CCQM-K95.1
Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea

OAWG Track A Key Comparison

Final Report
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SUMMARY

Extraction, chromatographic separation, and quantification of low-concentration organic compounds in complex matrices 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 made by national metrology institutes (NMIs) and designated institutes (DIs). To enable NMIs and DIs to update or establish their claims, in 2014 the Organic Analysis Working Group (OAWG) initiated CCQM-K95.1 "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea". This was a follow-on comparison from CCQM-K95 which was completed in 2014.

The polycyclic aromatic hydrocarbons (PAHs) benz[*a*]anthracene (BaA) and benzo[*a*]pyrene (BaP) are considered priority pollutants by U.S. Environmental Protection Agency and are regulated contaminants in food, pose chromatographic separation challenges, and for which exist well-characterized measurement procedures and standard materials. BaA and BaP in a smoked tea were therefore selected as representative target measurands for CCQM-K95.1. Ten NMIs participated in CCQM-K95.1. The consensus summary mass fractions for the two PAHs are in the range of (50 to 70) ng/g with relative standard deviations of (6 to 10) %.

Successful participation in CCQM K95.1 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molar mass of 100 g/mol to 500 g/mol and having polarity $pK_{ow} < -2$, in a botanical matrix ranging in mass fraction from 10 ng/g to 1000 ng/g: (1) value assignment of primary reference standards (if in-house purity assessment carried out), (2) value assignment of single and/or multi-component organic solutions, (3) extraction of analytes of interest from the matrix, (4) cleanup and separation of analytes of interest from interfering matrix or extract components, and (5) separation and quantification using gas chromatography or liquid chromatography.

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ACRONYMS

ASE	accelerated solvent extraction
BaA	benz[*a*]anthracene
BAM	Bundesanstalt fuer Materialforschung und –pruefung, DI: Germany
BaP	benzo[*a*]pyrene
BCR	Bureau Communautaire de Référence (precursor to IRMM)
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, DI: Germany
CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CENAM	Centro Nacional de Metrologia, NMI: Mexico
CIL	Cambridge Isotope Laboratories, Inc.
CMC	Calibration and Measurement Capability
CRM	certified reference material
CV	coefficient of total variation, expressed in %: CV = 100·s/$\bar{x}$
CV_{btw}	between-packet coefficient of variation, expressed in %: CV = 100·s/$\bar{x}$
CV_{wth}	within-packet coefficient of variation, expressed in %: CV = 100·s/$\bar{x}$
DI	designated institute
DoE	degrees of equivalence
EXHM	Chemical Metrology Laboratory, DI: Greece
GCxGC	two-dimensional gas chromatography
GC-HRMS	gas chromatography with high-resolution mass spectrometry detection
GC-IT-MS	gas chromatography with ion trap mass spectrometry detection
GC-MS	gas chromatography with mass spectrometry detection
GC-MS/MS	gas chromatography with tandem mass spectrometry detection
GC-TOFMS	gas chromatography with time-of-flight mass spectrometry detection
GLHK	Government Laboratory, Hong Kong, DI: Hong Kong
GPC	gel permeation chromatography
HPLC-DAD	high pressure liquid chromatography with diode array detection
HSA	Health Sciences Authority, DI: Singapore
ID	isotope dilution
INRIM	Istituto Nazionale di Ricerca Metrologica, NMI: Italy
IRMM	Institute for Reference Materials and Measurements, international organization
JCTLM	Joint Committee for Traceability in Laboratory Medicine
KC	Key Comparison
KCRV	Key Comparison Reference Value
KEBS	Kenya Bureau of Standards, NMI: Kenya
KRISS	Korea Research Institute of Standards and Science, NMI: Republic of Korea
LC	liquid chromatography
LNE	Laboratoire National de Métrologie et d'Essais, NMI: France
MRM	multiple reaction monitoring
NIM	National Institute of Metrology, NMI: China
NIST	National Institute of Standards and Technology, NMI: USA
NMI	national metrology institute
NMIJ	National Metrology Institute Japan, NMI: Japan
NMISA	National Metrology Institute South Africa, NMI: South Africa
NMR	nuclear magnetic resonance spectroscopy

OAWG	Organic Analysis Working Group
PAH	polycyclic aromatic hydrocarbon
pK_{ow}	negative logarithm of the octanol-water partition coefficient
PSE	pressurized solvent extraction
qNMR	quantitative nuclear magnetic resonance spectroscopy
QuEChERS	"Quick, Easy, Cheap, Effective, Rugged, Safe" liquid/solid extraction
RMP	Reference Measurement Procedure
SIM	selected ion monitoring
SPE	solid phase extraction
SRM	Standard Reference Material, a NIST CRM
UME	National Metrology Institute of Turkey, NMI: Turkey
VNIIM	D.I. Mendeleyev Institute for Metrology, NMI: Russia
VSL	VSL Dutch Metrology Institute, NMI: The Netherlands
v/v	volume fraction

SYMBOLS

di	degree of equivalence: xi - KCRV
%di	percent relative degree of equivalence: 100·di/KCRV
k	coverage factor: U(x) = k·u(x)
MAD_E	median absolute deviation from the median (MAD)-based estimate of s: MAD_E = 1.4826·MAD, where MAD = median(|xi-median(xi)|)
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: $\bar{x} = \sum_{i=1}^{n} x_i/n$
z_i	z-score, a standardized quantity value: $z_i = (x_i - \bar{x})/s$

INTRODUCTION

Extraction, chromatographic separation, and quantification of low-concentration organic compounds in complex matrices 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).

In 2012, the Organic Analysis Working Group (OAWG) of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) sponsored the Key Comparison (KC) CCQM-K95 "Mid-Polarity Analytes in Food Matrix: Mid-Polarity Pesticides in Tea" [1]. CCQM-K95 was designed to assess participants' capabilities for determination of mid-polarity contaminants in a food matrix. Results from eight of the 18 participants were considered technically flawed for various reasons including incomplete extraction of the analytes from the matrix. Follow up analyses by several participants confirmed that pre-wetting the matrix was necessary to completely extract the target analytes from the dry tea matrix. To enable CCQM-K95 participants to update their competency claims and allow other NMIs and DIs to demonstrate their competencies, in 2014 the OAWG initiated CCQM-K95.1 "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea".

PAHs result from combustion sources and are ubiquitous in environmental samples. Some smoked teas, a sample matrix relatively similar to that of the CCQM-K95 samples, are known to have measurable concentrations of PAHs. The PAHs benz[*a*]anthracene (BaA) and benzo[*a*]pyrene (BaP) are considered to be priority pollutants by U.S. Environmental Protection Agency and are regulated contaminants in food, pose chromatographic separation challenges, and for which exist well-characterized measurement procedures and standard materials. BaA and BaP in a smoked tea were therefore selected as representative target measurands for CCQM-K95.1. BaA and BaP are also measurands in the OAWG sponsored CCQM-K131 "Low-Polarity Analytes in a Multicomponent Organic Solution: Polycyclic Aromatic Hydrocarbons (PAHs) in Acetonitrile". The CCQM-K95.1 and CCQM-K131 were designed as complementary and were conducted over the same time frame.

BaA and BaP can be successfully evaluated using either gas chromatography (GC) or liquid chromatography (LC) in conjunction with various detection methods including but not limited to isotope dilution mass spectrometry (IDMS). PAHs must be removed from solid matrices by solvent extraction. Some cleanup of the extract is necessary to prepare the sample for analysis. The method(s) used by participants in CCQM-K95.1 are intended to represent the way they deliver quantification of contaminants in complex food matrices to their customers.

The following sections of this report document the timeline of CCQM-K95.1, the measurands, study material, participants, results, and the measurement capability claims that participation in CCQM-K95.1 can support. The Appendices reproduce the official communication materials and summaries of information about their results provided by the participants.

TIMELINE

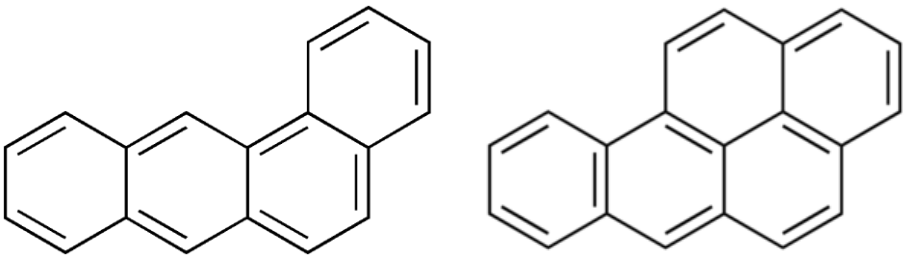
Table 1 lists the timeline for CCQM-K95.1.

Table 1: Timeline for CCQM-K95.1

Date	Action
Apr 2014	Proposed to CCQM
Apr 2015	Draft protocol presented to OAWG as potential Track A Key Comparison
May 2015	OAWG authorized CCQM-K131 as a Track A Key Comparison; protocol approved
Jul 2015	Call for participation to OAWG members
Dec 2015 to Apr 2016	Study samples shipped to participants. The range in shipping times reflects delays from shipping and customs.
Sep 2016	Results due to coordinating laboratory: revised from Feb 2016 to accommodate participants who received samples late or experienced equipment difficulties
Sep 2016	Draft A report distributed to OAWG
Apr 2017	Draft B report distributed to OAWG
Apr 2018	(Draft) Final report delivered to OAWG Chair

MEASURANDS

Recent European Union requirements for the analysis of contaminants in food include the PAHs BaA, BaP, and benzo[*ghi*]perylene. Participants at the October 2014 OAWG meeting decided to focus the CCQM-K95.1 study on BaA, a four-ring *cata*-condensed PAH of molar mass 228 g/mol and BaP, a five-ring *peri*-condensed PAH of molar mass 252 g/mol. Figure 1 displays the molecular structure of these compounds.



Benz[*a*]anthracene
BaA

Benzo[*a*]pyrene
BaP

Figure 1: Structures of Benz[*a*]anthracene and Benzo[*a*]pyrene

STUDY MATERIALS

Some brands of yerba mate tea, particularly those produced in South America, undergo a long smoking process and therefore contain PAHs at quantifiable concentrations. The CCQM-K95.1 study material is a commercial yerba mate tea. This commercial tea, which contains stems and leaves, was ground in an ultracentrifugation mill. Particle size analysis at NIST indicated that 70 % of the material had a particle size less than 100 µm. The powdered tea was heat-sealed in nitrogen-flushed 4-mil (0.004 inch, ≈ 0.10 mm) thick plastic bags which were then heat-sealed inside a nitrogen-flushed Mylar bag with two packets of silica gel as a desiccant. Each packet contains 10 g ± 0.1 g of powdered tea.

Each participant received six packets: three packets were required for analysis with three packets available for practice, screening analysis, and/or moisture determination. The recommended minimum sample amount for analysis was at least 300 mg. Measurement results were to be reported on a dry-mass basis.

Moisture Determination

Participants at the October 2014 OAWG meeting recommended that all CCQM-K95.1 participants use the same method for determining moisture. The recommended protocol was for a minimum of three subsamples of the tea, sample size 1 g each, to be dried over anhydrous phosphorous pentoxide (P_2O_5) in a desiccator at room temperature until a constant mass was reached. Moisture determination was to be performed concurrent with the analysis of the test sample portions. The correction used for dry-mass conversion was to be reported in addition to the required PAH mass fractions.

Homogeneity Assessment of Study Material

The homogeneity of the tea material was assessed at NIST by analyzing duplicate 300 mg subsamples from each of 10 packets of tea. The material was extracted by Soxhlet extraction with hexane/acetone (1:1 volume fraction) for 20 h. The extracts were concentrated to 5 mL and centrifuged. The supernatant was eluted through an amino SPE cartridge with dichloromethane/hexane (1:4 volume fraction) and the eluent concentrated to 0.5 mL. The samples were analyzed by GC-MS; SRM 2260a Aromatic Hydrocarbons in Toluene was used as a calibrant. Based on the measurements from the homogeneity assessment, the target mass fraction ranges were as follows: BaA (50 to 80) ng/g and BaP (50 to 80) ng/g.

One-way ANOVA was used to evaluate whether there were significant between-packet differences in the concentration of either of the two proposed measurands. Results of the homogeneity study are summarized in Table 2. The between-packet coefficient of variations expressed as a percent of the mean values, CV_{btw}, were both less than 1 % and the probabilities of falsely rejecting the null hypothesis that all packets have the same concentrations are both well above the usual 5 % threshold. We therefore conclude that at least for the two PAHs to be measured, there are no significant between-packet concentration differences.

The within-packet coefficient of variations expressed as a percent of the mean values, CV_{wth}, were both less than 5 %. These estimates include all sources of variability other than between-packet differences. Assuming measurement results are reported as the mean of three

independent replicates using methods like that used in the homogeneity assessment, the expected measurement standard uncertainties are on the order of $5/\sqrt{3} = 3$ %.

Table 2. Results of the homogeneity assessment for target PAHs in yerba mate tea

ANOVA Estimate	BaA	BaP
Within-packet, CV_{wth}	4.2 %	3.9 %
Between-packet, CV_{btw}	0.8 %	0.0 %
Total analytical variability, CV	4.3 %	3.9 %
Probability of falsely rejecting the hypothesis that all packets have the same concentration	46 %	85 %

Stability Assessment of Study Material

NIST has not performed a formal stability study for this tea material. However, NIST has not observed any stability issues in more than 30 years' experience with PAHs in other natural matrix CRMs (e.g., sediment, diesel and atmospheric particulate matter, and fish and mussel tissue).

PARTICIPANTS AND INSTRUCTIONS

The call for participation was distributed in Jul-2015 with the intent to distribute samples in Sep-2015, receive results in Dec-2015, and discuss results at the Spring OAWG meeting, Apr-2016. Appendix A reproduces the Call for Participation; Appendix B reproduces the study Protocol. Due to shipping practicalities, sample shipping was delayed to Dec-2015. Due to customs issues, the last set of materials was delivered in Apr-2016. Because of these delays, the deadline for submission of results was several times postponed with a final deadline of 16-Sep-2016 to enable discussion of results at the Fall 2016 OAWG meeting, 3-Oct-2016.

Table 3 lists the institutions that received CCQM-K95.1 samples.

Table 3: Institutions Receiving CCQM-K95.1 Sample Materials

NMI or DI	Code	Contact
Bundesanstalt fuer Materialforschung und –pruefung	BAM	Rosemarie Philipp
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	BVL	Rudolf Hackenberg
Centro Nacional de Metrologia	CENAM	Mariana Arce Osuna
Chemical Metrology Laboratory EXHM/GCSL-EIM	EXHM	Charalampos Alexopoulos
Health Sciences Authority, Singapore	HSA	Tang Lin Teo
Kenya Bureau of Standards	KEBS	Luvonga Caleb
National Institute of Metrology, China	NIM	Tang Hua
National Institute of Metrology, Thailand	NIMT	Jintana Nammoonnoy
National Institute of Standards and Technology	NIST	Jacolin Murray
National Metrology Institute of South Africa	NMISA	Laura Quinn
National Metrology Institute of Turkey TÜBİTAK UME	UME	Ahmet Ceyhan Gören

RESULTS

Participants were requested to report a single estimate of the mass fraction (ng/g) for the two target PAHs based on measurements for one subsample from each of three packets of the tea material (i.e., three independent replicates) on a dry-mass basis. The moisture content of the mate tea sample was to be determined and reported.

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

Methods Used by Participants

Participants were instructed to base their measurement method on either gas chromatography (GC) or liquid chromatography (LC). The methods employed in this study are intended to represent the way the NMI delivers this measurement service. However, participants were informed that the Key Comparison Reference Values (KCRV) for the measurands would be based only on results from IDMS methods.

CCQM-K95.1 results were received from ten of the eleven institutions that received samples; KEBS withdrew from participation because of equipment difficulties.

All participants based their analyses on some form of GC-MS with IDMS quantification. Brief descriptions of the analytical methods used by the participants, including sample preparation, analytical technique, calibrants, and quantification approach are summarized in Appendix F. The participants' approaches to estimating uncertainty are provided in Appendix G. The participants' results as reported are provided in Appendix H.

Calibration materials Used by Participants

Participants established the metrological traceability of their results using certified reference materials (CRMs) with stated traceability and/or commercially available high purity materials for which they determined the purity. Table 4 lists the CRMs that were used. Table 5 lists how participants established traceability. If through their own measurements, Table 5 lists the material, its assigned purity, the method used, and how the participant had demonstrated their competence in the use of the method(s).

Table 4: 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[b]
SRM 1647f	NIST	BaA	5.16 ± 0.07	99.84 ± 0.11	DSC
		BaP	6.22 ± 0.11	99.3 ± 0.7	qNMR, DSC
SRM 2260a	NIST	BaA	4.415 ± 0.078	99.79 ± 0.21	DSC
		BaP	4.71 ± 0.17	99.5 ± 0.5	qNMR, DSC
CRM 4213-a	NMIJ	BaP	99.2 ± 3.0	99.23 ± 3.83	DSC, FPD

a Stated as Value ± U_{95}(Value)

b DSC: Differential scanning calorimetry
FPD: Freezing point depression
qNMR: Quantitative nuclear magnetic resonance

Table 5: Metrological Traceability of Participants' Results

NMI/DI	Analyte	Source of Traceability	Material	Mass Fraction[a] Purity, %	Purity Techniques[b]	Evidence of Competence
BAM	BaA,BaP	SRM 2260a	N/A			
BVL	BaA,BaP	SRM 2260a	N/A			
CENAM	BaA BaP	CENAM	Supelco Ultrascientific	98.68 ± 0.12 99.12 ± 0.76	MB	Successful participation in CCQM-P20.b,c,f CCQM-K55.a,b
EXHM	BaA,BaP	SRM 2260a	N/A			
GLHK	BaA,BaP	SRM 2260a	N/A			
HSA	BaA BaP	SRM 1647f	N/A			
		HSA	Cerilliant SCB-007	99.38 ± 0.51	MB	Successful participation in CCQM-K55.b-d
NIM	BaA BaP	NIM	Cerilliant Cerilliant	99.69 ± 0.37 99.30 ± 0.29	qNMR, GC-FID, HPLC-DAD	Successful participation in CCQM-P20.a,c-f, CCQM-K55.a-d
NIMT	BaA BaP	NIMT	BCR 271 AccuStandard	99.84 ± 0.35 99.74 ± 0.35	MB	Successful participation in CCQM-K55.b
NIST	BaA,BaP	SRM 2260a	N/A			
NMISA	BaA BaP	SRM 2260a CRM 4213-a	N/A			
UME	BaA BaP	UME	Supelco 4-8563 Supelco 4-8564	98.08 ± 0.30 94.12 ± 0.52	qNMR	Successful participation in CCQM-K55.a-d

a Stated as Value ± U_{95}(Value)

b DSC: Differential scanning calorimetry
GC-FID: Gas chromatography with flame ionization detection
HPLC-DAD: High pressure liquid chromatograph with diode-array detection
MB: Mass balance
qNMR: Quantitative nuclear magnetic resonance

Participant Results

The results for CCQM-K95.1 for the determination of BaA and BaP are detailed in Table 6 and presented graphically in Figure 2.

