

Report of the ninth interlaboratory comparison organised by the European Union Reference Laboratory for Heavy Metals in Feed and Food

IMEP-109: Total cadmium, lead, arsenic and mercury as well as methylmercury and inorganic arsenic in seafood

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EUR 24560 EN - 2010

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JRC 61114

EUR 24560 EN

ISBN 978-92-79-17198-7

ISSN 1018-5593

DOI 10.2787/31613

Luxembourg: Publications Office of the European Union

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arsenic in seafood



September 2010

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1 Abstract

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate General of the European Commission, operates the European Union Reference Laboratory for Heavy Metals in Feed and Food (EU-RL-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 ninth proficiency test (PT) of the EU-RL-HM which focused on the determination of total cadmium, lead, arsenic and mercury as well as methylmercury and inorganic arsenic in seafood.

The test material used in this exercise was the Certified Reference Material (CRM) DOLT-4, dogfish liver of the National Research Council of Canada (CNRC). The material was relabelled to prevent recognition by the participants and was dispatched the second half of May 2010. Each participant received one bottle containing approximately 20 g of test material. Thirty-eight laboratories from 27 countries registered to the exercise of which 38 reported results for total Cd, 36 for total Pb, 33 for total As, 36 for total Hg, five for methylmercury and 10 for iAs. The assigned values for total Cd, Pb, As, Hg and methylmercury are the certified values taken from the DOLT-4 certificate. An attempt was made to establish an assigned value for inorganic As (iAs) using the results provided by a group of five laboratories expert in the field, following a similar approach to that used in IMEP-107¹, a PT on total and inorganic arsenic in rice. Contrary to what was observed in IMEP-107, the results obtained by the expert laboratories for iAs was method dependent, therefore no assigned value could be established.

The uncertainties of the assigned values, u_{ref} , were taken directly from the CRM certificate as provided by the producer for total Cd, Pb, As, Hg and methylmercury. Participants were invited to report the uncertainty of their measurements. This was done by the majority of the laboratories taking part in this exercise.

Laboratory results were rated with z- and ζ -scores (zeta-scores) in accordance with ISO 13528². Since the concentration of iAs seems to be method dependent according to the results obtained by the expert laboratories, no scoring was provided to the laboratories that submitted results for iAs. The standard deviation for proficiency assessment (also called target standard deviation) was fixed to 15% by the advisory board of this ILC, on the basis of the outcome of previous ILCs organised by the EU-RL-HM and on the state-of-the-art in this field of analysis.

Between 80 and 97.5 % of the laboratories performed satisfactory for total Cd, As, Hg and methylmercury. Regarding total Pb, 70 % of the laboratories scored satisfactory.

2 Introduction

From a toxicological point of view metal speciation is of paramount importance since in most cases different species have different toxicologies. For instance, methylmercury is more toxic than the inorganic mercury compounds³ while inorganic arsenic is more toxic than the organic species of arsenic, with arsenosugars and arsenobetaine not being toxic¹. The mentioned differences in toxicology depending on the species in which a metal is present in food should be taken into consideration when fixing maximum levels in legislation.

In Europe only maximum levels for total mercury in food are given in legislation⁴, varying from 0.5 to 1 mg kg⁻¹ for different seafood, but no maximum level exists for methylmercury. The U.S. Food and Drug Administration established a guideline for methylmercury in seafood at a level of 1 mg kg⁻¹.

No maximum levels have been settled, so far, for arsenic in the European legislation, due to a lack of reliable analytical methods for the determination of iAs in different food commodities and due to the general belief among scientists that results for iAs are method dependent.

To support policy makers, the EU-RL-HM has organised along the four years that it has been operational several activities in the area of metal speciation analysis. In 2008 the EU-RL-HM organised a PT, IMEP-104, for the determination of heavy metals in seafood, including methylmercury as measurand. The aim of that exercise was to check the state-of-the-art among NRLs regarding methylmercury determinations. At that time only four NRLs had a method in place and reported results for methylmercury. In 2009, the EU-RL-HM organised a proficiency test, IMEP-107, for the determination of total and iAs in rice, in which laboratories from all around the world, not only NRLs, took part. In autumn 2008 a training on metal speciation was organised for the network of NRLs in which special attention was dedicated to iAs and methylmercury determinations.

In 2010 the EU-RL-HM organised a PT, IMEP-109, for the determination total Cd, Pb, As and Hg as well as methylmercury and iAs in seafood with two main purposes:

- to test whether the measurement capabilities of the NRLs have improved since IMEP-104 was conducted.
- to evaluate the performance of NRLs with regard to total and iAs determinations in a food matrix, seafood, which could pose more analytical problems than rice due to the co-existence of a wide spectrum of arsenic species.

This report summarises the outcome of IMEP-109.

3 Scope

As stated in Regulation No 882/2004 of the European Parliament and the Council⁵, one of the core duties of the EU-RL-HM is to organise interlaboratory comparisons for the benefit of staff from National Reference Laboratories. The scope of this PT is to test the competence of the appointed

NRLs to determine the total concentration of Cd, Pb, As and Hg as well as methylmercury and iAs in seafood.

The assessment of the measurement results is undertaken on the basis of requirements laid down in legislation^{6,7} and follows the administrative and logistics procedures of IMEP, the International Measurement Evaluation Programme. This programme is accredited according to ISO Guide 43-1. The designation of this PT is IMEP-109.

4 Time frame

This proficiency test was agreed upon by the NRLs network at the fourth EU-RL-HM workshop held on 1-2 October 2009. Invitation letters were sent to the participants on 21 April 2010 (cf Annex 1). The samples were dispatched to the participants on 18th May 2010. Reporting deadline was 15th June 2010.

5 Material

5.1 Preparation

The commercially available CRM DOLT-4 (dogfish liver Certified Reference Material for Trace Elements) was used for this PT. The material was 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 website⁸ (cf Annex 2).

The CNRC dispatched about 40 bottles of test materials at room temperature by courier to IRMM.

5.2 Homogeneity and stability

Information on the homogeneity and stability of the test material was gathered from the certificate of the CRM. According to it, uncertainties related to possible between-bottle variation (u_{hom}) are included in the overall uncertainty of the certified value. In the experience of the CRM producer, uncertainty components for long and short term stability were considered negligible and are thus not included in the uncertainty budget. No homogeneity and stability tests were conducted for iAs because based on previous experience¹, iAs is homogeneous if total As is homogeneous and iAs is stable at room temperature for the four weeks that elapsed between the dispatch of the test material and the deadline for reporting of results.

5.3 Distribution

The samples were dispatched to the participants by IRMM on 18th May 2010. Each participant received: a) one glass bottle containing approximately 20 g of test material, b) an accompanying letter

with instructions for sample handling and reporting (cf. Annex 3) and c) a form that had to be send back after receipt of the test material to confirm its arrival (cf. Annex 4).

6 Instructions to participants

Details on this intercomparison were discussed with the NRLs at the fourth workshop organised by the CRL-HM, held in Geel on 1-2 October 2009. 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 and Hg as well as methylmercury and iAs in seafood".

Laboratories were asked to perform two or three independent measurements and to report the mean of them, the uncertainty associated to the mean, the coverage factor and the technique that has been used to perform the measurements. The measurement results were to be corrected for recovery and for humidity, following a procedure described in the accompanying letter which has been optimised at IRMM. Participants were asked to follow their routine procedures. The results were to be reported in the same manner (eg. 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 specific questionnaire was attached to this on-line form. The questionnaire was intended to provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in Annex 5.

The laboratory codes were given randomly and communicated to the participants in a confidential letter that was sent to each participant together with the report.

7 Reference values and their uncertainties

The CRM certificate provided certified values for all the measurands included in this study (not for iAs). 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 and individual subsample and they were taken as the expanded uncertainties of the assigned values (U_{ref}).

In order to establish the assigned values for iAs, a group of five expert laboratories in the field performed analysis on the test material. The expert laboratories involved in the establishment of the assigned values were:

- Institute of Agricultural Chemistry and Food Technology (CSIC), Spain
- Institute of Chemistry, Karl-Franzens University Graz, Austria
- The Food and Environment Research Agency (FERA), United Kingdom
- New Technical University of Denmark (DTU), Denmark

Department of Analytical Chemistry, University of Barcelona, Spain

The experts were asked to use the method of their choice and no further requirements were imposed regarding methodology. The experts were also asked to report their results together with the measurement result uncertainty and a description of the method used.

The means reported by the expert laboratories and their associated standard uncertainties (u_{char}) for iAs are shown in Table 1.

The methods applied by the expert laboratories are summarised in Table 2.

Table 1, indicates there are strong discrepancies among the results reported by the expert laboratories, contrary to what was observed in IMEP-107 (total and iAs in rice). The concentration of iAs found by the different expert laboratories in IMEP-109 did not allow to establish any assigned value for iAs. It was therefore decided not to score laboratories having reported results for this measurand.

The assigned reference values (X_{ref}) for the remaining measurands, total Cd, Pb, As, Hg and methylmercury, and their respective estimated uncertainties are summarised in Table 3.

Table 1: Values for iAs and their associated uncertainties as reported by the expert laboratories.

| Certifier | X_n (mg kg ⁻¹) | U_n (k=2) (mg kg ⁻¹) | u_n (mg kg ⁻¹) |
|-------------|------------------------------|------------------------------------|------------------------------|
| Certifier 1 | < 0.040* | | |
| Certifier 2 | not detected [#] | | |
| Certifier 3 | 0.047 | 0.012 | 0.006 |
| Certifier 4 | 0.075 | 0.010 | 0.005 |
| Certifier 5 | 0.152 | 0.020 | 0.010 |

* 0.040 mg kg⁻¹ is the LoQ (on dry matter content basis) of the method used.

LoQ of the method used is 0.031 mg kg⁻¹ for arsenite and 0.084 mg kg⁻¹ for arsenate.

Table 2: Methods used by the expert laboratories for sample pre-treatment in the determination of total and inorganic As.

| Certifier | Sample treatment | Detection |
|--------------------|--|-------------|
| Certifier 1 | 0.2 gram of samples and 10 mL 0.07 mol L ⁻¹ HCl in 3 % H ₂ O ₂ were placed in μ -wave digestion vessels. Microwaves were applied for 20 min keeping the temperature at 90°C. The extract was centrifuged and filtered (0.45 μ m) prior to analysis with anion-exchange chromatography HPLC-ICP-MS | HPLC-ICP-MS |
| Certifier 2 | The inorganic arsenic (As(III) + As(V)) was evaluated from the speciation carried out after application of suitable extraction method. A sample of seafood (0.2 g from freeze-dried sample) and 20 ml of a methanol/water solution (1:1, v/v) were placed in the digestion vessels. 40 W of focused microwaves was applied for 10 min. After decanting, the extract was centrifuged at 2500 rpm for 10 min and the liquid phase was evaporated to remove the methanol under an IR lamp (T<40 °C) for approximately 4 h. The extract was then diluted in water up to 20 mL and filtered through a nylon membrane of 0.2 μ m porosity. The filtrate was then defatted by clean-up with a C18 cartridge, which had been previously conditioned by passing methanol (5 ml) and water (5 ml) at 1 ml min ⁻¹ . The extract was passed through the cartridge (1 ml min ⁻¹). The first 2–3 ml, mainly arising from conditioning solutions, were discarded. Finally, an aliquot of the cleaned up extract was made up to a fixed volume. Two chromatographic modes were used for separation of the As species. Arsenite, arsenate, DMA, MA, PO ₄ -sug, SO ₄ -sug and SO ₃ -sug were analyzed by anion-exchange chromatography on the Hamilton PRP-X100 column using an aqueous solution of 20 mM NH ₄ H ₂ PO ₄ at pH 5.6 as mobile phase. AB, AC, TMAO and Gly-sug were analysed in the extracts by cation-exchange chromatography on the Zorbax 300-SCX column with a mobile phase (1.5 ml min ⁻¹) of 20 mM pyridine (pH=2.6, adjusted with formic acid). | HPLC-ICP-MS |
| Certifier 3 | 0.250 g + 5 mL 1 mol L ⁻¹ trifluoroacetic acid. Sonicate for 10 min and let stand overnight. Add 50 μ L H ₂ O ₂ to reduce arsenite to arsenate. Microwave in an argon atmosphere (max temp. 95 °C) | HPLC-ICP-MS |
| Certifier 4 | 1 g of sample + 4.1 mL of H ₂ O + 18.4 mL of HCl agitated for 15 hours, let stand overnight. Add a reducing agent (2 mL HBr + 1 mL of hydrazine sulphate). Add 10 mL of chloroform and shake for 3 min. Separate the two phases centrifuging at 2000 rpm for 5 min. Repeat the extraction another two times. Eliminate remnants of organic As with a Whatman GD/X syringe filters with 25 mm PTFE membrane. Back extract into 10 mL of 1 mol L ⁻¹ HCl. Add 2.5 mL of 20 % w/v Mg(NO ₃) ₆ H ₂ O and 2 % w/v MgO) + 10 mL of 14 mol L ⁻¹ HNO ₃ . Evaporate to dryness at 425 °C for 12 h. Dissolve the ash in 5 mL of 6 mol L ⁻¹ HCl reduce with 5 mL reducing solution (5 % w/v KI + 5 % w/v ascorbic acid). After 30 min, filter the solution through Whatman No. 1 filter paper and dilute with 6 mol L ⁻¹ HCl. | FI-HG-AAS |
| Certifier 5 | Same approach than certifier 4 with some modifications, namely: No filtration through Whatman GD/X syringe filters was done before extracting into 1 mol L ⁻¹ HCl and no ashing step was applied; the 1 mol L ⁻¹ HCl was directly introduced in the HR-ICP-MS tuned to a resolution of at least 12,000. | HR-ICP-MS |

Table 3: 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 | 24.3 | 0.8 | 0.4 |
| Total Pb | 0.16 | 0.04 | 0.02 |
| Total As | 9.66 | 0.62 | 0.31 |
| Total Hg | 2.58 | 0.22 | 0.11 |
| Methylmercury | 1.33 | 0.12 | 0.06 |
| iAs | Not known | Not known | Not known |

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-eight laboratories from 27 countries registered to the exercise. Thirty-eight laboratories reported results for total Cd, 36 for total Pb (3 out of the 36 reported "less than" values), 33 for total As, 36 for total Hg, 5 for methylmercury and 11 for iAs (4 out of the 11 reported "less than" values).

