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IMEP-112: Total and inorganic arsenic in wheat, vegetable food and algae

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

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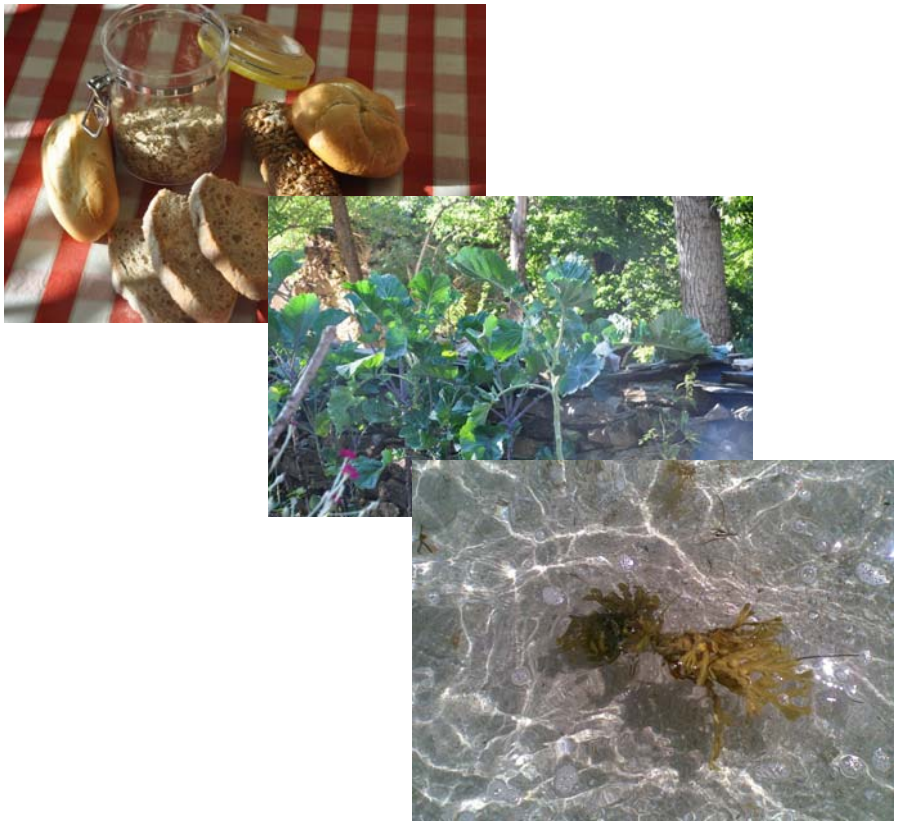

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The mission of the JRC-IRMM is to promote a common and reliable European measurement system in support of EU policies.

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Summary

The Institute for Reference Materials and Measurands (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). Two of its core tasks are to provide advice to the Directorate General for Health and Consumers (DG SANCO) on scientific matters and to organise interlaboratory comparisons (ILC) among appointed National Reference Laboratories (NRLs). This report presents the results of the twelfth ILC of the EU-RL-HM (former CRL-HM) which focused on the determination of total and inorganic As in wheat, vegetable food and algae. The test items used in this exercise are: wheat, spinach (SRM 1570a from NIST) and an algae candidate reference material. The test items were processed (in the case of wheat), bottled and labelled at IRMM and dispatched to the participants the second week of May 2011. Each participant received three bottles containing approximately 20 g of wheat, 15 g of spinach and 5 g of algae, respectively. Participation in this exercise was not limited to the NRLs but was open to laboratories from all around the world, to be able to judge the state-of-the-art of the determination of total and, more in particular, inorganic As in several food commodities. Seventy-four laboratories from 31 countries registered to the exercise, of which
– 64 reported results in wheat, 49 in spinach and 51 in algae for **total As**, and
– 43 reported results in wheat, and 40 in spinach and in algae for **inorganic arsenic**.
Thirty of the participants were NRLs of the EU-RL-HM network, out of which 13 reported values for inorganic As.

The assigned values for IMEP-112 were provided by the certificates when available and otherwise by a group of seven laboratories expert in the field. The uncertainties of the respective assigned values *(u_{ref})* were derived from the standard deviation of the means provided by the experts *(u_{char})* and from the contribution for homogeneity *(u_{bb})* and stability (u_{st}).

