APMP.QM-S19/P40
Toxic Elements in Seafood

APMP Supplementary Comparison / Pilot Study

Study Protocol (Revised)
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INTRODUCTION

Seafood is one of the major food resources for human consumption in the world. CODEX Alimentarius Commission [12.1] and many jurisdictions have set maximum levels of metallic contaminants in seafood. The use of reliable methods for measurement of metallic contaminants is important in safeguarding the quality of these products and the public health.

The last CCQM or RMO key comparison / supplementary comparison in the area of metallic contaminants in seafood was organized by the Government Laboratory, Hong Kong, China (GLHK) in 2011 (APMP.QM-S5 Essential and Toxic Elements in Seafood). Hence, it is timely to organize another comparison that covers different measurands. This APMP supplementary comparison (APMP.QM-S19) offers different analytical challenges (e.g. in analysis of mercury and different range of measurands) as compared to the previous comparison. Moreover, this will be a good opportunity for the National Metrology Institutes / Designated Institutes (NMIs/DIs) which did not participate in the previous comparison to demonstrate their measurement competencies.

The supplementary comparison aims to enable participating NMIs/DIs to demonstrate their competence in the determination of toxic elements at mg/kg levels in seafood matrix. It will also enable NMIs/DIs with the relevant services, upon successful completion, to submit Calibration and Measurement Capabilities (CMCs) claims under the Mutual Recognition Arrangement of the International Committee for Weights and Measures (CIPM MRA).

TIMELINE

Table 1 lists the timeline for the proposed study.

Table 1. Programme Schedule.

Action	**Date**	
	APMP.QM-S19	**APMP.QM-P40**
Call for participation	July 2021	16 November 2021
Deadline for registration	30 September 2021	30 November 2021
Distribution of samples	October 2021	By end November 2021
Deadline for submission of results	1 April 2022	
Presentation of first report in APMP TCQM meeting	Nov/Dec 2022	

MEASURANDS

The comparison will cover arsenic, cadmium, mercury and lead in a seafood matrix. The expected mass fractions of the measurands (on a dry mass basis) are given in Table 2.

Table 2. Measurands and expected mass fraction.

Measurand	**Expected mass fraction (mg/kg)**
Arsenic	0.2 – 50
Cadmium	0.04 – 10
Mercury	0.02 – 5
Lead	0.04 – 10

STUDY MATERIALS

Dried shrimps were purchased from the local market in Hong Kong. The shrimps were soaked in a spike solution containing the target analytes for several hours, freeze-dried, blended into powder, and subjected to a sieving process through two calibrated sieves (200 and 100 m respectively). The sieved powder (particle size: 100–200 µm) was thoroughly homogenized in a 3-dimensional mixer for 5 days. The material was irradiated using a gamma source at a dose of about 10 kGy for disinfection. The irradiated material was packed into high-density polyethylene bottles, each of about 30 g. The bottles were purged with nitrogen and stored at room temperature (20 ± 5 °C).

Each participant will receive with **TWO** bottles of sample, each containing approximately 30 g of dried shrimp material. Measurement results are to be reported on a dry-mass basis.

Homogeneity Assessment of Study Material

Ten bottles of sample were randomly selected for homogeneity study. Two test portions of 0.5 g each were taken from each bottle for analysis. The test portions were digested using microwave-assisted acid digestion and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) with gravimetric standard additions. ANOVA at 95% level of confidence was applied to assess the between-bottle homogeneity in accordance with ISO Guide 35:2017 [12.2], the comparison material was found to be sufficiently homogeneous. The results are summarized in Table 3.

Table 3. Results of the homogeneity assessment for the measurands.

Measurand	ANOVA test		Relative standard uncertainty due to between-bottle (in)homogeneity, u_{bb} (%)
	F-statistics	Critical value	
Arsenic	1.50	3.02	0.7
Cadmium	0.93	3.02	0.6
Mercury	1.95	3.02	1.6
Lead	0.59	3.02	1.0

The graphical representation of the homogeneity data for individual measurand(s) are provided in Figures 1 to 4.

