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IMEP-115: Determination of Methylmercury in Seafood

A Collaborative Trial Report

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IMEP-115: Determination of Methylmercury in Seafood

Collaborative Trial Report

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Contents

1 Executive Summary

A collaborative trial study, IMEP-115, was conducted in accordance with international protocols to determine the performance characteristics of an analytical method for the determination of methylmercury in seafood.

The present exercise was organised in support to the Commission Regulations 1881/2006 and 882/2004. The method is based on a double liquid-liquid extraction, first with an organic solvent and then with a cysteine solution. The final quantification is done with a direct mercury analyzer.

Fifteen laboratories, from ten European countries, registered for participation. All were experienced in the direct determination of mercury.

Five test items, covering a reasonable concentration range, were selected. The five test items were certified reference materials: dogfish liver (NRCC, DOLT-4), lobster hepatopancreas (NRCC, TORT-2), organics in freeze-dried mussel tissue (NIST, SRM 2974a), oyster tissue (NIST, SRM 1566b) and tuna fish (EC-JRC-IRMM, ERM CE464). BCR (also IRMM) 463, tuna fish, was used as pre-test item for training purposes.

The repeatability relative standard deviation (RSD_r) ranged from 3.9 to 12.3 % while the reproducibility relative standard deviation (RSD_R) ranged from 8.4 to 24.8 %.

The method demonstrates to have acceptable precision for all test materials, thus it fit for its intended analytical purpose.

2 Introduction

Mercury is an environmental contaminant present in fish and seafood mostly in the form of methylmercury. According to the Scientific Opinion of the European Food Safety Agency (EFSA) published in 2003 [1], the major source of methylmercury intake in humans is fish and seafood products. Specifically, large predatory fish which are at the top of the food chain, such as swordfish and tuna, contain high levels of methylmercury and are significant sources of human exposure to that contaminant. Microorganisms are able to convert inorganic mercury to organic forms whereby the mercury in its methylated form more easily can enter the food-chain. Bacteria that methylate mercury have been isolated from the mucous material on the surface of fish but such bacteria are mainly present in sediments, especially in fresh water systems and estuaries. 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, but in industrially contaminated waters, methymercury levels in fish muscle may exceed 10 mg kg^{-1} [2]. The significant bioaccumulation of methylmercury in seafood has resulted in a serious food safety problem. Methylmercury can accumulate 100-fold in fish muscle, which can lead to dangerously elevated levels of mercury in seafood even in regions with typical aquatic mercury levels [3].

Methylmercury is highly toxic particularly to the nervous system, and the developing brain (foetus) is thought to be the most sensitive target organ [1]. The exposure of young children to methylmercury is an intermediate case between foetus and adults because their nervous systems are still developing and thus are more sensitive to these compounds [4].

In 2003 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg kg^{-1} body weight. The National Research Council of the United States of America established an intake limit of 0.7 µg kg^{-1} body weight per week. In Europe the estimated intakes of methylmercury vary from country to country depending on the amount and type of fish consumed [1]. So far no maximum limit has been introduced in the European legislation for contaminants although the European Commission recommends pregnant women, breast feeding women and children to limit their consumption of big predatory fish.

From an analytical point of view, methylmercury determination is frequently performed by coupling gas chromatography (GC) [5] or high performance liquid chromatography (HPLC) [6] to different detectors such as electron impact-mass spectrometry (EI-MS) [7] inductively coupled plasma-mass spectrometry (ICP-MS) [8], microwave induced plasma-atomic emission spectrometry (MIP-AES) [9], cold vapour-atomic absorption spectrometry (CV-AAS) [10] and cold vapour-atomic fluorescence spectrometry (CV-AFS) [5]. When gas chromatography is used for the separation of the species, derivatisation of methylmercury is needed to convert them into volatile species. Grignard reagents [9], sodium tetraethylborate and sodium tetraphenylborate [11] are frequently used as

derivatisating agents [12]. Papers summarising and discussing the different analytical approaches used to determine methylmercury have been published [12, 13]. So far, three analytical methods have been standardised for the determination of methylmercury in seafood, all of them based on the use of gas or liquid chromatography [14].

Mandated by the General Directorate for Health and Consumers (DG SANCO) the European Committee for Standardisation (CEN) is in the process of validating a method for the determination of methylmercury in seafood based on the use of GC-ICP-MS. DG SANCO also requested the European Union Reference Laboratory for Heavy Metals in Feed and Food (EURL-HM) to validate a method which could be used by laboratories which do not run many methylmercury analyses per year and which do not have sophisticated hyphenated techniques at their disposal.

The EURL-HM selected for validation an existing method based on a double liquid-liquid extraction, first with an organic solvent and then with a cysteine solution and final quantification with a direct mercury analyzer (DMA) [15] which had been successfully used by the *Instituto Português do Mar e da Atmosfera* (IPMA) [16], the Portuguese National Reference Laboratory for the analysis of heavy metals in fish, and which is run under the scope of accreditation of the *Laboratori Agència Salut Pública de Barcelona*. It should be emphasized that the method applied here is an operationally defined method where the response for the resulting inorganic mercury in the detection system can be correlated to the certified concentrations of methylmercury in these reference materials. The selective extraction of methylmercury using cysteine must be able to discriminate between inorganic mercury and methylmercury. The analyte is therefore only measured indirectly.

This report summarises the outcome of the collaborative trial, IMEP-115, run by the EURL-HM to validate the above mentioned method.

3 IMEP support to EU policy

The International Measurement Evaluation Programme (IMEP®) is operated by the Joint Research Centre - *Institute for Reference Materials and Measurements*. IMEP provides support to the European measurement infrastructure in the following ways:

IMEP **disseminates metrology** from the highest level down to the field laboratories. These laboratories can benchmark their measurement result against the IMEP certified reference value. This value is established according to metrological best practice.

IMEP helps laboratories to assess their estimate of **measurement uncertainty**. The participants are invited to report the uncertainty on their measurement results. IMEP integrates the estimate into the scoring, and provides assistance for the interpretation.

IMEP **supports EU policies** by organising interlaboratory comparisons in the frame of specific EU Directives, or on request of a specific Directorate-General. IMEP-115 was carried out in the frame of the mandate of the European Union Reference Laboratory for

Heavy Metals in Feed and Food (EURL-HM) as requested by Directorate-General for Health and Consumers (DG SANCO) and in support to the Commission Regulation 1881/2006 [17].

4 Scope and aim

The scope of this collaborative trial (CT) was to establish the performance characteristics of a method to be used in the determination of methylmercury in seafood. The statistical scrutiny of the results was done following ISO 5725-2:1994 [18]. Furthermore, the administrative and logistic procedures of IMEP® were respected. IMEP is accredited accordingly to ISO 17043:2010 [19].

5 Invitation, registration and distribution

The exercise was announced via the IMEP web page on the 6th of June 2012 (Annex 1). Additionally, the exercise was announced to National Reference Laboratories (NRLs) which belong to the EURL-HM network, having experience in the direct determination of mercury. This announcement was made on the 30th April 2012 (Annex 2).

Registration was opened till the 15th June 2012. Fifteen participants from ten different EU Member States registered to the exercise. Test items were dispatched on the 4th of July 2012. Each participant received one package containing:

a) A "Sample accompanying letter" (Annex 3),

b) 10 bottles containing, each approximately 2.5 g (sample 3) or 5 g (samples 1, 2, 4 and 5) of the test items (two bottles from each test material),

c) A copy of the standard operational procedure which must be followed strictly,

d) A bottle of a pre-test item (containing 15 g of material),

e) A "Confirmation of Receipt" form (Annex 4),

The pre-test item, dispatched to registered participants, resembled most of the other test items. It allowed participants to become familiar with the method under investigation before starting the collaborative trial. Results for the pre-test item were reported directly to the project coordinator by e-mail. The accompanying letter clearly mentioned that the standard operating procedure was to be strictly followed.

The sample accompanying letter described the measurand, the number of independent replicates required per bottle and provided, for each test item, detailed instructions for the moisture determination and how to report results.

The deadline for reporting results was the 10th September 2012. Dispatch was followed by the messenger's parcel tracking system on the internet. Participants received an individual code to access the online reporting interface, to report their measurement results and to complete the related questionnaire. The questionnaire was used to extract all relevant information related to measurements and laboratories (Annex 5).

5.1 Procedure to apply

The standard operating procedure (SOP) was provided by the *Laboratory of Public Health Agency of Barcelona* (LPHA, Barcelona, Spain), based on a protocol used by the *Instituto Português do Mar e da Atmosfera* (IPMA, Lisbon, Portugal).

