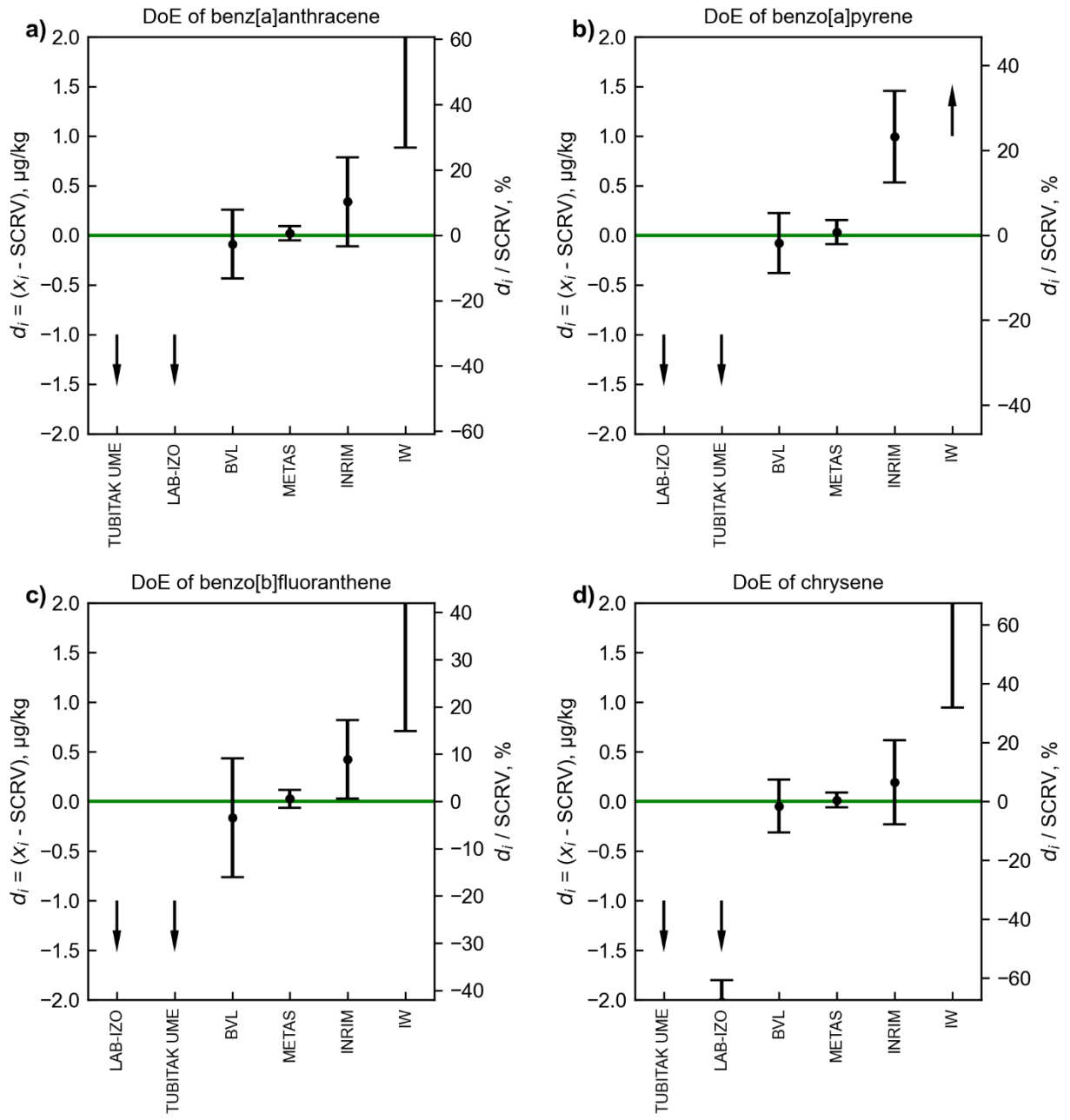


Figure 8: Enlarged plots of the Degrees of Equivalence (DoEs) associated with the SCRVs. Panels a) to d) display the DoEs for the uncertainty-weighted mean SCRVs for BaA, BaP, BbF and Chr. All results are sorted by increasing *d*. The axis to the left edge of each panel displays the absolute DoE, *d*, in µg/kg. The axis to the right edge of each panel displays the relative DoE, 100·*d*/SCRV, as percent. Dots represent the *d*, bars their approximate 95 % expanded uncertainties, $U_{95}(d)$. The thick green horizontal line denotes perfect agreement with the SCRV.

USE OF EURAMET.QM-S15 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in EURAMET.QM-S15 demonstrates the measurement capabilities for determining the mass fraction of low polarity ($pK_{ow} < -2$) organic compounds, with molecular masses between 150 g/mol and 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in a high protein food matrix. Beside value assignment, the demonstration of measurement capabilities includes extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.

Core Competency Statements and CMC support

Table 17 to Table 22 list the core competencies claimed by the participants in EURAMET.QM-S15. The information in these tables is as provided by the participants; however, the presentation of many entries has been condensed and standardized. Details of the analytical methods used by each participant in this study are provided in Appendix G.

The results of TUBITAK UME and LAB-IZO for BaA, BaP, BbF and Chr are not consistent with the SCRV for these measurands and yield DoEs that do not cross zero. This inconsistency could be traced back to inefficient extraction of the PAHs out of the matrix due to the usage of the nonpolar solvent n-hexane.

The results of INRIM for BaP and BbF and of IW for BaA, BaP, BbF and Chr are not consistent with the SCRV for these measurands and yield DoEs that do not cross zero. This inconsistency could possibly be attributed to the calibrants used and other methodological reasons. However, no additional investigation was carried out by the two institutes.

Table 17: Core competencies demonstrated in EURAMET.QM-S15 by BVL

EURAMET.QM-S15	***BVL***	**PAHs in Protein Matrix**
Scope of Measurement: Participation in this supplementary comparison would provide the opportunity to demonstrate participant's capabilities in determining low polarity (pKow < -2) organic compounds with molecular masses of about 150 – 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NIST SRM 1647f
Identity verification of analyte(s) in calibration material. #	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s). #	N/A	
For calibrants which are a calibration solution: Value-assignment method(s). #	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Identification via retention time, ratio of response quantifier to qualifier ions, high-resolution mass spectra
Extraction of analyte(s) of interest from matrix	✓	HUPsSE (Heat-Ultrasonic-Pressure supported Solvent Extraction)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-HRMS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) external standard calibration with internal standard b) multi-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

Table 18: Core competencies demonstrated in EURAMET.QM-S15 by INRIM

EURAMET.QM-S15	***INRIM***	**PAHs in Protein Matrix**
Scope of Measurement: Participation in this supplementary comparison would provide the opportunity to demonstrate participant's capabilities in determining low polarity (pKow < -2) organic compounds with molecular masses of about 150 – 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Supelco (Merck) RTC - CRM47940 Lot LRAD2034 (calibration solution)
Identity verification of analyte(s) in calibration material.	×	Not performed
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).	×	Not performed
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time
Extraction of analyte(s) of interest from matrix	✓	QuEChERS
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Centrifugation
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	✓	a) External standard b) 6-point calibration curve (area ratio vs. internal standard)
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

The INRIM results for BaP and BbF are not consistent with the SCRV for these measurands and yield DoEs that do not cross zero. This inconsistency could possibly be attributed to the calibrant used. No other reasons could be determined.

Table 19: Core competencies demonstrated in EURAMET.QM-S15 by IW

EURAMET.QM-S15	***IW***	**PAHs in Protein Matrix**
Scope of Measurement: Participation in this supplementary comparison would provide the opportunity to demonstrate participant's capabilities in determining low polarity (pKow < -2) organic compounds with molecular masses of about 150 – 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	PAH-Mix 9 100 µg/mL in Acetonitrile, DRE-XA20950009AL, Lot G1126284AL, (Dr. Ehrensdorfer)
Identity verification of analyte(s) in calibration material.	×	Not performed
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).	×	Not performed
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	
Extraction of analyte(s) of interest from matrix	✓	QuEChERS
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	HPLC DAD/FLD
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) external standard calibration b) multi-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

The IW results for BaA, BaP, BbF and Chr are not consistent with the SCRV for these measurands and yield DoEs that do not cross zero. This inconsistency could possibly be attributed to the calibrant used. No other reasons could be determined.

Table 20: Core competencies demonstrated in EURAMET.QM-S15 by LAB-IZO

EURAMET.QM-S15	***LAB-IZO***	**PAHs in Protein Matrix**
Scope of Measurement: Participation in this supplementary comparison would provide the opportunity to demonstrate participant's capabilities in determining low polarity (pKow < -2) organic compounds with molecular masses of about 150 – 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NIST SRM 1647f
Identity verification of analyte(s) in calibration material.	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Chromatographic retention time
Extraction of analyte(s) of interest from matrix	✓	Ultrasonic extraction with n-hexane (3 repetitions)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	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 of analyte(s) in matrix	✓	a) External standard b) 4-point calibration curve (standard addition)
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

The LAB-IZO results for BaA, BaP, BbF and Chr are not consistent with the SCRV for these measurands and yield DoEs that do not cross zero. This inconsistency could be traced back to inefficient extraction of the PAHs out of the matrix duo to the nonpolar solvent n-hexane.

Table 21: Core competencies demonstrated in EURAMET.QM-S15 by METAS

EURAMET.QM-S15	***METAS***	**PAHs in Protein Matrix**
Scope of Measurement: Participation in this supplementary comparison would provide the opportunity to demonstrate participant's capabilities in determining low polarity (pKow < -2) organic compounds with molecular masses of about 150 – 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NIST SRM 1647f
Identity verification of analyte(s) in calibration material.	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	✓	ASE
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) linear regression IDMS b) 6-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

Table 22: Core competencies demonstrated in EURAMET.QM-S15 by TUBITAK UME

EURAMET.QM-S15	***TUBITAK UME***	**PAHs in Protein Matrix**
Scope of Measurement: Participation in this supplementary comparison would provide the opportunity to demonstrate participant's capabilities in determining low polarity (pKow < -2) organic compounds with molecular masses of about 150 – 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NIST SRM 1647f
Identity verification of analyte(s) in calibration material.	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention Time, Mass Spec Ion Ratio
Extraction of analyte(s) of interest from matrix	✓	PSE with n-hexane
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) 8-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	.

TUBITAK UME results for BaA, BaP, BbF and Chr are not consistent with the SCRV for these measurands and yield DoEs that do not cross zero. This inconsistency could be traced back to inefficient extraction of the PAHs out of the matrix due to the nonpolar solvent n-hexane.

CONCLUSIONS

Participants in EURAMET.QM-S15 demonstrated their ability to determine the mass fractions of low polarity (pKow < -2) organic compounds, with molecular masses between 150 g/mol and 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in a high protein food matrix. Four results were excluded from SCRV calculation due to identified issues with their methodologies (poor extraction of the PAHs out of the matrix) or metrological traceability (not in accordance with CIPM MRA and OAWG Practices and Guidelines document). The SCRV was calculated with the results of two laboratories using the uncertainty-weighted mean as SCRV estimator.

ACKNOWLEDGEMENTS

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

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APPENDIX A: Additional Information to Support the SCRV

To support and justify the approach to calculating the SCRV, additional information about the study material is provided in this Appendix. The additional information refers to calculation of gravimetrically prepared mass fractions from production data and an ancillary interlaboratory comparison (ILC) study with external expert laboratories (commercial and official food control laboratories). The additional information is documented in detail in [8]

Gravimetrically prepared mass fractions

The following summary of the material production was adapted from [8].The protein-rich food matrix consisted of whey protein powder. Because industrially manufactured whey protein powder was shown to be free of the target PAHs (< 0.05 µg/kg), liquid whey was contaminated before further processing. The liquid whey (raw material) was a retentate from membrane filtration with approx. 0.32 g/g of dry matter, which was supplied by a Swiss dairy product producer from one of their conventional production streams. A quantity of 5 kg of this liquid whey raw material was contaminated with the four PAHs BaA, BaP, BbF, and Chr using a spike solution in acetonitrile. The spike solution was prepared from a PAHs stock solution that was in turn prepared by dissolving CRMs of BaA (Sigma-Aldrich, Buchs, Switzerland, Supelco® 75451), BaP (Sigma-Aldrich, Buchs, Switzerland, Supelco® 51968), BbF (Sigma-Aldrich, Buchs, Switzerland, Supelco® 30958), and Chr (Sigma-Aldrich, Buchs, Switzerland, Supelco® 94035) in acetonitrile. After spiking, the contaminated liquid whey was stirred for 15 min. at room temperature with a cup stirrer before it was transferred back to the Swiss dairy product producer. There, the contaminated liquid whey was mixed with another 85 kg portion of uncontaminated liquid whey raw material, resulting in a total of 90 kg of contaminated liquid whey. This mixture was then spray-dried using an industrial pilot plant yielding 23 kg of whey protein powder with approx. 0.77 g/g of protein (0.81 g/g of protein in dry matter), 0.10 g/g of carbohydrates, 0.06 g/g of fat, and 0.05 g/g of water. After sieving, the final bulk product was filled into pre-cleaned amber glass bottles in 30 g portions. Figure A-1 shows a schematic representation of the production steps of the whey protein powder.