Table 6: Reported Results for Moisture, BaA, and BaP

NMI/DI	% Moisture	Benz[*a*]anthracene, ng/g				Benzo[*a*]pyrene, ng/g			
		x	*u*(*x*)	*k*	*U*(*x*)	*x*	*u*(*x*)	*k*	*U*(*x*)
BAM	4.44	64.20	1.40	2.00	2.90	49.50	1.20	2.00	2.40
BVL	5.05	64.75	1.24	2.00	2.48	45.25	0.87	2.00	1.75
CENAM	4.63	62.07	2.06	2.00	4.13	58.63	1.66	2.00	3.32
EXHM	4.01	68.57	1.46	2.00	2.92	52.49	1.17	2.00	2.34
HSA	4.65	68.90	3.14	2.00	6.30	53.90	2.44	2.00	4.90
NIM	4.70	67.03	2.06	2.00	4.13	52.03	1.81	2.00	3.61
NIMT	5.03	63.60	2.70	2.08	5.50	46.60	2.20	2.01	4.40
NIST	3.99	67.51	1.46	2.00	3.00	58.78	2.72	2.00	6.00
NMISA	5	57.9	3.8	2	7.6	47.4	3.3	2	6.6
UME	5.53	69.00	4.00	2.00	8.00	56.00	4.20	2.00	8.40
n	10	10				10			
$\bar{x}$	4.70	65.35				52.06			
s	0.48	3.57				4.85			
$\bar{u}$		2.53				2.37			
CV	10.2	5.5				9.3			

n = number of results included in summary statistics; $\bar{x}$ = mean; *s* = standard deviation;
CV = $100 \cdot s/\bar{x}$; $\bar{u} = \sqrt{\sum_i^n u^2(x_i)/n}$, the "average" reported standard uncertainty

NMISA provided analyte recovery-adjusted results for both BaA and BaP after the April 2017 CCQM meeting, however they have chosen **not** to withdraw either of their original results. Their adjusted values are displayed as open diamonds in Figure 2; these adjusted values are not considered in the KCRV or degree of equivalence (DoE) calculations.

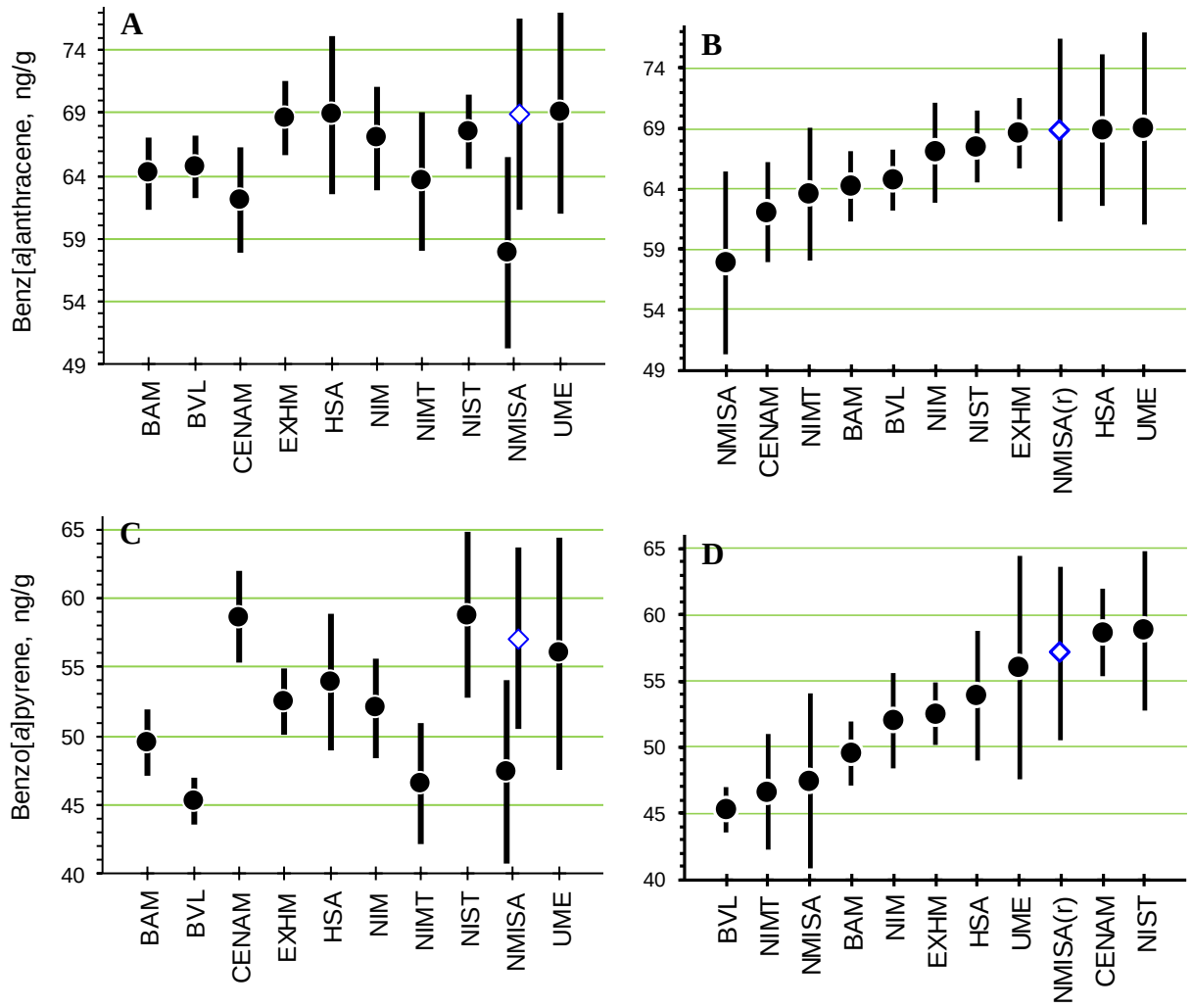


Figure 2: Dot-and-Bar Display of Reported Results, ng/g

Panels A and B display the reported results for benz[*a*]anthracene (BaA); panel A displays the results sorted alphabetically by NMI acronym, panel B displays results sorted by increasing reported value. Panels C and D display the reported results for benzo[*a*]pyrene (BaP); panel C displays the results sorted alphabetically by NMI acronym, panel D displays results sorted by increasing reported value. Dots represent the reported mean values, x; bars their 95 % expanded uncertainties, $U(x)$; open diamonds represent the recovery-adjusted results reported by NMISA. The thin horizontal gridlines are provided for visual guidance.

Within- and Between-Measurand Comparisons

Comparison of measurement results for two measurands in the same sample can help differentiate systematic measurement system biases from sample-specific issues or measurement imprecision. When systematic biases are dominant, the correlation between pair sets of results should be strongly positive. The square of the correlation coefficient, R^2, directly estimates the fraction of the between-participant covariance that is attributable to the systematic biases. Figure 3 presents a Youden-style [2] comparison of the BaA, BaP, and moisture content results.

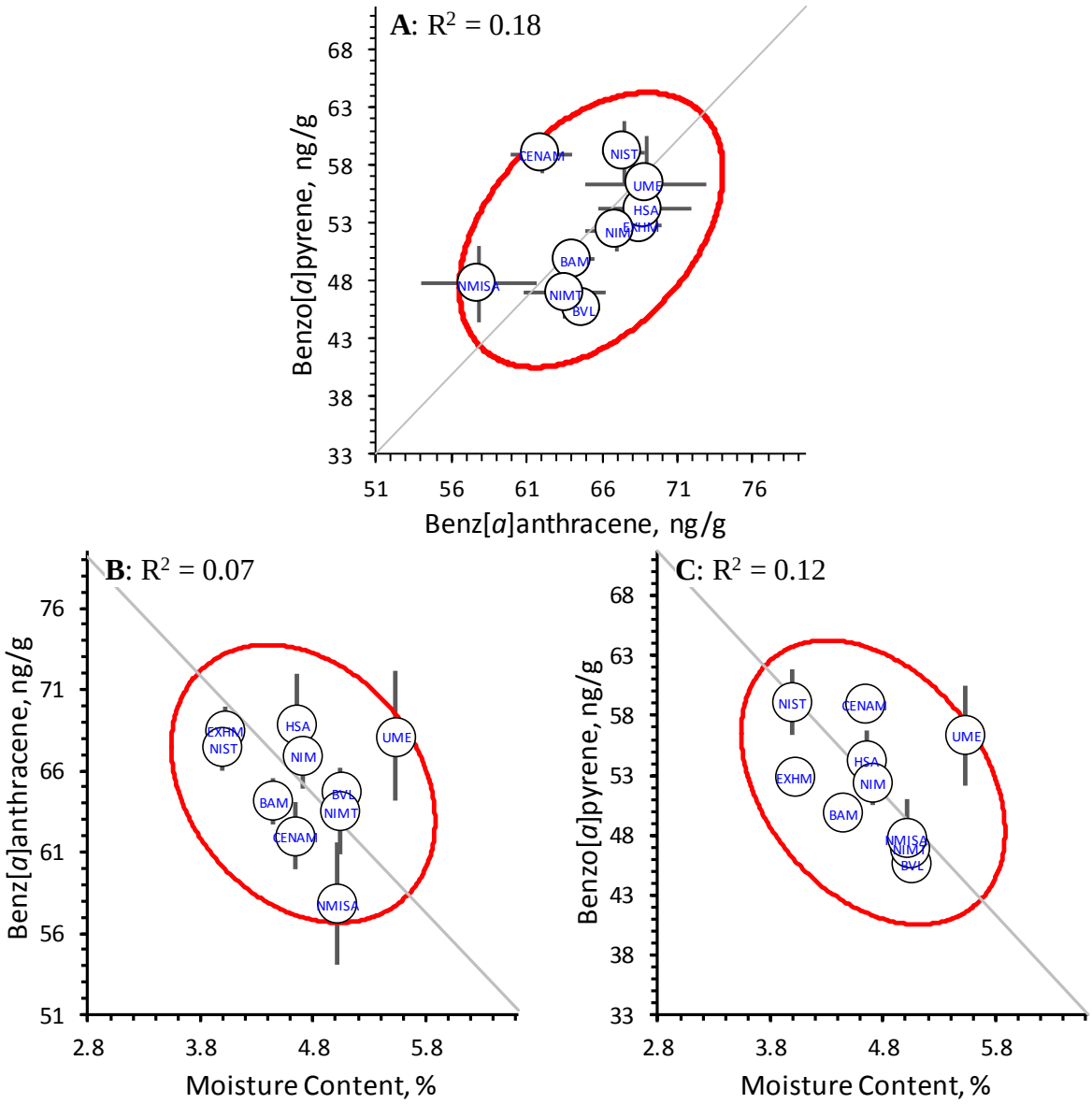


Figure 3: Comparison Between Measurands

Each panel displays the bivariate distribution of the reported results for two measurands. Panel A displays results for BaA (X-axis) and BaP (Y-axis), B for moisture content (X-axis) and BaA (Y-axis), and C for moisture content (X-axis) and BaP (Y-axis). Open circles each represent a {measurand-X, measurand-Y} pair; bars span $x_i \pm u(x_i)$. The ellipses bound approximate 95 % bivariate distributions. The diagonal line in panel A marks where the pairs would be expected to lie if the measurements of the two measurands were perfectly correlated; in panels B and C the lines mark where the pairs would be expected to lie if the measurements were perfectly anti-correlated.

Hypothesis: Origin of Correlation between Moisture and PAH Content

Excepting results submitted by UME, panels B and C of Figure 3 suggest that there may be significant negative correlation between moisture content (as estimated by mass loss on drying) and the BaA and BaP results: the greater the reported moisture content, the lower the reported PAH levels. To check whether this relationship is explained by the differing values reported for moisture content, Figure 4 redisplays the panels with the PAH results adjusted to an as-received basis: $PAH_{\text{as received}} = PAH_{\text{dry}} \times (100\text{-}\%\text{Water})/100$.

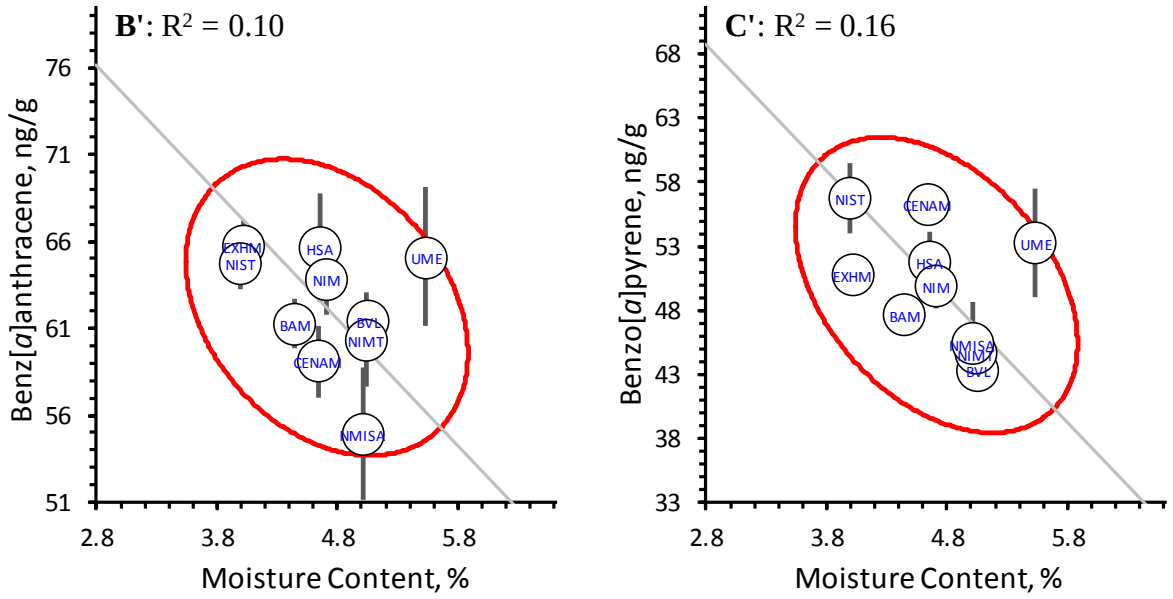


Figure 4: Relationship Between Adjusted PAH and Water Content Results

Panels B' and C' are analogous to Figure 3 panels B and C, but with the as-reported BaA and BaP mass fractions adjusted to reflect "as received" values. Otherwise the formats are identical to that in Figure 3.

The major effect of this adjustment is to decrease the PAH results by about 5 %. But rather than weakening the correlation between moisture and PAH content, the adjustment to an "as-received" basis slightly strengthens it (slight increase in R^2 values).

Panel A of Figure 5 summarizes NIST's study of moisture content in the CCQM-K95.1 source materials as determined by mass loss on drying. Four drying methods were evaluated: in a desiccator over $Mg(ClO_4)_2$, in a desiccator over P_2O_5, a drying oven for 2 hours at 90 °C, and two seven-day freeze drying cycles. While providing the lowest value for moisture content, drying over P_2O_5 approached a constant value reasonably quickly and was deemed the procedure likeliest to provide uniform results across participants. The moisture determination protocol therefore specified that "A minimum of three subsamples (recommended sample size of 1 g each) of the tea should be dried over anhydrous phosphorous pentoxide in a desiccator at room temperature until a constant mass is reached." The protocol did not indicate the diameter of the vessel containing the subsample (which impacts the exposed surface area) nor how long this process was expected to take. Panel B of Figure 5 summarizes a drying study performed by UME. Unfortunately, it is apparent that the specified protocol did not provide the desired uniformity.

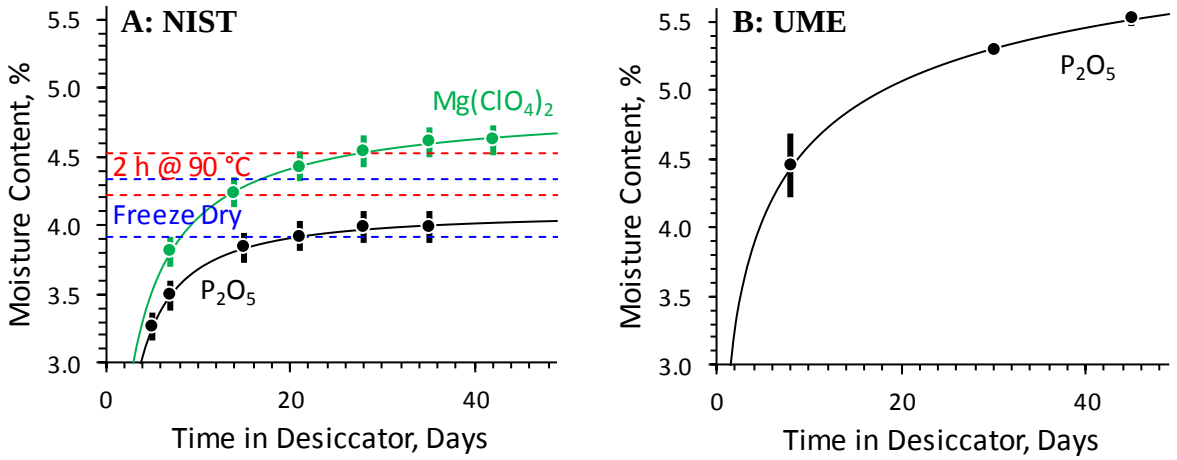


Figure 5: Studies of Mass Loss on Drying

Panel A summarizes studies conducted at NIST; panel B summarizes a study done at UME. In both panels, the dots&bars represent the mean and standard deviation of desiccator-drying measurements on multiple independent subsamples. The horizontal dashed red lines in panel A represent the [mean - standard deviation, mean + standard deviation] range of two-hour oven drying at 90 °C. The horizontal dashed blue lines in Panel A represent the [mean - standard deviation, mean + standard deviation] range after two seven-day freeze drying cycles.

UME chose to base their moisture content on their observed loss at 45 days. Excluding their long-duration UME results from the calculation, the squared-correlation (R^2) between moisture content and BaA rises to 0.41 and that to BaP to 0.50. Since the R^2 between an independent and dependent variable is a direct measure of explained variance, the variability in water content accounts for more than 40 % of the variability in the measured PAH mass fractions. This strongly suggests a relationship between measured water content and the PAH mass fractions. It does not, however, identify what that relationship could be.

If the sample materials were heated or dried at any time prior to extraction, the PAHs in the sample material may have been rendered resistant to extraction. If so, the water content as measured by P_2O_5 drying could be an alias for extraction resistance. However, we do not have any information on how the sample materials were handled during shipping to the participants and after receipt by the participants.

Hypothesis: Origin of Correlation Between BaA and BaP Content

The modest positive correlation between the BaA and BaP mass fractions is suggestive of some common cause. Given the independence of the chromatographic separation and mass-spectrometric quantification of BaA and BaP, the most likely commonality is extraction completeness. Figure 6 redisplays the data of Figure 3 panel A, annotated with extraction technique, temperature, and duration. The diversity of techniques, solvent systems, and conditions forestalls any quantitative analysis, but in general the higher temperature and/or longer duration methods extracted somewhat more of both BaA and BaP. Note that NMISA's recovery-adjusted results plot in the upper-right corner of the figure.

Appendix I provides details of NIST's extraction efficiency studies. This information was requested by the OAWG at its April 2017 meeting and was sent to CCQM-K95.1 participants 8-May-2017.