Laboratory results were rated with z-and ζ-scores (zeta-scores) in accordance with ISO 13528. The standard deviations for proficiency assessment (also called target standard deviation) were fixed 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 to:

- 15 % for total and inorganic arsenic in **wheat**.

- 22% for total arsenic and 25 % for inorganic arsenic in **vegetable food**, to account for the difficulty introduced by the relatively low concentration of both measurands in this test material.

- 15 % for total arsenic and 22 % for inorganic arsenic in **algae**.

Most of the participants performed satisfactorily for total arsenic and for inorganic arsenic in vegetable food (75 and 85 %); 60 % did for inorganic arsenic in wheat but only 20 % of the laboratories reported satisfactory results in the algae test material.

Contents

1 Introduction

From a toxicological point of view, arsenic speciation plays an important role, as inorganic arsenic species are more toxic than the organic ones. Absorption of arsenic is affected by the type of arsenic compounds: As (V) is more readily absorbed than As (III), and inorganic more that organic. As (V) is excreted faster than As (III) compounds and organic arsenic compounds are excreted faster than the inorganic ones.

The highest **total arsenic (totAs)** levels had been measured in the following food commodities: fish and seafood, products or supplements based on algae (especially hijiki), cereals and cereals products, with particular high concentrations in rice grains, rice-based products, bran and germ. Nevertheless, not all of these foods also contain high levels of **inorganic arsenic (iAs)**. In fish and seafood, for example, iAs levels were low because in aquatic species, arsenic is found in the form of stable, non-toxic organic compounds such as arsenosugars and arsenobetaine.

According to the Scientific Opinion on As in food of the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain [1], the following food sub classes were identified as largely contributing to the iAs daily exposure in the general European population: cereal grains and cereal based products, followed by foods for special dietary uses, bottled water, coffee and beer, rice grains and rice based products, fish and vegetables.

At European level only one standard method was published in 2008 and it deals with the determination of iAs in seaweed [2]. In China a standard for determination of totAs and abio-arsenic in foods exists since 2003 [3].

The EU-RL-HM organised in the second half of 2009 a proficiency test (PT) for the determination of total and iAs in rice (IMEP-107). The two main conclusions were that the concentration of iAs in rice is not dependent on the analytical method applied and that the introduction of maximum levels for iAs in legislation could be considered.

The Directorate General for Health and Consumers of the European Commission requested the EU-RL-HM to expand the study initiated with IMEP-107 to other food matrices and to evaluate the performance of European laboratories with regard to total and inorganic arsenic determinations in cereals (other than rice), vegetables and algae in view of future discussions on the need for possible regulatory measures.

Therefore, the EU-RL-HM organised a PT on the determination of total and inorganic arsenic in wheat, vegetable food and algae, open to laboratories worldwide with analytical capabilities in the field.

2 The IMEP support

The organisation of IMEP-112 follows the administrative and logistic procedures of the International Measurement Evaluation Programme (IMEP), accredited according to ISO Guide 43.

IMEP is a registered trade mark owned by IRMM. IMEP provides support to the European measurement infrastructure by:

- **providing metrological traceability** from the highest level down to the field laboratories. These laboratories can benchmark their measurement result against the IMEP certified reference value. This value is established according to metrological best practice.
- helping laboratories to assess their estimate of **measurement uncertainty**. The participants are invited to report the uncertainty on their measurement result. IMEP integrates the estimate into the scoring, and provides assistance for the interpretation.

IMEP supports EU policies by organising intercomparisons in the frame of specific EU Directives, or on request of a specific Directorate General. In the case of the IMEP-112, it was realised in the context of the European legislation on contaminants in food [4, 5] and in support to the activities of the EU-RL-HM [6]. IMEP-112 provided specific support to the following stakeholders:

- The European Co-operation for Accreditation (EA) in the frame of a Memorandum of Understanding (MoU) on a number of metrological issues, including the organisation of intercomparisons. National accreditation bodies were invited to nominate a limited number of laboratories for participation in IMEP-112. Mrs. Kirsten Jebjerg from the Danish Accreditation and Metrology Fund (DANAC) liaised between EA and IMEP for this PT. This report does not discern the EA nominees

from the other participants. Their results are however summarised in a separate report to EA.