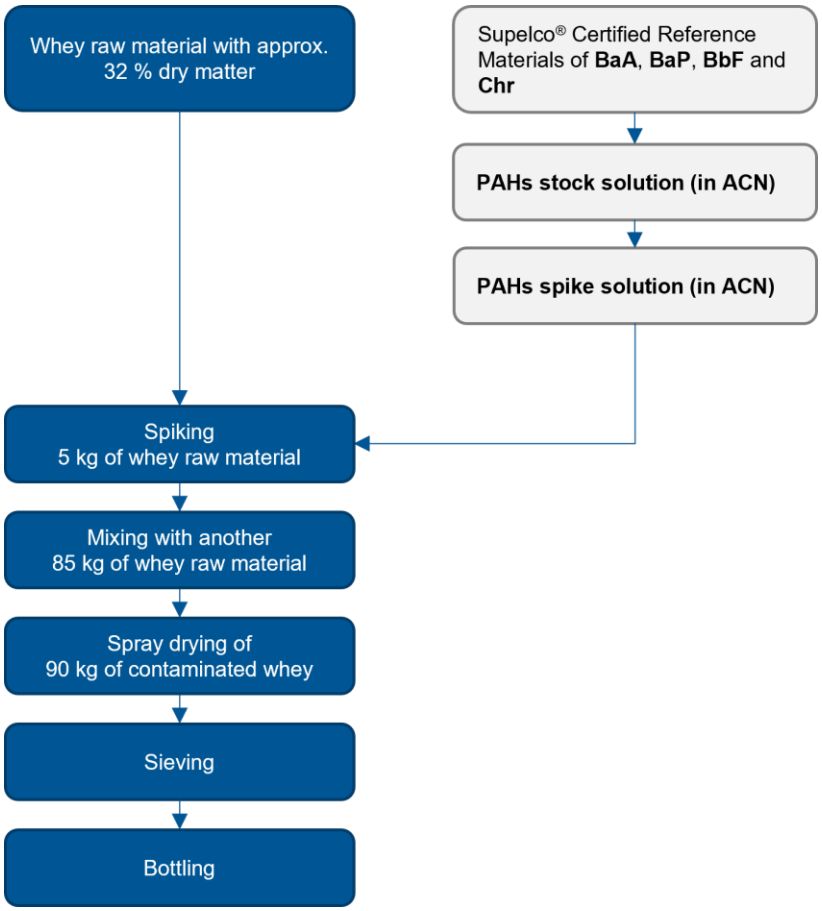

Figure A-1: Schematic representation of the production steps of the whey protein powder.

All production steps were carried out gravimetrically using air buoyancy correction. This production approach allowed the evaluation of gravimetrically prepared mass fractions, which are given in Table A-1.

Table A-1: Gravimetrically prepared mass fraction

PAH	$x_{grav.}$	$u(x_{grav.})$
BaA	3.230	0.031
BaP	4.331	0.042
BbF	4.937	0.077
Chr	2.906	0.028

In addition to Figure 5, Figure A-1 compares the SCRVs with the gravimetrically prepared mass fractions. The gravimetrically prepared mass fractions show a very good agreement with the SCRVs.

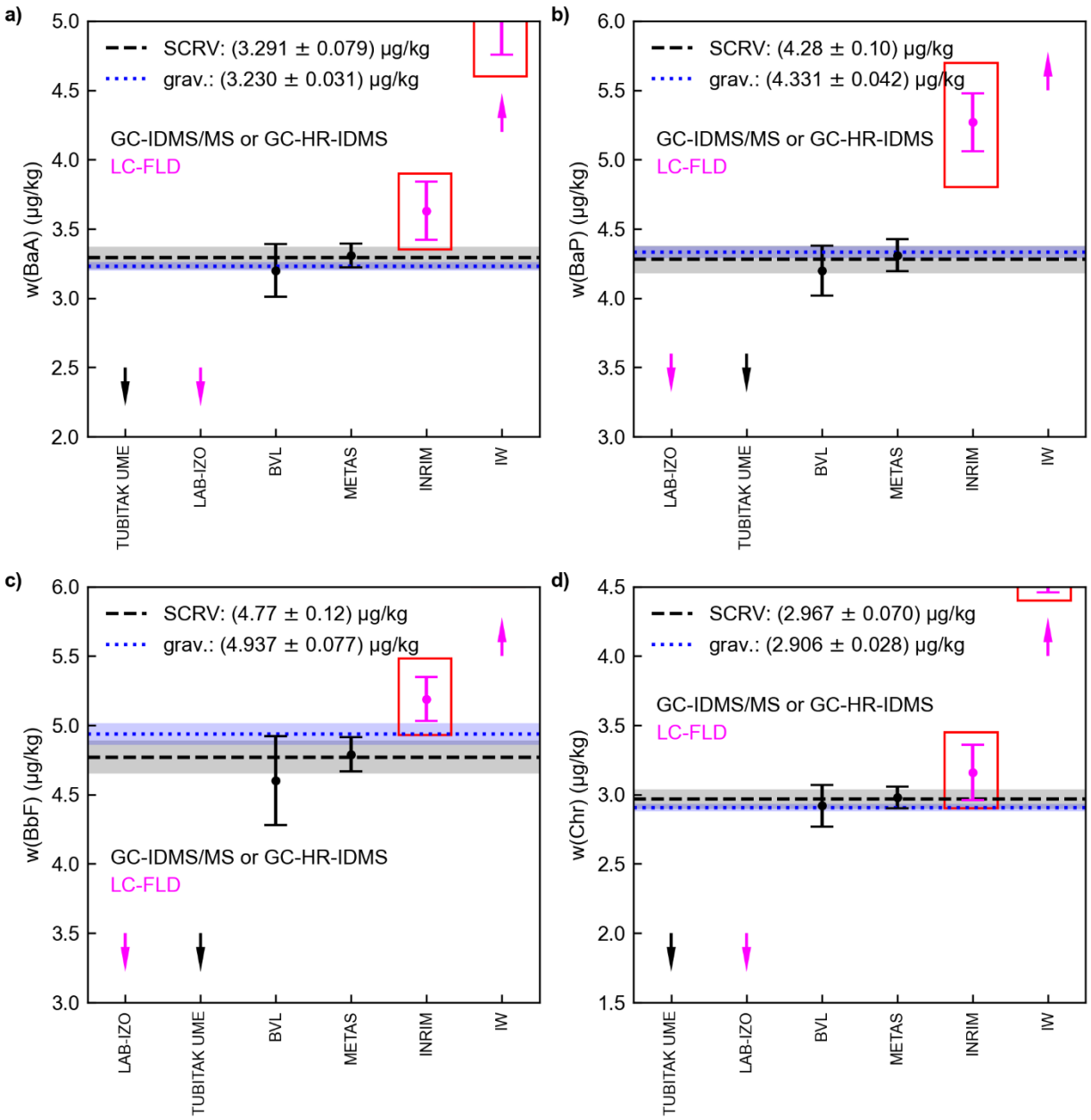

Figure A-2: Comparison of SCRV and gravimetrically prepared mass fractions.
Panels a) to d) display the SCRVs relative to the reported results for BaA, BaP, BbF and Chr and are compared to gravimetrically prepared mass fractions. In all panels, the results are sorted by increasing reported value. Dots represent the reported mean values, x; bars their standard uncertainties, $u(x)$. The black and blue horizontal lines denote the SCRVs and the gravimetrically prepared mass fractions, respectively. The grey and blue bands denote their associated standard uncertainties.

Ancillary study with external expert laboratories

To gain a better understanding of the material, an additional interlaboratory comparison (ILC) with external expert laboratories (commercial and official food control laboratories) was organized. Figures A-3 and A-4 compare the results reported by the laboratories with the results of the Supplementary Comparison as well as with the SCRVs and the gravimetrically prepared mass fractions.

As it was the case in the Supplementary Comparison study, laboratories using nonpolar or polar aprotic solvents for extraction reported very low results that were close or below 1 µg/kg. These laboratories are framed in blue in Figures A-3 and A-4. Apart from laboratory nine, all other laboratories reported results, which are located around the SCRVs and gravimetrically prepared mass fractions. Laboratories 5 and 9, which are colored in grey, did not send any experimental information even after request.

One half of the laboratories, which participated in the ancillary ILC used GC-IDMS, GC-IDMS/MS or GC-HR-IDMS whereas the other half used LC-FLD. No patterns were evident that would suggest that significantly better results were obtained with one technique than the other in this ILC on the level of commercial and official food control laboratories.

Both the gravimetrically prepared mass fractions and the ancillary ILC with external expert food control laboratories support the SCRVs calculated in this report. This additional information also justifies the calculation of the SCRVs with the results of only two laboratories.

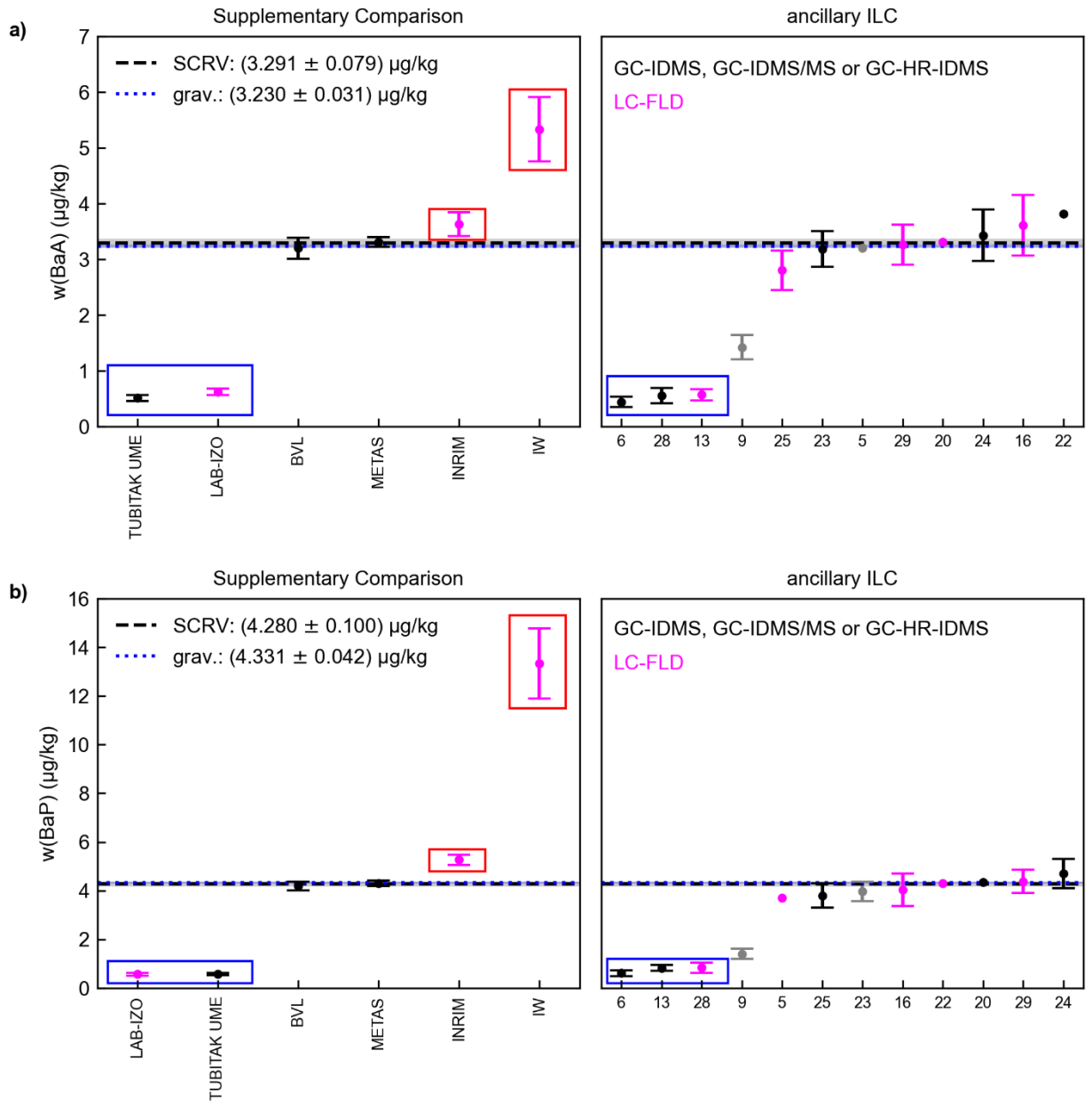

Figure A-3: Comparison of ancillary ILC results with the results of the Supplementary Comparison, the SCRVs and the gravimetrically prepared mass fractions.
Panels a) to b) display the SCRVs relative to the reported results for BaA and BaP and are compared to gravimetrically prepared mass fractions. In all panels, the results are sorted by increasing reported value. Dots represent the reported mean values, x; bars their standard uncertainties, $u(x)$. The results of the black dots were obtained with GC-IDMS, GC-IDMS/MS or GC-HR-IDMS and the results of the magenta dots with LC-FLD. The black and blue horizontal lines denote the SCRVs and the gravimetrically prepared mass fractions, respectively. The grey and blue bands denote their associated standard uncertainties.