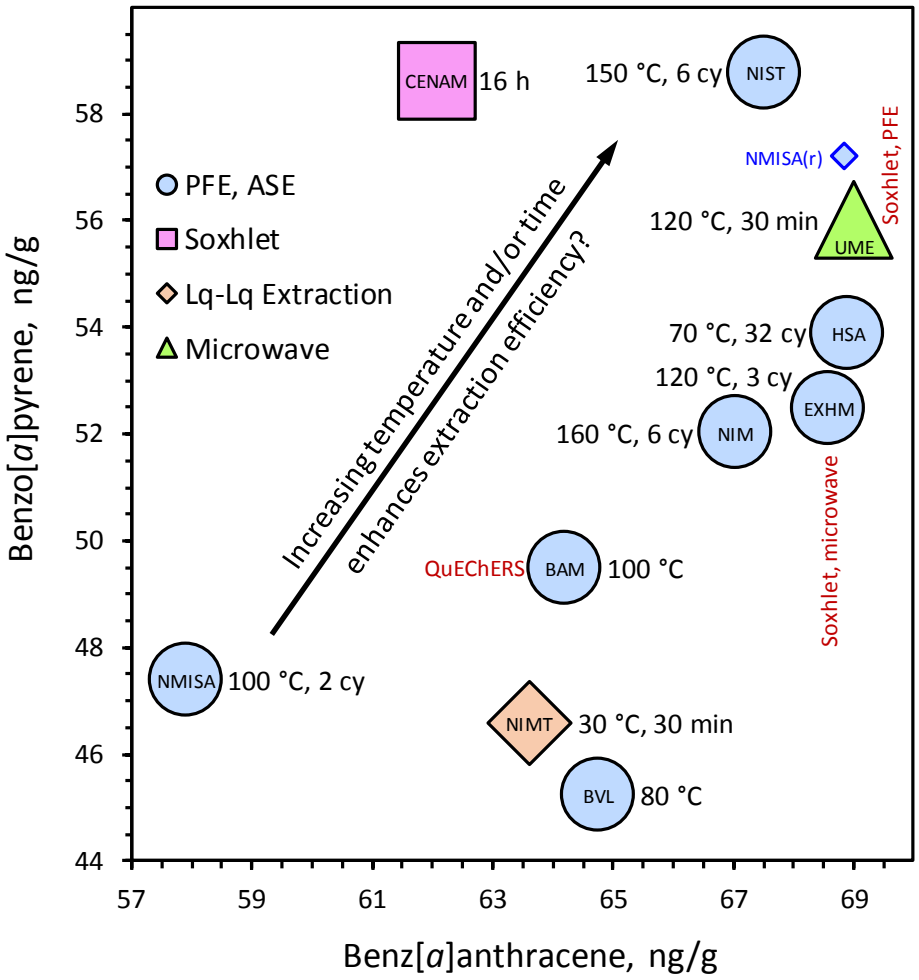


Figure 6: Possible Origin of Correlation Between BaA and BaP Results

Symbols identify the primary extraction technique used by each participant. The available temperature and extraction duration information for the method are provided in black font. Secondary extraction techniques used by some participants are specified in small red font.

The protocol for CCQM-K95.1 (Appendix B) reminded participants that "the presence of trace amounts of water in the solvent or wetting of the sample prior to extraction was critical for complete extraction of the beta-endosulfan and endosulfan sulphate from the tea matrix". However, only three participants report including water in their extraction protocols (Appendix F) for CCQM-K95.1: BAM, HSA, and NIMT. BAM explicitly studied the issue using two methods, one with additional water and one without, and found no significant difference. The different response to including water in the extraction methods does not appear related to the intrinsic mass-fraction moisture content of the botanical materials used: about 7 % in CCQM-K95, 5 % in CCQM-K95.1. It can reasonably be concluded that extraction completeness is a complex function of the matrix and the analytes, with new materials presenting potentially unique challenges.

Hypothesis: Correlation Between CCQM-K95.1 and CCQM-K131 Results
BaA and BaP were also measurands in the parallel CCQM-K131 KC that addressed quantification of PAH calibration solutions. Nine of the ten participants in CCQM-K95.1 also participated in CCQM-K131, although not all analysts in K95.1 were involved in K131.

Five of the nine participants used the same or similar calibrants in both studies: BVL, CENAM, HSA, NIM, and UME. If the analysts at each of the participating NMI used a common approach to calibration in the two studies, systematic differences between participants would be expected to cause the measurement results to be positively correlated. Figure 7 compares the results for the two PAHs. For BaP, panel B provides some evidence for this. For BaA, panel A also provides some evidence of systematic effects; however, the correlation is unexpectedly negative. This behavior is unexplained.

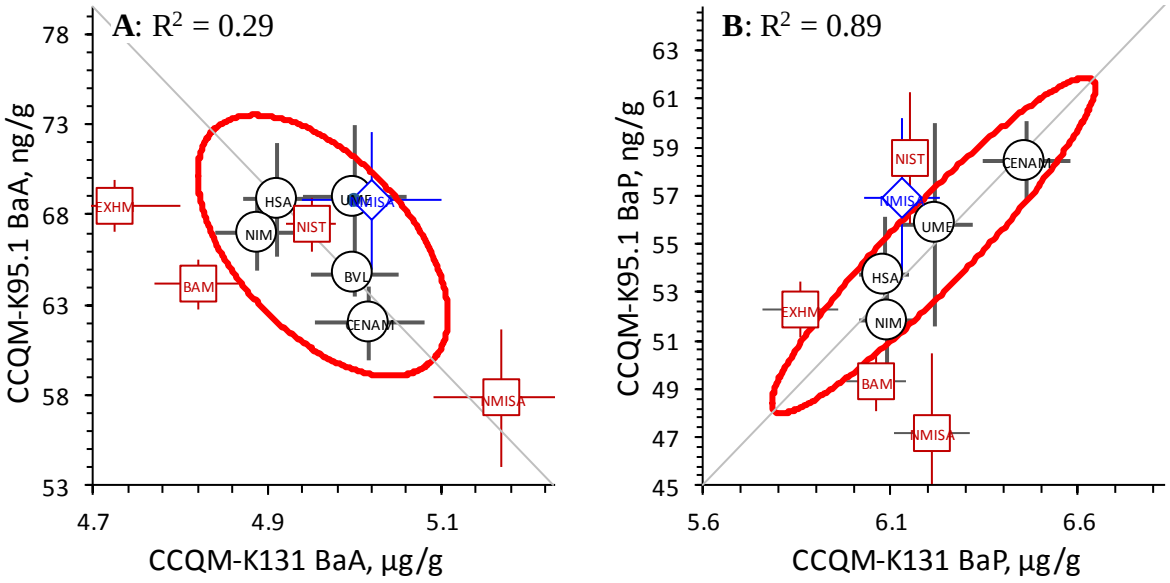


Figure 7: Comparison Between CCQM-K131 and CCQM-K95.1 Results

Each panel displays the bivariate distribution of the reported results for one measurand in the two KCs, with the results in CCQM-K131 along the X-axis and in CCQM-K95.1 along the Y-axis. Panel A displays results for BaA; panel B for BaP. Open symbols each represent a {CCQM-K131, CCQM-K95.1} pair. Black circles denote the NMIs that reported using the same calibrants in the two studies; dark red squares denote NMIs that did not report using the same calibrants; the blue diamonds denote NIMSA's corrected K131 and recovery-adjusted K95.1 results. The bars span $x_i \pm u(x_i)$. The ellipses bound approximate 95 % bivariate distributions about the NMIs that used the same calibrants. The diagonal line in panel A marks where the pairs would be expected to lie if the measurements of the two measurands were perfectly anti-correlated; in panel B the line marks where the pairs would be expected to lie if the measurements were perfectly correlated. BVL withdrew their BaP result for CCQM-K131 and so is not displayed in panel B.

Performance Relative to Past Studies
Figure 8 displays CCQM-K95 and CCQM-K95.1 participant results.

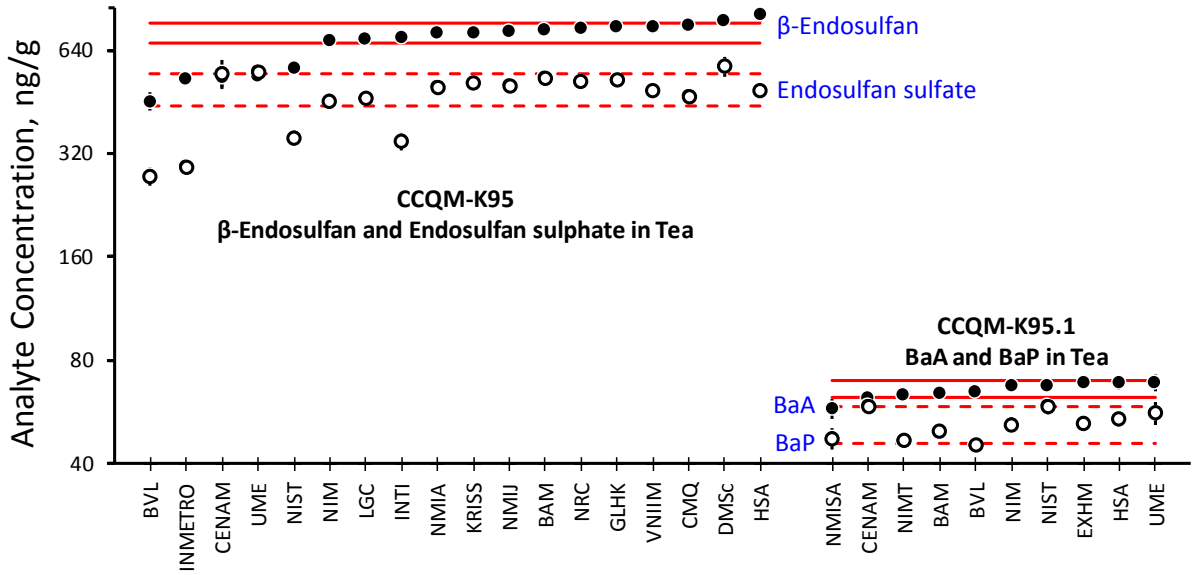


Figure 8: Participant Results in CCQM-K95 and CCQM-K95.1

Figure 9 displays summary estimates of measurement dispersion, estimated as the robust MAD_E, as a function of measurement value, estimated as the robust median, relative to the "Horwitz Curve" and a constant CV of 9 %. The empirical Horwitz function describes the inter-laboratory variability expected for a given mass fraction of an arbitrary analyte in an arbitrary matrix.

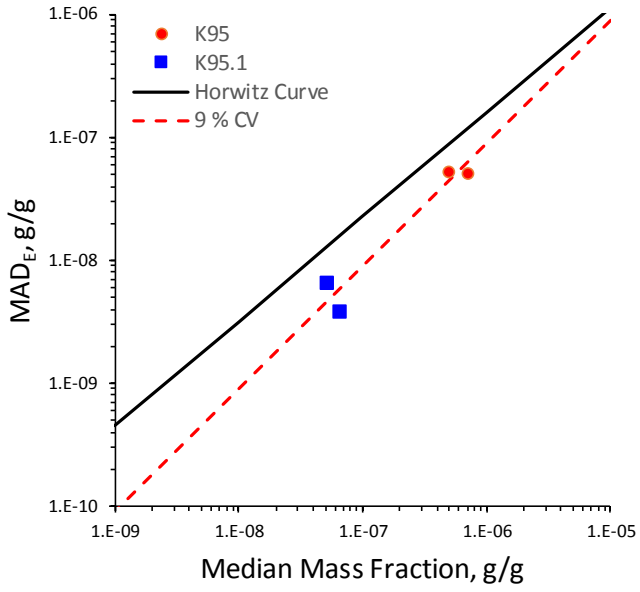


Figure 9: Summary Performance Relative to the Horwitz Curve and 9 % CV

The CCQM-K95.1 summary results are consistent with the measurement performance demonstrated in CCQM-K95.

KEY COMPARISON REFERENCE VALUE (KCRV)

The lack of overlap among the $U(x)$ 95 % expanded uncertainties for both BaA and BaP implies that the reported uncertainties do not fully account for the between-participant variance, often termed "excess variance" or "dark uncertainty" [3,4]. However, for both analytes all values appear to be drawn from compact uniform distributions with no obvious "outliers." For such data, use of robust estimators of location and dispersion such as the median and adjusted median absolute deviation from the median (MAD_E) are inappropriate [3]. The choice of appropriate estimators for the KCRV therefore depends upon whether or not the reported measurement uncertainties are deemed credible for the purpose of establishing the KCRV: 1) if not all are credible, the simplest appropriate estimator is the equally-weighted arithmetic mean and its standard deviation-based uncertainty and 2) if most are considered credible, recommended estimators that properly account for excess variance include the DerSimonian-Laird variance-weighted mean (DL-mean) [3] and hierarchical Bayesian analysis (Bayes) [5].

Table 7 lists the candidate locations, X, and standard uncertainties, $u(X)$, calculated using the relevant equations in [3] for the arithmetic mean and DL-mean and the NIST Consensus Builder [6] for Bayes. The approximate 95 % expanded uncertainties, $U_{95}(X)$, on the mean and DL-mean are estimated as: $U_{95}(X) = t_s \cdot u(X)$, where $t_s \approx 2.26$ is the Student's t two-tailed expansion factor for 9 degrees of freedom and 95 % coverage. The $U_{95}(X)$ for the Bayes location is estimated as one-half of the location's 95 % credible interval.

Table 7: Candidate Key Comparison Reference Values

		Benz[*a*]anthracene, ng/g			Benzo[*a*]pyrene, ng/g		
Estimator	u?[a]	X	$u(X)$	$U_{95}(X)$[b]	X	$u(X)$	$U_{95}(X)$[b]
Mean	No	65.35	1.13	2.56	52.06	1.53	3.47
DL-Mean	Yes	65.59	0.85	1.92	51.85	1.55	3.51
Bayes	Yes	65.57	0.96	1.94	51.85	1.76	3.51

a) Does the estimator utilize the information in the reported uncertainties?
b) $U_{95}(X) = t_s \cdot u(X)$, where t_s is the appropriate two-tailed Student's t critical value for 95 % coverage.

At the September 2017 meeting in Ottawa, Canada, the OAWG agreed that there were no identifiable "outlier" values and that the reported uncertainties were mostly credible. Following recommendations in [3], the OAWG elected to use the DL-Mean for both measurands. Figure 10 displays the KCRV values and uncertainties for BaA and BaP.

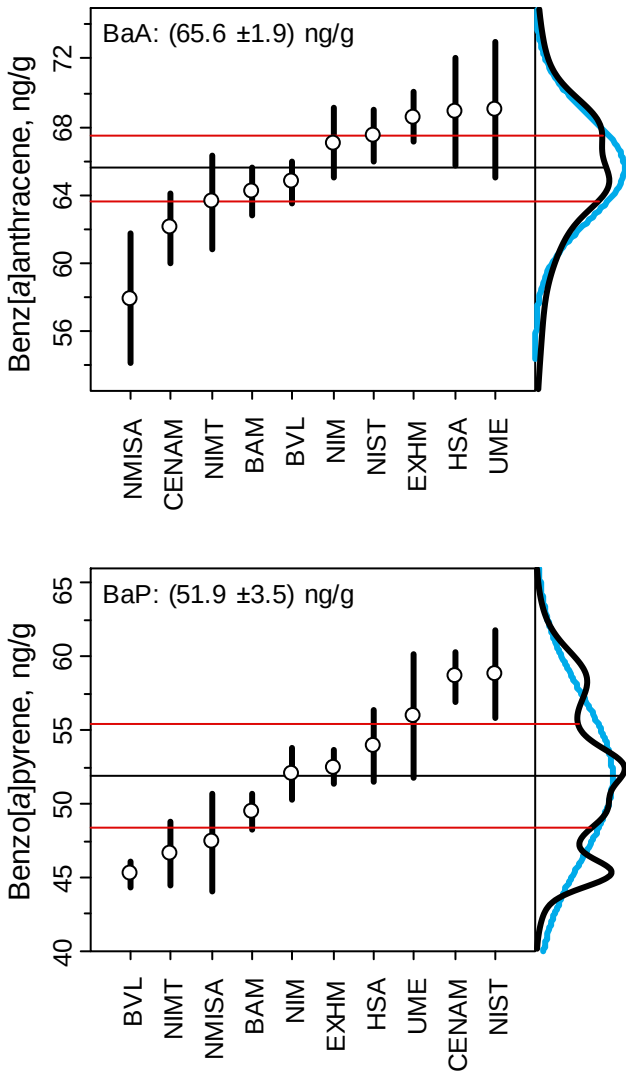


Figure 10: KCRVs

These panels display KCRV relative to the reported results for benz[*a*]anthracene (BaA) and benzo[*a*]pyrene (BaP). In both 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 KCRV. The bracketing red lines denote the approximate 68 % level of confidence interval (± 1 *u*(KCRV)) about the KCRV. The black curve along the right edge of each panel is the empirical probability density function for the reported values. The blue curve along the right edge represents the probability density function for a normal distribution having mean KCRV and standard deviation U_{95}(KCRV)/2.

DEGREES OF EQUIVALENCE (DoE)

The absolute degrees of equivalence for the participants in CCQM-K95.1 are estimated as the signed difference between the combined value and the KCRV: $d_i = x_i - \text{KCRV}$. Since the KCRV is estimated from consensus of all results, the nominal k=2 expanded uncertainty on the d_i, $U_{k=2}(d_i)$, is estimated as twice the square root of the sum of the squares of the standard uncertainties of the two components minus twice the covariance between the x_i and the KCRV:

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

To enable comparison with the degrees of equivalence estimates from other studies, it is convenient to express the d_i and $U_{k=2}(d_i)$ as percentages relative to the KCRV: $\%d_i = 100 \cdot d_i/\text{KCRV}$ and $U_{k=2}(\%d_i) = 100 \cdot U_{k=2}(d_i)/\text{KCRV}$. Table 8 lists the numeric values of d_i, $U_{k=2}$ (d_i), d_i, and $U_{k=2}(d_i)$ for all participants in CCQM-K95.1 for both BaA and BaP. Figure 11 displays the estimated DoE for the two CCQM-K95.1 measurands relative to the KCRVs. Each panel displays both the absolute $d_i \pm U_{k=2}(d_i)$ and relative $\%d_i \pm U_{k=2}(\%d_i)$.

Table 8: Degrees of Equivalence for Measurands

	Benz[*a*]anthracene, ng/g				Benzo[*a*]pyrene, ng/g			
NMI/DI	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$	*d*	$U_{k=2}(d)$	*%d*	$U_{k=2}(\%d)$
BAM	-1.4	4.2	-2.1	6.5	-2.4	9.1	-4.5	17.5
BVL	-0.8	4.0	-1.3	6.2	-6.6	8.9	-12.7	17.3
CENAM	-3.5	5.2	-5.4	7.9	6.8	9.4	13.1	18.1
EXHM	3.0	4.3	4.5	6.6	0.6	9.1	1.2	17.5
HSA	3.3	7.0	5.1	10.7	2.0	10.0	4.0	19.4
NIM	1.4	5.2	2.2	7.9	0.2	9.5	0.3	18.3
NIMT	-2.0	6.3	-3.0	9.6	-5.3	9.8	-10.1	18.9
NIST	1.9	4.3	2.9	6.6	6.9	10.3	13.4	19.9
NMISA	-7.7	8.2	-11.7	12.6	-4.5	11.0	-8.6	21.2
UME	3.4	8.6	5.2	13.1	4.1	12.1	8.0	23.4

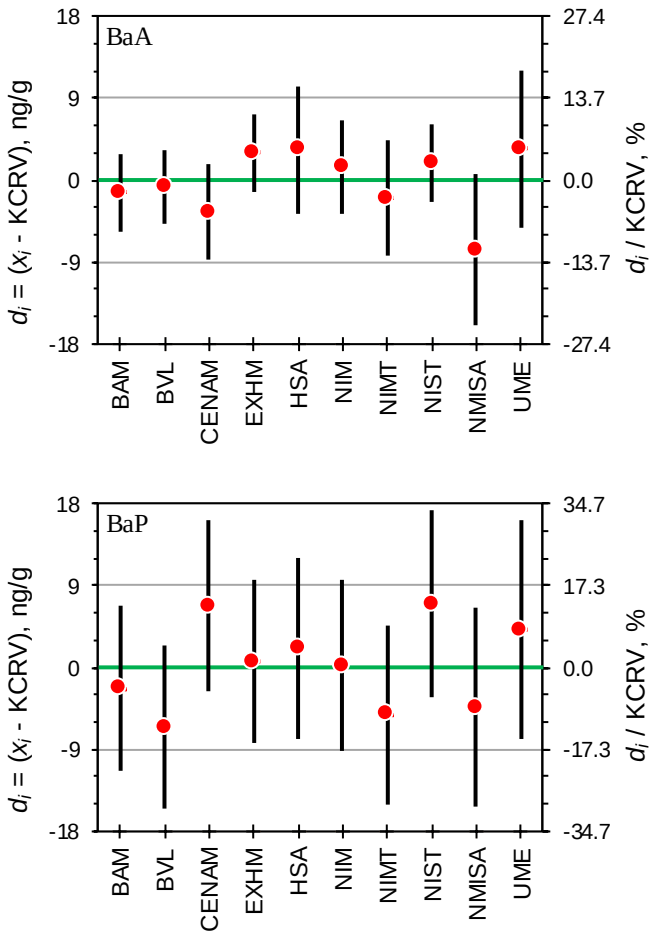


Figure 11: Degrees of Equivalence

These panels display the Degrees of Equivalence (DoE) for benz[*a*]anthracene (BaA) and benzo[*a*]pyrene (BaP) basing the KCRV on the DerSimonian-Laird variance-weighted mean (DL-Mean). Results are sorted alphabetically by participant acronym. Dots represent the DoE, bars their approximate 95 % expanded uncertainties, U_{95}(DoE).

