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Report of the fourth interlaboratory comparison organised by the Community Reference Laboratory for Heavy Metals in Feed and Food

Total Cd, Pb, As, Hg and methylmercury in seafood

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Heavy Metals
in Feed and Food

Report of the fourth interlaboratory comparison

Total Cd, Pb, As, Hg and methylmercury in seafood

IMEP®

September 2008

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Table of Contents

1 Summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the fourth ILC of the CRL-HM which focused on the determination of total Cd, Pb, As, Hg and methylmercury (related to dry mass) in seafood.

The test material used in this exercise was the Certified Reference Material (CRM) TORT-2, lobster hepatopancreas, of the National Research Council of Canada. The material was rebottled and relabelled to prevent recognition by the participants and it was dispatched on the first half of May 2008. Each participant received one bottle containing approximately 20 g of test material. Thirty-four participants from twenty-six countries registered to the exercise of which 33 sets of results were reported for Cd and total mercury, 32 for Pb, 27 for arsenic and 4 for methylmercury. One laboratory reported two sets of values for Cd, Pb, Hg and As obtained with two different techniques, respectively. The Assigned values were the certified values taken from the TORT-2 certificate.

The uncertainties of the respective assigned values, u_{ref}, were taken directly from the CRM certificate as provided by the producer. Participants were invited to report the uncertainty on their measurements. This was done by all the laboratories taking part in this exercise.

Laboratory results were rated with z and zeta scores in accordance with ISO 13528[1]. Standard deviation for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation[2] and were 9.76 % for total Cd, 18. 74 % for total Pb, 10 % for total As, 19.48 % for total Hg and 21.24 % for methylmercury.

2 Introduction

Organomercury compounds are characterised by a very high toxicity. In general, exposure to organic mercury can cause brain damage to a developing foetus[3]. The exposure is more dangerous for young children than for adults because their nervous systems are still developing and thus are more sensitive to these compounds[4]. For this reason the European Commission recommends pregnant women, breast feeding women and children to limit their consumption of big fish predators in which high contents of methylmercury are known to be present.

Organomercury cations form salts with inorganic and organic acids and react readily with biological important ligands, notably sulfhydryl groups. They also pass easily across biological membranes perhaps because the halides (e.g. CH_3HgCl) and dialkylmercury are lipid soluble.

Agricultural soils contain mercury levels between 0.06 and 0.2 mg kg^{-1}. There is probably a root barrier preventing mercury uptake by plants. Nevertheless, there is some accumulation of mercury in mushrooms (1 or more mg kg^{-1} of which 40 µg kg^{-1} methylated), carrots and potatoes.

Terrestrial animals rarely have levels of mercury in muscle exceeding 50 µg kg^{-1}.

Microorganisms are able to convert inorganic mercury to organic forms. Bacteria that methylate mercury have been isolated from the mucous material on the surface of fish, as well as from fresh and salt water sediments.

Methylmercury does not undergo a rapid biotransformation in body tissues. Reported half-times for methylmercury vary from about 70 days in humans up to 700-1000 days in some species of fish and shellfish. The concentration of methylmercury in fish is generally related to size and ecological niche. Concentrations of 1 mg kg^{-1} have been reported for open ocean predators such as sword fish and tuna, but in industrially contaminated waters, methylmercury levels in fish muscle may exceed 10 mg kg^{-1} [5]. The significant bioaccumulation of methylmercury in seafood has resulted in a serious food safety problem. Methylmercury can accumulate in fish muscle by a 105 or 107 factor, which can lead to dangerously elevated levels of mercury in seafood even in regions with typical aquatic mercury levels[6].

From an analytical point of new, Methylmercury determination is performed by coupling gas chromatography (GC)[7] or high performance liquid chromatography (HPLC)[8] to different detectors such as electronic impact-mass spectrometry (EI-MS)[9], inductively coupled plasma-mass spectrometry (ICP-MS)[10], microwave induced plasma-atomic emission spectrometry (MIP-AES)[11], cold vapour-atomic absorption spectrometry (CV-AAS)[12] and cold vapour-atomic fluorescence spectrometry (CV-AFS)[7]. When gas chromatography is used for the separation of the species, derivatisation of methylmercury is needed to convert them into volatile species. Grignard reagents, sodium tetraethylborate and sodium tetraphenylborate[13] are frequently used as derivatisating agents[14]. Papers summarising and discussing the different approaches have been published in recent years[14,15].

Regarding maximum levels, the U.S. Food and Drug Administration established a guideline for methylmercury in seafood at a level of 1 mg kg^{-1}. In Europe only maximum levels for total mercury in food are given in legislation[16], varying from 0.5 to 1 mg kg^{-1} for different seafood species, but not for methylmercury. The reason for this being that fully validated methods for speciation between organic and inorganic mercury compounds are currently not available. In order to improve this situation and with a view to get more precise data on methylmercury in fish, the Community Reference Laboratory for Heavy Metals in Feed and Food organised, under request from the Directorate General for Health and Consumer Protection (DG SANCO), a proficiency test exercise for the determination of total Pb, Cd, As, Hg and methylmercury in seafood for its network of National Reference Laboratories.

3 Scope

As stated in regulation 882/2004 of the European Parliament and of the Council[17], one of the core duties of the CRL-HM is to organise interlaboratory comparisons for the benefit of staff from national Reference Laboratories. The scope of this ILC is to test the competence of the appointed NRLs to determine the total concentration of Cd, Pb, As, Hg and methylmercury in seafood.

The assessment of the measurement results is undertaken on the basis of requirements laid down in legislation[16,18], and follows the administrative and logistic procedures of IMEP, the International Measurement Evaluation Programme, of IRMM. This programme is accredited according to ISO Guide 43-1. The designation of this ILC is IMEP-104.

4 Time frame

This interlaboratory comparison was agreed upon by the NRLs network at the second CRL-HM workshop held on 24-25 September 2007. Invitation letters were sent to the participants on 22 April 2008 (cf Annex 1). The samples were dispatched to the participants on 7 May 2008. Reporting deadline was 15 June 2008.

5 Test Material

5.1 Preparation

The commercially available CRM TORT-2 (lobster hepatopancreas Reference Material for trace metals) was used for this ILC. The material was rebottled and relabelled to avoid identification by the participants as an existing CRM. Comprehensive information on the preparation of the CRM can be found on the certification report on the CNRC website[19].

5.2 Homogeneity and stability

According to the producer of the CRM used in this exercise, TORT-2 is an old CRM produced by the CNCR prior to their adoption of the Guide 35 protocols. For that reason, no independent assessment of homogeneity (or its uncertainty) was undertaken during the certification campaign. Under request, the CNRC informed the CRL-HM that *"uncertainties provided in the certificate* (cf. Annex 2) *represent 95 % confidence limits and the data sets for each element would comprise some 10-15 determinations by three or four independent techniques on a relatively large population of randomly selected bottles of material"*.

Regarding stability, based on the experience of the CNRC with TORT-2, *"there should be no component of uncertainty related to stability"*.

5.3 Distribution

The samples were dispatched to the participants by IRMM on 7 May 2008. Each participant received: a) a bottle containing approximately 20 g of test material, b) an accompanying letter with instructions on sampling handling and reporting (cf. Annex 3) and c) a form that had to be sent after receipt of the sample to confirm its arrival (cf. Annex 4).

6 Instructions to participants

Details on this intercomparison were discussed with the NRLs at the second workshop organised by the CRL-HM, held in Geel on 24-25 September 2007. Concrete instructions were given to all participants in a letter that accompanied the test material. The measurands and matrix were defined as "total Cd, Pb, As, Hg and methylmercury in seafood".

Laboratories were asked to perform two or three independent measurements and report them, together with the mean of the results and its associated uncertainty. The measurement results were to be corrected for moisture (following a procedure described in the accompanying letter, procedure that was optimised at IRMM by the Reference Materials Unit) and recovery. Participants were asked to follow their routine procedures. The results were to be reported in the same manner (e.g., number of significant figures) as those normally reported to the customer.

The results were to be reported in a special on-line form for which each participant received an individual access code. A special questionnaire was attached to this on-line form. The

questionnaire is intended to provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in Annex 5.

7 Reference values and their uncertainties

The CRM certificate provided certified values for all the measurands included in this study. Those certified values were used as assigned values (X_{ref}) for this intercomparison.

The certificate was valid during the time frame of the intercomparison.

The uncertainties provided in the certificate of the CRM represent 95 % confidence limits for an individual subsample and they were taken as the expanded uncertainties of the assigned values (U_{ref}). As already mentioned, no contribution to u_{ref} ($u_{ref} = U_{ref}/2$) for stability was necessary according to the CRM provider. No contribution for between-bottle homogeneity to u_{ref} was considered due to the lack of an independent assessment of homogeneity. However, considering that a relatively large number of randomly selected bottles was used during the certification campaign, it can be assumed that the uncertainty provided in the certificate already includes some component for heterogeneity.

The assigned values (X_{ref}) and their respective estimated uncertainties are summarised in Table 1.

Table 1: Assigned values and their associated standard uncertainties for the measurands of this ILC.

Measurand	X_{ref} (mg kg^{-1})	U_{ref} (mg kg^{-1})	u_{ref} (mg kg^{-1})
Total Cd	26.7	0.6	0.3
Total Pb	0.35	0.13	0.07
Total As	21.6	1.8	0.9
Total Hg	0.27	0.06	0.03
Methylmercury	0.152	0.013	0.007

X_{ref} is the certified value and u_{ref} is the corresponding standard uncertainty; U_{ref} is the estimated expanded uncertainty; with a coverage factor k=2, corresponding to a level of confidence of about 95 %.

8 Evaluation of results

8.1 General observations

Thirty-four laboratories from 26 countries registered for participation in this exercise. One laboratory out of the 34 did not submit results. Thirty-two laboratories reported values for Cd and total Hg, 31 for Pb, 26 for As and 4 for methylmercury (one out of the four reported "<than". Thirty-two laboratories reported two or more measurement values per measurand. One laboratory submitted two sets of results, each of them obtained with a different technique. For each of the two sets, only one value accompanied by an uncertainty statement was reported. Thirty-two laboratories responded to the questionnaire included in the on-line reporting from.

