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IMEP-41: Determination of inorganic As in food

Collaborative Trial Report

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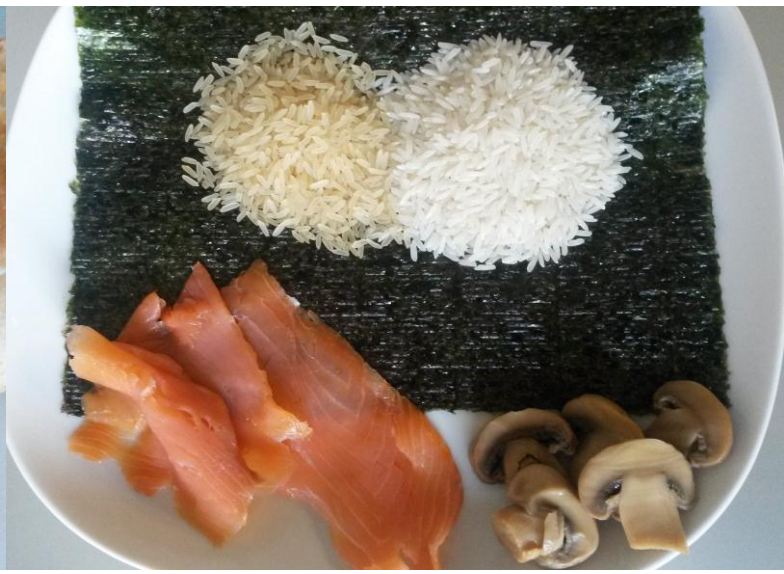
January 2015



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Determination of inorganic arsenic in food by flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS).

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IMEP®

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Executive Summary

A collaborative trial, named IMEP-41, was conducted in accordance with international protocols to determine the performance characteristics of an analytical method for the quantification of inorganic arsenic (iAs) in food.

The method is based on (i) solubilization of the protein matrix with concentrated hydrochloric acid to denature proteins and allow the release of all arsenic species into solution, and (ii) the subsequent extraction of the inorganic arsenic present in the acid medium using chloroform. The final detection and quantification is done by flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS).

Thirteen laboratories, from nine EU countries, registered for participation. All were experienced in the analysis of inorganic arsenic in various food commodities using FI-HG-AAS.

The seven test items used in this exercise were reference materials (certified reference materials or former IMEP test items) covering a broad range of matrices and concentrations: mussel tissue (EC-JRC-IRMM, ERM-CE278k), cabbage (IAEA, IAEA-359), seaweed (Hijiki) (NMIJ, CRM 7405a), fish protein (NRC, DORM-4), rice (EC-JRC-IRMM, IMEP-107), wheat (EC-JRC-IRMM, IMEP-112), mushrooms (EC-JRC-IRMM, IMEP-116) and rice (EC-JRC-IRMM, ERM-BC211) used as pre-test item for training purposes.

The relative standard deviation for repeatability (RSD_r) ranged from 4.1 to 10.3 %, while the relative standard deviation for reproducibility (RSD_R) ranged from 6.1 to 22.8 %. The obtained performance characteristics (i.e. precision and trueness) clearly demonstrate the fitness-for-purpose of the investigated analytical method for the determination of iAs in food, for iAs mass fractions ranging from 0.074 to 7.55 mg kg^{-1}.

1 Introduction

Arsenic is a widely found contaminant which occurs both naturally and as a result of human activity. Since the late 1960s, scientific evidence has been building showing that high levels of As may cause skin lesions with carcinogenic [1-3] or non-carcinogenic effects [4, 5]. However, final correlations have not been established yet. Until recently, most studies focused on the determination of total As in the diet [6]. Based on their chemical properties, the arsenic species are categorized as lipid-soluble or water-soluble arsenicals, both including inorganic and organic compounds. [7]. However, inorganic arsenic (iAs) species (As(III) and As(IV)) are the more toxic forms [8].

There is a worldwide concern about dietary iAs exposure and the associated health risks have been emphasized in recent evaluations by the European Food Safety Authority (EFSA) and the Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) [9-11].

The recognition that the chemical form of As is critical for assessing risk, coupled with advances in analytical methods, has resulted in a significant expansion of the amount of published scientific studies on As speciation [4]. The determination of the iAs levels in food and the calculation of typical intakes are critical to establish background exposure levels to As and to understand elevated risks from excess of natural or anthropogenic sources [12]. For this reason there has been increasing interest in robust and reliable methods to determine the iAs concentration in a range of food commodities, that trigger the inclusion of iAs maximum levels in the next revision of the Commission Regulation (EC) 1881/2006 [13].

The most commonly applied analytical methods for As speciation are based on high performance liquid chromatography hyphenated with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) [14, 15]. However, chemical separation of arsenic species with subsequent AAS determination are easier and more cost-effective than the respective HPLC ones [16, 17]. Hydride generation (HG) is one of the most straightforward approaches, with a high selectivity due to the hydride formation of only few arsenic species. [18, 19].

The European Committee for Standardization (CEN TC 327/WG 4) standardised a method (EN 16278) for the determination of iAs in animal feeding stuffs by HG-AAS after microwave extraction and separation by solid phase extraction (SPE) [20]. This method was ring-trial validated in the frame of the IMEP-32 project [21]. However, the limit of quantification of the method was found not low enough for the analysis of food commodities taking into consideration the maximum limits set by legislation. Currently, CEN TC 275/WG 10 is validating a method for the selective determination of iAs in food based on HPLC-ICP-MS.

The International Measurement Evaluation Program (IMEP) of the Joint Research Centre (JRC) of the European Commission, organised a collaborative trial (IMEP-41) for the validation of an alternative method to determine iAs in food, based on the selective extraction of iAs into chloroform and further determination by HG-AAS. The experimental

protocol was developed and successfully implemented by the Trace Elements Group of the Institute of Agrochemistry and Food Technology (IATA) of the Spanish National Research Council (CSIC) [8].

This report summarises the outcome of the collaborative trial, IMEP-41.

2 Scope and aim

The scope of this collaborative trial (CT) is to establish the trueness and precision of a method to be used in the determination of iAs in food. The statistical data treatment was performed according to ISO 5725-2:1994 [22]. Furthermore, the IMEP® administrative and logistic procedures were followed.

3 Invitation, registration and distribution

IMEP-41 was announced on December 12, 2013 (Annex 1) to National Reference Laboratories (NRLs) of the network of the European Union Reference Laboratory for Heavy Metals in Feed and Food (EURL-HM). The exercise was also announced on the IRMM web site on January 15, 2014 (Annex 2).

Registration was opened until February 2, 2014. Thirteen participants from nine different EU Member States registered to this exercise. Test items were dispatched on February 13, 2014. Each participant received one package containing:

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

b) Sixteen bottles containing, each approximately 20 g (samples 1 and 2), 8 g (sample 4), 5 g (samples 3, 5, 6, 7 and 8) of the test items (two bottles from each test item);

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

d) A copy of the standard operating procedure, SOP (Annex 4);

e) A "Confirmation of Receipt" form (Annex 5).

The accompanying letter clearly stated that the SOP was to be strictly followed. It also included information about the analyte to be measured in all the samples, the number of independent replicates required per bottle and detailed instructions on how to determine the moisture in every test item and how to report results.

The deadline for reporting results was set to April 16, 2014. 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 to collect relevant information about measurements and laboratories (Annex 6).

3.1 Procedure to apply

The SOP (Annex 4) was drafted by the Trace Elements Group of the Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC) (Valencia, Spain).

4 Test items

4.1 Food commodities covered

The seven food commodities used in this collaborative trial are listed in Table 1. Two of the distributed samples were identical (S1 and S3) to ensure that the analysis of one sample does not affect the subsequent measurement on another sample. A pre-test sample (ERM-BC211) was sent to participants allowing them to confirm the proper implementation of the method under investigation, before starting the collaborative trial.

Table 1 – List of reference materials used in the IMEP-41 exercise. S4, S7, S8 and pre-test are CRMs certified for total As. S7 and pre-test are also certified for iAs.

Sample ID	Reference material	Food commodity
S1 & S3	IMEP-107	Rice
S2	IMEP-112	Wheat
S4	ERM-CE278k	Mussels
S5	IAEA-359	Cabbage
S6	IMEP-116	Mushroom
S7	NMIJ-7405a	Seaweed
S8	DORM-4	Fish
pre-test	ERM-BC211	Rice

4.2 Preparation

The pre-test item and the test items S1, S2, S4 and S6 bottles were relabelled by removing the previous labels and sticking the new ones with the appropriate code (IMEP-41, material number and sample number). For the test items S3, S7 and S8, the supplied units 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 thorough mixing and re-homogenisation. The materials were then refilled in labelled vials using a vibrating feeder and a balance in a clean-cell equipped with a HEPA filter. For cabbage (S5), a handful of Teflon balls were added during mixing to break up agglomerates since the material was clogged upon delivery. In order to break the agglomerates the material was forced to go through a 500 µm mesh. It was then transferred into a 5 L acid-washed plastic drum and subsequently in a 3D-mixer for 30 minutes, before filling in vials using a vibrating feeder and a balance in the clean-cell.

Care was taken to avoid cross-contamination between the different materials; any two powders were never handled at the same time. Every material was mixed and filled only after thorough cleaning of the whole equipment used.

4.3 Homogeneity and stability

Based on previous experience, it was assumed that the same homogeneity and stability characteristics would apply to total and inorganic arsenic. Therefore, no further investigations were deemed necessary since all the homogeneity and stability studies were performed by the CRM producers or in the frame of the IMEP projects.

5 Assigned values and associated uncertainties used to evaluate the trueness of the method.

In order to determine the trueness of the method, assigned values for iAs mass fractions in all the test items were determined using methods different from the one under validation. The iAs certified values and uncertainties in the ERM-BC211 pre-test item and NMIJ-7045a (S7) were provided by the respective CRM producers. For the remaining samples the iAs mass fraction was determined by five expert laboratories, selected on the basis of their demonstrated measurement capabilities, listed hereafter:

- *Instituto Superiore di Sanità (Rome, Italy);*
- *Institut für Chemie, Bereich Analytische Chemie, Karl-Franzens Universität (Graz, Austria);*
- *Technical University of Denmark, National Food Institute – DTU (Søborg, Denmark);*
- *Department of Chemistry, University of Aberdeen (Aberdeen, UK);*
- *Department of Analytical Chemistry, Faculty of Chemistry, University of Barcelona (Barcelona, Spain)*

Every expert laboratory received two bottles of each sample. They were requested to perform three independent measurements per bottle (under repeatability conditions) on two different days (one bottle/day) following the method of their choice. They had to report the values obtained for the six independent measurements, the corresponding mean and associated expanded measurement uncertainty (95 % confidence interval). They were informed about the food commodity contained in each bottle, because HPLC-based methods might need to be adapted depending on the matrix to be analysed.

The analytical methods used by the expert laboratories are summarized in Table 1. The order of these methods does not correspond to the list of expert laboratories given above. One of them analysed the test items using two different techniques, based on HG-ICP-MS and HPLC-ICP-MS (C4A and C4B, respectively).

Table 2 – Analytical protocols, as described by the expert laboratories.

C1: Microwave assisted extraction was used to solubilize iAs. Samples (0.35 g) were mixed with 10 mL of 1 % (v/v) HNO_3 and 1 % (v/v) H_2O_2 and left to stand overnight. Microwave irradiation was applied with the following temperature profile: 3 min ramp to 55 °C, 10 min at 55°C, 2 min ramp to 75 °C, 10 min at 74 °C, 2 min ramp to 95°C, 30 min at 95 °C. The extracts were centrifuged (10 min, 8000 rpm, 4 °C) and the supernatants filtered through a 0.22 µm filter. With the extraction procedure used, As(III) is converted to As(V), which appears as a well separated peak in the anion exchange HPLC-ICP-MS chromatogram. Therefore inorganic arsenic was measured as As(V), i.e., arsenate.
C2: About 0.5 g of powder was weighed with a precision of 0.1 mg into 50 mL polypropylene tubes, and a solution (10 mL) of 20 mmol·L^{-1} trifluoracetic acid containing 1 % (v/v) of a 30 % H_2O_2 solution was added. Samples were extracted with a GFL-1083 shaking water bath (Gesellschaft für Labortechnik, Burkwedel, Germany) at 95 °C for 60 min. After cooling to room temperature the extracts were centrifuged for 15 min at 4700 g. An aliquot of 1 mL was transferred to Eppendorf vials and centrifuged for 15 min at 8900 g. The supernatant was used directly for HPLC-ICP-MS analysis.
C3: For the determination of iAs subsamples of approximate 0.200 g were weighed into plastic tubes and 10.00 mL of 0.1 mol L^{-1} nitric acid (Merck) in 3 % hydrogenperoxide (Merck) was added. The solutions were placed in a water bath at 90 °C for 60 min. Then the solutions were allowed to cool to room temperature and centrifuged at approximately 4000 rpm for 10 min and subsequently filtered (0.45 µm) prior to analysis. The determination of inorganic arsenic was done using anion exchange HPLC-ICP-MS. The method is currently being evaluated as a future European standard method by CEN.
C4A and C4B: 0.1 g sample (by weight) were diluted with 10 g extraction solution containing 2 % (v/v) nitric acid and 3 % (v/v) hydrogen peroxide in 50 mL Falcon tubes. The solution was mixed and heated (loosely capped) in a microwave oven for 50 min total (temperature program: ramp in 2 min to 50 °C, 5 min at 50 °C, ramp in 2 min to 75 °C, 5 min at 75 °C, ramp in 4 min to 95 °C, 30 min at 95 °C). The cooled solution was weighed and then centrifuged at 4200 rpm for 10 min and the supernatant separated from the residue. The supernatant for samples 4 and 8 was further diluted by a factor of 5 and sample 7 by a factor of 10 using extraction solution. The dilution of samples 4 and 8 was required due to excessive foaming of sample during hydride generation. The solutions were then analysed by **A)** HG-ICP-MS and **B)** HPLC-ICP-MS. Two sets of data were delivered.
C5: The samples were accurately weighed in PTFE vessels and then extracted by adding 10 mL of 0.2 % (w/v) HNO_3 and 1 % (w/v) H_2O_2 solution in a microwave digestion system. The temperature was raised first to 55 °C (and held for 10 min) then to 75 °C (and held for 10 min) and finally the digest was taken up to 95 °C and maintained for 30 min. Samples were cooled to room temperature and centrifuged at 3500 rpm for 12 min. The supernatant was filtered through PET filters (pore size 0.45 µm). Arsenic speciation was carried out in the extracts by LC-ICP-MS.

