APMP.QM-S11
Organochlorine Pesticides in Ginseng Root

Supplementary Comparison

Final Report
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Della W. M. Sin, W. H. Fung and W. F. Wong
Government Laboratory, Hong Kong, China

With contributions from:

Klich Hanen
National Institute of research and Physical chemical analysis (INRAP)
Tunisia

Boniface Mbithi Muendo, Geoffrey Murrira Karau
Kenya Bureau of Standards (Chromatography laboratory) (KEBS)
Kenya

Ana Silva
Laboratorio Tecnológico del Uruguay (LATU)
Uruguay

Qinghe Zhang
National Institute of Metrology, P. R. China (NIM)
China

Nittaya Sudsiri
National Institute of Metrology (Thailand)/ Organic Analysis (NIMT)
Thailand

Dyah Styarini
Research Center for Metrology (RCM-LIPI)
Indonesia

Wai-fun Wong, Wai-hing Lam, Wai-hong Fung
Government Laboratory, Hong Kong (GLHK)
Hong Kong, China

SUMMARY

Ginseng is one of the most important traditional herbal medicines for health care and treatment of diseases. Trading of ginseng and related products is a multi-million dollar business. Four major countries including South Korea, China, Canada and the United States are the biggest producers and account for more than 99% of the total ginseng production around the world (i.e. about 80,000 tons) [1]. The Commission Regulation of European Union sets up that the maximum residue level (MRL) for hexachlorocyclohexane (sum of alpha, beta and delta isomers, except lindane) is 0.02 mg/kg and that for lindane is 1 mg/kg in ginseng [2]. The use of reliable methods for measurement of these organochlorine pesticides is important in safeguarding the quality of ginseng and related products and the public health.

The Government Laboratory, Hong Kong (GLHK) previously coordinated and completed CCQM-K95 "Mid-polarity Analytes in Food Matrix: Mid-polarity Pesticides in Tea" [3]. Two organochlorine pesticide residues including beta-endosulfan and endosulfan sulfate were selected for analysis. It is noteworthy that participating institutes in CCQM-K95 found that wetting of test samples prior to extraction was crucial for complete extraction of the incurred analytes in the test material of dried tea. It is apparent that sample extraction is a real technical challenge to the analysis of dried plant material.

The ginseng root is collected after years of plantation [4, 5]. It represents a higher level of analytical challenge for the participating national metrology institutes (NMIs) and designated institutes (DIs) in measuring the incurred organochlorine pesticides in dried ginseng/ginseng root, where the pesticides have been gradually accumulated in the plant material for several years. In this regard, GLHK proposed a new APMP supplementary comparison on determination of organochlorine pesticides in ginseng root at the APMP TCQM meeting in November 2015. The supplementary comparison was further discussed at the CCQM OAWG meeting in April 2016. The Chair of APMP TCQM eventually approved the proposed supplementary comparison for 2016/17 with a study number of APMP.QM-S11 in May 2016. To allow wider participation, a pilot study APMP.QM-P32, was run in parallel with this supplementary comparison.

Evidence of successful participation in formal, relevant international comparisons is needed to document calibration and measurement capability claims (CMCs) made by national metrology institutes (NMIs) and designated institutes (DIs).

Seven of NMIs/DIs participated in this Supplementary Comparison APMP.QM-S11 Organochlorine pesticides in ginseng root. Participants were requested to evaluate the mass fractions, expressed in μg/kg, of alpha-hexachlorocyclohexane (α-BHC, CAS No. 319-84-6) and gamma-hexachlorocyclohexane (Lindane, CAS No. 58-89-9) in a relatively complex food/plant material, termed ginseng root. The purpose of the comparison is to enable participating laboratories to demonstrate their capability on the determination of organochlorine pesticides in a relatively complex food/plant material. All participating laboratories performed wetting before

extraction. Different extraction methods such as soxhlet extraction, accelerated solvent extraction, ultrasonic extraction, QuEChERS technique, shaking and vortex were used among the participants. For the instrumental analysis, all laboratories employed GC technique for chromatographic separation and most laboratories used MS related techniques for detection and quantification. For α-BHC, the consensus mean was 413 µg/kg with standard deviation of 35.3 µg/kg from 4 participating institutes' results. For lindane, the consensus mean was 104 µg/kg with standard deviation of 10.9 µg/kg from 5 participating institutes' results.

Successful participation in APMP.QM-S11 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in food/plant matrices.

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ACRONYMS

ASE	accelerated solvent extraction
CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CMC	Calibration and Measurement Capability
CRM	certified reference material
CV	coefficient of variation, expressed in %: CV = $100 \cdot s/\bar{x}$
DI	designated institute
DoE	degrees of equivalence
GC-ECD	gas chromatography with electron capture detection
GC-MS	gas chromatography with mass spectrometry detection
GC-MS/MS	gas chromatography with tandem mass spectrometry detection
HPLC	high performance liquid chromatography
ID	isotope dilution
IDMS	isotope dilution mass spectrometry
KC	Key Comparison
LC	liquid chromatography
MADe	median absolute deviation from the median (MAD)-based estimate of s: MADe = $1.4826 \cdot$MAD, where MAD = median($\lvert x_i$-median(x_i)$\rvert$)
MRM	multiple reaction monitoring
NMI	national metrology institute
NMR	nuclear magnetic resonance spectroscopy
OAWG	Organic Analysis Working Group
pKow	logarithm of the octanol-water partition coefficient
PSE	pressurized solvent extraction
qNMR	quantitative nuclear magnetic resonance spectroscopy
QuEChERS	“Quick, Easy, Cheap, Effective, Rugged, Safe” liquid/solid extraction
SC	Supplementary Comparison
SCRV	Supplementary Comparison Reference Value
SIM	selected ion monitoring
SPE	solid phase extraction

SYMBOLS

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

INTRODUCTION

Ginseng is one of the most important traditional herbal medicines for health care and treatment of diseases. Trading of ginseng and related products is a multi-million dollar business. Four major countries including South Korea, China, Canada and the United States are the biggest producers and account for more than 99% of the total ginseng production around the world (i.e. about 80,000 tons). The Commission Regulation of European Union sets up that the maximum residue level (MRL) for hexachlorocyclohexane (sum of alpha, beta and delta isomers, except lindane) is 0.02 mg/kg and that for lindane is 1 mg/kg in ginseng. The use of reliable methods for measurement of these organochlorine pesticides is important in safeguarding the quality of ginseng and related products and the public health.

The Government Laboratory, Hong Kong (GLHK) previously coordinated and completed CCQM-K95 "Mid-polarity Analytes in Food Matrix: Mid-polarity Pesticides in Tea". Two organochlorine pesticide residues including beta-endosulfan and endosulfan sulfate were selected for analysis. It is noteworthy that participating institutes in CCQM-K95 found that wetting of test samples prior to extraction was crucial for complete extraction of the incurred analytes in the test material of dried tea. It is apparent that sample extraction is a real technical challenge to the analysis of dried plant material.

The ginseng root is collected after years of plantation. It will be a higher level of analytical challenge for the participating national metrology institutes (NMIs) and designated institutes (DIs) in measuring the incurred organochlorine pesticides in dried ginseng/ginseng root, where the pesticides have been gradually accumulated in the plant material for several years.

The determination of organochlorine pesticides in a relatively complex food/plant material 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 calibration and measurement capability claims (CMCs) made by NMIs and DIs.

GLHK proposed a new APMP supplementary comparison on determination of organochlorine pesticides in ginseng root at the APMP TCQM meeting in November 2015. The supplementary comparison was further discussed at the CCQM OAWG meeting in April 2016. The Chair of APMP TCQM eventually approved the proposed supplementary comparison for 2016/17 with a study number of APMP.QM-S11 in May 2016. APMP.QM-S11 was designed to assess participants' capabilities for the determination of organochlorine pesticides in a relatively complex food/plant material, ginseng root. Alpha-hexachlorocyclohexane (α-BHC, CAS No. 319-84-6) and gamma-hexachlorocyclohexane (Lindane, CAS No. 58-89-9), which are commonly used organochlorine pesticides for the growth of ginseng, are selected as the analytes in this comparison.

According to the information from the BIPM Key Comparison Database (KCDB), only a few NMIs have made Calibration and Measurement Capabilities (CMCs) claims related to the analysis of α-BHC/lindane in ginseng. This APMP supplementary comparison will facilitate NMIs and DIs in making claims on the analysis of relevant organochlorine pesticide residues in appropriate low fat, low protein food/plant matrices (e.g. ginseng/ginseng root).

The following sections of this report document the timeline of APMP.QM-S11, the measurands, study material, participants, results, and the measurement capability claims that participation in APMP.QM-S11 can support. The Appendices reproduce the official communication materials and summaries of information about the results provided by the participants.

TIMELINE

Table 1 lists the timeline for APMP.QM-S11.

Table 1: Timeline for APMP.QM-S11

Date	Action
Nov 2015	Presentation of the proposed APMP supplementary comparison at the APMP TCQM meeting
April 2016	Update on progress and sample preparation for the proposed comparison at the CCQM OAWG meeting
Oct/Nov 2016	Presentation of the results of the homogeneity and stability studies for the proposed comparison at the CCQM OAWG meeting and APMP TCQM meeting
Nov 2016	Call for participation to OAWG members and APMP TCQM members
Nov – Dec 2016	Study samples shipped to participants. The range in shipping times reflects delays from shipping and customs.
May 2017	Results due to coordinating laboratory
Sep/Nov 2017	Presentation of the participants' results and proposed reference values for the supplementary comparison at the CCQM OAWG meeting and APMP TCQM meeting
Apr 2018	Discussion of the reference values for the supplementary comparison at the CCQM OAWG meeting
Oct – Nov 2018	Draft A report distributed to OAWG and APMP TCQM members
Apr 2019	Draft B report distributed to OAWG
June 2019	Final report approved by OAWG

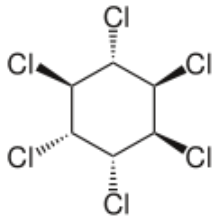
MEASURANDS

Mass fractions (μg/kg) of two incurred organochlorine pesticides, namely alpha-hexachlorocyclohexane and gamma-hexachlorocyclohexane, in ginseng root were to be determined. The general information of the two analytes and their expected mass fractions as determined by gas chromatography with mass spectrometry are listed in Table 2.

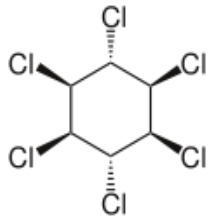
Table 2: General information of the two analytes

Analyte	Molecular weight	-log $P_{(octanol\text{-}water)}$	Expected mass fraction (μg/kg)
α-BHC	290.831	-3.8	10 – 1000
Lindane	290.831	-3.72	10 – 1000

Figure 1 below displays the molecular structure of these compounds.



alpha-hexachlorocyclohexane
α -BHC
CAS No.: 319-84-6
MW: 290.831
pK_{OW} -3.8



gamma-hexachlorocyclohexane
Lindane
CAS No.: 58-89-9
MW: 290.831
pK_{OW} -3.72

Figure 1: Structures of analytes

STUDY MATERIALS

A batch of about 12 kg of dried ginseng root confirmed to have the incurred organochlorine pesticides was purchased from the local market. The raw ginseng root was washed with distilled water to remove dirt and other foreign matters where necessary, and freeze-dried for 7 days. The dried material was blended to give a powder. The ginseng root powder was subjected to a sieving process through two calibrated sieves (200 and 100 µm respectively). The sieved powder (particle sizes: 100–200 µm) was thoroughly homogenised in a 3-dimensional mixer for 5 days. The material was irradiated using gamma source at a dose of about 1 kGy for disinfection. The irradiated material was packed into pre-cleaned and nitrogen-flushed amber glass bottles, each of about 25 g. Finally, each bottle of sample was vacuum-sealed in a polypropylene bag. All prepared bottles of sample were stored in a freezer (about -20 ^{o}C) prior to distribution or use.

Each participant received one bottle containing about 25 g of ginseng root powder. The recommended minimum sample amount for analysis was at least 1 g. Measurement results were to be reported on a dry-mass basis.

Dry Mass Determination

For the determination of dry mass correction, a minimum of three separate portions (recommended size to be about 1 g each) of the sample shall be taken and placed over anhydrous calcium sulphate (DRIERITE®) in a desiccator at room temperature for a minimum of 20 days until a constant mass is reached. Dry mass correction shall be carried out at the same time as the test sample portions are to be analysed.

Homogeneity Assessment of Study Material

The homogeneity study was conducted after the testing material was bottled and irradiated. 10 bottles of the test material (conditioned at about -20 ^{o}C) were randomly selected from the whole lot of bottles prepared. Two test portions of 1.0 g were taken from each bottle for analysis. The test portions were spiked with known amounts of labelled internal standards and then undergone a wetting process. The analytes were extracted from the sample by soxhlet extraction and then clean-up with Envi-Carb/NH_2 SPE and florisil SPE. The extracts were analysed using GC-NCI-MS using the calibration curve approach. ANOVA technique was applied to assess the between-bottle homogeneity in accordance with ISO Guide 35:2006 [6].

The results are summarised in Table 3. The homogeneity study results indicated that no significant inhomogeneity was observed in the test material. The test material was considered fit for the purpose of the supplementary comparison.

Table 3. Results of the homogeneity assessment.

Analyte	ANOVA test		Relative standard uncertainty due to between-bottle inhomogeneity, u_{bb} (%)
	F-statistics	Critical value	
α-BHC	1.38	3.02	0.716
Lindane	1.13	3.02	0.980

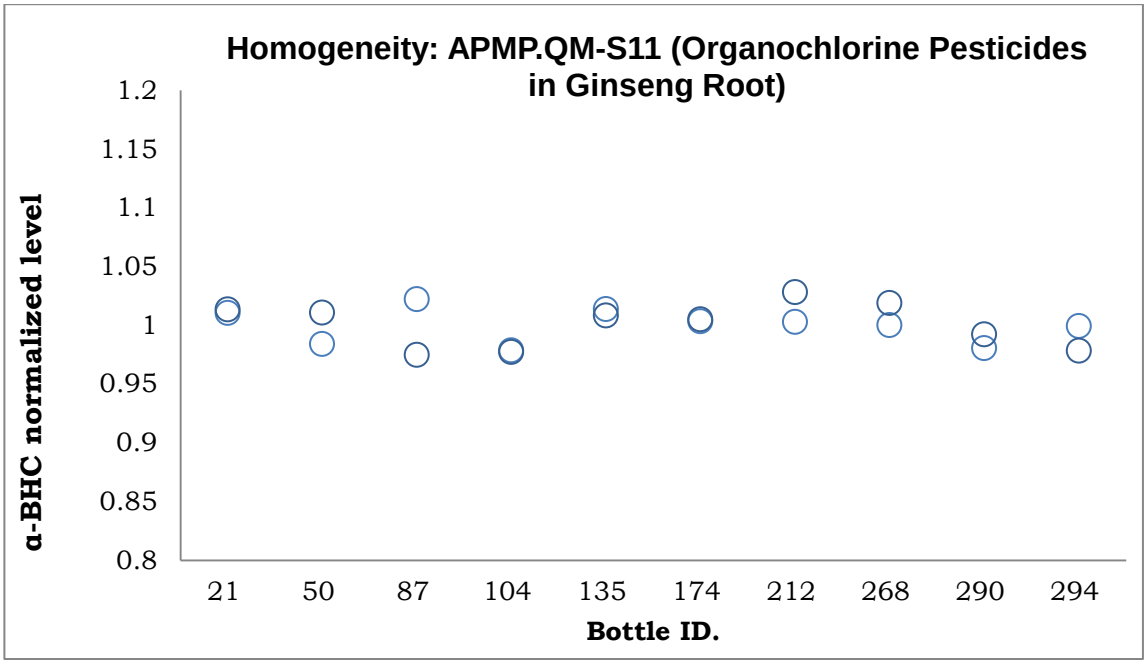


Figure 2 Graphical presentation of homogeneity results for α-BHC.