8.2 Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and zeta scores in accordance with ISO 13528[1] and the International Harmonised Protocol[20].

$$z = \frac{x_{lab} - X_{ref}}{\hat{\sigma}} \qquad \text{Eq. 2}$$

$$zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u^2_{ref} + u^2_{lab}}} \qquad \text{Eq. 3}$$

where:

x_{lab} is the measurement result reported by a participant
X_{ref} is the certified reference value (assigned value)
u_{ref} is the standard uncertainty of the reference value
u_{lab} is the standard uncertainty reported by a participant
$\hat{\sigma}$ is the standard deviation for proficiency assessment

The z score compares the participant's deviation from the reference value with the standard deviation accepted for the proficiency test, $\hat{\sigma}$. Very frequently, in the area of food and feed $\hat{\sigma}$ is derived from the improved Horwitz equation[2]. The values for $\hat{\sigma}$ obtained for this exercise when applying the improved Horwitz equation were: 9.76 % for total Cd, 18. 74 % for total Pb, 10 % for total As, 19.48 % for total Hg and 21.24 % for methylmercury.

Should participants feel that these $\hat{\sigma}$ values are not fit for their purpose they can recalculate their scorings with a standard deviation matching their requirements. x_{lab} is the mean of the individual measurement results calculated by the ILC organiser. If sigma hat is regarded as satisfactory, the z-score can be interpreted as:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

Zeta score state if the laboratory result agree with the assigned value within the respective uncertainties. The interpretation of the zeta score is similar to the interpretation of the z-score:

$\|zeta\| \leq 2$	satisfactory result
$2 < \|zeta\| \leq 3$	questionable result
$\|zeta\| > 3$	unsatisfactory result

An unsatisfactory zeta-score might be due to an underestimation of the uncertainty, or to a large error causing a large deviation from the reference value, or to a combination of the two factors. A laboratory with an unsatisfactory zeta-score has an estimation of the uncertainty of

its measurements which is not consistent with the laboratory's deviation from the reference value.

The standard uncertainty of the laboratory (u_{lab}) was calculated dividing the reported expanded uncertainty by the reported coverage factor (*k*). When *k* was not specified, the reported expanded uncertainty was considered as the half-width of a rectangular distribution; u_{lab} was then calculated by dividing this half-width by √3, as recommended by Eurachem and CITAC[21].

8.3 Laboratory results and scorings

The results, as reported by the participants, are summarised in Table 2 a-e for total Cd, total Pb, total As, total Hg and methylmercury, respectively, together with the z- and zeta scores. Laboratory codes were given randomly.

Three sets of figures are provided for Cd, Pb, As, Hg and methylmercury (Figure 1-5). Each set includes (a) the Kernel density plot, (b) individual mean value and associated expanded uncertainty, (c) the z- and zeta scores. For methylmercury, the Kernel density plot is not shown due to the reduced number of laboratories which reported values for that measurand. The solid line represents the assigned value, the dashed lines delimit the reference interval ($X_{ref} \pm 2\ u_{ref}$) and the dotted lines delimit the target interval ($X_{ref} \pm 2\sigma$). The Kernel plots were obtained using a software tool developed by AMC[22].

Regarding z and zeta scores, the results for total Cd, Pb, As and Hg are summarised in Table 3. There is quite a good agreement in the number of laboratories in the different groups, satisfactory, questionable and unsatisfactory, between the z and zeta scores. Actually, most results with $|z| \leq 2$ also have a $|zeta| \leq 2$. In the same way a high percentage of the results with an unsatisfactory zeta score also have an unsatisfactory z score. This indicates that most laboratories have made a realistic estimation of their uncertainties for the different measurands. The unusually large uncertainties reported by L28 for most of the measurands could be due to an error in the units in which uncertainties were reported.

Methylmercury deserves a more detailed discussion. Only four laboratories reported values for methylmercury. One laboratory reported < 1 mg kg^{-1}. The maximum limits given by European Legislation[16] for total mercury are in the range 0.5-1 mg kg^{-1} depending on the fish species. Methylmercury can account for almost 100 % of mercury in some fish species, but not always, as it is the case in the test material used in this exercise. For this reason it would be advisable to have methods implemented with detection limits lower than 1 mg kg^{-1}. The three remaining laboratories which provided results for methylmercury obtained satisfactory results as it regards the z score. One out of the three laboratories had a questionable zeta score, the other two obtained a $|zeta| \leq 2$. Due to the reduced number of laboratories reporting results for methylmercury no statistic evaluation for this measurand is included in Table 3.

Additional information was gathered from the questionnaire that participants were asked to fill in (Annex 5). Eleven laboratories have corrected their results for recovery. Most of the recovery factors applied fell in the range 90-110 %. There were some exceptions to that rule: L20 applied a correction factor of 138 % for total Hg determinations, L 33 also corrected the concentrations found for total Hg by 118.8 % and l23 applied correction factors of 155 and 73 % for total Cd and Pb determinations, respectively.

When asked about the level of confidence reflected by the reported coverage factor (k) for their uncertainty estimates eighteen laboratories answered 95 %, one reported a level of 99.5, one of 100, one of 10, one of 5 and one of 2 %. Nine laboratories did not provide any figure. For uncertainty estimates, various combinations of two or more options (question 3 of the questionnaire) were given. Twenty laboratories use uncertainty calculated during the in-house validation of the method. Eleven laboratories use precision data. Eight laboratories make uncertainty calculations according to ISO-GUM. Five laboratories use intercomparison data. Four laboratories reported the known uncertainties of the standard method. One laboratory uses expert "guesstimate". Two laboratories have reported to use data from control charts.

Twenty laboratories provide uncertainty statements to their customers for this type of analysis, eleven do not and one did not answer this question.

Thirty-one laboratories corrected their results for moisture and one indicated that correction for moisture was not performed because analyses were carried out on a dry sample. Twenty-seven laboratories reported values for moisture in the range 7.1-9.7 %. One laboratory reported 1.3 % and three laboratories reported 91.15, 91.53 and 99 %, respectively, which seem to be answers to a wrongly interpreted question.

Fourteen laboratories have used official methods for the analyses. Information about methods of analysis which have been applied in this exercise are summarised in Annex 6.

Thirty laboratories carry out this type of analysis on a routine basis. The distribution of them in terms of samples analysed per year is shown in Annex 7. In the particular case of methylmercury analysis, only one laboratory reported to carry out analyses on a routine basis (0-50 per year). Other five laboratories also reported to determine methylmercury in 0-50 samples per year. One laboratory did not answer to this question.

All participants filling in the questionnaire but one have a quality system in place. In 29 cases the quality system is set according to ISO 17025, in one case according to ISO 9000 and in one case according to the two previously mentioned standards. When asked about accreditation none of the participants is accredited for methylmercury analysis, 11 are not accredited for total As determination, four are not accredited for total Hg and Cd analysis and three are not accredited for total Pb determinations.

Thirty laboratories participate regularly in proficiency tests for this type of analyses. Two laboratories did not answer to this question. When the same information was asked about the individual measurands covered by IMEP-104 only one laboratory participates regularly in PTs for the determination of methylmercury, 25 participate in PTs for arsenic determination, 30 participate in ILCs for total mercury determination and all laboratories takes part regularly in intercomparisons for total Cd and Pb determinations.

Twenty-eight laboratories use certified reference materials for this type of analysis. Twenty-seven out of the twenty-eight use the CRM for validation purposes and seven out of the twenty-eight use CRMs for calibration of instruments. Three laboratories use TORT-2 for their internal quality control.