6 Test material

6.1 Preparation

The supplied units of each CRM were opened, pooled into a 5 l acid-washed plastic drum and placed in a 3D-mixer for 30 minutes (Dynamix CM200, WAB, Basel, CH) for careful mixing and re-homogenisation. For one CRM a handful of Teflon balls were added during mixing to break up agglomerates since the material was found to be severely clogged upon delivery (TORT-2). The five selected certified reference materials (CRMs), Table 1, were then refilled in vials using a vibrating feeder and a balance in a HEPA-filter clean-cell and labelled to avoid easy identification by the participants. Vials containing 5 g (materials 1, 2, 4 and 5) or 2.5 g (material 3) were then dispatched to the participants. Care was taken to avoid cross-contamination between the CRMs which would jeopardize the whole study. Two CRM-powders were therefore never handled at the same time. Each CRM was mixed and filled and the subsequent material was only filled after thorough cleaning of the equipment.

6.2 Homogeneity and stability study

The selected CRMs used in the present collaborative trial were adequately homogeneous and stable for the purpose of the exercise, as stated in their respective certificates. Therefore, no additional homogeneity and stability studies were carried out for the materials used.

7 Reference values and their uncertainties

The certified values and their corresponding expanded uncertainties, derived from the respective CRM certificate, are presented in Table 1. They were used as reference values

for the present collaborative trial. All the calculations were done expressing the methylmercury mass fractions as Hg (in mg kg^{-1}). The method trueness (expressed as an analytical recovery) for each test material and at the concentration level given by the certificate, was estimated.

Table 1 – Certified ranges (X_{ref} ± U_{ref}, k = 2, all values of methylmercury expressed as Hg, in mg kg^{-1})

Sample	X_{ref}	U_{ref} (k = 2)	Reference
1 (DOLT-4)	1.33	0.12	[20]
2 (TORT-2)	0.152	0.013	[21]
3 (SRM 2974a)	0.0691	0.0008	[22]
4 (SRM 1566b)	0.0132	0.0007	[23]
5 (ERM CE464)	5.12	0.34	[24]

8 Results and evaluation

Pre-test item

The pre-test item used during the feasibility study consisted on the BCR® 463 reference material [25] which allowed participants to become familiar with the SOP. Results from this pre-test item were reported to the collaborative trial organiser. Laboratories having reported results in agreement with the certified value (2.83 ± 0.32, k = 2, in mg kg^{-1}) were requested to analyse the test items used in the collaborative trial. Laboratories having reported significantly biased results were requested to initiate a route cause analysis and take proper corrective actions to prevent further biases.

Collaborative trial

Results were received from 12 of the 15 registered laboratories. Three independent measurements per bottle were requested to be reported (measured under repeatability conditions). This process should be repeated, on two different days (one bottle/day) following the SOP.

Annexes 6 to 10 present all the reported results and the corresponding Kernel density plots for each sample. The Kernel density plots were obtained using software provided by the Statistical Subcommittee of the Analytical Methods Committee of the UK Royal Society of Chemistry [26, 27].

8.1 Statistical analysis for method performance assessment

Statistical evaluation of the data was performed following international standard recommendations (ISO 5725-2:1994 [18]).
Laboratory L02 reported not having used L-cysteine standard solution on the calibration standards. The decision was taken after careful check that no significant matrix effects were observed while measuring a certified reference material (DORT-2). However, L-cysteine standard solution was used for all samples, hence the Advisory Board of the CT decided not to exclude the results from this participant for the statistical scrutiny of the proposed method.

The following tests were performed:

i) Analysis of variance, ANOVA, to confirm that no statistically significant difference existed, for any of the test items, between the two individual bottles provided to the participants, analysed on different days. Since this was the case, all six replicated measurements were pooled for further calculations;

ii) Check for outliers in the laboratory precision (variance) applying the Cochran test. This test compares the highest laboratory internal repeatability variance with the sum of reported variances from all the participants;

iii) Check for laboratory outliers within the series of independent replicates applying the Grubbs-internal test (repeatability). This test is of particular relevance for laboratories being flagged as stragglers by the Cochran test;

iv) Check for outliers in the laboratory mean applying the Grubbs test. This test checks for laboratory means deviating significantly from the total mean calculated from data reported from all participants.

Method performance characteristics related to the method (or laboratory) precision were estimated after the identification and elimination (if applicable) of outlier results. Accordingly to ISO 5725-2 Ch. 7.2.5, erroneous data should be investigated and discarded. Reported results from laboratory L12 were identified as outliers for test samples 1 and 2 and reported a "lower than" for test samples 3 and 4. As too many abnormal test results were reported from the same laboratory, it was considered reasonable to discard all the results reported by this laboratory because enough evidence was available to demonstrate that this laboratory did not have the method under control [18].

Table 2 provides an overview of the outlier identification for all test samples. Laboratories having reported their within-laboratory variability significantly larger than that of the remaining laboratories were identified using the Cochran test (L12 for samples 1 and 2, L06 for samples 3, 4 and 5). Laboratories for which their calculated mean (of its

corresponding six replicates) was identified as an outlier by the Grubbs test (with 99 % confidence level) were also identified.
Other cases are as illustrated in Table 2.

Table 2 – Statistical data evaluation (scrutinizing for outlier identification)

Sample	Laboratory	N° Outliers (replicates)	Outlier type
1 (DOLT-4)	L12 **	6	Cochran and Grubbs
2 (TORT-2)	L12 **	6	Cochran and Grubbs
	L06 **	1	Grubbs internal (lowest)
3 (SRM 2974a)	L11 **	1	Grubbs internal (lowest)
	L12 [a]	6	
	L06 **	6	Cochran
4 (SRM 1566b)	L04 **	6	Cochran
	L06 **	6	Cochran
	L12 [a]	6	
5 (ERM CE464)	L06 **	6	Cochran and Grubbs

** The test statistics is greater than its 1 % critical value and the laboratory (or the single replicate value) is considered as an outlier.

Grubbs internal outlier refers to a single replicate being statistically significantly different from the other replicates within the same laboratory.

[a] L12 reported "lower than" values

All the remaining measurements were used to evaluate the performance characteristics of the method under investigation, related to trueness and precision. Table 3 provides:

- the number of laboratories used to assess the performance characteristics of the method (after outlier exclusion),
- the number of outlier laboratories and replicates,
- the certified values and their associated expanded uncertainties (X_{ref}, U_{ref}),
- the overall observed mean (after the outlier rejection, X_{obs}) and their respective expanded uncertainty, expressed as the reproducibility standard deviation (S_R) multiplied by a coverage factor of 2, which approximates to a 95 % confidence interval),
- the repeatability standard deviation (S_r) the repeatability limit *r (computed as 2.8 S_r)* and the repeatability relative standard deviation, or within-laboratory variability, RSD_r),
- the reproducibility standard deviation (S_R), the reproducibility limit *R* (computed as 2.8 S_R) and the RSD_R,
- the Horwitz ratio expressed as the ratio between the observed RSD_R value divided by the RSD_R value calculated from the Horwitz equation [28],

- the E_N number [19] computed as follows:

$$E_N = \frac{X_{obs} - X_{ref}}{\sqrt{\left(2S_R^2 + U_{ref}^2\right)}} \qquad \text{Eq. 1}$$

The analytical method should be considered as unbiased if $E_N < 1$.
Where X_{obs}, X_{ref} , S_R and U_{ref} were defined as before.

- the overall analytical recovery R, calculated as follows:

$$R = \frac{X_{obs}}{X_{ref}} 100 \qquad \text{Eq. 2}$$

And its associated uncertainty (u_R) was estimated as [29]:

$$u_R = R \cdot \sqrt{\left(\frac{u_{obs}}{X_{obs}}\right)^2 + \left(\frac{u_{ref}}{X_{ref}}\right)^2} \qquad \text{Eq. 3}$$

Where: R is the analytical recovery (Eq. 2),

u_{obs} is the estimated standard deviation under reproducibility conditions (S_R),

u_{ref} is the standard uncertainty of the certified value,

X_{obs} and X_{ref} have the same meaning as in Eq. 1.

Considering the estimated uncertainty of the analytical recovery as a confidence interval ($2u_R$, which corresponds to approximately 95 % confidence level) no statistically significant difference could be identified between the overall observed mean and the certified values for each respective test sample. It could be concluded, no significant bias could be identified for any of the test samples, i.e. $R \pm 2u_R$ covers the value 100 %.