8.2 Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z- and ζ -scores in accordance with ISO 13528².

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}} \quad \text{Eq. 1}$$

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

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
- σ is the standard deviation for proficiency assessment

The assigned reference values (X_{ref}), and their respective estimated uncertainties are summarised in Table 3.

The interpretation of the z- and ζ -score is done as follows:

| | |
|-----------------------------|-----------------------|
| $ \text{score} \leq 2$ | satisfactory result |
| $2 < \text{score} \leq 3$ | questionable result |
| $ \text{score} > 3$ | unsatisfactory result |

The ζ -score states if the laboratory result agrees with the assigned value within the respective uncertainties. The denominator of Eq. 1 is the combined uncertainty of the assigned value and the measurement uncertainty as stated by the laboratory. The ζ -score is therefore the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value (assigned value), its uncertainty and the unit of the result as well as the uncertainty of the reported

values. An unsatisfactory ζ -score can either be caused by an inappropriate estimation of the concentration or of its uncertainty.

The standard uncertainty of the laboratory, u_{lab} , was estimated by dividing the reported expanded uncertainty by the reported coverage factor, k . When no uncertainty was reported, it was set to zero ($u_{\text{lab}} = 0$). 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 $\sqrt{3}$, as recommended by Eurachem and CITAC [9].

Uncertainty estimation is not trivial; therefore an additional assessment was provided to each laboratory reporting uncertainty, indicating how reasonable their uncertainty estimate is. The standard uncertainty from the laboratory (u_{lab}) is most likely to fall in a range between a minimum uncertainty (u_{min}), and a maximum allowed (u_{max}). u_{min} is set to the standard uncertainty of the reference value. It is unlikely that a laboratory carrying out the analysis on a routine basis would measure the measurand with a smaller uncertainty than the expert laboratories chosen to establish the assigned value. u_{max} is set to the target standard deviation ($\hat{\sigma}$) accepted for the PT. If u_{lab} is smaller than u_{min} , the laboratory may have underestimated its uncertainty. Such a statement has to be taken with care as each laboratory reported only measurement uncertainty, whereas the uncertainty of the reference value also includes contributions of homogeneity and stability. If those are large, measurement uncertainties smaller than u_{min} are possible and plausible. If $u_{\text{lab}} > u_{\text{max}}$, the laboratory may have overestimated the uncertainty. An evaluation of this statement can be made when looking at the difference of the reported value and the assigned value: if the difference is small and the uncertainty is large, then overestimation is likely. If, however, the deviation is large but is covered by the uncertainty, then the uncertainty is properly assessed even if large. It should be pointed out that u_{max} is not a normative criterion: it is up to the customer of the respective result to decide which uncertainty is acceptable for a certain measurement.

The z-score compares the participant's deviation from the reference value with the target standard deviation for proficiency assessment ($\hat{\sigma}$) used as common quality criterion. $\hat{\sigma}$, is defined by the PT organiser as the maximum acceptable standard uncertainty. Based on feedback from experts, on the state-of-the-art and on discussions among the members of the advisory board of this PT, values for $\hat{\sigma}$ were set as 15 % of the assigned value for all the measurands for which z-scores were provided.

8.3 Laboratory results and scorings

The results as reported by the participants for total Cd, Pb, As, Hg, methylmercury and iAs are summarised in Annexes 6 to 11, respectively, together with the z- and ζ -scores (no scores were provided for iAs). Annexes 6 to 11 also include figures in which the individual mean values and associated expanded uncertainties are shown. The Kernel distribution plots, obtained using a software

tool developed by AMC¹⁰ are shown in Annex 12. For methylmercury and iAs no Kernel density are presented, due to the low number of results.

Two of the expert laboratories are NRLs and their results for iAs are included in Annex 11.

Regarding the z- and ζ -scores, the results for total Cd, Pb, As, Hg and methylmercury are summarised in Table 4. Taking into consideration the z-score, between 80 and 97.5 % of the laboratories performed satisfactory for total Cd, As, Hg and methylmercury. The percentage of laboratories that scored satisfactory for total Pb was 70 %. The lower concentration of total Pb in the sample in comparison with those of total Cd, As, Hg and methylmercury, could explain the lower percentage of laboratories that scored satisfactory for total Pb. When the associated uncertainties are taken into account the percentage of laboratories that scored satisfactorily was around 80 % for all the measurands but total As.

Seven laboratories out of the ten having unsatisfactory z-scores for total As underestimated the concentration of this analyte in the test material. According to the information obtained from the expert laboratories having performed speciation analyses on the test material used in IMEP-109, most of the arsenic is present in the form of arsenobetaine. It is known that digestion of arsenobetaine is difficult and requires high temperatures (more than 280 °C). Such a difficulty should not be a problem for laboratories using ICP-MS because the temperature in the plasma is high enough to mineralise arsenobetaine but could explain the unsatisfactory underestimated results reported by four laboratories using HG-AAS.

Laboratory 25 acknowledged to have reported total arsenic in the field for iAs. Their results have not been considered and consequently no scoring was provided.

Table 4: Number and percentages of laboratories reporting results not "less than" with satisfactory, questionable and unsatisfactory scores.

| | Total Cd | | Total Pb | | Total As | | Total Hg | | Methylmercury | |
|---------------------------|----------|------|----------|----|----------|----|----------|----|---------------|----|
| | N° | % | N° | % | N° | % | N° | % | N° | % |
| z | | | | | | | | | | |
| S | 37 | 97.5 | 23 | 70 | 28 | 85 | 34 | 97 | 4 | 80 |
| Q | 1 | 2.5 | 5 | 15 | 1 | 3 | 0 | 0 | 0 | 0 |
| U | 0 | 0 | 5 | 15 | 4 | 12 | 1 | 3 | 1 | 20 |
| ζ | | | | | | | | | | |
| S | 30 | 79 | 27 | 82 | 22 | 67 | 29 | 83 | 4 | 80 |
| Q | 4 | 10.5 | 2 | 6 | 1 | 3 | 4 | 11 | 0 | 0 |
| U | 4 | 10.5 | 4 | 12 | 10 | 30 | 2 | 6 | 1 | 20 |

N°: Number of laboratories, S: Satisfactory, Q: Questionable, U: Unsatisfactory.

As mentioned earlier no assigned values could be attributed for iAs and therefore no scores could be given due to the dispersion of the results provided by the expert laboratories. The similar scatter of results was observed for values reported by participants to the proficiency test. Only 7 NRLs reported values for iAs other than “less than” thus making impossible any conclusion about distribution of results. However, a parallel PT (IMEP-30) was organised using the same test material. IMEP-30 was open to all laboratories willing to take part in the exercise (while only NRLs could participate in IMEP-109). Figure 1 shows the results reported for iAs both in IMEP-109 and IMEP-30. Information about the results reported by the participants in IMEP-30 will be available from the IMEP-30 final report¹¹.

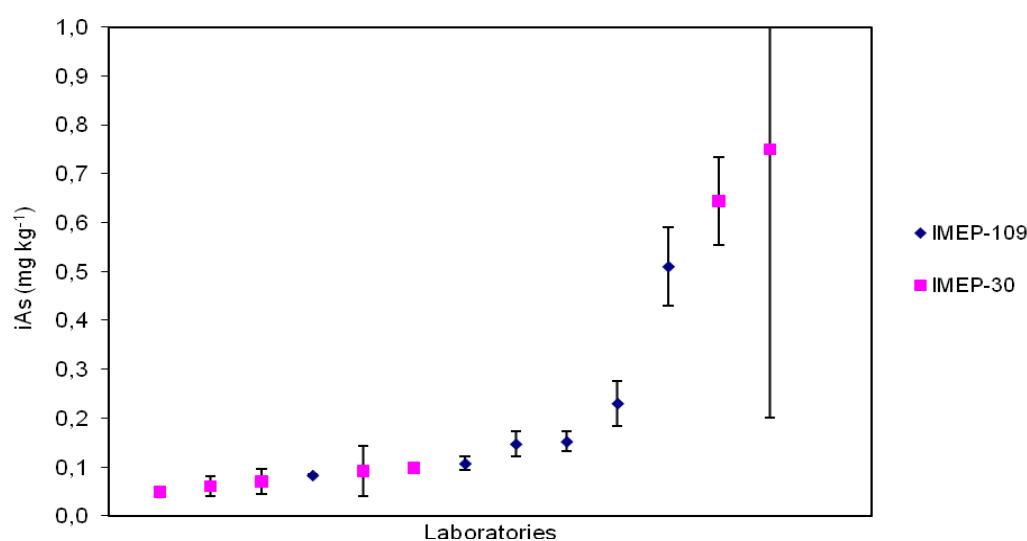


Figure 1: Results reported for iAs by participants in IMEP-109 and in IMEP-30.

Three laboratories (one in IMEP-109 and two in IMEP-30) reported values higher than 1 mg kg^{-1} (5.75 ± 0.7 , 5.29 ± 0.07 and $3.23 \pm 0.60 \text{ mg kg}^{-1}$) and are not shown in Figure 1. Four NRLs reported values “less than”, Annex 11. Similarly, four participants in IMEP-30 reported “less than” values (<0.010 , <0.040 , <0.100).

With such a scatter of results it is not possible to derive any conclusion about the concentration of iAs in this test material. However, 20 laboratories agree on the fact the percentage of iAs in this seafood is very low, between 0.5 and 2 % of the total arsenic. Eight laboratories were not able to detect it. Four participants found concentrations of iAs far above the mentioned range. It is difficult to elucidate whether these high percentages are due to a really high content of iAs in the test material, to an intrinsic problem of iAs determination in the sample, or to a poor performance of the laboratory. The laboratory taking part in IMEP-30 that reported $3.23 \pm 0.60 \text{ mg kg}^{-1}$ used the standard method EN 15517:2008. The same method was used by laboratory 35 in IMEP-109 to obtain $0.51 \pm 0.08 \text{ mg kg}^{-1}$ iAs. Such a large difference could indicate that the standard EN 15517:2008 validated for the determination of iAs in seaweed might not be adequate for seafood of animal origin. Even laboratories with large experience in this field of analysis such as certifiers 4 and 5 and which, within their

uncertainties, agreed on the concentration of iAs in rice¹, have obtained very discrepant results in IMEP-109, despite having used the same method of analysis.

8.4 Additional information extracted from the questionnaire

Additional information was gathered from the questionnaire that participants were asked to fill in (Annex 5).

8.4.1 Sample treatment related questions

Sixteen laboratories analysed total Cd, Pb, As and Hg following an official method. Only one laboratory used an official method for iAs analysis and none for the determination of methylmercury. The information provided by the laboratories for their methods of analysis for total Cd, Pb, As and Hg is summarised in Annex 13, for methylmercury in Annex 14 and for iAs in Annex 15. No Influence of the techniques used (mainly ZETAAS, HG-AAS and ICP-MS) was detected for any of the measurands covered in this PT. The low number of results submitted for methylmercury did not allow to perform any study about the influence of the method or the instrumental approach used. Regarding iAs a number of laboratories used HPLC-based methods with ICP-MS detection and some other used HG-AAS. Several laboratories followed an identical approach (see method used by certifiers 4 and 5 in Table 2), but even among them large discrepancies (up to 2-fold) were observed.

Twenty-nine laboratories corrected their results for recovery, following one of the following options (or a combination of): 20 laboratories calculated the recovery factor using a certified reference material and 11 adding a known amount of the same analyte to be measured (spiking). One laboratory added a known amount of analyte to a reagent blank, which was taken through the whole procedure. One participant answered that they do not correct the results in heavy metal analysis, one did not correct for recovery because he considered that recovery is included in the uncertainty.

Two laboratories did not correct the results for the water content, one of them because the water content was found to be negligible. The moisture content reported by the laboratories that applied a correction factor for it ranged from 0.03 up to 13 %. The way how the water content of the material was to be determined was described in detail in the accompanying letter (Annex 3) and was optimised at IRMM to obtain the same results as with Karl-Fisher titration.

8.4.2 Uncertainty related questions

Various approaches were used to scrutinise the measurement uncertainty, (Figure 2).

Twenty-eight laboratories usually report uncertainty to the customers, 8 do not and 2 did not answer to this question.

When asked about the level of confidence covered by the reported coverage factor (k), most of the participants reported 95 %, one reported 95.4 % and 11 did not reply to this question.

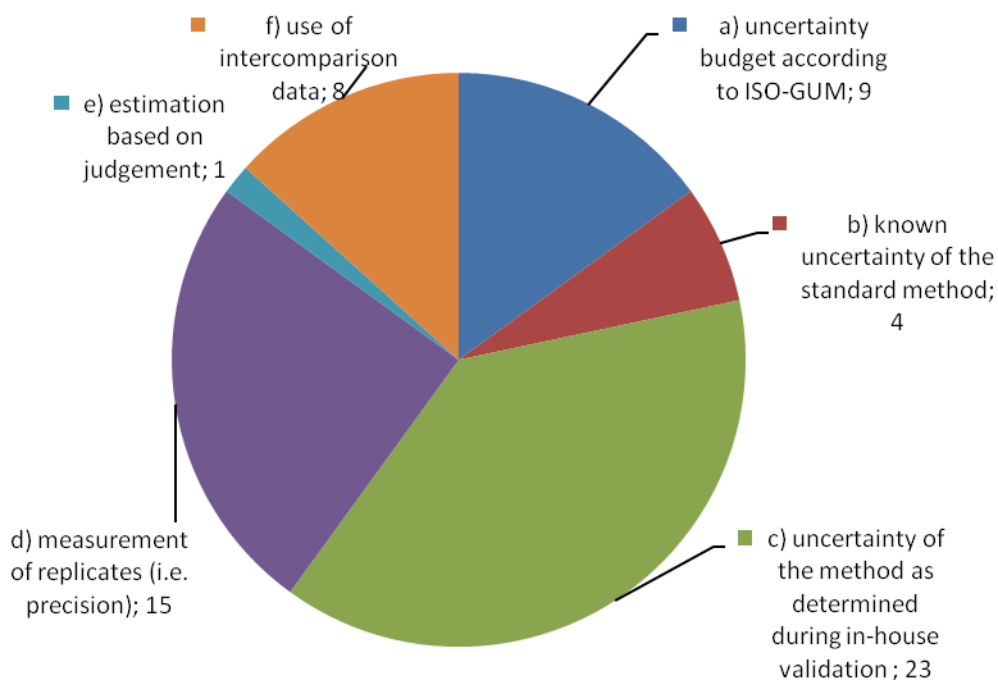


Figure 2: Different approaches used by the participants in IMEP-109 to estimate the uncertainty of their measurements.