The mean of the means provided by the expert laboratories was used, after removal of outliers, to derive the assigned values of the collaborative trial (X_{CT}), according to ISO Guide 35 [23].

In all cases (except for S5 and S8) the expert laboratories reported values with overlapping expanded uncertainties (Annexes 7 – 13). The uncertainty contribution due to characterization (u_{char}) was calculated according to ISO/IEC Guide 98:2008 [24].

$$u_{char} = \frac{1}{p}\sqrt{\sum_1^p u_i^2} \qquad \text{Eq. 1}$$

where "*p*" refers to the number of expert laboratories used to assign the X_{CT}, while "u_i" is the associated combined uncertainty reported by the experts.

In the case of S5 and S8, expert laboratories reported values, which did not overlap within their respective expanded measurement uncertainties (Annexes 10, 12). u_{char} was then calculated according to ISO Guide 35 [23]:

$$u_{char} = \frac{s}{\sqrt{p}} \qquad \text{Eq. 2}$$

where "*s*" refers to the standard deviation of the means obtained by the expert laboratories.

The uncertainties of the reference values (u_{CT}) were then estimated combining the uncertainty of the characterization (u_{char}) with the contributions for homogeneity (u_{bb}) and stability (u_{st}) in compliance with ISO/IEC Guide 98:2008 (GUM) [25].

$$u_{CT} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{st}^2} \qquad \text{Eq. 3}$$

For S7, u_{CT} was provided in the NMIJ certificate. For the former IMEP test items (S1, S2, S6) u_{bb} and u_{st} were extracted from the corresponding IMEP reports to participants. As for the remaining samples (S4, S5, S8) u_{bb} and u_{st} were derived from those reported for total As by the respective CRM producers.

Table 3 presents the assigned values and expanded uncertainties (X_{CT} and U_{CT} = 2 u_{CT}). In the case of sample S7 (Seaweed) the reference values of the CRM were used.

Table 3 – Assigned values for iAs mass fraction in the various test items, expressed in mg kg^{-1} (X_{CT}, reference value; UCT, expanded measurement uncertainty of the reference value; RSU (relative expanded uncertainty)

Sample	$X_{CT} \pm U_{CT}$ (k = 2)	RSU	Ref.
S1 – Rice (IMEP-107)	0.108 ± 0.011	10.2 %	[26]
S2 – Wheat (IMEP-112)	0.165 ± 0.021	12.7 %	[27]
S4 – Mussels (ERM-CE278k)	0.0863 ± 0.008x	9.3 %	[28]
S5 - Cabbage (IAEA-359)	0.091 ± 0.016	17.6 %	[29]
S6 – Mushroom (IMEP-116)	0.321 ± 0.026	8.1 %	[30]
S7 – Seaweed (CRM 7405a)	10.10 ± 0.50	5.0 %	[31]
S8 – Fish (DORM-4)	0.271 ± 0.061	22.5 %	[32]

6 Results and evaluation

6.1 Pre-test item

CRM ERM-BC211 [33] was the pre-test item used during the feasibility study to allow participants to check the proper implementation of the SOP in their laboratory. The results obtained analysing this sample were reported to the collaborative trial coordinator. Laboratories having reported results in agreement with the certified value (0.124 ± 0.011, k = 2, in mg kg^{-1}) were allowed to continue with the analyses of the other test items. Laboratories having reported significantly biased results were requested to initiate a root-cause analysis and take proper corrective actions to prevent further biases. Only when the bias was properly identified and corrected for, laboratory could continue with the analysis of the remaining items.

Laboratory L05 failed to analyse correctly the pre-test sample and was not able to provide adequate route-cause analysis. Furthermore, L05 reported unsatisfactory results for total As in the frame of another proficiency testing exercise organised by the EURL-HM the same year. It was assumed that the problem may be attributed to a bias in the total As determination and not to the analytical method under validation. Hence, L05 was not excluded from the exercise.

6.2 Collaborative trial

Thirteen laboratories from 9 countries registered to this collaborative trial. L05 failed to analyse correctly the test-sample. L04 did not report any results due to failure of his instrumentation. L06 modified the SOP and used ICP-MS instead of the prescribed HG-AAS; this set of data was excluded from the statistical calculations. Ten participants reported results that were further evaluated. Laboratories were requested to perform three independent measurements per bottle under repeatability conditions. This process was to be repeated on two different days (one bottle/day) following the SOP.

6.3 Statistical analysis for method performance assessment

The statistical evaluation of data was performed following the international standard recommendations set by ISO 5725-2:1994 [18]. The same statistical approach was used for the evaluation of the results reported by the expert laboratories.

The following sequence of statistical tests was applied:

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. This test could not be applied to the results of L07 because this laboratory analysed only one bottle on one single day.

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 overall mean (X_{obs}) calculated from data reported from all participants.

Trueness and precision of the method were estimated after identification and rejection (when applicable) of outliers. Table 4 provides an overview of the identified outliers for all test items.

According to ISO 5725 an outlying results should be investigated and rejected only when an explanation is found for their anomaly. Results should not be discarded only on the basis of statistical analysis.

Only the results reported by L03 for S4 were flagged as Grubbs outliers; all the others were Cochran outliers. The comments made by the laboratories in the questionnaire (Table 6) were scrutinised to understand the discrepancies of the results reported for some of the test items.

- L01 mentioned at the time of its registration that the instrument to be used for the analysis was old. It was not equipped with a flow injection system and it needed to be operated in the batch mode. The laboratory was not sure about the quality of the results that could be obtained with this instrument. The very large scatter of reported results for all matrices confirmed his concern. Hence, the results of L01 were not included in the statistical evaluation.

- The results reported by L07 for S4 were identified as Cochran outliers despite having a repeatability variance comparable to that of other sets of data. This mathematical artefact was due to the fact that the laboratory analysed only one bottle on one single day, thus having less degrees of freedom. It was therefore decided to retain these results for further statistical evaluation.
- L03 did not filter the chloroform phase. Filtering the chloroform phase is a crucial clean-up step necessary to avoid any traces of the HCl initially used to extract all As species from the matrix (cf. Point 9.3 of the SOP, Annex 4). Residues of the concentrated HCl in the chloroform phase may introduce contamination with organic arsenic species. Such a contamination would be particularly important in samples in which iAs represents a small fraction of the total As. This is the case of S4 and S8, where the iAs mass fractions (0.086 ± 0.008 mg kg^{-1} and 0.271 ± 0.061 mg kg^{-1}, respectively), represent 1 and 4 % of the total As mass fraction. (6.7 ± 0.4 mg kg^{-1} in S4 and 6.80 ± 0.64 mg kg^{-1} in S8). While seaweeds (S7) also contain high levels of several organic arsenic species, the iAs mass fraction (10.1 ± 0.5 mg kg^{-1}) represents 28 % of the total As mass fraction (35.8 ± 0.9 mg kg^{-1}). This could explain the high values reported by L03 for S4 as well as the high dispersion of data for S8 and S6. As indicated in the IMEP-116 report to participants [30] about half of the total As mass fraction corresponds to organic compounds. Even though L12 did not filter the chloroform phase it was not flagged as outlier for any of the test items, proving that sound results can still be obtained when the organic phase is carefully sampled. Therefore, L03 was excluded from the statistical evaluation only when the results were flagged as outliers.

L08 reported having many problems with S4 and S8, while L13 had problems with S5 and S8 due to the formation of emulsion during the back extraction (point 9.4 of the SOP, Annex 4). Laboratory L02 did not apply the final filtration step 9.5.7 of the SOP which did not have a significant influence on the reported results

Regarding the results reported by the expert laboratories it is interesting to mention that the results obtained by HG-ICP-MS (C4A) for S4, S5 and S8 were not in agreement with the results reported by the experts using HPLC-ICP-MS within their respective standard uncertainties (95 % confidence level). Only the results reported by C4 (for both methods) for S4 were flagged as Grubbs outliers. This expert reported that: "*Sample 8 produced highly divergent results between HPLC and HG-ICP. The samples have been done several times with the same results; the reason for this is not clear. Sample 4 contains an organic As-compound eluting very near to As(V), which may co-elute with As(V) depending on column conditions used.*" In addition for S4 and S8 the expert laboratory reported that the sample had to be diluted after extraction to avoid formation of foam during hydride generation (Table 2). Some organic compounds, for instance methylated species of As, are known to form arsine and could therefore interfere with the determination of iAs when present in a matrix. This could be the case of mussels (S4). For S8 the value obtained by HG-ICP-MS was twice as high as that obtained by the same laboratory using HPLC-ICP-MS, although it was not flagged as an outlier.

Table 4– List of identified outliers for the different matrices

Sample	Laboratory (number of outlying results)	Outlier type **
S1 – Rice (IMEP-107)	L13 (6)	Cochran
S4 – Mussels (ERM-CE278k)	C4A (6)	Grubbs
	C4B (6)	Grubbs
	L08 (5)	Cochran
	L03 (6)	Grubbs
S5 - Cabbage (IAEA-359)	L13 (6)	Cochran
S6 – Mushroom (IMEP-116)	L08 (6)	Cochran
	L03 (6)	Cochran
S8 – Fish (DORM-4)	L03 (6)	Cochran

All the remaining measurement results were used to evaluate trueness and precision. Table 5 provides:

- the number of laboratories used to assess the performance characteristics of the method (after outlier exclusion);
- the number of outlier laboratories and replicates excluded;
- the assigned values and associated expanded measurement uncertainties (X_{CT}, U_{CT});
- the overall observed mean (after outlier rejection, X_{obs}) and their respective expanded uncertainty, expressed as the reproducibility standard deviation (S_R) multiplied by a coverage factor of 2, to approximate 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 (HorRat) expressed as the ratio between the observed RSD_R value divided by the predicted reproducibility relative standard deviation ($PRSD_R$) value calculated from the Horwitz equation [34]; and
- the overall analytical recovery R, calculated as:

$$R = 100\,\frac{X_{obs}}{X_{CT}} \qquad \text{Eq. 4}$$

while the associated uncertainty (u_R) was estimated as [29]:

$$u_R = R.\sqrt{\left(\frac{u_{obs}}{X_{obs}}\right)^2 + \left(\frac{u_{CT}}{X_{CT}}\right)^2} \qquad \text{Eq. 5}$$

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

u_{CT} is the standard uncertainty of the X_{CT}.