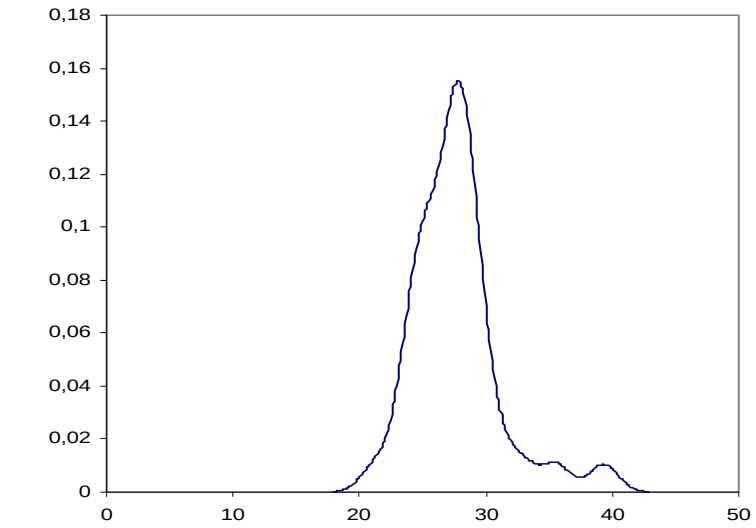

Figure 1a
Total Cd in seafood
Kernel density,
all results

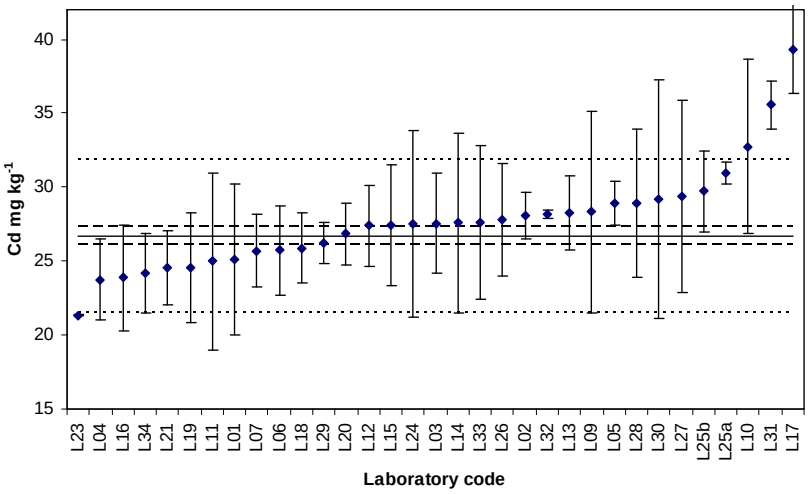

Figure 1b
Reported results and corresponding expanded uncertainties

$X_{ref} = 26.7$
$u_{ref} = 0.3$
$\hat{\sigma} = 2.61$ (9.76 %)
in mg kg^{-1}

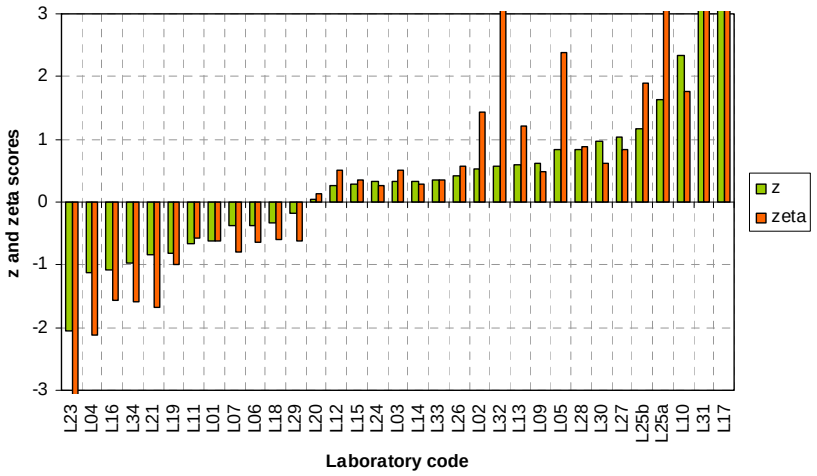

Figure 1c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

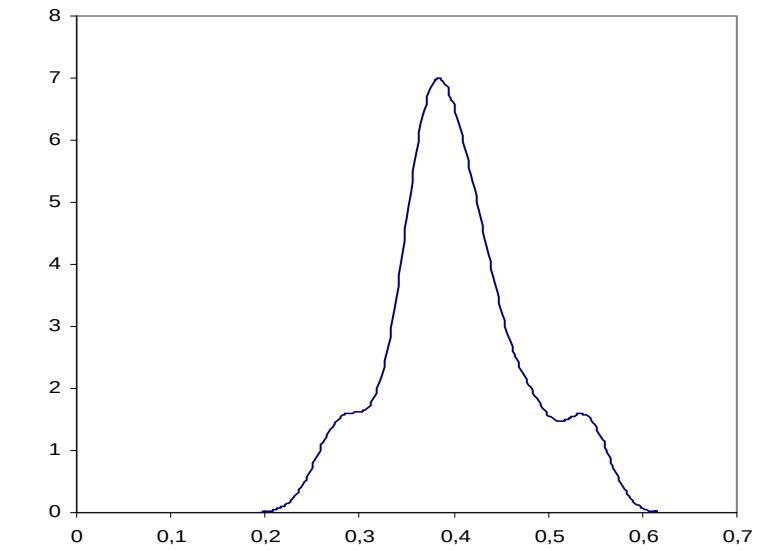

Figure 2a
Total Pb in seafood
Kernel density,
all results

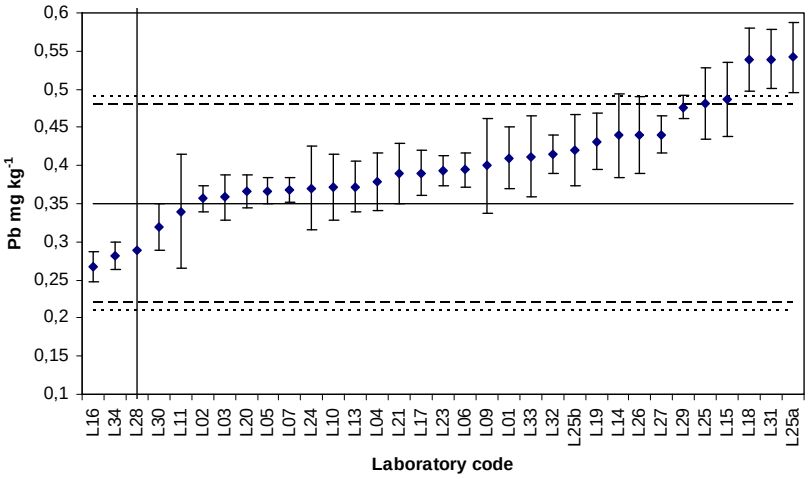

Figure 2b
Reported results and corresponding expanded uncertainties

$X_{ref} = 0.35$
$u_{ref} = 0.065$
$\hat{\sigma} = 0.07$ (18.74 %)
in mg kg^{-1}

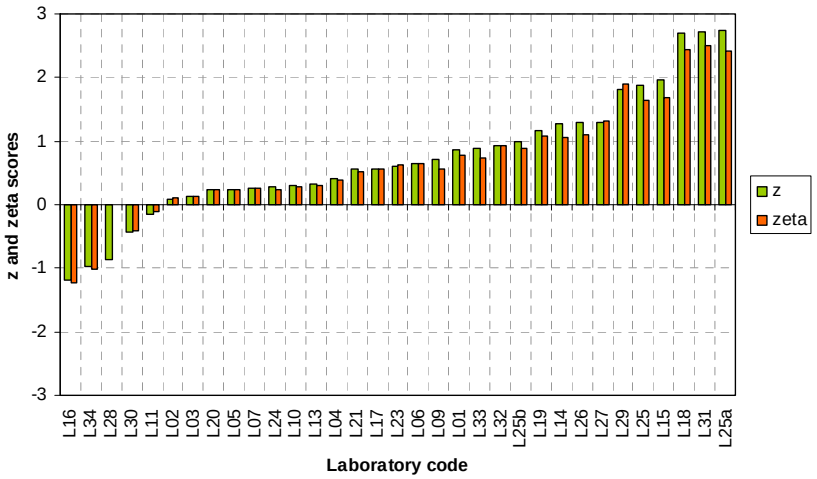

Figure 2c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

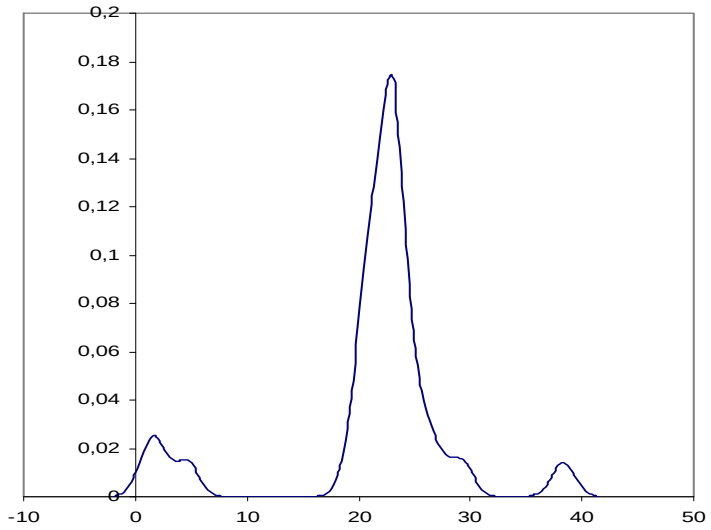

Figure 3a
Total As in seafood
Kernel density,
all results

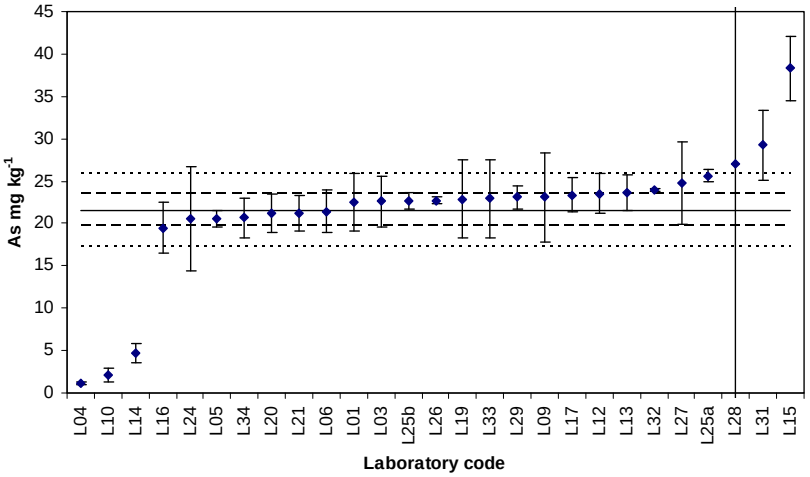

Figure 3b
Reported results and corresponding expanded uncertainties

$X_{ref} = 21.6$
$u_{ref} = 0.9$
$\hat{\sigma} = 2.18$ (10 %)
in mg kg^{-1}

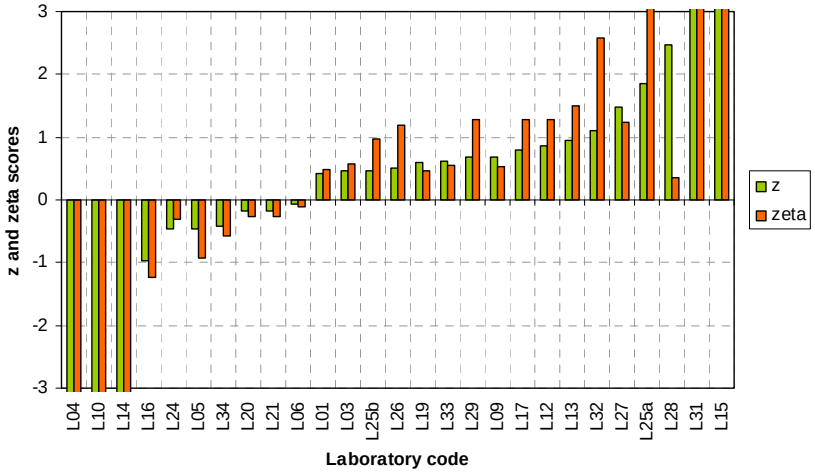

Figure 3c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

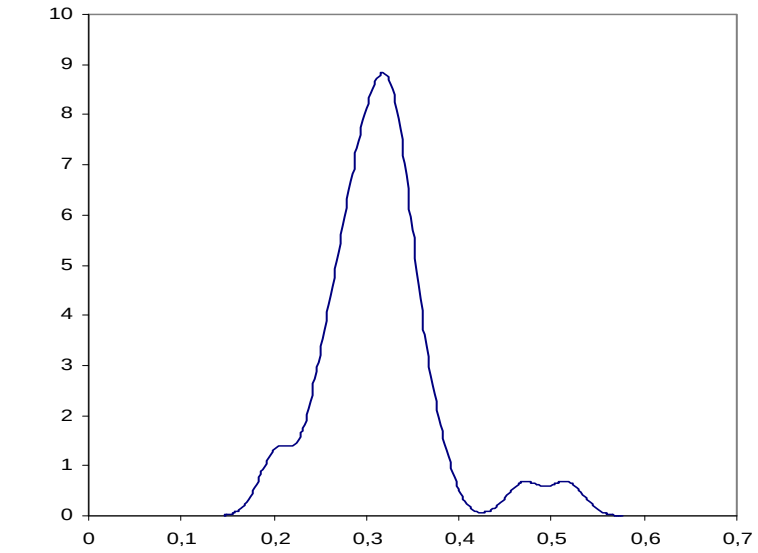

Figure 4a
Total Hg in seafood
Kernel density,
all results

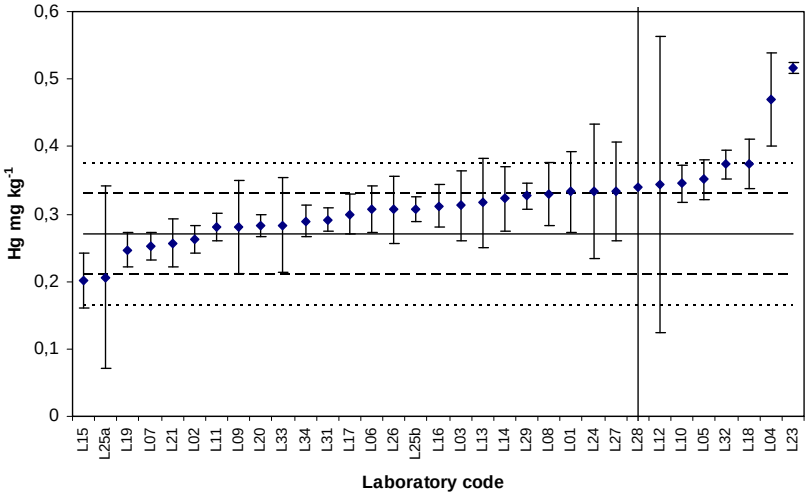

Figure 4b
Reported results and corresponding expanded uncertainties

$X_{ref} = 0.27$
$u_{ref} = 0.03$
$\hat{\sigma} = 0.05$ (19.48 %)
in mg kg^{-1}

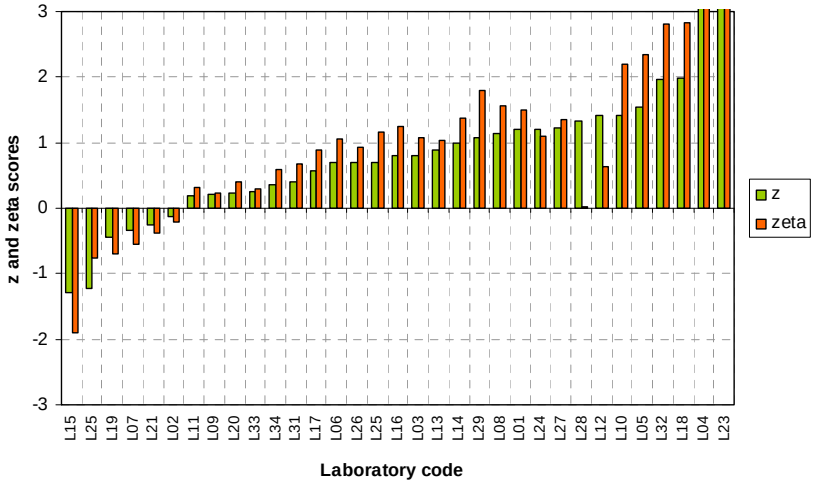

Figure 4c
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

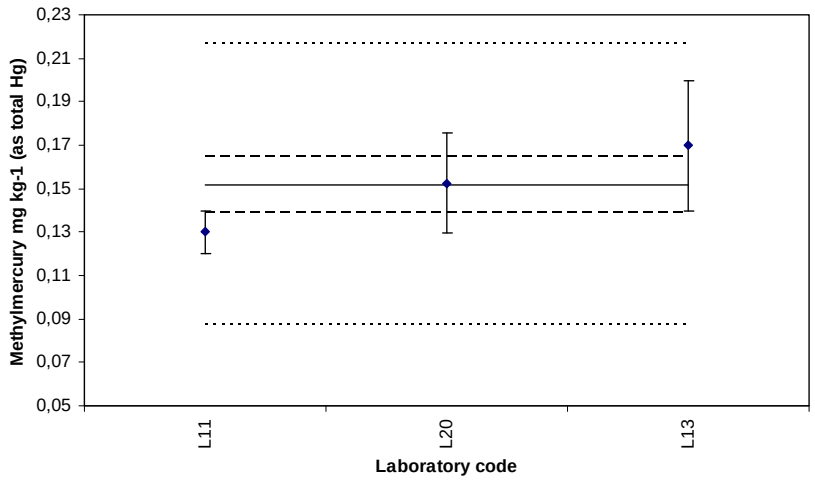

Figure 5a
Methylmercury in seafood
Reported results and corresponding expanded uncertainties

$X_{ref} = 0.152$
$u_{ref} = 0.0065$
$\hat{\sigma} = 0.03$ (21.24 %)
in mg kg^{-1}

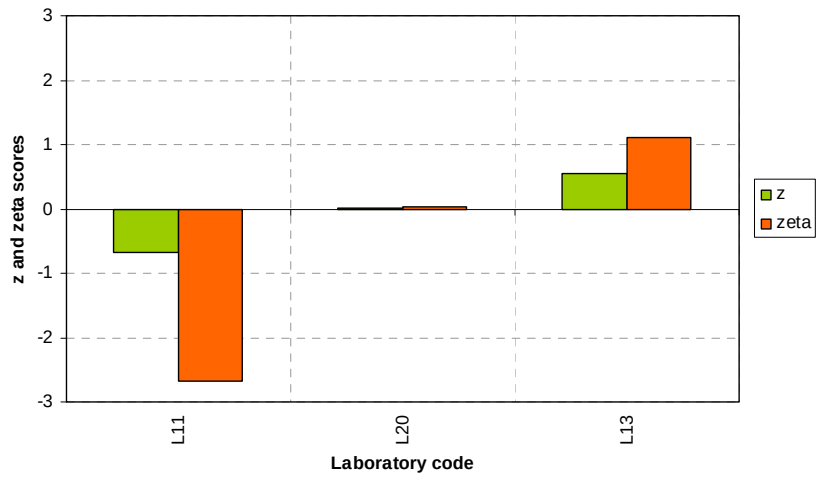

Figure 5b
Performance evaluation
$z = (x_{lab}-X_{ref})/ \hat{\sigma}$
$zeta = (x_{lab}-X_{ref})/ \sqrt{(u_{lab}^2+u_{ref}^2)}$