8.4.3 Quality assurance related questions

Thirty-seven of the 38 participating laboratories regularly take part in PTs, although not all of them for all the measurands. The distribution for participation in PTs by measurand, is shown in Figure 3.

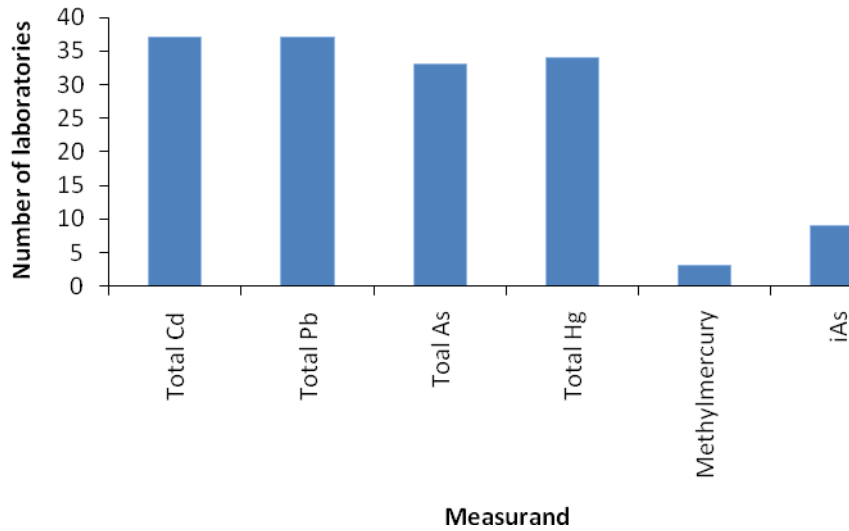


Figure 3: Numer of participants that take part regularly in PTs for the different measurands covered in IMEP-109.

Participants were asked whether they make use of CRMs in their laboratories and for which purpose (validation and/or calibration). Thirty-two use CRMs, 3 do not and 3 did not answer. Thirty-two use the CRM during the validation procedure and 10 for calibration purposes.

8.4.4 Questions related to the experience of the laboratories in this field of analysis

Only 2 laboratories perform methylmercury analysis on a routine basis (between 0 and 50 analysis per year), 5 laboratories analyse iAs routinely (4 of them between 0 and 50 samples per year and one between 50 and 250 samples per year). Thirty-four participants analyse total Cd, Pb, As and Hg on a routine basis, 1 does not and 3 did not reply to this question. The distribution in terms of number of analysis per year is shown in Figure 4.

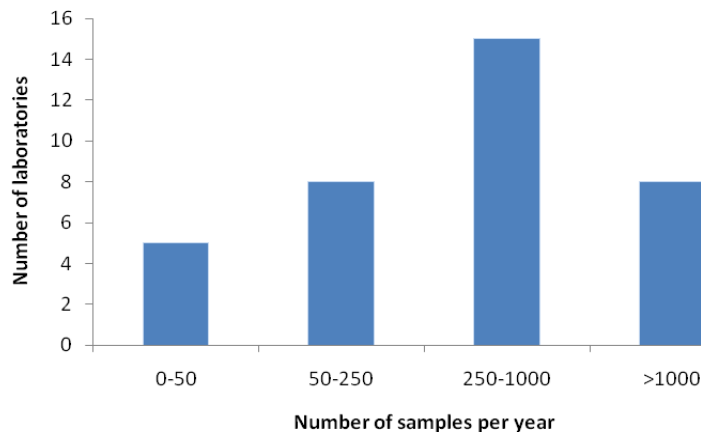


Figure 4: Participants experience in the analysis of total Cd, Pb, As and Hg expressed as number of analysis per year.

8.4.5 Quality system related questions

Thirty-six laboratories have a quality system in place. One did not reply to the question. Most of them have a quality system based on ISO 17025, three of them have a quality system also based not only on ISO 17025 but also on the ISO 9000 series.

9 Conclusions

The main conclusion that can be derived from this study is that determination of iAs in the seafood used as test material in this exercise, presents serious analytical problems, contrary to what had been observed previously in a rice matrix. The expert laboratories could not agree on a value for iAs within a reasonable uncertainty and the same dispersion of results was observed for the values found by the participants in the PT.

The results reported by the expert laboratories and by a high percentage of the participants (pooling together the results of IMEP-109 and of IMEP-30) indicate that the amount of iAs (between 0.047 to 0.75 mg kg⁻¹ which corresponds to 0.5 and 8 % of the total As respectively) in the test sample is very low, despite the high content of total As. Three participants have reported values for iAs which would account for up to 50 % of the total As. However, considering the low number of reported data it is difficult to make any statement on the results.

The second conclusion that can be drawn from MEP-109 is that, unfortunately, no real increase took place since 2008 in the number of NRLs with capacity for methylmercury analysis in seafood, with only five laboratories reporting results for this measurand. During the discussions within the NRL network on the methylmercury issue, some NRLs pointed out that their laboratories do not have the instrumentation required (hyphenated techniques) to perform methylmercury analysis. For those laboratories that find themselves in this situation, the method used by laboratory 7 (hydrolisation with hydrobromic acid followed by extraction with toluene and further separation of MeHg with cysteine, Annex 14) could be an elegant solution for the problem because it does not require the use of any chromatographic set up for the separation of methylmercury from the other mercury species.

10 Acknowledgements

P. Connely from the Reference Materials Unit is acknowledged for his support in optimising the drying method. I. Baer, F. Cordeiro are thanked for the fruitful discussions about the organisation of the IMEP-109 exercise and the thorough revision of this paper. A. M. Jensen and F. Ulberth are acknowledged for revising the manuscript.

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

| Organisation | Country |
|--|----------------|
| AGES - Austrian Agency for Health and Food Safety | Austria |
| AGES | Austria |
| Scientific Institute of Public Health | Belgium |
| CODA-CERVA | Belgium |
| Central Laboratory of Veterinary Control and Ecology | Bulgaria |
| State General Laboratory | Cyprus |
| State Veterinary Institute Olomouc | Czech republic |
| CISTA | Czech republic |
| National Food Institute (DTU Food) | Denmark |
| The Danish Plant Directorate | Denmark |
| Agricultural Research Centre | Estonia |
| Veterinary and Food Laboratory | Estonia |
| Evira | Finland |
| AFSSA | France |
| Laboratoire SCL de Bordeaux | France |
| Federal Office of Consumer Protection and Food Safety (BVL) | Germany |
| Ministry Of Rural Development And Food | Greece |
| General Chemical State Laboratory | Greece |
| Central Agricultural Office | Hungary |
| Central Agricultural Office, Food and Feed Safety Directorate | Hungary |
| Health Service Executive (HSE-South) | Ireland |
| Istituto Superiore Sanita' | Italy |
| Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta | Italy |
| Institute of Food Safety, Animal Health and Environment -BIOR | Latvia |
| National food and veterinary risk assessment institute | Lithuania |
| Public Health Laboratory Malta | Malta |
| Food and Consumer Product Safety Authority | Netherlands |
| RIKILT | Netherlands |
| National Veterinary Research Institute | Poland |
| National Institute of Public Health-National Institute of Hygiene | Poland |
| INRB, I.P./L-IPIMAR | Portugal |
| Institute of Hygiene and Veterinary Public Health | Romania |
| State veterinary and food institute - Kosice | Slovakia |
| National Veterinary Institute | Slovenia |
| Laboratorio Arbitral Agroalimentario | Spain |
| National Food Administration | Sweden |
| The Food and Environment Research Agency | United kingdom |

11 References

-
- ¹ M.B. de la Calle, T. Linsinger, H. Emteborg, J. Charoud-Got, I. Verbist, "Report of the seventh interlaboratory comparison organised by the European Union-Reference Laboratory for Heavy Metals in Feed and Food", EUR 24314 EN, (2010).
- ² ISO 13528:2005; Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons.
- ³ M.B. de la calle, D. Vendelbo, A. Bernreuther, H. Emteborg, I. Verbist, P. Taylor, "Report of the fourth interlaboratory comparison organised by the Community Reference Laboratory for Heavy Metals in Feed and Food", EUR 23505 EN, (2008).
- ⁴ Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
- ⁵ Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.
- ⁶ Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.
- ⁷ Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
- ⁸ <http://www.nrc-cnrc.gc.ca/eng/services/inms/reference-materials.html>
- ⁹ Eurachem/CITAC guide; Quantifying Uncertainty in Analytical Measurements, 2000 (www.eurachem.ul.pt).
- ¹⁰ The software to calculate Kernel densities is provided by the Statistical Subcommittee of the Analytical Methods Committee (AMC) of the Royal Society of Chemistry and described in the AMC Technical Brief "Representing data distributions with Kernel density estimates" (2006), see www.rsc.org/amc
- ¹¹ http://irmm.jrc.ec.europa.eu/html/interlaboratory_comparisons/imep/about_imep/index.htm

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Annex 1: Invitation letter



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

Geel, 21 April 2010
JRC.DDG.D6/BCa/ive/ARES(2010)207362

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

Dear Madam / Sir,

Inter-laboratory comparison for EU-RL Heavy Metals in Feed and Food

On behalf of the EU-RL Heavy Metals in Feed and Food, I would like to invite you to participate in the Proficiency Test [IMEP-109] for the "**Determination of total Cd, Pb, As, Hg and methylmercury in seafood**".

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=459>
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.**

The **deadline for registration is 12 May 2010**. Samples will be sent to participants during the second half of May. The deadline for submission of results is 18 June 2010.

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

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

Yours sincerely

A handwritten signature in black ink, appearing to read 'M.B. de la Calle', with a long horizontal flourish extending to the right.

Dr. M.B. de la Calle
Operating Manger EU-RL-HM

Cc: Franz Ulberth

Annex 2: Certificate of the CRM used in IMEP-109

National Research
Council CanadaConseil national
de recherches Canada

Certified Reference Material

DOLT-4

Dogfish Liver Certified Reference Material for Trace Metals

This reference material is primarily intended for use in the calibration of procedures and the development of methods for the analysis of marine fauna and materials with a similar matrix.

Elements for which certified values have been established for this dogfish (*Squalus acanthias*) liver CRM, along with their expanded uncertainty ($U_{\text{CRM}} = k u_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [1] and $k=2$ is the coverage factor) are listed in Table 1. It is intended that U_{CRM} encompasses every aspect that reasonably contributes to the uncertainty of the certified mass fraction [2]. Values are based on dry mass.

Table 1. Certified Values for DOLT-4

| Element | Mass Fraction (mg/kg) | |
|-----------------------------------|--------------------------|--------|
| Arsenic (d,e,h) | 9.66 | ± 0.62 |
| Cadmium (d,e,i,p) | 24.3 | ± 0.8 |
| Copper (d,e,i,p) | 31.2 | ± 1.1 |
| Iron (d,i) | 1833 | ± 75 |
| Lead (d,e,p) | 0.16 | ± 0.04 |
| Mercury (c,d,p) | 2.58 | ± 0.22 |
| Nickel (d,e,i,p) | 0.97 | ± 0.11 |
| Selenium (e,h) | 8.3 | ± 1.3 |
| Silver (d,e,p) | 0.93 | ± 0.07 |
| Zinc (d,i,p) | 116 | ± 6 |
| CH ₃ Hg (as Hg)(g,s,t) | 1.33 | ± 0.12 |

Coding

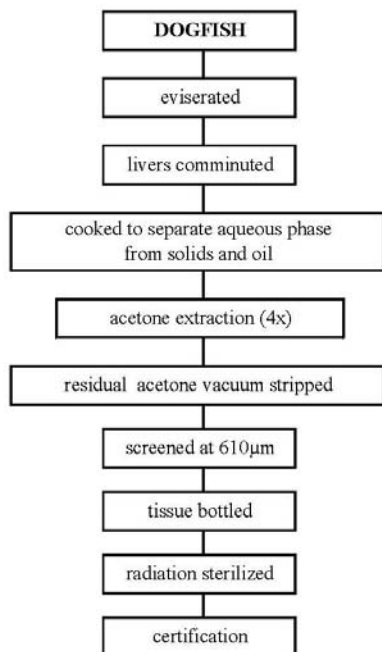
The coding refers only to the instrumental method of determination of the measurand.

- | | |
|---|---|
| c - Cold vapour atomic absorption spectrometry. | i - Inductively coupled plasma atomic emission spectrometry. |
| d - Inductively coupled plasma mass spectrometry. | p - Isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS). |
| e - Electrothermal vaporization atomic absorption spectrometry (ETAAS). | s - SPME isotope dilution gas chromatography ICPMS. |
| g - Solid phase microextraction (SPME) isotope dilution gas chromatography mass spectrometry. | t - Ethylation cold vapor atomic fluorescence spectrometry. |
| h - Hydride generation atomic absorption spectrometry. | |

NRC - CNRC

Preparation of DOLT-4

This reference material was processed at the Guelph Food Technology Center, Guelph Ontario. The preparation sequence is illustrated below.



The material was sterilized by gamma irradiation (minimum dose of 25 kGy) at the Canadian Irradiation Centre, Laval, Québec

Sampling

A sample mass of 250 mg of material (dry mass basis) is the minimum sample intake for which the established uncertainty is valid.

Instructions for Drying

Moisture content should be determined using a separate sub-sample. DOLT-4 can be dried to constant mass 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.