Table 5 – Method performance characteristics from the collaborative study (following ISO 5725-2:1994 [22])

	Units	S1 – Rice (IMEP-107)	S2 – Wheat (IMEP-112)	S4 – Mussels (ERM-CE278k)	S5 - Cabbage (IAEA-359)	S6 – Mushroom (IMEP-116)	S7 – Seaweed (CRM 7405a)	S8 – Fish (DORM-4)
N° Laboratories (after outlier rejection)		**8**	**9**	**6**	**8**	**7**	**9**	**8**
N° Outlier Lab (test used)		**1 (C)**	**-**	**1 (C), 1 (G)**	**1 (C)**	**2 (C)**	**-**	**1 (C)**
N° Replicates excluded		**6**	**-**	**11**	**6**	**12**	**-**	**6**
Assigned value $X_{CT} \pm U_{CT}$ **(k = 2)**	mg kg^{-1}	**0.108 ± 0.011**	**0.165 ± 0.021**	**0.086 ± 0.008**	**0.091 ± 0.016**	**0.321 ± 0.026**	**10.1 ± 0.5**	**0.271 ± 0.061**
Overall mean $X_{obs} \pm 2S_R$	mg kg^{-1}	**0.096 ± 0.030**	**0.146 ± 0.032**	**0.133 ± 0.048**	**0.074 ± 0.033**	**0.275 ± 0.034**	**7.548 ± 2.301**	**0.295 ± 0.134**
S_r	mg kg^{-1}	**0.007**	**0.015**	**0.011**	**0.007**	**0.011**	**0.357**	**0.030**
r	mg kg^{-1}	**0.021**	**0.041**	**0.032**	**0.020**	**0.031**	**1.001**	**0.085**
RSD_r	%	**7.8**	**10.1**	**8.6**	**9.6**	**4.1**	**4.7**	**10.3**
S_R	mg kg^{-1}	**0.015**	**0.016**	**0.024**	**0.016**	**0.017**	**1.151**	**0.067**
R	mg kg^{-1}	**0.042**	**0.045**	**0.068**	**0.046**	**0.047**	**3.222**	**0.188**
RSD_R	%	**15.6**	**10.9**	**18.2**	**22.1**	**6.1**	**15.2**	**22.8**
Hor_{Rat}		**0.71**	**0.52**	**0.83**	**1.02**	**0.32**	**1.29**	**1.18**
Recovery $Rec \pm 2u_{Rec}$ ***(~ 95 %)***	%	**88.9 ± 29.4**	**88.7 ± 22.5**	**153.7 ± 57.6**	**81.6 ± 38.7**	**85.8 ± 12.6**	**74.7 ± 23.1**	**108.8 ± 55.4**

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

No statistically significant difference could be identified between the overall observed mean and the assigned values for all test items when taking into account the estimated expanded uncertainty of the analytical recovery ($2u_R$, to approximate the 95 % confidence interval). Therefore, no significant bias could be identified for the matrices investigated.

Furthermore, the method is considered fit for its intended purpose, since the HorRat ratios are below 2 in all cases.

No significant difference was observed for the two identical samples (S1 and S3), where the following ranges (expressed as $X_{obs} \pm 2\ S_R$) were obtained: 0.096 ± 0.03 mg kg^{-1} for S1 and 0.089 ± 0.022 mg kg^{-1} for S3.

6.4 Discussion of the results

The graphs in Annexes 7 to 13 clearly show that the mean values of the expert laboratories and those of the participants do overlap within their respective expanded measurement uncertainties.

It should be mentioned that the expert laboratories were informed about the kind of material they were analysing while the participants were not. This could explain why the expert laboratories agreed in the results reported for S7 within 5 %, even when seaweeds are known to be a challenging matrix for iAs speciation. The test item used as S7 was the only seaweed CRM in the market with a certified value for iAs and is widely known by laboratories working in this field. Most likely, NMIJ-7405a, was already used by some of the expert laboratories to validate their HPLC methods. Otherwise, it is difficult to explain a U_{CT} for S7 of 5 %, when the respective U_{CT} obtained for S1, a rice sample considered a much simpler matrix from the analytical point of view, was 10 %.

In addition, expert laboratories have used their methods of choice for the analysis while the participants were required to use the SOP. The results of the expert laboratories were also statistically evaluated and only in the case of the mussel sample (S4) two values were excluded as Grubbs outliers (Annex 9). It is interesting to mention that the results obtained by HG-ICP-MS (CA4) especially in the case of mussels (S4), cabbage (S5) and fish (S8) were not in agreement with the rest of the results delivered by the experts using HPLC-ICP-MS within their respective uncertainties.

The selective determination of iAs seems to be challenging in food of marine origin, this being particularly true for shell-fish, such as mussels. Even expert laboratories experienced problems in the analysis of those samples. This is probably due to the complex profile of organic species of As in those matrices, which could interfere and/or hinder the identification of the iAs species.

Direct determination of iAs by HG-ICP-MS seems to be affected by serious interferences in samples with a high percentage of organic species of As, if no previous separation of those species is done, for instance using liquid-liquid extraction or solid phase extraction (SPE). We acknowledge the fact that only one expert laboratory used HG-ICP-MS and so a limited amount of data was available. However, this observation was in agreement with

what was recorded in the IMEP-112 study where results obtained with the standard methods EN 15517:2008 and GB/T 5009.11-2003 (based on direct HG-AAS) resulted in unsatisfactory z-scores due to overestimation of the iAs mass fraction, when applied to algae.

In addition to the submission of results, participants were asked to answer a number of questions related to the standard operating procedure and to how the participants ensured the quality of their reported results.

These questions were considered relevant 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.

Accreditation

Two laboratories L08 and L011, did not hold accreditation. Laboratory L08 provided dispersed results in all cases and was identified as a Cochran outlier twice (S4, S6) and Cochran straggler once (S2). No outlying results were reported by the second non-accredited lab (L11).

Experience with iAs analysis

Five laboratories (L07, L08, L09, L10, L12) were not familiar with the specific analytical method. Participants L07, L08 do not include iAs as part of their routine analyses.

The comments collected by the participants regarding the method or specific issues of the exercise are shown in Table 6.

Point 9.3.1 of the SOP seems to be critical, certainly in samples with high fat content such as S4 and S8. Some laboratories could not separate the two phases properly due to formation of an emulsion. For this reason a sentence has been included in the SOP indicating that the centrifugation time or speed may be increased if needed to achieve a clear separation of the two phases.

Table 6 – Comments of the laboratories participating in the IMEP-41 CT

Lab	Do you have any comments? Please let us know...
L07	We do not recommend this method for routine analysis because it is very time consuming, quite complicated and because of the chemicals used. This method takes much more time than the one with SPE. The filtration step is complicated for some samples, and it was not possible to filter sample 8 (the filter was blocked).
L08	we have found many difficulties in the procedure of samples 4 and 8
L11	The blanks are different between days, and are different for each bottle of sample. It´s not correct to calculate a blank mean for each sample. The blank values obtained varied between 0.288 and 0.414 µg L^{-1}
L12	1. The MgO, we had got, contained As that may have significant effect on the results or its uncertainty. 2. We have a suggestion to use only Mg-nitrate in higher concentration instead of MgO. 3. We guess that less time in muffle furnace is enough (e.g. 3 h) 4. In some cases the chloroform phase and acid phase were not clearly separated by 5

	minutes centrifugation.
L13	At the stage of back-extraction (point 9.4 SOP) in some cases (samples No. 5 and 8) a scum layer appeared between the phase boundaries after shaking and centrifuging

7 Conclusion

The method validated in IMEP-41 is robust and does not require any adaptation depending on the matrix to be analysed. On the basis of the statistical evaluation of the present collaborative trial study, the proposed method is considered fit for its intended analytical purpose, i.e. determination of iAs in different food commodities. The method proves to have adequate trueness and precision for the iAs mass fraction range 0.074 to 7.5 mg kg^{-1}, provided that the SOP is respected.

The validated method is a low-cost alternative for laboratories which do not have HPLC-ICP-MS instrumentation at their premises, and may be applied directly for the analysis of a wide range of food commodities.

The results obtained by HG-ICP-MS (CA4) especially in the case of mussels (S4), cabbage (S5) and fish (S8) were not in agreement with the rest of the results delivered by the experts using HPLC-ICP-MS within their respective uncertainties. Direct determination of iAs by HG-ICP-MS seems to be affected by serious interferences in samples with a high percentage of organic species of As, if no previous separation of those species is done.

The chromatographic resolution of iAs seems to be particularly challenging in food of marine origin, especially in the case of shell-fish, such as mussels.

Acknowledgements

Participating laboratories (listed below) are kindly acknowledged. Furthermore, F. Ulberth and J. Charoud-Got are acknowledged for reviewing the manuscript.

Organisation	Country
AGES GmbH	Austria
Croatian National Institute of Public Health	Croatia
State Veterinary Institute Olomouc	Czech Republic
Laboratoire SCL de Bordeaux	France
General Chemical State Laboratory	Greece
National Food Chain Safety Office	Hungary
Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise	Italy
ARPA Marche	Italy
National Institute of Public Health - National Institute of Hygiene (NIPH - NIH)	Poland
Lab. Salud Publica Bizkaia	Spain
Universidad Miguel Hernández de Elche	Spain
University of Valencia	Spain
Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC)	Spain

Abbreviations

ILC	Interlaboratory Comparison
CT	Collaborative Trial (collaborative study)
IMEP	International Measurement Evaluation Programme
JRC	Joint Research Centre
IAEA	International Atomic Energy Agency
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
NMIJ	National Metrology Institute of Japan
NRCC	National Research Council of Canada
NIST	National Institute of Standards and Technology
SOP	Standard Operating Procedure

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ANNEXES

Annex 1: Announcement to NRLs

Announcement of IMEP-41 collaborative trial to NRLs

FIAMEGKOS Ioannis (JRC-GEEL)

You forwarded this message on 18/12/2013 16:13.

Sent: Thu 12/12/2013 13:16
To: JRC IRMM EURL HEAVY METALS
Cc: DE LA CALLE GUNTINAS Maria Beatriz (JRC-GEEL); CORDEIRO RAPOSO Fernando (JRC-GEEL); ROBOUCH Piotr (JRC-GEEL)

Dear colleagues,
During the last workshop organised by the EURL for Heavy Metals in Feed and Food, you were informed that IMEP will organise a collaborative trial (IMEP-41) with the purpose of validating a method for the selective determination of inorganic arsenic (iAs) in food.
Currently, CEN TC 275/WG 10 is in the process of validating a method for the selective determination of iAs in food which is based on the use of HPLC-ICP-MS. The method which IMEP will try to validate in a collaborative trial is based on selective extraction of iAs with an organic solvent and further determination by Hydride Generation Atomic Absorption Spectrometry (HG-AAS). This method, if successfully validated, will be complementary to the one standardised by CEN since it will be an alternative for laboratories which do not have an ICP-MS in their premises or which are not familiar with hyphenation.
Those of you with experience in HG-AAS and who are interested in taking part in IMEP-41, please contact us before the end of 2013. The collaborative trial will be run in the first half of 2014. We will distribute the SOP of the candidate method in January 2014. Those laboratories which will register in IMEP-41 will receive a test sample before starting the collaborative trial so that they can get familiar with the SOP.
If you know some laboratories in your country which are currently using this method or a similar one and which could be interested in taking part in IMEP-41, please let them know. Keep in mind that the method implies the use of HG-AAS.

Looking forward to hearing from you

With kindest regards

Dr. IOANNIS FIAMEGKOS
EU-RL Heavy Metals / IMEP

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DG JRC-IRMM
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B-2440 Geel/Belgium
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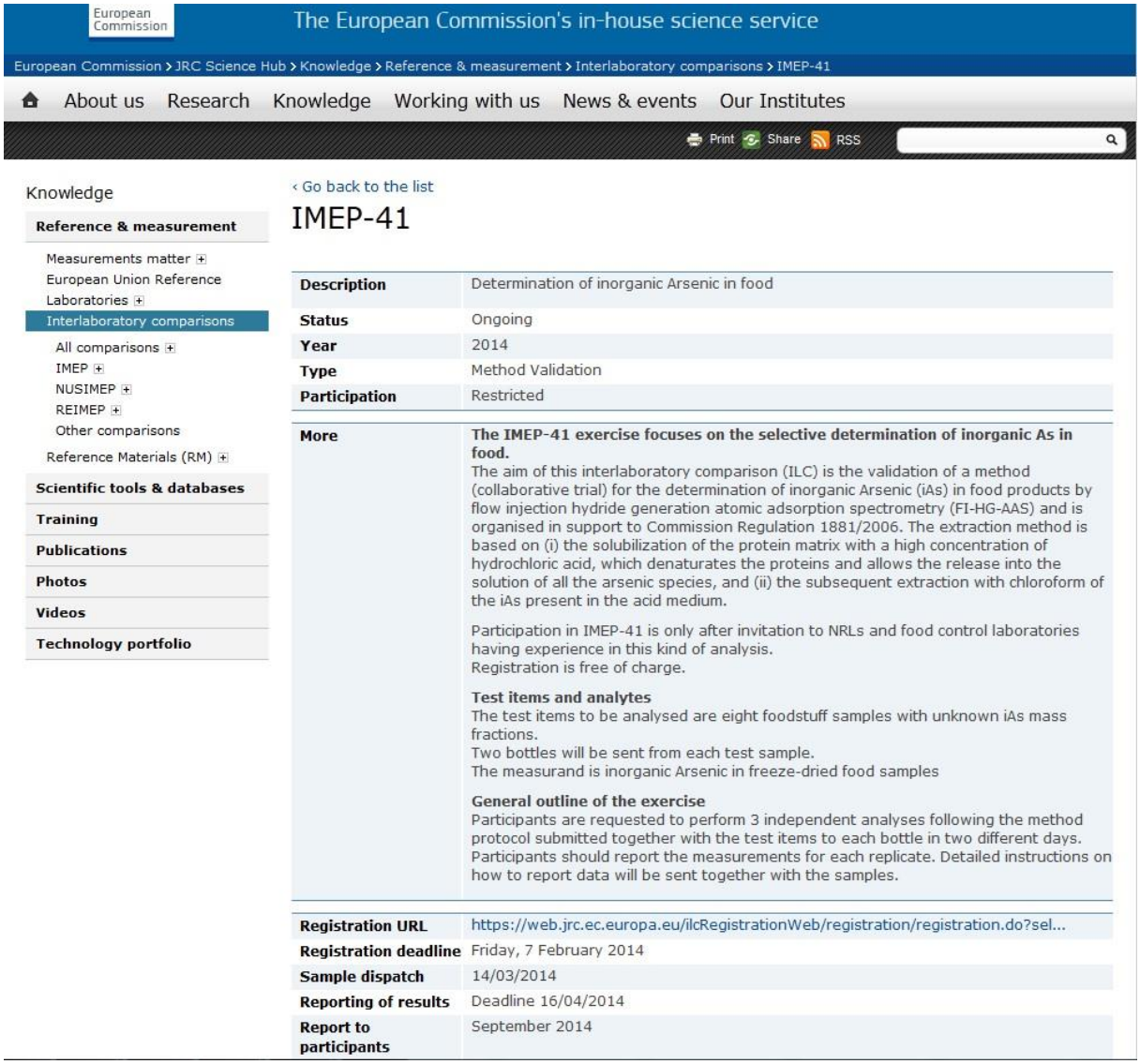
Annex 2: IRMM – IMEP web announcement