The axis to the left edge of each panel displays the absolute DoE, *d*, in units of µg/g. The axis to the right edge displays the relative DoE, %*d* = 100·*d*/KCRV, as percent. The thick green horizontal line denotes perfect agreement with the KCRV.

Composite Degrees of Equivalence for Participants

All participants in CCQM-K95.1 reported results for two measurands. A combined relative DoE, %*D*, can be estimated from the relative degrees of equivalence, %d_i, of the measurands they reported using the NICOB "Linear Pool" estimator [6]. This estimator models each %d_i as a N(%d_i, u^2(%d_i)) normal (Gaussian) distribution and combines them into a single distribution.

Table 9 lists the %*D* values for the ten participants. The composite distributions are not necessarily unimodal or symmetric, so are best characterized using their (2.5, 50, and 97.5) % percentiles: $D_{2.5\%}$, $D_{50\%}$, and $D_{97.5\%}$. The median, $D_{50\%}$, is a robust estimate for the participant DoE; the interval from $D_{2.5\%}$ to $D_{97.5\%}$ is its 95 % uncertainty interval.

If a symmetric 95 % expanded uncertainty for %D is required, a conservative estimate is

$$U_{95}(\%D) = \mathrm{MAX}(D_{50\%} - D_{2.5\%},\ D_{97.5\%} - D_{50\%})$$

where "MAX" is the function "return the maximum of the two values." Figure 12 displays the %D and 95 % uncertainty interval for each participant.

Table 9: Composite Relative Degrees of Equivalence for Participants, %D

NMI/DI	$\%D_{2.5\%}$	$\%D_{50\%}$	$\%D_{97.5\%}$	$U_{95}(\%D)$
BAM	-19.0	-2.8	9.9	16
BVL	-27.0	-4.3	4.6	23
CENAM	-12.0	0.2	28.0	28
EXHM	-13.2	3.6	15.6	17
HSA	-12.3	4.7	20.2	17
NIM	-14.6	1.7	15.5	16
NIMT	-25.6	-5.4	7.2	20
NIST	-4.3	5.5	29.6	24
NMISA	-26.8	-10.6	9.0	20
UME	-12.0	6.2	27.5	21

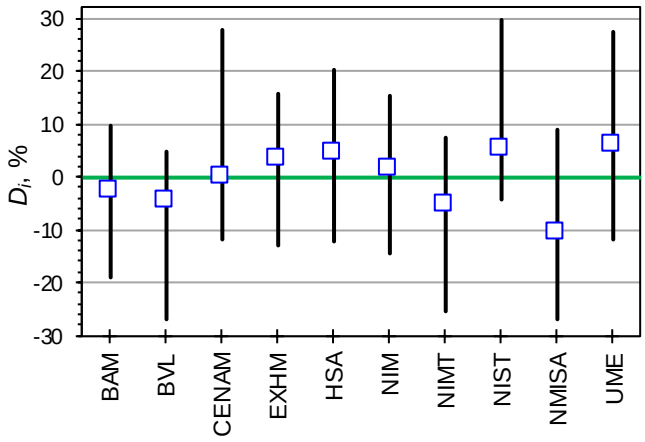


Figure 12: Composite Relative Degrees of Equivalence for Participants

The open squares represent the relative Degrees of Equivalence for each participant, estimated from the composite distribution of the relative DoE, %d = 100×d/KCRV, for the analytes reported by the participant. The bars span the central 95 % of the composite distribution. Results are sorted alphabetically by participant acronym.

USE OF CCQM-K95.1 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in CCQM-K95.1 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molar mass of 100 g/mol to 500 g/mol and having polarity $pK_{ow} < -2$, in a botanical matrix ranging in mass fraction from 10 ng/g to 1000 ng/g:

1) value assignment of primary reference standards (if in-house purity assessment carried out),
2) value assignment of single and/or multi-component organic solutions,
3) extraction of analytes of interest from the matrix,
4) cleanup and separation of analytes of interest from interfering matrix or extract components,
5) separation and quantification using gas or liquid chromatographic analytical systems.

Core Competency Statements

Tables 10a to 10j list the Core Competencies claimed by the participants in CCQM-K95.1. 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 F.

CCQM is considering the application of "broader-scope" Calibration and Measurement Capabilities (CMCs). Appendix J presents a possible prototype "broader-scope" CMC that could be claimed based on successful participation in CCQM-K95.1 and relevant previous CCQM Key Comparisons.

Table 10a: Core Competencies Demonstrated in CCQM-K95.1 by BAM

CCQM-K95.1	***BAM***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**

Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.

Competency	**✓, ×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Calibration solution, NIST SRM 2260a
Identity verification of analyte(s) in calibration material	✓	GC-MS, mass spectra and retention time
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	✓	GC-MS, mass spectra and retention time
Extraction of analyte(s) of interest from matrix	✓	Pressurized liquid extraction (ASE), liquid/solid extraction (modified QuEChERS)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Gel permeation chromatography (GPC), Solid phase extraction (SPE), QuEChERS
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) internal standard (deuterated or ^{13}C labelled) b) 5- to 8-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample	N/A	
Other	N/A	

Table 10b: Core Competencies Demonstrated in CCQM-K95.1 by BVL

CCQM-K95.1	***BVL***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**
Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.		
Competency	**✓, ×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Calibration solution, NIST SRM 2260a
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, mass ratio
Extraction of analyte(s) of interest from matrix	✓	ASE (*n*-hexane/acetone 50:50)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	1) SPE (silica) 2) SPE (styrene-divinylbenzene polymeric sorbent)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-HRMS (R=10,000)
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) 4-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample	N/A	
Other	N/A	

Table 10c: Core Competencies Demonstrated in CCQM-K95.1 by CENAM

CCQM-K95.1	***CENAM***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**

Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.

Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Highly pure substances BaA: Supelco; BaP: Ultrascientific
Identity verification of analyte(s) in calibration material	✓	GC-MS/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s)	✓	Mass Balance approach: organic impurities; GC FID with two columns, water content; Karl Fischer titration
For calibrants which are a calibration solution: Value-assignment method(s)	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and MRM ion pairs
Extraction of analyte(s) of interest from matrix	✓	Automated Soxhlet Extraction, 1:1 hexane:acetone
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Filtration with PVDF acrodisc of 0.2 μm
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	NA	
Analytical system	✓	GC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample	N/A	
Other	N/A	

Table 10d: Core Competencies Demonstrated in CCQM-K95.1 by EXHM

CCQM-K95.1	***EXHM***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**
Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Calibration solution, NIST SRM 2260a
Identity verification of analyte(s) in calibration material	✓	GC-IT-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s)	N/A	
For calibrants which are a calibration solution: Value-assignment method(s)	✓	used NIST assigned values
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	retention time, MRMs, ion ratios
Extraction of analyte(s) of interest from matrix	✓	ASE (also Soxhlet, saponification, ultrasound)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	l-l extraction – SPE with molecularly imprinted polymers
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-IT-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS single-point exact matching
Verification method(s) for value-assignment of analyte(s) in sample	✓	in-house prepared PAH solutions
Other	N/A	

Table 10e: Core Competencies Demonstrated in CCQM-K95.1 by HSA

CCQM-K95.1	***HSA***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**
Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.		
Competency	**✓, ×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Highly pure substances BaA:BCR-271, IRMM BaP: SCB-007, Cerilliant
Identity verification of analyte(s) in calibration material	✓	Comparison with NIST SRM 2260a based on retention time and m/z ratio
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s)	✓	The purity of the BaP was determined in-house using mass balance approach. The purity of BaA was verified with NIST SRM 1647f.
For calibrants which are a calibration solution: Value-assignment method(s)	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention times, SIM mode with one ion on GC-MS.
Extraction of analyte(s) of interest from matrix	✓	Accelerated solvent extraction (ASE) *n*-hexane/acetone (1:1, v/v)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Solid phase extraction (SPE)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	Agilent 7890A/5975C GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS single-point exact-matching
Verification method(s) for value-assignment of analyte(s) in sample	✓	ERM-CZ100, IRMM Fine Dust analyzed in parallel with each tea sample for quality control
Other	N/A	

Table 10f: Core Competencies Demonstrated in CCQM-K95.1 by NIM

CCQM-K95.1	***NIM***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**
Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Highly-pure substance BaA & BaP from Cerilliant
Identity verification of analyte(s) in calibration material	✓	GC-MS, NMR
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s)	✓	GC-FID, HPLC-DAD, qNMR
For calibrants which are a calibration solution: Value-assignment method(s)	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Comparison of both retention time and mass spectrum in GC-MS
Extraction of analyte(s) of interest from matrix	✓	ASE was used as extraction method.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	GPC and SPE were used as cleanup method
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	HPLC-DAD and GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	GC-IDMS single point with BaA-$^{13}C_6$, BaP-$^{13}C_4$ as IS.
Verification method(s) for value-assignment of analyte(s) in sample	N/A	
Other	N/A	

Table 10g: Core Competencies Demonstrated in CCQM-K95.1 by NIMT

CCQM-K95.1	***NIMT***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**
Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		highly-pure substances BaA: BCR-271 BaP: AccuStandard
Identity verification of analyte(s) in calibration material	✓	GC-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s)	✓	mass balance using normalized GC, HPLC, TGA, KFT
For calibrants which are a calibration solution: Value-assignment method(s)	✓	Gravimetric
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Comparison GC-MS retention times and m/z with BCR-271 BaA and BaP standards.
Extraction of analyte(s) of interest from matrix	✓	Liquid-liquid extraction with 1:1 (v/v) hexane/acetone
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Silica SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) 6-point calibration curve, the isotope ratio in sample blends were controlled to be close to 1.0.
Verification method(s) for value-assignment of analyte(s) in sample	N/A	
Other	N/A	

Table 10h: Core Competencies Demonstrated in CCQM-K95.1 by NIST

CCQM-K95.1	***NIST***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**
Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.		
Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		calibration solution, NIST SRM 2260a
Identity verification of analyte(s) in calibration material	✓	GC-MS (retention time and mass spectra)
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s)	N/A	
For calibrants which are a calibration solution: Value-assignment method(s)	✓	used NIST SRM 2260a assigned values, confirmed with NIST SRM 1647f
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention times and MS (SIM mode with presence of quantitation ion and confirmation ion)
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
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS with IS concentrations similar to the samples using BaA-d_{12} & BaP-d_{12} b) average response factor of six bracketing calibrants
Verification method(s) for value-assignment of analyte(s) in sample	✓	NIST SRM 2585 as a control.
Other	N/A	

Table 10i: Core Competencies Demonstrated in CCQM-K95.1 by NMISA

CCQM-K95.1	***NMISA***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**

Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.

Competency	**✓,×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Calibration solutions: BaA; IRMM BCR-271 BaP; NMIJ CRM 4213-a NIST SRM 2260a
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 spectrum ion ratio's relative to NIST SRM 1647f
Extraction of analyte(s) of interest from matrix	✓	ASE using 3:1 Dichloromethane and hexane
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	ASE using in-cell clean-up with activated florisil and alumina
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-TOFMS and GCxGC-TOFMS
Calibration approach for value-assignment of analyte(s) in matrix	✓	double IDMS and standard addition using deuterated PAHs and various NMI-CRMs
Verification method(s) for value-assignment of analyte(s) in sample	✓	IDMS using the QuEChERS extraction technique was used for confirmation. NIST SRM 1944 New York/ New Jersey Waterway sediment as well as spiked green tea was used as quality control samples.
Other	N/A	

Table 10j: Core Competencies Demonstrated in CCQM-K95.1 by UME

CCQM-K95.1	***UME***	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**
Scope of Measurement: Participation in Track A study "Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea" is intended to demonstrate measurement capabilities similar to those demonstrated by participation in CCQM-K95, including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study can be used to demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.		
Competency	**✓, ×, or N/A**	**Specific Information**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Highly pure substances BaA, Supelco 4-8563 BaP, Supelco 4-8564
Identity verification of analyte(s) in calibration material	✓	GCMS/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s)	✓	qNMR, traceability through UME CRM 1301 Chloramphenicol Primary Calibrant NIST SRM 2260a was used for confirmation
For calibrants which are a calibration solution: Value-assignment method(s)	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, Parent/Product Ion
Extraction of analyte(s) of interest from matrix	✓	Soxhlet, Microwave Assisted Extraction, Ultrasonic, PSE
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE ($LCNH_2$)
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) 6-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample	✓	NIST SRM 2585 was used for method validation
Other	N/A	

CONCLUSIONS

Participants in CCQM-K95.1 demonstrated their ability to identify and quantify low volatility non-polar organic compounds in a botanical matrix at mass fractions of a few ng/g. All 20 reported results had degrees of equivalence that overlapped zero. The central 50 % of all reported results were within ± 5 % of the consensus KCRVs with a median 95 % expanded uncertainty of 15 %.

Participant results for BaA and BaP do not overlap within their stated 95 % expanded uncertainties, the distributions of the results for the two measurands appear to be rectangular, and the results for BaA and BaP are somewhat correlated with each other. While results for both measurands are anti-correlated with water content, the relationship is not directly related to the as-received to dry-mass adjustment. There is some evidence that the between-participant differences are related to extraction completeness.

The best-fit relationship between the robust averages, estimated as the median, and the robust standard deviations, estimated by the MAD_E, for the CCQM-K95 and CCQM-K95.1 measurands is a CV of 9 % over mass fractions from (50 to 900) ng/g.

ACKNOWLEDGEMENTS

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

REFERENCES

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APPENDIX A: Call for Participation

From: Lindsey.Mackay@measurement.gov.au
Date: Tue, July 21, 2015 8:14 AM -0400

To: [OAWG contact list]

Subject: Comparison protocols for CCQM-K131 and K95.1 (PAHs in solution and tea)

Dear OAWG colleagues

Please find attached the protocols and associated documentation for two upcoming key comparisons. Many thanks to Steve Wise at NIST for providing them and for coordinating these studies.

CCQM-K95.1 is a repeat of the Track A comparison for pesticides in tea, the specific measurand in this case is PAHs in tea. Only laboratories that would like to improve upon their performance in the original CCQM-K95 comparison need to participate.
The second comparison is a Track A comparison for non-polar analytes in organic solutions, in this case the measurand is PAHs in Acetonitrile. All NMIs/DIs with CMCs related to the How Far the Light Shines statement for this Track A comparison would be expected to participate.
Both comparisons will occur in the Sep – Dec 2015 timeframe. Please complete the attached registration forms and return them to stephen.wise@nist.gov by 28 August.

Best regards
Lindsey

Attachments:
CCQM 95_1 Participant Registration form.docx
CCQM K95_ 1 PAH in Mate Tea protocol July2015.docx
K95_1 Core Competency Table .doc
Reporting Form CCQM-K95_1.xlsx
CCQM K131 PAH in Acetonitrile protocol July2015.docx
CCQM K131 Participant Registration.docx
K131 Core Competency Table .doc
Reporting Form CCQM-K131.xlsx

APPENDIX B: Protocol

CCQM-K95.1 Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea

Key Comparison
Track A

Coordinating Laboratory: NIST
Study Protocol
July 20, 2015

Introduction

CCQM-K95 "Mid-Polarity Analytes in Food Matrix: Mid-Polarity Pesticides in Tea", which was co-organized by the Government Laboratory of Hong Kong and the National Metrology Institute of China, was conducted in 2012. CCQM-K95 was intended as an OAWG core competency study to assess capabilities for determination of mid-polarity contaminants in a food matrix. The intended food-matrix was tea, and the mid-polarity analytes of interest were *beta*-endosulfan and endosulfan sulphate. The study results were first reported at the April 2012 meeting of the OAWG, and the final report for this study was presented at the OAWG meeting in October 2014. Of the 18 participants in the study, results from almost half of the participants were not used in the determination of the KCRV for *beta*-endosulfan and endosulfan sulphate for various reasons including incomplete extraction of the analytes from the matrix. Discussion at the OAWG meeting in April 2012 indicated that the presence of trace amounts of water in the solvent or wetting of the sample prior to extraction was critical for complete extraction of the *beta*-endosulfan and endosulfan sulphate from the tea matrix. Several laboratories performed follow up analyses after pre-wetting the matrix and confirmed that their extractions were incomplete. Because of these difficulties and the need to demonstrate measurement capabilities for measurements of contaminants in food, a number of laboratories expressed interest in participating in another similar or related key comparison study.

NIST is currently in the process of developing a certified reference material for polycyclic aromatic hydrocarbons (PAHs) in yerba mate tea. This candidate CRM would be a suitable material for a follow up key comparison. This study would represent low-polarity analytes (PAHs) in a botanical matrix (yerba mate tea).

Study Material

Some brands of yerba mate tea, particularly those produced in South America, undergo a long smoking process, and therefore contain PAHs at quantifiable levels. The candidate CRM is a commercial yerba mate tea. The commercial tea, which contained stems and leaves, was ground in an ultracentrifugation mill. Particle size analysis at NIST indicated that 70 % of the material had a particle size less than 100 µm. The powdered tea was heat-sealed in nitrogen-flushed 4-mil plastic bags, which were then heat-sealed inside a nitrogen-flushed Mylar bag with two packets of silica gel as a desiccant. Each packet contains 10 g ± 0.1 g of powdered tea.

Measurands

At the April 2014 OAWG meeting, the appropriate PAH measurands were discussed. M. Ricci (IRMM) suggested that recent EU requirements for PAHs in food be considered, which include benz[*a*]anthracene, benzo[*a*]pyrene, and benzo[*ghi*]perylene. At the October OAWG meeting, the decision was made to focus on only two PAHs for the study: benz[*a*]anthracene and benzo[*a*]pyrene, which represent a four-ring *cata*-condensed PAH of molar mass 228 g/mol and a five-ring *peri*-condensed PAH of molar mass 252 g/mol.

Homogeneity and Stability Assessment

The homogeneity of the tea material was assessed at NIST by analyzing duplicate 300 mg subsamples from each of 10 packets of tea (n = 20). The material was extracted using Soxhlet extraction using hexane/acetone (1:1, v:v) for 20 h. The extracts were concentrated to 5 mL and centrifuged. The supernatant was eluted through an amino SPE cartridge with 20 % dichloromethane in hexane and the eluent concentrated to 0.5 mL. The samples were analyzed by using GC-MS; SRM 2260a Aromatic Hydrocarbons in Toluene was used as a calibrant. Based on the measurements from the homogeneity assessment, the target mass fraction ranges are as follows: benz[*a*]anthracene (50 ng/g – 80 ng/g) and benzo[*a*]pyrene (50 ng/g – 80 ng/g). Results of the homogeneity study are summarized in Table 1.