Information Values

Table 2 presents information values for elements which could not be certified because of insufficient information to accurately assess uncertainties.

Table 2. Information Values for DOLT-4

| Element | Mass Fraction, (mg/kg) |
|---------|------------------------|
| Na | 6800 |
| Mg | 1500 |
| Al | 200 |
| K | 9800 |
| Ca | 680 |
| V | 0.6 |
| Cr | 1.4 |
| Co | 0.25 |
| Sr | 5.5 |
| Mo | 1 |
| Sn | 0.17 |

Storage and Handling

This material should be kept in the original bottle tightly closed and stored in a cool location, away from any significant radiation sources such as ultraviolet lamps and sunlight. The contents should be well mixed by rotation and shaking prior to use, and the bottle tightly closed immediately after sampling.

Calculation of Certified Values

DOLT-4 was provided as an unknown sample to a group of laboratories participating in an annual intercomparison for trace metals in marine samples coordinated by NRCC [3]. Data generated by NRCC were also included in the pool of intercomparison results.

Laboratories were requested to provide triplicate results using an analytical method of choice based on total digestion of the sample. DOLT-3 was provided as a quality control sample.

Data were returned to NRCC for evaluation. Results from a select sub-group of participants were used for the certification of DOLT-4. Such laboratories were selected based on their performance history in previous intercomparisons.

The certified values were calculated from the unweighted means of the results. Data were first examined for outliers using the Dixon and Grubb's Tests. Testing of variances was conducted using the Cochran and Bartlett's Tests.

Included in the overall uncertainty estimate are uncertainties in the batch characterisation (u_{char}) and uncertainties related to possible between-bottle variation (u_{hom}). Expressed as standard uncertainties these components can be combined as:

$$u_{c(CRM)}^2 = u_{char}^2 + u_{hom}^2$$

Based on NRC's experience with similar materials, uncertainty components for long and short term stability were considered negligible and are thus not included in the uncertainty budget.

Results for the various uncertainty components used to calculate the certified values are summarized in Table 3.

Table 3. Statistical Data for DOLT-4

| Element | data sets | u_{char} , (mg/kg) | u_{hom} , (mg/kg) |
|--------------------|-----------|----------------------|---------------------|
| As | 10 | 0.22 | 0.21 |
| Cd | 12 | 0.25 | 0.31 |
| Cu | 10 | 0.31 | 0.46 |
| Fe | 10 | 22 | 30 |
| Pb | 8 | 0.016 | 0.013 |
| Hg | 8 | 0.014 | 0.11 |
| Ni | 9 | 0.024 | 0.049 |
| Se | 9 | 0.18 | 0.63 |
| Ag | 8 | 0.017 | 0.028 |
| Zn | 11 | 2 | 2 |
| CH ₃ Hg | 3 | 0.016 | 0.057 |

Expiration of Certificate

A predecessor CRM, DOLT-2, has been periodically analyzed for more than nine years and found to be both physically and chemically stable over this time interval. We expect similar characteristics from DOLT-4. The stability of this CRM will continue to be monitored and any significant irregularity will be posted on our web site.

The certified values for DOLT-4 are considered valid until April 2014, provided the CRM is handled and stored in accordance with instructions herein.

References

- [1] Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9, 1st ed. ISO, Geneva, Switzerland (1993).
- [2] ISO Guide 35:2006, Reference materials — General and statistical principles for certification Geneva, Switzerland (2006)
- [3] S. Willie, Twentieth Intercomparison for Trace Elements in Marine Sediments and Biological Tissues, NRC No. 50099, October 2007.

Acknowledgements

The following staff members of the Institute for National Measurement Standards, National Research Council Canada, participated in the certification: P. Maxwell, C. Scriver, L. Yang and S. Willie.

The cooperation of I. Britt and A. Mannen of the Guelph Food Technology Centre, Guelph, ON, Canada in the preparation of this material is gratefully acknowledged.

The following laboratories participated in the certification of DOLT-4:

ALS Environmental
Vancouver, B.C. V5L 1K5

Australian Institute of Marine Sciences
Queensland, 4810, Australia

Australian Nuclear Science and Technology
Organization, Environmental Science Program
Menai, N.S.W., Australia

Battelle Pacific Northwest
Sequim Bay Road, Sequim, WA 98382

Massachusetts Water Resources Authority
Central Laboratory, Winthrop, MA 02152

NOAA, National Ocean Service,
Hollings Marine Laboratory, Charleston, SC 29412

San Francisco Public Utilities Commission
Southeast Laboratory, San Francisco, CA,

Texas A. & M.
College of Veterinary Medicine
Trace Element Research Laboratory
College Station, TX 77843-4458

Texas Parks and Wildlife
Environmental Contaminants Laboratory
San Marcos, TX 78666

U.S. Customs Laboratory
Savannah, GA 31408

USGS-WRD
3039 Amwiler Road, Atlanta, GA 30360-2824

Updates

Users of this material should ensure that the certificate in their possession is current. Please consult our web site at http://inms-ienm.nrc-cnrc.gc.ca/calserv/chemical_metrology_e.html for any new information.

As additional data become available, the certified values may be updated and reliable values assigned to additional measurements.

Certificate issued May 2008.

The results presented 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. NRCC CRM's are 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 Canada
Institute for National Measurement Standards
M-12, 1500 Montreal Road
Ottawa, Ontario, Canada K1A 0R6**

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

Également disponible en français sur demande.

Canada

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

Geel, May 2010
D04-IM(2008)BdlC/ive/D/26282

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

Participation to IMEP-109, a proficiency test exercise for the "Determination of total Cd, Pb, As and Hg as well as methylmercury and inorganic arsenic in seafood".

Dear «TITLE» «SURNAME»,

Thank you for participating in the IMEP-109 intercomparison for the determination of **total Cd, Pb, As and Hg as well as methylmercury and inorganic arsenic 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 (not more than 18 °C) until analysis.

The measurands are: **total Cd, Pb, As and Hg as well as methylmercury and inorganic arsenic in seafood**.

We would like to take this opportunity to continue the study on inorganic As determination that was initiated with IMEP-107 (Total and inorganic As in rice) and to extend it to a new type of food matrix, in this case seafood, which is known to be one of the main contributors of As to the human diet. For this reason, I would appreciate if those of you with measurement capabilities for inorganic As (certainly those that reported values for inorganic As in IMEP-107) make again an effort in helping us to elucidate whether the content of inorganic As found in food commodities is method dependent or not.

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

«PARTKEY»

For the determinations of all the covered measurands use a method that resembles 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 report the **corrected mean** on the reporting website. The results should be reported in the same form (e.g., number of significant figures) as those normally reported to the customer.

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 10 ± 1 min at 80 ± 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!**

Note 3: **it is crucial that you respect the procedure described above to determine the water content. If you warm up the test material longer than 10 min or at temperatures higher than 80 °C, losses of volatile compounds other than water will occur.**

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

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 measurand the **mean** of your two or three measurement results, the **uncertainty of the mean**, the **coverage factor** and the **technique** you used. After entering all results, please complete also the relating questionnaire. Do not forget to save, 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 15/06/2010.

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

A handwritten signature in black ink, appearing to read 'M.B. de la Calle', with a long horizontal line extending to the right.

Dr. M.B. de la Calle
IMEP-109 Co-ordinator

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

Cc: F. Ulberth

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

Annex to JRC.DDG.D6/BCa/ive/ARES(2010)/265235

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

CRL-HM-09 / IMEP-109

total Cd, Pb, As and Hg as well as MeHg and inorganic As 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-109 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

Annex 5: Questionnaire

Comparison for IMEP-109

This questionnaire is offline.

Please fill in the questionnaire.

Submission Form

Recovery factor (R, in %)

What are the recovery factors you applied to correct your measurement results? Please complete the table below.

| Questions/Response table | As | Cd | Pb | Hg | MeHg | Inorganic As |
|--------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| R (%) | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |

1. How did you determine the recovery factor (R)? By:

a) adding a known amount of the same analyte to be measured (spiking)

b) using a certified reference material

c) other

1.1. If other, please specify:

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

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

a) uncertainty budget according to ISO-GUM

b) known uncertainty of hte 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

3.1. If other, please specify

4. Do you usually provide an uncertainty statement to your customer 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 determine MeHg according to an official method?

No

Yes

6.1. If yes, which one?

6.2. If no, please describe in max 150 characters your :

6.2.1. sample pre-treatment

6.2.2. digestion step

6.2.3. extraction / separation step

6.2.4. derivatization

6.2.5. instrument calibration

7. Did you determine inorganic As according to an official method?

- No
 Yes

7.1. If yes, which one?

7.2. If no, please describe in max 150 characters your:

7.2.1. sample pre-treatment

7.2.2. digestion step

7.2.3. extraction / separation step

7.2.4. instrument calibration step

8. Did you determine total As, Cd, Pb and Hg according to an official method?

- No
 Yes

8.1. If yes, which one?

8.2. If no, please describe in max 150 characters your:

8.2.1. sample pre-treatment

8.2.2. digestion step

8.2.3. extraction / separation step

8.2.4. instrument calibration step

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

- No
 Yes

9.1. 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

10. Does your laboratory carry out inorganic As analysis on a routine basis?

- No
 Yes

10.1. 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

11. Does your laboratory carry out total As, Cd, Pb and Hg analysis on a routine basis?

- No
 Yes

11.1. 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

12. Does your laboratory have a quality system in place?

No
 Yes

12.1. If yes, which:

ISO 17025
 ISO 9000 series
 Other

12.1.1. If other, please specify:

13. Does your laboratory take part in an interlaboratory comparison on a regular basis for the analysis of: (multiple answers possible)

MeHg
 inorganic As
 total As
 total Cd
 total Hg
 total Pb

13.1. Which ILC scheme(s)?

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

No
 Yes

14.1. If yes, which one?

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

No
 Yes

14.3. Is the material used for calibration of instruments?

No
 Yes

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

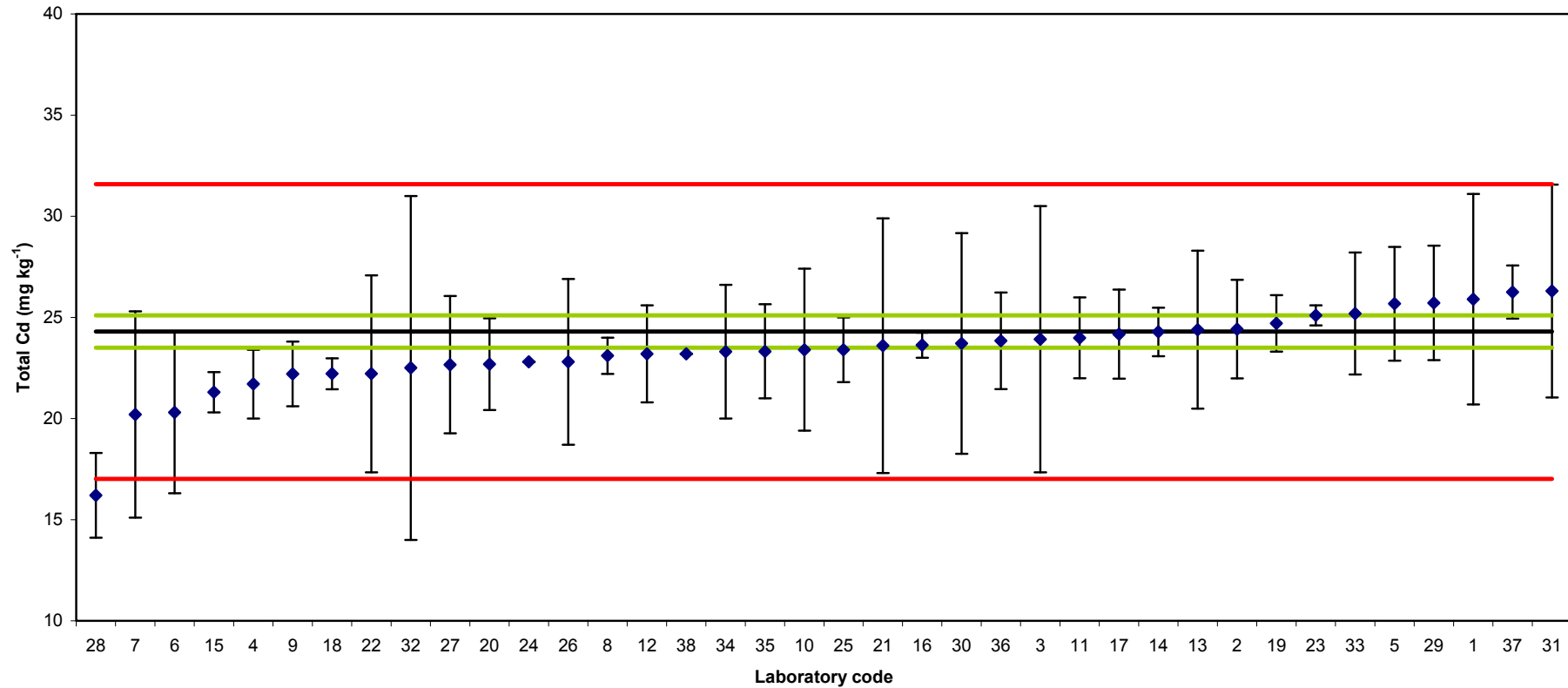
Annex 6: Total Cd in seafood

$X_{ref} = 24.3 \pm 0.8 \text{ mg kg}^{-1}$ (k=2)