European Commission

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IMEP-41

Description	Determination of inorganic Arsenic in food
Status	Ongoing
Year	2014
Type	Method Validation
Participation	Restricted
More	**The IMEP-41 exercise focuses on the selective determination of inorganic As in food.** The aim of this interlaboratory comparison (ILC) is the validation of a method (collaborative trial) for the determination of inorganic Arsenic (iAs) in food products by flow injection hydride generation atomic adsorption spectrometry (FI-HG-AAS) and is organised in support to Commission Regulation 1881/2006. The extraction method is based on (i) the solubilization of the protein matrix with a high concentration of hydrochloric acid, which denaturates the proteins and allows the release into the solution of all the arsenic species, and (ii) the subsequent extraction with chloroform of the iAs present in the acid medium. Participation in IMEP-41 is only after invitation to NRLs and food control laboratories having experience in this kind of analysis. Registration is free of charge. **Test items and analytes** The test items to be analysed are eight foodstuff samples with unknown iAs mass fractions. Two bottles will be sent from each test sample. The measurand is inorganic Arsenic in freeze-dried food samples **General outline of the exercise** Participants are requested to perform 3 independent analyses following the method protocol submitted together with the test items to each bottle in two different days. Participants should report the measurements for each replicate. Detailed instructions on how to report data will be sent together with the samples.
Registration URL	https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?sel...
Registration deadline	Friday, 7 February 2014
Sample dispatch	14/03/2014
Reporting of results	Deadline 16/04/2014
Report to participants	September 2014

Annex 3: Samples accompanying letter

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Geel, 13 February 2014
JRC.D5/IF/acs/ Ares(2014)361384

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

Participation in IMEP-41, a collaborative trial for the validation of a method for the determination of inorganic Arsenic in food.

Dear «Title» «Surname»,

Thank you for participating in the IMEP-41 a collaborative trial for the validation of a method to determine inorganic arsenic in food. 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) 16 bottles containing, each approximately 20 g (sample 1 and 2), 8 g (sample 4), 5 g (samples 3, 5, 6, 7 and 8) of the test items (two bottles from each test material),
b) A bottle of a pre-test item (containing 15 g of test item) named "test sample"
c) A copy of the standard operational procedure which must be followed strictly
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 °C) until analysis.

The measurand is inorganic As in eight different food matrices.

Before starting with the analyses of Samples 1-8, apply the method to be validated as described in the Standard Operational Procedure to the Sample Test item. Send to jrc-irmm-imep@ec.europa.eu the result that you have obtained for that sample **(do this by 12th March 2014)**, wait till you will have received an e-mail from the IMEP coordinator saying that you can continue with the analysis of Samples 1-8. 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 the **Sample Test**, follow the standard Operational Procedure that you have received. Report the mean value for inorganic Arsenic together with its associate expanded uncertainty (k=2). Express the results in mg kg^{-1} of As following the instructions given in paragraph 12.4 of the SOP.

For the analysis of **Samples 1-8**, 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} of As following the instructions given in paragraph 12.4 of the SOP.

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 moisture. **To calculate the moisture content in the test materials, please apply the following procedures:**

Test Sample. Weigh accurately at least 0.2 g of test material in a glass container (preferably with a lid). Place it in an oven for 18 h at 85 ± 2 °C. Allow the glass container (covered with the lid) to cool down for about 30 min in a desiccator before weighing.

Sample 1. Weigh accurately ca. 1 g of test material in a glass container of 5-7 cm diameter (preferably with a lid). Place it in an oven for 18 h at 85 ± 2 °C. Allow the glass container (covered with the lid) to cool down for about 30 min in a desiccator before weighing.

Sample 2. Weigh accurately ca. 1 g of test material in a glass container of 5-7 cm diameter (preferably with a lid). Place it in an oven for 60 ± 5 min at 120 ± 2 °C. Allow the glass container (covered with the lid) to cool down for about 30 minutes in a desiccator before weighing.

Sample 3. Weigh accurately ca. 1 g of test material in a glass container of 5-7 cm diameter (preferably with a lid). Place it in an oven for 18 h at 85 ± 2 °C. Allow the glass container (covered with the lid) to cool down for about 30 min in a desiccator before weighing.

Sample 4. Weigh accurately ca. 0.2 g of test material and dry the sample in the oven at atmospheric pressure at 103 ± 2 °C until constant mass is attained.

Sample 5. Weigh accurately ca. 0.5 g of test material and dry the sample in the oven at atmospheric pressure at 100 °C until constant mass is attained. Subsequent weighing should differ by less than 5 mg.

Sample 6. Weigh accurately ca 0.5 g of test material in a petri-dish of 3.5 cm diameter (preferably with a lid). The thickness of the powder-layer should be about 3-4 mm covering the bottom of the dish. Place it in an oven for 60 ± 2 minutes at 90 ± 2 °C. Allow the glass container (covered with the lid) to cool down for about 30 minutes in a desiccator before weighing.

Sample 7. Weigh accurately ca. 1.0 g of test material in a glass container and dry the sample in the oven at atmospheric pressure for 12 – 16 h at 85 °C. Allow the glass container (covered with the lid) to cool down in a silica desiccator before weighing.

Sample 8. Weigh accurately ca. 0.5 g of test material in a glass vessel (preferably with a lid). Place it in an oven for 2 h at 102 ± 2 °C. 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 moisture content in **duplicate**.
Note 2: Do not use for the inorganic arsenic determination the same aliquots of test material that you have used for the moisture 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 **11/04/2014**.

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. Ioannis Fiamegkos
IMEP-41 Co-ordinator

Enclosures:

1) Two bottles containing the test material for each test sample (16 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: Standard Operating Procedure (SOP)

PROTOCOL FOR DETERMINATION OF INORGANIC ARSENIC IN FOOD SAMPLES

1. SCOPE

This standard operating procedure specifies a method for the determination of inorganic arsenic (iAs) content in various food commodities by flow injection hydride generation atomic adsorption spectrometry (FI-HG-AAS).

The calibration curve ranges from 0.5 to 10 µg $L^{-1}$of As.

The quantification limit is 0,010 mg kg^{-1} of iAs.

> Note: Samples with arsenic concentrations above this level should be diluted appropriately (or reanalyse diluting in a larger volume) to ensure measurement within the calibration range.

2. PRINCIPLE

The extraction method is based on (i) the solubilization of the protein matrix with a high concentration of hydrochloric acid, which denatures the proteins and allows the release into the solution of all the arsenic species, and (ii) the subsequent extraction with chloroform of the inorganic arsenic (iAs) present in the acid medium.

3. EQUIPMENTS

3.1 Centrifuge.

3.2 Mechanical shaker.

3.3 Sand bath.

3.4 Muffle furnace.

3.5 Atomic absorption spectrometer equipped with a flow injection system.

4. REAGENTS

4.1 Deionized water (18.2 MΩ cm).

4.2 Hydrochloric acid (HCl) concentrated, not less than 37 % m/v, c(HCl) = 12 mol L^{-1}, having a density of approximately ρ (HCl) 1,15 g L^{-1}.

4.3 Nitric acid (HNO_3) concentrated, not less than 65 % m/v, c(HNO_3) = 14 mol L^{-1}, having a density of approximately ρ (HNO_3) 1,38 g L^{-1}.

4.4 Chloroform ($CHCl_3$).

4.5 Hydrogen bromide (HBr), not less than 48 % m/v.

4.6 Hydrazine sulphate ($N_2H_6SO_4$).

4.7 Magnesium nitrate hexahydrate [$Mg(NO_3)$ $6H_2O$].

4.8 Magnesium oxide (MgO).

4.9 Potassium iodide (KI).

4.10 Ascorbic acid ($C_6H_8O_6$).

4.11 Sodium hydroxide (NaOH).

4.12 Sodium borohydride ($NaBH_4$).

4.13 Arsenic(V) standard solution, 1000 mol L^{-1}. Use traceable certified standard solutions commercially available.

5. SOLUTIONS

5.1 Hydrochloric acid 6 mol L^{-1}, to be prepared: pipette 100 mL of concentrated hydrochloric acid (4.2) in a 200 mL volumetric flask and fill to the mark with deionized water (4.1).

5.2 Hydrochloric acid 1 mol L^{-1}, to be prepared: pipette 8.33 mL of concentrated hydrochloric acid (4.2) in a 100 mL volumetric flask and fill to the mark with deionized water (4.1).

5.3 Nitric acid 50 % v/v, to be prepared: pipette 100 mL of concentrated nitric acid (4.3) in a 200 mL volumetric flask and fill to the mark with deionized water (4.1).

5.4 Nitric acid 10 % v/v, to be prepared: pipette 10 mL of concentrated nitric acid (4.3) in a 100 mL volumetric flask and fill to the mark with deionized water (4.1).

5.5 Nitric acid 5 % v/v, to be prepared: pipette 5 mL of concentrated nitric acid (4.3) in a 100 mL volumetric flask and fill to the mark with deionized water (4.1).

5.6 Hydrazine sulphate (15 mol L^{-1}). Suspend 150 mg of Hydrazine sulphate (4.6) in 10 mL of deionized water (4.1).

5.7 Ashing aid solution. Suspend 20 g of magnesium nitrate hexahydrate (4.7) and 2 g of magnesium oxide (4.8) in 100 mL of deionized water (4.1).

5.8 Pre-reducing solution: Dissolve 5 g of potassium iodide (4.9) and 5 g of ascorbic acid (4.10) in 100 mL of deionized water (4.1).

5.9 As(V) standard solution 10 mg L^{-1}, to be prepared: pipette 1 mL of the arsenic(V) standard solution 1000 mol L^{-1} (4.13) in a 100 mL volumetric flask and fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

5.10 As(V) standard solution 0.1 mg L^{-1}, to be prepared: pipette 1 mL of the arsenic(V) standard solution 10 mg L^{-1} (5.9) in a 100 mL volumetric flask and fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

5.11 As(V) standard solution 25 µg L^{-1}, to be prepared: pipette 25 mL of the arsenic(V) standard solution 0.1 mg L^{-1} (5.10) in a 100 mL volumetric flask and fill to the mark with hydrochloric acid (5.1).

6. CALIBRATION STANDARD SOLUTIONS OF As(III)

6.1 As(III) calibration standard solution 10 µg L^{-1}, to be prepared: pipette 20 mL of the As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

6.2 As(III) calibration standard solution 7.5 µg L^{-1}, to be prepared: pipette 15 mL of the As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

6.3 As(III) calibration standard solution 5 µg L^{-1}, to be prepared: pipette 10 mL of the As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

6.4 As(III) calibration standard solution 2.5 µg L^{-1}, to be prepared: pipette 5 mL the As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

6.5 As(III) calibration standard solution 1.0 µg L^{-1}, to be prepared: pipette 2 mL the As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

6.6 As(III) calibration standard solution 0.5 µg L^{-1}, to be prepared: pipette 1 mL the As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

6.7 Calibration blank, to be prepared: pipette 10 mL of hydrochloric acid (5.1), add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

Note1: All As(III) calibration standard solutions shall be freshly prepared before each calibration.

Note2: Other volumes of preparation are suitable provided that they maintain the proportions described above.