Table 1. Results of the homogeneity assessment for target PAHs in yerba mate tea

	Benz[*a*]anthracene	Benzo[*a*]pyrene
SD (%)	4.2	4.0
SD (Mean) %	1.0	0.7
S_{btw}	0.8	0.0
S_{wth}	4.2	4.6
F	1.07	0.49
P-value	0.45	0.85

The results of the homogeneity assessment indicated that SD (mean) was less than 1 % for the target benz[*a*]anthracene and benzo[*a*]pyrene. One-way ANOVA was used to test whether there were significant between-packet differences in the concentration of either of the two proposed measurands. All of the estimated within-replicate standard deviations, S_{wth}, proved to be larger than the between-packet standard deviations, S_{btw}. The values of the relevant F-test ratios, F, are uniformly small. Under the assumption that all packets have the same composition, the probability of observing each ratio, P-value, is comfortably larger than the usual critical 0.05 confidence level. We therefore conclude that at least for the two PAHs to be measured, there are no significant between-packet concentration differences and that the material is suitably homogeneous for this study.

NIST has not performed a formal stability study for this tea material. However, we have not observed any stability issues with PAHs in other natural matrix CRMs (e.g., sediment, atmospheric particulate matter, and diesel particulate matter). Therefore, we anticipate that the PAHs would be stable in the tea material during the period of the study.

Methods
PAHs must be removed from the yerba mate tea matrix by solvent extraction, and some cleanup of the extract will be necessary to prepare the sample for analysis. Participants are anticipated to perform measurements by isotope-dilution gas chromatography-mass spectrometry (GC-MS); however, other techniques such as liquid chromatography (LC) may be used.

Reference Standards Available
Solution CRMs for the target PAHs are available from NIST (SRM 2260a PAHs in Dichloromethane) and SRM 1647f Priority Pollutant PAHs in Acetonitrile (both benz[*a*]anthracene and benzo[*a*]pyrene, IRMM (ERM-AC213) (benz[*a*]anthracene only), and NMIJ (CRM 4313) (benzo[*a*]pyrene only). Isotopically-labeled (deuterium or carbon-13) PAHs for use as internal standards are commercially available from a number of sources.

There are no suitable plant-matrix CRMs available for determination of PAHs. However, there are several particulate matter CRMs available with certified values for the four target PAHs. Examples of such CRMs include: ERM CZ100 PM_{10}-like Fine Dust (IRMM), SRM 1649b Urban Dust (NIST), SRM 2786 Fine Particulate Matter (<4 µm) (NIST), SRM 2787 Fine Particulate Matter (<10 µm) (NIST), SRM 2585 House Dust (NIST). However, it should be noted that the mass fractions of PAHs in these CRMs are 10 to 50 times higher than the mass fractions in the tea material. It is recommended that laboratories analyze a matrix CRM in conjunction with the study for quality control and report the results of these analyses as part of the study.

Study Guidelines
Each participant will receive six packets, each containing 10 g of tea (three packets will be required for analysis and three packet are available for practice, screening analysis, and/or moisture determination. Samples can be stored at room temperature. The minimum sample amount for analysis should be at least 300 mg.

Participants are requested to report a single estimate of the mass fraction (ng/g) for the two target PAHs based on measurements for one subsample from each of three packets of the tea material (i.e., three independent replicates). Result should be reported on a dry-mass basis. Therefore the moisture content of the mate tea sample must be determined and reported (see below). Participants may use their preferred laboratory procedures; however, only results based on isotope dilution mass spectrometry will be used to calculate the KCRV.

Moisture Determination
At the October 2014 OAWG meeting, the recommendation was made that all study participants use the same protocol for determination of moisture. After discussions at the meeting, the recommendation was to use phosphorus pentoxide (P_2O_5) as the desiccant rather than calcium sulphate as used in the K95 study due to the long time required to reach constant mass. A minimum of three subsamples (recommended sample size of 1 g each) of the tea should be dried over anhydrous phosphorous pentoxide in a desiccator at room temperature until a constant mass is reached. The moisture determination should be performed concurrent with the analysis of the test sample portions. The correction used for dry-mass conversion should be reported.

Submission of Results

Each participant must provide results using the reporting sheet provided with the samples including a core competency table. The results should be sent via email to the study coordinator (stephen.wise@nist.gov) before the submission deadline. Submitted results are considered final and no corrections or adjustments of analytical data will be accepted unless approved by the OAWG. The results must include: (1) a single estimate of the mass fraction for each of the two PAHs on a dry-mass basis (dry-mass correction should be reported), and (2) the standard and expanded uncertainties with detailed description of the full uncertainty budget. A description of the analytical procedure (extraction, clean-up, GC-MS column and conditions, quantification approach) must be provided in the reporting forms. Details must also be provided concerning calibration and internal standards used with appropriate purity statement and/or laboratory assessment.

How Far Does the Light Shine?

Participation in this Track A study "Low-Polarity Analytes in Botanical Matrix: PAHs in Tea" is intended to demonstrate the same measurement capabilities as CCQM-K95 "Mid-polarity Analytes in Food Matrix: Mid-Polarity Pesticides in Tea". This study would provide the opportunity to demonstrate measurement capabilities similar to those demonstrated by participating in CCQM-K95 including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study would demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.

Time Schedule

This study will be conducted in parallel with K131 Low-Polarity Analytes in a Multicomponent Organic Solution: Polycyclic Aromatic Hydrocarbons (PAHs) in Acetonitrile. Call for participants in K95.1 will be in August 2015. Samples for K95.1 and K131 would be distributed in September 2015. The deadline for submission of results would be December 15, 2015. The first discussion of the results would be during the OAWG meeting in Paris in April 2016.

Update, 24-Feb-2016

From: Wise, Stephen A. [mailto:stephen.wise@nist.gov]
Sent: Wednesday, February 24, 2016 6:15 PM
To: [CCQM-K95.1 participant list]
Cc: Mackay, Lindsey <Lindsey.Mackay@measurement.gov.au>; Lippa, Katrice <katrice.lippa@nist.gov>

Subject: NEW Deadline for CCQM K95.1 PAHs in Tea and K131 PAHs in Acetonitrile

To All:

When we sent the samples for K131 and K95.1 in late December 2015, we proposed a tentative deadline as April 1, 2016 for reporting the results of these studies. However, we have had a shipment delay with one lab and another lab has requested that the deadline be push later due to laboratory renovations. I have discussed this situation with Lindsey Mackay, and we have now moved the deadline for submission of results for both studies until MAY 20, 2016. I hope that this change will give everyone adequate time to complete the study and submit their results. If you have any questions, please let me know.

Thanks

Steve

Stephen A. Wise, Ph.D.
Chemical Sciences Division
National Institute of Standards and Technology (NIST)
100 Bureau Drive MS 8390
Gaithersburg, MD 20899

Phone: 301-975-3112

Update, 25-Apr-2016

From: Duewer, David L. Dr. (Fed)
Sent: Monday, April 25, 2016 3:34 PM
To: [CCQM-K95.1 participant list]
Cc: Mackay, Lindsey <Lindsey.Mackay@measurement.gov.au>; Lippa, Katrice <katrice.lippa@nist.gov>

Subject: NEWEST Deadline for CCQM-K95.1 PAHs in Tea and -K131 PAHs in Acetonitrile

The attached .pptx is the "Update presentation" for the CCQM-K95.1 and –K121 KCs. There are two important bits:

1. Due to shipping/customs issues, the timelines for submitting results have again been pushed back for both studies. The new "must be submitted by" dates are:
   a. CCQM-K95.1: June 20, 2016
   b. CCQM-K131: August 15, 2016

2. Dr. Wise has retired; I am now the acting "Point of Contact" for these two studies and Katrice Lippa is my backup. Please send your measurement results to me, david.duewer@nist.gov, with a CC to katrice.lippa@nist.gov.

I would very much appreciate receiving your results as soon as they are complete!

Dave

David Lee Duewer
Research Chemometrician
Chemical Sciences Division
Materials Measurement Laboratory
National Institute of Standards and Technology
Gaithersburg, MD 20899-8390
david.duewer@nist.gov

Update, 16-Jun-2016

From: Duewer, David L. Dr. (Fed) [mailto:david.duewer@nist.gov]
Sent: Thursday, June 16, 2016 1:16 PM
To: [CCQM-K95.1 participant list]
Cc: Mackay, Lindsey <Lindsey.Mackay@measurement.gov.au>; Lippa, Katrice <katrice.lippa@nist.gov>

Subject: CCQM-K95.1 (PAH in tea): Deadline extended to Aug 15, 2016

Due to shipping delays and reported instrument problems, the deadline for reporting CCQM-K95.1 results is extended to August 15, 2016, the same as for CCQM-K131.

To enable everyone to have a chance to confirm my transcription of the information you provide prior to the Fall meeting, I would VERY MUCH appreciate getting your result forms AS SOON AS POSSIBLE.

Thanking you in advance for not waiting until the last minute,

Dave

APPENDIX C: Registration Form

Registration Form

CCQM-K95.1 Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea

ORGANIZATION / DEPARTMENT / LABORATORY

FULL ADDRESS FOR SHIPMENT OF SAMPLES (no PO box)

CONTACT PERSON

E-MAIL AND TELEPHONE

Will you also be participating in CCQM-K131 Low-Polarity Analytes in a Multicomponent Organic Solution: Polycyclic Aromatic Hydrocarbons (PAHs) in Acetonitrile?

Yes _____ No _____

Date _____

Please complete the form and send it back to stephen.wise@nist.gov **before August 28, 2015**.

APPENDIX D: Reporting Form

The original form was distributed as an Excel workbook. The following are pictures of the relevant portions of the workbook's three worksheets.

"Participant Details" worksheet

CCQM-K95.1

Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea

Data Submission Form

Please complete all pages of the reporting form and submit it by email before December 15, 2015 to:
stephen.wise@nist.gov

Reporting Date	
Institute	
Submitted by (name)	
E-mail address	

"Results" Worksheet

CCQM-K95.1 RESULTS

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene				
Benzo[*a*]pyrene				

"Analytical Information" Worksheet

Information about the analytical procedure

Sample amount used for analysis g

Sample pre-treatment (if applicable)

Extraction method/conditions
(e.g., Soxhlet, ASE, Ultrasonic, solvent)

"Analytical Information" Worksheet (Continued)

Clean-up Procedure
(e.g., SPE, LC)

Analytical instrumentation used
(e.g., GC-MS, LC-MS)

MS Settings

Chromatographic Column
(i.e., specify tpe and manufacturer)

Chromatographic Conditions
(e.g., GC temperature program, LC mobile phase and gradient)

Calibration type / details
(e.g., single-point, bracketing /
external calibration, internal standard calibration, IDMS)

Calibration standards
(e.g., source, purity, and traceability of standards)

"Analytical Information" Worksheet (Continued)

Internal standards used (if applicable) (Please specify the compounds, sourse, and at which stage of the analysis were the internal standards added)	
Purity assessment of the calibrant (if applicable) (e.g. methods used for value assignment/verification)	
Estimation of impurities (if applicable) (e.g. type of impurity, mass fraction, uncertainty)	
Indicate ion/MRM monitored in Mass Spectrometer	
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	
Additional Comments or Observations	

APPENDIX E: Core Competency Table Form

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

CCQM-K95.1	*NMI*	**Low-Polarity Analytes in a Botanical Matrix: Polycyclic Aromatic Hydrocarbons (PAHs) in Tea**

Scope of Measurement: Participation in this study would provide the opportunity to demonstrate measurement capabilities similar to those demonstrated by participating in CCQM-K95 including: (1) value assignment of primary reference standards; (2) value assignment of single and/or multi-component calibration solutions; (3) extraction of analytes of interest from the matrix; (4) cleanup and separation of analytes of interest from other interfering matrix or extract components; (5) separation and quantification using gas chromatography/mass spectrometry (GC-MS). This study would demonstrate the laboratory's capabilities in determining mass fraction of contaminants in a range from 10 ng/g to 1000 ng/g of analytes with molar mass of 100 g/mol to 500 g/mol having polarity $pK_{ow} < -2$ in low fat, low protein, plant matrices.

Competency	✓, ×, or N/A	Specific Information as Provided by *NMI/DI*
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		*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 F: Summary of Participants' Analytical Information

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

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

Disclaimer

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 National Institute of Standards and Technology or other participant in this Key Comparison, nor does it imply that the equipment, instruments, or materials identified are necessarily the best available for the purpose.

Table F-1: Summary of Sample Size, Extraction, and Cleanup for CCQM-K95.1

Institute	Pre-treatment	Extraction Method	Sample Size (g)	Clean-up
BAM Method 1	mixing samples with 0.5 g of diatomaceous earth, addition of internal standard solution	accelerated solvent extraction (ASE); 100 °C, 140 bar, 10 min static, 2 cycles 2:1 acetone:cyclohexane	0.5	2 times shaking with water to remove acetone centrifugation gel permeation chromatography (GPC) SPE, 1g silica gel, elution with cyclohexane evaporation to 1 mL
BAM Method 2		Liquid/Solid extraction (Modified QuEChERS extraction); Weigh sample in falcon tube (50 mL), add internal standard gravimetrically, incubate overnight in the fridge with lightly opened lid, add 5 mL water, mix carefully and let soak for 10 minutes, add 5 mL 1:1 (v/v) acetone:cyclohexane, mix thoroughly and extract for 60 min (30 min ultrasonic bath + 30 min shaker)	0.5	Post-extraction (QuEChERS method) Add 2 g MgSO4 and 0.5 g NaCl to the extract and shake manually (1 minute), centrifuge (3000xg, 5 minutes, 20 °C), transfer 3 mL of the supernatant to a fresh vial and evaporate down to 1.5 mL (N2-stream), adjust volume to 3 mL with cyclohexane and repeat the evaporation process twice gel permeation chromatography (GPC): Apply 3 mL of the crude extract to GPC purification, evaporate the GPC-extract down to 1 mL (N2-stream) Silica SPE: Rinse SPE cartridges (3 mL, 0.5 g silica, MN CHROMABOND) with 6 mL cyclohexane, load 1 mL of GPC-extract onto cartridge and elute with 5 mL cyclohexane, evaporate down to 1 mL
BVL		ASE, n-hexane/acetone 50:50, 80 °C	1.00	1: SPE silica cartridges 2: SPE cartridges with polymeric sorbent, HR-X, Macherey&Nagel
CENAM		Automatic Soxhlet extraction, 1:1 acetone:hexane, 16 h, 5 cycles/h	1	Evaporation to 10 mL, centrifugation at 4 °C for 15 min at 5000 rpm; evaporation to 0.5 mL filtration through 0.2 µm PVDF filter

Table F-1: Summary of Sample Size, Extraction, and Cleanup for CCQM-K95.1 (Continued)

Institute	Pre-treatment	Extraction Method	Sample Size (g)	Clean-up
EXHM		ASE 1:1 hexane:acetone, 100 atm, static time 10 min, 120 °C, 3 cycles Also evaluated and found 'equivalent' * Soxhlet 1:1 hexane:acetone, 20 h * Saponification methanol NaOH, 1 h * Ultrasonic, 1:1 hexane:acetone, 750 W, 20 min, 80 mL	2	solvent evaporation and redilution in cyclohexane; sequential liquid-liquid extraction with Na_2CO_3 saturated water, NaCl saturated water, water solvent evaporation; SPE on cartridges containing molecularly imprinted polymers
HSA	Tea weighed into a cellulose thimble held in an ASE cell. IS solutions were weighed using microsyringes then dispensed onto the tea sample. The ASE cell was then capped and equilibrated overnight at 18 °C to 25 °C.	ASE 1:1 v/v *n*-hexane/acetone, 32 extraction cycles, 70 °C ,over 18 h. In each cycle, extraction cell heated at 70 °C for 5 min before solvent released into the cell and heating continued for another 20 min. Solvent then released into collection vials. The collected extracts were combined, evaporated to 1 mL, exchanged twice with 2 mL *n*-hexane then concentrated to 1 mL.	1	SPE: Cleanert® BaP SPE cartridges conditioned with 5 mL dichloromethane followed by 5 mL *n*-hexane. Sample and calibration blends was loaded, washed with 10 mL of *n*-hexane, eluted with at least 8 mL of dichloromethane. Eluent evaporated to 1 mL, exchanged twice with 2 mL *n*-hexane then concentrated to 1 mL. Extract filtered with a 0.22 µm PTFE syringe filter before injecting into the GC-MS.
NIM	1g of tea and 1g of fine diatomite mixed and added to ASE extraction cell. 250µL mixed internal standard solution added to the cell	ASE 2:5 v/v hexane- dichloromethane, 6 extraction cycles, 160 °C, static extraction: 5 min, purge 100 s; flush volume: 80 %	1.0	GPC: 3.5 ml/min, 1:1 v/v hexane-dichloromethane, eluant of (22 to 33) min collected and further cleaned with SPE LC-Si 1 g/6 mL tubes. PAHs eluted with 6 mL 7:3 v/v hexane-dichloromethane then concentrated using a nitrogen gas stream

Table F-1: Summary of Sample Size, Extraction, and Cleanup for CCQM-K95.1 (Continued)

Institute	Pre-treatment	Extraction Method	Sample Size (g)	Clean-up
NIMT		liquid-liquid extraction. Sample was spiked with the ISs, 2 mL of DI water and 2.5 mL of hexane: acetone 1:1 v/v, vortexed at room temperature overnight then mixed at 30 °C for 30 min under ultrasonication. This process was repeated twice, replacing the mixed solvent with hexane. The organic layer was collected, dried using 0.2 $MgSO_4$, gently vortexed for 1 min, then centrifuged for 5 min at 3000 x g. The extract was evaporated to dryness using a gentle stream of nitrogen gas in a water bath at 50 °C. One mL hexane was added prior to SPE clean-up.	0.5	Silica SPE cartridges (VertipakTM, 6 mL, 500 mg). The cartridges were conditioned with 6 mL of hexane. One milliliter of the extract was loaded onto the cartridge and eluted with 5 mL of a mixture of hexane:dichloromethane 7:3 v/v. Eluted sample evaporated to dryness under stream of nitrogen gas in a water bath at 50 °C. The sample was then reconstituted with 1 mL of hexane and filtered through 0.22 µm PTFE syringe filter prior to GC-MS analysis.
NIST		Pressurized fluid extraction using dichloromethane. Extraction cell 150 °C 5 min with 6 cycles. Rinse volume 100 %, purge of 90 s. For each cell, method was run twice resulting in two collection vials per sample. The two extracts were combined and concentrated to a volume of 0.5 mL after solvent exchange to hexanes.	0.5	SPE using silica classic Sep-Pak cartridges conditioned and eluted with 20 mL of 5 % dichloromethane in hexane (v %).