| Lab ID | x_{lab} (mg kg ⁻¹) | u_{lab} (mg kg ⁻¹) | k | u_{lab} (mg kg ⁻¹) | Technique | z | ζ | Qual _u |
|--------|----------------------------------|----------------------------------|------|----------------------------------|-----------|------|---------|-------------------|
| 1 | 25,9 | 5,2 | 2 | 2,6 | ICP-MS | 0,4 | 0,6 | a |
| 2 | 24,42 | 2,44 | 2 | 1,22 | ETAAS | 0,0 | 0,1 | a |
| 3 | 23,92 | 6,578 | 2 | 3,289 | ICP-MS | -0,1 | -0,1 | a |
| 4 | 21,7 | 1,7 | 2 | 0,9 | ETAAS | -0,7 | -2,8 | a |
| 5 | 25,67 | 2,81 | 2 | 1,41 | AAS | 0,4 | 0,9 | a |
| 6 | 20,3 | 4 | √3 | 2,3 | ICP-OES | -1,1 | -1,7 | a |
| 7 | 20,2 | 5,1 | √3 | 2,9 | ETAAS | -1,1 | -1,4 | a |
| 8 | 23,1 | 0,9 | √3 | 0,5 | FAAS | -0,3 | -1,8 | a |
| 9 | 22,2 | 1,6 | 2 | 0,8 | ETAAS | -0,6 | -2,3 | a |
| 10 | 23,4 | 4 | 2 | 2 | ICP-MS | -0,2 | -0,4 | a |
| 11 | 23,99 | 2,0 | 2 | 1,0 | ETAAS | -0,1 | -0,3 | a |
| 12 | 23,2 | 2,4 | 2 | 1,2 | ICP-MS | -0,3 | -0,9 | a |
| 13 | 24,39 | 3,90 | 2 | 1,95 | ICP-MS | 0,0 | 0,0 | a |
| 14 | 24,28 | 1,2 | 2 | 0,6 | ICP-MS | 0,0 | 0,0 | a |
| 15 | 21,3 | 1,0 | 2 | 0,5 | ICP-MS | -0,8 | -4,7 | a |
| 16 | 23,625 | 0,622 | 3,18 | 0,196 | FAAS | -0,2 | -1,5 | a |
| 17 | 24,169 | 2,2 | 2 | 1,1 | ETAAS | 0,0 | -0,1 | a |
| 18 | 22,21 | 0,762 | √3 | 0,440 | ICP-MS | -0,6 | -3,5 | a |
| 19 | 24,7 | 1,4 | 2 | 0,7 | | 0,1 | 0,5 | a |
| 20 | 22,69 | 2,27 | 2 | 1,14 | ETAAS | -0,4 | -1,3 | a |
| 21 | 23,6 | 6,3 | 2 | 3,2 | ICP-MS | -0,2 | -0,2 | a |
| 22 | 22,21 | 4,87 | 2 | 2,44 | ETAAS | -0,6 | -0,8 | a |
| 23 | 25,1 | 0,5 | 2 | 0,3 | ICP-MS | 0,2 | 1,7 | b |
| 24 | 22,8 | 0,004 | 2 | 0,002 | ICP-MS | -0,4 | -3,7 | b |
| 25 | 23,4 | 1,6 | 2 | 0,8 | ICP-MS | -0,2 | -1,0 | a |
| 26 | 22,8 | 4,10 | 2 | 2,05 | ICP-OES | -0,4 | -0,7 | a |
| 27 | 22,66 | 3,40 | 2,02 | 1,68 | ZETA-AAS | -0,4 | -0,9 | a |
| 28 | 16,2 | 2,1 | 2 | 1,1 | ICP-MS | -2,2 | -7,2 | a |
| 29 | 25,71 | 2,83 | 2 | 1,42 | ETAAS | 0,4 | 1,0 | a |
| 30 | 23,71 | 5,45 | 2 | 2,73 | ETAAS | -0,2 | -0,2 | a |
| 31 | 26,3 | 5,26 | 2 | 2,63 | ICP-MS | 0,5 | 0,8 | a |
| 32 | 22,5 | 8,5 | 2 | 4,3 | ICP-MS | -0,5 | -0,4 | c |
| 33 | 25,19 | 3,02 | 2 | 1,51 | ETAAS | 0,2 | 0,6 | a |
| 34 | 23,3 | 3,3 | 2 | 1,7 | ETAAS | -0,3 | -0,6 | a |
| 35 | 23,32 | 2,33 | 2 | 1,17 | ETAAS | -0,3 | -0,8 | a |
| 36 | 23,84 | 2,38 | 2 | 1,19 | ICP-MS | -0,1 | -0,4 | a |
| 37 | 26,25 | 1,31 | 2 | 0,66 | ICP-MS | 0,5 | 2,5 | a |
| 38 | 23,20 | 0 | √3 | 0 | ETAAS | -0,3 | -2,8 | b |

Qual_u: qualitative information about u_{lab} : **a**: $u_{ref} < u_{lab} < \hat{\sigma}$; **b**: $u_{lab} < u_{ref}$; **c**: $\hat{\sigma} < u_{lab}$. For further information on these codes, please read chapter 8.2.

IMEP-109: results for total Cd
 Certified range: $24.3 \pm 0.8 \text{ mg kg}^{-1}$ ($k=2$)



This graph displays all measurements results and their associated uncertainties.
 The uncertainties are shown as reported, with various expansion factors and levels of confidence.
 The black line represents X_{ref} , the green lines delimit the reference interval ($X_{\text{ref}} \pm 2u_{\text{ref}}$: $24.3 \pm 0.8 \text{ mg kg}^{-1}$), the red lines delimit the target interval ($X_{\text{ref}} \pm 2\sigma$: $24.3 \pm 7.3 \text{ mg kg}^{-1}$)



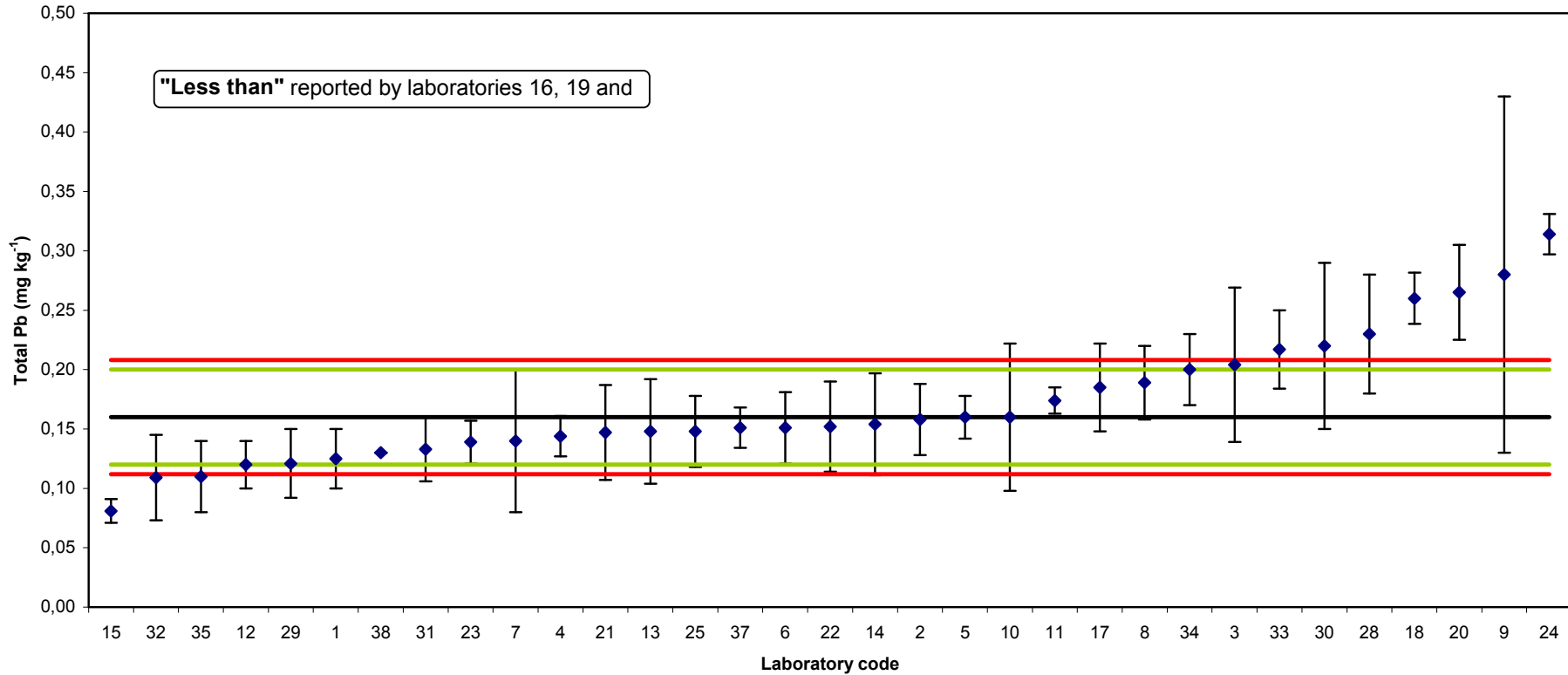
Annex 7: Total Pb in seafood

$$X_{\text{ref}} = 0.16 \pm 0.04 \text{ mg kg}^{-1} \text{ (k=2)}$$

| Lab ID | x_{lab} (mg kg ⁻¹) | u_{lab} (mg kg ⁻¹) | k | u_{lab} (mg kg ⁻¹) | Technique | z | ζ | Qual _u |
|--------|---|---|----|---|-----------|------|---------|-------------------|
| 1 | 0,125 | 0,025 | 2 | 0,013 | ICP-MS | -1,5 | -1,5 | b |
| 2 | 0,158 | 0,030 | 2 | 0,015 | ETAAS | -0,1 | -0,1 | b |
| 3 | 0,204 | 0,065 | 2 | 0,033 | ICP-MS | 1,8 | 1,2 | c |
| 4 | 0,144 | 0,017 | 2 | 0,009 | ICP-MS | -0,7 | -0,7 | b |
| 5 | 0,160 | 0,018 | 2 | 0,009 | AAS | 0,0 | 0,0 | b |
| 6 | 0,151 | 0,030 | √3 | 0,017 | ICP-OES | -0,4 | -0,3 | b |
| 7 | 0,14 | 0,06 | √3 | 0,035 | ETAAS | -0,8 | -0,5 | c |
| 8 | 0,189 | 0,031 | √3 | 0,018 | ETAAS | 1,2 | 1,1 | b |
| 9 | 0,28 | 0,15 | 2 | 0,08 | ETAAS | 5,0 | 1,5 | c |
| 10 | 0,16 | 0,062 | 2 | 0,031 | ICP-MS | 0,0 | 0,0 | c |
| 11 | 0,174 | 0,011 | 2 | 0,006 | ETAAS | 0,6 | 0,7 | b |
| 12 | 0,12 | 0,02 | 2 | 0,01 | ICP-MS | -1,7 | -1,8 | b |
| 13 | 0,148 | 0,044 | 2 | 0,022 | ICP-MS | -0,5 | -0,4 | a |
| 14 | 0,154 | 0,043 | 2 | 0,022 | ICP-MS | -0,3 | -0,2 | a |
| 15 | 0,081 | 0,010 | 2 | 0,005 | ICP-MS | -3,3 | -3,8 | b |
| 16 | <0,1 | | | | FAAS | | | |
| 17 | 0,185 | 0,037 | 2 | 0,019 | FAAS | 1,0 | 0,9 | b |
| 18 | 0,260 | 0,0216 | √3 | 0,0125 | ICP-MS | 4,2 | 4,2 | b |
| 19 | <0,5 | | | | | | | |
| 20 | 0,265 | 0,04 | 2 | 0,02 | ETAAS | 4,4 | 3,7 | a |
| 21 | 0,147 | 0,04 | 2 | 0,02 | ICP-MS | -0,5 | -0,5 | a |
| 22 | 0,152 | 0,038 | 2 | 0,019 | ETAAS | -0,3 | -0,3 | b |
| 23 | 0,139 | 0,018 | 2 | 0,009 | ICP-MS | -0,9 | -1,0 | b |
| 24 | 0,314 | 0,017 | 2 | 0,009 | ICP-MS | 6,4 | 7,1 | b |
| 25 | 0,148 | 0,03 | 2 | 0,02 | ICP-MS | -0,5 | -0,5 | a |
| 28 | 0,23 | 0,05 | 2 | 0,03 | ICP-MS | 2,9 | 2,2 | c |
| 29 | 0,121 | 0,029 | 2 | 0,015 | ETAAS | -1,6 | -1,6 | b |
| 30 | 0,22 | 0,07 | 2 | 0,04 | ETAAS | 2,5 | 1,5 | c |
| 31 | 0,133 | 0,027 | 2 | 0,014 | ICP-MS | -1,1 | -1,1 | b |
| 32 | 0,109 | 0,036 | 2 | 0,018 | ICP-MS | -2,1 | -1,9 | b |
| 33 | 0,217 | 0,033 | 2 | 0,017 | ETAAS | 2,4 | 2,2 | b |
| 34 | 0,20 | 0,03 | 2 | 0,02 | ETAAS | 1,7 | 1,6 | a |
| 35 | 0,11 | 0,03 | 2 | 0,02 | ETAAS | -2,1 | -2,0 | a |
| 36 | <0,3 | | | | ICP-MS | | | |
| 37 | 0,151 | 0,017 | 2 | 0,009 | ICP-MS | -0,4 | -0,4 | b |
| 38 | 0,13 | 0 | √3 | 0 | ETAAS | -1,3 | -1,5 | b |

Qual_u: qualitative information about u_{lab} : **a**: $u_{\text{ref}} < u_{\text{lab}} < \hat{\sigma}$; **b**: $u_{\text{lab}} < u_{\text{ref}}$; **c**: $\hat{\sigma} < u_{\text{lab}}$. For further information on these codes, please read chapter 8.2.