7. QUALITY CONTROL SOLUTIONS

Quality Control solutions are used to ensure the validity of the calibration. The quantification limit solution (QC1) ensures that the quantification at low concentration level is correct, while intermediate level solution (QC2) – placed at the end of the analytical sequence - confirms whether the response is stable at higher concentrations with no significant drift with time. Both solutions shall be freshly prepared before each analytical sequence.

7.1 Quantification limit solution QC1 (1 µg L^{-1} As(III)), to be prepared: pipette 2 mL of As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid (5.1).

7.2 Intermediate level solution QC2 (5 µg L^{-1} As(III)), to be prepared: pipette 10 ml of As(V) standard solution 25 µg L^{-1} (5.11) in a 50 mL volumetric flask, add 10 mL of pre-reducing solution (5.8), wait for 30 minutes, then fill to the mark with hydrochloric acid (5.1).

8. MATERIAL

8.1 Polypropylene centrifuge (PC) tubes (50 mL) with screw cap.

8.2 Syringe filters with hydrophobic PTFE membrane (25 mm diameter).

8.3 Pyrex glass beaker (tall form, 250 mL), capable of withstanding 500 ºC.

8.4 Watch glasses.

8.5 Volumetric flasks (10, 25, 100 or 200 mL), Class A

8.6 Plastic funnels.

8.7 Whatman nº 1 paper or equivalent.

Note: All glassware shall be treated with Nitric acid (5.4) for 24 h and rinsed three times with deionized water (4.1) before use.

9. PROCEDURE

9.1 Hydrolysis

9.1.1 Weigh accurately, ca. 0.5 to 1 g, of lyophilized sample (or freshly homogenised sample) in a PC tube (8.1)

9.1.2 Add 4.1 mL of deionized water (4.1)

9.1.3 Agitate with mechanical shaker (3.2) until the sample is completely wet (ca. 5 min)

9.1.4 Add 18.4 mL of hydrochloric acid (4.2)

9.1.5 Agitate with mechanical shaker (3.2) for 15 min

9.1.6 Leave to rest for 12-15 hours (overnight)

Sample of algae hydrolised overnight

Note: At least two reagent blanks shall undergo the whole analytical process, starting from Step 9.1.2 onwards.

9.2 Extraction

9.2.1 Add 2 mL of hydrogen bromide (4.5) and 1 mL of hydrazine sulphate (5.6) to the hydrolysed sample

9.2.2 Shake for 30 seconds with the mechanical shaker (3.2)

9.2.3 Add 10 mL of chloroform (4.4)

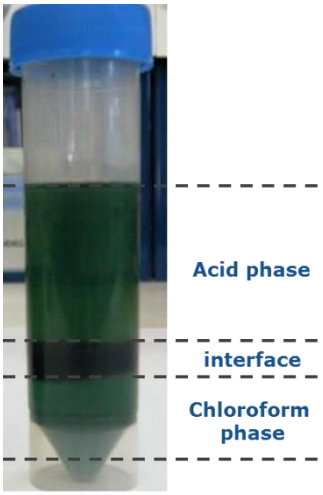
9.2.4 Shake for 5 min with the mechanical shaker (3.2)

9.2.5 Centrifuge (3.1) for 5 min at 2000 rpm (805 g)

9.2.6 Pipette the chloroform (lower) phase and pour into another PC tube (8.1)

9.2.7 Repeat steps 9.2.3- 6 one more time. Ca. 20 mL of chloroform should be collected

Note: Due care must be ensured to avoid cross-contamination



9.3 Clean-up of the chloroform phase

9.3.1 Centrifuge (3.1) the pooled chloroform phases for 5 min at 2000 rpm (805 g)

9.3.2 Remove **all** acid phase residues (drops) remaining on the surface of the chloroform with a 1 mL pipette

9.3.3 Filter (syringe) through hydrophobic membrane (8.2) the remaining solid or acid phase residues present in the chloroform phase

Filtration of chloroform phase after centrifugation

Note1: Step 9.3.1 The centrifugation time or speed may be increased if needed to achieve a clear separation of the two phases.

Note2: Step 9.3.2 is crucial. Any acid phase residues remaining in the chloroform phase will lead to overestimated iAs results

Note3: Practical tip - drill a hole in the cap of the PC tube and insert the syringe tip in order to collect directly the filtered solution (see photo above). Other set-ups may also be used.

9.4 Back-extraction

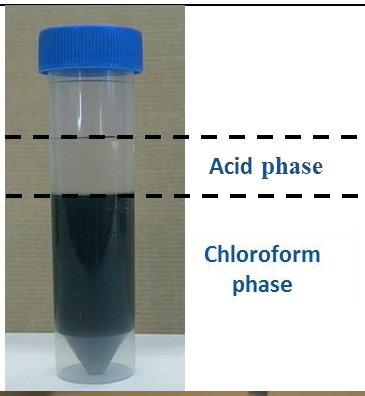
9.4.1 Add 10 mL of hydrochloric acid 1 mol L^{-1} (5.2) to the clean chloroform phase collected after the filtration step in the PC tube (8.1)

9.4.2 Shake for 5 min with the mechanical shaker (3.2)

9.4.3 Centrifuge for 5 min at 2000 rpm (805 g).

9.4.4 Pipette the (upper) back-extraction phase and pour into a tall Pyrex glass beaker (8.3) for mineralization.

9.4.5 Repeat steps 9.4.1-4 once more



Transfer the acid phases into a beaker by pipetting

9.5 Sample mineralization

9.5.1 Add 2.5 mL of the ashing aid (5.7) to the Pyrex glass beaker (8.3)

Note: The ashing aid must be added while the suspension is shaken in order to avoid precipitation.

9.5.2 Add 10 mL of concentrated nitric acid (4.3) and evaporate in a sand bath (3.3) to dryness.

Note1: Avoid any projections in the sand bath.

Note2: In order to verify that the samples are totally dry, place a watch glass on top of the Pyrex glass beaker and check that no condensation is formed.

9.5.3 Place the flask in the muffle furnace at an initial temperature not exceeding 150 °C, cover it with a watch glass, and progressively increasing the temperature to 425 ± 25 °C at the rate of 50 °C/h. Maintain at 425 ºC for 12 hours.

Note: This step is critical. To avoid any projections the rate of increase in temperature must be strictly implemented.

9.5.4 Allow the ashes to cool down

9.5.5 Add 0.5 mL of deionized water (4.1) to wet the ash and then add 5 mL of hydrochloric acid 6 mol L^{-1} (5.1). Dissolve the ash completely, shake if necessary.

Note: Due care should be taken to recuperate all the ash from the walls of the Pyrex glass beaker

9.5.6 Add 5 mL of pre-reducing agent (5.8), and wait 30 minutes to complete the reduction of iAs to As(III).

9.5.7 Filter the solution through the Whatman paper or equivalent (8.7) and collected in a 25 mL volumetric flask. Rinse the Pyrex glass beaker twice with hydrochloric acid (5.1), to avoid losses. Include the washing liquids, after filtration, to the solution then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).
Note1: The filter must be decontaminated before use. Wash it with nitric acid 5% m/v (5.5) and rince it twice with deionized water (4.1).

When the expected iAs concentration is close or below the quantification limit of 0,010 mg kg^{-1} the mineralisation step shall be modified as follows:

9.5.4 Allow the ashes to cool down

9.5.5b Add 0.5 mL of deionized water (1) to wet the ash and then add 2 mL of hydrochloric acid 6 mol L^{-1} (5.1). Dissolve the ash completely, shake if necessary.

9.5.6b Add 2 mL of pre-reducing agent (5.8), and wait 30 minutes to complete the reduction of the iAs.

9.5.7b Filter the solution through the Whatman paper (8.7) and collected in a 10 mL volumetric flask. Rinse the Pyrex glass beaker twice with hydrochloric acid (5.1), to avoid losses. Include the washing liquids, after filtration, to the solution then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

When the iAs concentration is expected to be high, the mineralisation step shall be modified as follows:

9.5.4 Allow the ashes to cool down

9.5.5c Add 0.5 mL of deionized water (1) to wet the ash and then add 10 mL of hydrochloric acid 6 mol L^{-1} (5.1). Dissolve the ash completely, shake if necessary.

9.5.6c Add 10 mL of pre-reducing agent (5.8), and wait 30 minutes to complete the reduction of the iAs.

9.5.7c Filter the solution through the Whatman paper (8.7) and collected in a 50 mL volumetric flask. Rinse the Pyrex glass beaker twice with hydrochloric acid (5.1), to avoid losses. Include the washing liquids, after filtration, to the solution then fill to the mark with hydrochloric acid 6 mol L^{-1} (5.1).

Note: Re-dissolved and pre-reduced samples are stable for 24 hours at 4 °C.

10. CALIBRATION

Calibration shall be performed by means of external calibration. It is important that the measurements are made in the linear range of the instrument. All calibration standard solutions mentioned under 6.2 shall be used (from blank to 10 µg L-1).

11. ANALYTICAL SEQUENCE

Use the following sequence as a guide:

- Quantification level solution QC1 (7.1).
- Reagent blank (9.1 Note)
- (several) Samples.
- Intermediate level solution QC2 (7.2)
-

12. DETERMINATION

Optimise the instrumental parameters of the equipment in accordance with the recommendations described in the manufacture's manual for the apparatus.

12.1 Example of instrumental conditions for HG-AAS quantification:

Flow injection hydride generation	• Loop sample: 0.5 mL Note: to be adapted when the reconstitution volume of the final pre-reducing solution is different from 25 mL. • Reducing agent: 0.2 % (w/v) NaBH4 in 0.05 % (w/v) NaOH; 5 mL min^{-1} flow rate • HCl solution 10 % (v/v), 10 mL min^{-1} flow rate • carrier gas argon, 100 mL min^{-1} flow rate
Atomic absorption spectrometer:	• wavelength: 193.7 nm • spectral band-pass: 0.7 nm • electrode-less discharge lamp system 2 • lamp current setting: 400 mA • cell temperature 900 °C

12.2 Information to register

All data required to ensure traceability of results shall be registered according to the EN 13804:2013 standard. The following information is considered to be the minimum for a report, but additional information may be required, depending on the circumstances.

a) information for the complete identification of the sample; b) description of the test portion analysed and prepared part of the sample; c) the test method used, d) the test results obtained and the units in which they are specified; e) date when the analysis was finished; f) whether the requirement of the repeatability limit has been fulfilled; g) all operating details not specified in this document, or regarded as optional, together with details of any incidents occurred when performing the method which may have influenced the test results.

12.3 Calculation

Results are obtained in mg kg^{-1} of As in the extract, applying the following equation.

$$iAs\ (mg\ kg^{-1}) = \frac{V \cdot (C_x - C_{Bl})}{1000 \cdot w}$$

Where:

- C_x: Concentration in the extract (µg L^{-1}), extrapolated from the calibration curve
- C_{Bl}: Concentration in the reagent blank sample (µg L^{-1}), extrapolated from the calibration curve
- V: final volume of the sample mineralisation step (9.5.7), usually V = 25 mL
- w: weight of sample (in grams)

12.4 Expression

Results of iAs and the corresponding expanded measurement uncertainty (k = 2) are expressed in mg kg^{-1}. Three decimal figures are to be reported for concentrations below 0,25 mg kg^{-1}, and two decimal figures from greater values. Results below quantification limit will be reported as "< LOQ (0,010 mg kg^{-1})", as recommended by EN 13804:2013.

12.5 Internal control for quality evaluation

Results will be considered acceptable only when the following criteria will be met:

12.5.1 Instrument verification:

Instrument verification is performed with control solutions at the beginning and the end of the analytical sequence.

Acceptance criteria for results obtained for QC1: 0,85 – 1,15 µg L^{-1}

Acceptance criteria for results obtained for QC2: 4,25 – 5,75 µg L^{-1}

12.5.2 Calibration curve:

Plot the relative residual error for the range of concentrations covered by the calibration standard solutions.