Moreover, a significance test (t_{cal}) can be performed to test whether the analytical recovery differs significantly from unity (from 100 % if represented as a percentage). This test should be performed according to equation 4 [29]:

$$t_{cal} = |R\text{-}1| \,/\, u_R \qquad \text{Eq. 4}$$

Where: t_{crit} is the critical t-value for a confidence level of 95 % and for n-1 degrees of freedom (with n referring to the number of all measurements used to estimate R).

The following conditions apply:

- if $t_{cal} > t_{crit}$ R differs significantly from unity, i.e., the method is biased
- if $t_{cal} \leq t_{crit}$ R does not differ significantly from unity, the method is not biased.

Taking the worst case (the test sample for which the analytical recovery showed the lowest associated uncertainty, i.e. for test sample 5) R was estimated as 87.3 % with an associated standard uncertainty u_R of 7.9 % (n = 60). Applying eq. 4 a t_{cal} of 1.61 was computed. The t_{crit} is obtained from statistical tables and it is 2.00.

Thus, regarding trueness, it can be concluded that the method does not show any evidence of any significant bias ($E_N < 1$ for all samples).

Regarding precision, the method should be considered fit for its intended purpose since the Horrat ratio is less than 2.

Table 3 – Method performance characteristics from the collaborative study (following ISO 5725-2:1994 [18])

	Units	Sample 1 (DOLT-4)	Sample 2 (TORT-2)	Sample 3 (SRM 2974a)	Sample 4 (SRM 1566b)	Sample 5 (ERM CE464)
N° Laboratories (after outlier rejection)		**11**	**11**	**10**	**9**	**10**
N° Outlier Lab (test used)		**1 (C and G)**	**1 (C and G)**	**1 (C)**	**2 (C)**	**1 (C)**
N° Replicates excluded		**6**	**6 + 1 (GI)**	**6 + 1 (GI)**	**6 + 6**	**6**
Reference value $X_{ref} \pm U_{ref}$ (k = 2)	**mg kg^{-1}**	**1.33 ± 0.12**	**0.152 ± 0.013**	**0.069 ± 0.0008**	**0.0132 ± 0.0007**	**5.12 ± 0.34**
Overall mean $X_{obs} \pm 2S_R$	**mg kg^{-1}**	**1.13 ± 0.40**	**0.147 ± 0.030**	**0.071 ± 0.016**	**0.019 ± 0.010**	**4.47 ± 0.76**
S_r	**mg kg^{-1}**	**0.06**	**0.009**	**0.005**	**0.002**	**0.18**
r	**mg kg^{-1}**	**0.16**	**0.03**	**0.014**	**0.007**	**0.49**
RSD_r	**%**	**5.2**	**6.1**	**5.1**	**12.3**	**3.9**
S_R	**mg kg^{-1}**	**0.21**	**0.015**	**0.008**	**0.005**	**0.38**
R	**mg kg^{-1}**	**0.57**	**0.04**	**0.023**	**0.013**	**1.05**
RSD_R	**%**	**18.2**	**10.0**	**11.5**	**24.8**	**8.4**
Hor_{Rat}		**1.16**	**0.47**	**0.48**	**0.85**	**0.66**
E_N		**-0.5**	**-0.2**	**0.1**	**0.6**	**-0.8**
Recovery **$R \pm 2u_R$ (~ 95 %)**	**%**	**84.6 ± 31.8**	**96.4 ± 21.0**	**103.3 ± 23.6**	**143.1 ± 71.4**	**87.3 ± 15.8**

C = Cochran test, G = Grubbs test (applied to laboratory means), GI = Grubbs internal test (applied to replicates within a laboratory).

8.2 Further information extracted from the questionnaire

In addition to the submission of results, participants were asked to answer a number of questions related to:

i) The standard operational procedure

ii) How the participants ensure the quality of their reported results

These questions were considered the most relevant, having in mind the SOP distributed, to identify any potential source of variability among the reported results. Issues that may be relevant to the outcome of the collaborative trial exercise are discussed below.

Instead of investigating the effect of each answer to the questionnaire in a so called single-variable effect, a multivariate approach was carried out by establishing a multivariate linear relationship between the methylmercury reported value (for each test sample) and the set of responses gathered from the questionnaire, once transformed into numerical variables (valuing 1 if answered positively and 0 if answered negatively). Partial least square regression models (PLS-R) were used. The statistical data treatment was performed using The Unscrambler X 10.1 (CAMO Software AS, Norway).

The multivariate approach provides a graphical interpretation of the results obtained by interlaboratory comparison exercises and/or collaborative trials, allowing the differentiation of laboratories according to their different procedures.

One PLS-R model was constructed for each test sample. Due to relatively low number of reported values (max. 12 for each sample) all models were cross-validated recurring to a randomly selected set of segments. All models succeed to "explain" the majority of the total covariance relating reported value (for each sample) and the set of answers to the questionnaire (ranging from 95 to 99 % when using the first three principal components of the PLS-R model). Furthermore, all models appear to have a model error similar or lower than the overall observed variability of all the reported values for each sample (expressed as one standard deviation). The above two statements prove the validity of the models, ensuring the validity of the interpretations made.

From all the five independent PLS-R models some questions (related to quality assurance or experimental parameters) could be identified as the most influencing for each respective model, ultimately explaining the reasons for their multivariate relationship with the respective reported values. Among these the following criteria could be identified as positively correlated with the reported values (hence, the majority of the laboratories which have answered positively to these queries reported, in general, a more accurate value:

i) Use of a CRM for validation or instrument calibration which best possible match with the material under investigation (e.g. the use of TORT-2 (lobster hepatopancreas) or DORM-3 (an agglomerate of fish protein) for instrument calibration was responsible for lower reported values for sample 1, DOLT-4 (dogfish liver)),

ii) Analysis of a relatively high number of test samples per year (regularly), i.e. having more experience on the direct determination of mercury (> 50 / year),

iii) Use of longer drying and decomposition times,

Similarly, the following answers to the questions enable to identify the following issues related with the SOP under scrutiny which negatively correlated with reported values (in other words laboratories which answered positively to those questions have, in general, reported lower (less accurate) values:

i) Use of a CRM for validation/instrument calibration which does not match the test material under investigation,

ii) Analysis of relatively few samples per year (20 – 50 / year),

iii) Use of a different batch of standard to prepare the external standard solution,

Table 4 presents the list of participants who have stated they have modified the SOP and the reasons why. When pooling the information extracted from the multivariate models, the modifications to the SOP as stated by some of the participants (illustrated in Table 4), and the reported values (Annexes 6 to 10) one can conclude that respecting the acceptable range provided in the protocol enhances the trueness of the method.

Table 4 – List of participants who stated to have introduced modifications to the SOP

Participant	Have you introduced any modification to the protocol?
L01	The centrifugation times were 20 minutes.
L02	We used HCl instead of HBr as precised in the protocol. We did not add L-cysteine in calibration std (no change with our reference, DOLT-4)
L04	Instrumental condition, lower volume of analysis, drying and decomposition time
L06	Sample preparation: after adding 15 mL toluene (2nd extraction): instead of trying to take the whole remaining upper organic phase, we took only 15 mL of the organic phase. In the calculations we corrected for the fact that we sampled (15 mL +15 mL)/45 mL
L12	0.1 g of lyophilised sample, 5 ml HBr, 17 ml toluene
L13	Our analyzer (DMA-80 TriCell of Milestone) has three cells. Using the calibration standards described in the analytical protocol (1 – 100 µg/L) our instrument only calibrates two cells, so our calibration range arrives only up to 25 ng (50 µg/L). So, we prepare two additional calibration standards (150 and 200 µg/L 75 and 100 ng)
L15	Centrifuge 15 minutes at 10.000 G ~ 9600 rpm

L02 use L-cysteine standard solution for all samples as described in 8.1.

9 Conclusion

As a result of the statistical evaluation of the present collaborative trial study, the proposed method fits its intended analytical purpose for the determination of methylmercury by direct analysis in different seafood test samples. The method proves to have adequate trueness and precision for the methylmercury determination (expressed as Hg) ranging from 0.012 to higher than 5 mg kg^{-1}, provided that the SOP is respected.

All method performance characteristics related to trueness and precision estimated in the present collaborative trial show values which are within acceptance levels as laid down in European legislation.

Furthermore, multivariate models are a good tool for interlaboratory comparison organisers to identify the major reasons for differences among participants based on experimental factors and quality assurance procedures.