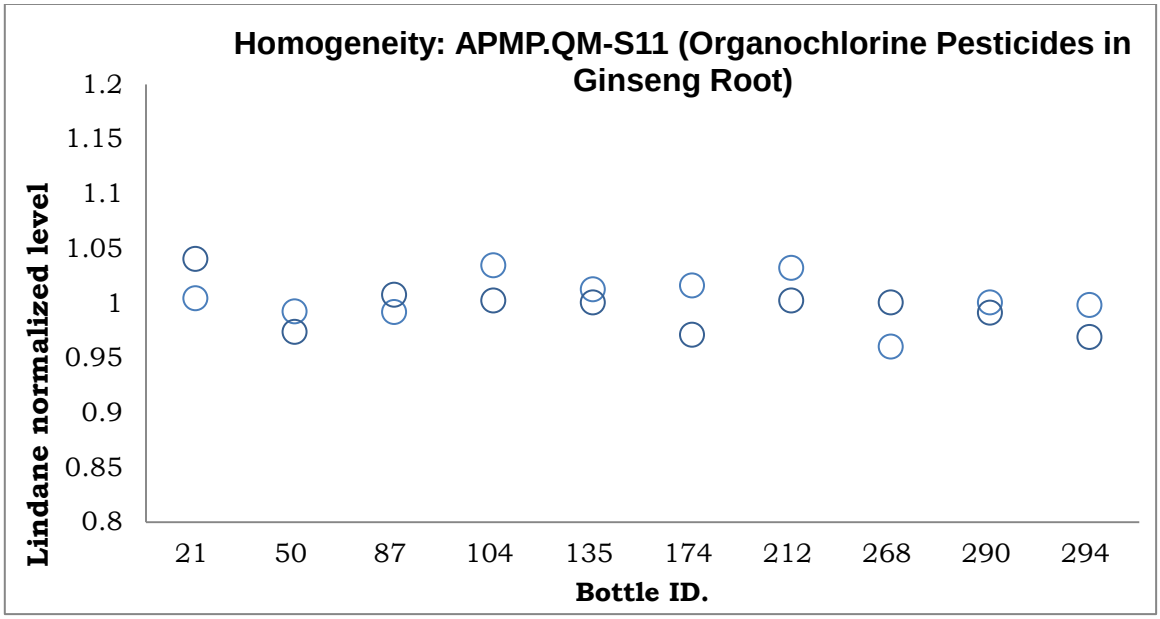


Figure 3 Graphical presentation of homogeneity results for lindane.

Stability Assessment of Study Material

The stability studies were conducted for the test material using the same analytical procedures as for the homogeneity study. For the short-term stability (i.e. stability of the test material under "transport conditions"), the study was conducted on the isochronous approach over a period of 4 weeks at a simulated transport temperature (conditioned at 30 ± 5 ^{o}C, 35 ± 5 ^{o}C and 40 ± 5 ^{o}C) against the reference temperature at about -70 ^{o}C. Two bottles of sample were randomly taken from the storage temperature (about -20 °C) to the simulated transport temperature on three occasions (1, 2 and 4 weeks) over the study period. Each bottle of sample was analysed in duplicate for monitoring the sample instability. The trend-analysis technique proposed by ISO Guide 35:2006 was applied to assess the stability of the test material at 30 ^{o}C, 35 ^{o}C and 40 ^{o}C. The results are summarised in Tables 4 and 5 and graphically presented in Figure 4.

Table 4. Summary of short-term study results of α-BHC

Duration	28 days		
Design	30ºC	35ºC	40ºC
Mean ($\bar{y}$) (µg/kg)	422.9	424.5	420.5
Slope of the regression line (b_1)	-2.171	-0.2774	-3.518
Intercept of the regression line (b_0)	426.7	425.0	426.7
Variance of the points (s^2)	17.35	17.63	19.04
Standard deviation of the points (s)	4.166	4.198	4.364
Uncertainty associated with slope [$s(b_1)$]	1.408	1.419	1.475
Student's t-test ($t_{0.95, n-2}$)	4.303	4.303	4.303
Critical value of b_1 [$t_{0.95, n-2} \times s(b_1)$]	6.059	6.107	6.348

Table 5. Summary of short-term study results of lindane

Duration	28 days		
Design	30ºC	35ºC	40ºC
Mean ($\bar{y}$) (µg/kg)	109.6	109.6	108.2
Slope of the regression line (b_1)	0.03159	-0.3507	-0.4580
Intercept of the regression line (b_0)	109.6	110.3	109.0
Variance of the points (s^2)	1.128	0.07972	4.133
Standard deviation of the points (s)	1.062	0.2823	2.033
Uncertainty associated with slope [$s(b_1)$]	0.3590	0.09545	0.6873
Student's t-test ($t_{0.95, n-2}$)	4.303	4.303	4.303
Critical value of b_1 [$t_{0.95, n-2} \times s(b_1)$]	1.545	0.4107	2.957

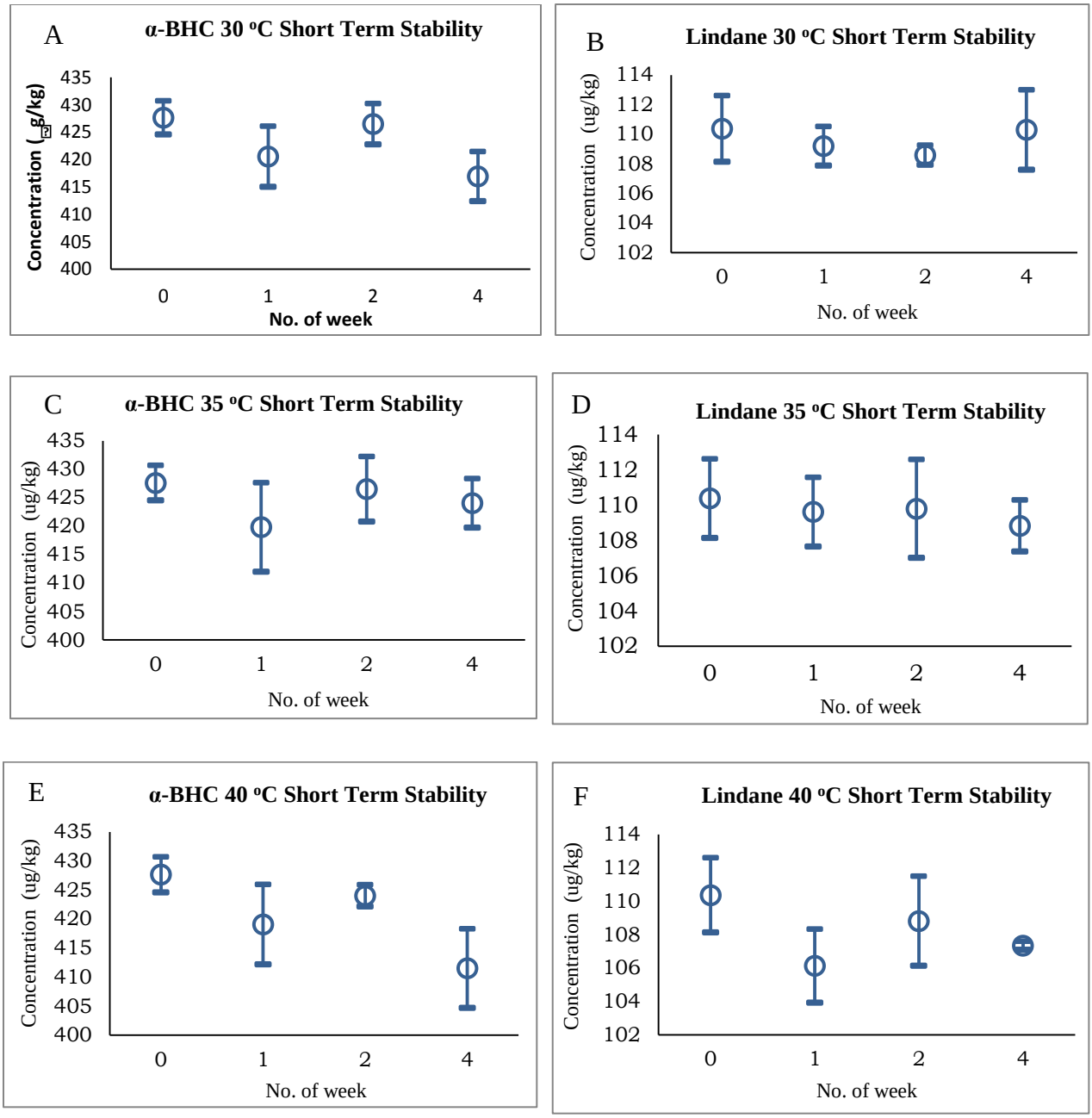


Figure 4: Short Term Stability Results

Note: Panel A displays the short term stability results for α-BHC at 30 °C. Panel B displays the short term stability results for lindane at 30 °C. Panel C displays the short term stability results for α-BHC at 35 °C. Panel D displays the short term stability results for lindane at 35 °C. Panel E displays the short term stability results for α-BHC at 40 °C. Panel F displays the short term stability results for lindane at 40 °C.

The statistical results shown in Tables 4 and 5 indicated that no significant trend at 95% confidence level was detected as the absolute values of b_1 (i.e. slope of the regression line) were smaller than the critical values of b_1 which were the uncertainty associated with the slope of the

regression line for the stability at different temperatures for 4 weeks. Hence, the instability of the material was insignificant at the study temperature under "transport conditions".

The stability of the study material was also evaluated through ANOVA test on the regression with results summarised in Table 6.

Table 6. Summary of p-value for short-term study results

Analyte	p-value for the slope		
	30 ºC	35 ºC	40 ºC
α-BHC	0.263	0.863	0.140
Lindane	0.940	0.067	0.574

All p-values were greater than 0.05, it was thus concluded that the corresponding slope was not significantly deviated from zero at 95% level of confidence. In other words, no instability was observed for the test material at 30 ºC, 35 ºC and 40 ºC during the testing period.

For the long-term stability (i.e. stability of the test material under "storage conditions"), the study is conducted on the classical approach covering the period from "the planned date of distribution of test samples to participants" to "the deadline for submission of results" at the storage temperature (conditioned at about -20 °C). The results are summarised in Tables 7 and 8 and graphically presented in Figure 5.

Table 7. Summary of long-term study results

Duration	266 days at -20°C (as at 8 May 2017)	
Design	α-BHC	Lindane
Mean ($\bar{y}$) (µg/kg)	430.3	106.4
Slope of the regression line (b_1)	-0.02097	-0.01366
Intercept of the regression line (b_0)	432.8	107.5
Variance of the points (s^2)	8.426	5.437
Standard deviation of the points (s)	2.903	2.332
Uncertainty associated with slope [$s(b_1)$]	0.01338	0.01002
Student's t-test ($t_{0.95, n-2}$)	3.182	3.182
Critical value of b_1 [$t_{0.95, n-2} \times s(b_1)$]	0.04257	0.03189

Table 8. Summary of p-value for long-term study results

Analyte	p-value for the slope
	266 days (as at 8 May 2017)
α-BHC	0.215
Lindane	0.222

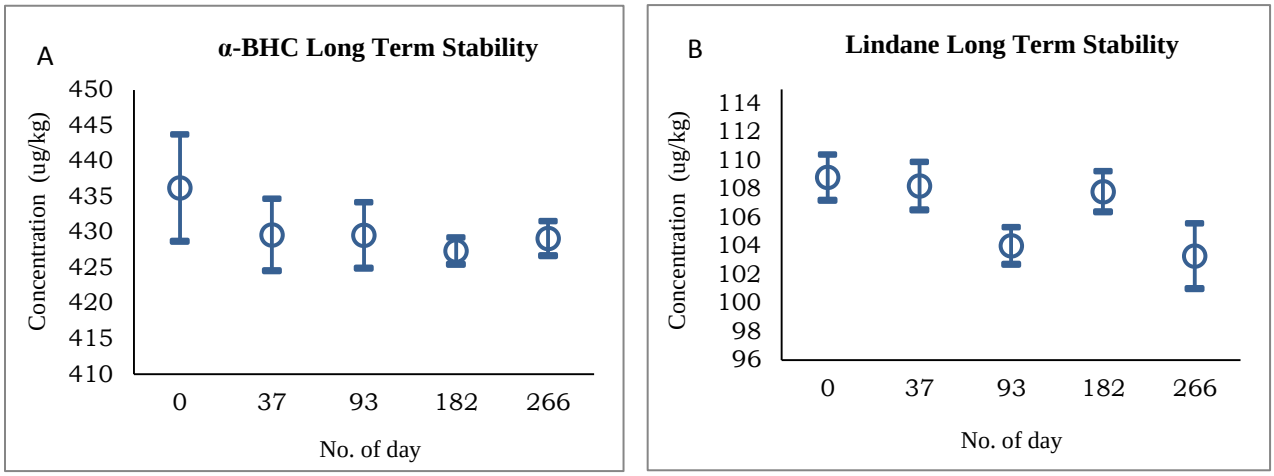


Figure 5: Long Term Stability Results

Note: Panel A displays the long term stability results for α -BHC. Panel B displays the long term stability results for lindane.

The statistical results shown in Table 7 indicated that no significant trend at 95% confidence level was detected as the absolute values of b_1 (i.e. slope of the regression line) were smaller than the critical values of b_1 which were the uncertainty associated with the slope of the regression line for the stability at storage temperatures for 266 days. Hence, the instability of the material was insignificant throughout the programme. Moreover, all p-values were greater than 0.05, it was concluded that the corresponding slope was not significantly deviated from zero at 95% level of confidence. In other words, no instability was observed for the test material at the storage temperature of -20°C during the testing period. The test material was considered fit for the purpose of the supplementary comparison.

PARTICIPANTS, INSTRUCTIONS AND SAMPLE DISTRIBUTION

The call for participation was distributed in November 2016 with the intent to distribute samples in December 2016, receive results in 31 March 2017. Request for extension of deadline of result submission from participant(s) was received in March and April due to delay on shipment of

standard/chemicals, personnel shortage and other technical reasons. The deadline for results submission was then extended from 31 March 2017 to 15 May 2017. The results were discussed at the Ottawa OAWG meeting, September 2017 and APMP TCQM Meeting. See Table 1 for study timeline. Appendix A reproduces the Call for Participation; Appendix B reproduces the Technical Protocol.

Table 9 lists the institutions that registered for APMP.QM-S11

Table 9: Institutions Registered for APMP.QM-S11

NMI or DI	Code	Country	Contact
Kenya Bureau Of Standards-Chromatography Laboratory	KEBS	Kenya	Mr. Boniface Mbithi Muendo mbithib@kebs.org
National Institute of Metrology (Thailand)/ Organic Analysis	NIMT	Thailand	Ms. Nittaya Sudsiri nittayas@nimt.or.th
Research Center for Metrology	RCM-LIPI	Indonesia	Dyah Styarini dyah.styarini@lipi.go.id
National Institute of Metrology, China	NIM	China	Dr. Qinghe Zhang zhangqh@nim.ac.cn
Government Laboratory, Hong Kong	GLHK	Hong Kong	Dr. Wai-fun Wong wfwong@govtlab.gov.hk
Laboratorio Tecnológico del Uruguay	LATU	Uruguay	Ms. Ana Inés Silva asilva@latu.org.uy
National Institute of research and Physical chemical analysis	INRAP	Tunisia	Ms Klich Hanen klich_hanen@yahoo.fr

Seven NMIs/DIs (Table 9) registered for participation in APMP.QM-S11. One bottle of sample each containing about 25 g of the ginseng root powder with cold packs in foam box were sent to all participants via courier at the end of December 2016. A temperature strip was attached on each bottle for the purpose of monitoring the maximum temperature exposure during the transportation. Relevant documents were sent to participants by e-mail. Participants were asked to check the physical conditions of the sample upon receipt of the sample pack. All samples were received by the participants in good condition not later than mid-January 2017.