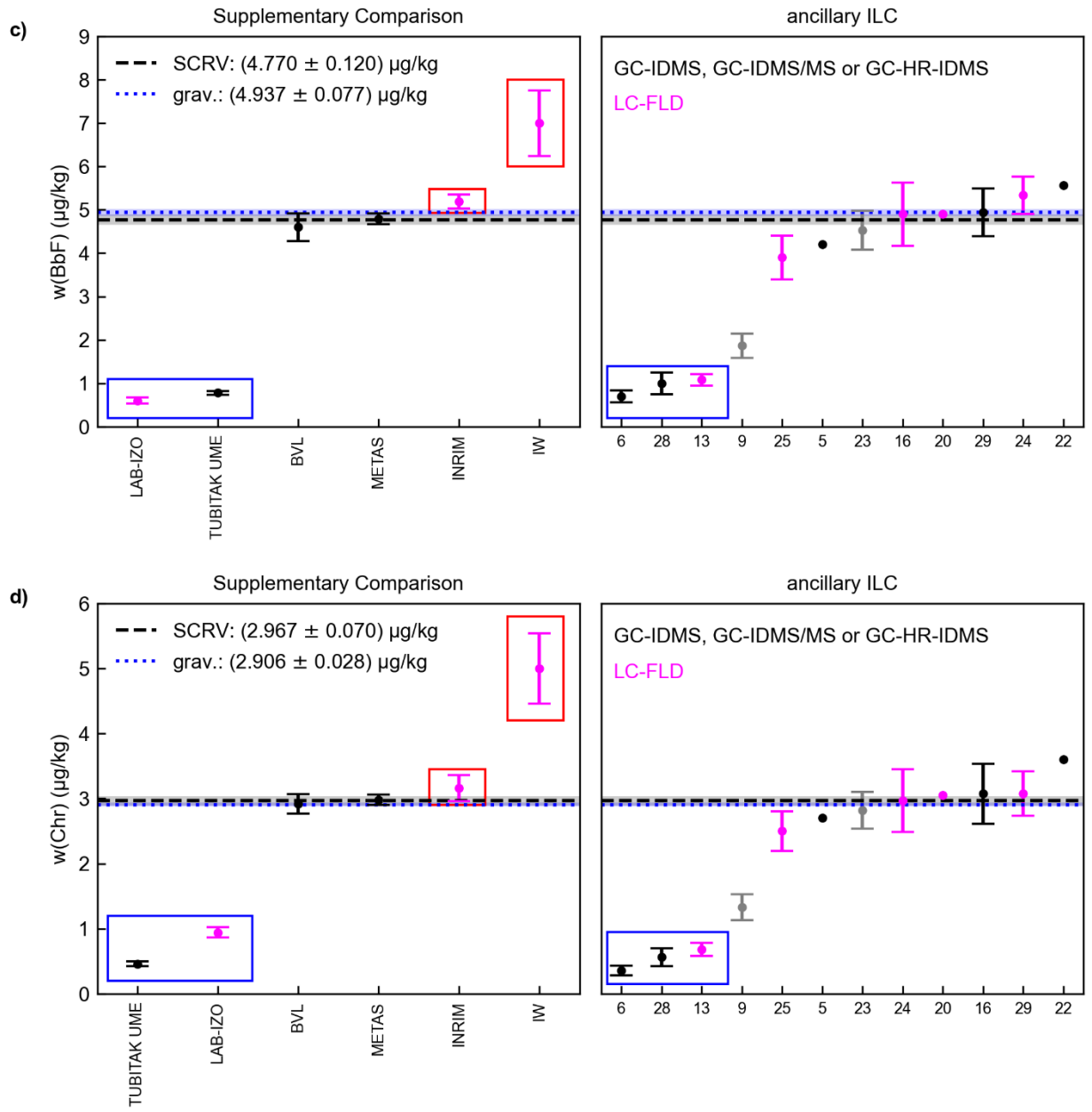

Figure A-4: Comparison of ancillary ILC results with the results of the Supplementary Comparison, the SCRVs and the gravimetrically prepared mass fractions.
Panels c) to d) display the SCRVs relative to the reported results for BbF and Chr and are compared to gravimetrically prepared mass fractions. In all panels, the results are sorted by increasing reported value. Dots represent the reported mean values, x; bars their standard uncertainties, $u(x)$. The results of the black dots were obtained with GC-IDMS, GC-IDMS/MS or GC-HR-IDMS and the results of the magenta dots with LC-FLD. The black and blue horizontal lines denote the SCRVs and the gravimetrically prepared mass fractions, respectively. The grey and blue bands denote their associated standard uncertainties.

APPENDIX B: Call for Participation

At the EURAMET SCBOA meeting on 2 February 2021 and at the CCQM OAWG meeting on 5 May 2021, METAS made a statement of intent for the organization of a supplementary comparison for the determination of PAHs in a high protein matrix.

At the EURAMET SCBOA meeting on 1 February 2022 and at the CCQM OAWG meeting on 16/17 June 2022, the supplementary comparison EURAMET.QM-S15 was officially presented.

An e-mail (see below), containing the protocol of the supplementary comparison, was sent to interested NMIs. The NMIs then registered directly by e-mail.

From: Umbricht Gisela METAS
Sent: Friday, 14 October, 2022 14:21
To: [interested NMIs]
Subject: Supplementary comparison PAH

Dear all,

A while ago you indicated your interest in participating to the proposed supplementary comparison about PAHs in a protein matrix.

We, METAS together with TÜBITAK UME (Mine Bilsel), can now propose to you to participate in a supplementary comparison for the determination of the mass fraction of 4 different PAHs (benz(a)anthracene (BaA), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), and chrysene (Chr)) in a protein-rich matrix.
Please find attached the protocol.
If you are interested to participate, please let me know. In this case, could you please also indicate the exact address, where we shall send the sample.

I am looking forward to working with you on this interesting comparison.

Best regards

Gisela

Attachments: Protocol_Supplementary_Comparison_PAH_in_Protein_Final.pdf

APPENDIX C: Protocol

The initial protocol "Protocol_Supplementary_Comparison_PAH_in_Protein_Final.pdf" was modified on 30 November 2022: the sample distribution date was changed from November 2022 to December 2022. The protocol shown below corresponds to the following version: "Protocol_Supplementary_Comparison_PAH_in_Protein_Final_V2.pdf".

Extensions of the deadline for the submission of the results were communicated on 13 April 2023 and 7 July 2023 via email (see below).

From: Umbricht Gisela METAS
Sent: Thursday, 13 April 2023, 10:05
To: [participants]
Subject: Supplementary Comparison PAHs

Dear all

Due to problems encountered by a participant laboratory the delay for submission of the results was postponed for one month, until the end of May 2023.

Thank you for your understanding and your participation.

Best regards

Gisela

From: Umbricht Gisela METAS
Sent: Friday, 7 July 2023, 15:26
To: [participants]
Subject: Supplementary Comparison PAHs

Dear all

Due to problems encountered by a participant laboratory the delay for submission of the results was postponed until the end of August 2023.

Thank you for your understanding and your participation.

Best regards

Gisela

EURAMET Supplementary Comparison EURAMET.QM-S15
Measurement of PAHs in Protein Matrix

Supplementary Comparison

Study Protocol
July 2022

Proposed dates
09/2022 to 09/2023

Coordination Laboratory
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METAS Contact Points
Simon Lobsiger (simon.lobsiger@metas.ch)
Silvia Mallia (silvia.mallia@metas.ch)

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are a class of chemicals, consisting of hydrocarbons with at least two connected aromatic ring systems. They may be produced during the processing of foodstuffs, such as smoking. They may, however, also find their way into foodstuffs unintentionally, e.g. from mineral oils. Because they may have negative effects on health – they are associated with cancer and other diseases – threshold values have been set for PAHs. Accurate analyses regarding these chemicals are therefore called for.

There were other comparisons previously conducted for the determination of PAHs. CCQM conducted key comparisons with final reports published in 2018 (CCQM-K95.1: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea) and 2020 (CCQM-K146: Benzo[a]pyrene in Olive Oil). So far, however, no comparison of PAHs in a protein-rich matrix has been carried out.

In this comparison the target PAHs are benz(a)anthracene (BaA), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), and chrysene (Chr) in protein-rich matrix.

Successful participation in this supplementary comparison will demonstrate the measurement capabilities in determining mass fractions of low polarity organic compounds with molecular masses of about 150 - 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg (as received) in a protein-rich matrix.

TIMELINE

Table 1 lists the time schedule for the proposed study.

Table 1: Proposed study schedule

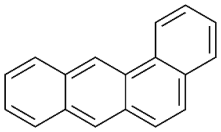
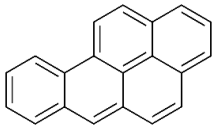
Date	**Action**
December 2020	Sample preparation
From March to May 2022	Homogeneity and stability testing
September 2022	Call for participation
December 2022	Sample distribution
April 2023	Deadline for submission of results
June 2023	Preliminary discussion of results

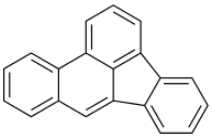
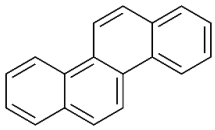
MEASURANDS

The measurands are the mass fractions (as received) of BaA, BaP, BbF and Chr in high-protein powder with the assigned value expressed in µg/kg. For each measurand, the indicative value for the mass fraction is 0.1 µg/kg to 50 µg/kg.

Table 2 below contains information about these compounds.

Table 2: Information of PAHs investigated in this supplementary comparison

	Benz(a)anthracene (BaA)	**Benzo(a)pyrene (BaP)**
CAS	56-55-3	50-32-8
Molecular formula	$C_{18}H_{12}$	$C_{20}H_{12}$
Molecular weight	228.3	252.3
Structure		

	Benzo(b)fluoranthene (BbF)	**Chrysene (Chr)**
CAS	205-99-2	218-01-9
Molecular formula	$C_{20}H_{12}$	$C_{18}H_{12}$
Molecular weight	252.3	228.3
Structure		

STUDY MATERIAL

The study material is a high-protein powder, spiked with the aforementioned PAHs. The material was produced by spiking fresh liquid protein concentrate with a solution of PAHs in acetonitrile. After mixing, the liquid protein concentrate was subjected to spray drying. A powder with about 80 % of protein was obtained. The powder was then bottled into pre-cleaned glass-amber bottles, each containing 30 g of material.

Each participant will receive one bottle containing approximately 30 g of material. Measurement results were to be reported on an as-received basis.

Recommended Minimum Sample Amount

The recommended minimum sample amount for analysis is at least 1.0 g.

Homogeneity Assessment of Study Material

For the homogeneity assessment, 10 bottles covering the whole bottling range were randomly selected. Three independent test portions of each bottle were analyzed. The measurements were performed under repeatability conditions, using a validated method and according to a random sequence to prevent possible trends in analytical sequence and filling order. According to ISO Guide 35:2017 [1], the assessment of the homogeneity was carried out by a one-way analysis of variance (ANOVA). For all four PAHs, the observed F-values ($MS_{between\ bottles}/MS_{within\ bottles}$) were lower than the critical F-values, indicating that the variances of the measured values within and between the bottles do not differ significantly at a 95 % confidence level. No evidence of statistically significant inhomogeneity was therefore observed. The results of the

homogeneity study and the estimated uncertainties for potential inhomogeneity (u_{bb}) are given in table 3 and figure 1.

Table 3: Results of the homogeneity assessment

PAH	s_{wb} (%)	s_{bb} (%)	u^*_{bb} (%)	u_{bb} (%)[a)]	F_{obs}	F_{crit}
BaA	2.7	0.5	0.9	0.9	1.099	2.393
BaP	4.3	n/a	1.4	1.4	0.147	2.393
BbF	3.2	n/a	1.1	1.1	0.222	2.393
Chr	3.2	0.4	1.1	1.1	1.051	2.393

a) For the estimation of u_{bb} the higher value of u^*_{bb} and s_{bb} was taken. u^*_{bb} and s_{bb} were calculated according to [2].

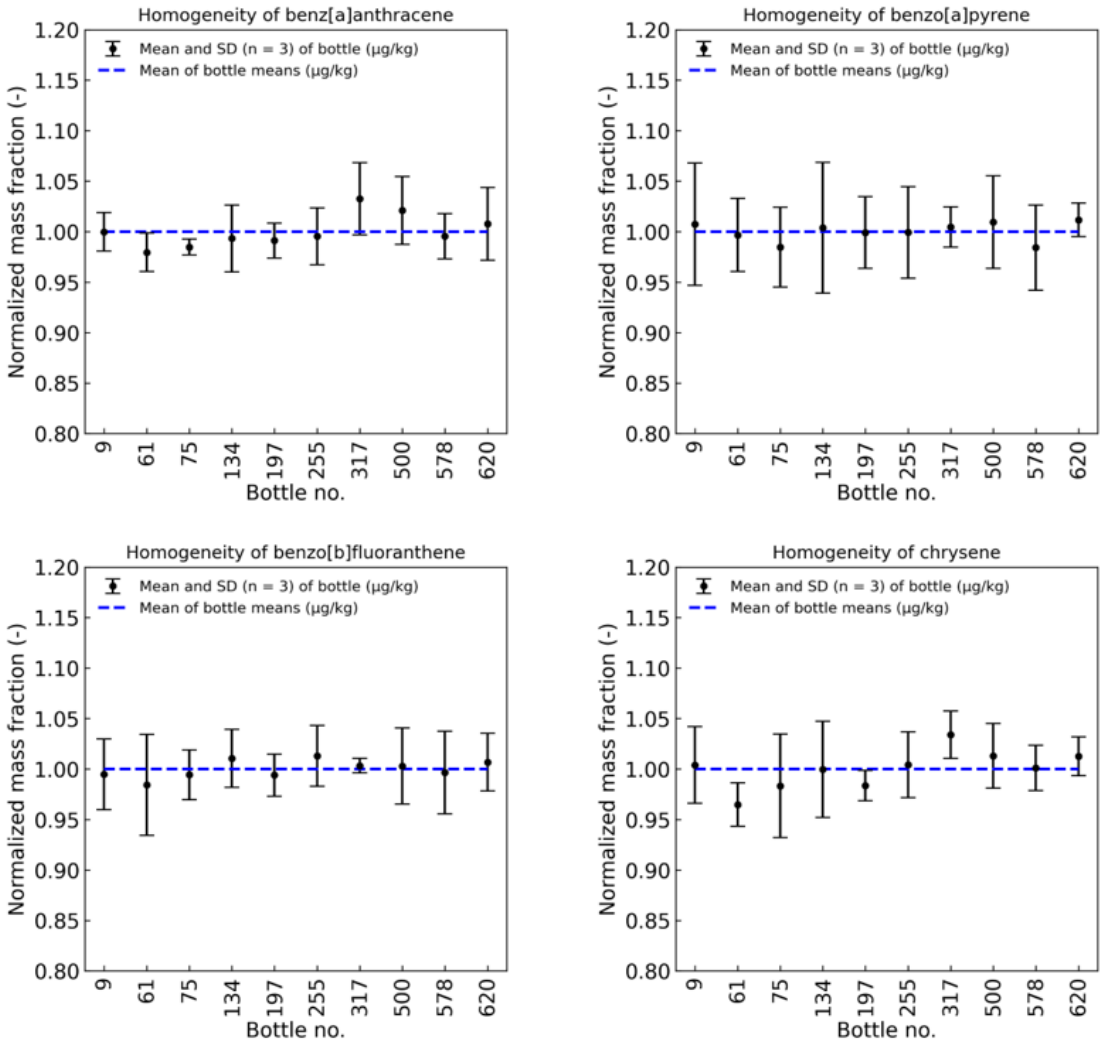


Figure 1: Homogeneity study of the four PAHs BaA, BaP, BbF and Chr: mean values of 10 selected bottles with their corresponding standard deviations (n = 3).