10 Acknowledgements

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Organisation	Country
CODA-CERVA	BELGIUM
FAVV - FLVVG	BELGIUM
State Veterinary Institute Olomouc	CZECH REPUBLIC
CISTA	CZECH REPUBLIC
Laboratoire SCL de Bordeaux	FRANCE
Service Commun des Laboratoires	FRANCE
Istituto Zooprofilattico Sperimentale	ITALY
National Institute of Public Health - National Institute of Hygiene	POLAND
Instituto Português do Mar e da Atmosfera	PORTUGAL
Laboratorio Arbitral Agroalimentario (MAGRAMA)	SPAIN
Centro de Salud Publica de Alicante	SPAIN
Laboratori Agència Salut Pública de Barcelona	SPAIN

Abbreviations

ILC	Interlaboratory Comparison
CT	Collaborative Trial (collaborative study)
IMEP	International Measurement Evaluation Programme
JRC	Joint Research Centre
IRMM	Institute for Reference Materials and Measurements
ISO	International Organisation for Standardisation
IUPAC	International Union for Pure and Applied Chemistry
EURL-HM	European Union Reference Laboratory for Heavy Metals in Food and Feed
ERM	European Certified Reference Material
NRCC	National Research Council of Canada
NIST	National Institute of Standards and Technology
PLS-R	Partial Least Squares Regression
SOP	Standard Operating Procedure

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Annexes

Annex 1: Publication on IRMM website

IMEP-115: Determination of methylmercury in seafood

The IMEP-115 focuses on the determination of methylmercury in seafood.

This interlaboratory comparison (ILC) aims for the validation of a method (collaborative trial) for the determination of methylmercury in seafood and is organised in support to the Commission Regulation 1881/2006. The method is based on a double liquid-liquid extraction, first with an organic solvent and then with a cysteine solution. The final quantification is done with a direct mercury analyzer.

Participation in IMEP-115 is mandatory for all NRLs having experience in direct mercury analysis. Due to limitations in the amount of the test material available for the exercise, only 15 registrations will be accepted. This number covers all NRL's having experience in this type of analysis and leaves some extra samples for control laboratory that may wish to participate. **Keep in mind that the aim of this exercise is to test the performance of the method not of the laboratory. The protocol must be strictly followed.**

Registration is free of charge.

Please register using the following link:

https://web.jrc.er.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=823

Test materials and analytes

The test material to be analysed is five seafood foodstuff samples with unknown concentrations (concentration range 0.010-1.000 mg as Hg kg^{-1}) will be sent out for the statistical validation of the method (10g in each bottle). Two bottles will be sent from each test sample.

The measurand is methylmercury in freeze-dried seafood samples.

General outline of the exercise

Participants are requested to perform 1-3 independent analyses following the method protocol submitted together with the test items to each bottle in two different days. Participants should report the mean (for each bottle), its expanded uncertainty and coverage factor *k*. Detailed instructions will be sent together with the sample.

Shedule

Registration	Sample Dispatch	Reporting of results	Report to participants
Deadline 15/06/2012	End of June 2012	Deadline 7 September 2012	First quarter of 2013

Annex 2: Invitation to expert laboratories

Invitation for participation in a collaborative study:
Determination of Methylmercury in Fish
by Direct Mercury Analyzer

Geel, 16th April 2012

Dear Madam, Sir,

The European Union Reference Laboratory for Heavy Metals in Feed and Food (EU-RL-HM) will organize an interlaboratory comparison (ILC) for the validation of a method (collaborative trial) for the determination of methylmercury in fish (IMEP-115) in support to the Commission Regulation 1881/2006 [1]. The method is based on a double liquid-liquid extraction, first with an organic solvent and then with a cysteine solution. The final quantification is done with a direct mercury analyzer.

In the Commission Regulation 1881/2006 setting maximum levels for certain contaminants in foodstuffs [1] a maximum tolerable weekly intake of 1.6 µg kg^{-1} (BW) has been set for mercury. The importance of a reliable determination of methylmercury is stressed when it states that "methylmercury is the chemical form of most concern and can make up more than 90 % of the total mercury in fish and seafood".

Participation in IMEP-115 is mandatory for all NRLs having experience in direct mercury analysis. Due to limitations in the amount of test material available for the exercise, only 15 registrations will be accepted. This number covers all NRLs having experience in this type of analysis and leaves some extra samples for control laboratory that may wish to participate.
Keep in mind that the aim of this exercise is to test the performance of the method not of the laboratory. The protocol must be strictly followed.

The set-up and execution of the collaborative study will be done according to the IUPAC protocol for the design, conduct and interpretation of method-performance studies [2]. According to this guideline a minimum of 5 different samples should be analysed by (at least) 8 (valid) laboratory results. Statistical evaluation will be conducted following ISO 5725-2 [3].

Five fish foodstuff samples with unknown concentrations (concentration range 0.010 – 1.000 mg as Hg kg^{-1}) will be sent out for the statistical validation of the method. Furthermore, for training purposes, one test sample will be sent to the participating laboratories before starting IMEP-115, as a "hands-on" exercise.

The required equipment and reagents are described in the protocol attached to this invitation letter.

Time schedule:

- Dispatch of samples: **end of May 2012**
- Deadline for submission of results: **6th July 2012.**
- Reports to participants will be sent out after statistical evaluation.

I hope you will find it attractive to participate in the validation of a future standard method for food control. Further information can be found hereunder and if interested please fill in and send the registration form. Your efforts are very much appreciated. Thanks in advance.

Laboratories interested in taking part in this exercise should register via:
https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=823

If you have any questions please send a mail to: JRC-IRMM-IMEP@ec.europa.eu or call +32 14 571687

Best regards,
ILC Co-ordinator
Dr. Fernando Cordeiro (International Evaluation Measurement Programme)
E-mail: fernando.cordeiro-raposo@ec.europa.eu
Phone: +32 14 571687
Institute for Reference Materials and Measurements
Retieseweg 111
2440 Geel
Belgium

References:

[1] Commission Regulation 1881/2006, OJ L 364/5

[2] Protocol for the design, conduct and interpretation of method-performance studies, Pure Appl. Chem., Vol. 67, N° 2, p 331-343, (1995)

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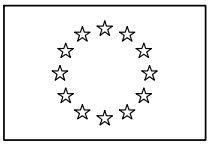
Annex 3: Sample accompanying letter



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Food Safety & Quality

IMEP®

Geel, 4 July 2012
JRC.D5/FCR/bk/ARES(2012)/

«Title» «Firstname» «Surname»«
Organisation»
«Department»
«Address»
«Address2»
«Zip» «Town»
«Country»

Participation in IMEP-115, a collaborative trial for the validation of a method for the determination of methylmercury in seafood.

Dear «Title» «Surname»,

Thank you for participating in the IMEP-115 a collaborative trial for the validation of a method to determine methylmercury in seafood. This exercise is organised in support to the Commission Regulation 1881/2006 which sets maximum levels for certain contaminants in foodstuffs.
Please keep this letter. You need it for reporting your results.

This parcel contains:

a) 10 bottles containing, each approximately 2.5 g (sample 3) or 5 g (samples 1, 2, 4 and 5) of the test items (two bottles from each test material),

b) A copy of the standard operational procedure which **must** be followed strictly

c) A bottle of a pre-test item (containing 15 g of test item)

d) A "Confirmation of Receipt" form,

e) This accompanying letter.

Please check whether the bottles containing the test material remained undamaged during transport. Then, send the "Confirmation of receipt" form back (fax: +32-14-571865, e-mail: jrc-irmm-imep@ec.europa.eu). You should store the samples in a dark and cool place (at 4 ºC) until analysis.

The measurand is methylmercury (as total Hg) in five different seafood matrices.

Before starting with the analyses of Samples 1-5, apply the method to be validated as described in the Standard Operational Procedure to the Pre-test item. Send to jrc-irmm-imep@ec.europa.eu the result that you have obtained for that sample **(do this by 13th July 2012)**, wait till you will have received an e-mail from the EU-RL-HM saying that you can continue with the analysis of Samples 1-5. The purpose of this pre-test is to make sure that you are implementing the method correctly and to avoid that a wrong interpretation of the standard operational procedure will nullify the whole collaborative trial.

For the analysis of Samples 1-5, perform three independent measurements per bottle (under repeatability conditions) on two different days (one bottle/day) following the standard Operational Procedure that you have received. Report the values obtained for the six independent measurements on the reporting website. Express the results in mg kg^{-1} **(as Hg)**. The results should be reported with two decimals (or more if necessary to avoid a series of identical values).

Test materials should be re-homogenised by shaking manually the bottle before taking the test portion.