Each participant was requested to determine the mass fractions (in µg/kg) of the two pesticides on a dry mass basis with their preferred methods. A minimum sample size of 1 g for testing was recommended with the following protocol for determination of moisture content:

(i) a minimum of three separate portions (recommended size of 1 g each) of the sample should be taken;

(ii) place the portions over anhydrous calcium sulphate (DRIERITE®) in a desiccator at room temperature for a minimum of 20 days until a constant mass is reached; and

(iii) perform moisture determination at the same time as the test sample portions are to be analysed.

The participants were requested to fill in the test results, extraction methods, post-extraction clean-up method and transformation procedures, analytical instrumental details, measurement equation, source(s) of calibrant(s) and internal standard(s), uncertainty estimation details and additional observation(s), if any, in the Analyte Matrix Core Competency Template and the Report Form and send the completed Form to the organiser by e-mail before the extended deadline for submission of results on 15 May 2017.

RESULTS

Each participant was requested to report the mass fractions (in μg/kg) of α-BHC and lindane on a dry mass basis

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

5 results of α-BHC and 7 results of lindane were received from 7 institutions that received samples.

Calibration Materials Used by Participants

Participants were required to establish the metrological traceability of their results using certified reference materials (CRMs) with stated traceability. Table 10 lists the CRMs that were used. Table 11 lists how participants established traceability.

KEBS did not establish a proper metrological traceability for the calibrant used. KEBS did not perform purity assessment on the lindane neat standard from commercial source and the result was therefore not included in the SCRV calculation.

Table 10: Certified Reference Materials Used

CRM	Provider	Analyte	Mass Fraction[a] Delivered	Mass Fraction[a] Source Material, %	In-house Purity Methods Used to Value-Assign Source Material[b]
SRM 2275	NIST	α-BHC	3.00 ± 0.15 mg/kg	Not provided	GCFID, DSC
GBW(E) 060081	NIM China	α-BHC	50.2 ± 0.5 μg/ml	99. 9 ± 0.5 %	MB, ^{1}H NMR
NMIA P1332	NMIA	lindane	NA	99. 7 ± 0.4 %	MB, ^{1}H NMR, elemental microanalysis
GBW(E) 060083	NIM China	lindane	50.1 ± 0.5 μg/ml	99. 9 ± 0.3	MB, DSC
SRM 2261	NIST	lindane	3.012 ± 0.15 mg/kg	Not provided	GCFID, DSC

Notes:
a Stated as Value ± U_{95}(Value)
b GC-FID: Capillary gas chromatography with flame ionization detection
DSC: Differential scanning calorimetry
MB: Mass balance
^{1}H NMR: Proton nuclear magnetic resonance

Table 11: Metrological Traceability of Participants' Results

NMI/DI	Analyte	Source of Traceability	Material	Mass Fraction[a] Purity, %	In-house Purity Techniques used to assess material	Evidence of Competence
KEBS	lindane	Nil	Sigma Aldrich	98.5 %	Nil	N/A
NIMT	α-BHC	SRM 2275	N/A			
	lindane	NMIA P1332				
RCM-LIPI	α-BHC	SRM 2275	N/A			
	lindane	NMIA P1332				
NIM	α-BHC	GBW(E)060081	N/A			
	lindane	GBW(E)060083				
GLHK	α-BHC	SRM 2275	N/A			
	lindane	NMIA P1332				
LATU	lindane	NMIA P1332	N/A			
INRAP	α-BHC	SRM 2275	N/A			
	lindane	SRM 2261				

a Stated as Value ± U_{95}(Value)

Methods Used by Participants

The methods for sample pre-treatment, extraction, clean-up and instrumental analysis used by participating laboratories are summarized in Tables 12-14.

All participating laboratories performed wetting before extraction. The wetting time ranged from 0.2 to 16 hours. The ratio of sample size to amount of water for wetting ranged from 1:2 to 1:5.

Different extraction methods for the analytes were used among the participants. GLHK and LATU used Soxhlet extraction and NIM employed accelerated solvent extraction method. Ultrasonic extraction was adopted by KEBS and NIMT. KEBS and NIMT also used other extraction techniques such as shaking and vortex. INRAP employed QuEChERS technique and RCM-LIPI used vortex to extract the analyte from the sample matrix. For clean-up procedures, most laboratories applied solid phase extraction (SPE) or dispersive SPE. For the instrumental analysis, all laboratories employed GC technique for chromatographic separation and most laboratories used MS related techniques for detection and quantification, either via quadrupole GC-MS or GC-MS/MS. NIMT, RCM-LIPI, GLHK and NIM used isotope dilution mass spectrometry (IDMS) for calibration. LATU and INRAP used internal standard for quantitation. KEBS quantified the analyte by using external standard calibration.

Table 12 Summary of extraction and clean-up methods used by participants

NMI/DI	Sample size (g)	Wetting method	Extraction method	Extraction Solvent	Clean-up method
KEBS	1	5 g water for 5 hours	Sonication, vortex, shaking and centrifugation for 1 hour at 24 ^{o}C	Acetonitrile, HPLC Water, Sodium Chloride, Magnesium Sulfate, Silica gel, Primary Secondary Amine (PSA)	Solid phase extraction (SPE), Dispersive SPE, Centrifugation
NIMT	1	4 mL water for 0.5 hours	Sonication, liquid/ Liquid extraction, vortex and shaking. 30 min for sonication extraction at 35 °C.	Acetonitrile	Solid phase extraction (SPE): GCB/PSA SPE (condition: 3 column of acetone, load: 1 ml of sample, elute: 12 ml of 3:1 acetone: toluene); Dispersive SPE: 50 mg PSA, 150 mg MgSO4, 50 mg C18; Centrifugation: 4000 rpm for 10 min. and 10000 rpm for 10 min.
RCM-LIPI	2	10 g water for 2 hours	Vortex for 1 min at room temperature	Acetonitrile	Solid phase extraction (SPE): 1 g of florisil with 10 ml of n-hexane/diethyl ether (85/15) mixture as eluent.
NIM	0.4	0.8 g water for 2 hours	Accelerated solvent extraction for 75min (include heat time and purge time) at 140 °C	ethyl acetate: petroleum ether (7:3; v/v)	Centrifugation: 12000 rpm; concentrated sulfuric acid and copper powder
GLHK	1	5 g water for 12 hours	Soxhlet extraction for 16 hours	Ethyl acetate	Solid phase extraction (SPE): Envi-Carb/NH2 SPE, Florisil SPE
LATU	1	4 g water for 16 hours	Soxhlet extraction for 6 hours	Ethyl acetate	Sample extracts were cleaned-up with Solid phase extraction (SPE) containing 2 g of florisil, 1g of primary secondary amine sorbent (PSA), 1 g C18 and 0,5 g of Na2SO4. SPE column was conditioned with 10 ml of hexane:acetone 80:20 (v/v), 2 g of concentrated extract was added. Clean extract was eluted with 15 mL of hexane:acetone 80:20.
INRAP	2	10 g water for 0.2 hours	Quechers for 2 hours at ambient temperature	Acétonitrile, grade HPLC; Water HPLC quality; Sulfate de magnésium, anhydre; Chlorure de sodium; Citrate de sodium dibasique sesquihydraté; Citrate de sodium tribasique dihydraté	Dispersive SPE: Using MgSO4, C18, PSA, noir de carbone graphité

Table 13 Summary of analytical instrument used by participants

NMI/DI	Analytical instrument(s) used	Chromatographic column	Chromatographic condition	mobile phase/ carrier gas	flow rate
KEBS	GC-MS, HPLC-UV	For HPLC (C18), (Size 150*4.6 mm)	LC-Oven temperature (40-80 ^{0}C), Wavelength 254 nm, Run time 10 Minutes, GC-MS (0-60 ^{0}C, Hold for 6 minutes, 60-180 ^{0}C @ 25 ^{0}C/min, 180-240 ^{0}C @ 4 ^{0}C/min)	HPLC-UV, Acetonitrile:water (50:50), For GC-MS Helium gas	HPLC (2.0 ml/Min), GC-MS (Total flow 30.0 ml/min
NIMT	GC-MS	DB5-MS (5% phenyl 95% methylsiloxane)	Initial temp: 105 ^{o}C, initial time 1.00 min. The column was maintained at 105 ^{o}C and ramped at 10 ^{o}C/min up to 130 ^{o}C, then ramped at 4 ^{o}C/min up to 230 ^{o}C, kept for 5 min, and finally at 40 ^{o}C/min up to 290 ^{o}C	He gas	1.0 ml/min
RCM-LIPI	GC-MS	HB-5 MS UI (30m x 0.250mm x 0.25 Micron)	The oven temperature was set at 70 ^{o}C as initial temperature and hold for 2 min. The temperature was then increased to 150 ^{o}C at rate of 25 ^{o}C/min without holding. Then the temperature was increased to 200 ^{o}C at rate of 3 ^{o}C/min without holding and increased to 280 ^{o}C at rate of 8 ^{o}C/min, hold for 10 min.	Helium	1.0 ml/min
NIM	GC-MS/MS	DB-17MS (30m×0.25mm×0.25µm)	Injector temperature at 250 ℃, oven temperature at 100 ℃ for 1 min, ramped to 180 ℃ at 40 ℃/min and held for 3 min, then to 210 ℃ at 30 ℃/min and held for 8 min, finally to 300 ℃ at 90 ℃/min and held 8 min. The transfer line was set at 230 ℃, and the ion source was set at 230 ℃. The ionization energy was under electron ionization mode at 70 eV.	He	1 mL/min
GLHK	GC-MS	DB-17MS (30m x 0.25mm x 0.25µm)	PTV injector temperature was set at 90 ℃. Oven temperature was set at 90 ℃ for 2 min, ramped to 150 ℃ at 25 ℃/min, then to 180 ℃ at 1.5 ℃/min, finally to 280 ℃ at 40 ℃/min and held 3 min. The transfer line was set at 250 ℃, and the ion source was set at 200 ℃. The CI gas flow was set at 1mL/min.	Helium	1mL/min
LATU	GC-ECD	Rtx-5MS, 30 m x 0,25 mm ID x 0,25 µm df.	120 ºC (5 minutes) to 235 ºC (1 minute) at 4,5 ºC/min, then to 285 ºC (1 minute) at 4,5 ºC/min.	He	2.19 mL/min
INRAP	GC-ECD	HP-5MS (5% Phényl Méthyl Siloxane) : 30m*250um*0.25um	50 °C-1mn, 25 °C/min to 100 °C, 5°C/min to 300 °C (5min)	Helium	1mL/min

Table 14 Summary of analytical instrument and quantitation method used by participants

NMI/DI	Ionisation mode of MS	Ions/MRM transitions monitored	Type of calibration	Method of quantitation	Matrix match calibration?	Method for ID of analyte
KEBS	EI (For GC-MS)		Multi-level calibration curve	External standard	No	Retention time
NIMT	ESI	Alpha-BHC : 181 (Q) 219 217 , Internal standard; 13C6-alpha- BHC à 187 (Q) 225 223 Lindane : 181 (Q) 219 217 , Internal standard; 13C6-alpha- BHC à 187 (Q) 225 223	Multi-level calibration curve	Isotope dilution mass spectrometry	Yes	SIM 3 ions for confirmation
RCM-LIPI	EI	α-BHC: $^{12}C_6$: m/z 181, 183 and 219 $^{13}C_6$: m/z 187, 189 and 225 Lindane : $^{12}C_6$: *m/z* 181, 183 and 254 $^{13}C_6$: *m/z* 187, 189 and 260	Single-point calibration	Isotope dilution mass spectrometry	No	Retention time and ratio of 3 ions.
NIM	EI	a-BHC: m/z 181/145 (quantitation), 181/109; 13C6-a-BHC: m/z 187/151 (quantitation), 187/115 Lindane: m/z 181/145 (quantitation), 181/109. 13C6-Lindane: m/z 187/151 (quantitation), 187/115	Single-point calibration	Isotope dilution mass spectrometry	No	Methods used for identification of the analyte in sample (e.g. retention time, MRM ratio of 3 ion transitions, etc.): Analytes identified through comparison against standard reference materials retention time and mass spectrum ion ratios of 2 independent multiple reaction monitoring (MRM) transitions by tandem GC-MS/MS
GLHK	NCI	α-BHC: 254.9 (Q), 252.9, 256.9, 260.9 (Q), 262.9, 264.9 Lindane: 254.9 (Q), 252.9, 256.9, 260.9 (Q), 262.9, 264.9	Bracketing calibration	Isotope dilution mass spectrometry	No	Retention time and mass ratio of 2 qualifier ions
LATU	N/A		Bracketing calibration	Internal standard	Yes	Retention time
INRAP			Multi-level calibration curve	Internal standard	Yes	Retention time

Participant Results for α-BHC and lindane

Five results of α-BHC and seven results of lindane were received from the seven institutions that received samples. The relative expanded uncertainties of INRAP's results were over 35% which were relatively significantly larger than other participating institutes. The reported result for lindane from KEBS was one order of magnitude lower than the other participating institutes. There was no significant trend in the results for different extraction or analytical techniques used.

The results for APMP.QM-S11 for the determination of α-BHC and Lindane are detailed in Table 15-16 and presented graphically in Figure 6-7 respectively.

Table 15: Reported Results for α-BHC

	α-BHC, µg/kg					
NMI	x	$u(x)$	$u(x)$ %	k	$U(x)$	$U(x)$ %
GLHK	430	15	3.49	2.00	30	6.98
INRAP	*428.6*	*81.427*	*19.0*	*2.00*	*162.8*	*38.0*
NIM	407	10.5	2.58	2.00	21	5.16
NIMT	366.9	24.002	6.54	2.06	50	13.6
RCM-LIPI	449	12	2.67	2.00	24	5.35
n	4					
$\bar{x}$	413					
s	35.3					
CV	8.55					

Results in *red italic font* have been withdrawn from statistical consideration
n = number of results included in summary statistics; $\bar{x}$ = mean; s = standard deviation;
CV = $100 \cdot s/\bar{x}$

The results for INRAP and NIMT do not align with the OAWG guidance document for significant figures with respect to their quoted values for u(x), they would be better reported as 81.4 µg/kg and 24.0 µg/kg, respectively. The same issue was observed for the lindane results for these two participants.