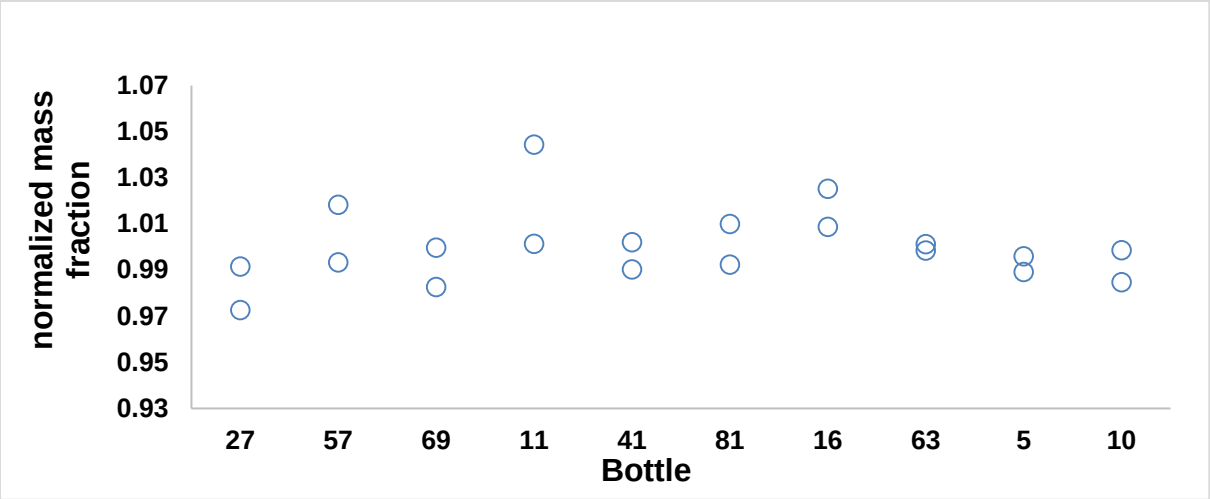

Figure 1. Homogeneity evaluation for Arsenic.

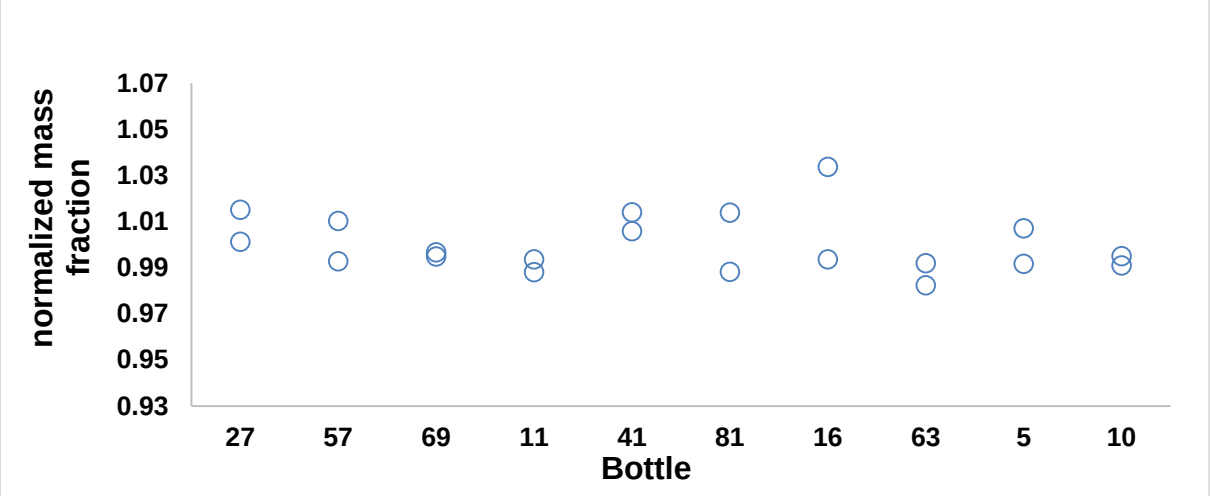

Figure 2. Homogeneity evaluation for Cadmium.