Table 2a: Total Cd, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total Cd content: 26.7 ± 0.6 mg kg^{-1}

Lab code	x1	x2	x3	Ulab	k	Mean-calc	Technique	z	zeta
L01	25,3	25,4	24,6	5,1	2	25,1	ICP-AES	-0,6	-0,6
L02	27,3	28,9	28,1	1,6	**a**	28,1	AAS flame	0,5	1,4
L03	28	28,1	26,6	3,4	2	27,6	ICP-MS	0,3	0,5
L04	20,9	23,7	26,7	2,7	2	23,8	ET-AAS	-1,1	-2,1
L05	28,78	28,88	29	1,5	**a**	28,89	ICP-MS	0,8	2,4
L06	26,4	25,9	24,9	3	2	25,7	ICP-MS	-0,4	-0,6
L07	25,11	25,78	26,2	2,44	2	25,70	AAS	-0,4	-0,8
L09	28,206	28,332	28,445	6,799	2	28,328	ICP-MS	0,6	0,5
L10	33,2	32,7	32,4	5,9	**a**	32,8	GFAAS	2,3	1,8
L11	25	25		6	2	25	GFAAS	-0,7	-0,6
L12	24,43	28,47	29,29	2,74	2	27,40	Zeeman electrothermal AAS	0,3	0,5
L13	28,08	27,68	29,07	2,51	2	28,28	ICP-MS	0,6	1,2
L14	28,08	28,23	26,44	6,07	2	27,58	ETAAS	0,3	0,3
L15	26,8	28,2	27,3	4,1	2	27,4	ICP-AES	0,3	0,4
L16	23,9	24	23,7	3,58	2	23,87	ICP-AES	-1,1	-1,6
L17	41	39	38	3	2	39	FAAS	4,8	8,3
L18	25,73	25,94	25,92	2,37	**a**	25,86	ETAAS	-0,3	-0,6
L19	24,5	24,77	24,42	3,68	**a**	24,56	Flame AAS	-0,8	-1,0
L20	26,6	28	25,9	2,1	2	26,8	ETAAS	0,1	0,1
L21	24,8	24,4	24,4	2,5	2	24,5	FAAS	-0,8	-1,7
L23	23,1	19,6		0,01	2	21,4	GF-AAS	-2,1	-17,8
L24	27,11	27,962		6,333	2	27,536	ETAAS	0,3	0,3
L25a	30,936			0,747	**a**	30,936	ICP-MS	1,6	8,1
L25b	29,728			2,73	**a**	29,728	Flame AAS	1,2	1,9
L26	28	27,7	27,7	3,8	2	27,8	FAAS	0,4	0,6
L27	29,9	29,7	28,6	6,5	2	29,4	ICP-MS	1,0	0,8
L28	28,8	28,8	29,1	5	2	28,9	ETAAS	0,8	0,9
L29	26,4	26,2	26,1	1,4	2	26,2		-0,2	-0,6

Lab code	x1	x2	x3	Ulab	k	Mean-calc	Technique	z	zeta
L30	28,7	29,7		8,01	2	29,2	ETAAS	1,0	0,6
L31	35,0054	36,4418	35,2231	1,5839	2	35,5568	ICP-MS	3,4	10,5
L32	27,85	28,55		0,29	1	28,20	ICP-AES	0,6	3,6
L33	27,6	27,3	28	5,2	2	27,6	ETAAS	0,4	0,4
L34	23,503	24,785	24,263	2,711	**a**	24,184	ICP-MS	-1,0	-1,6

All results expressed in mg kg^{-1}

a) k not reported; u = U/√3

Table 2b: Total Pb, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total Pb content: 0.35 ± 0.13 mg kg^{-1}