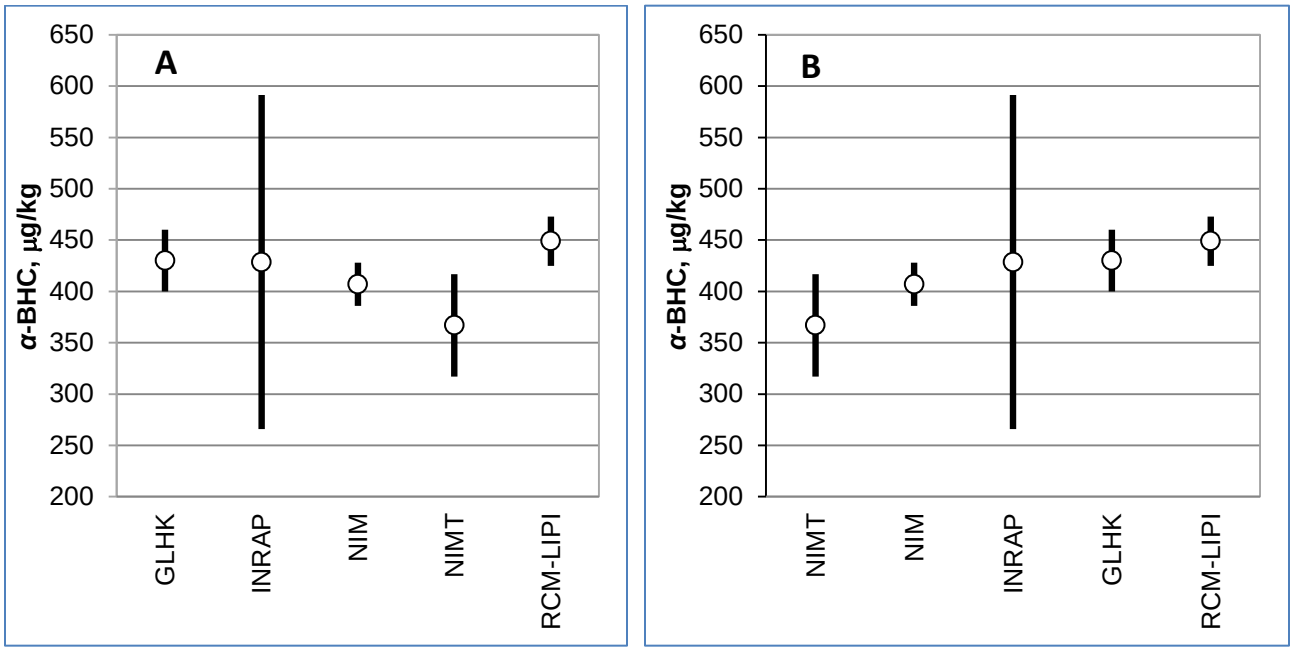


Figure 6: Illustrated Reported Results for α-BHC, µg/kg

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

Table 16: Reported Results for lindane

	lindane, µg/kg					
NMI	x	$u(x)$	$u(x)$ %	k	$U(x)$	$U(x)$ %
GLHK	108	2.4	2.22	2.00	4.8	4.44
INRAP	*164.79*	*32.609*	*19.8*	*2.00*	*65.21*	*39.6*
KEBS	*13.676*	*0.154*	*1.13*	*2.00*	*0.31*	*2.27*
LATU	120	6.5	5.42	2.00	13	10.8
NIM	102	3.5	3.43	2.00	7	6.86
NIMT	91.3	6.496	7.12	2.36	16	17.5
RCM-LIPI	98	4	4.08	2.00	8	8.16
n	5					
$\bar{x}$	104					
s	10.9					
CV	10.5					

Results in *red italic font* have been withdrawn from statistical consideration
n = number of results included in summary statistics; $\bar{x}$ = mean; s = standard deviation;
CV = $100 \cdot s/\bar{x}$

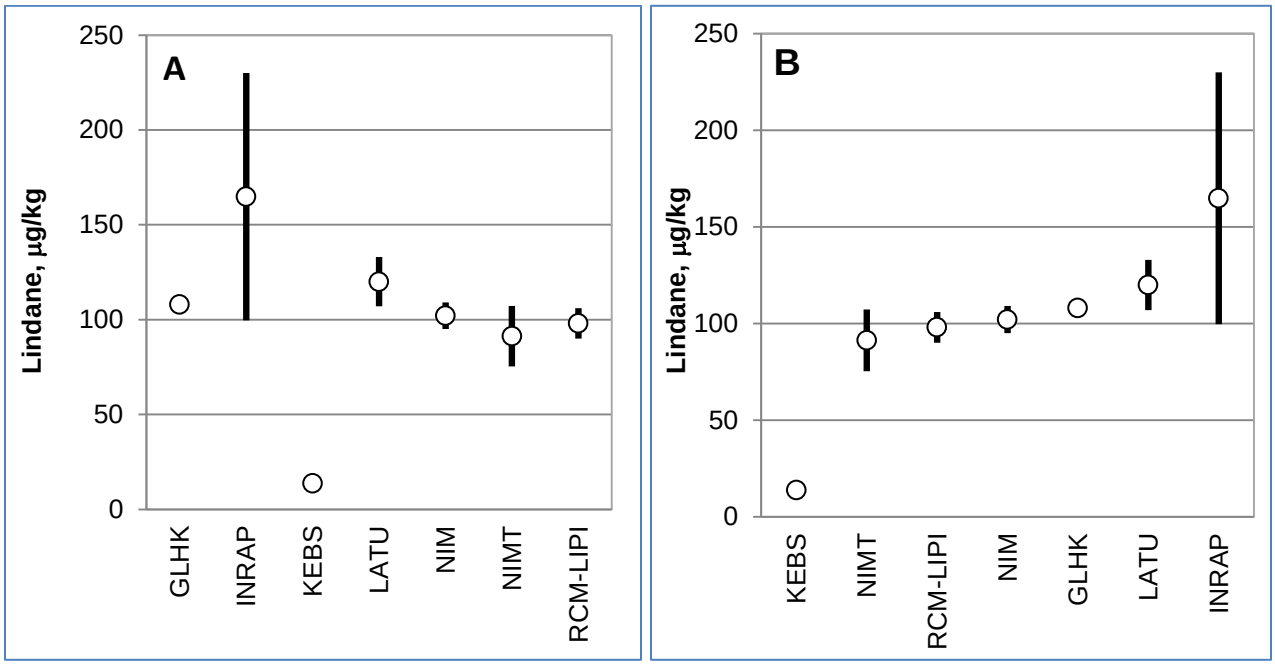


Figure 7: Illustrated Reported Results for lindane, µg/kg

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

Discussion of Results

An "Initial Result Summary" was prepared and circulated to the participants on 5 July 2017. Participating institutes were requested to check any transcription errors produced by the coordinating laboratory. They were also requested to review their own results and inform the coordinating laboratory, together with reasons, if they identify any measurement problems which explain errors on the reported results.

KEBS used a commercial neat standard as calibrant for the analysis of lindane in the returned Report Form. The coordinating laboratory clarified with KEBS the traceability of the calibrant used and KEBS's response on 25 July 2017 confirmed that they did not perform purity assessment on the lindane neat standard used. KEBS also reported they had dilution problem during analysis which led to a biased low result for lindane. KEB's expanded uncertainties for lindane appeared to be significantly under estimated and it is recommended they review their method and the uncertainty budget.

INRAP's reply on 6 July 2017 commented that they had made a transcription error in calculating the measurement uncertainties. They revised their reported MU for both measurands as listed in Table 17. These results were also reported with too many significant figures.

Table 17: Revised Reported Results from INRAP

	Mass fraction (μg/kg) (on dry mass basis)	Combined standard uncertainty (μg/kg)	Revised combined standard uncertainty (μg/kg)
α-BHC	428.6	81.427	13.607
lindane	164.79	32.609	6.858

Participating institutes in CCQM-K95 found that wetting of test samples prior to extraction was crucial for complete extraction of the incurred analytes (beta-endosulfan and endosulfan sulphate) in the test material of dried tea. It is apparent that sample extraction is a real technical challenge to the analysis of dried plant material. All the participating institutes in APMP.QM-S11 performed wetting before sample extraction. The wetting time ranged from 0.2 to 16 hours and the ratio of sample size to amount of water for wetting ranged from 1:2 to 1:5. There was no significant trend on the results against the wetting time or amount of water for wetting. The inclusion of wetting procedures prior to extraction could also achieve complete extraction of incurred organochlorine pesticides α-BHC and lindane in another dried plant material of ginseng root.

SUPPLEMENTARY COMPARISON REFERENCE VALUE (SCRV)

KEBS did not establish a proper metrological traceability for the calibrant used and had problem in dilution. KEBS's result for lindane was therefore excluded in the SCRV calculation. INRAP had revised their reported MU and their results were also excluded based on technical grounds in the calculation of SCRV.

The SCRV for α-BHC was calculated from 4 participants (NIMT, NIM, GLHK, RCM-LIPI) and that for lindane was calculated from 5 participants (NIMT, NIM, GLHK, RCM-LIPI, LATU).

The standard uncertainty of SCRV of arithmetic mean is calculated as follows:

$$\text{standard uncertainty} = \frac{\text{standard deviation}}{\sqrt{\text{n}}}$$

where n is the participants' results included in the calculation.

The standard uncertainty of SCRV of median is calculated as follows:

$$\text{standard uncertainty} = 1.25 \times \frac{\text{MADe}}{\sqrt{\text{n}}}$$

where n is the participants' results included in the calculation

The results were first discussed at the CCQM OAWG meeting in September 2017, Ottawa. Two approaches, mean and median, for the calculation of SCRVs were presented. Other statistical methods for SCRVs were suggested during the meeting. The participating institutes' results were further processed using the NIST Consensus Builder. The candidate SCRVs for α-BHC and lindane from different statistical approaches were shown in Tables 18 and 19 respectively. The approximate 95 % expanded uncertainties, U_{95}(SCRV) are estimated as: U_{95}(SCRV) = $t_s \times u$(SCRV), where t_s is the Student's t two-tailed expansion factor for corresponding measurand degrees of freedom and 95 % coverage.

Table 18: Candidate Supplementary Comparison Reference Values for α-BHC

α-BHC (n=4)	SCRV (μg/kg)	u(SCRV) (μg/kg)	u(SCRV) (%)	U_{95} (SCRV) (μg/kg)	U_{95}(SCRV) (%)
Arithmetic Mean	413	18	4.3	56	14
Median	419	20	4.7	62	15
DerSimonian-Laird Mean	417	16	3.9	52	13
Hierarchical Bayes	417	21	5.0	67	16
Linear Pool	413	35	8.4	110	26

U_{95}(SCRV) = $t_s \times u$(SCRV), where t_s is the appropriate two-tailed Student's t critical value for 3 degrees of freedom and 95 % coverage (3.18).

Table 19: Candidate Supplementary Comparison Reference Values for lindane

lindane (n=5)	SCRV (μg/kg)	u(SCRV) (μg/kg)	u(SCRV) (%)	U_{95} (SCRV) (μg/kg)	U_{95}(SCRV) (%)
Arithmetic Mean	104	4.9	4.7	14	13
Median	102	5.0	4.9	14	13
DerSimonian-Laird Mean	104	4.3	4.2	12	12
Hierarchical Bayes	104	5.0	4.8	14	13
Linear Pool	104	11	11	30	29

U_{95}(SCRV) = $t_s \times u$(SCRV), where t_s is the appropriate two-tailed Student's t critical value for 4 degrees of freedom and 95 % coverage (2.78).

Expert advice from the OAWG Chair and NIST was sought. Mike Nelson from NIST had produced a draft guidance document on the rationale for different statistical approaches to KCRV selection for the OAWG and this was discussed in conjunction with the APMP.QM-S11 results. This suggested that the Hierarchical Bayes mean and arithmetic mean were both suitable estimators for the SCRVs. The Hierarchical Bayes approach was selected as the SCRVs' estimator with the following rationale.

Among the five approaches, the DerSimonian-Laird procedure is most appropriate to combine measurement results from ten or more laboratories which is NOT suitable in this study. There were only four data points for α-BHC and five data points for lindane. The linear pool approach is a conservative approach which would be suitable if it was felt the laboratories may be measuring different materials or if there is a large degree of heterogeneity in the test material. In this study, there was just a single matrix (ginseng root) and the relative standard uncertainty due to between-bottle inhomogeneity, u_{bb} was 0.72% and 0.98% for α-BHC and lindane, respectively. There was no evidence of large degree of heterogeneity in the test material. The linear pool model would then

be overly conservative and is not suitable. The arithmetic mean and median model are more simple estimators for the SCRVs but they do not include the individual laboratory uncertainties which may underestimate the SCRV uncertainty. The Hierarchical Bayes procedure is the most suitable estimator for both measurands, which essentially gives the weighted mean as the SCRV for α-BHC and the mean as the SCRV for lindane, both having uncertainty that accounts for laboratory-specified uncertainties and "dark uncertainty".

Figure 8 below displayed the Hierarchical Bayes mean as SCRVs to the reported data.

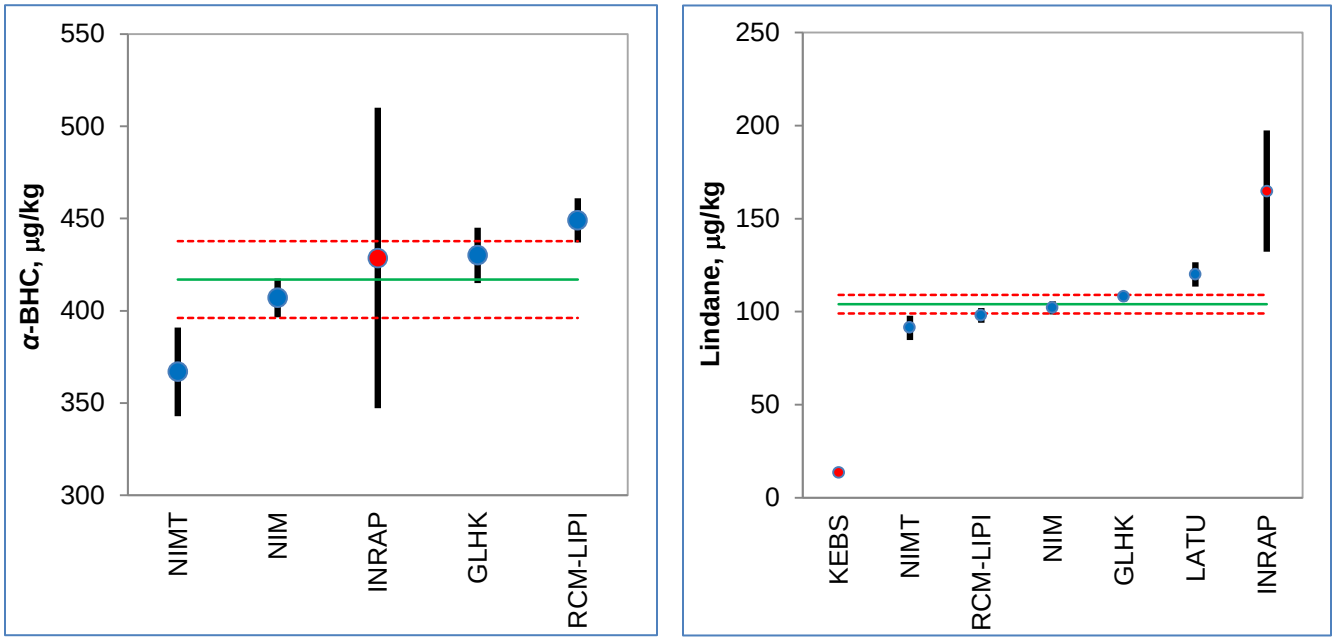


Figure 8: SCRVs for APMP.QM-S11

Notes: The results are sorted by increasing reported value. Dots represent the reported values, x; bars their standard uncertainties, $u(x)$. The green horizontal line denotes the SCRV. The bracketing red lines denote the standard uncertainty of the SCRV.

DEGREES OF EQUIVALENCE (DoE)

The absolute degrees of equivalence for the participants in APMP.QM-S11 are estimated as the signed difference between the combined value and the SCRV: $d_i = x_i$ – SCRV.

The following paragraph provides an explanation of how the uncertainty of the DoE ($U(d_i)$) is determined. The expanded uncertainty at 95% confidence interval on the d_i, $U(d_i)$ for the NMI/DIs with results included in the SCRV calculation is calculated using the NIST Consensus Builder. Since the NIST Consensus Builder currently does not provide the uncertainty of the DoE for laboratories that are excluded in the SCRV calculation, advice was consulted from NIST. The nominal k=2 expanded uncertainty on the d_i, $U(d_i)$ for the NMI/DIs with results excluded in the SCRV calculation is calculated using the following equation:

$$U(d_i) = 2\sqrt{u^2(x_i) + \tau^2}$$

where τ is the "dark uncertainty". For α-BHC, τ=25.13. For lindane, τ=6.7.

To enable comparison with the degrees of equivalence estimates from other studies, it is convenient to express the d_i and $U(d_i)$ as percentages relative to the SCRV: $\%d_i = 100 \cdot d_i$ / SCRV and

$U(\%d_i) = 100 \cdot U(d_i)$ / SCRV.