Stability Assessment of Study Material

For the stability assessment, an isochronous approach was used. The investigated bottles (one for each stability point) were stored for 1.5, 3, 6 and 12 months at different temperatures: -20 °C (reference temperature), 4 °C, room temperature (ca. 20 °C) and 45 °C (up to 3 months only). After the storage time was reached for a certain stability point, the corresponding bottle was stored at the reference temperature before it was analyzed three times. For t = 0, data from the homogeneity study were used. According to ISO Guide 35:2017 [1] and Linsinger et al. [3], the stability was assessed by applying a linear regression model. The slope b_1 and intercept b_0 were fit to the stability data. Using a two-tailed t-test, $t_{b1} = |b_1|/s(b_1)$, it could be shown that the slopes for all PAHs at all investigated temperatures do not differ significantly from 0 at a 95 % confidence level. Therefore, since no evidence of statistically significant instability was found at the various temperatures during the investigated storage times, b_1 was set to 0 for further calculations.

For the estimation of the uncertainties for potential long-term instability (u_{lts}) at the storage temperature of -20 °C the extrapolation model $u_{lts} = u(b_1=0) \cdot t$, with t = 24 months, was used [1, 3]. The results are given in table 4 and figure 2. The long-term stability, including 2 years and 4 years stability points, will be further investigated. For storage temperatures at 4 °C and room temperature (ca. 20 °C) similar uncertainty estimates were obtained as for -20 °C. This indicates that the long-term stability is also given at temperatures up to room temperature.

Table 4: Results of the long-term stability assessment at a storage temperature of -20 °C

PAH	**b_1 (months^{-1})**	**b_0 (-)**	**$s(b_1)$ (-)**	**t_{b1}**	**t_{crit}**	**$s(b_1=0)$ (-)**	**u_{lts} (%)**
BaA	-0.001275	1.005737	0.001948	0.654	2.160	0.001908	4.6
BaP	-0.001408	1.006334	0.002029	0.680	2.160	0.002029	4.9
BbF	0.001560	0.992980	0.001963	0.795	2.160	0.001937	4.7
Chr	-0.002210	1.009946	0.002151	1.027	2.160	0.002156	5.2

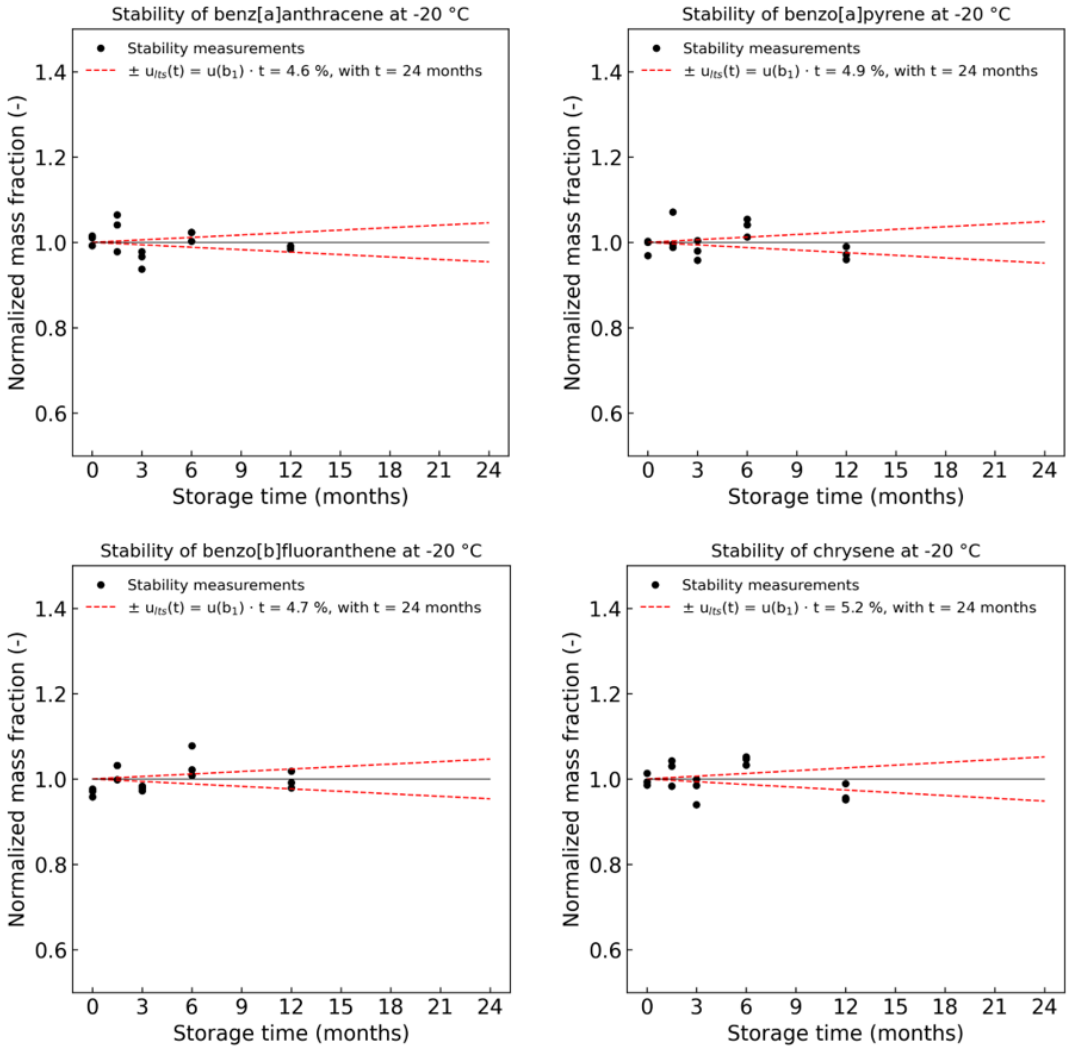


Figure 2: Long-term stability study of the four PAHs BaA, BaP, BbF and Chr with estimated uncertainty u_{lts} contributions.

The short-term stability was evaluated at a storage temperature of 45 °C. For the estimation of the uncertainty (u_{sts}) for potential instability when the material is exposed to higher temperatures during transportation a similar extrapolation model as for the long-term stability was applied. An exposure time of 2 weeks (t = 0.5 months) was used. The results are presented in table 5.

Table 5: Results of the short-term stability assessment at a storage temperature of 45 °C

PAH	**b_1 (months^{-1})**	**b_0 (-)**	**$s(b_1)$ (-)**	**t_{b1}**	**t_{crit}**	**$s(b_1=0)$ (-)**	**u_{sts} (%)**
BaA	-0.005666	1.008499	0.007300	0.776	2.364	0.007116	0.36
BaP	-0.000824	1.001237	0.006728	0.123	2.364	0.006300	0.32
BbF	0.003833	0.994251	0.005405	0.709	2.364	0.005234	0.27
Chr	0.008454	0.987319	0.005267	1.605	2.364	0.005762	0.29

INSTRUCTIONS AND SAMPLE DISTRIBUTION

Sample distribution

Each participant will receive one bottle containing approximately 30 g of powder. The sample will be shipped dry ice cooled and should be stored at -20 °C until analysis and not exposed to intense direct light and ultraviolet radiation. 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 samples should be equilibrated to room temperature before analysis. The sample preparation should be carried out immediately after opening the bottles.

Methods

The study will require solvent extraction, separation of the target analytes from interfering matrix components (clean-up), analytical separation, and selective detection of the target analytes in high protein matrix. Participants are anticipated to perform measurements by isotope-dilution (ID) gas chromatography mass spectrometry (GC-MS). However, other techniques such as liquid chromatography (LC) may be used.

RESULTS

Reporting of results

Each participant must send the results using the reporting excel sheet provided. The results should be sent via email to the study coordinator (gisela.umbricht@metas.ch) before the submission deadline. Submitted results are considered final and no corrections or adjustments of analytical data will be accepted unless approved by EURAMET. The results must include the mass fractions of the four PAHs in protein on an as-received basis and their associated standard and expanded (95 % level of confidence) uncertainties.

Participants will be requested to report a single estimate of the mass fractions in µg/kg for BaA, BaP, BbF and Chr in protein. The reported mass fractions will be the overall mean from replicate measurements. Reporting should include the values of the individual replicates in addition to the overall mean.

In addition to the quantitative results, participants will be instructed to describe their analytical methods and their approach to uncertainty estimation.

Evaluation of results

All results of the supplementary comparison will be evaluated against the supplementary comparison reference value (SCRV). The SCRV will be determined from the results of all NMIs/DIs participating in the supplementary comparison that have used appropriately validated methods with demonstrated metrological traceability.

Available Calibration Materials

Participants may establish the metrological traceability of their results using certified reference materials (CRMs) with stated traceability and/or commercially available high purity materials for which they determined the purity.

Solution CRMs including all target PAHs are available from NIST, SRM 2260a and SRM 1647f. Other solution and solid CRMs containing only one of the target PAHs are, to the best of our knowledge, available from the suppliers given in table 6.

Table 6: Certified reference materials available for use

CRM	Provider	Analyte
SRM 2260a	NIST	Aromatic hydrocarbons in toluene including all four target PAHS
SRM 1647f	NIST	Priority pollutant polycyclic aromatic hydrocarbons in acetonitrile including all four target PAHs
4213-a	NMIJ	Benzo(a)pyrene in 2,2,4-trimethylpentane
HRM-1017A	HSA	Benzo(a)pyrene
GBW08733	NIM	Benz(a)anthracene in acetonitrile
GBW08734	NIM	Benzo(a)pyrene in acetonitrile
GBW08728	NIM	Benzo(b)fluoranthene in acetonitrile

Isotopically labeled (deuterium or carbon-13) PAHs for use as internal standards are commercially available from various sources.

USE OF THE SUPPLEMENTARY COMPARISON EURAMET.QM-S15 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in this supplementary comparison will demonstrate the measurement capabilities in determining mass fractions of low polarity organic compounds with molecular masses of about 150 - 500 g/mol in the range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering

Core Competency Statements and CMC support

The Core Competencies template that will be used to claim competencies by the participants in this study is given in Appendix B.

REFERENCES

[1] ISO Guide 35:2017, Reference materials - Guidance for characterization and assessment of homogeneity and stability, International Organization (ISO), Geneva, 2017.

[2] Linsinger et al., Homogeneity and stability of reference materials, Accred. Qual. Assur., 2001, 6, 20.

[3] Linsinger et al., Estimating the uncertainty of stability for matrix CRMs, Fresenius J. Anal. Chem., 2001, 370, 183.

APPENDIX D: Registration Form

Interested NMIs registered directly by e-mail. No registration form was distributed.

APPENDIX E: Reporting Form

"Participant_Information" worksheet

Reporting Form for Supplementary Comparison EURAMET.QM-S15, PAHs in Protein Matrix

Participiant Information

Please complete all pages of the reporting form and submit it by e-mail to:
gisela.umbricht@metas.ch

Reporting date (YYYY-MM-DD)	
Institute and address	
Submitted by (name)	
E-mail address	
Analyst(s)	
Bottle No.	

"Results" worksheet

Reporting Form for Supplementary Comparison EURAMET.QM-S15, PAHs in Protein Matrix

Summary of Results

Analyte	Abbreviation	Mass fraction w (µg/kg)	Combined standard uncertainty u_c (µg/kg)	Coverage factor k	Expanded uncertainty U (µg/kg)	Number of replicates n
Benz[a]anthracene	BaA					
Benzo[a]pyrene	BaP					
Benzo[b]fluoranthene	BbF					
Chrysene	Chr					

Results of each replicate

Replicate n	Mass fraction w of PAH (µg/kg)			
	BaA	BaP	BbF	Chr
1				
2				
3				
5				
6				
7				
8				
9				
Mean				
Standard deviation				

Additional comments	

"Analytical_Information" worksheet

Reporting Form for Supplementary Comparison EURAMET.QM-S15, PAHs in Protein Matrix

Information about the analytical procedure

Sample amount used for analysis (g)	
Sample pre-treatment (if applicable)	
Extraction method/conditions Briefly describe the extraction procedure (e.g. Soxhlet, ASE, Saponification, …) and the conditions (solvents, volume, time, temperature, …)	
Clean-up procedure Briefly describe the post extraction clean-up procedure (e.g. SPE, GPC, …)	
Analytical instrumentation used Specify the analytical instrument(s) you used (e.g. GC-MS/MS, LC-FLD, …) including the model (manufacturer, number, …). Please also specify the type of injector you used in case of GC-MS/MS.	
Chromatographic (pre)column(s) Specify the chromatographic pre and main columns (e.g. type, dimensions, manufacturer, …)	
Chromatographic conditions Describe the chromatographic conditions (e.g. mobile phase gradient for LC, temperature program for GC)	
Method of quantification Describe the method of quantification you used (e.g. external calibration, internal calibration, IDMS, ...)	

Calibration type Describe the type of calibration you used (e.g. single-point, bracketing, multi-point, matrix-matched, …)	
Calibration standards used Specify the standards you used for calibration (e.g. source, certified value, uncertainty, traceability, …)	
Internal standards used (if applicable) Specify the internal standards you used (e.g. compunds, source, ...)	
At which stage of the analytical process were the internal standards added? (if applicalbe)	
Purity assessment of calibrant (if applicable) If you determined the purity yourself, please describe the purity assessment (e.g. confirmation of identity, value and uncertainty assignment, analytical methods used, ...)	
MS method used Specify the MS method you used for ion detection (SIM, MRM/SRM, …)	
Ions monitored in MS Specify the ions monitored for native (calibrant) and isotopically labelled compounds (e.g. precursor and detection ions in MRM/SRM mode, collision energy, …)	
Detection methods other than MS (if applicable) Please specify your measurement procedure if you did not use an MS detector (e.g. fluorecence detection FLD).	
Additional comments and observations Please give additonal comments concerning the analytical procedure and share any special observations you made when analyzing the material.	

"Uncertainty_Budget" worksheet

Reporting Form for Supplementary Comparison EURAMET.QM-S15, PAHs in Protein Matrix

Information about the uncertainty budget

Give the complete equation(s) for the calculation of the mass fractions of each of the PAHs

Please provide details of all input quantities listed in the equation(s) and indicate how they were determined. Please give a complete description of how the estimates were obtained and combined to the overall uncertainty. The table below can be used to provide the uncertainty budget.