Results should be reported referring to dry mass, thus corrected for humidity. **To calculate the water content in the test materials, please apply the following procedures:**

Sample 1 – Weigh accurately 1 g of test material in a glass container of 5-7 cm diameter (preferably with a lid). Place it in an oven at 80 ± 2 °C for 10 ± 1 minutes. Allow the glass container (covered with the lid) to cool down for about 30 minutes in a desiccator before weighing.

Samples 2 **and 3**– Weigh accurately 0.8 g of test material in a glass container of 5-7 cm diameter (preferably with a lid). Place it in an oven at 105 ± 1 °C for 2 hours. Allow the glass container (covered with the lid) to cool down for about 30 minutes in a desiccator before weighing.

Sample 4 – Weigh accurately 0.8 g of test material in a glass container of 5-7 cm diameter (preferably with a lid). Place it in an oven at 105 ± 1 °C for 1 hour. Allow the glass container (covered with the lid) to cool down for about 30 min. in a desiccator before weighing.

Sample 5 and pre-test sample – Weigh accurately 100 mg of test material in a glass container of 5-7 cm diameter (preferably with a lid). Place it in an oven at 102 ± 2 °C for 3-4 hours. Allow the glass container (covered with the lid) to cool down for about 30 min. in a desiccator before weighing.

> Note 1: Perform the measurements for the water content in **duplicate**.
> **Note 2: Do not use the for the methyl mercury determination the same aliquots of test material that you have used for the water determination.**

You can find the reporting website at https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do

To access the webpage you need a personal password key, which is: **«Part_key»**. The system will guide you through the reporting procedure.

Please report:

- the result for **each replicate** (mg kg^{-1})
- the associated expanded **uncertainty** (mg kg^{-1}),
- the **coverage factor**

After entering all results, please complete also 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 **10/09/2012.**

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.

Note that the aim of this exercise is to test the performance of the method not of the laboratory. The standard operating procedure must be strictly followed.

Your participation in this project is greatly appreciated. If you have any remaining questions, please contact me by e-mail: jrc-irmm-imep@ec.europa.eu

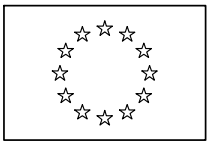
With kind regards

Dr. Fernando Cordeiro Raposo
IMEP-115 Coordinator

Enclosures: 1) Two bottles containing the test material for each test sample (10 bottles);
2) One bottle of a pre-test sample;
3) A copy of the standard operational procedure,
4) Confirmation of receipt form; 5) Accompanying letter.

Cc: F. Ulberth

Annex 4: 'Confirmation of receipt' forms



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for reference materials and measurements
Food Safety & Quality

Annex to JRC.D5/FCR/bk/ARES(2012)/806283

«Title» «Firstname» «Surname»«
Organisation»
«Department»
«Address»
«Address2»
«Zip» «Town»
«Country»

IMEP-115

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. Fernando Cordeiro

IMEP-115 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

Fax : +32-14-571865
e-mail : jrc-irmm-imep@ec.europa.eu

Annex 5: Questionnaire

Milc questionnaire

Comparison for Collaborative trial IMEP-115

Determination of methylmercury in foods by elemental mercury analizer. "THIS IS A STUDY OF THE METHOD NOT OF THE LABORATORY. THE METHOD MUST BE STRICTLY FOLLOWED AS DESCRIBED" Please feel the questionnaire!

Submission Form

1. **Have you introduced any modification to the proposed protocol?**

☐ No

☐ Yes

1.1. If yes, please briefly describe it.

2. **Instrumental conditions**

2.1. Volume of analysis

2.2. Decomposition temperature

2.3. Decomposition time

2.4. Drying temperature

2.5. Drying time

3. **Which range (µg/l) was used for the calibration standard solutions?**

3.1. Highest

3.2. Lowest

4. **Have you used an external standard solution?**

- ☐ No
- ☐ Yes

4.1. How did you prepare the external std solution?

- ☐ a) using a different brand?
- ☐ b) using a different batch?
- ☐ c) using a CRM?

5. **Which was your recovery factor ((in %)**

5.1. How did you estimate the recovery factor?

- ☐ a) Using a CRM?
- ☐ b) Adding a known amount of the analyte (spiking)?

6. **Which volume was used to spike the test sample?**

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

- ☐ No
- ☐ Yes

8. **What is the basis of your uncertainty estimate ? (multiple answers possible)**

- ☐ a) uncertainty budget according to ISO-GUM
- ☐ b) known uncertainty of the standard method
- ☐ c) uncertainty of the method as determined during in-house validation
- ☐ d) measurement of replicates (i.e. precision)
- ☐ e) estimation based on judgement
- ☐ f) use of intercomparison data
- ☐ g) other

8.1. If other, please specify :

9. **What is the level of confidence reflected by coverage factor k reported with your results ? (in %)**

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

- ☐ No
- ☐ Yes

10.1. If yes, which one ?

- ☐ ISO 17025
- ☐ ISO 9000 series
- ☐ Other

10.1.1. If other, please specify :

10.2. Are you accredited ?

- ☐ No
- ☐ Yes

10.2.1. If yes, by which accreditation body ?

11. **Does your laboratory carry out this type of analysis on a regular basis ?**

- ☐ No
- ☐ Yes

11.1. If yes, please estimate the number of samples :

- ☐ a) < 10 samples/year
- ☐ b) 10-20 samples/year
- ☐ c) 20-50 samples/year
- ☐ d) > 50 samples/year

12. **Does your laboratory take part in similar interlaboratory comparisons on a regular basis ?**

- ☐ No
- ☐ Yes

12.1. Which ILC scheme(s) ?

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

☐ No

☐ Yes

13.1. If yes, which one ?

13.2. Is the material used for the validation of procedures ?

☐ No

☐ Yes

13.3. Is the material used for instrument calibration?

☐ No

☐ Yes

14. **How have you heard about this exercise ?**

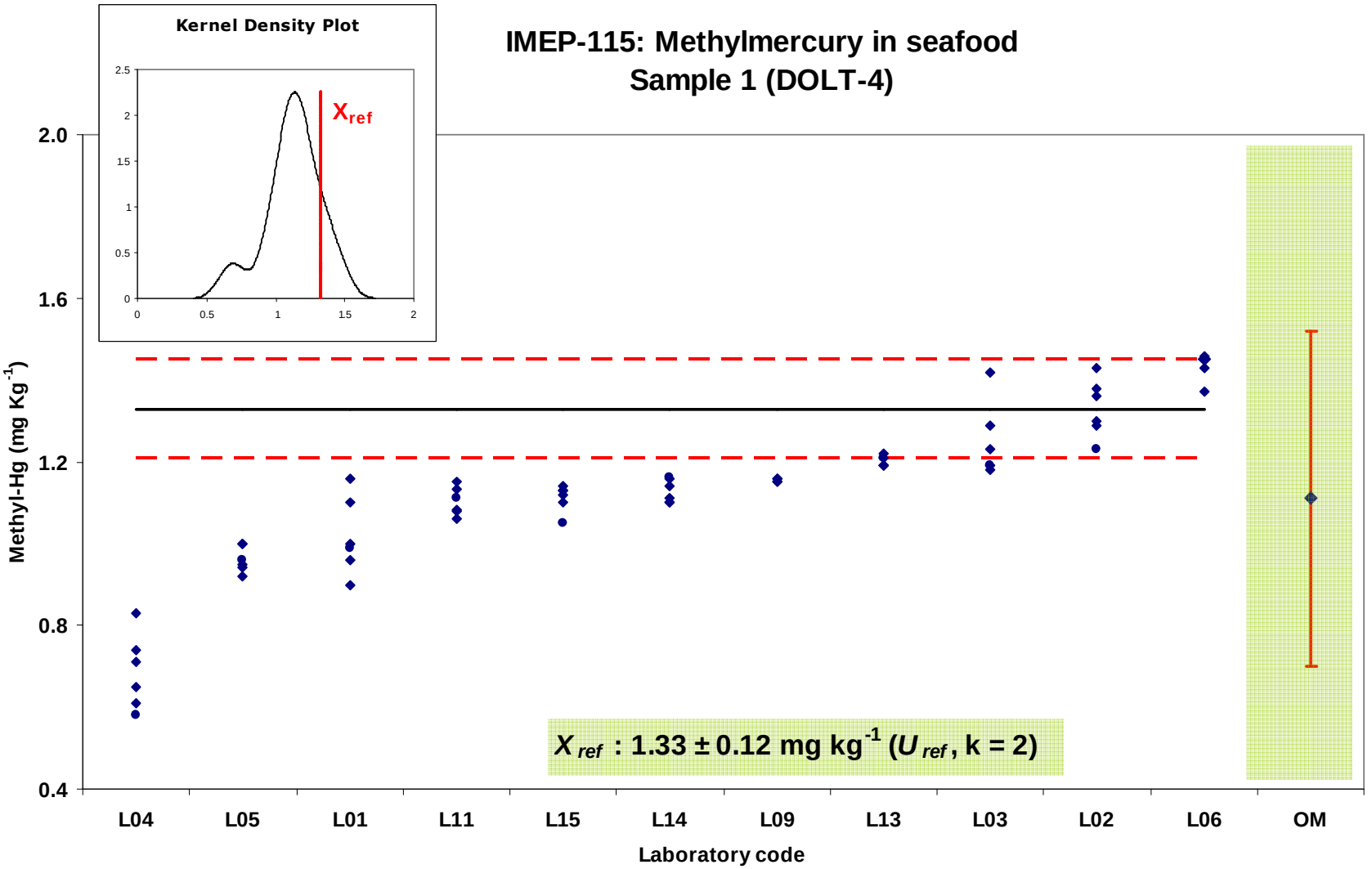
15. **Do you have any comments ? Please, let us know ...**