Table 20 below lists the numeric values of d_i, $U(d_i)$, d_i, and $U(d_i)$ for all participants in APMP.QM-S11 for both α-BHC and lindane.

Table 20: Degrees of Equivalence for α-BHC and lindane

	α-BHC, μg/kg				lindane, μg/kg			
NMI	*d*	*U*(*d*)	*%d*	*U*(%*d*)	*d*	*U*(*d*)	*%d*	*U*(%*d*)
GLHK	13	96	3.1	23	4.1	24	3.9	23
INRAP	11	170	2.7	41	61	67	59	64
KEBS	-	-	-	-	-90	13	-87	13
LATU	-	-	-	-	16	26	16	25
NIM	-10	92	-2.4	22	-1.9	24	-1.8	23
NIMT	-50	100	-12	24	-13	25	-12	24
RCM-LIPI	32	91	7.6	22	-5.9	24	-5.7	24

Figure 9 below graphically illustrates both the absolute and relative DoEs for two measurands using the KCRVs (Hierarchical Bayes approach).

α-BHC Lindane

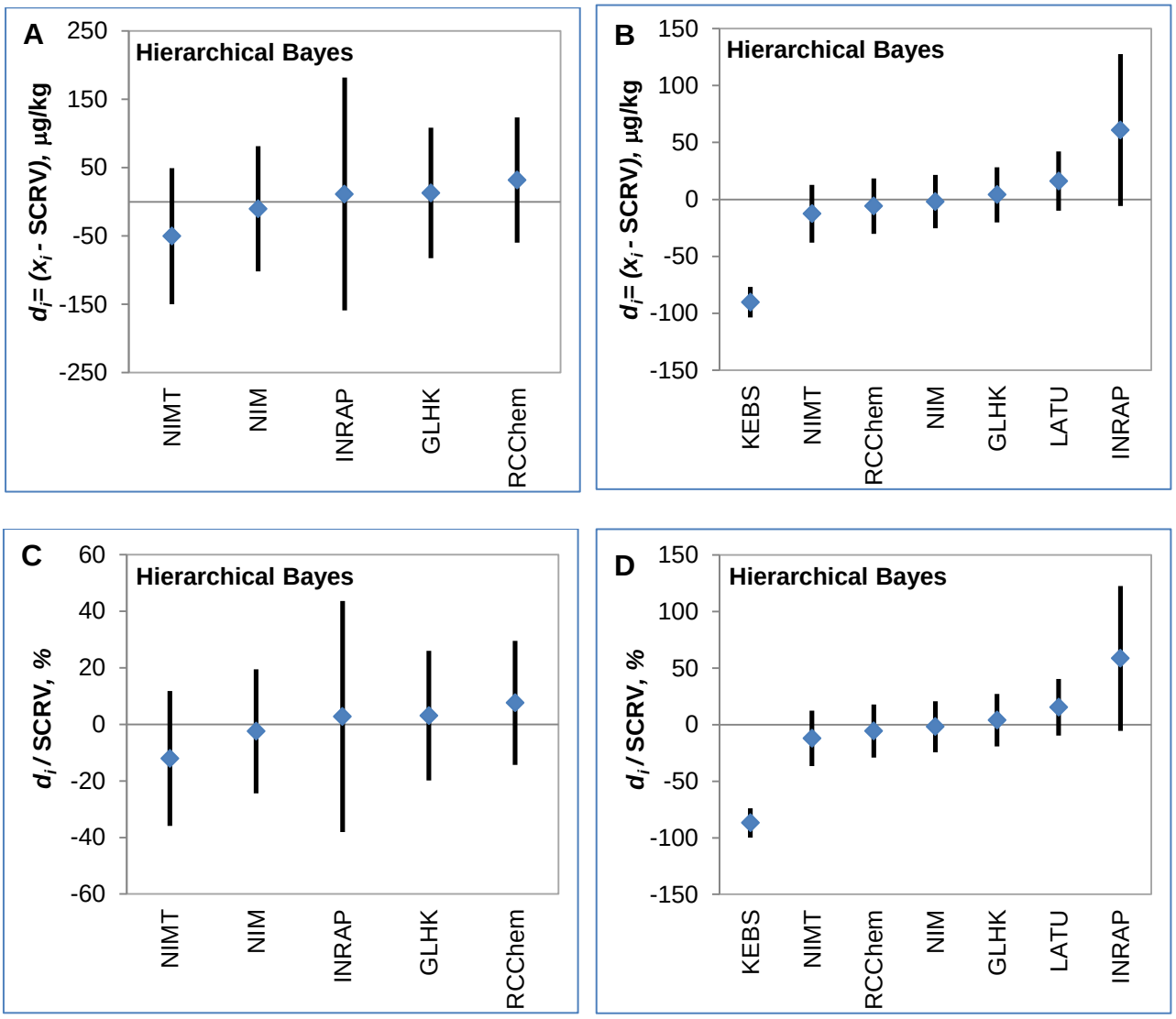


Figure 9: Degrees of Equivalence

Notes: Panels A and C display the DoE for α-BHC; panels B and D display them for lindane. All results are sorted by increasing x. The axis of panels A and B displays the absolute DoE, d, in units µg/kg. The axis of panels C and D displays the relative DoE, $100 \cdot d/\text{SCRV}$, as percent. Dots represent the d, bars their approximate 95 % expanded uncertainties, $U_{95}(d)$.

USE OF APMP.QM-S11 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

Successful participation in APMP.QM-S11 demonstrates the following measurement capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.

Core Competency Statements and CMC support

Tables 21 to 27 list the Core Competencies claimed by the participants in APMP.QM-S11. The information in these Tables is as provided by the participants.

Table 21: Core Competency Demonstrated in APMP.QM-S11 by GLHK

APMP.QM-S11	***GLHK***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrix. Generally, it provides demonstration of a laboratory's capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		α-HCH: NIST SRM 2275 Chlorinated Pesticide Solution II in *Iso*-octane (calibration solution) γ-HCH: NMIA P1332 Lindane (pure standard)
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	✓	Analytes identified through retention time of calibration standard, relative retention time of internal standard and mass ratio of 2 qualifier ions
Extraction of analyte(s) of interest from matrix	✓	5mL water was added to 1g sample, vortex mix well until the sample is fully immersed in water, equilibrate for about 12 hours. Add approximately 1.4g absorbent polymer / celite (1:1 *w/w*). The sample was then standard for 4 hours. Transfer the chemically dried sample into a thimble. Start soxhlet Extraction with 200mL Ethyl acetate for 16 hours.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Solid-phase extraction Clean-up 1) Supelco Envi-Carb/NH_2 500mg/ 6mL SPE Tube After concentrate the extract from soxhlet extraction to near dryness, reconstitute the dry residue with 10mL acetonitrile/toluene (3:1 v/v). Join 2 Envi-Carb/NH_2 SPE Tube 2 SPE tubes Load the sample into Envi-Carb/NH_2 SPE Tubes and rinse twice with 5mL acetonitrile/toluene (3:1 *v/v*). Concentrate the eluate using a rotary evaporator to just dryness and reconstitute it with 1mL *n*-hexane. 2) Alltech Extract Clean ™ Florisil 500mg/4mL SPE Load the sample to Florisil SPE, rinse with 2mL *n*-hexane twice, Elute the florisil SPE with 2mL *n*-hexane.

Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✘	
Analytical system	✓	GC-MSD (NCI)
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) bracketing
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	GC-MS/MS
Other	N/A	

Table 22: Core Competency Demonstrated in APMP.QM-S11 by INRAP

APMP.QM-S11	***INRAP***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrix. Generally, it provides demonstration of a laboratory's capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance"or calibration solution?		We use a reference material from LGC and CRM from NISTfor the calibration: -SRM 2261 from NIST, chlorinated pesicides in hexane: cas number lindane :58-89-9 -SRM 2275 from NIST, chlorinated pesicide solution -II in isooctane : cas number alpha-HCH: 319-84-6 - 14071000 alpha-HCH (LGC):cas number 319-84-6 -14073000 gamma-HCH (LGC):cas number 58-89-9 - 14073000 gamma-HCH
Identity verification of analyte(s) in calibration material.[#]	X	
For calibrants which are a highly-pure substance: Value-assignment / Purity Assessment method(s).[#]	X	The purity estimations of the chlorinated pesticide components were based on NIST analyses using capillary GC with flame ionization detection (FID), the purity assay information from the component suppliers, and, where appropriate, differential scanning calorimetry. The purity estimations of the chlorinated pesticide components were based on NIST analyses using capillary GC with flame ionization
For calibrants which are a calibration solution: Value-assignment method(s).[#]	✓	Gravimetric
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and GCMSD
Extraction of analyte(s) of interest from matrix	✓	Quechers
Cleanup- separation of analyte(s) of Interest from other interfering matrix components (if used)	✓	Dispersive purification
Transformation-conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	Not used

Analytical system	✓	GC-ECD
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) quantification mode used : internal standard b) calibration mode used: x-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Confirmation by GCMSD (SIM mode)
Other		

Table 23: Core Competency Demonstrated in APMP.QM-S11 by KEBS

APMP.QM-S11	***KEBS***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrix. Generally, it provides demonstration of a laboratory's capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		Pure standard from Sigma Aldrich
Identity verification of analyte(s) in calibration material.[#]	✓	Retention time, m/z
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	N/A
For calibrants which are a calibration solution: Value-assignment method(s).[#]	✓	HPLC-UV, GC-MS
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, m/z
Extraction of analyte(s) of interest from matrix	✓	QuECHERS METHOD- Mixing with shaker, vortex, centrifugation, shaking by hand
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	HPLC-UV, GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) EXTERNAL STANDARD b) X-POINT CALIBRATION
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other		Calculation of STDEV, uncertainty of measurements

The results for lindane are not consistent with the SCRV as the 95% confidence intervals for the DoE does not cross zero. KEBs have not identified a specific reason for this deviation.

Table 24: Core Competency Demonstrated in APMP.QM-S11 by LATU

APMP.QM-S11	***LATU***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrix. Generally, it provides demonstration of a laboratory's capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		*Pure material por NMIA P1332 – lindane.*
Identity verification of analyte(s) in calibration material.[#]	✓	*GC-µECD (retention time) and GC-MS.*
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	*Retention time.*
Extraction of analyte(s) of interest from matrix	✓	*Soxhlet extraction.*
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	*SPE*
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	*GC-µECD*
Calibration approach for value-assignment of analyte(s) in matrix	✓	*Bracketing with internal standard.*
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	

Other	N/A	

Table 25: Core Competency Demonstrated in APMP.QM-S11 by NIM

APMP.QM-S11	***NIM***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrix. Generally, it provides demonstration of a laboratory's capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		CRM GBW(E) 060081 a-HCH 50µg/mL , *U*=1%, *k*=2 CRM GBW(E) 060083 γ-HCH 50µg/mL , *U*=1%, *k*=2
Identity verification of analyte(s) in calibration material.[#]	√	GC-MS/MS, comparison to independent reference material retention time and mass spectrum.
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	√	Analytes identified through comparison against standard reference material's retention time and mass spectrum ion ratios of 2 independent multiple reaction monitoring (MRM) transitions by tandem CG-MS/MS
Extraction of analyte(s) of interest from matrix	√	The extraction was performed using an accelerated solvent extractor (ASE 350, Dionex) with 34 mL ASE extraction cells. The homogenates were extracted with ethyl acetate: petroleum ether (7:3; v/v). ASE conditions: 140 ℃, static time: 15 min; flush volume: 80%; purge: N_2, 80 s; number of cycles: 4.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	Concentrated sulfuric acid was added to clean up the sample. The high purity copper powder (99.90%, sigma) was added to remove the residual of sulfuric acid.
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) single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	.
Other	N/A	

Table 26: Core Competency Demonstrated in APMP.QM-S11 by NIMT

APMP.QM-S11	***NIMT***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrix. Generally, it provides demonstration of a laboratory's capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		SRM 2275 for alpha-BHC and NMIA P1332 for Lindane
Identity verification of analyte(s) in calibration material.[#]	✓	GC-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	✓	Gravimetric
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	The analytes in the samples were identified against SRM 2275 for alpha-BHC standard and NMIA P1332 for Lindane standards by comparing their retention times and m/z of GC-MS.
Extraction of analyte(s) of interest from matrix	✓	Liquid-liquid extraction with 10 mL acetonitrile per gram of ginseng
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	GCB/PSA 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, isotope ratios in the sample blends were controlled to be closed to 1.0.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

Table 27: Core Competency Demonstrated in APMP.QM-S11 by RCM-LIPI

APMP.QM-S11	***RCM-LIPI***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrix. Generally, it provides demonstration of a laboratory's capabilities in determining mass fraction of organic compounds, with molecular mass of 100 g/mol to 500 g/mol, having low polarity pKow < -2, in mass fraction range from 10 µg/kg to 1000 µg/kg in a food/plant matrix.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		We used : 1. "Highly-pure substance" Lindane from NMIA, Australia (P 1332). Purity of Lindane 99.7 ± 0.4 %) 2. Calibration solution containing α-BHC from NIST (SRM NIST 2275). Concentration of α-BHC is 3 ± 0.15 mg/kg.
Identity verification of analyte(s) in calibration material.[#]	X	-
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	X	-
For calibrants which are a calibration solution: Value-assignment method(s).[#]	X	-
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	The identification of analyte(s) in sample was done by comparing the retention time and the mass spec ion ratios on GCMS.
Extraction of analyte(s) of interest from matrix	√	Wetting with distilled water continued with extraction by using acetonitrile.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	1 g of florisil with 10 ml of n-hexane/diethyl ether (85/15) mixture as eluent.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	X	-
Analytical system		GCMS

Calibration approach for value-assignment of analyte(s) in matrix		IDMS with one-point exact-matching calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)		ID-GCMS multi point calibration
Other		CRM matrix Ginseng Powder KRISS CRM 108-10-013 was used as quality control material for evaluating the performance of the analytical method.

CONCLUSIONS

The majority of participants in APMP.QM-S11 demonstrated their capability on the determination of organochlorine pesticides (α-BHC and lindane) in a relatively complex food/plant material of ginseng root. One result was excluded from use in defining the SCRV of α-BHC and two results were excluded from use in defining the SCRV of lindane for identified causes.

ACKNOWLEDGEMENTS

The study coordinators thank the participating laboratories for providing the requested information used in this study. The study coordinators also thank Mike Nelson of NIST for providing advices on the statistical methods for SCRVs calculation.

REFERENCES

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2. Commission Regulation (EC) No. 396/2005.

3. CCQM-K95 Final Report, *Metrologia*, 2015, **52**, Tech. Suppl., 08007.

4. Hong Kong Chinese Materia Medica Standards, Volume 1, Radix Ginseng, (http://www.cmd.gov.hk/hkcmms/vol1/Docs/radix_ginseng.pdf)

5. Hong Kong Chinese Materia Medica Standards, Volume 3, Radix Panacis Quinquefolii, (http://www.cmd.gov.hk/hkcmms/vol3/Vol3_pdf_English/D_Monographs/P177-194_rpq_E.pdf)

6. International Standards Organization, ISO Guide 35: Reference materials – General and statistical principles for certification, Geneva, Switzerland, 2006.

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

APPENDIX A: Call for Participation

Date: 29/11/2016 09:51
Subject APMP.QM-S11 on Organochlorine Pesticides in Ginseng Root - Call for participation

Dear OAWG colleagues
Please find attached the call for participation for the APMP comparison for organochlorine pesticides in ginseng root. Could interested parties register their interest with the Hong Kong laboratory by **9 December 2016.**
Many thanks

Lindsey

Attachments:
APMP.QM-S11_Technical Protocol_Organochlorine Pesticides in Ginseng Root_161118.pdf
APMP.QM-S11_Registration Form_Oorganochlorine Pesticides in Ginseng Root_161118.doc
APMP.QM-S11_Report Form_Oorganochlorine Pesticides in Ginseng Root_161118.pdf

Cc:
Date: 22/11/2016 17:31
Subject: Call participation of APMP.QM-S10 on Elements in Food Supplement and APMP.QM-S11 on Organochlorine Pesticides in Ginseng

Dear all,

It's good meeting every one of you in Da Nang,and thanks for the contribution to TCQM from every one of you!