Lab code	x1	x2	x3	Ulab	k	Mean-calc	Technique	z	zeta
L01	0,41	0,41	0,41	0,08	2	0,41	ICP-AES	0,9	0,8
L02	0,373	0,345	0,352	0,029	**a**	0,357	ETAAS	0,1	0,1
L03	0,381	0,358	0,337	0,06	2	0,359	ICP-MS	0,1	0,1
L04	0,363	0,377	0,396	0,074	2	0,379	ETAAS	0,4	0,4
L05	0,374	0,362	0,364	0,03	**a**	0,367	ICP-MS	0,3	0,2
L06	0,402	0,386	0,396	0,045	2	0,395	ICP-MS	0,7	0,6
L07	0,373	0,36	0,37	0,033	2	0,368	AAS	0,3	0,3
L09	0,3874	0,3949	0,4162	0,124	2	0,3995	ICP-FD-MS	0,8	0,6
L10	0,377	0,452	0,286	0,074	**a**	0,372	GFAAS	0,3	0,3
L11	0,36	0,32		0,15	2	0,34	GFAAS	-0,2	-0,1
L13	0,371	0,386	0,359	0,066	2	0,372	ICP-MS	0,3	0,3
L14	0,453	0,427	0,438	0,109	2	0,439	ETAAS	1,4	1,1
L15	0,493	0,475	0,493	0,097	2	0,487	ICP-AES	2,1	1,7
L16	0,26	0,267	0,273	0,04	2	0,267	ETAAS	-1,3	-1,2
L17	0,41	0,37		0,06	2	0,39	FAAS	0,6	0,6
L18	0,544	0,564	0,509	0,072	**a**	0,539	ETAAS	2,9	2,4
L19	0,4099	0,441	0,4434	0,0647	**a**	0,4314	ETAAS	1,2	1,1
L20	0,372	0,36		0,044	2	0,366	ETAAS	0,2	0,2
L21	0,4	0,37	0,4	0,08	2	0,39	ETAAS	0,6	0,5
L23	0,343	0,442		0,04	2	0,393	GFAAS	0,6	0,6
L24	0,35	0,39		0,11	2	0,37	ETAAS	0,3	0,2
L25a	0,542			0,08	**a**	0,542	ICP-MS	2,9	2,4
L25b	0,42			0,08	**a**	0,42	Flame AAS	1,1	0,9
L26	0,42	0,46		0,1	2	0,44	ETAAS	1,4	1,1
L27	0,446	0,421	0,455	0,048	2	0,441	ICP-MS	1,3	1,3
L28	0,295	0,322	0,252	25	2	0,290	ETAAS	-0,9	0,0
L29	0,51	0,46	0,46	0,03	2	0,48	ICP-MS	1,9	1,9

Lab code	x1	x2	x3	Ulab	k	Mean-calc	Technique	z	zeta
L30	0,33	0,31		0,061	2	0,32	ETAAS	-0,5	-0,4
L31	0,5851	0,5148	0,5193	0,0789	2	0,5397	ICP-MS	2,9	2,5
L32	0,419	0,411		0,026	1,037	0,415	GF-AAS	1,0	0,9
L33	0,484	0,371	0,38	0,107	2	0,412	ETAAS	0,9	0,7
L34	0,297	0,258	0,289	0,0316	**a**	0,281	ICP-MS	-1,0	-1,0

All results expressed in mg kg^{-1}

a) k not reported; u = U/√3

Table 2c: Total As, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total As content: 21.6 ± 1.8 mg kg^{-1}

Lab code	x1	x2	x3	Ulab	k	Mean-calc	Technique	z	zeta
L01	22,7	22,9	22	3,4	2	22,5	ICP-AES	0,4	0,5
L03	21,2	24,4	22,2	3	2	22,6	ICP-MS	0,5	0,6
L04	0,95	1,09	1,34	0,13	2	1,13	HG-AAS	-9,4	-22,7
L05	20,5	21	20,3	1	**a**	20,6	HG-AAS	-0,5	-0,9
L06	22,2	21,4	20,7	2,5	2	21,4	ICP-MS	-0,1	-0,1
L09	23,067	22,948	23,267	5,311	2	23,094	ICP-MS	0,7	0,5
L10	2,54	2,72	2,54	0,83	**a**	2,16	HG-AAS	-8,9	-19,1
L12	22,97	23,73	23,77	2,35	2	23,49	Zeeman electrothermal AAS	0,9	1,3
L13	23,77	23,5	23,78	2,1	2	23,68	ICP-MS	1,0	1,5
L14	4,64	4,57	4,8	1,17	2	4,67	HG-AAS	-7,8	-15,8
L15	42	37,2	35,8	3,8	2	38,3	HG-ICP-AES	7,7	8,0
L16	19,6	19,2	19,7	2,92	2	19,5	ICP-AES	-1,0	-1,2
L17	24	23	23	2	2	23	ICP-MS	0,8	1,3
L19	22,49	23,03	23,17	4,58	**a**	22,90	HG-AAS	0,6	0,5
L20	21,1	21,1	21,4	2,3	2	21,2	HG-AAS	-0,2	-0,3
L21	21,2	21,3	21,2	2,1	2	21,2	HG-AAS	-0,2	-0,3
L24	19,87	21,06	20,81	6,17	2	20,58	ETAAS	-0,5	-0,3
L25a	25,636			0,779	**a**	25,636	ICP-MS	1,9	4,0
L25b	22,614			0,941	**a**	22,614	HG-AAS	0,5	1,0
L26	22,6	22,8		0,4	2	22,7	ETAAS	0,5	1,2
L27	25,2	25	24,2	4,9	2	24,8	ICP-MS	1,5	1,2
L28	26,4	22,8	31,7	30	2	27,0	HG-AAS	2,5	0,4
L29	22,8	23,1	23,3	1,4	2	23,1		0,7	1,3
L31	28,5351	29,8807	29,3143	4,0941	2	29,2434	ICP-MS	3,5	3,4
L32	22,54	22,37		0,21	1,068	22,46	GF-AAS	0,4	0,9
L33	25,2	21,8	21,9	4,6	2	23,0	ETAAS	0,6	0,6
L34	21,242	20,769	19,986	2,378	**a**	20,666	ICP-MS	-0,4	-0,6

All results expressed in mg kg^{-1}
a) k not reported; u = U/√3

Table 2d: Total Hg, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.
Total Hg content: 0.27 ± 0.06 mg kg^{-1}

Lab code	x1	x2	x3	x4	Ulab	k	Mean-calc	Technique	z	zeta
L01	0,34	0,33	0,33		0,06	2	0,33	ETAAS	1,2	1,5
L02	0,342	0,34	0,35		0,02	**a**	0,263	CV-AAS	-0,1	-0,2
L03	0,315	0,311	0,312		0,052	2	0,313	ICP-MS	0,8	1,1
L04	0,48	0,46			0,07	2	0,47	HG-AAS	3,8	4,3
L05	0,334	0,366	0,353		0,03	**a**	0,351	ICP-MS	1,5	2,3
L06	0,309	0,303	0,308		0,035	2	0,307	ICP-MS	0,7	1,1
L07	0,256	0,242	0,26		0,02	2	0,253	HG-AAS	-0,3	-0,5
L08	0,331	0,326	0,342	0,319	0,047	2	0,330	CV-AAS	1,1	1,6
L09	0,2837	0,2776			0,07	2	0,2807	ICP-FD-MS	0,2	0,2
L10	0,358	0,337	0,34		0,028	**a**	0,345	CV-AAS	1,4	2,2
L11	0,27	0,29			0,02	2	0,28	Thermal decomposition, amalgamation and AAS	0,2	0,3
L12	0,362	0,333	0,337		0,22	1,96	0,344	Thermal decomposition, amalgamation and AAS	1,4	0,6
L13	0,307	0,317	0,325		0,066	2	0,316	CV-AAS	0,9	1,0
L14	0,322	0,317	0,329		0,048	2	0,323	AAS AMA 254/Mercury Analyser	1,0	1,4
L15	0,19	0,2	0,215		0,04	2	0,202	CV-ICP-AES	-1,3	-1,9
L16	0,309	0,312	0,315		0,031	2	0,312	CV-AAS	0,8	1,2
L17	0,31	0,29	0,3		0,03	2	0,30	ICP-MS	0,6	0,9
L18	0,364	0,376	0,382		0,037	**a**	0,374	CV-AAS	2,0	2,8
L19	0,2425	0,2514			0,0247	**a**	0,2470	CV-AAS	-0,4	-0,7
L20	0,282	0,283			0,017	2	0,283	CV-AAS	0,2	0,4
L21	0,255	0,265	0,251		0,036	2	0,257	CV-AAS	-0,2	-0,4
L23	0,599	0,434			0,009	2	0,517	HG-AAS	4,7	8,1
L24	0,344	0,323			0,1	2	0,334	AFS	1,2	1,1
L25a	0,206				0,135	**a**	0,206	ICP-MS	-1,2	-0,8
L25b	0,307				0,019	**a**	0,307	CV-AAS	0,7	1,2
L26	0,28	0,32	0,32		0,05	2	0,31	CV-AAS	0,7	0,9
L27	0,348	0,334	0,32		0,073	2	0,334	ICP-MS	1,2	1,4
L28	0,332	0,348			10	2	0,340	CV-AAS	1,3	0,0

Lab code	x1	x2	x3	x4	Ulab	k	Mean-calc	Technique	z	zeta
L29	0,31	0,35	0,32		0,02	2	0,33		1,1	1,8
L31	0,2905	0,2914	0,292		0,017	2	0,2913	Advanced Mercury Analyser AMA 254	0,4	0,7
L32	0,382	0,378	0,369		0,021	0,989	0,376	AFS	2,0	2,9
L33	0,282	0,289	0,28		0,07	2	0,284	CV-AFS	0,3	0,3
L34	0,2885	0,2922	0,2875		0,0232	**a**	0,2894	ICP-MS	0,4	0,6

All results expressed in mg kg^{-1}

a) k not reported; u = U/√3

Table 2e: Methylmercury, quantitative information reported by the participants plus the laboratory scorings provided by the organiser.

Methylmercury content: 0.152 ± 0.013 mg kg^{-1}

Lab code	x1	x2	x3	x4	Ulab	k	Mean-calc	Technique	z	zeta
L09	<1	<1	<1					GC-ECD		
L11	0,13	0,13			0,01	2	0,13	Thermal decomposition, amalgamation and AAS	-0,7	-2,7
L13	0,17	0,17	0,172	0,168	0,03	2	0,17	GC-ICP-MS	0,6	1,1
L20	0,158	0,147			0,023	2	0,153	HPLC-ICP-MS	0,0	0,0

All results expressed in mg kg^{-1}

Table 3: Number and percentage of laboratories with satisfactory, questionable and unsatisfactory scores.

	Total Cd		Total Pb		Total As		Total Hg	
z	**No labs**	**Labs (%)**	**No labs**	**Labs (%)**	**No labs**	**Labs (%)**	**No labs**	**Labs (%)**
Satisf.	29	88	29	91	21	78	30	91
Quest.	2	6	3	9	1	4	1	3
Unstat.	2	6	0	0	5	18	2	6
zeta								
Satisf.	26	79	30	94	21	78	27	82
Quest.	2	6	2	6	0	0	4	12
Unstat.	5	15	0	0	6	22	2	6

9 Conclusions

As a general conclusion it can be stated that the National Reference Laboratories taking part in this exercise performed well as it is supported by the high percentage of laboratories that obtained satisfactory scores.