Table F-1: Summary of Sample Size, Extraction, and Cleanup for CCQM-K95.1 (Continued)

<table>
<tr><th>Institute</th><th>Pre-treatment</th><th>Extraction Method</th><th>Sample Size (g)</th><th>Clean-up</th></tr>
<tr><td>NMISA</td><td></td><td>Accelerated solvent extraction, ASE
<table><tr><th>Parameter</th><th>Variable</th></tr><tr><td>Solvent (v/v)</td><td>3:1 (Dichloromethane: Hexane)</td></tr><tr><td>Temperature (°C)</td><td>100</td></tr><tr><td>Static extraction time (s)</td><td>5</td></tr><tr><td>Number of static cycles</td><td>2</td></tr><tr><td>Purge volume (%)</td><td>80</td></tr><tr><td>Purge time (s)</td><td>120</td></tr><tr><td>Extraction cell size (mL)</td><td>100</td></tr><tr><td>Filters</td><td>Cellulose</td></tr><tr><td>Extraction time (min)</td><td>± 20</td></tr><tr><td>Total volume (mL)</td><td>± 15</td></tr><tr><td>Sample size (g)</td><td>1</td></tr></table></td><td>1</td><td>In cell ASE clean-up procedure.
Two cellulose filters were placed at the bottom of the cell followed by Na2SO4, neutral alumina and Florisil® layers.
The sample mixed with diatomaceous earth was placed above the Florisil® layer.</td></tr>
<tr><td>UME</td><td></td><td>1) Soxhlet, BUCHI 811, 20 h, 150 mL 1:1 acetone:hexane
2) Soxhlet, BUCHI 811, 20 h, 150 mL toluene
3) Ultrasonic, 80 °C, 150 mL, 5 h, toluene
4) PSE Applied Seperations,100 °C, 100 bar, 3 cycles 10 min, dichloromethane
5) Microwave, Milestone, 30 min, 120 °C, 1:1 acetone:hexane
Results from 1) and 5) used for BaA
Results from 5) used for BaP.</td><td>1) 1
2) 1
3) 0.5
4) 0.5
5) 0.5</td><td>Na_2SO_4 added, extracts filtered through filter paper, concentrated by rotary evaporator to ≈1-2 mL. SPE cleanup with SUPELCO LCNH2, eluted with 20 mL 98:2 hexane:dichloromethane. Concentrated to ≈5 mL by rotary, then to 1 mL by nitrogen stream, solvent exchanged to toluene and concentrated to 1 mL for 1 g sample or to 0.5 mL for 500 mg sample.</td></tr>
</table>

Table F-2: Summary of Analytical Techniques for CCQM-K95

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
BAM Method 1	GC-MS	DB-EUPAH 60 m×0.25 mm×0.25 µm	**MS**: electron impact 70 eV, quad 150 °C source 230 °C, transfer 320 °C **GC**: He, 1 mL/min 60 °C (1 min), 45 °C/min to 200 °C, 10 °C/min to 320 °C (33 min)	BaA: 228; BaA-d_{12}: 240 BaP: 252 BaP-$^{13}C_4$: 256
BAM Method 2	GC-MS AGILENT GC 6890N + AGILENT MSD 5975B	AGILENT DB-17MS 60 m×0.25 mm×0.25 µm	**MS**: electron impact 70 eV, quad 150 °C source 230 °C, transfer 320 °C, solvent delay 6 min **GC**: He, 1 mL/min 60 °C (1 min), 45 °C/min to 200 °C, 10 °C/min to 320 °C (33 min)	BaA: 228.1/226.1 BaA-d_{12}: 240.2/241.2 BaP: 252.1/253.1 BaP-$^{13}C_4$: 256.2/257.2
BVL	GC-HRMS	Optima 35 MS (Macherey & Nagel), precolumn deactivated, 30 m×0.25 mm×0.25 µm	**MS**: Resolution R=10 000, source temp. 260 °C **GC**: He, 1 ml/min, splitless 280 °C, 80 °C (2 min), 30 °C/min to 240 °C, 3.0 °C/min to 270 °C, 2.0 °C/min to 285 °C, 8 °C/min to 330 °C	BaA: 228.0933 BaA-d_{12}: 240.1687 BaP: 252.0933 BaP-d_{12}: 264.1687
CENAM	GC-MS/MS Agilent GC 7890 MS 7000QQQ	HP-50 60 m×250 µm×0.25 µm	100 °C (1 min); 40 °C/min; 280 °C (45 min) 1.1 mL/min,	BaA: 228.1/226.1; BaA-d_{12}: 240.1/236.1 BaP: 252.1/250.1 BaP-d_{12}: 264.1/260.1

Table F-2: Summary of Analytical Techniques for CCQM-K95 (Continued)

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
EXHM	GC-IT-MS Thermo Trace Ultra GC PolarisQ ion trap MS	Agilent J&W DB-35 ms 30 m×0.25 mm×0.25 µm	**MS**: source 230 °C, transfer 280 °C, damping gas 2 mL/min, excitation 5.6 V, max energy 0.43 **GC**: He, 1 mL/min 0-15 min, 0.1 mL/min to 2 mL/min (9 min) PTV injector,10 µL, 85 °C, split flow 100 mL/min, 160 kPa, 25 mL/min Evaporation: 15 °C/s to 85 °C (0.5 min), 15 °C/s to 300 °C, 14.5 °C/s to 320 °C (28 min) oven: 80 °C (3 min), 25 °C/min to 230 °C, 10 °C/min to 250 °C, 3 °C/min to 310 °C (5 min)	BaA: 228 – 226/224 BaA-d_{12}: 240 – 236/232 BaP: 252 – 250/246 BaP-d_{12}: 264 - 260/258
HSA	GC-MS Agilent 7890A GC, 5975C MS	Restek Rxi-PAH, 40 m×0.18 mm×0.07 µm	**MS**: transfer 280 °C, source 280 °C, Quad 180 °C **GC**: He, 1 ml/min, 1.5 µL, splitless 275 °C, 110 °C (1 min), 45 °C/min to 230 °C, 3 °C/min to 260 °C, 11 °C/min to 300 °C (3 min).	BaA: 228.1; BaA-$^{13}C_6$: 234.1 BaP: 252.1 BaP-$^{13}C_4$: 256.1
NIM Method 1	GC-MS Agilent 6890N-5975)	Agilent DB-XLB 60 m×0.25 mm×0.25 µm	**MS**: SIM mode, EI, source 250 °C, auxilliary 280 °C **GC**: Ramp flow 1 mL/min (15 min), 1.8 mL/min (20 min), 2.2 mL/min (10 min), 1.8 mL/min (23 min) 60 °C (1 min), 25 °C/min to 180 °C, 10 °C/min to 200 °C, 4 °C/min to 220 °C, 2 °C/min to 240 °C, 1.5 °C/min to 280 °C, 5 °C/min to 300 °C (15 min)	BaA: 228,226 BaA-$^{13}C_6$: 234,232 BaP: 252,250 BaP-$^{13}C_4$: 256,254

Table F-2: Summary of Analytical Techniques for CCQM-K95 (Continued)

Institute	Analytical Technique	Chromatographic Column	Chromatographic and Mass Spectrometry Conditions	ion/MRM monitored
NIM Method 2	GC-MS/MS ThermoFisher TSQ Quantum XLS	Agilent DM-5 50 m×0.25 mm×0.25 µm	**MS**: SIM mode, EI, source 250 °C, auxilliary 280 °C **GC**: Ramp flow 0.8 mL/min (10 min), 2.4 mL/min (30 min), 1.2 mL/min (17 min) 60 °C (1 min), 25 °C/min to 180 °C, 10 °C/min to 200 °C, 3 °C/min to 230 °C, 1.5 °C/min to 240 °C, 1 °C/min to 265 °C, 20 °C/min to 300 °C (20 min)	BaA: 228,226 BaA-$^{13}C_6$: 234,232 BaP: 252,250 BaP-$^{13}C_4$: 256,254
NIMT	GC-MS	DB5-MS (5 % phenyl 95 % methyl siloxane)	**MS**: SIM, quad 150 °C, source 250 °C **GC**: inlet 280 °C, flow 1.0 mL/min 100 °C (1 min), 20 °C/min to 250 °C(1 min), 5 °C/min to 290 °C (5 min)	BaA: 226, 228, 229 BaA-$^{13}C_6$: 232, 234, 235 BaP: 250, 252, 253 BaP-$^{13}C_4$: 254, 256, 257
NIST	GC-MS Agilent 6890/5973	Restek Rxi-PAH 60 m×0.25 mm ×0.15 µm	**MS**: SIM, quad 200 °C, source 250 °C, transfer 340 °C **GC**: On-column injections using oven tracking mode. 70 °C (1 min), 10 °C/min to 100 °C, 4 °C/min to 330 °C (30 min)	BaA: 228, 226 BaA-d_{12}: 240 BaP: 252 BaP-d_{12}: 264
NMISA	GC-TOFMS	Restek Rxi-PAH 60 m×0.25 mm×0.10 µm	**MS**: 1800 V, 10 spectra/s, energy -70V, source 250 °C, transfer 275 °C **GC**: inlet 300 °C, He, 1.5 mL/min 65 °C (30 s), 5 °C/min to 170 °C, 3 °C/min to 250 °C, 10 °C/min to 340 (10 min)	BaA: 228 BaA-d_{12}: 240 BaP: 252 BaP-d_{12}: 264
UME	GC-MS/MS Thermo Scientific TSQ	Agilent, DB EUPAH 60 m×0.25 mm×0.25 µm	**MS**: injection 1 µL, flow 1 mL/min, source 275 °C, inlet 300 °C, transfer 300 °C **GC**: 60 °C (1.30 min), 45 °C/min to 200 °C, 10 °C /min to 320 °C (33 min)	BaA: 228/226 BaA-d_{12}: 240/236 BaP: 252/250 BaP-d_{12}: 264/260

Table F-3: Summary of Calibrants and Standards for CCQM-K95.1

Institute	Type of Calibration	Calibrants	Internal Standards
BAM Method 1	internal standard 5 calibration points linear regression	NIST SRM 2260a	BaA-d_{12} & BaP-d_{12} Dr Ehrenstorfer PAH Mix 9 BaP-$^{13}C_4$ CIL (BaP-d_{12} and BaP-$^{13}C_4$ gave similar results) spiked on the samples before extraction
BAM Method 2	internal standard 8 calibration points Linear	NIST SRM 2260a	BaA-d_{12} & BaP-d_{12} Dr Ehrenstorfer BaP-$^{13}C_4$ Cerillant (BaP-d_{12} & BaP-$^{13}C_4$ gave similar results) added directly after weighing the tea sample
BVL	internal standard	NIST 2260a	BaA-d_{12} & BaP-d_{12} Promochem, added before extraction
CENAM	single point IDMS	Commercial sources	BaA-d_{12} 98.7 % D CIL BaP-d_{12} 98 % D CDN isotopes
EXHM	single point, exact matching IDMS	NIST SRM 2260a	BaA-d_{12} & BaP-d_{12}, Chem Service added to the 2 g sample, equilibrated for 2 h
HSA	single point, exact matching IDMS	BaP Cerilliant SCB-007, (993.8 ± 5.1) mg/g BaA BCR-271 IRMM, (998.4* ± 0.9) mg/g * The purity was verified with NIST SRM 1647f	BaA-$^{13}C_6$ CIL CLM-3602-1.2, BaP-$^{13}C_4$ CIL CLM-2722-1.2 Both IS diluted with toluene to ≈ 0.26 µg/mL. A volume appropriate for achieving equal amounts IS and native analytes weighed then dispensed into the weighed tea sample contained in an ASE thimble.
NIM	IDMS	BaA Cerillant 99.69 % BaP Cerillant 99.30 %	BaA-$^{13}C_6$ & BaP-$^{13}C_4$ from CIL IS added to sample before extraction process
NIMT	6-point calibration curve IDMS	BaA BCR-271 IRMM BaP AccuStandard, (997.4 ± 3.5) mg/g	BaA-$^{13}C_6$, BaP-$^{13}C_4$
NIST	Internal standard, bracketing	NIST SRM 2260a	BaA-d_{12} & BaP-d_{12}
NMISA	double IDMS, (bracketing) standard addition.	NIST SRM 2260a NMIJ CRM 4213-a IRMM BCR-271	BaA-d_{12} & BaP-d_{12}, NIST SRMs 2269 and 2270

Institute	Type of Calibration	Calibrants	Internal Standards
UME	6 points from 10 ng/g to-100 ng/g, IDMS	BaA, Supelco 4-8563 (98.084 ±0.297) % BaP, Supelco 4-8564 (94.118±0.517) %	BaA-d_{12} 98 % D CIL DLM-610-0.1 BaP-d_{12} 97 % D CIL DLM-258-0.1

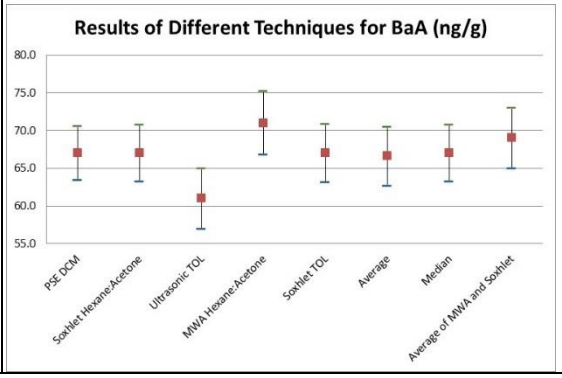
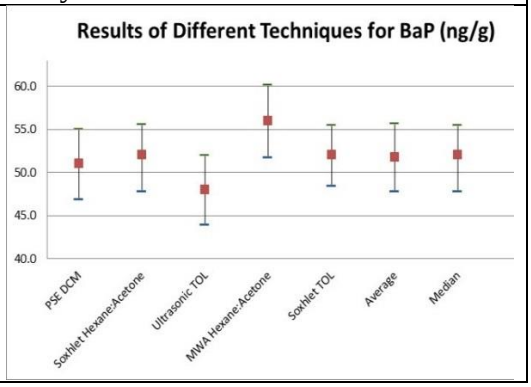
Table F-4 Assessment and Verification Methods for CCQM-K95.1

Institute	Purity Assessment	Result Verification
CENAM	Karl Fischer titration and GC-FID with two different columns	
EXHM		In-house prepared PAH solutions
HSA	In-house evaluation of BaP Structurally related organics: HPLC-DAD with Eclipse PAH LC column, confirmed using Kinetex C18, using relative peak area approach. Mass fraction: 5.85 mg/g. Moisture: Mettler Toledo Karl Fisher Coulometer, validated using NIST SRM 2890. Mass fraction: 0.32 mg/g. Volatile organics: Headspace GC-MS with capillary DB-624 column identified small amount of dichloromethane. TGA result below 2.3 mg/g LOD. Mass fraction: 0 mg/g with associated uncertainty estimated from the LOD. Total non-volatiles: TGA result below 5.0 mg/g LOD. Mass fraction: 0 mg/g with associated uncertainty estimated from the LOD. BaA purity verified by comparison with NIST SRM 1647f	The IRMM Fine Dust (ERM-CZ100) was analysed in parallel with each tea sample for quality control (QC). Other than the sample size taken (0.1 g instead of 1g in tea sample), each QC was subjected to the same sample treatment and cleanup as the tea sample. The same CB was used for both the SBs and QCs. The QC results were all within the expanded uncertainty of the certified values for BaA and BaP. Liquid-liquid extraction (LLE) was also performed on additional subsamples. After the addition of internal standards, the blends were soaked overnight in water. The wetted blends were shaken with n-hexane/acetone (1:1 v/v), followed by the addition of QuEChERS unbuffered salt. The organic layer was taken after a centrifugation step. The extraction was repeated another four times with the organic solvents. The extracts were concentrated then subjected to the same clean-up procedure described above. The results were used to estimate the uncertainty in sample preparation methods (LLE vs ASE). Subsamples of the tea samples subjected to ASE were analysed with both the GC-MS and GC-HRMS (Thermo Scientific DFSTM Magnetic Sector). The results were used to estimate the uncertainty in the use of different instruments.

Table F-4 Assessment and Verification Methods for CCQM-K95.1 (Continued)

Institute	Purity Assessment	Result Verification
NIM	GC-FID, HPLC-DAD, and NMR	1g fine diatomite was analysed together with CCQM-K95.1 sample for monitoring any contamination that may be induced in whole analytical process. 1g fine diatomite spiked with BaA & BaP to same level as the CCQM-P95.1 sample was used as quality control sample in parallel analysis with CCQM-K95.1. CCQM-K95.1 samples were analysed by two columns in two GC-MS instruments.
NIMT	Mass balance by GC, HPLC, TGA, and KFT	
NIST		NIST SRM 2585
NMISA		IDMS using the QuEChERS extraction technique was used for confirmation. NIST SRM 1944 New York/ New Jersey Waterway sediment as well as spiked green tea were used as quality control samples.
UME	Purity determined at UME by qNMR, traceability to UME CRM 1301 Chloramphenicol Primary Calibrant	NIST SRM 2585

Table F-5: Additional Comments for CCQM-K95.1

Institute	Additional Comments
BAM	Results are the means o six determinations. Blank samples were processed together with the tea samples, blank values were negligible. Similar results were obtained using deuterated B(a)P instead of ^{13}C for the quantification of B(a)P and when water was added to the samples before extraction
CENAM	Four subsamples of 1 g each of the tea were dried over anhydrous magnesium perchlorate in a desiccator at room temperature until a constant mass was obtained.
NIMT	Five portions of one gram of tea sample was placed over phosphorus pentoxide in a desiccator at room temperature for 20 days to reach a constant mass.
NIST	Attempted pressurized fluid extraction adding the internal standard prior to extraction but observed large variability for BaP that was not observed in past PAH analysis. Suspect the lot of hydromatrix used. Also attempted Soxhlet extraction and obtained slightly lower values for both analytes.
UME	 

APPENDIX G: Summary of Participants' Uncertainty Estimation Approaches

The following are pictures of the uncertainty-related information provided by the participants in the "Analytical Information" worksheet of the "Reporting Form" Excel workbook. Information is grouped by participant and presented in alphabetized acronym order.

Uncertainty Information from BAM

measurement equation:

$$x_{sample} = \frac{r - i_c}{sl} \cdot \frac{m_{is}}{m_{sample}} \cdot F_{purity}$$

symbol	parameter description
r	area ratio native/internal std
i_c	intercept of calibration line
sl	slope of calibration line
m_is	mass of internal standard added to sample
m_sample	mass sample
F_purity	purity correction factor
x_sample	**result**

coverage factor k = 2

uncertainty estimate		u_rel Nap	u_rel B(a)A	u_rel B(a)P
residual scatter of calibration		0.015	0.005	0.006
estimate combining u(ic), u(sl), and covar		0.002	0.004	0.003
SD of replicate weighings, converted to u_rel		0.003	0.003	0.003
SD of replicate weighings of mass standard, converted to u_rel		0.001	0.001	0.001
certificate uncertainty NIST SRM 1647f, includes purity		0.007	0.007	0.009
		0.017	**0.009**	**0.012**
	expanded relative uncertainty U:	0.033	0.019	0.023
	expanded uncertainty U µg/g:	0.845	0.091	0.142
	standard uncertainty u µg/g	0.423	0.046	0.071

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

uncertainty components:
weighing (sample, internal standard, calibrant)
variation of response factor
uncertainty of calibrant (see certificate NIST 2260a)
combined uncertainty = sqrt (sum of squared standard uncertainties)

Uncertainty budget K131 (PAH in solution)

standard uncertainties, relative values u(x)/X

	BaA [%]	BaP [%]	Comment
Weighing processes			
weighing of sample	0.66	0.66	76 +/- 0,5 mg
addition of internal standard solution	0.23	0.23	220 +/- 0,5 mg
Calibration			
Uncertainty of calibrant	0.59	1.20	NIST 2260a, see certificate
weighing NIST 2260a	0.057	0.057	876 +/- 0,5 mg
variation response factor	0.3	1.1	accounts for injection and integration bias
combined standard uncertainty [%]	0.96	1.77	
expanded uncertainty (k=2) [%]	**1.9**	**3.5**	

mean RF	std. dev.	CV [%]	
1.024	0.003	0.3	(BaA)
1.126	0.012	1.1	(BaP)

Uncertainty Information from CENAM

$$w_x = \frac{m_{Ix} \cdot R_x \cdot m_0}{m_x \cdot R_0 \cdot m_{I0}} \cdot w_0$$

m_0	Mass of measurand for calibration solution
m_{I0}	Mass of labeled compound for calibration solution
m_x	Sample mass
m_{Ix}	Mass of labeled solution for sample
R_0	Area ratio for calibration solution
R_x	Area ratio for sample
w_0	Mass fraction of measurand in calibration solution
w_x	Mass fraction of measurand in sample

Several uncertainty sources were combined: The budget of each uncertainty by ampule is shown un next figure.The expanded uncertainty was obtained by multiplying the combined standards uncertainty by the cover factor with a 95 % level of confidence k =2.