I have been asked by GLHK to circulate the attached documents and advise you that participation in APMP.QM-S10 on Elements in Food Supplement and APMP.QM-S11 on Organochlorine Pesticides in Ginseng Root which had been registed in BIPM KCDB.

Please find the attached documents and contact with the coordinators.

Best regards,

Ma Liandi
TCQM Chair

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致
礼!

马联弟

中国计量科学研究院
化学计量与分析科学研究所 副所长
(国家标准物质研究中心)
地址：北京市朝阳区北三环东路18号
邮编：100029
电话：010-64223987 010-64524704
传真：010-64223987

Attachments:
APMP.QM-S10_Technical Protocol_Elements in food supplement_161118.pdf
APMP.QM-S10_Registration Form_Elements in Food Supplement_161118.doc
APMP.QM-S10_Report Form_Elements in Food Supplement_161118.pdf
APMP.QM-S11_Technical Protocol_Organochlorine Pesticides in Ginseng Root_161118.pdf
APMP.QM-S11_Registration Form_Oorganochlorine Pesticides in Ginseng Root_161118.doc
APMP.QM-S11_Report Form_Oorganochlorine Pesticides in Ginseng Root_161118.pdf

APPENDIX B: Protocol

APMP.QM-S11
APMP Supplementary Comparison
Organochlorine Pesticides in Ginseng Root

Technical Protocol

1. Introduction

Ginseng is one of the most important traditional herbal medicines for health care and treatment of diseases. Trading of ginseng and related products is a multi-million dollar business. Four major countries including South Korea, China, Canada and the United States are the biggest producers and account for more than 99% of the total ginseng production around the world (i.e. about 80,000 tons) [15.1]. The Commission Regulation of European Union sets up that the maximum residue level (MRL) for hexachlorocyclohexane (sum of alpha, beta and delta isomers, except lindane) is 0.02 mg/kg and that for lindane is 1 mg/kg in ginseng [15.2]. The use of reliable methods for measurement of these organochlorine pesticides is important in safeguarding the quality of ginseng and related products and the public health.

The Government Laboratory, Hong Kong (GLHK) previously coordinated and completed CCQM-K95 "Mid-polarity Analytes in Food Matrix: Mid-polarity Pesticides in Tea" [15.3]. Two organochlorine pesticide residues including beta-endosulfan and endosulfan sulfate were selected for analysis. It is noteworthy that participating institutes in CCQM-K95 found that wetting of test samples prior to extraction was crucial for complete extraction of the incurred analytes in the test material of dried tea. It is apparent that sample extraction is a real technical challenge to the analysis of dried plant material.

The ginseng root is collected after years of plantation [15.4, 15.5]. It will be a higher level of analytical challenge for the participating national metrology institutes (NMIs) and designated institutes (DIs) in measuring the incurred organochlorine pesticides in dried ginseng/ginseng root, where the pesticides have been gradually accumulated in the plant material for several years. In this regard, GLHK proposed a new APMP supplementary comparison on determination of organochlorine pesticides in ginseng root at the APMP TCQM meeting in November 2015. The supplementary comparison was further discussed at the CCQM OAWG meeting in April 2016. The Chair of APMP TCQM eventually approved the proposed supplementary comparison for 2016/17 with a

study number of APMP.QM-S11 in May 2016. The purpose of the comparison is to enable participating laboratories to demonstrate their capability on the determination of organochlorine pesticides in a relatively complex food/plant material (e.g. ginseng root). Alpha-hexachlorocyclohexane (α-BHC, CAS No. 319-84-6) and gamma-hexachlorocyclohexane (Lindane, CAS No. 58-89-9), which are commonly used organochlorine pesticides for the growth of ginseng, are selected as the analytes in this comparison.

According to the information from the BIPM Key Comparison Database (KCDB), only a few NMIs have made Calibration and Measurement Capabilities (CMCs) claims related to the analysis of α-BHC/lindane in ginseng. This APMP supplementary comparison will facilitate participating national metrology institutes (NMIs) and designated institutes (DIs) in making claims on the analysis of relevant organochlorine pesticide residues in appropriate low fat, low protein food/plant matrices (e.g. ginseng/ginseng root).

2. Objectives

The objective of the study is to enable participating NMIs and DIs to demonstrate their capabilities in determining the mass fractions of two analytes (α-BHC and Lindane) at μg/kg levels in a test sample of ginseng root by various analytical techniques. The mass fractions of the analytes reported on a dry mass basis will be used for comparability purpose.

This supplementary comparison facilitates claims by participants on the Calibration and Measurement Capabilities (CMCs) as listed in Appendix C of the Key Comparison Database (KCDB) under the Mutual Recognition Arrangement of the International Committee for Weights and Measures (CIPM MRA).

3. Co-ordinating laboratory

The supplementary comparison is co-ordinated by the Government Laboratory, Hong Kong (GLHK) (Address: 7/F., Homantin Government Offices, 88 Chung Hau Street, Homantin, Kowloon, Hong Kong). GLHK takes responsibility for all tasks in the development and operation of the comparison, including preparation and distribution of samples, data analysis and evaluation of results, preparation of reports, and communications with participants.

4. Test material

A batch of about 12 kg of dried ginseng root confirmed to have the incurred organochlorine pesticides was purchased from the local market. The raw ginseng root was washed with distilled water to remove dirt and other foreign matters where necessary, and freeze-dried for 7 days. The dried material was blended to give powder. The ginseng root powder was subjected to a sieving process through two calibrated sieves (200 and 100 μm respectively). The sieved powder (particle sizes: 100 – 200 μm) was thoroughly homogenised in a 3-dimensional mixer for 5 days. The material was irradiated using gamma source at a dose of about 1 kGy for disinfection. The irradiated material was packed into pre-cleaned and nitrogen-flushed amber glass bottles, each of about 25 g. Finally, each bottle of sample was vacuum-sealed in a polypropylene bag. All prepared bottles of sample were stored in a refrigerator (about -20 °C) prior to distribution or use.

The homogeneity study was conducted after the testing material was bottled and irradiated. 10 bottles of the test material (conditioned at about -20 °C) were randomly selected from the whole lot of bottles prepared. Two test portions of 1.0 g were taken from each bottle for analysis. The test portions were undergone a wetting process [15.3] followed by soxhlet extraction. Following validated procedures, the extracts were analysed using isotope dilution gas chromatography mass spectrometry (ID-GC-MS). ANOVA technique was applied to assess the between-bottle homogeneity in accordance with ISO Guide 35:2006 [15.6]. The results are summarised in Table 1.

Table 1

Analyte	ANOVA test		Relative standard uncertainty due to between-bottle (in)homogeneity, u_{bb} (%)
	F-statistics	Critical value	
α-BHC	1.38	3.02	0.716
Lindane	1.13	3.02	0.980

The homogeneity study results indicated that no significant inhomogeneity was observed in the test material. The test material was considered fit for the purpose of the supplementary comparison.

The stability studies were conducted for the test material using the same analytical procedures as for the homogeneity study. For the short-term stability (i.e. stability of the test material under "transport conditions"), the study was conducted on the isochronous approach over a period of 4 weeks at a simulated transport temperature (conditioned at 30 ± 5 °C, 35 ± 5 °C and 40 ± 5 °C) against the reference temperature at about -70 °C. Two bottles of sample were randomly taken from the storage temperature (about -20 °C) to the simulated transport temperature on three occasions (1, 2 and 4 weeks) over the study period. Each bottle of sample was analysed in duplicate for monitoring the sample instability. The trend-analysis technique proposed by ISO Guide 35:2006 was applied to assess the stability of the test material at 30 °C, 35 °C and 40 °C. The results are summarised in Table 2.

Table 2

Analyte	p-value for the slope		
	30 °C	35 °C	40 °C
α-BHC	0.263	0.863	0.140
Lindane	0.940	0.067	0.574

All p-values were greater than 0.05, it was concluded that the corresponding slope was not significantly deviated from zero at 95% level of confidence. In other words, no instability was observed for the test material at 30 °C, 35 °C and 40 °C during the testing period. The test material was considered fit for the purpose of the supplementary comparison.

For the long-term stability (i.e. stability of the test material under "storage conditions"), the study is conducted on the classical approach covering the period from "the planned date of distribution of test samples to participants" to "the deadline for submission of results" at the storage temperature (conditioned at about -20 °C).

5. Measurands

Participating laboratories will be provided with ONE bottle containing about 25 g of ginseng root powder. The general information of the two analytes and their expected mass fractions as determined by gas chromatography with mass spectrometry are listed in Table 1.

Table 1

Analyte	Molecular weight	-log P $_{(octanol-water)}$	Expected mass fraction (μg/kg)
α-BHC	290.831	-3.8	10 – 1000
Lindane	290.831	-3.72	10 – 1000

6. Methods/procedures

Participants shall carry out the analysis of the two analytes (i.e. α-BHC and Lindane) and submit the analytical results accordingly.

Participants shall use any analytical methods of their choice. Upon receipt, the sample shall be stored at about -20 °C prior to analysis. The temperature of the bottle shall reach the room temperature (20 ± 5 °C) before opening. The sample shall be mixed thoroughly before processing and the analysis shall be conducted with a recommended sample size of at least 1 g. It is noteworthy that wetting of test sample prior to extraction was crucial for complete extraction of the incurred analytes in dried food/plant matrices as learned from the key comparison CCQM-K95. Hence, it is recommended that participants should wet a test portion of the sample with an appropriate amount of water for sufficient time prior to extraction. Participants shall perform at least three independent measurements on three separate portions of the sample and determine the mass fractions of the analytes.

For the determination of dry mass correction, a minimum of three separate portions (recommended size to be about 1 g each) of the sample shall be taken and placed over anhydrous calcium sulphate (DRIERITE®) in a desiccator at room temperature for a minimum of 20 days until a constant mass is reached. Dry mass correction shall be carried out at the same time as the test sample portions are to be analysed.

7. Reporting and submission of results

- For each analyte, the mean value of at least three independent measurements on three separate portions of the sample and its associated measurement uncertainty shall be reported on a dry mass basis;
- Report the mass fractions of the analytes and associated uncertainties in μg/kg;
- Participants shall provide (i) description of analytical methods (including sample extraction methods, calibration methods and analytical instruments used) and (ii) details of the uncertainty estimation (including complete specification of the measurement equations and description of all uncertainty sources and their typical values); and
- Sources, purity and traceability of reference materials used for calibration purpose shall be provided.

Both the Analyte Matrix Core Competency Template and the Report Form shall be submitted to the GLHK (E-mail: ycyip@govtlab.gov.hk) before the scheduled deadline.

8. Measurement uncertainty

Measurement uncertainty is best estimated within the individual laboratory environment. An estimate of uncertainty of measurement is normally based on the combination of a number of influencing parameters (components of uncertainty) such as errors in reference values, instrument errors, repeatability, thermal effects, weighing errors, inhomogeneity etc. As stipulated in ISO Guide to the Expression of Uncertainty in Measurement [15.7], the influence of each component of uncertainty on the measurement result shall be quantified and expressed numerically as a standard deviation. These values are then combined according to the rules of the propagation of uncertainty to produce a combined standard deviation (combined standard uncertainty) and the combined standard uncertainty is multiplied by a coverage factor to produce an expanded uncertainty at the required level of confidence.

To facilitate in-depth performance evaluation, participants shall clearly identify and quantify those factors that are considered to contribute to the measurement uncertainty of the analysis.

9. Proposed programme schedule

The proposed time schedule for the various phases of the comparison is as follows:

Proposed time schedule	Phase
November 2015	Presentation of the proposed APMP supplementary comparison at the APMP TCQM meeting
April 2016	Update on progress and sample preparation for the proposed comparison at the CCQM OAWG meeting
October/November 2016	Presentation of the results of the homogeneity and stability studies for the proposed APMP supplementary comparison at the CCQM OAWG meeting and APMP TCQM meeting
October/November 2016	Call for Participation
30 November 2016	Deadline for registration
November/December 2016	Distribution of samples
31 March 2017	Deadline for submission of results
April 2017	Presentation of the participants' results and proposed reference values for the APMP supplementary comparison at the CCQM OAWG meeting
June 2017	Preparation of Draft A report for comments
October/November 2017	Presentation and confirmation of the reference values for the APMP supplementary comparison at the CCQM OAWG meeting and APMP TCQM meeting
December 2017	Preparation of Draft B report for comments

10. Requirements for participation

Participation in supplementary comparisons organised by the APMP is only open to laboratories that meet the requirements of Section 6 of the CIPM-MRA, and are listed in Appendix A of the CIPM-MRA, and the BIPM.

Participation is open to all interested NMIs or officially DIs under the APMP. NMIs or DIs from other RMOs are also welcome to join this supplementary comparison.

11. Registration

Please complete and return the Registration Form to GLHK (E-mail: ycyip@govtlab.gov.hk) for the participation of the proposed comparison on or before the deadline for registration. Successful registration will be notified by e-mail.

12. How Far does the Light Shine?

This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may require extraction, clean-up, analytical separation and selective detection in food/plant matrices. Generally, it provides demonstration of a laboratory's capabilities in determining the mass fractions in the range from 10 to 1000 µg/kg of analytes with the molecular mass range 100-500 a.m.u. and having low polarity in food/plant matrices.

13. Confidentiality

The participating laboratories will receive the reports giving all results for assessment/comments. They will be identified in the reports. The supplementary study is conducted in the belief that participants will perform the analysis and report results with scientific rigour. Collusion between participants or falsification of results is clearly against the spirit of this study.

14. Contact

For enquiries, participants may wish to contact the co-ordinating laboratory as follows:

Dr. Della W.M. SIN, GLHK
E-mail: wmsin@govtlab.gov.hk
Tel.: +852 2762 3701

Dr. Yiu-chung YIP, GLHK
E-mail: ycyip@govtlab.gov.hk
Tel.: +852 2762 3853

15. References

15.1. I. H. Baeg and S. H. So, *J. Ginseng Res.*, 2013, **37**, 1-7 "The world ginseng market and the ginseng (Korea)".

15.2. Commission Regulation (EC) No. 396/2005.

15.3. CCQM-K95 Final Report, *Metrologia*, 2015, **52**, Tech. Suppl., 08007.

15.4. Hong Kong Chinese Materia Medica Standards, Volume 1, Radix Ginseng, (http://www.cmd.gov.hk/hkcmms/vol1/Docs/radix_ginseng.pdf)

15.5. Hong Kong Chinese Materia Medica Standards, Volume 3, Radix Panacis Quinquefolii, (http://www.cmd.gov.hk/hkcmms/vol3/Vol3_pdf_English/D_Monographs/P177-194_rpq_E.pdf)

15.6 ISO Guide 35:2006 "Reference materials –General and statistical principles for certification", 2006, Geneva, Switzerland.

15.7 ISO/IEC Guide 98-3:2008 "Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)", 2008, Geneva, Switzerland.

APPENDIX C: Registration Form

APMP.QM-S11
APMP Supplementary Comparison
Organochlorine Pesticides in Ginseng Root

Registration Form

Institute/ Laboratory: _____

NMI/DI: National Metrology Institute (NMI) or Designated Institute (DI)*

Postal address: _____

_____

Zip/Postal code: _____

Authorised person: _____ _____ _____
Title Given name Surname

E-mail: _____

Telephone no.: _____

Alternative contact person and telephone no.: _____

Date: _____

Any particular local customs/ quarantine requirements / special permits for samples sent into your country are needed? Yes / No *

(Please delete where appropriate.)*

Confirmation of Participation

I, on behalf of my institute/laboratory, would like to participate in APMP.QM-S11 on determination of organochlorine pesticides in ginseng root. Please send the test material to the postal address.