The network of NRLs have reacted positively to the demand of providing results for total As, a measurand for which, so far, none of them has an official mandate. Some action is needed in the field of methylmercury determination. Even if only four laboratories reported results for this analyte, their performance was satisfactory and this should encourage the rest of the NRLs dealing with food analysis to go ahead with the implementation of methods for methylmercury determination in marine organisms. When asked about the reason why no results were reported for methylmercury, most laboratories indicated that either they do not have a method implemented or they are in the phase of implementation but the method is not yet fully validated. Most of them indicated that they look forward to receiving training on the matter. A list of official methods for heavy metal analysis in feed and food, among them some methods for methylmercury determination in shellfish, can be found in the web page of the CRL-HM[23]. The CRL-HM will organise a training on methylmercury, As and Se speciation in food and feed on the occasion of the third workshop for NRLs, which will take place on 25-26th September 2008 at IRMM.

A clear improvement is observed in the quality of uncertainty estimation made by the laboratories along the four ILCs organised so far by the CRL-HM.

The number of laboratories taking part in the proficiency tests organised by the CRL-HM increases constantly with every new exercise, including NRLs which do not have a mandate for the matrix covered and which participate on a voluntary basis.

10 Acknowledgements

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The NRLs participating in this exercise, listed below are kindly acknowledged.

Organisation	Country
• AGES Zentrum Analytik und Mikrobiologie	Austria
• AGES Competence Centre of Elements	Austria
• Institut Scientifique de Sante Publique	Belgium
• Central Laboratory of Veterinary Control and Ecology	Bulgaria
• State General Laboratory	Cyprus
• SVI Olomuc	Czech Republic
• National Food Institute	Denmark
• Agricultural Research Centre	Estonia
• Veterinary and Food Laboratory	Estonia
• Evira	Finland
• Service Commun des Laboratoires	France
• AFSSA	France
• Federal Office of Consumer Protection and Food Safety	Germany
• General Chemical State Laboratory	Greece
• Central Agricultural Office, Food and Feed Safety Directorate	Hungary
• Public Analyst's Laboratory	Ireland
• IZSTO	Italy
• National Diagnostic Center	Latvia
• National Veterinary Laboratory	Lithuania
• Public Health Laboratory	Malta
• National Veterinary Research Institute	Poland
• National Institute of Public Health-National Institute of Hygiene	Poland
• IPIMAR	Portugal
• Hygiene and Veterinary Public Health Institute	Romania
• State Veterinary and Food Institute	Slovakia
• National Veterinary Institute	Slovenia
• Laboratorio Arbitral Agroalimentario	Spain
• Spain Food Safety Nutrition Agency	Spain
• National Veterinary Institute	Sweden
• National Food Administration	Sweden
• Dutch Food and Consumer Product Authority	The Netherlands
• RIKILT	The Netherlands
• Central Science Laboratory	UK

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Annexes

Annex 1: Invitation letter to laboratories

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for
heavy metals in feed and food

CRL
Heavy Metals
in Feed and Food

Geel, 22 April 2008
D04-IM/BCa/ive(2008)D/10368

«Title» «M_1st_name» «last_name»
«Institute»
«Department»
«Address»
«ZIP» «City»
«COUNTRY»

Inter-laboratory comparison for CRL Heavy Metals in Feed and Food

Dear Madam / Sir,

On behalf of the CRL Heavy Metals in Feed and Food, I would like to invite you to participate in the Proficiency Test [IMEP-104] for the determination of **total** Cd, Pb, As, Hg and methylmercury in seafood. Determination of the total concentration of As was not originally in the 2008 work programme of the CRL Heavy Metals in Feed and Food, for this reason we leave it up to you to provide results for the mentioned measurand.

I would like to remind you that – according to Regulation (EC) No 882/2004 - you have the duty as NRL to participate in PTs organised by the CRL if you hold a mandate for the type of matrix investigated.

Please register electronically for this inter-laboratory comparison using the following link: https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=99

Your participation is free of charge.

Once you have submitted your registration electronically, please follow the procedure indicated: a) print your registration form; b) sign it; and c) fax it to us. **Your fax is the confirmation of your participation.**

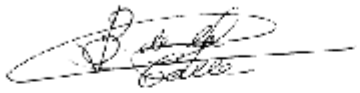
Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 299. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

The **deadline for registration is 30th April 2008**. Samples will be sent to participants during the first half of May. The deadline for submission of results is 15th June 2008.

I am the project leader for this inter-laboratory comparison. In case of questions/doubts, do not hesitate to contact me.

Yours sincerely

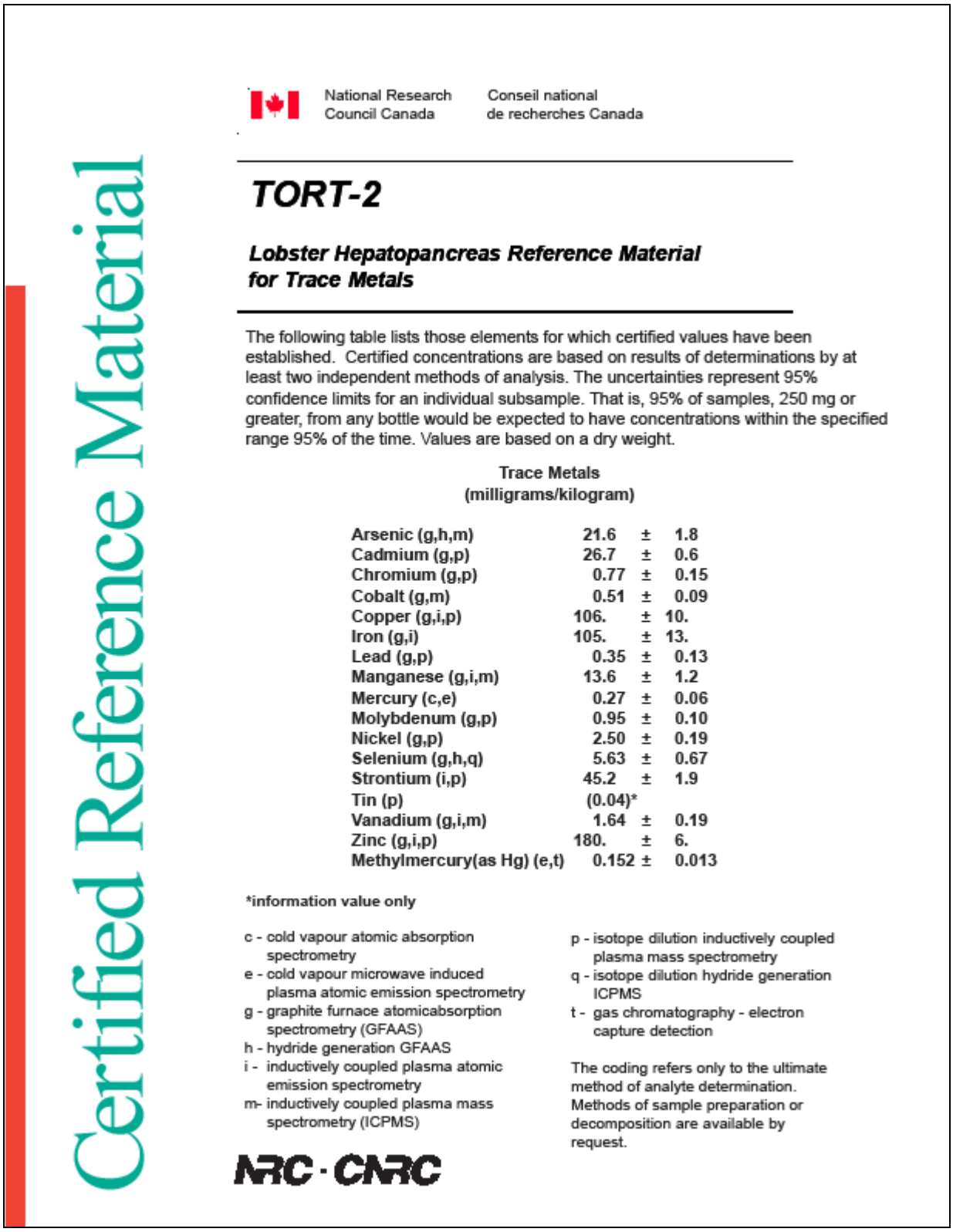


Dr. M.B. de la Calle
Deputy-Operating Manger CRL-HM

Cc: Philip Taylor

2

Annex 2: Certificate of the CRM used in IMEP-104



Certified Reference Material

National Research Council Canada | Conseil national de recherches Canada

TORT-2

Lobster Hepatopancreas Reference Material for Trace Metals

The following table lists those elements for which certified values have been established. Certified concentrations are based on results of determinations by at least two independent methods of analysis. The uncertainties represent 95% confidence limits for an individual subsample. That is, 95% of samples, 250 mg or greater, from any bottle would be expected to have concentrations within the specified range 95% of the time. Values are based on a dry weight.

Trace Metals
(milligrams/kilogram)

Arsenic (g,h,m)	21.6	±	1.8
Cadmium (g,p)	26.7	±	0.6
Chromium (g,p)	0.77	±	0.15
Cobalt (g,m)	0.51	±	0.09
Copper (g,i,p)	106.	±	10.
Iron (g,i)	105.	±	13.
Lead (g,p)	0.35	±	0.13
Manganese (g,i,m)	13.6	±	1.2
Mercury (c,e)	0.27	±	0.06
Molybdenum (g,p)	0.95	±	0.10
Nickel (g,p)	2.50	±	0.19
Selenium (g,h,q)	5.63	±	0.67
Strontium (i,p)	45.2	±	1.9
Tin (p)	(0.04)*		
Vanadium (g,i,m)	1.64	±	0.19
Zinc (g,i,p)	180.	±	6.
Methylmercury(as Hg) (e,t)	0.152	±	0.013

*information value only

c - cold vapour atomic absorption spectrometry
e - cold vapour microwave induced plasma atomic emission spectrometry
g - graphite furnace atomicabsorption spectrometry (GFAAS)
h - hydride generation GFAAS
i - inductively coupled plasma atomic emission spectrometry
m- inductively coupled plasma mass spectrometry (ICPMS)
p - isotope dilution inductively coupled plasma mass spectrometry
q - isotope dilution hydride generation ICPMS
t - gas chromatography - electron capture detection

The coding refers only to the ultimate method of analyte determination. Methods of sample preparation or decomposition are available by request.