Paramerter	Value	units	uncertainty source	standard uncertainty
Mass of measurand for calibration solution	**0.38670**	g	and calibration	0.00006
Mass of labeled compound for calibration solution	**0.38546**	g	and calibration	0.00006
Sample mass	**0.38422**	g	and calibration	0.00004
Mass of labeled solution for sample	**0.38786**	g	and calibration	0.00004
Area ratio for calibration solution	**1.0397**		Experimental, repeatibility	0.0088
Area ratio for sample	**1.0049**		Experimental, repeatibility	0.0059
Mass fraction of measurand in calibration solution	**5.1792**	mg/kg	repeatability and purity	0.0178

Uncertainty Information from EXHM

The measurement equation is:

$$w_{M,S} = w_{M,C} \frac{100}{Rec} \times \frac{1}{1 - H} \times \frac{m_{is,S}}{m_{M,S}} \times \frac{m_{M,C}}{m_{is,C}} \times \frac{R_S}{R_B}$$

where $w_{M,S}$ = dry mass fraction of the analyte (BaA or BaP) in the sample, ($\mu g/kg$)

$w_{M,C}$ = mass fraction of the analyte (BaA or BaP) in the calibration solution, ($\mu g/kg$)

H = sample moisture content (g/g)

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

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

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

$m_{M,C}$ = mass of the analyte (BaA or BaP) solution added to calibration blend, (g)

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

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

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

The equation used to estimate standard uncertainty is:

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

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

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

Benz[*a*]anthracene

uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	68,57	1,0000	0,81	0,0117	0,8053	0,6485
mass fraction of B[a]A in the calibration solution, (µg/kg)	699,93	0,0980	7,19	0,0103	0,7044	0,4961
sample moisture content, (g/g)	0,0401	-71,4384	0,0002	0,0044	-0,0127	0,0002
recovery (%)	100,00	-0,6857	1,437	0,0144	-0,9853	0,9707
mass of B[a]A-d_{12} solution added to sample blend, (*g*)	0,19416	353,1880	0,00007	0,0004	0,0247	0,0006
mass of test material in sample blend, (*g*)	1,98492	-34,5474	0,00007	0,0000	-0,0025	0,0000
mass of B[a]A solution added to calibration blend, (*g*)	0,04795	1430,1082	0,00003	0,0006	0,0429	0,0018
mass of B[a]A-d_{12} solution added to calibration blend, (*g*)	0,04764	-1439,4141	0,00003	0,0006	-0,0432	0,0019
measured peak area ratio of the selected ions in the sample blend	1,0493	65,3497	considered to be included in the			
measured peak area ratio of the selected ions in the calibration blend	1,0985	-62,4230	estimation of method precision			
result (ng/g)	68,57					
combined standard uncertainty (ng/g)	1,46					
relative standard uncertainty (%)	2,12					
effective degrees of freedom	58,2					
coverage factor	2,00					
expanded uncertainty (ng/g)	2,91					

Benzo[*a*]pyrene

uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	52,49	1,0000	0,56	0,0107	0,5603	0,3140
mass fraction of B[a]P in the calibration solution, (µg/kg)	643,18	0,0816	11,61	0,0180	0,9473	0,8974
sample moisture content, (g/g)	0,04010	-54,6840	0,00018	0,0044	-0,0098	0,0001
recovery (%)	100,00	-0,5249	0,762	0,0076	-0,4000	0,1600
mass of B[a]P-d_{12} solution added to sample blend, (*g*)	0,16166	324,7057	0,00007	0,0004	0,0227	0,0005
mass of test material in sample blend, (*g*)	1,98492	-26,4450	0,00007	0,0000	-0,0019	0,0000
mass of B[a]P solution added to calibration blend, (*g*)	0,04084	1285,2879	0,00003	0,0007	0,0386	0,0015
mass of B[a]P-d_{12} solution added to calibration blend, (*g*)	0,04063	-1291,9311	0,00003	0,0007	-0,0388	0,0015
measured peak area ratio of the selected ions in the sample blend	1,0571	49,6558	considered to be included in the			
measured peak area ratio of the selected ions in the calibration blend	1,1047	-47,5178	estimation of method precision			
result (ng/g)	52,49					
combined standard uncertainty (ng/g)	1,17					
relative standard uncertainty (%)	2,23					
effective degrees of freedom	20,4					
coverage factor	2,00					
expanded uncertainty (ng/g)	2,35					

Uncertainty Information from HSA

(i) Measurement equation used to determine the mass fraction of the measurands

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

where

C_Z = mass fraction of benz[a]anthracene/benzo[a]pyrene in the calibration standard solution used to prepare the calibration blend

m_Y = mass of internal standard solutions added to the sample blend

m_{Yc} = mass of internal standard solutions added to the calibration blend

m_{Zc} = mass of standard solutions added to the calibration blend

m_X = dry mass of tea sample in the sample blend

$m_x = (100\text{-}F_m) \times m'_x / 100$, where F_m represents the moisture content and m'_x represents the wet mass of tea sample

R_X = observed isotope abundance ratio in the tea sample

R_Y = observed isotope abundance ratio in the internal standard

R_Z = observed isotope abundance ratio in the calibration standard

R_B = observed isotope abundance ratio in the tea sample blend

R_{Bc} = observed isotope abundance ratio in the calibration blend

(ii) Uncertainty Budget

$$C_X = F_p.F_i.F_{sp}.F_r.C_Z \cdot \frac{m_Y \cdot m_{Zc}}{m_X \cdot m_{Yc}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$$

where

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

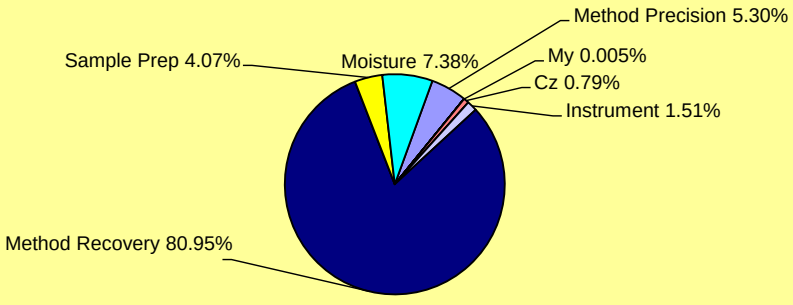
F_p = Factor representing method precision

F_i = Factor representing any bias in the result due to choice of instrument

F_{sp} = Factor representing any bias in the result due to choice of sample preparation method

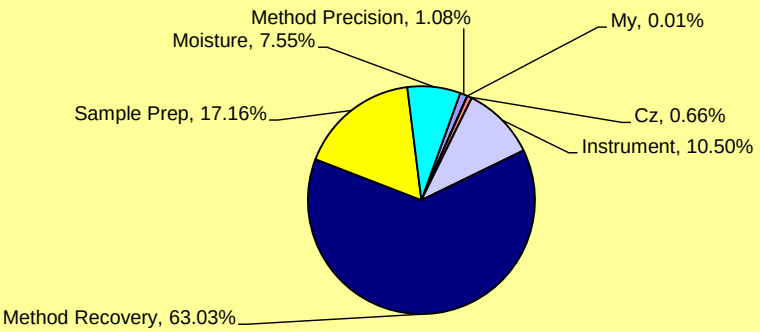
F_r = Factor representing method recovery

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



Parameter	x_i	u_{xi}	u_{xi}/x_i	Contribution	Sources of uncertainty
F_p	1	0.01051	1.0513%	5.303%	Standard deviation of the mean of three results (one subsample each from three packets).
F_i	1	0.00560	0.5603%	1.507%	Comparison of results obtained using GC-MS and GC-HRMS on the same subsamples.
F_{sp}	1	0.00921	0.9205%	4.066%	Comparison of results obtained using accelerated solvent extraction and liquid-liquid extraction.
F_r	1	0.04107	4.1072%	80.948%	Method recovery using IRMM Fine Dust CRM (ERM-CZ100) as
F_m	4.6492	0.05766	1.2402%	7.381%	Uncertainty in the moisture content estimated from the relative standard deviation of the the mean of moisture content determined from three subsamples.
C_Z	0.2977	0.00121	0.4049%	0.787%	Uncertainty in the purity value of benz[a]anthracene certified reference material (BCR-271). Uncertainty in weighing based on balance calibration certificate.
m_Y	0.2243	0.0000707	0.0315%	0.005%	Uncertainty in weighing based on balance calibration certificate.
m_{Yc}	0.4360	0.0000778	0.0178%	0.002%	
m_{Zc}	0.4329	0.0000778	0.0180%	0.002%	
m'_X	1.0180	0.0000707	0.0069%	0.000%	
R_X, R_Y, R_Z	Negligible				
R_B, R_{Bc}	Uncertainty included in method precision				

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



Parameter	x_i	u_{xi}	u_{xi}/x_i	Contribution	Sources of uncertainty
F_p	1	0.00470	0.4703%	1.085%	Standard deviation of the mean of three results (one subsample each from three packets).
F_i	1	0.01463	1.4630%	10.499%	Comparison of results obtained using GC-MS and GC-HRMS on the same subsamples.
F_{sp}	1	0.01871	1.8705%	17.164%	Comparison of results obtained using accelerated solvent extraction and liquid-liquid extraction.
F_r	1	0.03584	3.5845%	63.029%	Method recovery using IRMM Fine Dust CRM (ERM-CZ100) as
F_m	4.6492	0.05766	1.2402%	7.546%	Uncertainty in the moisture content estimated from the relative standard deviation of the the mean of moisture content determined from three subsamples.
C_Z	0.2962	0.00109	0.3679%	0.664%	Uncertainty in the purity value of benzo[a]pyrene (SCB-007) determined in-house. Uncertainty in weighing based on balance calibration certificate. Bias in the preparation of calibration blends.
m_Y	0.1739	0.0000707	0.0407%	0.008%	Uncertainty in weighing based on balance calibration certificate.
m_{Yc}	0.3363	0.0000778	0.0231%	0.003%	
m_{Zc}	0.3334	0.0000778	0.0233%	0.003%	
m'_X	1.0180	0.0000707	0.0069%	0.000%	
R_X, R_Y, R_Z	Negligible				
R_B, R_{Bc}	Uncertainty included in method precision				

Uncertainty Information from NIM

Measurement equation was showed as following

$$C_{sample} = \frac{R_{SM} \times C_{calib} \times f_{purity} \times M_{spike(sample)}}{R_{CM} \times M_{sample} \times f_{dry} \times C_{spike(calb)}}$$

R_{SM} : Area ratio of target compound and labeled compound in sample solution.
R_{CM} : Area ratio of target compound and labeled compound in calibration.
C_{calib}: Mass faction of standard solution,by weighing.

$M_{spike(sample)}$: Mass of labeled compound to added into sample, by weighing .
$C_{spike(calib)}$: Mass fractionof labeled compound to add into calibration soultion, by weighing.
M_{sample}: Sample mass, by weighing.

f_{purity} : Calibrate Purity
f_{dry} : Ratio of the sample mass before drying and after drying

Parameter of BaA	Standard Uncertainty (ng/g)	Degrees of freedom	Type
Method precision	0.42	5	A
Efficiency of extraction procedure	1.01	large	B
Purity of pure standard	0.28	large	A+B
Mass fraction of internal standard	0.05	large	A+B
Mass fraction of sample	0.01		A+B
Mass fraction calibration standard	0.05		A+B
Matrix effects in calibration blend	1.01	large	B
Influnce of peak seperation	1.41	large	B
Combined standard uncertainty	2.06		
Coverage factor	2.00		
Combined expanded uncertainty	4.13		

Parameter of BaP	Standard Uncertainty (ng/g)	Degrees of freedom	Type
Method precision	0.56	5	A
Recovery of extraction procedure	0.78	large	B
purity of pure standard	0.18	large	A+B
Mass fraction of internal standard	0.08		A+B
Mass fraction of sample	0.01		A+B
Mass fraction calibration standard	0.04		A+B
Matrix effects in calibration blend	0.78	large	B
Influnce of peak seperation	1.30	large	B
Combined standard uncertainty	1.81		
Coverage factor	2.00		
Combined expanded uncertainty	3.61		

Method precision:	reproducibility of sample determination
Efficiency of extraction procedure:	Comparison of results from different extraction techniques and different extraction time.
Purity of pure standard:	Type A uncertainty (combined uncertainty of 3 method for purity determination),type B uncertainty (respond factor, influnce from temperature and moisture)were combined.
Mass fraction of internal standard:	Type A uncertainty (reproducibility of weighing, n=6) and type B uncertainty (linearity of weighing, certificate of calibration and solvent evaporation) were combined.
Mass fraction of sample:	Type A uncertainty (reproducibility of weighing, n=6) and type B uncertainty (linearity of weighing, certificate of calibration and influnce from loss of moisture during weighing) were combined.
Mass fraction calibration standard:	Type A uncertainty (reproducibility of weighing, n=6) and type B uncertainty (linearity of weighing, certificate of calibration) were combined.
Matrix effects in calibration blend:	Comparison of results from calibration blends prepared from solvent and tea matrix
Influnce of peak seperation:	Influnce of interfernce peak to analyte peak area

Uncertainty Information from NIMT

$$w_x = F_{std} \cdot \frac{[w_0].w_{y(x)}}{F_{drymass}} \cdot \frac{m_{y(x)}}{m_x}$$

w_x= mass fraction of benz(a)anthracene or benzo(a)pyrene (ng/g) in test sample

w_0= Mass fraction ratio (between unlabeled/labeled) obtained from the calibration curve (ng/ng)

$w_{y(x)}$= Mass fraction of internal standard, ng/g

$m_{y(x)}$ =Mass of internal standard spiked into sample (g)

m_x=Mass of sample (g)

$F_{dry\ mass}$ = correction factor of drymass

F_{std} = given a value of 1 but accounting for the uncertainty including bias and random effects of calibration standard (type B and type A)

$$\frac{u(x)}{x} = \sqrt{\left(\frac{u(m_y)}{m_y}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(F_{drymass})}{F_{drymass}}\right)^2 + \left(\frac{u(w_0)}{w_0}\right)^2 + \left(\frac{u(F_{std})}{F_{std}}\right)^2 + \left(\frac{u(F_E)}{F_E}\right)^2 + \left(\frac{u(F_P)}{F_P}\right)^2 + \left(\frac{u(R)}{R}\right)^2}$$

$u(m_y)$, $u(m_x)$ = standard uncertainties due to weighing estimated from bias of balance

$u(F_{dry\ mass})$ = standard uncertainty of the dry mass correction factor which was estimated from the moisture content analysis.

$u(w_0)$= standard uncertainty of the Mass fraction ratio (between unlabeled/labeled) obtained from the calibration curve (ng/ng) estimated from the regression

$u(F_{std})$ = standard uncertainty of the calibration standard estimated from bias and random effects (type B and type A)

$u(F_E)$ = standard uncertainty of extraction

$u(F_P)$ = standard uncertainty of method precision

u(R) = standard uncertainty of recovery

Benz(a)anthracene: Uncertainty Analysis Results

Cx =	63.621	ng/g, Moisture Corrected
u(x) =	2.627	ng/g
u(x)/x =	4.13%	
Veff(total) =	21.547	
k=	2.08	(@ 95% level)
U(x) =	5.463	
%U(x) =	8.59%	

Factor	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Measurement equation factors			
Method Precision	1.00	0.022	2.18%
w0	1.21	0.012	1.02%
wy(x)	341.55	3.456	1.01%
my(x)	0.07	0.000057	0.08%
mx	0.48	0.000046	0.01%
Calibrant type B	593793.38	2076.293	0.35%
Calibrant type A	428.49	0.250	0.06%
$F_{Dry\ mass}$	0.95	0.001	0.13%
Extraction effects	1.00	0.030	3.00%
Recovery	0.99	0.010	1.05%

Benzo(a)pyrene: Uncertainty Analysis Results

Cx =	46.559	ng/g, Moisture Corrected
u(x) =	2.185	ng/g
u(x)/x =	4.69%	
Veff(total) =	49.120	
k=	2.01	(@ 95% level)
U(x) =	4.391	
%U(x) =	9.43%	

Factor	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Measurement equation factors			
Method Precision	1.00	0.014	1.38%
w0	0.93	0.027	2.91%
wy(x)	483.63	4.894	1.01%
my(x)	0.06	0.000057	0.09%
mx	0.51	0.000046	0.01%
Calibrant type B	638575.17	2787.687	0.44%
Calibrant type A	340.88	0.248	0.07%
$F_{Dry\ mass}$	0.95	0.001	0.13%
Extraction effects	1.00	0.030	3.00%
Recovery	1.02	0.012	1.20%

Uncertainty Information from NIST

For calibration solutions: RF=((ng PAH)/(ng labeled PAH))*((area labeled PAH)/(area PAH))
For mate tea samples ng/g PAH = (RF*ng labeled PAH * area PAH)/(area labeled PAH * g of tea)

Assignment of values and uncertainties

The estimated values are the means of the measurements available for each analyte.

The combined uncertainty incorporates Type A and Type B uncertainties related to measurement variability, response factor, stock calibrants, and moisture correction, consistently with the ISO GUM [1].

For BaA, the following relative uncertainties are incorporated:

- Measurement variation: 1.26 %
- Response factor: 0.98 %
- Stock calibrant: 0.59 %
- Moisture correction: 0.03 %

For BaP, the following relative uncertainties are incorporated:

- Measurement variation: 1.58 %
- Response factor: 3.67 %
- Stock calibrant: 1.20 %
- Moisture correction: 0.03 %

The relative uncertainties due to stock calibrant are taken from the Certificate of Analysis for SRM 2260a.

The relative uncertainties related to the gravimetric weighing of materials are not included because they would be too small relative to the other uncertainties listed to make any substantial difference.

Dry-mass values (moisture correction)

The dry-mass proportion (0. 0.9601 ± 0.0007) is determined from drying with P_2O_5 for 35 days at NIST. The uncertainty shown on this value is an expanded uncertainty. A relative uncertainty component of 0.03 % for the dry-mass proportion is incorporated in the uncertainties of the dry-mass basis values provided in this report.