Benz[a]anthracene (BaA)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)

w(BaA)	Mass fraction of BaA		**µg/kg**	
u_c[w(BaA)]	Combined standard uncertainty		**µg/kg**	
k	Coverage factor			
u[w(BaA)]	Expanded uncertainty		**µg/kg**	

Benzo[a]pyrene (BaP)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)

w(BaP)	Mass fraction of BaP		**µg/kg**	
u_c[w(BaP)]	Combined standard uncertainty		**µg/kg**	
k	Coverage factor			
u[w(BaP)]	Expanded uncertainty		**µg/kg**	

Benzo[b]fluoranthene (BbF)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)

w(BbF)	Mass fraction of BbF		**µg/kg**	
u_c[w(BbF)]	Combined standard uncertainty		**µg/kg**	
k	Coverage factor			
u[w(BbF)]	Expanded uncertainty		**µg/kg**	

Chrysene (Chr)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)

w(Chr)	Mass fraction of Chr		**µg/kg**
u_c[w(Chr)]	Combined standard uncertainty		**µg/kg**
k	Coverage factor		
u[w(Chr)]	Expanded uncertainty		**µg/kg**

APPENDIX F: Core Competency Tables

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

EURAMET.QM-S15	***NMI/DI***	**PAHs in Protein Matrix**
Scope of Measurement: Participation in this supplementary comparison would provide the opportunity to demonstrate participant's capabilities in determining low polarity (pKow < -2) organic compounds with molecular masses of about 150 – 500 g/mol in the mass fraction range from 0.1 µg/kg to 100 µg/kg in high protein matrix. Beside value assignment, the demonstration of measurement capabilities may include extraction of target analytes from the matrix, separation of target analytes from interfering matrix components (clean-up), chromatographic separation and quantification of target analytes.		
Competency	**✓, ×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		*Indicate if you used a "pure material" or a calibration solution. Indicate its source and ID, eg CRM identifier*
Identity verification of analyte(s) in calibration material. #		*Indicate method(s) you used to identify analyte(s)*
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s). #		*Indicate how you established analyte mass fraction/purity (i.e., mass balance (list techniques used), qNMR, other)*
For calibrants which are a calibration solution: Value-assignment method(s). #		*Indicate how you established analyte mass fraction in calibration solution*
Sample Analysis Competencies		
Identification of analyte(s) in sample		*Indicate method(s) you used to identify analyte(s) in the sample (i.e., Retention time, mass spec ion ratios, other)*
Extraction of analyte(s) of interest from matrix		*Indicate extraction technique(s) used, if any, (i.e. Liquid/liquid, Soxhlet, ASE, other)*
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)		*Indicate cleanup technique(s) used, if any (i.e., SPE, LC fractionation, other)*
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)		*Indicate chemical transformation method(s), if any, (i.e., hydrolysis, derivatization, other)*
Analytical system		*Indicate analytical system (i.e., LC-MS/MS, GC-HRMS, GC-ECD, other)*
Calibration approach for value-assignment of analyte(s) in matrix		*a) Indicate quantification mode used (i.e., IDMS, internal standard, external standard, other)* *b) Indicate calibration mode used (i.e., single-point calibration, bracketing, x-point calibration curve, other)*
Verification method(s) for value-assignment of analyte(s) in sample (if used)		*Indicate any confirmative method(s) used, if any.*
Other		*Indicate any other competencies demonstrated.*

Instructions:

- In the middle column place a tick, cross or say the entry is not applicable for each of the competencies listed (the first row does not require a response)
- Fill in the right hand column with the information requested in blue in each row
- Enter the details of the calibrant in the top row, then for materials which would not meet the CIPM traceability requirements the three rows with a # require entries.

APPENDIX G: Summary of Participants' Analytical Information

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

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

Certain commercial equipment, instruments, or materials are identified in these Tables to specify adequately experimental conditions or reported results. Such identification does not imply recommendation or endorsement by the participants in this Supplementary Comparison, nor does it imply that the equipment, instruments, or materials identified are necessarily the best available for the purpose.

Table G-1: Summary of sample size, extraction, pre-treatment and cleanup for EURAMET.QM-S15

NMI/DI	Sample size (g)	Pre-treatment	Extraction method	Clean-up
BVL	1.5	None	HUPsSE (Heat-Ultrasonic-Pressure supported Solvent Extraction), 3 separate extraction cycles with 6mL MeOH/TBME (1:1) each, extraction time 15 min. at 70°C ultrasonic bath, cooling in ice bath, centrifugation for 6min at 4000rpm at 4°C, combining extract of 3 cycles, evaporation of the extract under N_2-stream at 40°C	Two steps of SPE-Clean-Up 1: CHROMABOND HR-X cartridge (85µm, 6mL, 500mg) condition with isohexane, wash with isohexane and MeOH and isohexane, elute with dichloromethane, evaporation of the extract under N_2-stream at 40°C 2: ISOLUTE SI (6mL, 1g) condition with cyclohexane, elute with cyclohexane, evaporation of the extract under N_2-stream at 50°C, resolve in fluorochrysene standard solution (surrogate standard)
INRIM	1.03 ± 0.03	The sample was maintained at room temperature (23.4 °C) with RH(%) = 40) for one hour before the analysis	1 g of sample was weighed and then put in a 50 mL centrifuge tube, after 5 mL of water and 10mL of acetonitrile were added. The extraction was performed through a QuEChERS extraction pouch (4g MgSO4 + 1g NaCl), the sample was then acidified with 0.15 mL of formic acid. The tube was vigorously shaken with a vortex for five minutes.	The extracted sample was then centrifuged for 10 min at 4°C at 4500 rpm. 1 mL of supernatant was transferred in a 2 mL microcentrifuge tube and brought to dryness. At the end the sample was reconstituted with 0.1 mL of isopropanol.
IW	5	None	QuEChERs extraction; 10 mL acetonitrile, add buffer salt mixture and immediately shake vigorously	SPE

NMI/DI	Sample size (g)	Pre-treatment	Extraction method	Clean-up
LAB-IZO	1.5	N/A	Extraction with hexane: internal standard benzo(b)chrysene (5 ng in 50 µL) was added to 1.5 g of whey. 10 mL of hexane was added. The mixture was put to vortex for 2 minutes and then to ultrasound bath for 15 min. It was centrifuged (15 min at 5000 rpm). The hexane fraction was collected. The whey was extracted two more times in the same way. All three hexane fractions were collected together and dried under gentle steam of nitrogen. The residue was dissolved in 1 mL of hexane.	SPE cartridge (SUPELCLEAN LC-SI, 1 g, 6 mL, cat. no. 57051) was cleaned with dichloromethane, vacuum dried and washed with hexane. The extract was loaded and eluted with hexane/dichloromethane (70:30 v/v). The first 1 mL of eluate was discarded, next 7 mL of eluate were collected and dried under a nitrogen stream. The residue was dissolved in 1 mL of acetonitrile and injected into the HPLC apparatus.
METAS	3	N/A	ASE system EDGE (CEM Corporation). Extraction cycles: 2. Volume of extraction solvent per cycle: 30 mL. Extraction solvent: methanol : tert-butyl methyl ether (1/1, v/v). Temperature: 120 °C. Extraction time per cycle: 3 min.	Two step SPE clean-up: 1) Supelclean EZ-POP (Supelco 54341-U) 2) Affinimip SPE PAHs (Affinisep FS119-03-NG)
TUBITAK UME	2	2.0 g sample weighed in weighing paper. Transfered to PSE (Pressurized Solvent Extraction) thimble. 100 µL IS spiked.	PSE (Applied Separations) extraction by n-hexane at 100 °C, 120 bar, 2 cycle, 10 min. static time, 3 min nitrogen purge.	Extract evaporated under gentle nitrogen stream to 1 mL at 45 °C. LC-NH_2 500 mg 6 cc SPE cartridge conditioned with 6 mL n-hexane. Sample added to cartridge and eluted with 10 mL hexane. Eluate evaporated under gentle nitrogen stream at 45 °C till 500 uL, 1 ml ethyl acetate added and evaporated till 500 uL, then 1 mL acetonitrile added and evaporated till 200 uL left.

Table G-2: Summary of analytical techniques for EURAMET.QM-S15

NMI/DI	Analytical technique	Chromatographic column	Chromatographic and mass spectrometry conditions	MS mode/ion monitored FLD parameters
BVL	GC-HRMS (Thermo Fisher Scientific TriPlus RSH + PTV + Trace 1300 + DFS)	Precolumn: Restek Rxi Guard (5 m x 0,25 mm desactivated) Column: Agilent J&W Select PAH (30 m x 0,25 mm x 0,15 µm film thickness)	**GC** He (99.999%), column flow 2.0mL/min (constant flow), injection pulsed splitless (280kPa 0.5min), PTV temperature program: 95°C, heating rate 12°C / s, 350 °C (3 min), 1.2 min splitless, Injection volume 1µL, temperature program GC: 70°C (0.7min), 85 °C/min, 180 °C (0 min), 3,0 °C/min, 230 °C (7 min), 28 °C/min, 280 °C (20 min), 14 °C/min, 350 °C (8 min) **MS** GC-MS interface temperature: 325°C, ion source temperature: 260°C	SIM mode BaA (228,09335) BaA-$^{13}C_6$ (234,1142) Chr (228,0933) Chr-d_{12}(240,1687) BbF (252,0933) BbF-d_{12} (264,1687) BaP (252,0933) BaP-$^{13}C_4$ (256,1075)
INRIM	HPLC-FLD (Jasco) AS-4050 PU-4180 CO-4061 FP-4025	Kinetex 3.5µm PAH 100x4.6 mm Phenomenex	**LC** A: water, B: acetonitrile, C: isopropanol 2 min (60% A - 40% B) 15 min (100% B) 25min (100% B) 26 min (50% B - 50% C) 36min (50% B - 50% C) 37min (60% A - 40% B) 50min (60% A - 40% B)	Fluorescence detection FLD Excitation wavelength: 270 nm Emission wavelength: 404 nm

NMI/DI	Analytical technique	Chromatographic column	Chromatographic and mass spectrometry conditions	MS mode/ion monitored FLD parameters
IW	HPLC DAD/FLD	PAH column C18, Supelco, 5 cm	**LC** mobile phase: H_2O:CH_3CN; gradient: 0.01 min CH_3CN - 60%; 0.01 min flow-1; 0.50 min CH_3CN - 60%; 19.00 min CH_3CN - 100%; 19.01 min CH3CN - 60%; 22.00 min Stop	
LAB-IZO	HPLC (Agilent 1100, equipped with degasser, binary pump, thermostated injection and thermostated column department, FLD detector)	Precolumn: C18 4 x 3.0mm ID; Phenomenex, part no. AJ0-4287 Column: Phenomenex Kinetex PAH 3.5 µm 150 × 4.6 mm (P/N 00F-4764-E0; S/N H17-339823)	**LC** Flow: 1.2 mL/min Gradient: A-water; B-ACN 0 min – 50% B – 8 min – 100% B – 17.5 min – 100% B – 17.75 min – 50% B; eqiul. time: 5 min	FLD
METAS	GC-MS/MS (Thermo Scientific Trace 1310 and TSQ 8000 Evo). Injector: PTV.	Pre-column: Phenomenex Zebron Z-Guard, 5 m x 0.18 mm, deactivated fused silica (7AD-G000-00-GZ0) Column: Phenomenex ZB-PAH-EU, 20 m x 0.18 mm, 0.14 µm (7FD-G043-47)	**GC** GC temperature program: 60 °C (1 min.) --> (12 °C/min.) 210 °C --> (8 °C/min.) 280 °C (9.75 min.) --> (8 °C/min.) 320 °C (2 min.) **MS** Transfer line: 300 °C Source: 300 °C	SRM mode PAH: m/z precursor → m/z fragment (coll. energy (eV)) BaA: 228.1 → 226.1 (30) BaA-d_{12}: 240.2 → 236.1 (30) Chr: 228.1 → 226.1 (30) Chr-d_{12}: 240.2 → 236.1 (30) BbF: 252.1 → 250.1 (30) BbF-d_{12}: 264.2 → 260.1 (30) BaP: 252.1 → 250.1 (30) BaP-d_{12}: 264.2 → 260.2 (30)

NMI/DI	Analytical technique	Chromatographic column	Chromatographic and mass spectrometry conditions	MS mode/ion monitored FLD parameters
TUBITAK UME	GC-MS/MS (Thermo TSQ Quantum XLS)	Varian VF 17MS 60 m x 0.25 mm 0.25 um	**GC** Rate (°C/min) / Temp. (°C) / Hold (min) — / 70 / 1.70 20.0 / 180 / 0.00 8.0 / 280 / 5.00 4.0 / 320 / 20.00 **MS** Source temp.: 250 °C **Injection** PTV Large Volume Injection: 3 uL	GC-MS/MS using electron Impact (EI) in MRM mode. BaA & Chr: 228 → 226 CE: 35 BaA-d_{12} & Chr-d_{12}: 240 → 236 CE: 35 BbF & BaP: 252 → 250 CE: 38 BbF-d_{12} & BaP-d_{12}: 264 → 260 CE:38