Annex 6: Results for Sample 1 (DOLT-4)

X_{ref} = 1.33 and U_{ref} = 0.12 (k = 2); all values are given in mg kg^{-1} (expressed as Hg)

Laboratory	R_1	R_2	R_3	R_4	R_5	R_6	Mean
L01	1.16	1.1	0.96	0.9	1	0.99	1.02
L02	1.43	1.36	1.29	1.3	1.38	1.23	1.33
L03	1.23	1.29	1.42	1.18	1.19	1.19	1.25
L04	0.61	0.65	0.74	0.71	0.83	0.58	0.69
L05	1	1	0.92	0.95	0.94	0.96	0.96
L06	1.45	1.45	1.46	1.43	1.37	1.45	1.44
L09	1.15	1.15	1.16	1.16			1.16
L11	1.082	1.134	1.079	1.06	1.15	1.11	1.10
L12	*4.22*	*3.83*	*3.71*	*4.62*	*3.59*	*3.96*	*3.99 **C,G*
L13	1.19	1.21	1.19	1.19	1.22	1.21	1.20
L14	1.101	1.113	1.1	1.157	1.141	1.162	1.13
L15	1.12	1.1	1.13	1.14	1.13	1.05	1.11

**** C,G = Outlier identified by Cochran and Grubbs tests**



This plot shows all measurement results. The solid line refers to the X_{ref}. The dotted line the boundaries of X_{ref} (X_{ref} ± U_{ref}), OM refers to the overall mean (X_{Obs} ± $2S_R$)

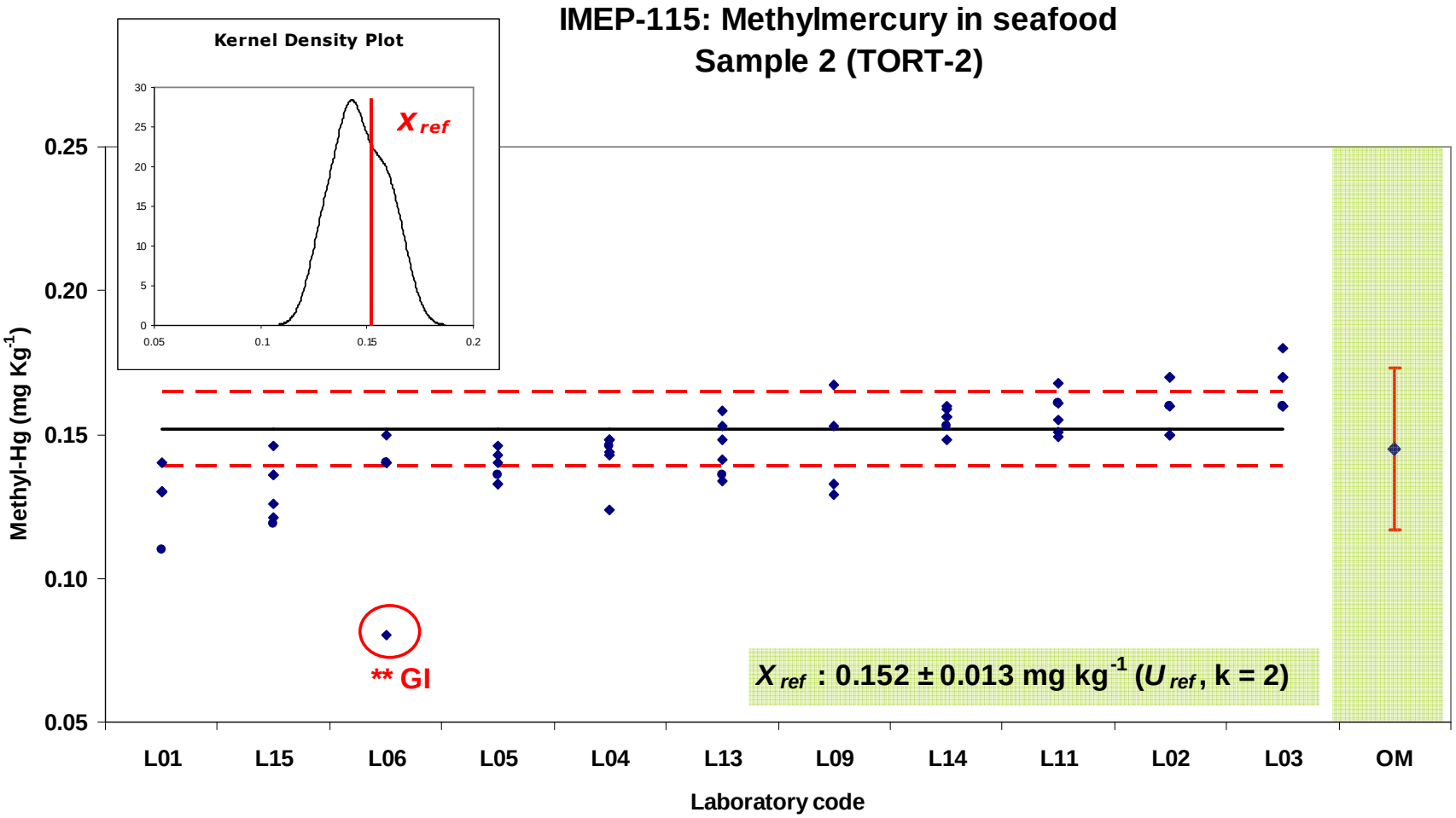
Annex 7: Results for Sample 2 (TORT-2)

X_{ref} = 0.152 and U_{ref} = 0.013 (k = 2); all values are given in mg kg^{-1} (expressed as Hg)

Laboratory	R_1	R_2	R_3	R_4	R_5	R_6	Mean
L01	0.13	0.13	0.13	0.13	0.14	0.11	0.128
L02	0.15	0.16	0.15	0.17	0.17	0.16	0.160
L03	0.16	0.18	0.17	0.17	0.16	0.16	0.167
L04	0.148	0.143	0.124	0.148	0.144	0.146	0.142
L05	0.143	0.14	0.146	0.133	0.133	0.136	0.139
L06	0.14	0.15	0.14	0.14	0.08 **GI	0.14	0.132
L09	0.167	0.153	0.133	0.129			0.146
L11	0.155	0.149	0.151	0.161	0.1676	0.161	0.157
L12	*0.79*	*0.61*	*0.57*	*0.7*	*0.71*	*0.52*	*0.65 **C,G*
L13	0.153	0.148	0.141	0.158	0.134	0.136	0.145
L14	0.156	0.159	0.156	0.148	0.16	0.153	0.155
L15	0.146	0.136	0.136	0.126	0.121	0.119	0.131