Notes: (i) Participation in APMP.QM-S11 is restricted to national metrology institutes and designated institutes. Please complete this form and return it to GLHK (E-mail: ycyip@govtlab.gov.hk) on or before the deadline for registration.

(ii) Please note that GLHK will NOT be responsible for any import taxes or charges due to the test samples.

APPENDIX D: Reporting Form

APMP.QM-S11
APMP Supplementary Comparison
Organochlorine Pesticides in Ginseng Root

Report Form

Institute/ Laboratory: _____

NMI/DI: National Metrology Institute (NMI) or Designated Institute (DI)*

Postal address: _____

_____

Authorised person: _____ _____ _____
Title Given name Surname

E-mail: _____

Telephone: _____

Date: _____

(Please delete where appropriate.)*

I. Analytical results and measurement uncertainties (reported on a dry mass basis)

Analytes	Mass fraction (Mean value of replicate measurements, μg/kg)	No. of replicate measurements	Combined standard uncertainty (μg/kg)	Coverage factor, *k* (95% level of confidence)	Expanded uncertainty (μg/kg)
e.g. α-BHC	500	3	10	2	20
Alpha-hexachlorocyclohexane (α-BHC)					
Lindane					

Please note that the study is conducted in the belief that participants will perform the analysis and report results with scientific rigour. Collusion and falsification of results are clearly against the spirit of this study.

II. Method information

A) α-BHC

Calibrant used

Pure substance/ Calibration solution *

Source: _____

Purity: _____

Concentration of calibration solution: _____

Traceability: _____

Reference material used for calibration is in compliance with the requirements for Traceability in CIPM MRA (Document No.: CIPM 2009-24; Latest update: Revised 13 October 2009): Yes / No *

** (please delete where appropriate)*

Internal Calibrant (IS) used Yes / No *

Source: _____

Purity: _____

Please state the stage of analysis were the internal standard added:

_____

** (please delete where appropriate)*

Analytical Method

Method accreditation: _____

Method reference: _____

Sample amount used (g) for analysis: _____

Extraction solvent(s) and reagents used: _____

Sample pre-treatment:

☐ Wetting with water (Duration: _____ hour(s); Amount of water: _____ gram(s))

☐ Others: _____

Extraction technique:

☐ Sonication ☐ Liquid/ Liquid extraction

☐ Soxhlet extraction ☐ Vortex

☐ Shaking ☐ Accelerated solvent extraction

☐ Others: _____

Extraction conditions (e.g. duration, temperature etc.):

☐ Duration: _____

☐ Temperature: _____

☐ Others: _____

Clean-up method: Description:

☐ Solid phase extraction (SPE) _____

☐ Dispersive SPE _____

☐ Gel permeation _____

☐ Centrifugation _____

☐ Others _____

Analytical instrument(s)

☐ GC-ECD ☐ GC-FID

☐ GC-MS ☐ GC-HRMS

☐ LC-MS ☐ LC-MS/MS

☐ Others: _____

Chromatographic conditions

(i) Chromatographic column(s): _____

(ii) Chromatographic condition(s):
(e.g. GC Oven temperature programme, LC mobile phase programme, etc.) _____

(iii) Mobile phase(s) / Carrier gas: _____

(iv) Flow rate _____

Ionisation mode(s) of MS detector *(if applicable)*

☐ EI

☐ CI (positive / negative)

☐ NCI (positive / negative)

☐ ESI (positive / negative)

☐ Others:

Ions/MRM transitions monitored in mass spectrometer *(Please specify the ion/transition used for quantitation)*

_____

Type of calibration :

☐ Single-point calibration

☐ Bracketing calibration

☐ Multi-level calibration curve

Method of quantification:

☐ External standard ☐ Isotope dilution mass spectrometry

☐ Internal standard ☐ Standard addition

Matrix matched calibration blend:

☐ Yes ☐ No

Methods used for identification of the analyte in sample (e.g. retention time, MRM ratio of 3 ion transitions, etc.):

Purity Assessment of Calibrant *(if applicable)*

Direct estimate of principle components:

☐ Gas chromatography – Flame Ionization Detection

☐ Gas chromatography – mass spectrometry

☐ Liquid chromatography – ultraviolet spectroscopy

☐ Liquid chromatography – mass spectrometry

☐ Others:

Method(s) used for identification (e.g. UV, FTIR, MS, etc):

Estimation of impurities:

	Nature of the impurities	Method(s) used
☐	Organic impurities	
☐	Inorganic impurities	
☐	Water/moisture	
☐	Residual solvent	
☐	Other impurities	

Measurement equations and uncertainties

The measurement equation(s) used to calculate the mass fraction of the measurand. Please provide details of all the factors listed in the equations and indicate how these values were determined.

Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Additional comments or observations

B) Lindane

Calibrant used

Pure substance/ Calibration solution *

Source: _____

Purity: _____

Concentration of calibration solution: _____

Traceability: _____

Reference material used for calibration is in compliance with the requirements for Traceability in CIPM MRA (Document No.: CIPM 2009-24; Latest update: Revised 13 October 2009): Yes / No *

* *(please delete where appropriate)*

Internal Calibrant (IS) used Yes / No *

Source: _____

Purity: _____

Please state the stage of analysis were the internal standard added:

_____

* *(please delete where appropriate)*

Analytical Method

Method accreditation: _____

Method reference: _____

Sample amount used (g) for analysis: _____

Extraction solvent(s) and reagents used: _____

Sample pre-treatment:

☐ Wetting with water (Duration: _____ hour(s); Amount of water: _____ gram(s))

☐ Others: _____

Extraction technique:

☐ Sonication ☐ Liquid/ Liquid extraction

☐ Soxhlet extraction ☐ Vortex

☐ Shaking ☐ Accelerated solvent extraction

☐ Others: _____

Extraction conditions (e.g. duration, temperature etc.):

☐ Duration: _____

☐ Temperature: _____

☐ Others: _____

Clean-up method:	Description:
☐ Solid phase extraction (SPE)	_____
☐ Dispersive SPE	_____
☐ Gel permeation	_____
☐ Centrifugation	_____
☐ Others	_____

Analytical instrument(s)

☐ GC-ECD ☐ GC-FID

☐ GC-MS ☐ GC-HRMS

☐ LC-MS ☐ LC-MS/MS

☐ Others: _____

Chromatographic conditions

(v) Chromatographic column(s): _____

(vi) Chromatographic condition(s):
(e.g. GC Oven temperature programme, LC mobile phase programme, etc.) _____

(vii) Mobile phase(s) / Carrier gas: _____

(viii) Flow rate _____

Ionisation mode(s) of MS detector *(if applicable)*

☐ EI

☐ CI (positive / negative)

☐ NCI (positive / negative)

☐ ESI (positive / negative)

☐ Others:

Ions/MRM transitions monitored in mass spectrometer *(Please specify the ion/transition used for quantitation)*

_____

Type of calibration :

☐ Single-point calibration

☐ Bracketing calibration

☐ Multi-level calibration curve

Method of quantification:

☐ External standard ☐ Isotope dilution mass spectrometry

☐ Internal standard ☐ Standard addition

Matrix matched calibration blend:

☐ Yes ☐ No

Methods used for identification of the analyte in sample (e.g. retention time, MRM ratio of 3 ion transitions, etc.):

Purity Assessment of Calibrant *(if applicable)*

Direct estimate of principle components:

☐ Gas chromatography – Flame Ionization Detection

☐ Gas chromatography – mass spectrometry

☐ Liquid chromatography – ultraviolet spectroscopy

☐ Liquid chromatography – mass spectrometry

☐ Others:

Method(s) used for identification (e.g. UV, FTIR, MS, etc):

Estimation of impurities:

	Nature of the impurities	Method(s) used
☐	Organic impurities	
☐	Inorganic impurities	
☐	Water/moisture	
☐	Residual solvent	
☐	Other impurities	

Measurement equations and uncertainties

The measurement equation(s) used to calculate the mass fraction of the measurand. Please provide details of all the factors listed in the equations and indicate how these values were determined.

Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Additional comments or observations

Note: Please complete this form and return it to GLHK (E-mail: ycyip@govtlab.gov.hk) on or before the deadline for submission of results.

APPENDIX E: Core Competency Tables

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

APMP.QM-S11	***NMI/DI***	**Organochlorine Pesticides in Ginseng Root**
Scope of Measurement: This study provides the means for assessing measurement capabilities for determination of low polarity measurands in a procedure that may requires extraction, clean-up, analytical separation and selective detection in food/plant matrices. Generally, it provides demonstration of a laboratory's capabilities in determining the mass fraction in range from 10 to 1000 µg/kg of analytes with the molecular mass range 100-600 500 a.m.u. and having low polarity in food/plant matrices.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		*Indicate if you used a "pure material" or a calibration solution. Indicate its source and ID, e.g. 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' Uncertainty Estimation Approaches

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

Uncertainty Information from GLHK

Uncertainties were estimated based on contribution from four components: 1) Calibrant, 2) Precision, 3) Method bias and 4) Moisture content. Detailed breakdowns are given as follows:

Purity of calibration standard u(Purity)	0.025000
Method precision u(Precision)	0.018790
Method Bias u(*Rm*)	0.013801
Moisture content u(moisture)	0.008646
Combined Relative std uncertainty U	0.0352601
coverage factor k	2
Expanded Uncertainty (%)	7.05

Uncertainties were estimated based on contribution from four components: 1) Calibrant, 2) Precision, 3) Method bias and 4) Moisture content. Detailed breakdowns are given as follows:

Purity of calibration standard u(Purity)	0.005562
Method precision u(Precision)	0.014145
Method Bias u(*Rm*)	0.013801
Moisture content u(moisture)	0.008646
Combined Relative std uncertainty U	0.0222764
coverage factor k	2
Expanded Uncertainty (%)	4.46

Uncertainty on the concentration ratio	**u (Rapport Concentrations)**
Uncertainty on the internal standard	**u (CEI)**
Uncertainty on the extraction volume	**u (Volume d'extraction)**
Uncertainty on the sample weight	**u (Masse prise d'essai)**

Uncertainty on the concentration ratio:

X= C_{PEST}/C_{EI} **Y=** Pic area PEST /Pic area EI.

$$X = \frac{Y - b}{a}$$

u (Rapport Concentrations X)

$$= \sqrt{\left(\frac{dx}{dY}\right)^2 u_Y{}^2 + \left(\frac{dx}{da}\right)^2 u_a{}^2 + \left(\frac{dx}{db}\right)^2 u_b{}^2 + 2 * \left(\frac{dx}{da}\right) * \left(\frac{dx}{db}\right) cov(a,b)}$$

UNCERTAINTY ON THE INTERNAL STANDARD CONCENTRATION

$$\mathbf{u\ (CEI)} = C_{EI} * \sqrt{\left(\frac{u_{Ci}}{C_i}\right)^2 + \left(\frac{u_{Vi}}{V_i}\right)^2 + \left(\frac{U_{Vf}}{V_f}\right)^2}$$

With $C_{EI} = \frac{C_i \times V_i}{V_f}$

u_{Vi} = Uncertainty on the micropipette of 1 mL used to take the V_i. it comes from the calibration report of the micropipettes divided by K.

u_{Vf} = Tolérance of the flask used to prepare the intermediate spiking internal standard solution

$$u_{Ci} = C_i * \sqrt{\left(\frac{u_{m\,EI}}{m_{EI}}\right)^2 + \left(\frac{u_{V\ sol\ mère}}{V_{sol\ mère}}\right)^2 + \left(\frac{u_{Pureté\ EI}}{Pureté_{EI}}\right)^2}$$

Avec

$V_{sol\ mère}$: Total Volume of internal standard mother solution SM_{EI} (L)

m_{EI} : weight of the internal standard to prepare the stock solution of internal standard (SM_{EI})

$u_{(mEI)}$: Uncertainty on m_{EI}

UNCERTAINTY ON THE EXTRACTION VOLUME

$$\mathbf{u\ (Volume\ d'extraction)} = \sqrt{2} \times \frac{\mathbf{Incertitude\ sur\ la\ micropipette\ de\ 5\ mL}}{\mathbf{2}}$$

UNCERTAINTY ON THE SAMPLE WEIGHT

$$\mathbf{u\ (Masse\ prise\ d'essai)} = \sqrt{(u_{IP})^2 + (u_{\frac{ncpa}{x}})^2 + s_{reproductibilité}{}^2 + u_{resolution}{}^2}$$

With:

u_{IP} : Uncertainty on the Weighing scales

$u_{ncpa/x}$: Uncertainty on the air pressure

$u_{résolution}$: Uncertainty associated to the instrument resolution .

$$u_{resolution} = \frac{resolution_{instrument\ de\ pesée}}{2 * \sqrt{3}}$$

Combined uncertainty : (uc)

$$u_{Tpest} = T_{pest} \sqrt{\frac{u^2(Rapport\ Concentrations)}{(Rapport\ Concentrations)^2} + \frac{u^2\ (CEI)}{(CEI)^2} + \frac{u^2\ (Volume\ d'\ extraction)}{(Volume\ d^{\wedge\prime}\ extraction)^2} + \frac{u^2\ (Masse\ prise\ d^{\wedge\prime}\ essai)}{(Masse\ prise\ d^{\wedge\prime}\ essai)^2}}$$

u(Concentration ratio)	0,090671936
u(weight of internal standard 1 mg)	0,00028882g
u(CEI)	3,70635E-06 mg/ml
u(sample weight)	0,0020325 g
u(extraction volume) (10 ml)	0,0019905 ml
u(amount)	0,081427321 mg/Kg
U (k=2)	0,16mg/Kg

Uncertainty on the concentration ratio	**u (Rapport Concentrations)**
Uncertainty on the internal standard concentration	**u (CEI)**
Uncertainty on the extraction volume	**u (Volume d'extraction)**

Uncertainty on the sample weight	u (Masse prise d'essai)

Uncertainty on the concentration ratio:

X= C_{PEST}/C_{EI} **Y**= Pic area PEST /Pic area EI.

$$X = \frac{Y - b}{a}$$

u (Rapport Concentrations X)

$$= \sqrt{\left(\frac{dx}{dY}\right)^2 u_Y{}^2 + \left(\frac{dx}{da}\right)^2 u_a{}^2 + \left(\frac{dx}{db}\right)^2 u_b{}^2 + 2 * \left(\frac{dx}{da}\right) * \left(\frac{dx}{db}\right) cov(a,b)}$$

UNCERTAINTY ON THE INTERNAL STANDARD CONCENTRATION

$$\mathbf{u\ (CEI)} = C_{EI} * \sqrt{(\frac{u_{Ci}}{C_i})^2 + (\frac{u_{Vi}}{V_i})^2 + (\frac{U_{Vf}}{V_f})^2}$$

With $C_{EI} = \frac{C_i \times V_i}{V_f}$

u_{Vi} = Uncertainty on the micropipette of 1 mL used to take the V_i. it comes from the calibration report of the micropipettes divided by K.

u_{Vf} = Tolérance of the flask used to prepare the intermediate spiking internal standard solution

$$u_{Ci} = C_i * \sqrt{(\frac{u_{m\,EI}}{m_{EI}})^2 + (\frac{u_{V\,sol\,mère}}{V_{sol\,mère}})^2 + (\frac{u_{Pureté\,EI}}{Pureté_{EI}})^2}$$

Avec

$V_{sol\ mère}$: Total Volume of internal standard mother solution SM_{EI} (L)

m_{EI} : weight of the internal standard to prepare the stock solution of internal standard (SM_{EI})