Table G-3: Summary of calibrants and standards for EURAMET.QM-S15

NMI/DI	Method of quantification	Type of calibration	Calibrants	Internal standards (ISTD)	Addition of ISTD
BVL	External standard calibration with internal standard	Multi-point	NIST Standard Reference Material 1647f (expanded uncertainty k=2): BaA: (5.16 ± 0,07) µg/g Chr: (4.67 ± 0,08) µg/g BbF: (5.29 ± 0,06) µg/g BaP: (6.22 ± 0,11) µg/g	BaA-$^{13}C_6$ Chr-d_{12} BbF-d_{12} BaP-$^{13}C_4$	Before extraction
INRIM	External calibration	6 multi-points concentrations in solution	Sigma-Aldrich RTC - CRM47940 Lot LRAD2034 Traceable to the SI and higher order standard from NIST through an unbroken chain of comparison. BaA: (9.99 ± 0.12) µg/mL BaP: (10.00 ± 0.11) µg/mL BbF: (9.99 ± 0.12) µg/mL Chr: (10.00 ± 0.14) µg/mL	Benzo(b)chrysene Producer: LGC Grade: CRM Concentration: 10.00 µg/mL Expanded uncertainty: 0.31 µg/mL	The internal standard was added after the weighing
IW	External calibration	Multi-point calibration	Certified value (CRM PAH mix 9 100 ng/µL in CH_3CN Lot G1126284AL, Dr Ehrenstorfer GmbH)		
LAB-IZO	External calibration	Four-point calibration curve	NIST SRM 1647f Certified values and expanded uncertainties (coverage factor of 2): BaA: (4.02 ± 0.07) mg/L Chr: (3.64 ± 0.07) mg/L BbF: (4.12 ± 0.06) mg/L BaP: (4.48 ± 0.10) mg/L	Benzo(b)chrysene - used only for recovery correction; added to sample before extraction	Before extraction

NMI/DI	Method of quantification	Type of calibration	Calibrants	Internal standards (ISTD)	Addition of ISTD
METAS	Linear regression IDMS	6-point calibration	NIST SRM 1647f, priority pollutant PAHs in acetonitrile (PAH: w ± U, with k = 2) BaA: (5.16 ± 0.07) mg/kg BaP: (6.22 ± 0.11) mg/kg BbF: (5.29 ± 0.06) mg/kg Chr: (4.67 ± 0.08) mg/kg	Deuterated IS All-in-one 16 EPA Priority PAHs mix (Chiron AS, Trondheim, Norway, S-4513-K-T) in toluene (1000 µg/mL) BaA-d_{12} BaP-d_{12} BbF-d_{12} Chr-d_{12}	Before extraction
TUBITAK UME	IDMS	Multi-point (8 point)	Calibration stock solutions prepared by gravimetric dilution of NIST 1647f. BaA: (5.16 ± 0.07) mg/kg Chr: (4.67 ± 0.08) mg/kg BbF: (5.29 ± 0.06) mg/kg BaP: (6.22 ± 0.11) mg/kg	Internal standard stocks gravimetrically prepared by dissolving solids with acetonitrile. IS materials supplied from Cambridge Isotope Laboratories: BaA-d_{12} DLM-258-0.05 Lot# 18301 Chr-d_{12} DLM-261-0.1 Lot# 24875 BbF-d_{12} DLM-2136-0.01 Lot# 26381 BaP-d_{12} DLM-258-0.05 Lot# 26635	At the beginning, directly on whey protein in PSE thimble.

Table F-G Assessment and verification methods for EURAMET.QM-S15

NMI/DI	Purity Assessment	Result Verification
BVL	N/A	N/A
INRIM	N/A	N/A
IW	N/A	N/A
LAB-IZO	N/A	N/A
METAS	N/A	N/A
TUBITAK UME	NIST 1647F used for calibration	N/A

Table G-5: Additional comments for EURAMET.QM-S15

NMI/DI	Additional Comments
BVL	N/A
INRIM	N/A
IW	N/A
LAB-IZO	N/A
METAS	N/A
TUBITAK UME	N/A

APPENDIX H: Summary of Participants' Uncertainty Estimation Approaches

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

Uncertainty Information from BVL

One factor analysis of variance (ANOVA) with Excel-Output

--> determine the mean squares of the difference between the groups (separate analysis days, number N, $MS_{between}$) and the difference within the groups (replicates per day, number n, MS_{within})

--> u_{ip}

absolute standard deviation of intermediate precision

$$s_{ip} = \sqrt{\frac{MS_{between} - MS_{within}}{n}}$$

absolute standard uncertainty of intermediate precision

$$u_{ip} = \frac{s_{ip}}{\sqrt{N}}$$

--> u_r

absolute standard deviation of repeatability

$$s_r = \sqrt{MS_{within}}$$

absolute standard uncertainty of repeatability

$$u_r = \frac{s_r}{\sqrt{n \cdot N}}$$

-> both converted to relative uncertainties (in %)

--> $u_{standard}$

-> expanded uncertainty (k=2, value in µg/g) from certificate of analysis converted to relative uncertainty (in % and unexpanded value)

--> $u_{balance}$

-> maximum expanded uncertainty (k=2) calculated from the smallest weight (R=28,76mg) in the preparation of the calibration standard solutions (equation from calibration certificate)

$u_{balance}$ = 3,39 * 10^-5 + 2,83 * 10^-6 * R

-> converted to relative uncertainty (in %) and to unexpanded value

--> u_{bias}

$$u_{bias} = \sqrt{bias^2 + \frac{u_{rec}{}^2}{n} + u_{Cref}{}^2}$$

-> relative bias of own result to assigned value of last PT (bias)

-> u_{Cref} = 1,25 * $s_R/\sqrt{p}$ (u_{Cref} = uncertainty of PT material, s_R = robust reproducibility standard deviation calculated according to Algorithm A, p = number of results in the PT)

-> u_{rec} = standard deviation of repeated measurements of the PT material

--> u_c (combined) calculated via error propagation

--> U expanded uncertainty calculated with coverage factor k=2

Benz[a]anthracene (BaA)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{ip}	intermediate precision		0.25	%	A
u_r	repeatability		0.25	%	A
$u_{standard}$	calibration standard		0.68	%	B
$u_{balance}$	balance		0.06	%	B
u_{bias}	sum of method and laboratory bias		5.99	%	B

w(BaA)	Mass fraction of BaA	3.20	µg/kg
u_c[w(BaA)]	Combined standard uncertainty	0.19	µg/kg
k	Coverage factor	2.00	
U[w(BaA)]	Expanded uncertainty	0.39	µg/kg

Benzo[a]pyrene (BaP)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{ip}	intermediate precision		0.26	%	A
u_r	repeatability		0.22	%	A
$u_{standard}$	calibration standard		0.88	%	B
$u_{balance}$	balance		0.06	%	B
u_{bias}	sum of method and laboratory bias		4.18	%	B

w(BaP)	Mass fraction of BaP	4.20	µg/kg
u_c[w(BaP)]	Combined standard uncertainty	0.18	µg/kg
k	Coverage factor	2.00	
U[w(BaP)]	Expanded uncertainty	0.36	µg/kg

Benzo[b]fluoranthene (BbF)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{ip}	intermediate precision		0.46	%	A
u_r	repeatability		0.32	%	A
$u_{standard}$	calibration standard		0.57	%	B
$u_{balance}$	balance		0.06	%	B
u_{bias}	sum of method and laboratory bias		6.97	%	B

w(BbF)	Mass fraction of BbF	4.60	µg/kg
u_c[w(BbF)]	Combined standard uncertainty	0.32	µg/kg
k	Coverage factor	2.00	
U[w(BbF)]	Expanded uncertainty	0.65	µg/kg

Chrysene (Chr)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{ip}	intermediate precision		0.84	%	A
u_r	repeatability		0.16	%	A
$u_{standard}$	calibration standard		0.86	%	B
$u_{balance}$	balance		0.06	%	B
u_{bias}	sum of method and laboratory bias		4.95	%	B

w(Chr)	Mass fraction of Chr	2.92	µg/kg
u_c[w(Chr)]	Combined standard uncertainty	0.15	µg/kg
k	Coverage factor	2.00	
U[w(Chr)]	Expanded uncertainty	0.30	µg/kg

Uncertainty Information from INRIM

$$C_{PAHs} = d_f \times C_{calc}$$

$$d_f = \frac{w_{(sample)}}{V_{(extraction)}} \times \frac{V_{(supernatant)}}{V_{(reconstitution)}}$$

$$C_{calc} = \frac{Analyte\ peak\ area\ /\ ISTD\ peak\ area}{m_{(external\ calibration\ curve\ slope)}}$$

C_{PAHs} = mass fraction of single PAH

D_f = diluition factor calculated
Calculated as
[Weight of sample/Volume of extraction solution (10 mL)]*[Volume of sample solution picked up (1mL)/ reconstitution volume 0.10 mL]

C_c= Concentration calculated
(Area under the peak of the analyte/Area under the peak of internal standard)/slope of external calibration curves

Overall uncertainty:

- Weight of sample = X ± 0.08 mg
- Volume of extraction solution = X ± 0.13%
- Volume of sample solution picked up = X ± 0.10% -
Reconstitution volume = X ± 0.23%

- Slope of external calibration curves = The calibration curve was calculated by weighted and weighted total least-squares method.
To buil up the calibration curve was necessary to calculate the uncertainties of concentration for the six differnt points:
CRM standard uncertainties combined with the uncertainties of dilution performed by micropipettes (see above) and volumetric flask.
- Average uncertainties of CRM 0.13 µg/mL
- Volumetric flask = 0.07 mL

For the instrumental signals:
Propagation of errors for the ratio between the average of the analyte's area and the average IST area

Benz[a]anthracene (BaA)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
$w_{(sample)}$	weightening on analitical balance	1	0.00008	g	B
$V_{(extraction)}$	dilution by micropipette	10	0.013	mL	B
$V_{(supernatant)}$	aliqutation by micropipette	1	0.001	mL	B
$V_{(reconstitution)}$	reconstitution by micropipette	0.1	0.00026	mL	B
(A_{sample}/A_{ISTD})	instrumental response	0.1	0.001	Arbitrary Unit	B
Purity of CRMs	analysis certificate	9.99	0.12	µg/mL	B
$C_{(stock\ solution)}$	Flask volumetric dilution	100	1.22	ng/mL	B
$C_{(calibration\ point)}$	dilution by micropipette	10	0.14	ng/mL	B

w(BaA)	Mass fraction of BaA	3.63	**µg/kg**
u_c[w(BaA)]	Combined standard uncertainty	0.21	**µg/kg**
k	Coverage factor	2	
u[w(BaA)]	Expanded uncertainty	0.42	**µg/kg**

Benzo[a]pyrene (BaP)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
$w_{(sample)}$	weightening on analitical balance	1	0.00008	g	B
$V_{(extraction)}$	dilution by micropipette	10	0.013	mL	B
$V_{(supernatant)}$	aliqutation by micropipette	1	0.001	mL	B
$V_{(reconstitution)}$	reconstitution by micropipette	0.1	0.00026	mL	B
(A_{sample}/A_{ISTD})	instrumental response	0.1	0.001	Arbitrary Unit	B
Purity of CRMs	analysis certificate	10.00	0.11	µg/mL	B
$C_{(stock solution)}$	Flask volumetric dilution	100	1.13	ng/mL	B
$C_{(calibration point)}$	dilution by micropipette	10	0.11	ng/mL	B

w(BaP)	Mass fraction of BaP	5.27	**µg/kg**
u_c[w(BaP)]	Combined standard uncertainty	0.21	**µg/kg**
k	Coverage factor	2	
u[w(BaP)]	Expanded uncertainty	0.42	**µg/kg**

Benzo[b]fluoranthene (BbF)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
$w_{(sample)}$	weightening on analitical balance	1	0.00008	g	B
$V_{(extraction)}$	dilution by micropipette	10	0.013	mL	B
$V_{(supernatant)}$	aliqutation by micropipette	1	0.001	mL	B
$V_{(reconstitution)}$	reconstitution by micropipette	0.1	0.00026	mL	B
(A_{sample}/A_{ISTD})	instrumental response	0.1	0.001	Arbitrary Unit	B
Purity of CRMs	analysis certificate	9.99	0.12	µg/mL	B
$C_{(stock solution)}$	Flask volumetric dilution	100	1.23	ng/mL	B
$C_{(calibration point)}$	dilution by micropipette	10	0.12	ng/mL	B

w(BbF)	Mass fraction of BbF	5.19	**µg/kg**
u_c[w(BbF)]	Combined standard uncertainty	0.16	**µg/kg**
k	Coverage factor	2	
u[w(BbF)]	Expanded uncertainty	0.32	**µg/kg**

Chrysene (Chr)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
$w_{(sample)}$	weightening on analitical balance	1	0.00008	g	B
$V_{(extraction)}$	dilution by micropipette	10	0.013	mL	B
$V_{(supernatant)}$	aliqutation by micropipette	1	0.001	mL	B
$V_{(reconstitution)}$	reconstitution by micropipette	0.1	0.00026	mL	B
(A_{sample}/A_{ISTD})	instrumental response	0.1	0.001	Arbitrary Unit	B
Purity of CRMs	analysis certificate	10.00	0.14	µg/mL	B
$C_{(stock solution)}$	Flask volumetric dilution	100	1.42	ng/mL	B
$C_{(calibration point)}$	dilution by micropipette	10	0.14	ng/mL	B

w(Chr)	Mass fraction of Chr	3.16	**µg/kg**
u_c[w(Chr)]	Combined standard uncertainty	0.20	**µg/kg**
k	Coverage factor	2	
u[w(Chr)]	Expanded uncertainty	0.40	**µg/kg**

Uncertainty Information from IW

When calculating the measurement uncertainty, contributions from the purity of the standard, calibration curve, reproducibility and contribution from sample preparation were taken into account. For reproducibility, the standard solution of a certain concentration was analyzed 10 times and the standard deviation was calculated. The contribution from the sample preparation was obtained by soldering the sample with a certain standard concentration and calculating the recovery. $c=c_0 \times u_{std} \times u_{c0} \times u_R R$

where is: c_0 - the uncertainty of the calibration curve; u_{std}- the uncertainty from the purity of the standard; u_R - uncertainty from reproducibility; R - recovery factor (from sample preparation)

Benz[a]anthracene (BaA)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	the uncertainty of the calibration curve				
	the uncertainty from the purity of the standard				
	uncertainty from reproducibility				
	recovery factor				

w(BaA)	Mass fraction of BaA	**5.333**	**µg/kg**
u_c[w(BaA)]	Combined standard uncertainty	**0.575**	**µg/kg**
k	Coverage factor	**2**	
u[w(BaA)]	Expanded uncertainty	**1.449**	**µg/kg**

Benzo[a]pyrene (BaP)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	the uncertainty of the calibration curve				
	the uncertainty from the purity of the standard				
	uncertainty from reproducibility				
	recovery factor				

w(BaP)	Mass fraction of BaP	**13.333**	**µg/kg**
u_c[w(BaP)]	Combined standard uncertainty	**1.438**	**µg/kg**
k	Coverage factor	**2**	
u[w(BaP)]	Expanded uncertainty	**2.875**	**µg/kg**

Benzo[b]fluoranthene (BbF)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	the uncertainty of the calibration curve				
	the uncertainty from the purity of the standard				
	uncertainty from reproducibility				
	recovery factor				

w(BbF)	Mass fraction of BbF	**7.000**	**µg/kg**
u_c[w(BbF)]	Combined standard uncertainty	**0.755**	**µg/kg**
k	Coverage factor	**2**	
u[w(BbF)]	Expanded uncertainty	**1.51**	**µg/kg**

Chrysene (Chr)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	the uncertainty of the calibration curve				
	the uncertainty from the purity of the standard				
	uncertainty from reproducibility				
	recovery factor				

w(Chr)	Mass fraction of Chr	**5.000**	**µg/kg**
u_c[w(Chr)]	Combined standard uncertainty	**0.539**	**µg/kg**
k	Coverage factor	**2**	
u[w(Chr)]	Expanded uncertainty	**1.078**	**µg/kg**

Uncertainty Information from LAB-IZO

Four point linear calibration curve was constructed based on three replicates for each point.