- The Asia Pacific Laboratory Accreditation Cooperation (APLAC), in the frame of the collaboration with EA. Mr. Daniel Tholen, was invited to announce the exercise to the accreditation bodies in the APLAC network.
- The National Reference Laboratories for Heavy Metals in Feed and Food which were invited to distribute the information between control laboratories in their respective countries.

3 Scope

As stated in Regulation (EC) 882/2004 of the European Parliament and of the Council [6], two of the core duties of the EU-RL-HM are to provide advice on scientific matters to DG SANCO and to organise PTs for the benefit of staff from National Reference Laboratories. The scope of this PT was to test the competence of appointed NRLs to determine the total and inorganic arsenic content in wheat, vegetable food and algae. Furthermore it was requested to evaluate the state-of-the-art in the determination of iAs in the same food commodities by laboratories worldwide, with the aim to provide support to the EU policy makers in performing risk assessment studies and eventually in setting maximum levels for iAs in legislation. The designation of this PT is IMEP-112.

4 Set-up of the exercise

4.1 Time frame

This PT was agreed upon by the NRL network at the 5th EU-RL-HM workshop held on 24 September 2010. Invitation letters were sent to the NRLs on 21 March 2011 (Annex 1). The exercise was announced to EA in an invitation letter sent to the EA coordinator on 25 March 2011 (Annex 2) and to APLAC by an e-mail sent to Mr. Daniel Tholen on 18 March 2011 (Annex 3).

Laboratories could register until 26 April 2011. The samples were dispatched on 10-13 May 2011. The reporting deadline was first set to 10 June 2011 and later extended to 15 June 2011.

4.2 Confidentiality

EA was invited to nominate laboratories for participation. The following confidentiality statement was made to EA: "*Confidentiality of the participants and their results towards third parties is guaranteed. However, IMEP will disclose details of the participants that have been nominated by EA to the EA working group for ILCs in Testing. The EA accreditation bodies may wish to inform the nominees of this disclosure.*"

4.3 Distribution

On 10 – 13 May 2011 IRMM dispatched to participants parcels containing:

- three bottles containing approximately 20 g of wheat, 15 g of vegetable food and 5 g of algae, respectively;
- an accompanying letter with instructions on measurands, sample storage conditions, protocol for the moisture content determination, measurements, the individual access code for the result reporting website and the reporting deadline (Annex 4);
- a form that had to be sent back to IMEP after receipt of the test material to confirm its arrival (Annex 5).

4.4 Instructions to participants

Details of this PT were discussed with the NRLs and DG SANCO at the 5th workshop organised by the EU-RL-HM, held in Brussels on 24 September 2010. Concrete instructions were given to all participants in a letter accompanying the test material (Annex 4). The measurands and matrix were defined as *"total and inorganic Asin wheat, vegetable food and algae"*.

Laboratories were asked to perform two or three independent measurements and to report them, together with the mean of the results and its associated uncertainty. The measurement results were to be corrected for moisture (following a procedure described in the accompanying letter which had been cross-checked by Karl-Fisher titration at IRMM) and for recovery. Participants were asked to follow their routine procedures. The results

were to be reported in the same manner (e.g. number of significant figures) as those normally reported to the customers.

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 (Annex 6). The questionnaire was intended to provide further information on the measurements and the laboratories.

5 Test material

5.1 Preparation

The three test materials used in IMEP-112 were processed as follows:

• **Wheat:** Twenty eight kilos of wheat were stored at IRMM at -20 °C waiting for processing. The material was passed through a 5 mm sieve (Russell Finex industrial sieve, model 17300, London, United Kingdom) to remove the coarser pieces (mainly straw). The fine fraction was sieved on the same machine with a 2 mm sieve while sucking the lighter fraction away with a vacuum cleaner (chaff). In that way, 27 kg of wheat grains were collected. They were divided over six plastic drums placed in liquid nitrogen for pre-cooling. They were fed using a vibrating feeder into a cryogenic vibrating mill (Palla VM-KT, Humboldt-Wedag, Köln, Germany) cooled down to -196 °C prior to milling. Each milling cycle lasted about 45 minutes from -196 °C to about -90 °C. Milling was then interrupted and the mill was cooled again. Nineteen kilos of ground wheat grains were collected and stored at -20 °C. The material was then passed through a 500 µm sieve. The fraction below 500 µm was kept and homogenised for 30 minutes in a three-dimensional mixer (WAB, Dynamix CM-200, Basel, Switzerland). The homogenised product was tested for its particle size distribution using laser diffraction where it was confirmed that the top-particle size was below 610 µm, X_{50} was about 70 µm and X_{90} approximately 200 µm. A water content of 12 % (w/w) was determined by heating at 105 °C (Sartorius MA150, Göttingen, Germany). Such a high value is expected for materials like wheat and rice flours. It should be noted that commercially available wheat flour is about 15 % in water. Thereafter, 20 g powder portions were filled in 60 ml bottles with a PE-insert and screw-cap.

• **Vegetable food:** The commercially available SRM 1570a (spinach leaves) from the National Institute of Standards and Technology (NIST) was used for this PT. NIST

dispatched 30 bottles of test materials at room temperature by courier to IRMM. The material was rebottled and relabelled to avoid identification by the participants. Comprehensive information on the preparation of this material can be found in the certification report on the NIST website [7].

- **Algae:** 60 kg of seaweed were spread on nylon sieves placed on PTFE coated trays in an Elbanton drying cabinet (Elbanton, Kerkdriel, The Netherlands) at a temperature of 26 °C. The material was manually moved in the tray now and then to achieve a more uniform drying of the material. After two weeks of drying the material was taken out of the drying cabinet. The dry material (11.18 kg) was then stored in a plastic drum. Once the seaweed was dried and crushed, it was placed in stainless steel drums immersed in liquid nitrogen over night. The seaweed was then scooped up and fed slowly into a Palla vibrating mill (KHD Humboldt Wedag, Köln, Germany) cooled down to -196 °C with liquid nitrogen. All machine parts in contact with the material are made of high purity titanium to avoid any contamination with other trace metals. The obtained powder was passed through a 125 µm Nylon sieve using a Russel Finex Industrial sieve (Model 17300, London, United Kingdom). The fraction < 125 µm of the sieved powder was spread over 10 Teflon coated trays (600 – 800 g on each) and placed in a freeze-drier (Epsilon 2-D 85 Martin-Christ, Osterode, Germany). After freeze-drying the 6.3 kg of vacuum dried material was homogenised using a three-dimensional mixer in one run of 0.5 h on a Dyna-MIX CM200 mixer (WAB, Basel, Switzerland). Filling the seaweed powder into 20 mL amber glass vials was performed using an All Fill automatic filling machine (All Fill, Sandy, United Kingdom). All vials were acid washed (2% HNO_3 solution in water (m/m)) and rinsed with Milli-Q water before drying in a drying cabinet to remove possible point contamination of the glassware. The hopper containing the material (a large funnel) inside the filling machine and the auger (feeding screw) were from stainless steel. A positive ion blower (Sartorius/ Ion-care, Malmö, Sweden) was installed about 4 cm from the filling point to reduce problems with static electricity. Under these conditions each vial was reproducibly filled with slightly more than 5.0 g of material. Once the vials were filled, lyo-inserts were automatically pressed down in the neck of the vials by the filling machine. The vials were flushed with nitrogen before and after filling; the hopper was also continuously flushed with nitrogen providing an inert atmosphere above the material.

5.2 Homogeneity and stability

The homogeneity and stability studies for wheat were performed by The Food and Environment Research Agency (Fera). Homogeneity was evaluated according to ISO 13528 [8]. The material proved to be homogeneous for total and inorganic arsenic. The contribution from homogeneity *(u_{bb})* to the uncertainty of the reference value *(u_{ref})* was calculated using SoftCRM [9].

The stability study was conducted following the isochronous approach [10]. The evaluation of the stability of the test item was made using the software SoftCRM [11]. The material proved to be stable for both total and inorganic arsenic, even at 60 °C for the five weeks that elapsed between the dispatch of the samples and the deadline for submission of results, for both total and inorganic arsenic.

The analytical results and statistical evaluation of the homogeneity and stability studies are provided in Annex 7.