Acceptance criteria: Relative residual error below 15 % at 1 µg L^{-1} and below or equal to 10% for all other concentrations

Annex 5: Confirmation of receipt form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Tis document is an attachment to
JRC.D5/IF/acs/ Ares(2014)361384

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

IMEP-41

Determination of inorganic Arsenic in food

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:

Ioannis Fiamegkos

IMEP-41 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

Fax : +32-14-571865

e-mail : JRC-IRMM-IMEP@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 374, Fax: (32-14) 571 865

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

Annex 6: Questionnaire

Questionnaire questions

1. **Which instrument did you use for the analysis ?** *[Q:106941: TEXT]*

2. **Which range (µg/L) was used for the calibration curve ?** *[Q:106936: TEXT]*

3. **In case you have introduced any modifications to the proposed protocol, please describe it/them briefly** *[Q:106943: GROUP]*

   3.1. Hydrolysis *[Q:106970: TEXT]*

   3.2. Reduction *[Q:107033: TEXT]*

   3.3. Extraction *[Q:106971: TEXT]*

   3.4. Clean-up of the chloroform phase *[Q:106972: TEXT]*

   3.5. Back extraction *[Q:106973: TEXT]*

   3.6. Sample mineralization *[Q:106974: TEXT]*

   3.7. Other *[Q:106975: TEXT]*

4. **Specify the final dilution volume used if different from 25 mL.** *[Q:106944: CUSTOM TABLE]*

   Final dilution volume

Questions/Response table	Sample 1	Sample 2	Sample 3	Sample 4
volume (mL)				
Questions/Response table	Sample 5	Sample 6	Sample 7	Sample 8
volume (mL)				

5. **Which filter did you use for the clean-up of the chloroform phase ?** *[Q:106945: TEXT]*

6. **Did you use a blank concentration to calculate the iAs concentration using the equation of the paragraph 12.3 of the protocol ?**

   - No *[A:1236]*
   - Yes *[A:397]*

6.1. If Yes, which one ? *[Q:106978: CUSTOM TABLE]*

Blank concentration

Questions/Response table	Sample 1	Sample 2	Sample 3	Sample 4
Concentration (µg/L)				
Questions/Response table	Sample 5	Sample 6	Sample 7	Sample 8
Concentration (µg/L)				

7. **Where you familiar with this method before participating in this collaborative trial ?** *[Q:106947: CHECKBOX]*

- ☐ No *[A:1236]*
- ☐ Yes *[A:397]*

8. **Does your laboratory analyze inorganic As on a regular basis ?** *[Q:106953: CHECKBOX]*

- ☐ No *[A:396]*
- ☐ Yes *[A:397]*

8.1. If yes, please estimate the number of samples : *[Q:106954: CHECKBOX]*

- ☐ a) < 10 samples/year *[A:1116]*
- ☐ b) 10-20 samples/year *[A:1115]*
- ☐ c) 20-50 samples/year *[A:1117]*
- ☐ d) > 50 samples/year *[A:1118]*

8.2. If Yes, which method are you using for this kind of analysis *[Q:106976: TEXT]*

9. **Does your laboratory have a quality system in place ?** *[Q:106948: CHECKBOX]*

- ☐ No *[A:396]*
- ☐ Yes *[A:397]*

9.1. If yes, which one ? *[Q:106949: CHECKBOX]*

- ☐ ISO 17025 *[A:302]*
- ☐ ISO 9000 series *[A:301]*
- ☐ UV *[A:294]*

9.1.1. If other, please specify : *[Q:106950: TEXT]*

9.2. Are you accredited ? *[Q:106951: CHECKBOX]*

- ☐ No *[A:396]*
- ☐ Yes *[A:397]*

9.2.1. If yes, by which accreditation body ? *[Q:106952: TEXT]*

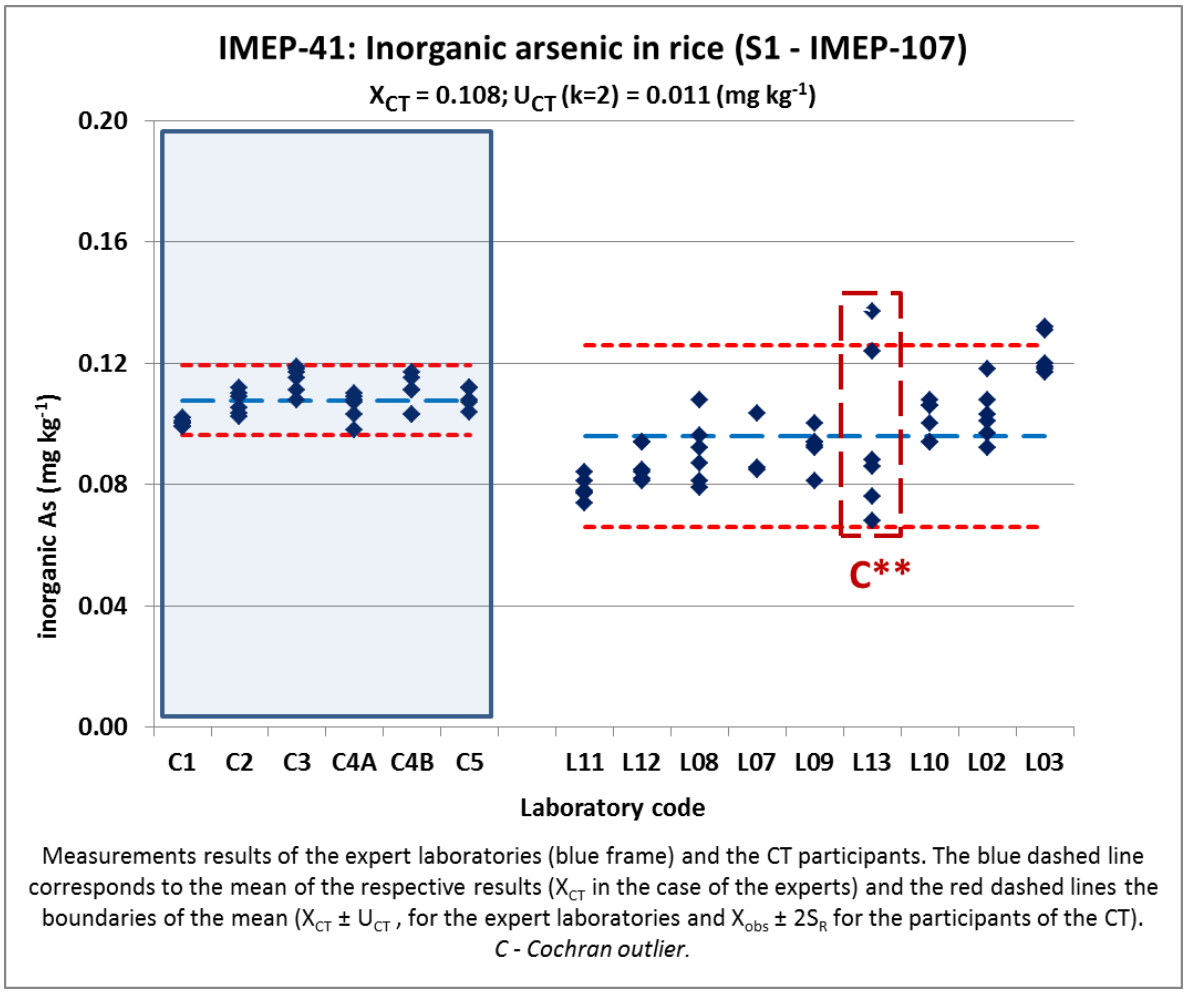
10. **Do you have any comments ? Please, let us know ...** *[Q:106962: TEXT]*

Annex 7: Results for Sample 1 - Rice (IMEP-107)

X_{CT} = 0.108 and U_{CT} = 0.011 (k=2); all values are given in mg kg^{-1}.

Lab code	R1	R2	R3	R4	R5	R6	mean
L11	0.078	0.074	0.077	0.084	0.081	0.077	**0.079**
L12	0.094	0.085	0.094	0.082	0.081	0.084	**0.087**
L08	0.081	0.087	0.096	0.108	0.092	0.079	**0.091**
L07	0.1034	0.0854	0.0847				**0.091**
L09	0.081	0.093	0.1	0.094	0.094	0.092	**0.092**
L13	**0.076**	**0.086**	**0.124**	**0.088**	**0.137**	**0.068**	**0.097 **C**
L10	0.094	0.094	0.106	0.106	0.108	0.1	**0.101**
L02	0.108	0.092	0.118	0.103	0.101	0.097	**0.103**

**C = Outlier identified by Cochran test



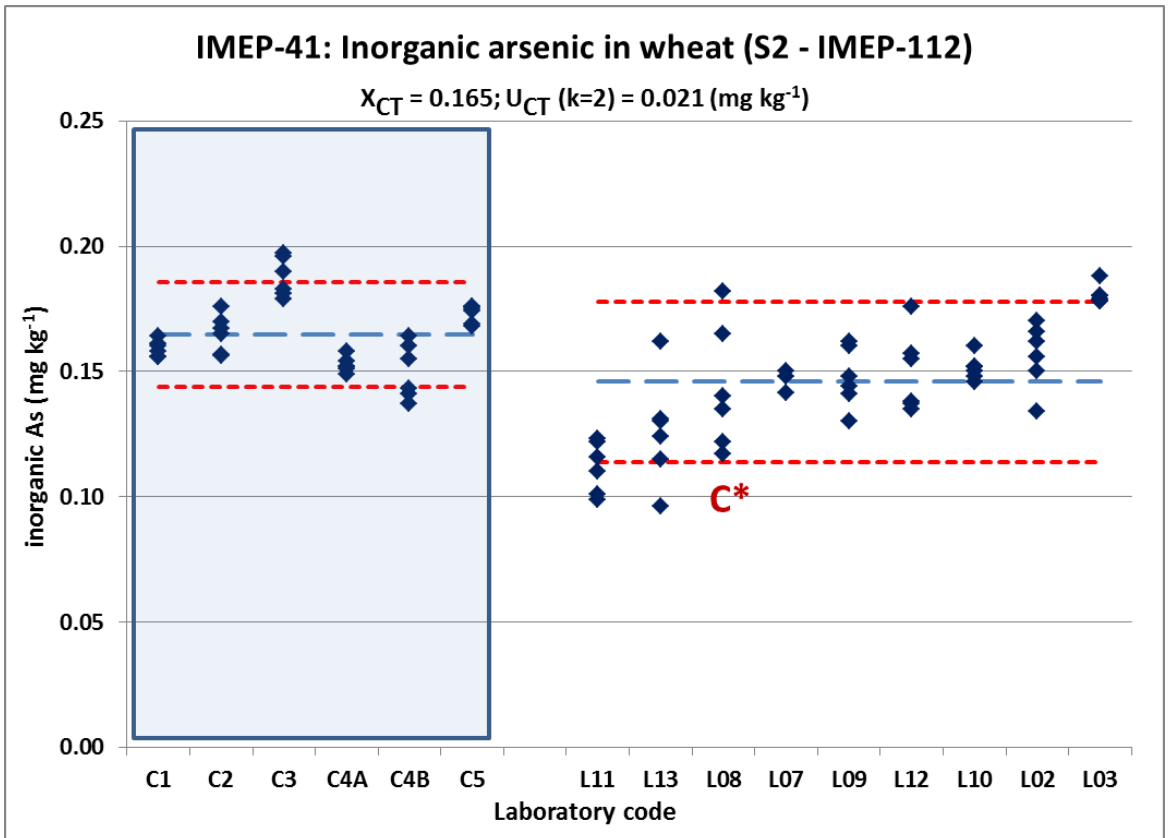
Measurements results of the expert laboratories (blue frame) and the CT participants. The blue dashed line corresponds to the mean of the respective results (X_{CT} in the case of the experts) and the red dashed lines the boundaries of the mean ($X_{CT} \pm U_{CT}$, for the expert laboratories and $X_{obs} \pm 2S_R$ for the participants of the CT).
C - Cochran outlier.

Annex 8: Results for Sample 2 - Wheat (IMEP-112)

X_{CT} = 0.165 and U_{CT} = 0.121 (k=2); all values are given in mg kg^{-1}.

Lab code	R1	R2	R3	R4	R5	R6	mean
L02	0.162	0.15	0.17	0.134	0.166	0.156	**0.156**
L03	0.18	0.18	0.179	0.188	0.178	0.178	**0.181**
L07	0.142	0.148	0.150				**0.147**
L08	**0.135**	**0.122**	**0.117**	**0.165**	**0.14**	**0.182**	**0.144 *C**
L09	0.16	0.148	0.144	0.162	0.13	0.141	**0.148**
L10	0.146	0.15	0.148	0.16	0.152	0.152	**0.151**
L11	0.122	0.116	0.123	0.11	0.101	0.099	**0.112**
L12	0.137	0.135	0.138	0.155	0.176	0.157	**0.150**
L13	0.162	0.13	0.124	0.115	0.131	0.096	**0.126**

*C = Straggler identified by Cochran test - The test statistics is greater than its 1 % critical value and the laboratory (or the single replicate value) is considered as an outlier.