Mass fraction PAH(µg/kg)=(c_{HPLC}×V_s×Rec)/(m_s)
Where:
X_{HPLC} is the amount of HPLC-FLD determined PAH according to 4-point calibration using the linear formula
X_{HPLC} (ng) = (y-b)/m
where y = peak area in arbitrary units
b = -0.0469726 (for BaA) or 0.00391594 ((Chr) or -0.133758 (BbF) or -0.189107 (BaP)
m = 2,437812(for BaA) or 1,85875 (Chr) or 1,85875 (BbF) or 4.09402 (BaP)
r^2 = 0.99973 (for BaA) or 0.99973 (Chr) or 0.99904 (BbF) or 0.99981 (BaP)
V_s is volume of extraxt solution, before injection in HPLC (1 mL)
Rec is recovery (amount of IS added to the sample prior to the extraction (ng))/(amount of IS found (ng))
m_s is mass of sample (g)

$u_c^2 = (u_{rep}^2 + u_{pur}^2 + u_{cal}^2)$
$U = k \times X \times u_C$
U = expanded uncertainty, k = coverage factor (2)
Xs = mean value of PAH in whey
u_C = combined relative uncertainty
u_{rep} = relative standard uncertiuiny of measurement: SD_{mean}/Xs
u_{pur} = relative standard uncertainty of purity of PAH, given in calibrant certificate
u_{cal} = relative standard uncertainty of calibration according to EURACHEM CITAC GUIDE

Benz[a]anthracene (BaA)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{rep}	standard deviation of the mean	0.619	0.042	µg/kg	A
u_{pur}	purity of calibrant	4.02	0.035	mg/L	B
u_{cal}	uncertainty of calibration acc. to EURACHEM CITAC Guide		0.0346	µg/kg	A

w(BaA)	Mass fraction of BaA	0.619	µg/kg
u_c[w(BaA)]	Combined standard uncertainty	0.055	µg/kg
k	Coverage factor	2	
u[w(BaA)]	Expanded uncertainty	0.109	µg/kg

Benzo[a]pyrene (BaP)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{rep}	standard deviation of the mean	0.568	0.037	µg/kg	A
u_{pur}	purity of calibrant	4.84	0.05	mg/L	B
u_{cal}	uncertainty of calibration acc. to EURACHEM CITAC Guide		0.0350	µg/kg	A

w(BaP)	Mass fraction of BaP	0.568	µg/kg
u_c[w(BaP)]	Combined standard uncertainty	0.051	µg/kg
k	Coverage factor	2	
u[w(BaP)]	Expanded uncertainty	0.102	µg/kg

Benzo[b]fluoranthene (BbF)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{rep}	standard deviation of the mean	0.600	0.015	µg/kg	A
u_{pur}	purity of calibrant	4.12	0.03	mg/L	B
u_{cal}	uncertainty of calibration acc. to EURACHEM CITAC Guide		0.0665	µg/kg	A

w(BbF)	Mass fraction of BbF	**0.600**	**µg/kg**
u_c[w(BbF)]	Combined standard uncertainty	**0.068**	**µg/kg**
k	Coverage factor	**2**	
u[w(BbF)]	Expanded uncertainty	**0.137**	**µg/kg**

Chrysene (Chr)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
u_{rep}	standard deviation of the mean	0.942	0.081	µg/kg	A
u_{pur}	purity of calibrant	3.64	0.035	mg/L	B
u_{cal}	uncertainty of calibration acc. to EURACHEM CITAC Guide		0.0300	µg/kg	A

w(Chr)	Mass fraction of Chr	**0.942**	**µg/kg**
u_c[w(Chr)]	Combined standard uncertainty	**0.087**	**µg/kg**
k	Coverage factor	**2**	
u[w(Chr)]	Expanded uncertainty	**0.174**	**µg/kg**

Uncertainty Information from METAS

Equations for linear regression IDMS (6-point calibration):

$$R_{bz,h} = b_1 \cdot \frac{w_{yz,h}(PAH)}{w_{yz,h}(y)} + b_0$$

$$w_{stock}(PAH) = w_{ref}(PAH) \cdot \frac{m_{ref}}{m_{toluene,stock} + m_{ref}}$$

$$w_{z,h}(PAH) = w_{stock}(PAH) \cdot \frac{m_{stock,h}}{m_{toluene,z,h} + m_{stock,h}}$$

$$w_{yz,h}(PAH) = w_{z,h}(PAH) \cdot \frac{m_{z,h}}{m_{z,h} + m_{yz,h} + m_{z,FBkF,h}}$$

$$w_{yz,h}(y) = \frac{m_{y,h}}{m_{yz,h} + m_{z,h} + m_{z,FBkF,h}}$$

$$w_i(PAH) = \left(\frac{R_{bx,i} - b_0}{b_1}\right) \cdot \frac{m_{yx,i}}{m_{x,i}}$$

u_{meas}[w(PAH) was estimated by linear propagation of standard uncertainties of the input quantities given above using METAS UncLib.

$$w(PAH) = \frac{1}{k} \cdot \sum_{i=1}^{k} w_i(PAH)$$

$$u_{rep}[w(PAH)] = s[w(PAH)] \cdot f_{exp}$$

$$u_c[w(PAH)] = w(PAH) \cdot \sqrt{u_{meas}^2[w(PAH)] + u_{rep}^2[w(PAH)]}$$

Benz[a]anthracene (BaA)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
w_{ref}(BaA)	Certified mass fraction of BaA in the standard reference material SRM NIST 1647f	5.16	0.035	mg/kg	B
m_{ref}	Mass of standard reference material SRM NIST 1647f for preparation of reference stock solution	0.98379	0.00009	g	A/B
$m_{toluene,\ stock}$	Mass of toluene for the preparation of reference stock solution	1.99199	0.00012	g	A/B
$m_{stock,\ h}$	Mass of reference stock solution for preparation of intermediate reference solution z, h (h = 1 to 6); typical value for h = 3	0.33120	0.00008	g	A/B
$m_{toluene,\ z,\ h}$	Mass of toluene for the preparation of the intermediate reference solution z, h (h = 1 to 6); typical value for h = 3	14.68851	0.00068	g	A/B
$m_{z,\ h}$	Mass of the intermediate reference solution z, h for preparation of the calibration blends zy, h (h = 1 to 6); typical value for h = 3	0.42825	0.00009	g	A/B
$m_{yz,\ h}$	Mass of the spike solution y, h for preparation of the calibration blends yz, h (h = 1 to 6); typical value for h = 3	0.34270	0.00008	g	A/B
$m_{z,\ FBkF,\ h}$	Mass of the injection standard FBkF for the preparation of the calibration bends yz, h (h = 1 to 6); typical value for h = 3	0.08557	0.00008	g	A/B
$m_{yx,i}$	Mass of the spike solution y for the preparation of the sample blend yx	0.17122	0.00008	g	A/B
$m_{x,i}$	Mass of the sample for the preparation of the sample blend yx	3.01069	0.00016	g	A/B
$R_{bx,i}$	Measured isotope ratio (peak area ratio) of the quantifier ions of BaA and BaA-d_{12} in the sample blend yx	1.20574	0.00000	-	A
$R_{bz,h}$	Measured isotope ratio (peak area ratio) of the quantifier ions of BaA and BaA-d_{12} in the calibration blend yz, h (h = 1 to 6); typical value for h = 3	0.98837	0.01018	-	A
u_{meas}	Uncertainty contribution from measurement steps (intermediate result)	3.3109	0.0251	µg/kg	A/B
s[w(BaA)]	Standard deviation of w_i(BaA) (i = 1 to 6)	3.3109	0.0731	µg/kg	A
f_{exp}	Expanding factor for the consideration of reporducibility	1.1316	-	-	A
u_{rep}	Uncertainty contribution from reproducibility (intermediate result)	3.3109	0.0828	µg/kg	A

w(BaA)	Mass fraction of BaA	3.31	µg/kg
u_c[w(BaA)]	Combined standard uncertainty	0.086	µg/kg
k	Coverage factor	2	
u[w(BaA)]	Expanded uncertainty	0.18	µg/kg

Benzo[a]pyrene (BaP)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
w_{ref}(BaP)	Certified mass fraction of BaP in the standard reference material SRM NIST 1647f	6.22	0.055	mg/kg	B
m_{ref}	Mass of standard reference material SRM NIST 1647f for preparation of reference stock solution	0.98379	0.00009	g	A/B
$m_{toluene,\ stock}$	Mass of toluene for the preparation of reference stock solution	1.99199	0.00012	g	A/B
$m_{stock,\ h}$	Mass of reference stock solution for preparation of intermediate reference	0.33120	0.00008	g	A/B
$m_{toluene,\ z,\ h}$	Mass of toluene for the preparation of the intermediate reference solution z, h (h = 1 to 6); typical value for h = 3	14.68851	0.00068	g	A/B
$m_{z,\ h}$	Mass of the intermediate reference solution z, h for preparation of the calibration blends zy, h (h = 1 to 6); typical value for h = 3	0.42825	0.00009	g	A/B
$m_{yz,\ h}$	Mass of the spike solution y, h for preparation of the calibration blends yz, h (h = 1 to 6); typical value for h = 3	0.34270	0.00008	g	A/B
$m_{z,\ FBkF,\ h}$	Mass of the injection standard FBkF for the preparation of the calibration bends yz, h (h = 1 to 6); typical value for h = 3	0.08557	0.00008	g	A/B
$m_{yx,i}$	Mass of the spike solution y for the preparation of the sample blend yx	0.17122	0.00008	g	A/B
$m_{x,i}$	Mass of the sample for the preparation of the sample blend yx	3.01069	0.00016	g	A/B
$R_{bx,i}$	Measured isotope ratio (peak area ratio) of the quantifier ions of BaP and BaP-d_{12} in the sample blend yx	1.88121	0.00000	-	A
$R_{bz,h}$	Measured isotope ratio (peak area ratio) of the quantifier ions of BaP and BaP-d_{12} in the calibration blend yz, h (h = 1 to 6); typical value for h = 3	1.42054	0.01492	-	A
u_{meas}	Uncertainty contribution from measurement steps (intermediate result)	4.3142	0.0373	µg/kg	A/B
s[w(BaP)]	Standard deviation of w_i(BaP) (i = 1 to 6)	4.3142	0.0603	µg/kg	A
f_{exp}	Expanding factor for the consideration of reporducibility	1.7896	-	-	A
u_{rep}	Uncertainty contribution from reproducibility (intermediate result)	4.3142	0.1079	µg/kg	A

w(BaP)	Mass fraction of BaP	4.31	µg/kg
u_c[w(BaP)]	Combined standard uncertainty	0.114	µg/kg
k	Coverage factor	2	
u[w(BaP)]	Expanded uncertainty	0.23	µg/kg

Benzo[b]fluoranthene (BbF)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
w_{ref}(BbF)	Certified mass fraction of BbF in the standard reference material SRM NIST 1647f	5.29	0.03	mg/kg	B
m_{ref}	Mass of standard reference material SRM NIST 1647f for preparation of reference stock solution	0.98379	0.00009	g	A/B
$m_{toluene, stock}$	Mass of toluene for the preparation of reference stock solution	1.99199	0.00012	g	A/B
$m_{stock, h}$	Mass of reference stock solution for preparation of intermediate reference solution z, h (h = 1 to 6); typical value for h = 3	0.33120	0.00008	g	A/B
$m_{toluene, z, h}$	Mass of toluene for the preparation of the intermediate reference solution z, h (h = 1 to 6); typical value for h = 3	14.68851	0.00068	g	A/B
$m_{z, h}$	Mass of the intermediate reference solution z, h for preparation of the calibration blends zy, h (h = 1 to 6); typical value for h = 3	0.42825	0.00009	g	A/B
$m_{yz, h}$	Mass of the spike solution y, h for preparation of the calibration blends yz, h (h = 1 to 6); typical value for h = 3	0.34270	0.00008	g	A/B
$m_{z, FBkF, h}$	Mass of the injection standard FBkF for the preparation of the calibration bends yz, h (h = 1 to 6); typical value for h = 3	0.08557	0.00008	g	A/B
$m_{yx,i}$	Mass of the spike solution y for the preparation of the sample blend yx	0.17122	0.00008	g	A/B
$m_{x,i}$	Mass of the sample for the preparation of the sample blend yx	3.01069	0.00016	g	A/B
$R_{bx,i}$	Measured isotope ratio (peak area ratio) of the quantifier ions of BbF and BbF-d_{12} in the sample blend yx	2.18260	0.00000	-	A
$R_{bz,h}$	Measured isotope ratio (peak area ratio) of the quantifier ions of BbF and BbF-d_{12} in the calibration blend yz, h (h = 1 to 6); typical value for h = 3	1.25555	0.01794	-	A
u_{meas}	Uncertainty contribution from measurement steps (intermediate result)	4.7938	0.0322	µg/kg	A/B
s[w(BbF)]	Standard deviation of w_i(BbF) (i = 1 to 6)	4.7938	0.0752	µg/kg	A
f_{exp}	Expanding factor for the consideration of reporducibility	1.5934	-	-	A
u_{rep}	Uncertainty contribution from reproducibility (intermediate result)	4.7938	0.1198	µg/kg	A

w(BbF)	Mass fraction of BbF	4.79	µg/kg
u_c[w(BbF)]	Combined standard uncertainty	0.124	µg/kg
k	Coverage factor	2	
u[w(BbF)]	Expanded uncertainty	0.25	µg/kg