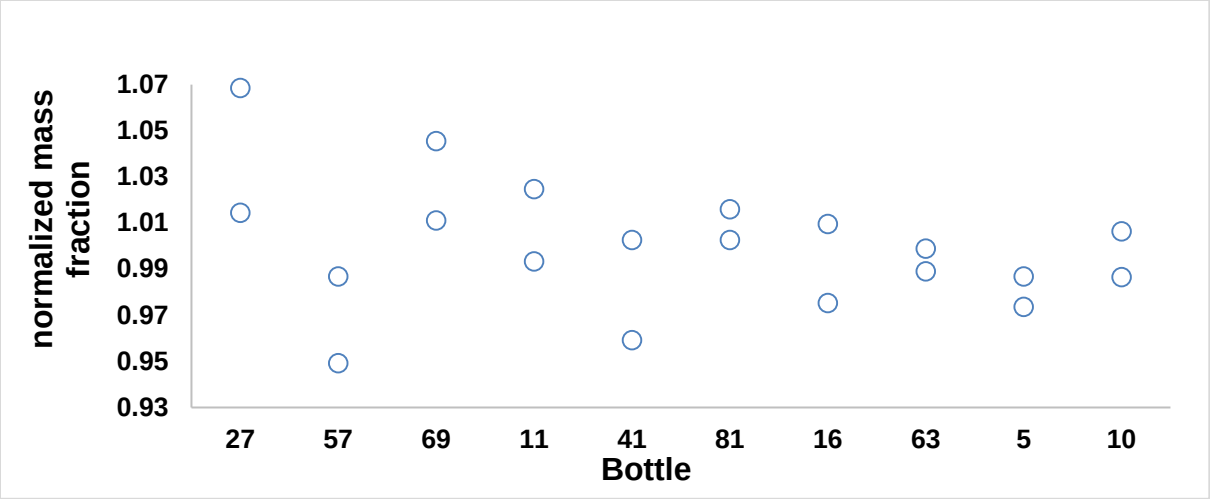

Figure 3. Homogeneity evaluation for Mercury.

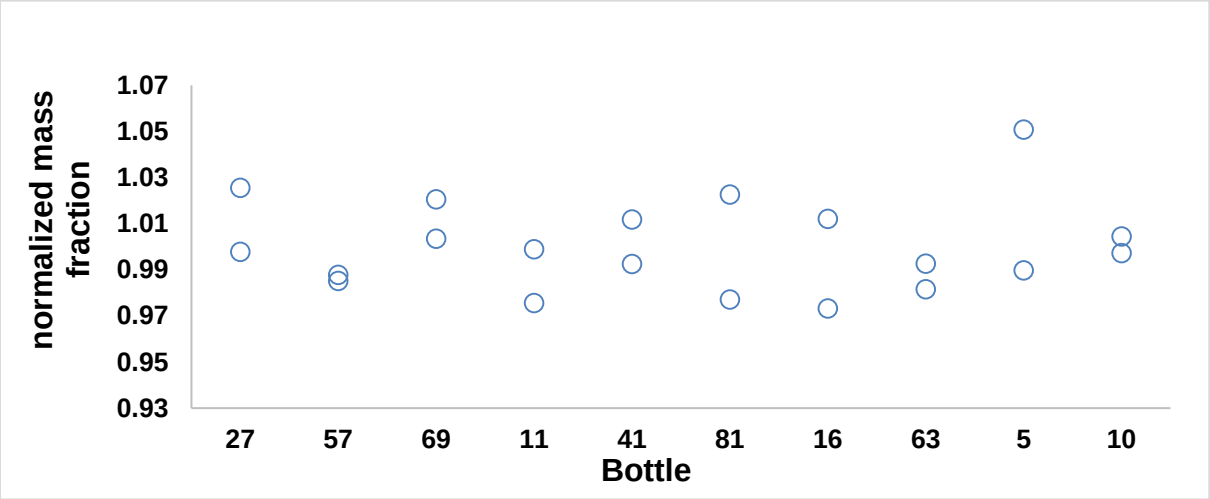

Figure 4. Homogeneity evaluation for Lead.

Stability Assessment of Study Material

The short-term stability of the measurands over a period of 4 weeks at 40 °C was accessed using isochronous approach, using the same analytical procedures as for the homogeneity study. Two randomly selected sample bottles were transferred from the storage condition (20 ± 5 °C) to 40 °C on three occasions (1, 2 and 4 weeks) over the study period. Two subsamples were then taken from each bottle. Using Student's *t*-test on the slope of the linear regression at 95% level of confidence, no significant instability of the measurands was observed upon exposure to 40 °C up to 4 weeks. The results are summarized in Table 4 and graphically represented in Figure 5.

Table 4. Results of the stability assessment (at 40 °C for 4 weeks) for the measurands.

Measurand	Student's *t*-test		*p*-value
	Calculated test statistics	Critical value	
Arsenic	0.047	4.303	0.859
Cadmium	0.201	4.303	0.479
Mercury	0.054	4.303	0.838
Lead	0.165	4.303	0.552