$u_{(mEI)}$: Uncertainty on m $_{EI}$

UNCERTAINTY ON THE EXTRACTION VOLUME

$$\mathbf{u\ (Volume\ d'extraction)} = \sqrt{2} \times \frac{\mathbf{Incertitude\ sur\ la\ micropipette\ de\ 5\ mL}}{\mathbf{2}}$$

UNCERTAINTY ON THE SAMPLE WEIGHT

$$\mathbf{u\ (Masse\ prise\ d'essai)} = \sqrt{(u_{IP})^2 + (\frac{u_{ncpa}}{x})^2 + s_{reproductibilité}{}^2 + u_{resolution}{}^2}$$

With:

u_{IP} : Uncertainty on the Weighing scales

$u_{ncpa/x}$: Uncertainty on the air pressure

$u_{résolution}$: Uncertainty associated to the instrument resolution .

$$u_{resolution} = \frac{resolution_{instrument\ de\ pesée}}{2 * \sqrt{3}}$$

Combined uncertainty : (uc)

$$u_{Tpest} = T_{pest}\sqrt{\frac{u^2(Rapport\ Concentrations)}{(Rapport\ Concentrations)^2} + \frac{u^2\ (CEI)}{(CEI)^2} + \frac{u^2\ (Volume\ d'\ extraction)}{(Volume\ d^{\wedge\prime}\ extraction)^2} + \frac{u^2\ (Masse\ prise\ d^{\wedge\prime}\ essai)}{(Masse\ prise\ d^{\wedge\prime}\ essai)^2}}$$

u(Concentration ratio)	0,055563278
u(weight of internal standard 1 mg)	0,00028882 g
u(CEI)	3,70635E-06 mg/mL
u(sample weight)	0,0020325 g
u(extraction volume) (10 ml)	0,0019905 mL
u(amount)	0,032609055 mg/Kg
U (k=2)	0,06 mg/Kg

Uncertainty Information from KEBS

Mean	13.67625				
std Dev	0.826995				
$U_{(Re)}$=	**0.826995**				
Relative Standard Uncertainty					
balance = ($U_{(w)}$/1)	1	$U_{(w)}$/1 =	6.9282E-05		($U_{(w)}$/1)² =
Standard purity = ($U_{(t)}$/98.5)					
reproducibility = ($U_{(Re)}$/14.676)	13.67625	$U_{(Re)}$/14.676 =	0.060469447		($U_{(Re)}$/14.676)² =
combined relative uncertainty					
Uc = √(($U_{(w)}$/1)²+($U_{(t)}$/98.5)²+($U_{(Re)}$/13.676)²) =				**0.154**	
expanded uncertainty = k x combined relative uncertainty at 95% CL (k=2) =				**0.31**	
		k = 2	2		

Uncertainty Information from LATU

The standard uncertainty was estimated considering all the components included in equation (1) as well as some additional factors: f_{bias} to account for method bias and f_{rep} to account for measurement repeatiblity. The uncertainty of these factors were then combined considering each sensitivity coefficients and then multiplied by a coverage factor of 2 to determine the expanded uncertainty at 95% confidence interval.

Parameter	Sources of uncertainty	Typical value	Unit	Standard uncertainty	Uncertainty contribution (%)
m_s	Balance calibration report	1.381818	g	0.000019	0.0
$m_t = m_s + m_{IS,s}$	Balance calibration report	1.475250	g	0.000026	0.0
$w_{IS,S}$	Balance calibration report	0.0505791	mg/kg	0.0000099	0.0
R_s	Standard deviation of area ratios	2.810	-	0.053	13.0
R_{cal1}	Standard deviation of area ratios	1.428	-	0.053	11.0
R_{cal2}	Standard deviation of area ratios	2.147	-	0.053	48.0
$w_{\gamma\text{-BHC, cal1}}$	Purity value from certificate and balance calibration report	0.009254	mg/kg	0.000047	0.2
$w_{\gamma\text{-BHC, cal2}}$	Purity value from certificate and balance calibration report	0.014226	mg/kg	0.000071	1.7
$w_{IS, cal1}$	Balance calibration report	0.009526	mg/kg	0.000012	0.0
$w_{IS, cal2}$	Balance calibration report	0.009447	mg/kg	0.000012	0.1
f_{bias}	Recovery of extraction procedure obtained from control samples	0.0000	mg/kg	0.0033	26.0
D	Dry mass factor, balance calibration report, repeatibility	0.9054	-	0.0014	0.1
f_{rep}	Standard deviation of result replicates	0.00000	mg/kg	0.00039	0.4

Uncertainty Information from NIM

Uncertainty of a-BHC	
Source of uncertainty	$u_{(x)}/(x)$
m_{ix}	0.03%
m	0.01%
m_{is}	0.03%
m_s	0.01%
C_s	0.5%
Method Precision	2.08%
f moisture content of the sample	0.12%
R_1/R_2	0.68%
Relative combined standard uncertainty (u_c)	2.25%
Coverage factor , *k*	2
Relative expanded uncertainty (Uc)	5%
Mass Fraction (µg/kg)	407
Expanded uncertainty, U (µg/kg)	21

Uncertainty of Lindane	
Source of uncertainty	$u_{(x)}/(x)$
m_{ix}	0.03%
m	0.01%
m_{is}	0.03%
m_s	0.01%
C_s	0.5%
Method Precision	2.50%
f moisture content of the sample	0.12%

R_1/R_2	0.89%
Relative combined standard uncertainty (u_c)	2.70%
Coverage factor , *k*	2
Relative expanded uncertainty (Uc)	6%
Mass Fraction (µg/kg)	102
Expanded uncertainty, U (µg/kg)	7

Uncertainty Information from NIMT

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

u(my), u(mx) = standard uncertainties due to weighing estimated from bias of balance

$u(F_{\text{dry mass}})$ = 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)

F_P = Standard uncertainty from method precision factor estimated from standard deviation of the mean of multiple results

F_E = Standard uncertainty from extraction effect estimated from extraction efficiency

Factor	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Measurement equation factors			
Method Precision	1.00	0.046	4.61%
w_0	0.98893	0.021	2.16%
$w_{y(x)}$	1022.763	0.076	0.01%
$m_{y(x)}$	0.33003	0.000055	0.02%
m_x	1.00252	0.000	0.0044%
Calibrant type B	3.000	0.075	2.50%
Calibrant type A	1642.12	41.053	3%
Dry mass	0.90	0.005	0.61%
Additional Factors			
Extraction effect	1.00	0.020	2.00%

Cx =	366.8	ng/g
u(x) =	24.002	ng/g
u(x)/x =	6.54%	
Veff(total) =	25.962	
k=	2.06	(@ 95% level)
U(x) =	49.433	
%U(x) =	13.48%	

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

u(my), u(mx) = standard uncertainties due to weighing estimated from bias of balance

$u(F_{\text{dry mass}})$ = 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 estimated from standard deviation of the mean obtained from multiple measurements

$u(F_P)$ = standard uncertainty of method precision estimated from extraction efficiency

Factor	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Measurement equation factors			
Method Precision	1.00	0.064	6.42%
w_0	0.66	0.014	2.16%
$w_{y(x)}$	991.74	5.784	0.58%
$m_{y(x)}$	0.11	0.000055	0.05%

m_x	1.01	0.000	0.00%
Calibrant type B	1.31	0.003	0.27%
Calibrant type A	0.0010	0.000	0.0045%
Dry mass	0.90	0.005	0.61%
Additional Factors			
Extraction effect	1.00	0.020	2.00%

Cx = 91.274 ng/g

u(x) = 6.496 ng/g

u(x)/x = 7.12%

Veff(total) = 7.507

k= 2.36 (@95%level)

U(x) = 15.361

%U(x) = 16.83%

Uncertainty Information from RCM-LIPI

The standard uncertainty of all components in the measurement equation was estimated and then combined by using respective derived sensitivity coefficient to get the combined standard uncertainty of the reported result. The combined standard uncertainty was then multiplied by a coverage factor of 2 to determine the expanded uncertainty at 95 % confidence interval. Other possible sources of uncertainty such as from precision (F_p) and different calibration blend (F_{CB}) are accounted for in the final uncertainty budget with the use of the following measurement equation:

$$C_x = C_z * \frac{m_y * m_{zc}}{m_x * m_{yc}} * \frac{R_B}{R_{Bc}} * \frac{1}{f_d} * F_p * F_{CB}$$

Uncertainty analysis results		
Cx	448.9	ug/kg
u(x)	12.1	ug/kg
u(x)/x	0.027	
U(x) with k=2	24.3	ug/kg
%U(x)	5.41	

Combination of Uncertainties						
Factor	Values	Uncertainties	u(x)/x	Sensitivity Coefficients		
	x	u(x)		δCx/δx	$c^2.u(x)^2$	#CTV
Measurement equation factors						
Method precision	1.00000	0.00966	0.00966	448.94401	18.80711	12.75870%
Mzc	0.24985	0.00002	0.00008	1796.83619	0.00145	0.00099%
My	0.16508	0.00002	0.00013	2719.59542	0.00333	0.00226%
Myc	0.16934	0.00002	0.00013	-2651.21808	0.00316	0.00215%
Mx	1.73616	0.00002	0.00001	-258.58459	0.00003	0.00002%
Cz	3.00	0.07500	0.02500	149.64800	125.96920	85.45719%
R'b	0.84358	Uncertainties captured in method precision				
R'bc	0.87126	Uncertainties captured in method precision				
fd	0.90770	0.000102	0.00011	-494.59515	0.00255	0.00173%
Additional factors						
Calibration blend	1.00000	0.00361	0.00361	448.94401	2.61938	1.77698%
			Total	2159.56982	147.40622	100%

The standard uncertainty of all components in the measurement equation was estimated and then combined by using respective derived sensitivity coefficient to get the combined standard uncertainty of the reported result. The combined standard uncertainty was then multiplied by a coverage factor of 2 to determine the expanded uncertainty at 95 % confidence interval. Other possible sources of uncertainty such as from precision (Fp), analytical method recovery (Frec) and different calibration blend (FCB) are accounted for in the final uncertainty budget with the use of the following measurement equation:

$$C_x = C_z * \frac{m_y * m_{zc}}{m_x * m_{yc}} * \frac{R_B}{R_{Bc}} * \frac{1}{f_d} * F_p * F_{\mathrm{Re}c} * F_{CB}$$

Uncertainty analysis results		
Cx	98.40	ng/g
u(x)	3.97	ng/g
u(x)/x	0.040	
U(x) with k=2	7.95	ng/g
%U(x)	8.08	

Combination of Uncertainties						
Factor	Values	Uncertainties		Sensitivity Coefficients		
	x	u(x)	u(x)/x	δCx/δx	$c^2.u(x)^2$	#CTV
Measurement equation factors						
Method precision	1.00000	0.01267	0.01267	98.39557	1.55391	9.84514%
Mzc	0.13944	0.00002	0.00015	705.64806	0.00022	0.00142%
My	0.14478	0.00002	0.00015	679.62908	0.00021	0.00132%
Myc	0.15024	0.00002	0.00014	-654.94436	0.00019	0.00122%
Mx	1.73616	0.00002	0.00001	-56.67428	0.00000	0.00001%
Cz	1.41	0.01084	0.00769	69.82956	0.57262	3.62795%
R'b	0.787	Uncertainties captured in method precision				
R'bc	0.961	Uncertainties captured in method precision				
Fd	0.90770	0.000102	0.00011	-108.40098	0.00012	0.00077%
Additional factors						
Calibration blend	1.00000	0.03337	0.03337	98.39557	10.78010	68.29970%
Method Recovery	0.983121597	0.016944838	0.01723575	100.0848382	2.87615	18.22247%
			Total	931.96306	15.78353	100%

APPENDIX G: Additional Comments from Participants

<table>
<tr><th>Institute</th><th>Additional Comments</th></tr>
<tr><td>KEBS</td><td>α-BHC was not qualitatively and quantitatively analysed as the laboratory did not received the standard on time. A request of the same standard was ordered but due to lengthy procurement procedures a delay to delivery was experienced.</td></tr>
<tr><td>INRAP</td><td>The uncertainties were reviewed and we found a transcription error concerning the uncertainty value due to a transcription error of the resolution of the balance used to weight the internal standard in the excel sheet (0.000001 g instead of 0.001 g) which affected the u(Cei) value and the final uncertainty value.
<table>
<tr><td>u(Concentration ratio)</td><td>0,090671936</td></tr>
<tr><td>u(weight of internal standard 1 mg)</td><td>0,00028882g</td></tr>
<tr><td>u(CEI)</td><td>4,65024E-07 mg/ml</td></tr>
<tr><td>u(sample weight)</td><td>0,0020325 g</td></tr>
<tr><td>u(extraction volume) (10 ml)</td><td>0,0019905 ml</td></tr>
<tr><td>u(amount)</td><td>0,013607806 mg/Kg</td></tr>
<tr><td>U (k=2)</td><td>0,027 mg/Kg</td></tr>
</table>
<table>
<tr><td>u(Concentration ratio)</td><td>0,055563278</td></tr>
<tr><td>u(weight of internal standard 1 mg)</td><td>0,00028882 g</td></tr>
<tr><td>u(CEI)</td><td>4,65024E-07 mg/ml</td></tr>
<tr><td>u(sample weight)</td><td>0,0020325 g</td></tr>
<tr><td>u(extraction volume) (10 ml)</td><td>0,0019905 mL</td></tr>
<tr><td>u(amount)</td><td>0,006858291 mg/Kg</td></tr>
<tr><td>U (k=2)</td><td>0,013 mg/Kg</td></tr>
</table></td></tr>
</table>

APPENDIX H: Participants' Quantitative Results as Reported

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

Quantitative Results from GLHK

Measurand	Mass Fraction (μg/kg)	No. of replicate measurements	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
α-BHC	430	15	15	2	30
Lindane	108	15	2.4	2	4.8

Quantitative Results from INRAP

Measurand	Mass Fraction (μg/kg)	No. of replicate measurements	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
α-BHC	428.6	6	81.427	2	162.8
Lindane	164.79	5	32.609	2	65.21

Quantitative Results from KEBS

Measurand	Mass Fraction (μg/kg)	No. of replicate measurements	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
α-BHC	-	-	-	-	-
Lindane	13.676	4	0.154	2	0.31

Quantitative Results from LATU

Measurand	Mass Fraction (μg/kg)	No. of replicate measurements	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
α-BHC	-	-	-	-	-
Lindane	120	3	6.5	2	13

Quantitative Results from NIM

Measurand	Mass Fraction (μg/kg)	No. of replicate measurements	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
α-BHC	407	10	10.5	2	21
Lindane	102	10	3.5	2	7

Quantitative Results from NIMT

Measurand	Mass Fraction (μg/kg)	No. of replicate measurements	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
α-BHC	366.9	14	24.002	2.06	50
Lindane	91.3	11	6.496	2.36	16

Quantitative Results from RCM-LIPI

Measurand	Mass Fraction (μg/kg)	No. of replicate measurements	Combined Standard Uncertainty (μg/kg)	Coverage Factor (k)	Expanded Uncertainty (μg/kg)
α-BHC	449	3	12	2	24
Lindane	98	3	4	2	8

APPENDIX I: Prototype Broader-Scope Core Competency Claim

Prototype Broader Category 11 Claims
for All Participants

Measurement service	Category 11. Food
Measurement service sub-category	Sub-category 11.2 Contaminants
Matrix	Low fat, low protein food/plant matrices
Measurand	Analyte or Component: low polarity pKow < -2 and organic analyte of molecular mass range (100 to 500 g/mol) Quantity: mass fraction, µg/kg
Dissemination range of measurement capability	From 10 to 1000 Unit: µg/kg
Range of expanded uncertainties as disseminated	From 4.44 to 13.6 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	Organochlorine pesticides of similar polarity
Supporting Evidence	Successfully participated in APMP.QM-S11