No homogeneity/stability tests were organised for the purpose of IMEP-112 for the vegetable food and the algae because according to the producers of those test materials (NIST and IRMM), the materials are homogenous and stable.

6 Reference values and their uncertainties

The NIST certificate provided the assigned value for totAs in the vegetable food. The assigned value for totAs in the algae test material was provided by the Studiecentrum voor Kernenergie (SCK-CEN) using neutron activation analysis. SCK has proven its measurements capabilities by successful participation in the Comité Consultative de la Quantité de Matière (CCQM) key comparisons.

The remaining assigned values for totAs and iAs (totAs and iAs in wheat, iAs in vegetable food and algae) were provided by a group of laboratories expert in the field:

- Institute of Agricultural Chemistry and Food Technology (CSIC), ES
- Institute of Chemistry, Karl-Franzens University Graz, AT
- Istituto Superiore di Sanità (ISS), IT
- The Food and Environment Research Agency (FERA), UK
- New Technical University of Denmark (DTU), DK

- Department of Analytical Chemistry, University of Barcelona, ES
- TESLA (Trace Element Speciation Laboratory), University of Aberdeen, UK

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 uncertainty and with a clear and detailed description on how uncertainty was calculated.

The mean of the independent means provided by the expert laboratories for total and inorganic arsenic, was used as assigned values (X_{ref}) for this PT according to ISO Guide 35 [12]. The standard uncertainties *(u_{ref})* associated to the assigned values were calculated using Eq. 1 and Eq. 2.

$$u_{ref} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2} \qquad \text{Eq.1}$$

Where: u_{ref} is the standard uncertainty associated to the assigned value
u_{char} is the standard uncertainty of characterisation by expert laboratories
u_{bb} is the standard uncertainty contribution for the between-bottle homogeneity
u_{st} is the standard uncertainty contribution derived from the stability study

u_{char} is calculated according to ISO Guide 35 [12]:

$$u_{char} = \frac{SD_{ExpertMeans}}{\sqrt{n}} \qquad \text{Eq.2}$$

Where: $SD_{ExpertMeans}$ is the standard deviation of the means reported by the expert laboratories
n is the number of expert laboratories

u_{bb} and u_{st} were set to zero for the vegetable food on the basis of the information given in the certificate by NIST. u_{bb} and u_{st} for the algae test material were provided by IRMM.

The means reported by the expert laboratories (certifiers) and their associated uncertainties *(u_{char})* for total and inorganic arsenic are listed in Table 1 and Table 2 together with the assigned values and their respective uncertainties.

The results reported by Cert. 5 for iAs in wheat and algae did not overlap with the results reported by the other certifiers, within their respective uncertainties. Concerning the wheat, Cert. 5 reported a recovery factor for iAs of 65 %. When looking at the provided chromatogram, only one peak corresponding to As(V) and some traces of DMA could be observed. Cert. 5 also reported having had problems with carbon interferences in the determination of iAs.

As for the algae, it was not possible to explain the discrepancy between Cert.5 and the other certifiers by the mass balance, due to the large amount of arsenic species (mainly organic) present in the algae test material. An alternative attempt was made to identify which step in the method of analysis used by Cert. 5 when analysing iAs could explain the bias observed for wheat and algae results. The methods used by the certifiers in the determination of total and inorganic arsenic are summarised in Table 4 and Table 5. While most of the certifiers using HPLC-based methods added H_2O_2 before the microwave digestion to enhance the efficiency of the digestion, Cert. 5 added H_2O_2 after the microwave digestion with the only purpose of oxidising As (III) to As (V). This could explain the bias of the results reported by Cert.5 for iAs in wheat and algae. It could also explain the carbon interference observed in the analysis of the wheat sample, due to the incomplete digestion in the absence of H_2O_2. It could be argued that the addition of H_2O_2 before the microwave digestion could induce degradation of the organic arsenic species present in the algae. According to Cert. 7 who performed a thorough investigation on the effect of several digestion conditions on the different species of arsenic, organic species could degrade down to DMA in the presence of H_2O_2 but no further degradation into iAs would occur.

It was therefore decided not to use the results reported by Cert. 5 for iAs in wheat and algae when establishing X_{ref}. Since the bias of Cert. 5 in wheat and algae were matrix related and no bias was detected in the results reported by that certifier for iAs in vegetable food, its results were included in the calculation of X_{ref} in that matrix.