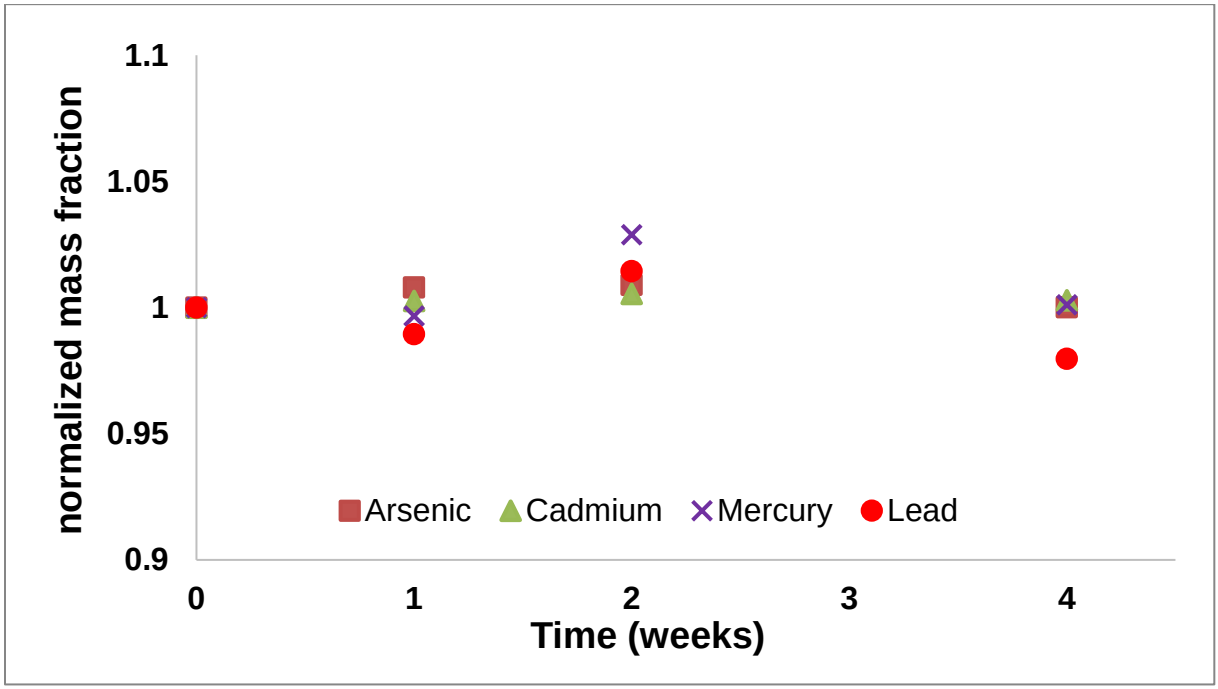

Figure 5. Short-term stabilities of the measurands at 40 °C for 4 weeks.

The long-term stability of the measurands in the comparison material at 20 ± 5 °C will be assessed using the same analytical procedures as for the homogeneity study. The testing will be carried out before sample dispatch and continuously monitored until completion of the supplementary comparison using the classical approach. For each occasion of the stability testing, at least two bottles will be randomly selected, and two subsamples will be taken from each bottle. Student's t-test on the slope of the linear regression at 95% level of confidence will be used for the evaluation of instability of the measurands.

Calibration Materials

Participants may establish the metrological traceability of their results to the SI using a direct realization via a primary method, certified reference materials (CRMs) from a NMI/DI having the required CMC claims, or by preparing their own calibration standards using commercially

available high purity materials for which they have determined the purity themselves. Commercial standards should not be employed if a supplementary comparison is to be used to support CMCs claims (See section 3 in CIPM MRA-G-13 for more information: https://www.bipm.org/utils/common/documents/CIPM-MRA/CIPM-MRA-G-13.pdf) [12.3].

INSTRUCTIONS AND SAMPLE DISTRIBUTION

The samples will be transported at room temperature (monitored by a temperature strip). Upon receipt, the samples should be stored at 20 ± 5 °C. A Sample Receipt Form will be provided to the participating NMIs/DIs for completion. The completed form should be sent to GLHK at your earliest convenience.

Recommended Minimum Sample Amount

The recommended minimum sample amount for analysis is at least 0.5 g. Participating NMIs/DIs should take at least 4 subsamples for the measurement of measurands. The bottle contents should be well mixed by rotation and shaking prior to use.

Dry Mass Determination

Dry mass determination shall be carried out, at the same time as the test portions are analyzed, by placing three separate portions (about 1 g each) of sample over anhydrous calcium sulphate (e.g. DRIERITE®) in a desiccator for at least 10 days until constant mass is reached. Do not use the sample, which was used for the determination of moisture content, for analysis.

RESULTS

Reporting and submission of results

A Report Form will be provided to the participating NMIs/DIs for completion. The participating NMIs/DIs are expected to report their results based on at least four subsamples for each measurand. Only one result, calculated from the average of the measurements, should be reported for each measurand. The results should be reported on a dry-mass basis in the unit of mg/kg, and should include standard and expanded uncertainties (95% level of confidence) for the mean of the replicate determinations.