NRC · CNRC

This reference material is primarily intended for use in the calibration of procedures and the development of methods used for the determination of trace metals in biological materials.

Storage

It is recommended that the reference material to be stored in a cool, clean location. The bottle should be well mixed by rotation and shaking prior to use, and tightly closed immediately thereafter.

Preparation of TORT-2

The starting material was freshly frozen "edible grade" lobster tomalley from Prince Edward Island. Prior to processing at the Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, the tomalley was stored at -30°C to inhibit protein/oil degradation. The tomalley was thawed, homogenized to produce a finely divided slurry and spray dried. Following extraction with acetone to remove the oil, the powder was vacuum dried. The material was then mixed in a rotary blender, screened through a 0.058" mesh nylon screen, reblended and bottled. After bottling, the samples were radiation sterilized at Nordion International, Laval, Quebec.

Instructions for drying

TORT-2 can be dried to constant weight by:

(1) drying at reduced pressure (e.g. 50 mm Hg) at room temperature in a vacuum desiccator over magnesium perchlorate for 24 hours.

(2) vacuum drying (about 0.5 mm Hg) at room temperature for 24 hours

Stability

Inorganic components certified in the predecessor to TORT-2 (TORT-1) have been periodically analyzed for more than seventeen years with no loss of integrity. Similar characterisitics are expected from TORT-2.

The methymercury content of TORT-2 is continually monitored by NRC. Studies indicate the methylmercury content in similar materials has been stable for twelve years. This is not expected to change, provided the material is stored in an appropriate manner.

Updates

It is possible that more data may become available and the established values may be updated and certified values assigned to more elements. These updates will be forwarded to all users of this reference material and posted on our website (http://inms-ienm.nrc-cnrc.gc.ca/calserv/chemical_metrology_e.html).

Canada

Acknowledgements

The majority of the analytical and certification work was performed at the Institute for National Measurement Standards, National Research Council of Canada. Several external expert laboratories cooperated in the certification exercises.

The following members of staff of the Institute for National Measurement Standards, National Research Council of Canada, participated in the analyses and certification: S.S. Berman, V.J. Boyko, V.P. Clancy, J. Lam, P.S. Maxwell, J. W. McLaren, B. Methven, K.W.M. Siu, S.N. Willie.

The cooperation of the following in the preparation and analysis of this material is gratefully acknowledged: C. Hotton, D.S. Singer. I.J. Britt and M.A. Tung, Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, Halifax, Nova Scotia.

D.C. Baxter, H. Emteborg and W. Frech, Department of Chemistry, University of Umeå, S-901 87 Umeå, Sweden.

C. Anderson, M. Deuth and B. Lasorsa, Marine Science Laboratories, Battelle Pacific Northwest, Sequim, Washington.

Date of issue: December 1994
Date of expiry: December 2011

The results listed in this certificate are traceable to the SI through gravimetrically prepared standards of established purity and international measurement intercomparisons. As such, they serve as suitable reference materials for laboratory quality assurance programs, as outlined in ISO/IEC 17025. This CRM is registered at the Bureau International des Poids et Mesures (BIPM) in Appendix C of the Comité International des Poids et Mesures database listing Calibration and Measurement Capabilities accepted by signatories to the Mutual Recognition Arrangement of the Metre Convention.

Comments, information and inquiries should be addressed to:

Dr. R.E. Sturgeon
National Research Council of Canada
Institute for National Measurement Standards
M-12, Montreal Road
Ottawa, Ontario, Canada K1A 0R6

Telephone (613) 993-2359
Facsimile (613) 993-2451
E-mail crm.inms@nrc-cnrc.gc.ca

Également disponible en français sur demande.

Annex 3: Accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for
heavy metals in feed and food

CRL
Heavy Metals
in Feed and Food

Geel, 6 May 2008
D04-IM(2008)BCa/ive/D/11294

«TITLE» «FIRSTNAME» «SURNAME»
«ORGANISATION»
«DEPARTMENT»
«ADDRESS»
«ADDRESS2»
«ADDRESS3»
«ADDRESS4»
«ZIP» «TOWN»
«COUNTRY»

Participation in IMEP-104, a proficiency test exercise for the determination of total Cd, Pb, As, Hg and methylmercury in seafood

Dear «TITLE» «SURNAME»,

Thank you for participating in the IMEP-104 intercomparison for the determination of **total** Cd, Pb, As, Hg and methylmercury in seafood. This exercise takes place in the frame of the CRL Heavy Metals in Feed and Food.

This parcel contains:
a) One glass bottle containing approximately 20 g of the test material
b) A "Confirmation of Receipt" form
c) This accompanying letter

Please check whether the bottle containing the test material remained undamaged during transport. Then fax (at +32-14-571865) or send the "Confirmation of receipt" form back. You should store the samples in a dark and cold place (4 °C) until analysis.

The measurands are: **total** Cd, Pb, As, Hg and methylmercury in seafood. The procedure used for the analyses should resemble as closely as possible the one that you use in routine sample analysis.

Please perform two or three independent measurements per measurand. Correct the measurement results for recovery and water content (following the procedure as described hereafter), and report the corrected values, plus their mean on the reporting website. The results should be reported in the same way (e.g., number of significant figures) as normally reported to the customer.

«PARTKEY»

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

The results are to be reported referring to dry mass and thus corrected for humidity. To calculate the water content in the test material, please apply the following procedure:

1. *Weigh accurately 1 g of test material in a glass container of 5-7 cm diameter, Preferably with a lid because when the prescribed drying time has passed, the glass container must cool down about 30 minutes in a desiccator before weighing.*
2. *Place it in an oven for 60 ± 5 min at 102 ± 2 °C.*
3. *Place the glass container covered with a lid in a desiccator and wait 30 min before weighing the test material again.*

Note 1: perform the measurements of the water content in triplicate.
Note 2: ***do not use for the heavy metal determinations the aliquots of test material that you have used for the water content determination!***

You can find the reporting website at www.irmm.jrc.be/imepapp/jsp/loginResult.jsp To access this webpage you need a personal password key, which is: «PARTKEY». The system will guide you through the reporting procedure. Please enter for each parameter the two or three measurement results plus the technique you used, but do not report the uncertainty for each individual measurement. In addition, please report the mean of the results with technique and with uncertainty information in the allocated space for "measurement 4". After entering all results, please also complete the relating questionnaire. **Do not forget to submit and confirm always when required**.

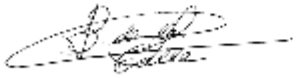
Directly after submitting your results and the questionnaire information online, you will be prompted to print the completed report form. Please do so, **sign the paper version and return it to IRMM by fax (at +32-14-571-865) or by e-mail**. Check your results carefully for any errors before submission, since this is your definitive confirmation.

The deadline for submission of results is 15/06/2008.

Please keep in mind that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to customers, accreditation bodies and analysts alike.

Your participation in this project is greatly appreciated. If you have any remaining questions, please contact me by e-mail:
JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu

With kind regards



Dr. M.B. de la Calle
IMEP-104 Co-ordinator

Enclosures: 1) one glass bottle containing the test material; 2) confirmation of receipt form; 3) accompanying letter.

Cc: P. Taylor

Annex 4: Acknowledgement of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Community reference laboratory for
heavy metals in feed and food

CRL
Heavy Metals
in Feed and Food

Annex to D04-IM(2008)BCa/ive/D/11294

«TITLE» «FIRSTNAME» «SURNAME»
«ORGANISATION»
«DEPARTMENT»
«ADDRESS»
«ADDRESS2»
«ADDRESS3»
«ZIP» «TOWN»
«COUNTRY»

CRL-HM-04 / IMEP-104

total Cd, Pb, As, Hg and methylmercury in seafood

Confirmation of receipt of the samples

Please return this form at your earliest convenience.
This confirms that the sample package arrived.
In case the package is damaged,
please state this on the form and contact us immediately.

ANY REMARKS .....
.....

Date of package arrival .....

Signature .....

Please return this form to:
Dr Beatriz de la Calle

IMEP-104 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

Fax : +32-14-571865
e-mail : JRC-IRMM-CRL-HEAVY-METALS@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 252. Fax: (32-14) 571 865.

E-mail: jrc-irmm-crl-heavy-metals@ec.europa.eu

IMEP

Annex 5: Questionnaire

Interlaboratory comparison system

This e-service is meant for registration in interlaboratory comparisons by JRC-IRMM and for reporting of results to the organiser.
If you agree to the terms of using this e-service, you can proceed to register your participation in the selected interlaboratory comparison below.

Questionnaire input for IMEP-104

Please complete this questionnaire

Questionnaire form

1. **Did you apply a recovery factor to correct your measurement results?**

- ○ no
- ○ yes

1.1. If Yes, what are the recovery factors (R, in %) you used:

1.1.1. for Cd (in %)

1.1.2. for Pb (in %)

1.1.3. for As (in %)

1.1.4. for Hg (in %)

1.1.5. for MeHg (in %)

1.2. If Yes, did you determine R by:

- ☐ 1. adding a known amount of the same analyte to the sample
- ☐ 2. using a certified reference material
- ☐ 3. other

1.2.1. if other, specify

2. **What is the level of confidence reflected by the coverage (k) factors stated above? (in %)**

3. **What is the basis of your uncertainty estimate (multiple answers are possible)**

- ☐ 1. uncertainty budget calculated according to iso-gum
- ☐ 2. known uncertainty of the standard method
- ☐ 3. uncertainty of the method as determined in-house validation
- ☐ 4. measurement of replicates (i.e. precision)
- ☐ 5. expert guestimate
- ☐ 6. use of intercomparison data
- ☐ 7. other

3.1. If other, please specify

4. **Do you usually provide an uncertainty statement to your custumers for this type of analysis?**

- ○ no
- ○ yes

5. **Did you correct for the water content of the sample?**

- ○ no
- ○ yes

5.1. If Yes, what is the water content (in % of the sample mass)?

5.2. If No, what was the reason not to do this?

6. **Did you analyse the sample according to an official method?**

- ○ no
- ○ yes

6.1. If no, please describe (in max 150 characters for each reply) your:

6.1.1. sample pre-treatment

6.1.2. digestion step

6.1.3. extraction / separation step

6.1.4. derivatization (for MeHg)

6.1.5. instrument calibration step

6.2. If yes, which:

7. **Does your laboratory carry out this type of analysis (as regards the measurands, matrix and methods) on a routine basis?**