**** GI = Outlier identified by Grubbs internal**

**** C,G = Outlier identified by Cochran and Grubbs tests**



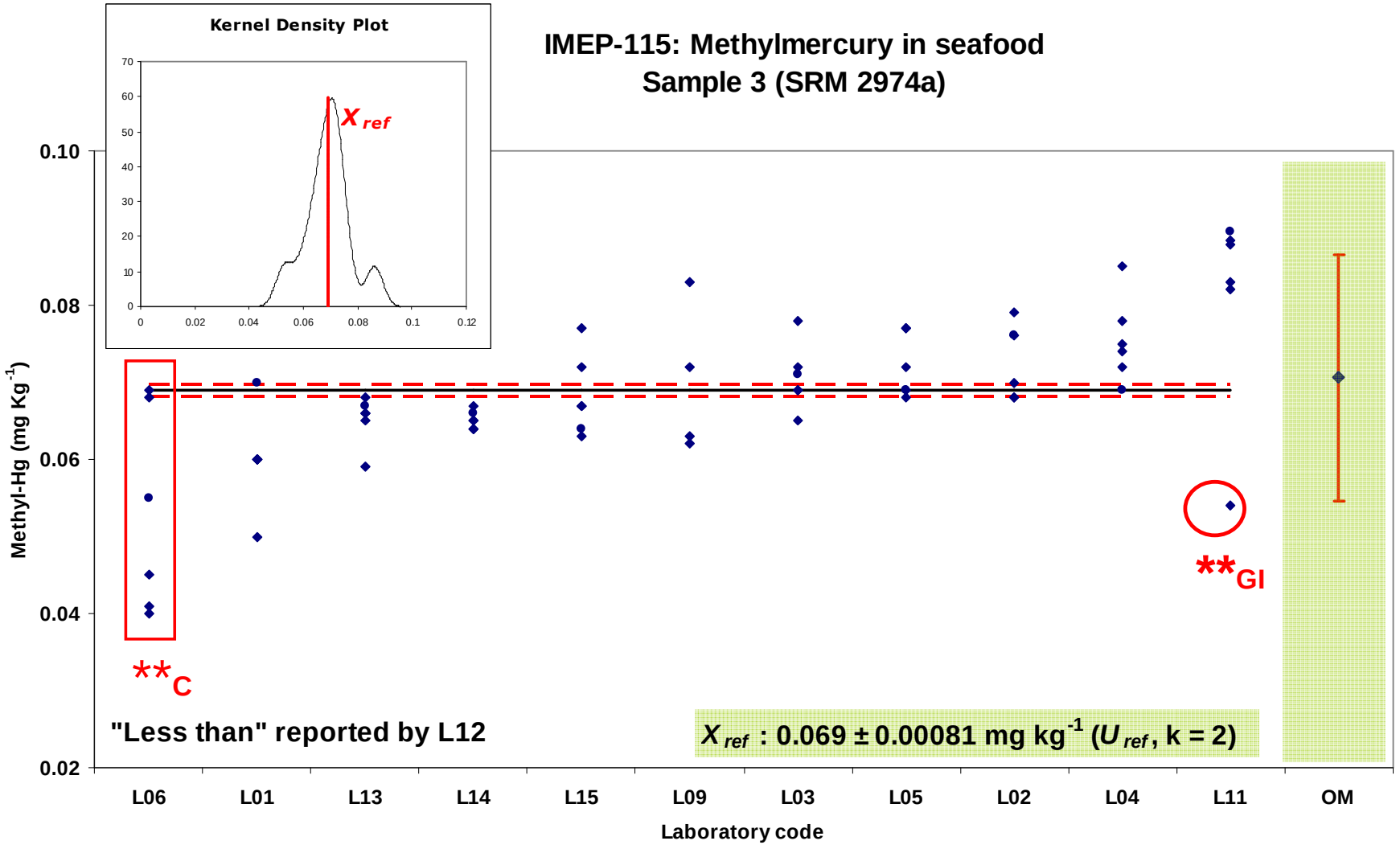
This plot shows all measurement results. The solid line refers to the X_{ref}. The dotted line the boundaries of X_{ref} ($X_{ref} \pm U_{ref}$), *OM refers to the overall mean* ($X_{Obs} \pm 2S_R$). Identified replicate was considered outlier.

Annex 8: Results for Sample 3 (SRM 2974a)

X_{ref} = 0.069 and U_{ref} = 0.00081 (k = 2); all values are given in mg kg^{-1} (expressed as Hg)

Laboratory	R_1	R_2	R_3	R_4	R_5	R_6	Mean
L01	0.06	0.05	0.06	0.06	0.06	0.07	0.060
L02	0.07	0.076	0.079	0.068	0.068	0.076	0.073
L03	0.065	0.078	0.072	0.069	0.069	0.071	0.071
L04	0.085	0.075	0.074	0.072	0.078	0.069	0.076
L05	0.072	0.077	0.077	0.069	0.068	0.069	0.072
L06	*0.045*	*0.041*	*0.04*	*0.068*	*0.069*	*0.055*	*0.053 **C*
L09	0.062	0.083	0.072	0.063			0.070
L11	0.083	0.082	*0.054 **GI*	0.0884	0.0878	0.0895	0.086
L13	0.066	0.066	0.065	0.068	0.059	0.067	0.065
L14	0.065	0.064	0.064	0.067	0.065	0.066	0.065
L15	0.072	0.067	0.067	0.077	0.063	0.064	0.068

*** C = Straggler identified by Cochran test**
**** GI = Outlier identified by Grubbs internal**



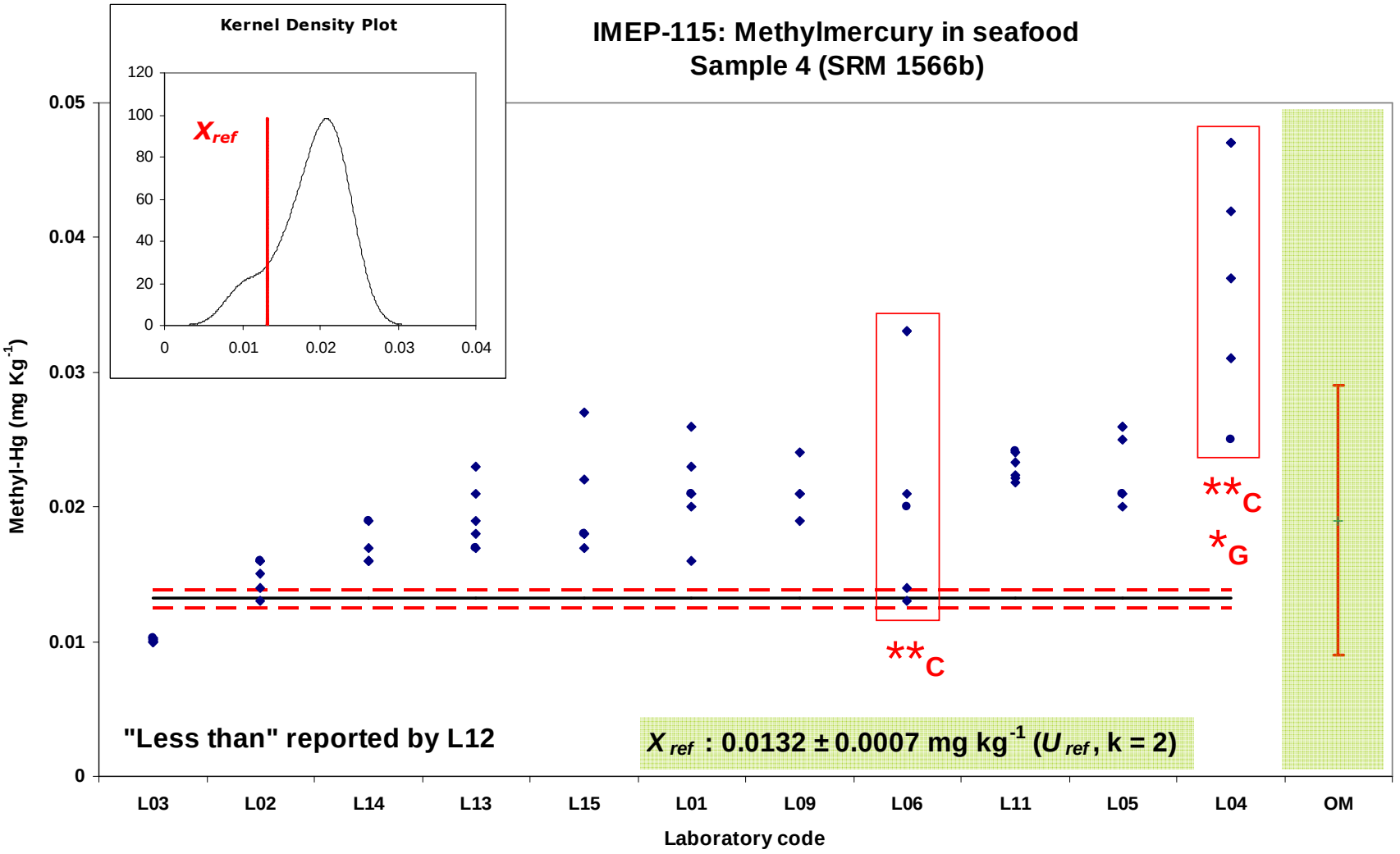
This plot shows all measurement results. The solid line refers to the X_{ref}. The dotted line the boundaries of X_{ref} (X_{ref} ± U_{ref}), *OM refers to the overall mean* (X_{Obs} ± $2S_R$). Identified laboratory and replicate were considered outliers.