IMEP-109: results for total Pb
 Certified range: $0.16 \pm 0.04 \text{ mg kg}^{-1}$ ($k=2$)



This graph displays all measurements results and their associated uncertainties.
 The uncertainties are shown as reported, with various expansion factors and levels of confidence.
 The black line represents X_{ref} , the green lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$: $0.16 \pm 0.04 \text{ mg kg}^{-1}$), the red lines delimit the target interval ($X_{ref} \pm 2\sigma$: $0.16 \pm 0.05 \text{ mg kg}^{-1}$)



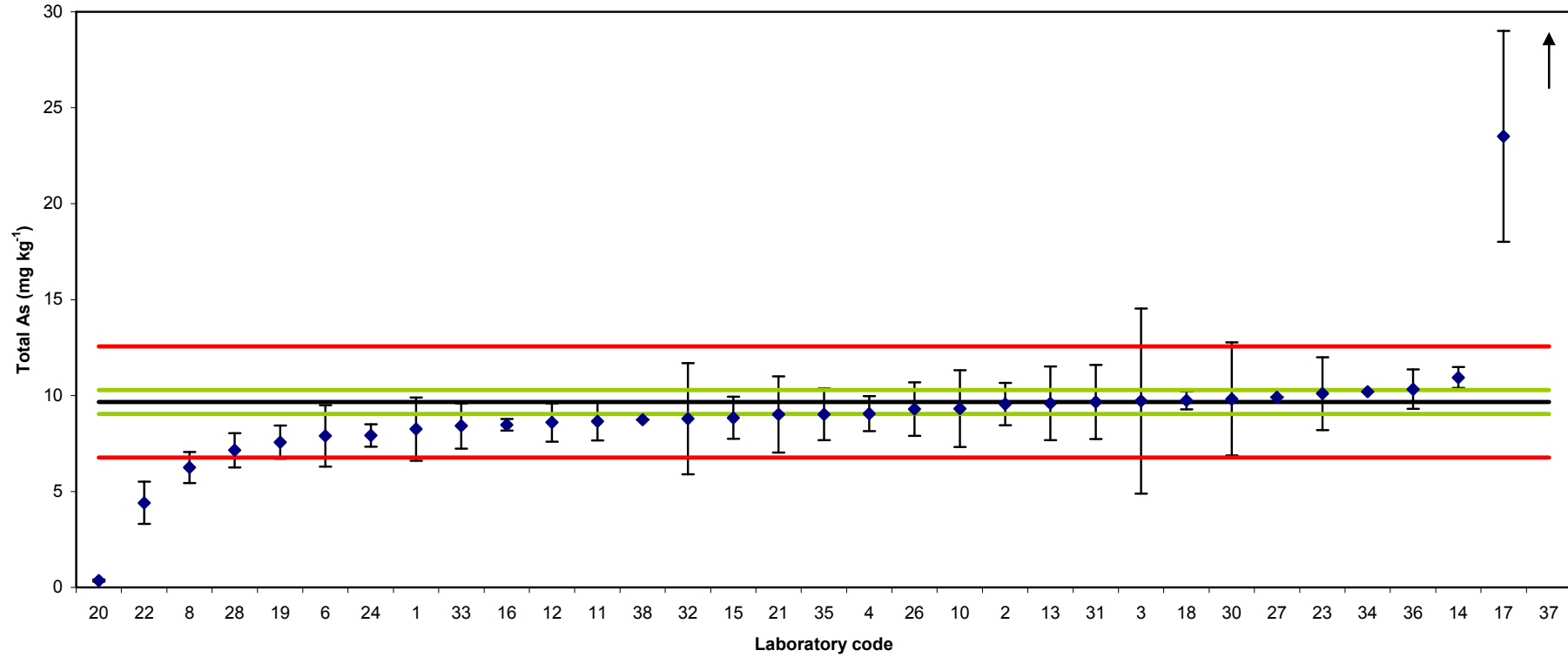
Annex 8: Total As in seafood

$X_{ref} = 9.66 \pm 0.62 \text{ mg kg}^{-1}$ ($k=2$)

| Lab ID | x_{lab} (mg kg ⁻¹) | u_{lab} (mg kg ⁻¹) | k | u_{lab} (mg kg ⁻¹) | Technique | z | ζ | Qual _u |
|--------|----------------------------------|----------------------------------|------|----------------------------------|-----------|-------|---------|-------------------|
| 1 | 8,25 | 1,65 | 2 | 0,83 | ICP-MS | -1,0 | -1,6 | a |
| 2 | 9,552 | 1,106 | 2 | 0,553 | ETAAS | -0,1 | -0,2 | b |
| 3 | 9,713 | 4,83 | 2 | 2,415 | ICP-MS | 0,0 | 0,0 | c |
| 4 | 9,06 | 0,91 | 2 | 0,46 | HG-AAS | -0,4 | -1,1 | b |
| 6 | 7,9 | 1,6 | √3 | 0,9 | ICP-OES | -1,2 | -1,8 | a |
| 8 | 6,25 | 0,81 | √3 | 0,47 | HG-AAS | -2,4 | -6,1 | b |
| 10 | 9,32 | 2,0 | 2 | 1,0 | ICP-MS | -0,2 | -0,3 | a |
| 11 | 8,66 | 1,0 | 2 | 0,5 | ICP-MS | -0,7 | -1,7 | b |
| 12 | 8,6 | 1,0 | 2 | 0,5 | ICP-MS | -0,7 | -1,8 | b |
| 13 | 9,60 | 1,92 | 2 | 0,96 | ICP-MS | 0,0 | -0,1 | a |
| 14 | 10,94 | 0,55 | 2 | 0,28 | ICP-MS | 0,9 | 3,1 | b |
| 15 | 8,84 | 1,10 | 2 | 0,55 | ICP-MS | -0,6 | -1,3 | b |
| 16 | 8,474 | 0,306 | 3,18 | 0,096 | HG-AAS | -0,8 | -3,7 | b |
| 17 | 23,511 | 5,5 | 2 | 2,750 | HG-AAS | 9,6 | 5,0 | c |
| 18 | 9,75 | 0,473 | √3 | 0,273 | ICP-MS | 0,1 | 0,2 | b |
| 19 | 7,57 | 0,86 | 2 | 0,43 | | -1,4 | -3,9 | b |
| 20 | 0,356 | 0,053 | 2 | 0,027 | HG-AAS | -6,4 | -29,9 | b |
| 21 | 9,02 | 1,99 | 2 | 1,00 | ICP-MS | -0,4 | -0,6 | a |
| 22 | 4,410 | 1,103 | 2 | 0,552 | HG-AAS | -3,6 | -8,3 | b |
| 23 | 10,1 | 1,9 | 2 | 1,0 | ICP-MS | 0,3 | 0,4 | a |
| 24 | 7,92 | 0,584 | 2 | 0,292 | ICP-MS | -1,2 | -4,1 | b |
| 26 | 9,29 | 1,39 | 2 | 0,70 | HG-AAS | -0,3 | -0,5 | a |
| 27 | 9,91 | 0 | √3 | 0 | ZETA-AAS | 0,2 | 0,8 | b |
| 28 | 7,15 | 0,89 | 2 | 0,45 | ICP-MS | -1,7 | -4,6 | b |
| 30 | 9,82 | 2,95 | 2 | 1,48 | ETAAS | 0,1 | 0,1 | b |
| 31 | 9,66 | 1,93 | 2 | 0,97 | ICP-MS | 0,0 | 0,0 | b |
| 32 | 8,8 | 2,9 | 2 | 1,5 | ICP-MS | -0,6 | -0,6 | b |
| 33 | 8,415 | 1,178 | 2 | 0,589 | HG-AAS | -0,9 | -1,9 | b |
| 34 | 10,2 | 0 | √3 | 0 | ETAAS | 0,4 | 1,7 | b |
| 35 | 9,02 | 1,35 | 2 | 0,68 | ETAAS | -0,4 | -0,9 | a |
| 36 | 10,33 | 1,03 | 2,00 | 0,52 | ICP-MS | 0,5 | 1,1 | b |
| 37 | 1447 | 176 | 2 | 88 | HG-AAS | 992,0 | 16,3 | c |
| 38 | 8,74 | 0 | √3 | 0 | ETAAS | -0,6 | -3,0 | b |

Qual_u: qualitative information about u_{lab} : **a**: $u_{ref} < u_{lab} < \hat{\sigma}$; **b**: $u_{lab} < u_{ref}$; **c**: $\hat{\sigma} < u_{lab}$. For further information on these codes, please read chapter 8.2.

IMEP-109: results for total As
 Certified range: $9.66 \pm 0.62 \text{ mg kg}^{-1}$ ($k=2$)



This graph displays all measurements results and their associated uncertainties.
 The uncertainties are shown as reported, with various expansion factors and levels of confidence.
 The black line represents X_{ref} , the green lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$: $9.66 \pm 0.62 \text{ mg kg}^{-1}$), the red lines delimit the target interval ($X_{ref} \pm 2\sigma$: $9.66 \pm 2.90 \text{ mg kg}^{-1}$)



Annex 9: Total Hg in seafood

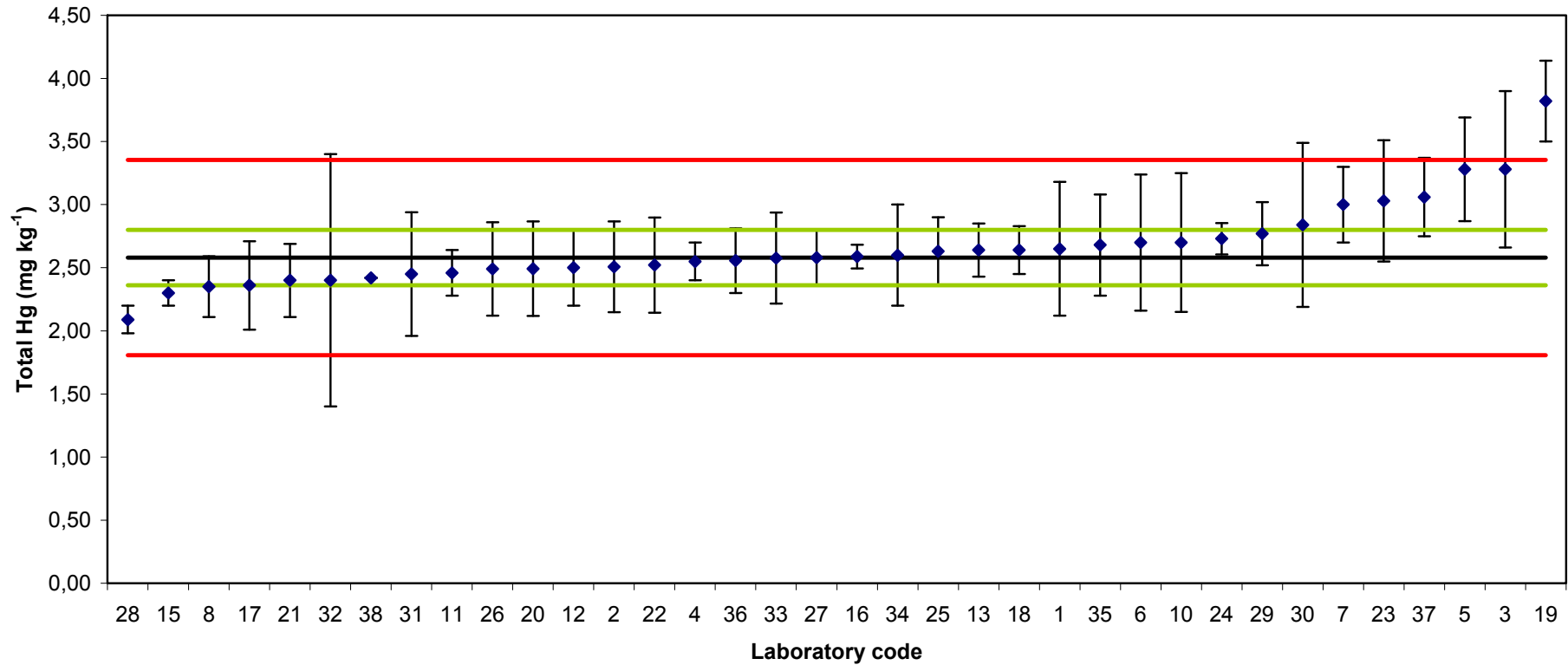
$X_{\text{ref}} = 2.58 \pm 0.22 \text{ mg kg}^{-1}$ (k=2)