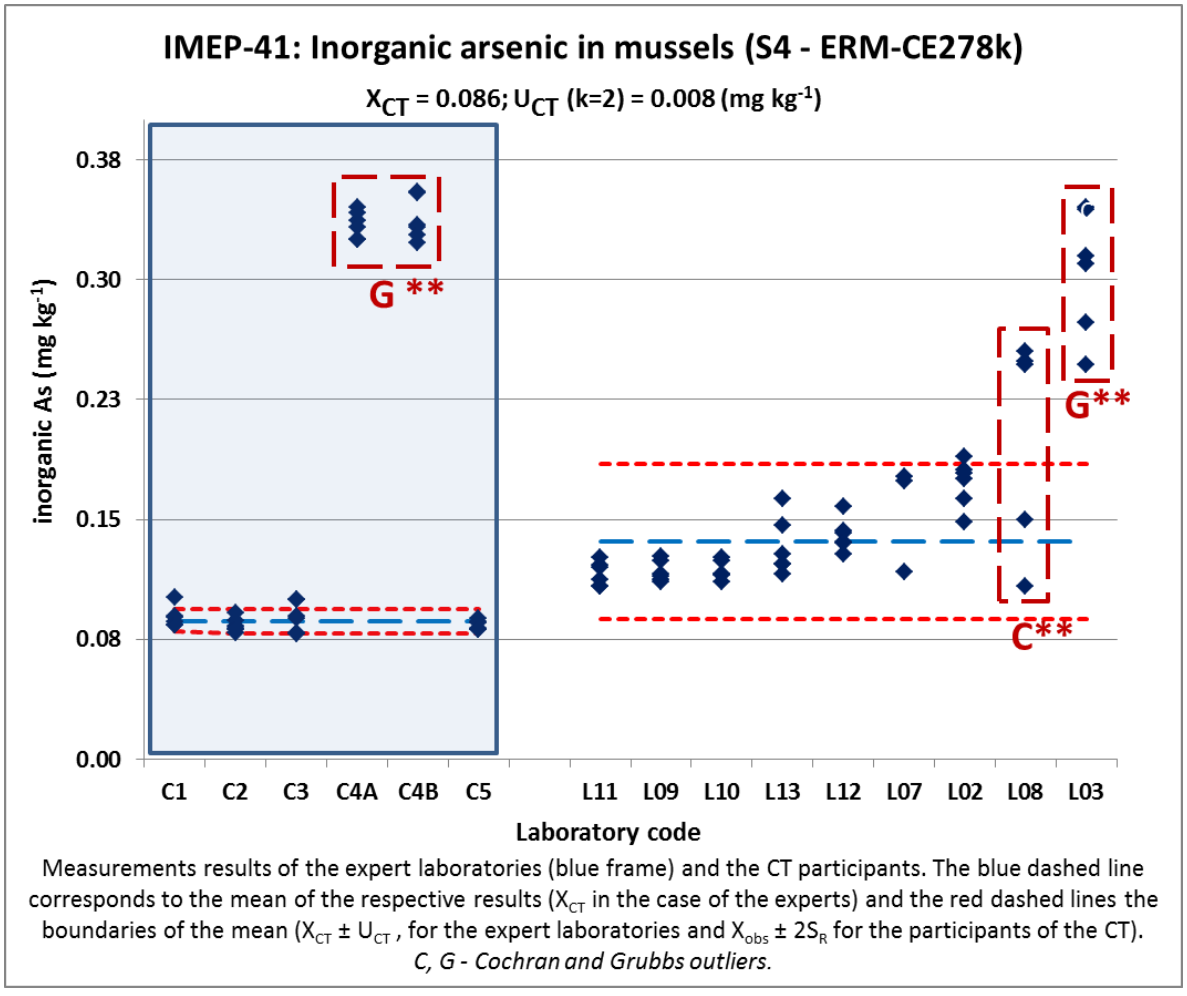
Measurements results of the expert laboratories (blue frame) and the CT participants. The blue dashed line corresponds to the mean of the respective results (X_{CT} in the case of the experts) and the red dashed lines the boundaries of the mean ($X_{CT} \pm U_{CT}$, for the expert laboratories and $X_{obs} \pm 2S_R$ for the participants of the CT).
C - Cochran straggler* - The test statistics is greater than its 1 % critical value and the laboratory (or the single replicate value) is considered as an outlier.

Annex 9: Results for Sample 4 - Mussels (ERM-CE278k)

X_{CT} = 0.086 and U_{CT} = 0.008 (k=2); all values are given in mg kg^{-1}.

Lab code	R1	R2	R3	R4	R5	R6	mean
L02	0.163	0.175	0.148	0.181	0.179	0.189	**0.173**
L03	**0.31**	**0.345**	**0.344**	**0.315**	**0.247**	**0.273**	**0.306 **G**
L07	0.117	0.174	0.177				**0.156**
L08	**0.108**	**0.15**		**0.247**	**0.249**	**0.255**	**0.202 **C**
L09	0.116	0.111	0.112	0.114	0.124	0.127	**0.117**
L10	0.124	0.116	0.116	0.111	0.126	0.115	**0.118**
L11	0.108	0.108	0.112	0.126	0.121	0.12	**0.116**
L12	0.143	0.141	0.128	0.135	0.136	0.158	**0.140**
L13	0.122	0.128	0.163	0.116	0.146	0.122	**0.133**

*G = Outlier identified by Grubbs test, **C = Outlier identified by Cochran test



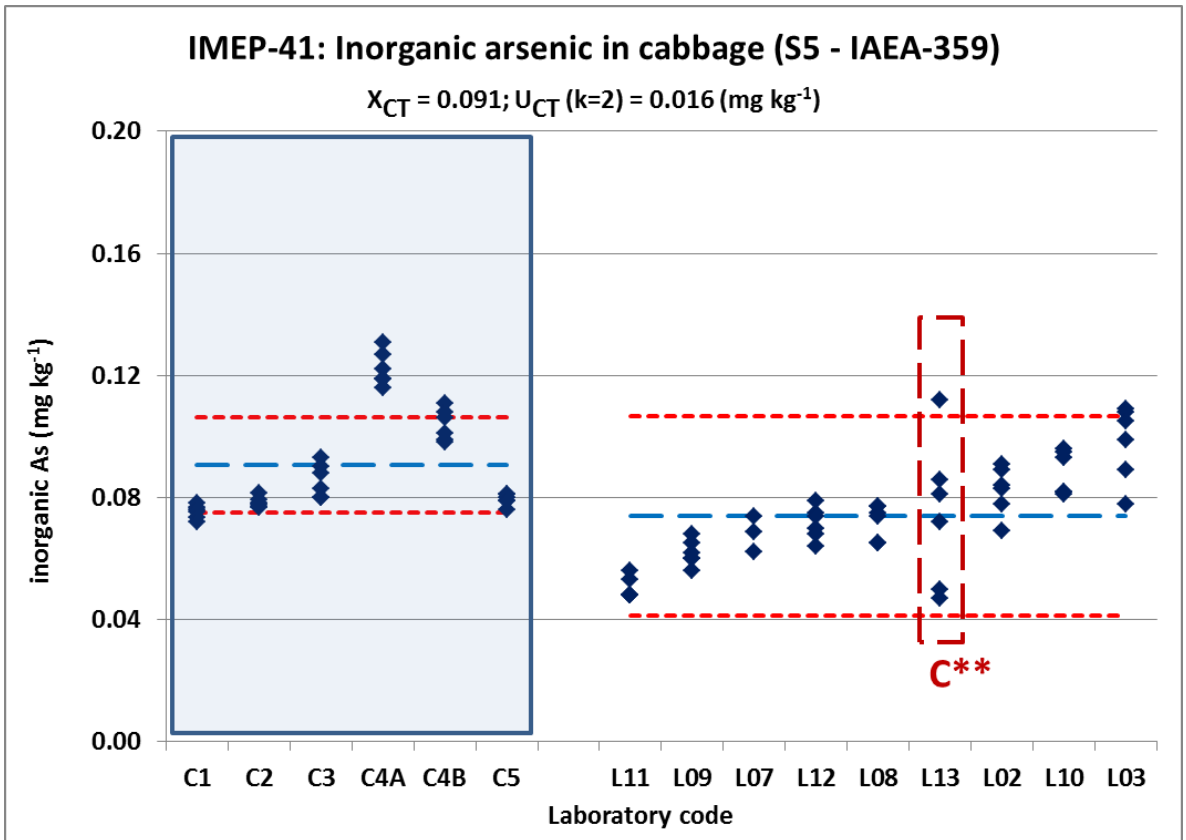
Measurements results of the expert laboratories (blue frame) and the CT participants. The blue dashed line corresponds to the mean of the respective results (X_{CT} in the case of the experts) and the red dashed lines the boundaries of the mean ($X_{CT} \pm U_{CT}$, for the expert laboratories and $X_{obs} \pm 2S_R$ for the participants of the CT).
C, G - Cochran and Grubbs outliers.

Annex 10: Results for Sample 5 - Cabbage (IAEA-359)

X_{CT} = 0.091 and U_{CT} = 0.016 (k=2); all values are given in mg kg^{-1}.

Lab code	R1	R2	R3	R4	R5	R6	mean
L02	0.078	0.083	0.069	0.089	0.084	0.091	**0.082**
L03	0.099	0.089	0.078	0.109	0.108	0.105	**0.098**
L07	0.0622	0.0688	0.0738				**0.068**
L08	0.065	0.065	0.075	0.077	0.077	0.074	**0.072**
L09	0.056	0.065	0.062	0.06	0.068	0.06	**0.062**
L10	0.093	0.096	0.095	0.082	0.082	0.081	**0.088**
L11	0.048	0.048	0.048	0.053	0.056	0.048	**0.050**
L12	0.075	0.074	0.07	0.068	0.064	0.079	**0.072**
L13	**0.081**	**0.112**	**0.086**	**0.047**	**0.072**	**0.05**	**0.075 **C**

**C = Outlier identified by Cochran test



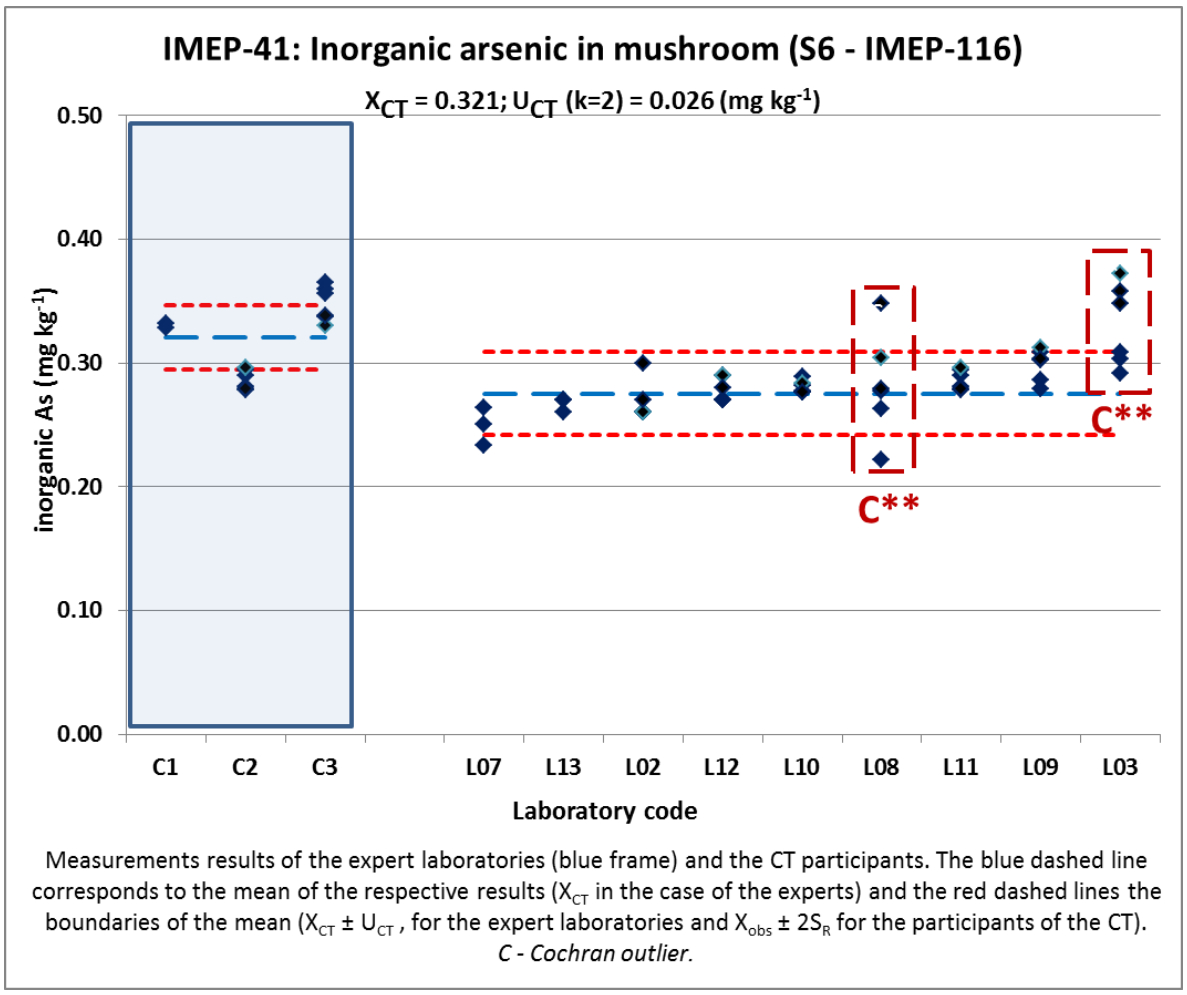
Measurements results of the expert laboratories (blue frame) and the CT participants. The blue dashed line corresponds to the mean of the respective results (X_{CT} in the case of the experts) and the red dashed lines the boundaries of the mean ($X_{CT} \pm U_{CT}$, for the expert laboratories and $X_{obs} \pm 2S_R$ for the participants of the CT). *C - Cochran outlier.*

Annex 11: Results for Sample 6 - Mushroom (IMEP-116)

X_{CT} = 0.321 and U_{CT} = 0.026 (k=2); all values are given in mg kg^{-1}.