Information on the measurement procedure (including the sample dissolution method, the calibration method, the internal standard, the quality control, the analytical instrument(s) used, etc), the calculation of the results, and the estimation of measurement uncertainty should be included in the Report Form. The completed form should be sent to GLHK on or before the scheduled deadline (1 April 2022). The submitted results will be considered as final.

To facilitate in-depth performance evaluation, participating NMIs/DIs shall clearly identify and quantify those factors that are considered to contribute to the measurement uncertainty of the analysis [12.4].

Reporting and submission of results

Results of all participating NMIs/DIs will be evaluated against the supplementary comparison reference value (SCRV). The SCRV and associated uncertainty will only be determined from results of NMIs/DIs that participate in the supplementary comparison using methods with demonstrated metrological traceability. The NIST Decision Tree approach (https://doi.org/10.6028/jres.126.007) [12.5] may be used for SCRV and Degree of Equivalence (DoE) calculations.

USE OF APMP.QM-S19 IN SUPPORT OF CALIBRATION AND MEASUREMENT CAPABILITY (CMC) CLAIMS

How Far the Light Shines

The comparison enables NMIs/DIs to demonstrate their capabilities in analyzing inorganic elements between the mass fraction range of 0.02 mg/kg to 50 mg/kg in seafood. The comparison will support CMCs for transition elements and metalloids/semi-metals in high organics content matrix, including seafood of animal origin and high protein food in category 11 (food).

Core Capability table

Analyte groups	Matrix challenges					
	Water	High Silica content (e.g. Soils, sediments, plants, ...)	High salts content (e.g. Seawater, urine, ...)	High organics content (e.g. high carbon) (e.g. Food, blood/serum, cosmetics, ...)	Difficult to dissolve metals (Autocatalysts, ...)	High volatile matrices (e.g. solvents, fuels, ...)
Group I and II: Alkali and Alkaline earth (Li, Na, K, Rb, Cs, Be, Mg, Ca, Sr, Ba)						
Transition elements (Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Y, Zr, Nb, Mo, Tc, Ag, Cd, Ta, W, Au, Hg, Al, Ga, In, Tl, Pb, Po)						
				Cd, Hg, Pb		
Platinum Group elements (Ru, Rh, Pd, Os, Ir, Pt)						
Metalloids / Semi-metals (B, Si, Ge, As, Sb, Te, Se)						
				As		
Non-metals (P, S, C, N, O)						
Halogens (F, Cl, Br, I)						
Rare Earth Elements (Lanthanides, Actinides)						

Low level (e.g. below 50 µg/kg)
High level (e.g. above 50 µg/kg)

REGISTRATION AND CONTACT DETAILS

The supplementary comparison is co-ordinated by the Government Laboratory, Hong Kong, China (GLHK) (Address: 7/F., Ho Man Tin Government Offices, 88 Chung Hau Street, Homantin, Kowloon, Hong Kong).

Participation is open to all NMIs/DIs under the APMP as listed in the CIPM MRA (https://www.bipm.org/en/cipm-mra/participation). NMIs/DIs from other RMOs are also welcome to join this supplementary comparison. Interested NMIs/DIs should complete the Registration Form and return it to Dr. Alvin W.H. Fung and Dr. Kelvin C.W. Tse before the deadline for registration on 30 September 2021.

Participation in the pilot study is open to 1) all laboratories eligible to join the supplementary comparison and 2) guest laboratories upon invitation. Interested NMIs/DIs/laboratories should complete the Registration Form and return it to Dr. Alvin W.H. Fung and Dr. Kelvin C.W. Tse before 30 November 2021.

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

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REFERENCES

12.1. CODEX STAN 193-1995 “General Standard for Contaminants and Toxins in Food and Feed”, Amendment: 2019, CODEX Alimentarius Commission.

12.2. ISO Guide 35:2017 “Reference materials — Guidance for characterization and assessment of homogeneity and stability”, 2017, Geneva, Switzerland.

12.3. CIPM MRA-G-13 “Calibration and measurement capabilities in the context of the CIPM MRA, Guidelines for their review, acceptance and maintenance”, Version 1.1, 30/03/2021.

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

12.5. Possolo, A., Koepke, A., Newton, D. and Winchester, M. (2021), Decision Tree for Key Comparisons, Journal of Research (NIST JRES), National Institute of Standards and Technology, Gaithersburg, MD, [online], https://doi.org/10.6028/jres.126.007.