Annex 9: Results for Sample 4 (SRM 1566b)

X_{ref} = 0.0132 and U_{ref} = 0.0007 (k = 2); all values are given in mg kg^{-1} (expressed as Hg)

Laboratory	R_1	R_2	R_3	R_4	R_5	R_6	Mean
L01	0.026	0.023	0.016	0.02	0.021	0.021	0.0212
L02	0.015	0.013	0.014	0.016	0.016	0.016	0.0150
L03	0.01	0.0102	0.01	0.0103	0.0101	0.0103	0.0102
L04	*0.047*	*0.047*	*0.042*	*0.037*	*0.031*	*0.025*	*0.038 **C*
L05	0.026	0.02	0.026	0.021	0.025	0.021	0.0232
L06	*0.033*	*0.014*	*0.013*	*0.033*	*0.021*	*0.02*	*0.022 **C*
L09	0.024	0.019	0.021	0.021			0.0213
L11	0.0224	0.0218	0.0221	0.0233	0.024	0.0242	0.0230
L13	0.018	0.021	0.019	0.023	0.017	0.017	0.0192
L14	0.017	0.019	0.016	0.016	0.016	0.019	0.0172
L15	0.022	0.018	0.017	0.027	0.018	0.018	0.0200

**** C = Outlier identified by Cochran test**



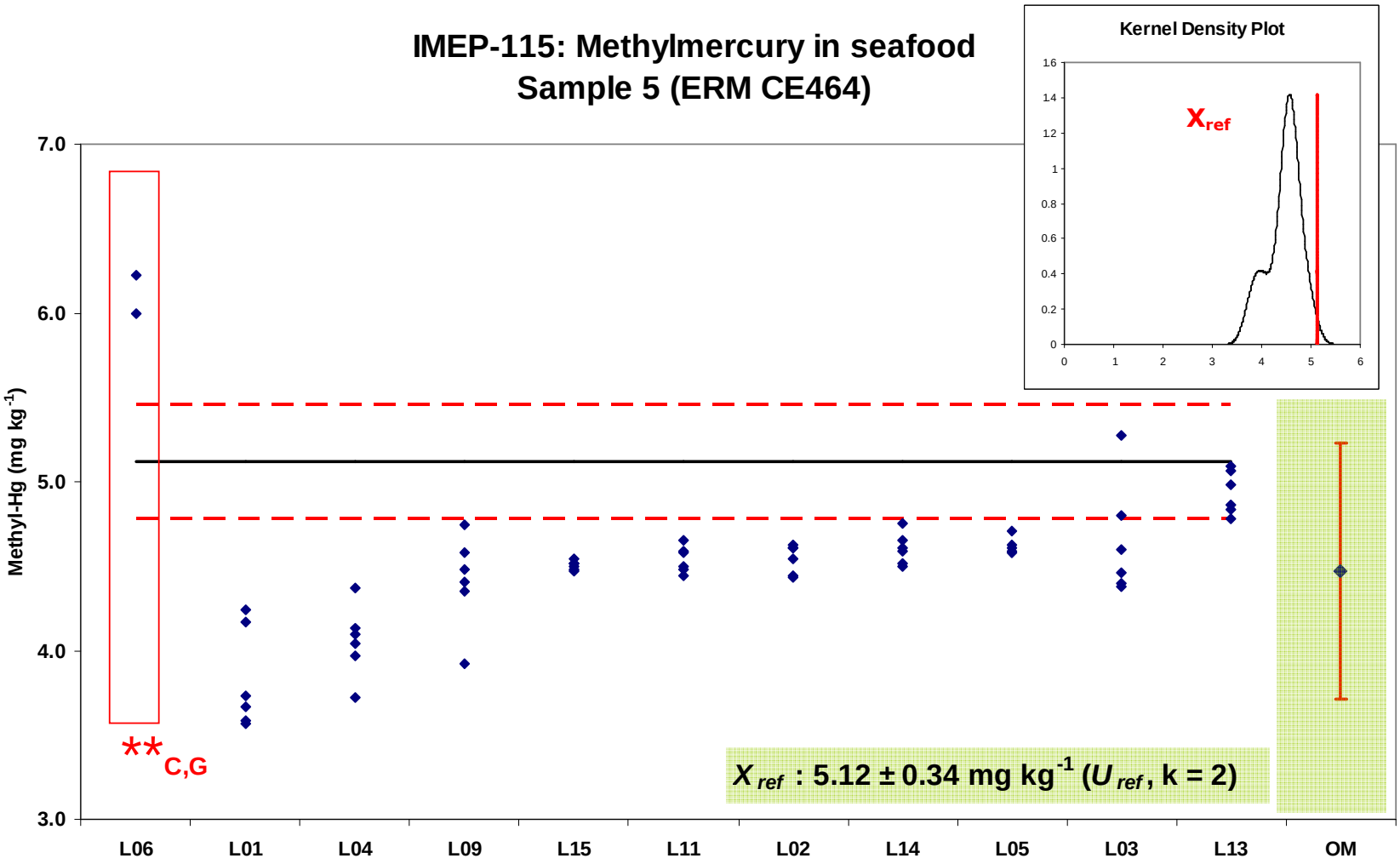
This plot shows all measurement results. The solid line refers to the X_{ref}. The dotted line the boundaries of X_{ref} (X_{ref} ± U_{ref}), *OM refers to the overall mean* (X_{Obs} ± $2S_R$). Identified laboratories were considered outliers.

Annex 10: Results for Sample 5 (ERM CE464)

X_{ref} = 5.12 and U_{ref} = 0.34 (k = 2); all values are given in mg kg^{-1} (expressed as Hg)

Laboratory	R_1	R_2	R_3	R_4	R_5	R_6	Mean
L01	4.17	4.24	3.57	3.58	3.73	3.67	3.83
L02	4.43	4.63	4.61	4.44	4.61	4.54	4.54
L03	4.38	4.46	4.4	4.6	4.8	5.27	4.65
L04	3.97	4.04	4.37	4.1	3.72	4.13	4.06
L05	4.61	4.71	4.58	4.59	4.63	4.58	4.62
L06	*0.19*	*0.13*	*0.2*	*6.22*	*6*	*0.31*	*2.18 **C,G*
L09	4.48	4.41	4.74	3.92	4.58	4.35	4.41
L11	4.5	4.44	4.48	4.653	4.587	4.583	4.54
L13	4.78	4.86	4.84	4.98	5.09	5.06	4.94
L14	4.5	4.59	4.61	4.75	4.52	4.65	4.60
L15	4.48	4.52	4.54	4.47	4.5	4.52	4.51

**** C,G = Outlier identified by Cochran and Grubbs tests**



This plot shows all measurement results. The solid line refers to the X_{ref}. The dotted line the boundaries of X_{ref} ($X_{ref} \pm U_{ref}$), *OM refers to the overall mean* ($X_{Obs} \pm 2S_R$). Identified laboratory was considered outlier.

European Commission
EUR 25830 – Joint Research Centre – Institute for Reference Materials and Measurements

Title: **IMEP-115: Determination of Methylmercury in Seafood – A collaborative trial report**

Author(s): F. Cordeiro, S. Gonçalves, J. Calderón, P. Robouch, H. Emteborg, P. Conneely, M.-F. Tumba-Tshilumba, B. Kortsen, B. de la Calle

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Abstract

A collaborative trial study, IMEP-115, was conducted in accordance with international protocols to determine the performance characteristics of an analytical method for the determination of methyl mercury in seafood.

The present exercise was organised in support to the Commission Regulations 1881/2006 and 882/2004.

Fifteen laboratories, from ten European countries, registered for participation. All of them are experienced in the direct determination of mercury.

Five test items have been selected. Their methyl mercury content was covering a reasonable wide range of concentration levels. All of them are certified reference materials from different producers:

The repeatability relative standard deviation (RSD_r) ranged from 4.5 to 12.6 % while the reproducibility relative standard deviation (RSD_R) ranged from 8.5 to 24.5 %.

The method demonstrates to have acceptable precision for all test materials, thus it should be considered that it fits its intended analytical purpose.

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