Chrysene (Chr)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
w_{ref}(Chr)	Certified mass fraction of Chr in the standard reference material SRM NIST 1647f	4.67	0.04	mg/kg	B
m_{ref}	Mass of standard reference material SRM NIST 1647f for preparation of reference stock solution	0.98379	0.00009	g	A/B
$m_{toluene, stock}$	Mass of toluene for the preparation of reference stock solution	1.99199	0.00012	g	A/B
$m_{stock, h}$	Mass of reference stock solution for preparation of intermediate reference solution z, h (h = 1 to 6); typical value for h = 3	0.33120	0.00008	g	A/B
$m_{toluene, z, h}$	Mass of toluene for the preparation of the intermediate reference solution z, h (h = 1 to 6); typical value for h = 3	14.68851	0.00068	g	A/B
$m_{z, h}$	Mass of the intermediate reference solution z, h for preparation of the calibration blends zy, h (h = 1 to 6); typical value for h = 3	0.42825	0.00009	g	A/B
$m_{yz, h}$	Mass of the spike solution y, h for preparation of the calibration blends yz, h (h = 1 to 6); typical value for h = 3	0.34270	0.00008	g	A/B
$m_{z, FBkF, h}$	Mass of the injection standard FBkF for the preparation of the calibration bends yz, h (h = 1 to 6); typical value for h = 3	0.08557	0.00008	g	A/B
$m_{yx,i}$	Mass of the spike solution y for the preparation of the sample blend yx	0.17122	0.00008	g	A/B
$m_{x,i}$	Mass of the sample for the preparation of the sample blend yx	3.01069	0.00016	g	A/B
$R_{bx,i}$	Measured isotope ratio (peak area ratio) of the quantifier ions of Chr and Chr-d_{12} in the sample blend yx	1.20057	0.00000	-	A
$R_{bz,h}$	Measured isotope ratio (peak area ratio) of the quantifier ions of Chr and Chr-d_{12} in the calibration blend yz, h (h = 1 to 6); typical value for h = 3	0.97964	0.01445	-	A
u_{meas}	Uncertainty contribution from measurement steps (intermediate result)	2.9846	0.0266	µg/kg	A/B
s[w(Chr)]	Standard deviation of w_i(Chr) (i = 1 to 6)	2.9846	0.0509	µg/kg	A
f_{exp}	Expanding factor for the consideration of reporducibility	1.4646	-	-	A
u_{rep}	Uncertainty contribution from reproducibility (intermediate result)	2.9846	0.0746	µg/kg	A

w(Chr)	Mass fraction of Chr	2.98	µg/kg
u_c[w(Chr)]	Combined standard uncertainty	0.079	µg/kg
k	Coverage factor	2	
u[w(Chr)]	Expanded uncertainty	0.16	µg/kg

Uncertainty Information from TUBITAK UME

$$\frac{u_c(Analyte)}{c_{Analyte}} = \sqrt{(\frac{u(m_{SI})}{m_{SI}})^2 + (\frac{u(c_{IS})}{c_{IS}})^2 + (\frac{u(c_{NSS})}{c_{NSS}})^2 + (\frac{u(r)}{r})^2 + (\frac{u(\mathrm{Re}c)}{\mathrm{Re}c})^2 + (\frac{u(Cal)}{C_0})^2}$$

SI: Sample intake
IS: Internal Spike
NSS: Native stock solution
r: Repeatibility
Rec: Recovery
Cal: Calibration curve

Benz[a]anthracene (BaA)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	Sample Intake	2.00525	0.00042	g	A
	IS spike	0.07735	9.50016E-05	g	A
	Native Stock Soln	389.7	2.6	µg/kg	B
	Recovery	100	0.74		A
	Repeatibility	0.51	0.051	µg/kg	A
	Calibration Graph	0.51	0.005	µg/kg	A

w(BaA)	Mass fraction of BaA	**0.51**	**µg/kg**
u_c[w(BaA)]	Combined standard uncertainty	**0.050**	**µg/kg**
k	Coverage factor	**2**	
u[w(BaA)]	Expanded uncertainty	**0.10**	**µg/kg**

Benzo[a]pyrene (BaP)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	Sample Intake	**2.00525**	**0.00042**	**g**	**A**
	IS spike	**0.07735**	**9.50016E-05**	**g**	**A**
	Native Stock Soln	**469.8**	**4.2**	**µg/kg**	**B**
	Recovery	**100**	**0.9**		**A**
	Repeatibility	**0.58**	**0.041**	**µg/kg**	**A**
	Calibration Graph	**0.58**	**0.007**	**µg/kg**	**A**

w(BaP)	Mass fraction of BaP	**0.58**	**µg/kg**
u_c[w(BaP)]	Combined standard uncertainty	**0.04**	**µg/kg**
k	Coverage factor	**2**	
u[w(BaP)]	Expanded uncertainty	**0.09**	**µg/kg**

Benzo[b]fluoranthene (BbF)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	Sample Intake	2.00525	0.00042	g	A
	IS spike	0.07735	9.50016E-05	g	A
	Native Stock Soln	399.6	2.3	µg/kg	B
	Recovery	100.0	0.6		A
	Repeatibility	0.78	0.044	µg/kg	A
	Calibration Graph	0.78	0.007	µg/kg	A

w(BbF)	Mass fraction of BbF	**0.78**	**µg/kg**
u_c[w(BbF)]	Combined standard uncertainty	**0.04**	**µg/kg**
k	Coverage factor	**2**	
u[w(BbF)]	Expanded uncertainty	**0.09**	**µg/kg**

Chrysene (Chr)

Input quantity	Source of uncertainty / determination	Typical value	Standard uncertainty	Unit	Type (A/B)
	Sample Intake	2.00525	0.00042	g	A
	IS spike	0.07735	9.50016E-05	g	A
	Native Stock Soln	352.7	3.0	µg/kg	B
	Recovery	100.0	1.1		A
	Repeatibility	0.46	0.0401	µg/kg	A
	Calibration Graph	0.46	0.0048	µg/kg	A

w(Chr)	Mass fraction of Chr	**0.46**	**µg/kg**
u_c[w(Chr)]	Combined standard uncertainty	**0.04**	**µg/kg**
k	Coverage factor	**2**	
u[w(Chr)]	Expanded uncertainty	**0.08**	**µg/kg**

APPENDIX I: Participants' Quantitative Results as Reported

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

Quantitative Results from BVL

Summary of Results

Analyte	Abbreviation	Mass fraction w (µg/kg)	Combined standard uncertainty u_c (µg/kg)	Coverage factor k	Expanded uncertainty U (µg/kg)	Number of replicates n
Benz[a]anthracene	BaA	3.20	0.19	2.00	0.39	15
Benzo[a]pyrene	BaP	4.20	0.18	2.00	0.36	15
Benzo[b]fluoranthene	BbF	4.60	0.32	2.00	0.65	15
Chrysene	Chr	2.92	0.15	2.00	0.30	15

Results of each replicate

Replicate n	Mass fraction w of PAH (µg/kg)			
	BaA	BaP	BbF	Chr
1 (day 1)	3.23	4.18	4.59	3.01
2 (day 1)	3.25	4.15	4.60	2.98
3 (day 1)	3.21	4.19	4.57	2.97
4 (day 2)	3.19	4.26	4.54	2.96
5 (day 2)	3.19	4.23	4.49	2.95
6 (day 2)	3.15	4.26	4.59	2.95
7 (day 3)	3.16	4.21	4.63	2.94
8 (day 3)	3.24	4.18	4.79	2.93
9 (day 3)	3.20	4.22	4.67	2.90
10 (day 4)	3.13	4.15	4.67	2.89
11 (day 4)	3.20	4.21	4.53	2.84
12 (day 4)	3.18	4.22	4.55	2.87
13 (day 5)	3.22	4.19	4.60	2.87
14 (day 5)	3.18	4.10	4.59	2.85
15 (day 5)	3.24	4.22	4.65	2.85
Mean	3.20	4.20	4.60	2.92
Standard deviation	0.03	0.04	0.07	0.05

Additional comments	15 samples measured in total over 5 days, 3 samples per day, separate extractions, comma (,) as decimal separator

Quantitative Results from INRIM

Summary of Results

Analyte	Abbreviation	Mass fraction w (µg/kg)	Combined standard uncertainty u_c (µg/kg)	Coverage factor k	Expanded uncertainty U (µg/kg)	Number of replicates n
Benz[a]anthracene	BaA	3.63	0.21	2	0.42	8
Benzo[a]pyrene	BaP	5.27	0.21	2	0.42	8
Benzo[b]fluoranthene	BbF	5.19	0.16	2	0.32	7
Chrysene	Chr	3.16	0.20	2	0.40	8

Results of each replicate

Replicate n	Mass fraction w of PAH (µg/kg)			
	BaA	BaP	BbF	Chr
1	3.94	5.79	5.00	3.40
2	3.36	4.99	5.46	3.29
3	3.78	5.32	4.91	3.11
5	3.60	5.02	5.29	2.99
6	3.39	5.37	5.48	3.18
7	3.66	5.53	4.94	3.30
8	3.72	4.88	5.22	2.91
9	3.57	5.25	---	3.11
Mean	3.63	5.27	5.19	3.16
Standard deviation	0.18	0.28	0.22	0.15

Additional comments	

Quantitative Results from IW

Summary of Results

Analyte	Abbreviation	Mass fraction w (µg/kg)	Combined standard uncertainty u_c (µg/kg)	Coverage factor k	Expanded uncertainty U (µg/kg)	Number of replicates n
Benz[a]anthracene	BaA	5.333	0.575	2	1.149	3
Benzo[a]pyrene	BaP	13.333	1.438	2	2.875	3
Benzo[b]fluoranthene	BbF	7.000	0.755	2	1.510	3
Chrysene	Chr	5.000	0.539	2	1.078	3

Results of each replicate

Replicate n	Mass fraction w of PAH (µg/kg)			
	BaA	BaP	BbF	Chr
1	4.000	14.000	6.000	4.000
2	6.000	12.000	8.000	6.000
3	6.000	14.000	7.000	5.000
5				
6				
7				
8				
9				
Mean	5.333	13.333	7.000	5.000
Standard deviation	1.155	1.155	1.000	1.000

Additional comments	

Quantitative Results from LAB-IZO

Summary of Results

Analyte	Abbreviation	Mass fraction w (µg/kg)	Combined standard uncertainty u_c (µg/kg)	Coverage factor k	Expanded uncertainty U (µg/kg)	Number of replicates n
Benz[a]anthracene	BaA	0.619	0.055	2	0.109	6
Benzo[a]pyrene	BaP	0.568	0.051	2	0.102	6
Benzo[b]fluoranthene	BbF	0.600	0.068	2	0.137	6
Chrysene	Chr	0.942	0.087	2	0.174	6

Results of each replicate

Replicate n	Mass fraction w of PAH (µg/kg)			
	BaA	BaP	BbF	Chr
1	0.633	0.902	0.616	0.556
2	0.593	0.884	0.573	0.575
3	0.602	1.076	0.603	0.576
4	0.699	0.985	0.596	0.631
5	0.600	0.953	0.611	0.545
6	0.589	0.854	0.600	0.524
7				
8				
Mean				
Standard deviation				

Additional comments	

Quantitative Results from METAS

Summary of Results

Analyte	Abbreviation	Mass fraction w (µg/kg)	Combined standard uncertainty u_c (µg/kg)	Coverage factor k	Expanded uncertainty U (µg/kg)	Number of replicates n
Benz[a]anthracene	BaA	3.31	0.086	2	0.18	6
Benzo[a]pyrene	BaP	4.31	0.114	2	0.23	6
Benzo[b]fluoranthene	BbF	4.79	0.124	2	0.25	6
Chrysene	Chr	2.98	0.079	2	0.16	6

Results of each replicate

Replicate n	Mass fraction w of PAH (µg/kg)			
	BaA	BaP	BbF	Chr
1	3.228	4.242	4.704	2.910
2	3.332	4.380	4.884	3.004
3	3.258	4.252	4.781	2.957
4	3.376	4.337	4.811	3.008
5	3.262	4.297	4.715	2.969
6	3.411	4.377	4.868	3.059
7				
8				
9				
Mean	3.311	4.314	4.794	2.985
Standard deviation	0.073	0.060	0.075	0.051

Additional comments	

Quantitative Results from TUBITAK UME

Summary of Results

Analyte	Abbreviation	Mass fraction w (µg/kg)	Combined standard uncertainty u_c (µg/kg)	Coverage factor k	Expanded uncertainty U (µg/kg)	Number of replicates n
Benz[a]anthracene	BaA	0.51	0.05	2	0.10	8
Benzo[a]pyrene	BaP	0.58	0.04	2	0.09	8
Benzo[b]fluoranthene	BbF	0.78	0.04	2	0.09	9
Chrysene	Chr	0.46	0.04	2	0.08	9

Results of each replicate

Replicate n	Mass fraction w of PAH (µg/kg)			
	BaA	BaP	BbF	Chr
1	0.51	0.58	0.76	0.48
2	0.48	0.60	0.78	0.46
3	0.57	0.61	0.80	0.49
4	0.54	0.53	0.82	0.49
5	0.54	0.56	0.81	0.45
6	0.47	0.57	0.74	0.44
7	0.50	0.59	0.76	0.43
8	0.46	0.61	0.78	0.45
9			0.75	0.43
Mean	0.51	0.58	0.78	0.46
Standard deviation	0.040	0.028	0.027	0.024

Additional comments