Lab code	R1	R2	R3	R4	R5	R6	mean
L02	0.26	0.26	0.26	0.3	0.26	0.27	**0.268**
L03	**0.292**	**0.303**	**0.309**	**0.358**	**0.372**	**0.348**	**0.330 **C**
L07	0.2331	0.2501	0.2635				**0.249**
L08	**0.222**	**0.263**	**0.277**	**0.279**	**0.304**	**0.348**	**0.282 **C**
L09	0.279	0.286	0.309	0.302	0.312	0.303	**0.299**
L10	0.289	0.282	0.277	0.276	0.284	0.276	**0.281**
L11	0.278	0.29	0.294	0.281	0.296	0.279	**0.286**
L12	0.27	0.27	0.27	0.29	0.29	0.28	**0.278**
L13	0.27	0.26	0.27				**0.267**

**C = Outlier identified by Cochran test

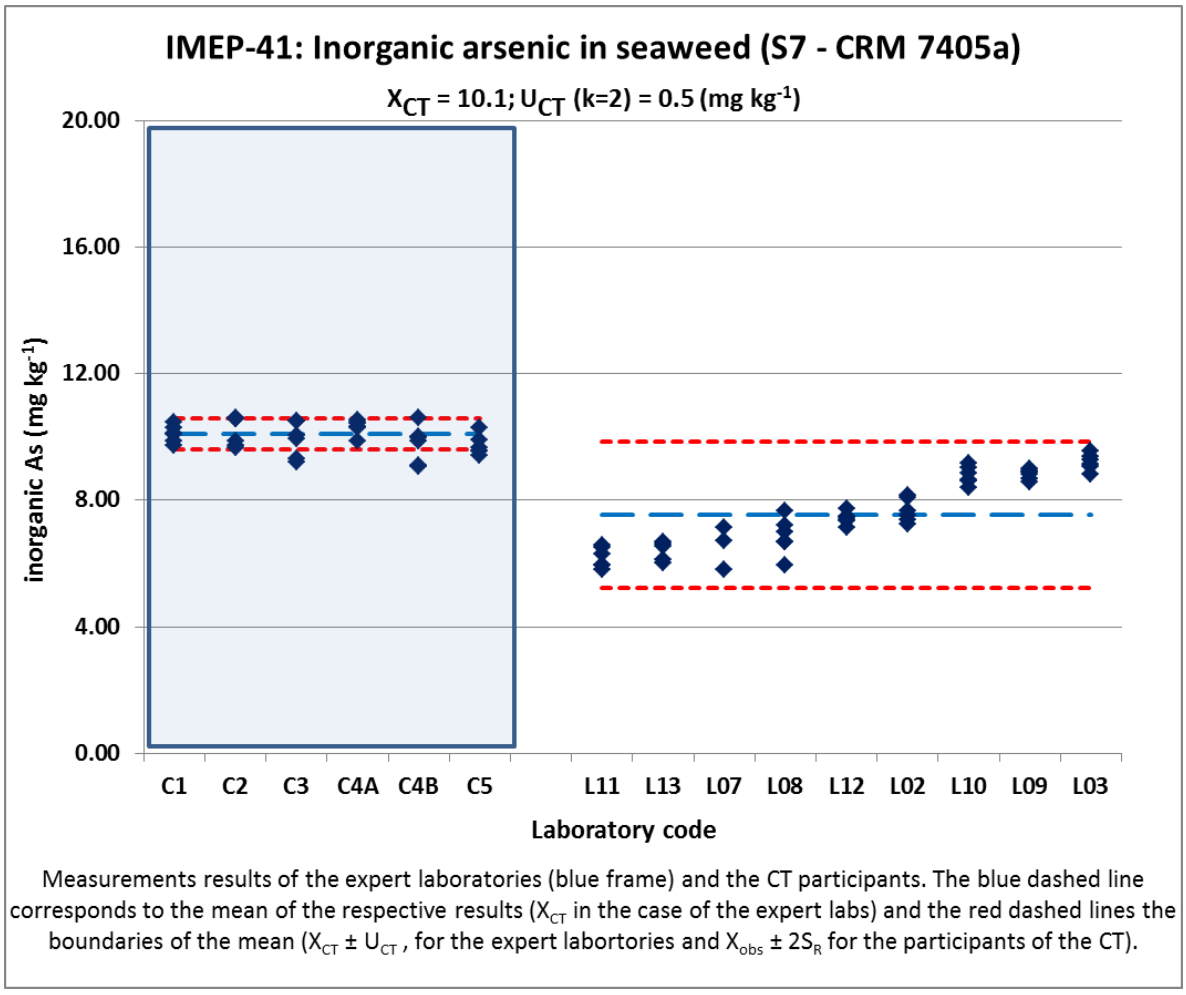


Measurements results of the expert laboratories (blue frame) and the CT participants. The blue dashed line corresponds to the mean of the respective results (X_{CT} in the case of the experts) and the red dashed lines the boundaries of the mean ($X_{CT} \pm U_{CT}$, for the expert laboratories and $X_{obs} \pm 2S_R$ for the participants of the CT).
C - Cochran outlier.

Annex 12: Results for Sample 7 - Seaweed (NMIJ-7405a)

X_{CT} = 10.1 and U_{CT} = 0.5 (k=2); all values are given in mg kg^{-1}.

Lab code	R1	R2	R3	R4	R5	R6	mean
L02	8.16	8.1	7.51	7.26	7.67	7.39	**7.682**
L03	8.82	9.14	9.07	9.26	9.55	9.4	**9.207**
L07	7.129	6.734	5.823				**6.562**
L08	5.941	6.683	6.69	7.007	7.672	7.215	**6.868**
L09	8.89	8.92	8.99	8.67	8.58	8.82	**8.812**
L10	8.392	8.663	8.599	9.015	9.16	8.867	**8.783**
L11	6.505	6.592	5.964	6.312	5.934	5.821	**6.188**
L12	7.5	7.47	7.73	7.35	7.37	7.15	**7.428**
L13	6.14	6.55		6.02	6.7	6.6	**6.402**



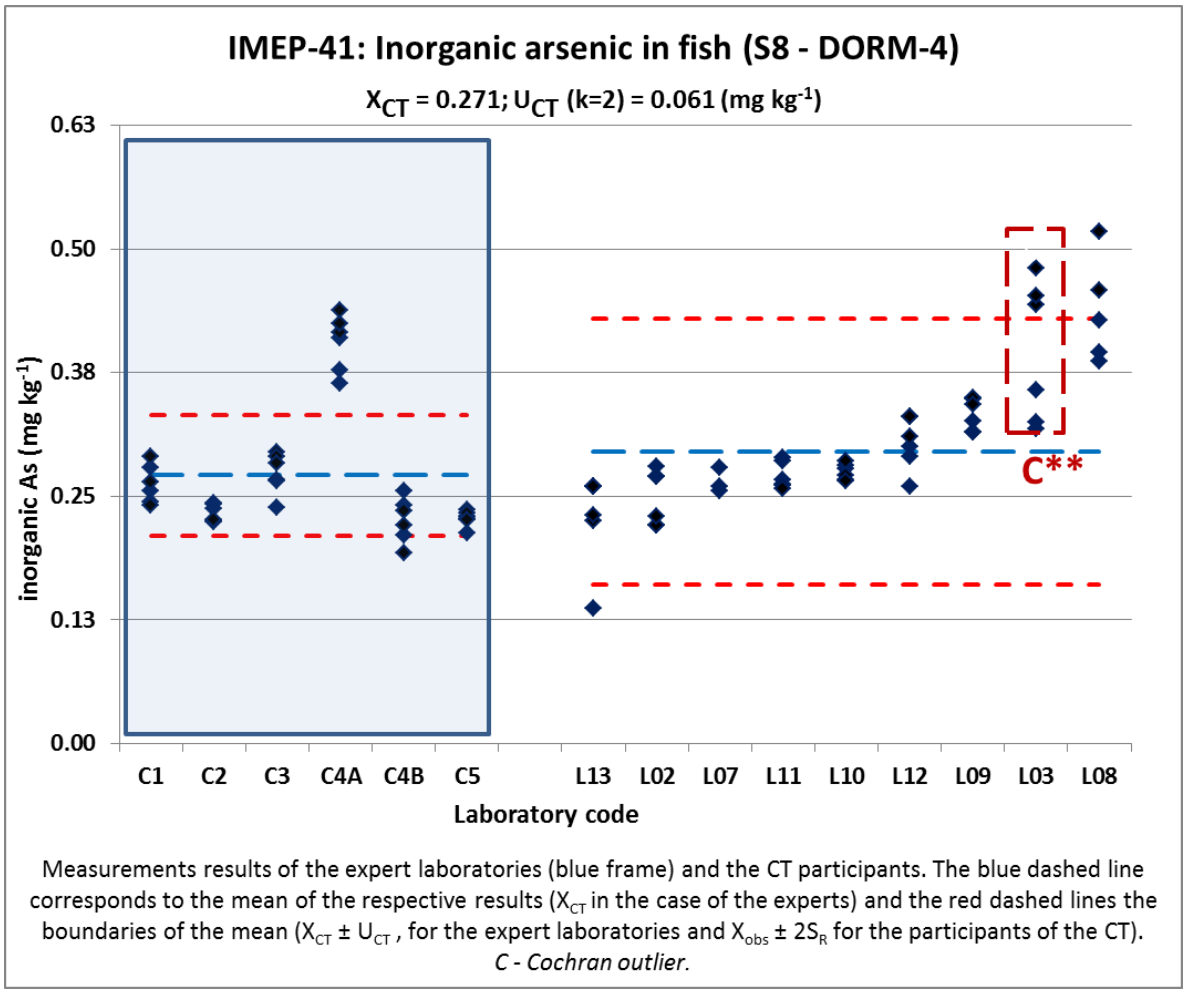
Measurements results of the expert laboratories (blue frame) and the CT participants. The blue dashed line corresponds to the mean of the respective results (X_{CT} in the case of the expert labs) and the red dashed lines the boundaries of the mean ($X_{CT} \pm U_{CT}$, for the expert labortories and $X_{obs} \pm 2S_R$ for the participants of the CT).

Annex 13: Results for Sample 8 - Fish (DORM-4)

X_{CT} = 0.271 and U_{CT} = 0.061 (k=2); all values are given in mg kg^{-1}.

Lab code	R1	R2	R3	R4	R5	R6	mean
L02	0.27	0.28	0.27	0.22	0.22	0.23	**0.248**
L03	**0.318**	**0.357**	**0.325**	**0.443**	**0.48**	**0.453**	**0.396 **C**
L07	0.279	0.255	0.2602				**0.265**
L08	0.428	0.395	0.386		0.517	0.458	**0.437**
L09	0.326	0.315	0.315	0.348	0.349	0.343	**0.333**
L10	0.267	0.278	0.281	0.285	0.271	0.265	**0.275**
L11	0.289	0.286	0.267	0.262	0.261	0.257	**0.270**
L12	0.3	0.29	0.26	0.33	0.33	0.31	**0.303**
L13	0.26	0.137	0.26	0.26	0.225	0.231	**0.229**

**C = Outlier identified by Cochran test



Measurements results of the expert laboratories (blue frame) and the CT participants. The blue dashed line corresponds to the mean of the respective results (X_{CT} in the case of the experts) and the red dashed lines the boundaries of the mean ($X_{CT} \pm U_{CT}$, for the expert laboratories and $X_{obs} \pm 2S_R$ for the participants of the CT).
C - Cochran outlier.

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

Title: **IMEP-41: Determination of inorganic As in food**

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Abstract

A collaborative trial, IMEP-41, was conducted with the aim to validate a method for the determination of iAs in food. The method is based on the selective extraction of iAs into chloroform and further determination by HG-AAS. Thirteen laboratories, from nine European countries, registered for participation. All participating laboratories were experienced in the analysis of iAs in various food commodities using FI-HG-AAS.
Seven test items (mussel, cabbage, seaweed, fish protein, rice, wheat and mushrooms), covering a reasonable concentration range, were selected.
The repeatability relative standard deviation (RSDr) ranged from 4.1 to 10.3 % while the reproducibility relative standard deviation (RSDR) ranged from 6.1 to 22.8 %. The method demonstrated to have acceptable precision and trueness for all test materials, thus it is fit for its intended analytical purpose.

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