| Lab ID | x_{lab} (mg kg ⁻¹) | u_{lab} (mg kg ⁻¹) | k | u_{lab} (mg kg ⁻¹) | Technique | z | ζ | Qual _u |
|--------|---|---|------|---|--|------|---------|-------------------|
| 1 | 2,65 | 0,53 | 2 | 0,27 | ICP-MS | 0,2 | 0,2 | a |
| 2 | 2,507 | 0,360 | 2 | 0,180 | CV-AAS | -0,2 | -0,3 | a |
| 3 | 3,280 | 0,62 | 2 | 0,31 | ICP-MS | 1,8 | 2,1 | a |
| 4 | 2,55 | 0,15 | 2 | 0,08 | CV-AAS | -0,1 | -0,2 | b |
| 5 | 3,28 | 0,41 | 2 | 0,21 | CV-AAS | 1,8 | 3,0 | a |
| 6 | 2,7 | 0,54 | √3 | 0,31 | Atomic Absorption by Amalgamation (AMA) | 0,3 | 0,4 | a |
| 7 | 3,0 | 0,3 | √3 | 0,2 | AAS with amalgamation (LECO) | 1,1 | 2,0 | a |
| 8 | 2,35 | 0,24 | √3 | 0,14 | CV-AAS | -0,6 | -1,3 | a |
| 10 | 2,7 | 0,55 | 2 | 0,28 | CV-AAS | 0,3 | 0,4 | a |
| 11 | 2,46 | 0,18 | 2 | 0,09 | AMA | -0,3 | -0,8 | b |
| 12 | 2,5 | 0,3 | 2 | 0,2 | ICP-MS | -0,2 | -0,4 | a |
| 13 | 2,64 | 0,21 | 2 | 0,11 | CV-AAS | 0,2 | 0,4 | a |
| 15 | 2,30 | 0,10 | 2 | 0,05 | ICP-MS | -0,7 | -2,3 | b |
| 16 | 2,589 | 0,094 | 2 | 0,047 | CV-AAS | 0,0 | 0,1 | b |
| 17 | 2,36 | 0,35 | 2 | 0,18 | CV-AAS | -0,6 | -1,1 | a |
| 18 | 2,64 | 0,189 | √3 | 0,109 | Atomic absorption spectrophotometer for mercury analysis (AMA) | 0,2 | 0,4 | a |
| 19 | 3,82 | 0,32 | 2 | 0,16 | | 3,2 | 6,4 | a |
| 20 | 2,493 | 0,374 | 2 | 0,187 | CV-AAS | -0,2 | -0,4 | a |
| 21 | 2,40 | 0,29 | 2 | 0,15 | AMA | -0,5 | -1,0 | a |
| 22 | 2,521 | 0,378 | 2 | 0,189 | AAS - AMA 254 | -0,2 | -0,3 | a |
| 23 | 3,03 | 0,48 | 2 | 0,24 | ICP-MS | 1,2 | 1,7 | a |
| 24 | 2,73 | 0,125 | 2 | 0,063 | ICP-MS | 0,4 | 1,2 | b |
| 25 | 2,63 | 0,27 | 2 | 0,14 | HG-AAS | 0,1 | 0,3 | a |
| 26 | 2,49 | 0,37 | 2 | 0,19 | CV-AAS | -0,2 | -0,4 | a |
| 27 | 2,580 | 0,224 | 2,01 | 0,111 | TDA-AAS | 0,0 | 0,0 | a |
| 28 | 2,09 | 0,11 | 2 | 0,06 | ICP-MS | -1,3 | -4,0 | b |
| 29 | 2,77 | 0,25 | 2 | 0,13 | CV-AAS | 0,5 | 1,1 | a |
| 30 | 2,84 | 0,65 | 2 | 0,33 | Hg analyser, fluorescence AAS | 0,7 | 0,8 | a |
| 31 | 2,45 | 0,49 | 2 | 0,25 | CV-AAS | -0,3 | -0,5 | a |
| 32 | 2,4 | 1,0 | 2 | 0,5 | ICP-MS | -0,5 | -0,4 | c |
| 33 | 2,577 | 0,361 | 2 | 0,181 | CV-AAS | 0,0 | 0,0 | a |
| 34 | 2,6 | 0,4 | 2 | 0,2 | CV-AAS | 0,1 | 0,1 | a |
| 35 | 2,68 | 0,40 | 2 | 0,20 | HG-AAS | 0,3 | 0,4 | a |
| 36 | 2,557 | 0,256 | 2 | 0,128 | AMA-254 | -0,1 | -0,1 | a |
| 37 | 3,06 | 0,31 | 2 | 0,16 | ICP-MS | 1,2 | 2,5 | a |
| 38 | 2,42 | 0 | √3 | 0 | Cold vapour fluorescence spectrometry | -0,4 | -1,5 | b |

Qual_u: qualitative information about u_{lab} : **a**: $u_{\text{ref}} < u_{\text{lab}} < \hat{\sigma}$; **b**: $u_{\text{lab}} < u_{\text{ref}}$; **c**: $\hat{\sigma} < u_{\text{lab}}$. For further information on these codes, please read chapter 8.2.

IMEP-109: results for total Hg

Certified range: $2.58 \pm 0.22 \text{ mg kg}^{-1}$ ($k=2$)



This graph displays all measurements results and their associated uncertainties.

The uncertainties are shown as reported, with various expansion factors and levels of confidence.

The black line represents X_{ref} , the green lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$: $2.58 \pm 0.22 \text{ mg kg}^{-1}$), the red lines delimit the target interval ($X_{ref} \pm 2\sigma$: $2.58 \pm 0.78 \text{ mg kg}^{-1}$)



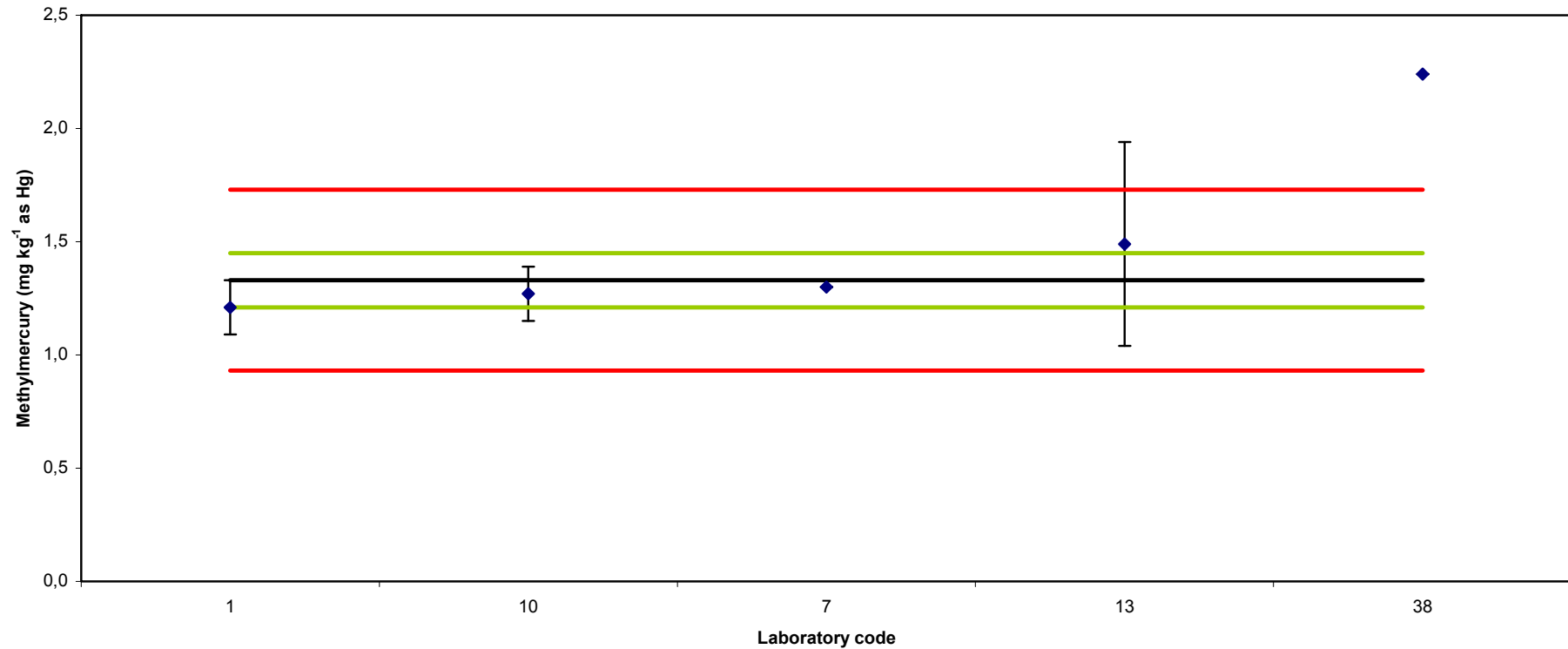
Annex 10: Methylmercury Hg in seafood

$X_{\text{ref}} = 1.33 \pm 0.12 \text{ mg kg}^{-1}$ (k=2)

| Lab ID | $x_{\text{lab}} \text{ (mg kg}^{-1}\text{)}$ | $U_{\text{lab}} \text{ (mg kg}^{-1}\text{)}$ | k | $u_{\text{lab}} \text{ (mg kg}^{-1}\text{)}$ | Technique | z | ζ | Qual _u |
|--------|--|--|------------|--|--|------|---------|-------------------|
| 1 | 1,21 | 0,12 | 2 | 0,06 | GC/ID-ICPMS | -0,6 | -1,4 | a |
| 7 | 1,3 | 0 | $\sqrt{3}$ | 0 | Extraction with toluene, followed by MeHg remotion with cysteine, followed by AAS with amalgamation (LECO) | -0,2 | -0,5 | b |
| 10 | 1,27 | 0,12 | 2 | 0,06 | GC-MS | -0,3 | -0,7 | a |
| 13 | 1,49 | 0,45 | 2 | 0,23 | LC-ICP-MS | 0,8 | 0,7 | c |
| 38 | 2,24 | 0 | $\sqrt{3}$ | 0 | HPLC ICPMS | 4,6 | 15,2 | b |

Qual_u: qualitative information about u_{lab} : **a**: $u_{\text{ref}} < u_{\text{lab}} < \hat{\sigma}$; **b**: $u_{\text{lab}} < u_{\text{ref}}$; **c**: $\hat{\sigma} < u_{\text{lab}}$. For further information on these codes, please read chapter 8.2.

IMEP-109: results for methylmercury.
Certified range: $1.33 \pm 0.12 \text{ mg kg}^{-1}$ ($k=2$)



This graph displays all measurements results and their associated uncertainties.

The uncertainties are shown as reported, with various expansion factors and levels of confidence.

The black line represents X_{ref} , the green lines delimit the reference interval ($X_{\text{ref}} \pm 2u_{\text{ref}}$: $1.33 \pm 0.12 \text{ mg kg}^{-1}$), the red lines delimit the target interval ($X_{\text{ref}} \pm 2\sigma$: $1.33 \pm 0.40 \text{ mg kg}^{-1}$)

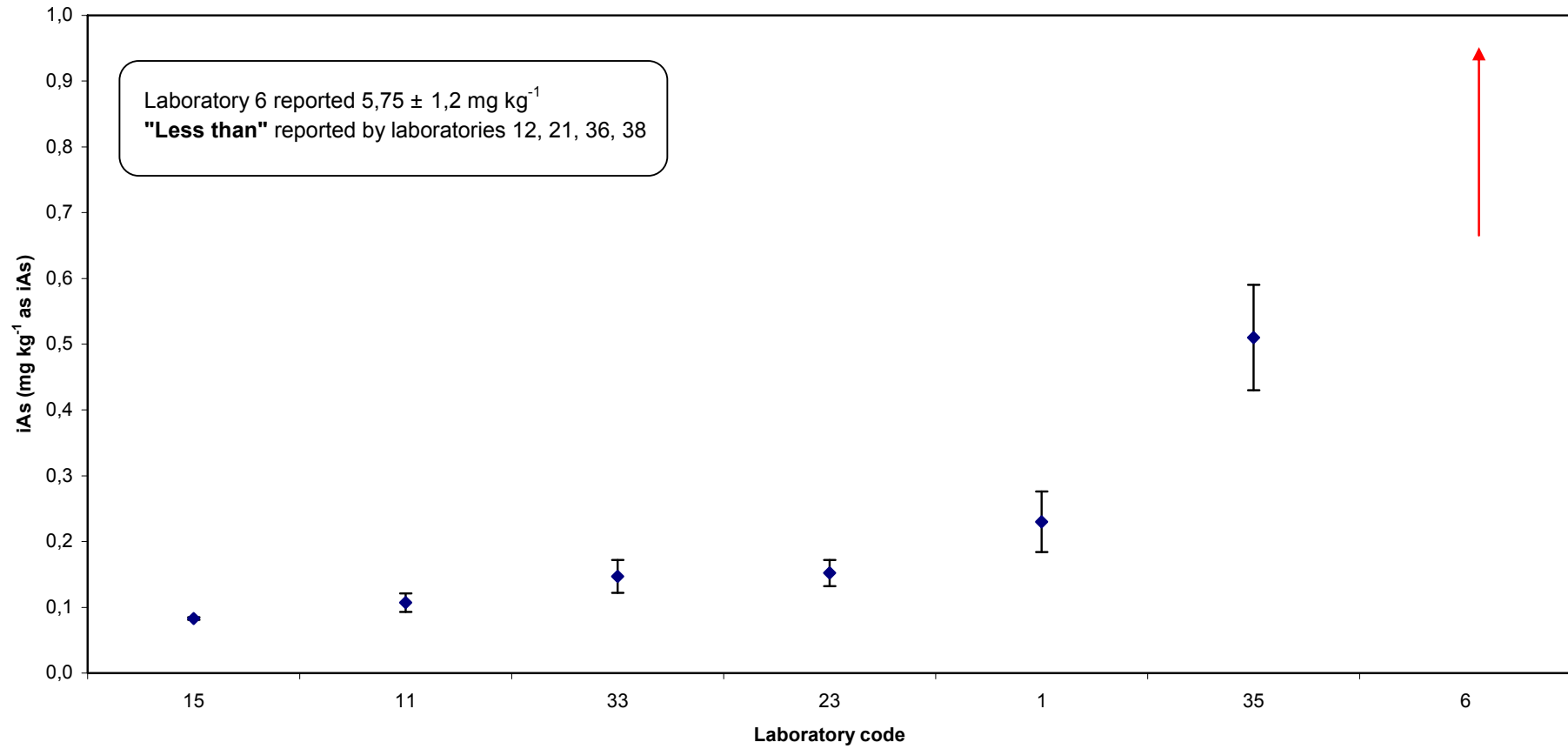


Annex 11: iAs in seafood

X_{ref} = not known

| Lab ID | $x_{lab}(mg\ kg^{-1})$ | $U_{lab}(mg\ kg^{-1})$ | k | $u_{lab}(mg\ kg^{-1})$ | Technique |
|--------|------------------------|------------------------|------------|------------------------|-------------|
| 1 | 0,23 | 0,046 | 2 | 0.023 | HPLC/ICP-MS |
| 6 | 5,75 | 1,2 | $\sqrt{3}$ | 0.7. | ICP-OES |
| 11 | 0,107 | 0,014 | 2 | 0,007 | HG-ICP-MS |
| 12 | <0,04 | | | | HPLC-ICPMS |
| 15 | 0,083 | 0,002 | 2 | 0,001 | HPLC-ICP-MS |
| 21 | <0,05 | | | | ICP-MS |
| 23 | 0,152 | 0,02 | 2 | 0,01 | HR-ICP-MS |
| 33 | 0,147 | 0,025 | 2 | 0,013 | HG-AAS |
| 35 | 0,51 | 0,08 | 2 | 0,04 | ETAAS |
| 36 | <0,2 | | | | HPLC-ICP-MS |
| 38 | <0,5 | | | | HPLC ICPMS |