Possible heterogeneity:
To address issues of possible inhomogeneity related to the three samples provided to NIST, analyses of variance with 5 % significance level and graphical analyses were run. There were no significant sample effects.

$$\bar{Y} = \frac{(Y_{dIDMSa} + Y_{IDMS\,b} + Y_{standard\ addition})}{2}$$

Where

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

f	Water correction factor
Y_{dIDMS}	Mass fraction of PAH in test portion µg/g
W_z	Mass fraction of PAH in calibration µg/g
m_z	Mass of CRM solution added to the calibration blend (g)
m_{yc}	Mass of isotope added to calibration blend (g)
m_y	Mass of isotope added to sample blend (g)
m_x	Mass of sample (g)
R'_B	Peak area ratio of analyte/ isotope in sample blend
R'_{BC}	Peak area ratio of analyte/ isotope in calibration blend

Level of confidence is approximately 95%

and

$$Y_{standard\ addition} = X_{intercept} * mass\ isotope$$

Where the $X_{intercept}$ is calculated as:

$$X_{intercept} = absolute\left(\frac{-c}{M}\right) \times f$$

derived from

$$y = mx + c$$

where in a linear regression of a calibration curve

y	Peak area ratio of analyte/ isotope
m	Slope
x	Mass of native/ mass isotope
c	y-intercept

$$U_{95}(\bar{Y}) = 2 \times \sqrt{\frac{\left(\sum_{j=1}^{N}(Y_j - \bar{Y})^2/N - 1\right) + \left(\sum_{j=1}^{N}\left[\frac{U_{95}(Y_j)}{2}\right]^2/N\right)}{N}}$$

Where j denotes the various methods used in determining the mean and
Y the concentration calculated (ng/g)

The following are examples of the uncertainty contributions included in the individual budgets

Standard addition example Benz[a]anthracene 59.94 ng/g is the expected value

		x	u	u/x	u/x^2	
CRM	Imported uncertainty	4.42	3.90E-02	8.83E-03	7.80E-05	
Mass of standard in calibration	Balance and mass uncertainty	0.16	1.02E-04	6.25E-04	3.90E-07	
Mass of sample used	Balance and mass uncertainty	1.03	5.41E-04	5.26E-04	2.77E-07	
Precision	Stdev of peak area ratio	1.61	1.79E-02	1.11E-02	1.24E-04	
Syx	Error of the calibration curve	1.61	1.13E-01	7.00E-02	4.90E-03	
					0.01	ng/g
					4.24	u
					8.31	U (k=1.96)
					14.00	Rel U

Bracketing example Benz[a]anthracene

		x	u	u/x	u/x2	
Wz	CRM uncertainty	4.42	3.90E-02	8.83E-03	7.80E-05	
mz	Weight of calibration blend (g)	0.11	1.02E-04	9.57E-04	9.15E-07	
my	Weight of Isotope added to sample (g)	0.10	1.02E-04	1.04E-03	1.08E-06	
myc	Weight of Isotope added to calibration blend (g)	0.10	1.93E-04	1.96E-03	3.86E-06	
mx	Weight of sampled (g)	1.00	2.00E-03	2.00E-03	4.00E-06	
R'B/R'BC	Ratio of peaks areas of native/ labelled in the samples	2.03	8.56E-02	4.21E-02	1.77E-03	
			Sum	0.06	0.002	ng/g
			Veff	20196136	2.37	u
			k	1.96	4.65	U
					8.45	Rel U

Uncertainty Information from UME

$$\frac{u_c(Analyte)}{c_{Analyte}} = \sqrt{\begin{array}{l}(\frac{u(m_{SI})}{m_{SI}})^2 + (\frac{u(c_{LSS})}{c_{LSS}})^2 + (\frac{u(c_{NSS})}{c_{NSS}})^2 + (\frac{u(V_{SLL})}{V_{SLL}})^2 \\ + (\frac{u(R_m)}{R_m})^2 + (\frac{u(r)}{r})^2 + \frac{u(Drymass)}{WaterContent} + (\frac{u(Cal)}{C_0})^2\end{array}}$$

Uncertainty Budget of BaA

Parameters	Value (X)	u(x)	u(x)/X
Mass of Sample Intake (SI)	1	0.0000132	1.32E-05
Labelled Stock Solution (LSS)	5981	41.95	7.01E-03
Native Stock Solution (NSS)	10280	27.83583	2.71E-03
Spiking of Labelled Stock Solution (SLL)	100.000	0.39333	3.93E-03
Recovery (R_m)	0.99	0.02544	2.57E-02
Repeatability (r)	69	0.76699	1.11E-02
Dry Mass Correction	5.5270777	0.021642665	3.92E-03
Calibration Graph (Cal)	50	2.11887432	4.24E-02
Result (ng/g)	69	4	0.052

Uncertainty Budget of BaP

Parameters	Value (X)	u(x)	u(x)/X
Mass of Sample Intake (SI)	0.5	0.0000080	1.60E-05
Labelled Stock Solution (LSS)	6597	49.44	7.49E-03
Native Stock Solution (NSS)	10290	37.12523	3.61E-03
Spiking of Labelled Stock Solution (SLL)	100,000	0.39333	3.93E-03
Recovery (R_m)	0.90	0.01065	1.18E-02
Repeatability (r)	50	0.40891	8.18E-03
Dry Mass Correction	2.7635	0.010821123	3.92E-03
Calibration Graph (Cal)	42	3.059162093	7.28E-02
Result (ng/g)	56	4.2	0.075

APPENDIX H: Participants' Quantitative Results as Reported

The following are pictures of the quantitative reults as provided by the participants in the "Results" worksheet of the "Reporting Form" Excel workbook. Information is grouped by participant and presented in alphabetized acronym order.

Quantitative Results from BAM

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	64.2	1.4	2	2.9
Benzo[*a*]pyrene	49.5	1.2	2	2.4

The result is the mean of means of 2 independent methods. Each method employs a different extraction principle.
Results, method parameters and uncertainty budgets for the 2 methods are in the Analytical Information sheets.
For B(a)P the uncertainty of the overall result was determined by propagation of the uncertainties of the 2 methods.
For B(a)A the uncertainty of the overall result is the standard deviation of the mean of the 2 methods (worst case estimate).

water content: (4.44+-0.01) %, 3 replicates, determined according to protocol, k=2

Quantitative Results from BVL

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	64.75	1.24	2	2.48
Benzo[*a*]pyrene	45.25	0.87	2	1.75

Dry mass of sample (mean of n=6): 94,95%; results are corrected for dry mass

Quantitative Results from CENAM

Analytes	Packet	Mass Fraction (μg/kg)	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
Benzo[a]anthracene	**1**	64.7	1.5	2.0	2.9
	2	60.1	1.8	2.0	3.6
	3	61.4	1.3	2.0	2.6
	Mean	62.1	2.1	2.0	4.1
Benzo[a]pyrene	**1**	57.3	1.4	2.0	2.9
	2	58.6	1.2	2.0	2.4
	3	59.9	1.8	2.0	3.6
	Mean	58.6	1.7	2.0	3.3

Moisture Content 0.04633 g/g

Four 1 g subsamples dried over anhydrous magnesium perchlorate at room temperature to constant mass

Quantitative Results from EXHM

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	68.57	1.46	2	2.92
Benzo[*a*]pyrene	52.49	1.17	2	2.34
moisture (%)	4.01	0.02	2	0.04

Quantitative Results from HSA

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	68.9	3.14	2	6.3
Benzo[*a*]pyrene	53.9	2.44	2	4.9

F_m	4.6492	0.05766	1.2402%	7.381%	Uncertainty in the moisture content estimated from the relative standard deviation of the the mean of moisture content determined from three subsamples.

Quantitative Results from NIM

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	67.03	2.06	2	4.13
Benzo[*a*]pyrene	52.03	1.81	2	3.61

Moisture content: 4.7 %

Quantitative Results from NIMT

Measurand	Mass Fraction (ng/g) dry-mass basis	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	63.6	2.7	2.08	5.5
Benzo[*a*]pyrene	46.6	2.2	2.01	4.4

Drying Factor used to determine dry mass basis	% Moisture	Drying Factor
	5.03	0.95

Quantitative Results from NIST

Measurand	Mass Fraction (ng/g) dry-mass basis	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	67.5	1.15	2.12	2.43
Benzo[*a*]pyrene	58.8	2.45	2.31	5.66

Drying Factor used to determine dry mass basis	% Moisture	Drying Factor
	3.99	0.9601

Quantitative Results from NMISA

Non-water corrected				
Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	55	3.6	2	7.2
Benzo[*a*]pyrene	45	3.2	2	6.3

Water corrected results where the water correction factor, *f* , is 1.05				
Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	57.9	3.8	2	7.6
Benzo[*a*]pyrene	47.4	3.3	2	6.6

Quantitative Results from UME

Measurand	Mass Fraction (ng/g)	Combined Standard Uncertainty (ng/g)	Coverage Factor (k)	Expanded Uncertainty (ng/g)
Benz[*a*]anthracene	69	4	2	8
Benzo[*a*]pyrene	56	4.2	2	8.4

Dry Mass Correction

Sample Number	8 days (% change)	30 days (% change)	45 days (% change)
1	4.35	5.30	5.52
2	4.33	5.29	5.52
3	4.38	5.26	5.53
4	4.40	5.28	5.57
5	4.35	5.26	5.53
6	4.36	5.36	5.60
7	4.97	5.32	5.42
Avg	4.45	5.30	5.53

Dry Mass Correction by P_2O_5 in desiccator in dark, 1 g sample used for each,

APPENDIX I: NIST measurements of Yerba Mate Tea (candidate SRM 3253)

Jacolin Murray and Michele Schantz

Before the initiation of measurements for the CCQM-K95.1 Key comparison study, NIST performed separate certification measurements on the candidate SRM 3253 Yerba Mate Tea using two extraction methods, Method 1 Soxhlet extraction, and Method 2 Pressurized fluid extraction (PFE). For the K95.1 study, results were reported from modified PFE Method 3, with a change of internal standards added *after* the extraction step. In addition to Method 3, a confirmatory Soxhlet extraction Method 4 with a different clean-up sorbent was used, however, results were not used in the K95.1 study. Method details for the four methods are described in Table I-1.

Table I-1: Method details for Methods 1 to 4.

	Method 1	Method 2	Method 3	Method 4
Date, Analyst	2013-05, Schantz	2015-07, Murray	2016-06, Murray	2016-06, Murray
Samples analyzed, #	20	6	9	3
Extraction Method	Soxhlet extraction using acetone:hexane (1:1 v:v). Extracted for 20 h.	PFE using dichloromethane. The extraction cell temperature was 150 °C with a static time of 5 min with 6 cycles. The rinse volume was 100 % and a purge of 90 s was used. For each extraction cell, the above method was run twice, resulting in two collection vials per sample.	PFE using dichloromethane. The extraction cell was heated to 150 °C with a static time of 5 min with 6 cycles. The rinse volume was 100 % and a purge of 90 s was used. For each extraction cell, the above method was run twice, resulting in two collection vials per sample.	Soxhlet extraction using acetone/hexane (1:1, v:v). Extracted for 16 h.
Clean-up procedure	SPE using NH_2 Sep-Pak cartridges. Eluting with 20 mL of 20 % dichloromethane in hexanes (v %)	SPE using NH_2 Sep-Pak cartridges. Eluting with 20 mL of 5 % dichloromethane in hexanes (v %).	SPE using silica classic Sep-Pak cartridges. Eluting with 20 mL of 5 % dichloromethane in hexanes (v %).	SPE using NH_2 Sep-Pak cartridges. Eluting with 20 mL of 5 % dichloromethane in hexanes (v %).
Analytical instrumentation used	GC-MS (Agilent 6890/5973)	GC-MS (Agilent 6890/5973)	GC-MS (Agilent 6890/5973)	GC-MS (Agilent 6890/5973)
MS settings	Select ion mode. Quant ions used were 228 and 252 for BaA and BaP, respectively.	Select ion mode. Quant ions used were 228 and 252 for BaA and BaP, respectively. Source temperature 250 °C, quad temperature was set to 200 °C.	Select ion mode. Quant ions used were 228 and 252 for BaA and BaP, respectively. Source temperature set to 250 °C, quad temperature set to 200 °C	Select ion mode. Quant ions used were 228 and 252 for BaA and BaP, respectively. Source temperature set to 250 °C, quad temperature set to 200 °C
Chromatographic column	Restek Rxi-PAH 60 m x 0.25 mm i.d. x 0.1 µm	DB-XLB 60 m x 0.25 mm i.d. x 0.25 µm	Restek Rxi-PAH 60 m x 0.25 mm i.d. x 0.15 µm	Restek Rxi-PAH 60 m x 0.25 mm i.d. x 0.15 µm
Chromatographic conditions	On-column injections using oven tracking mode. The oven was held at 60 °C (1 min) -40°C/min to 150 °C (5 min)- 2 °C/min to 300 °C (50 min).	Splitless injection in a 250 °C injector. The oven was held at 70 °C (1 min)-10°C/min to 100 °C-4°C/min to 330 °C (30 min).	On-column injections using oven tracking mode. The oven was set to 70 °C (1 min)-10 °C/min to 100 °C-4 °C/min to 330 °C (30 min).	On-column injections using oven tracking mode. The oven was set to 70 °C (1 min)-10 °C/min to 100 °C-4 °C/min to 330 °C (30 min).
Calibration type/details	A linear regression model (y=mx+b) calibration curve was used with the use of internal standards.	A linear regression model (y=mx+b) calibration curve was used with the use of internal standards.	Internal standard, bracketing at levels in samples. Used response factor: RF= (area PAH/area labeled PAH) *(ng labeled PAH/ng PAH).	Internal standard, bracketing at levels in samples. Used response factor: RF= (area PAH/area labeled PAH) *(ng labeled PAH/ng PAH).
Internal standards used	BaA-d_{12} and BaP-d_{12} (prepared from SRM 2269 and SRM 2270) added before extraction.	BaA-d_{12} and BaP-d_{12} (prepared from SRM 2269 and SRM 2270) added before extraction	BaA-d_{12} and BaP-d_{12} (prepared from SRM 2269 and SRM 2270) added after extraction (added to collection vessels).	BaA-d_{12} and BaP-d_{12} (prepared from SRM 2269 and SRM 2270) added before extraction

Figure I-1 shows the results for BaA and BaP using methods 1 to 4. The results are reported in ng/g on a dry mass basis based on the moisture that NIST reported in the CCQM-K95.1 study (3.99 % moisture with a drying factor of 0.9601). Figure 2 shows a linear opinion pooling [NIST Consensus Builder, https://consensus.nist.gov] from the four methods. For BaA, the consensus estimate of 69 ng/g with a standard uncertainty of 3.18 and the 95 % coverage internal ranges from 63 ng/g to 74.6 ng/g. For BaP, the consensus estimate is 56 ng/g with a standard uncertainty of 4.39 and a 95 % coverage interval from 47.8 ng/g to 63.4 ng/g.

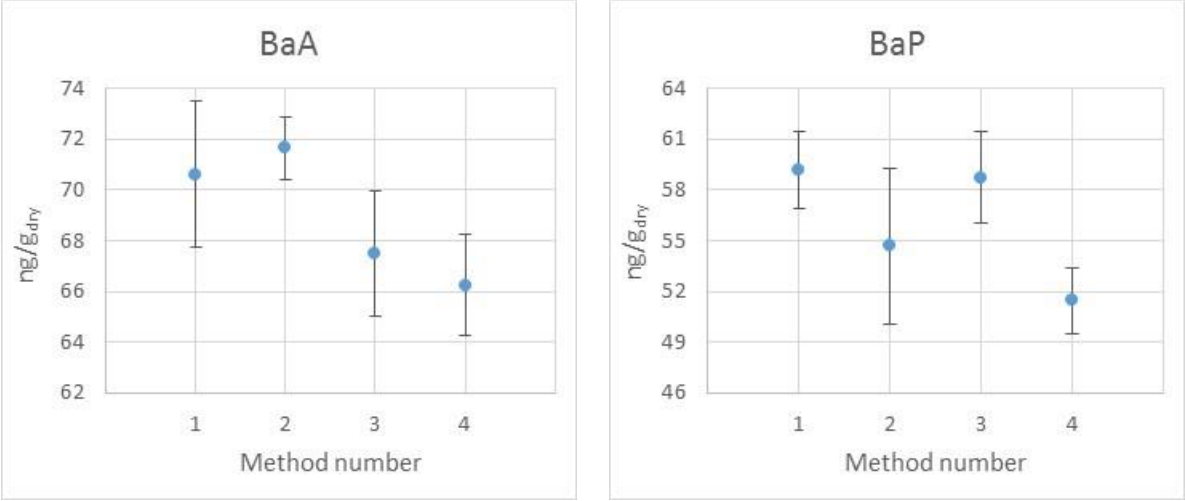


Figure I-1: BaA and BaP results (ng/g_{dry}) using methods 1 to 4.
Error bars represent ± *u*.

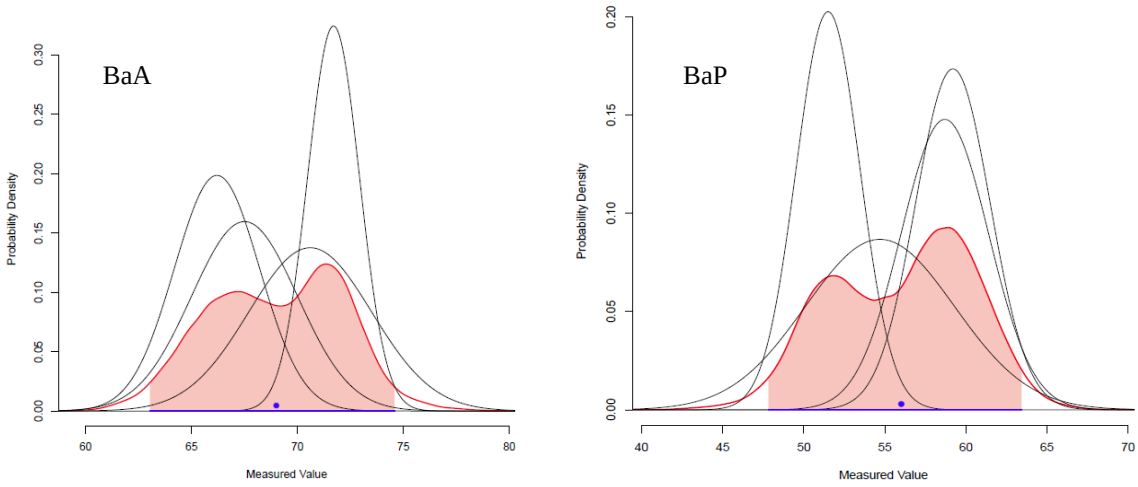


Figure I-2: Linear opinion pooling for BaA and BaP.
Blue dot marks the estimated consensus value. The blue line segment beneath the pink fill is a 95 % coverage interval.

APPENDIX J: Prototype Broader-Scope Core Competency Claim

Table J-1: Prototype Broader Category 10 Claims
for All Participants

Measurement service	Category 10. Biological fluids and materials
Measurement service sub-category	Botanical materials (10.6)
Matrix	Leaves
Measurand	Analyte or Component: low-polarity ($pK_{ow} < -2$) organic analyte of low molar mass (100 g/mol to 500 g/mol) Quantity: Mass fraction
Dissemination range of measurement capability	From 10 to 1000 Unit: ng/g
Dissemination position of expanded uncertainties	Demonstrated range: 6 % to 24 % (BaA and BaP) Unit: % Coverage factor: 2 or Student's $t_{1\text{-}0.95,n\text{-}1}$ Level of confidence: 95 % Expanded uncertainty is a relative one: Yes
Example measurands within this scope	PAHs, PCBs, PFOS, organochloride pesticides, steroids
Supporting Evidence	Successfully participated in CCQM-K95.1

Table J-2: Prototype Broader Category 1 Claims
for Participants Who Performed In-House Purity Assessment

Measurement service	Category 1. High purity chemicals
Measurement service sub-category	Organic compounds (1.2)
Matrix	High purity [individual primary component]
Measurand	Analyte or Component: low-polarity ($pK_{ow} < -2$) organic analyte of low molar mass (100 g/mol to 500 g/mol) Quantity: Mass fraction %
Dissemination range of measurement capability	From 92 to 100 [purity range of calibrant materials] Unit: %
Dissemination position of expanded uncertainties	Demonstrated range: 6 % to 24 % (BaA and BaP) Unit: % Coverage factor: 2 or Student's $t_{1\text{-}0.95,n\text{-}1}$ Level of confidence: 95 % Expanded uncertainty is a relative one: Yes
Example measurands within this scope	PAHs, PCBs, PFOS, organochloride pesticides
Supporting Evidence	Successfully participated in CCQM-K95.1 and participation in CCQM-K55 series