- ○ no
- ○ yes

7.1. If Yes, please estimate the number of samples (Cd, Hg, Pb measurements together):

- ○ a) 0-50 samples per year
- ○ b) 50-250 samples per year
- ○ c) 250- 1000 samples per year
- ○ d) more than 1000 samples per year

8. **Does your laboratory carry out MeHg analysis on a routine basis?**

- ○ no
- ○ yes

8.1. If Yes, please estimate the number of samples:

- ○ a) 0-50 samples per year
- ○ b) 50-250 samples per year
- ○ c) 250-1000 samples per year
- ○ d) more than 1000 samples per year

9. **Does your laboratory have a quality system in place?**

- ○ no
- ○ yes

9.1. If Yes, which:

- ☐ iso 9000 series
- ☐ iso/iec 17025
- ☐ other

9.1.1. If other, please specify

10. **Is your laboratory accredited for this type of analysis?**

Questions/Response Table	**no**	**yes**	*info*

MeHg *	○	○
total As *	○	○
total Hg *	○	○
total Pb *	○	○
total Cd *	○	○

11. **Does your laboratory take part in an interlaboratory comparison for this type of analysis on a regular basis?**

Questions/Response Table	**no**	**yes**	*info*
MeHg *	○	○	
total As *	○	○	
total Hg *	○	○	
total Pb *	○	○	
total Cd *	○	○	

12. **If you answered yes in question 11, which one:**

13. **Does your laboratory use a reference material for this type of analysis?**

○ no
○ yes

13.1. If YES, is the material used for the validation of procedures?

○ no
○ yes

13.2. If YES, is the material used for calibration of instruments?

○ no
○ yes

13.3. If YES, which one(s):

14. **Do you have any comments? Please let us know: ...**

Change results | Clear questionnaire | Save questionnaire | Submit questionnaire

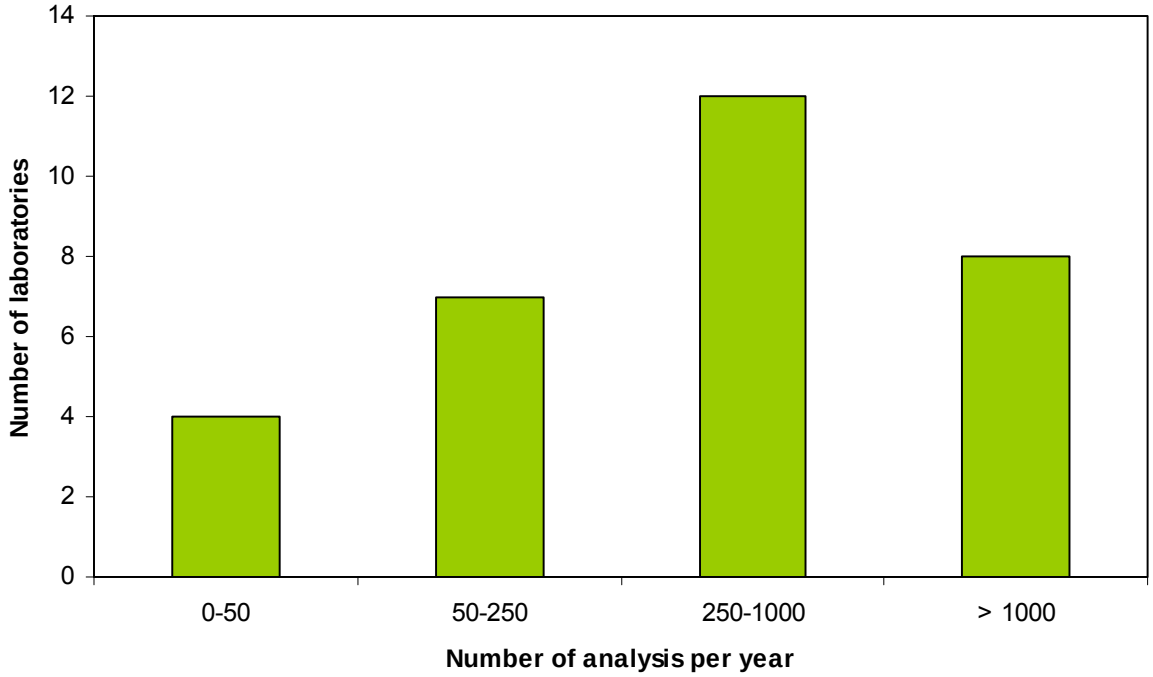
Annex 6: Experimental details.

Lab code	SOP?	Which SOP?	Sample pre-treatment	Digestion step	Extraction/ separation	Derivatis. for MeHg	Instrument calibration step
L01	No	Pb, Cd-AOAC 999.11/ Hg-AOAC 971,21	Weighing 0,5 g	With HNO_3	No		5 levels for ICP/AES except Hg (DMA55)
L02	Yes						
L03	No	This method is a prEN at the moment		HNO_3 and H_2O_2 in a microwave oven			ICP-MS is tuned. Calibration is done by external calibration measurements
L04	No			Nitric acid-microwave digestion			External standard-quality check with certified reference material
L05	No		High pressure microwave digestion	nitric acid and perhydrol	No		0,1, 0,5, 1,0, 2,0 ,5,0, 10,0, 20,0
L06	No			Samples of 0,3 to 1 g (wet mass) were weighed into closed quartz digestion vessels and wet-oxidized with 3 mL nitric acid 65%			External calibration in ICP-MS detection: The following m/z 75As. 111Cd, 114Cd,220Hg, 206Pb, 207Pb, 208Pb were measured in the standard mode
L07	No		Add 2 ml 65% HNO_3	microwave	Dilution		4 steps
L08	Yes						
L09	No		None	Acid digestion - nitric acid and hydrogen peroxide	None	HCl	Calibration standards
L10	Yes	ASU L 00.00-19/3; ASU L 00.00-19/4					
L11	Yes	EN 14084 to Pb and Cd; US EPA 7473 to Hg and MeHg					
L12	No		None	Digestion with H_2O_2 (30%) and HNO_3 conc. by microwave high pressure (for Cd, As)	None		Add. method; std solution Cd: 2 ppb; As: 20 ppb. Hg:square calibration with std. solution 25-50-100-150-300 ppb 1-2-5 ppm

Lab code	SOP?	Which SOP?	Sample pre-treatment	Digestion step	Extraction/ separation	Derivatis. for MeHg	Instrument calibration step
L13	No	Standards	None	Microwave	No	Yes	Yes
L14	No		Dry in oven	Ashing in mufle furnace at 450ºC, dissolved in 1 n HCL, Hg - AMA 254			Calibration curve for Pb (10-60 µg/l), Cd (1-10 µg/l), Hg (0.05-5 µg/l), As (3-25µg/l)
L15	No		To 1 g sample a mixture of HNO_3, $HClO_4$ and H_2SO_4 is added (Pb and Cd). For As and Hg only HNO_3 and $HClO_4$	Wet digestion in open vessels			External calibration, no standard addition
L16	Yes						
L17	No		Dry ashing	Dissolve the ash in diluted hydrochloric acid	Complexes of Pb and Cd with DDC are extracted into MIBK		Matrix matched calibration curve
L18	Yes	SR EN 13806:2003; SR EN 13805:2003; SR EN 14083:2003					
L19	Yes	EN 14084 (Digestion, GF-AAS), EN 13806 (CV-AAS), EN 14627 (HG-AAS)					
L20	Yes	AOAC					
L21	Yes	EN 14083:2004, EN 14546:2005					
L22							

Lab code	SOP?	Which SOP?	Sample pre-treatment	Digestion step	Extraction/ separation	Derivatis. for MeHg	Instrument calibration step
L23	No		None	0.5g sample microwave digested with 5ml HNO_3 at 200 Celcius for 15 min			
L24	Yes				None	Not applicable	Calibration of the monochromator
L25							
L26	No		None	Ashing for Pb, Cd, As Hg=Wet digestion	None	Not analysed	
L27	No		None	Microwave digestion - Aliquots (0.5 g) of test sample plus certified reference materials were digested in nitric acid using quartz high pressure closed vessels	None	Not analysed	External calibration + internal standards
L28	No		Dry ashing (arsenic, lead and cadmium) wet digestion (mercury)				Direct calibration
L29	No						
L30	Yes						
L31	No		Microwave digestion HNO_3	HNO_3 30 min at 180°C			Standard solutions
L32	Yes						4 point calibration with a similar matrix
L33	Yes		Homogenised by stirring	Microwave - HNO_3 65% - ramp to temperature 200C			First a standard curve proving linearity; samples are measured using standard addition
L34	Yes						

Annex 7: Number of samples analysed per year for total Cd, Pb, As and Hg.



European Commission

EUR 23505 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: Report of the fourth interlaboratory comparison organised by the Community Reference Laboratory Heavy Metals in Feed and Food: Total Cd, Pb, As, Hg and methylmercury in seafood.
Author(s): M.B. de la Calle, D. Vendelbo, I. Verbist, A. Bernreuther, H. Emteborg, P. Taylor
Luxembourg: Office for Official Publications of the European Communities
2008 – 32 pp. – 21 x 29.7 cm
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ISBN 978-92-79-09794-2
DOI 10.2787/70551

Abstract
The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the Community Reference Laboratory for Heavy Metals in Feed and Food (CRL-HM). One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs). This report presents the results of the fourth ILC of the CRL-HM which focused on the determination of total Cd, Pb, As, Hg and methylmercury (related to dry mass) in seafood.

The test material used in this exercise was the Certified Reference Material (CRM) TORT-2, lobster hepatopancreas, of the National Research Council of Canada. The material was rebottled and relabelled to prevent recognition by the participants and it was dispatched on the first half of May 2008. Each participant received one bottle containing approximately 20 g of test material. Thirty-four participants from twenty-six countries registered to the exercise of which 33 sets of results were reported for Cd and total mercury, 32 for Pb, 27 for arsenic and 4 for methylmercury. One laboratory reported two sets of values for Cd, Pb, Hg and As obtained with two different techniques, respectively. The Assigned values were the certified values taken from the TORT-2 certificate.

The uncertainties of the respective assigned values, u_{ref}, were taken directly from the CRM certificate as provided by the producer. Participants were invited to report the uncertainty on their measurements. This was done by all the laboratories taking part in this exercise.

Laboratory results were rated with z and zeta scores in accordance with ISO 13528. Standard deviation for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation and were 9.76 % for total Cd, 18. 74 % for total Pb, 10 % for total As, 19.48 % for total Hg and 21.24 % for methylmercury.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

JRC
EUROPEAN COMMISSION

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