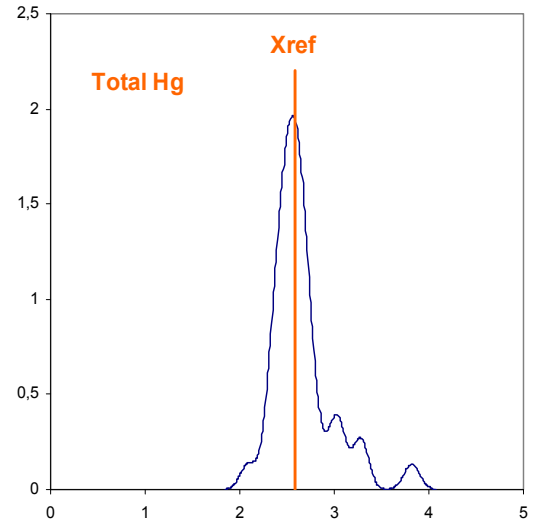
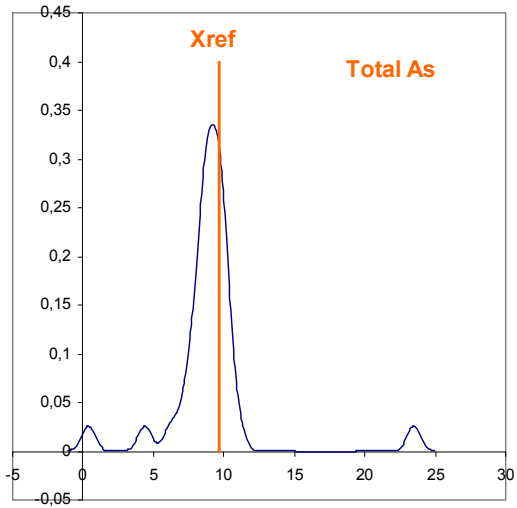
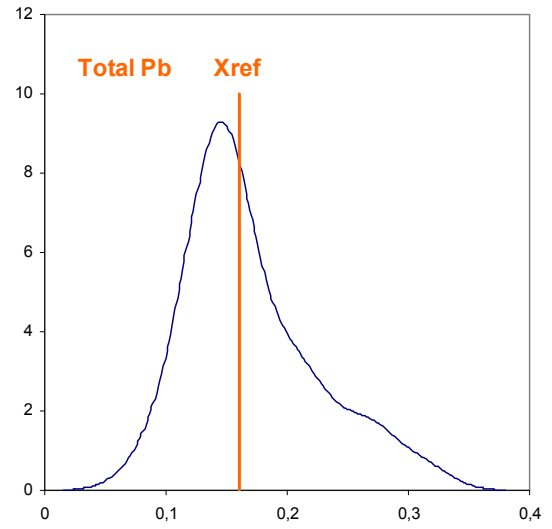
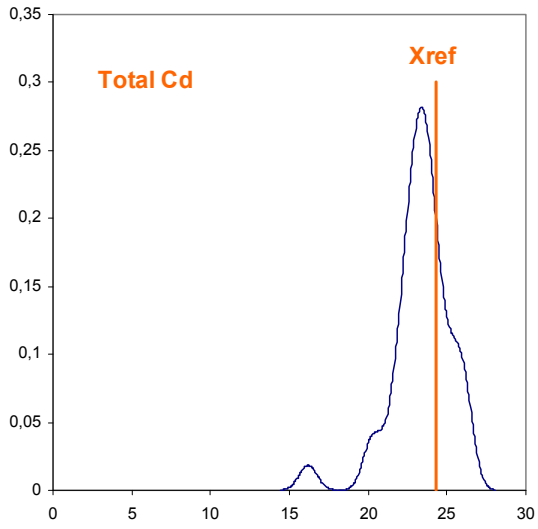
IMEP-109: results for iAs



This graph displays all measurements results and their associated uncertainties.
The uncertainties are shown as reported, with various expansion factors and levels of confidence.



Annex 12: Kernel distributions



Annex 13: Experimental details for total Cd, Pb, As and Hg determinations

| Lab ID | SOP? | If yes, which one? | Sample pre-treatment | Digestion step | Extraction/separation step | Instrument calibration step |
|--------|------|---|---|---|---|---|
| 1 | Yes | ----- MET 08 similar to NF EN 15763 | | | | |
| 2 | Yes | LST EN 14084:2003 (Cd, Pb); ASU L 00.00-19/4 (Hg); LST EN 14332:2004 (As) | | | | |
| 3 | No | | None | Acid Digestion in Microwave Digestor ((Nitric acid and Hydrogen Peroxide) | None (Dilution of digested solution with water) | Calibration of ICP-MS with standard solutions of As, Hg, Cd & Pb. |
| 4 | Yes | AOAC | | | | |
| 7 | Yes | EN 14084 (for Pb and Cd) EPA 7473 (for Hg) | | | | |
| 8 | Yes | Pb, Cd - AOAC 999.11; Hg - AOAC 971.21 | | | | |
| 9 | No | | None | Digestion using nitric acid | | |
| 10 | No | | | Microwave with ac. nitric | | ICP-MS |
| 11 | | | No | HNO ₃ and H ₂ O ₂ at T° of 180°C Open wet digestion | No | Calibration externe |
| 12 | No | | Mixing | µ-wave assisted acid digestion with conc HNO ₃ | | addition calibration |
| 14 | No | | Micro oven digestion with nitric acid and hydrogen peroxide | | | Direct calibration, internal standard |
| 15 | Yes | EN ISO 13805 and EN 15763 | | | | |
| 16 | Yes | Cd,Pb:EN 14082:2003; As:EN 14546:2005; Hg -No | | For Hg:HNO ₃ +H ₂ SO ₄ | | 0.200, 0.300, 0.400, 0.500 µg |
| 17 | Yes | SR EN - 14082:2003; 14083:2003; 13806:2003; 14546:2005 | | | | |
| 18 | Yes | Official Methods of Analysis AOAC | | | | |
| 19 | Yes | Cd + Pb meth. ICP 78 / 633 / EØF, As + Hg (in house) | | | | |
| 20 | Yes | As EN 14332:2004; Pb, Cd EN 14084:2003; Hg National Feed Codex | | | | |
| 21 | No | | Mixing | Micro-wave assisted 180°C + 4mL HNO ₃ conc. + 4 mL water | | ICP-MS (0 to 10 ppb) |

| Lab ID | SOP? | If yes, which one? | Sample pre-treatment | Digestion step | Extraction/separation step | Instrument calibration step |
|--------|------|---|---|---|--|--|
| 22 | No | | Dry in oven | Ashing in muffle furnace, dissolved in HCL, Hg - AMA 254 | | Calibration curve for Pb (10-60 ug/l); Cd (1-10 ug/L); Hg (0.05-5 ug/l); As (3-25ug/l) |
| 23 | No | | | Aliquots (0.5 g) of test sample were digested in conc. nitric acid (5 ml) using a high pressure microwave digestion system/ | Solutions were made up to volume (10 ml) with deionised water prior to a further dilution (5-fold) with internal standard (Rh/In). | Matrix-matched calibration standards were prepared using NIST-traceable element standard solution. |
| 24 | No | | Homogenise | Microwave digestion | n.a. | Calibration using certified standard solutions |
| 25 | No | | UNI EN 13804:2002 | UNI EN 13805:2002; Samples (equivalent to about 0,5 g dry weight) are weighed out into microwave digestion vessels; digestion solution (HNO ₃ :H ₂ O ₂ :H ₂ O=5:1:1) is added and samples are heated in a proprietary high pressure microwave digestion oven. | | UNI EN 13805:2003 (Hg) ; external curve have been used to read the other elements on ICP-MS (Rh has been used as internal standard) |
| 26 | Yes | EN 13806 (for Hg), EN 14332 (for As), LMBG 35 (for Cd) | | | | |
| 27 | No | | | Cd-As: microwave high pressure digestion with H ₂ O ₂ (30%) and HNO ₃ conc - Hg direct | | Cd: add. method, std solution 2 ppb; As: add. method, std solution 20 ppb; Hg non linearity calibration from 25 ppb to 5 ppm |
| 28 | No | | Addition of acid and hydrogen peroxide, let it stand for one hour | Microwave | Dilution | External standard |
| 29 | Yes | AOAC 999.10 (final action) | | | | |
| 31 | Yes | EN 13805 for digestion, EN 13806 for Hg, EN 15763 for As, Cd and Pb | | | | |
| 32 | Yes | SS-EN 15763:2009 | | | | |
| 33 | Yes | EN 14546:2005 (As); EN 14083:2004 (Cd, Pb); Hg - in-house method | | | | |
| 34 | No | | | 0.4 g of the sample were weighed in Teflon vessels. 10 mL HNO ₃ (65%) and 2 mL H ₂ O ₂ were added and the mixture was left at room temperature for 30 min. The samples were digested in a microwave oven. The digested samples were diluted to 20 mL by the addition of distilled water. | The diluted samples were filtered through PTFE membrane filters of 0.2 um. | For the determination of total Cd, Pb and Hg external calibration was used and for the determination of total As standard addition calibration was used. |

| Lab ID | SOP? | If yes, which one? | Sample pre-treatment | Digestion step | Extraction/separation step | Instrument calibration step |
|--------|------|--------------------|----------------------|---|----------------------------|---|
| 35 | No | | | Nitric acid, microwave digestion | | External standard, quality control using CRMs |
| 36 | No | | | | | ICP-MS |
| 37 | No | | | High pressure Microwave HNO ₃ H ₂ O ₂ | | External calibration, internal Standard Indium 0.1-20 ppb |

Annex 14: Experimental details for methylmercury determinations

| Lab ID | SOP? | Sample pre-treatment | Digestion step | Extraction/separation step | Derivatisation | Instrument calibration |
|--------|------|----------------------|--|---|-------------------|--|
| 1 | No | | 0.25 g and 5 ml TMAH and DIGIPREP | | Propylation | IDMS |
| 7 | No | No pre-treatment | Hydrolisation with hydrobromic acid | Extraction with toluene followed by remotion of Me Hg with cysteine | | External curve was made with standard solutions prepared by dilution from a 1000 mg/L mercuris nitrate standard solution |
| 10 | No | | | CIH 5 M | Tetrapropilborato | GC-ICP-MS |
| 11 | No | No pre-treatment | HNO ₃ and H ₂ O ₂ | No | No | Externe calibration |

Annex 15: Experimental details for iAs determinations

| Lab ID | SOP? | If yes, which one? | Sample pre-treatment | Digestion step | Extraction/separation step | Instrument calibration |
|--------|------|--------------------|---|--|--|--|
| 1 | No | | | 0.15 g with 10 ML OF 100% H ₂ O and MAE | | External calibration, IDMS |
| 7 | | | | | | External curve was made with standard solutions prepared by dilution from a 1000 mg/L mercuris nitrate standard solution |
| 11 | No | | No | Only with HNO ₃ at T° of 180°C Open wet digestion | No | Externe calibration |
| 12 | No | | Mixing | | µ-wave assisted extraction with HCl/H ₂ O ₂ | Matrix-matched external calibration curve |
| 21 | No | | Mixing | | Extraction with MeOH/H ₂ O | HPLC-ICP-MS calibrated with 5 species of As (0 to 25 ppb) |
| 23 | No | | None | Aliquots (0.25 g) of test sample, plus deionised water (2 ml), were solubilised in conc. HCl (10 ml) | Hydrobromic acid (1 ml) and hydrazinium sulphate (0.5 ml) were added, extracted into chloroform and back-extracted into dilute HCl. Internal standard (Ga) was added to all solutions. | Matrix-matched calibration standards were prepared using NIST-traceable arsenic standard solution. |
| 33 | No | | Hydrolysis in HCl, As (V) to As (III) reduction (HBr+hydrazine sulfate) | yes | Selective with CHCl ₃ ; back extraction with HCl | yas |
| 35 | Yes | EN 15517:2008 | | | | |
| 36 | No | | | | | HPLC-ICP-MS |

European Commission

EUR 24560 EN – Joint Research Centre – Institute for Reference Materials and Measurements

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Author(s): M.B. de la Calle, H. Emteborg, P. Robouch, I. Verbist

Luxembourg: Publications Office of the European Union

2010 – 51 pp. – 21 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1018-5593

ISBN 978-92-79-17198-7

DOI 10.2787/31613

Abstract

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate General of the European Commission, operates the European Union Reference Laboratory for Heavy Metals in Feed and Food (EU-RL-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 ninth proficiency test (PT) of the EU-RL-HM which focused on the determination of total cadmium, lead, arsenic and mercury as well as methylmercury and inorganic arsenic in seafood.

The test material used in this exercise was the Certified Reference Material (CRM) DOLT-4, dogfish liver of the National Research Council of Canada (CNRC). The material was relabelled to prevent recognition by the participants and was dispatched the second half of May 2010. Each participant received one bottle containing approximately 20 g of test material. Thirty-eight laboratories from 27 countries registered to the exercise of which 38 reported results for total Cd, 36 for total Pb, 33 for total As, 36 for total Hg, five for methylmercury and 10 for iAs. The assigned values for total Cd, Pb, As, Hg and methylmercury are the certified values taken from the DOLT-4 certificate. An attempt was made to establish an assigned value for inorganic As (iAs) using the results provided by a group of five laboratories expert in the field, following a similar approach to that used in IMEP-107, a PT on total and inorganic arsenic in rice. Contrary to what was observed in IMEP-107, the results obtained by the expert laboratories for iAs was method dependent, therefore no assigned value could be established.

The uncertainties of the assigned values, u_{ref} , were taken directly from the CRM certificate as provided by the producer for total Cd, Pb, As, Hg and methylmercury. Participants were invited to report the uncertainty of their measurements. This was done by the majority of the laboratories taking part in this exercise.

Laboratory results were rated with z - and ζ -scores (zeta-scores) in accordance with ISO 13528. Since the concentration of iAs seems to be method dependent according to the results obtained by the expert laboratories, no scoring was provided to the laboratories that submitted results for iAs. The standard deviation for proficiency assessment (also called target standard deviation) was fixed to 15% by the advisory board of this ILC, on the basis of the outcome of previous ILCs organised by the EU-RL-HM and on the state-of-the-art in this field of analysis.

Between 80 and 97.5 % of the laboratories performed satisfactory for total Cd, As, Hg and methylmercury. Regarding total Pb, 70 % of the laboratories scored satisfactory.

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