Cert. 6 reported "less than 0.100 mg kg^{-1}" for iAs in algae; the same laboratory had problems to detect iAs in seafood in a previous exercise (IMEP-109) [13] and so its results for iAs in algae were not taken into consideration.

The results reported by the certifiers are shown in Annexes 8, 9, 11 and 13 together with the results reported by the other participants. The results of the certifiers are placed on the left hand side of the graphs. In the case of iAs in vegetable food, two certifiers are outside the range $X_{ref} \pm U_{ref}$. The higher scatter of results in this matrix should be

interpreted while keeping in mind that the certified value for totAs as given in the certificate SRM 1570a has an associated uncertainty of 17 %. The uncertainty for iAs is unlikely to be lower, and both uncertainties are probably related to the low mass fractions in that material.

*Table 1 – Assigned vales for **totAs** and their associated expanded uncertainties (mg kg^{-1}).*

Certifier	**Wheat** $X_n \pm U_n$	**Vegetable food** $X_n \pm U_n$	**Algae** $X_n \pm U_n$
Certifier 1	0.188 ± 0.024		
Certifier 2	0.178 ± 0.008		
Certifier 3	0.195 ± 0.037		
Certifier 4	0.157 ± 0.005		
Certifier 5	0.175 ± 0.003		
Certifier 6	0.179 ± 0.011		
Certifier 7	0.166 ± 0.009		
X_{ref}	0.177	0.068	58.3
u_{char}	0.005	0.006	1.4
u_{bb}	0.003	0	0.9
u_{st}	0.002	0	3.1
u_{ref}	0.006	0.006	3.5
U_{ref} (k=2)*	0.012	0.012	7.0
$X_{ref} \pm U_{ref}$*	**0.177 ± 0.012**	**0.068 ± 0.012**	**58.3 ± 7.0**

*Table 2 – Assigned vales for **iAs** and their associated expanded uncertainties (mg kg^{-1})*

Certifier	Wheat $X_n \pm U_n$	Vegetable food $X_n \pm U_n$	Algae $X_n \pm U_n$
Certifier 1	0.183 ± 0.024	0.038 ± 0.005	0.161 ± 0.021
Certifier 2	0.176 ± 0.010	0.075 ± 0.004	0.205 ± 0.035
Certifier 3	0.194 ± 0.025	0.074 ± 0.010	0.194 ± 0.025
Certifier 4	0.154 ± 0.003	0.060 ± 0.002	0.190 ± 0.010
Certifier 5		0.055 ± 0.003	
Certifier 6	0.156 ± 0.022	0.034 ± 0.005	< 0.100
Certifier 7	0.152 ± 0.010	0.045 ± 0.003	0.188 ± 0.029
X_{ref}	0.169	0.054	0.188
u_{char}	0.007	0.006	0.007
u_{bb}	0.006	0	0.003
u_{st}	0.008	0	0.010
u_{ref}	0.012	0.006	0.013
U_{ref} (k=2)*	0.025	0.012	0.025
$X_{ref} \pm U_{ref}$*	**0.169 ± 0.025**	**0.054 ± 0.012**	**0.188 ± 0.025**

** U_{ref} is the estimated associated expanded uncertainty with a coverage factor k, corresponding to a level of confidence of about 95 %. The code attributed to the certifiers does not correspond to the order of listing at the beginning of Chapter 6.*

For years a debate has taken place within the scientific community on whether the iAs fraction in food commodities was or not dependent on the method used to perform the analysis. The expert laboratories that participated in the establishment of the assigned values in IMEP-112 used various method of analysis (with the exception of Cert. 1 and Cert. 3, Table 5). Nevertheless, all the results agree within a range of about 14 % for wheat, 22 % for vegetable food and 10 % for algae (95 % confidence interval). This indicates that the concentration of iAs is not method dependent in those matrices.

7 Evaluation Results

7.1 General observations

Seventy-four laboratories from 31 countries registered to the exercise (Fig 1), of which 65 reported results. Fourteen laboratories from the Asia-Pacific region have submitted results to IMEP-112. The precise number of results sets per measurand and matrix can be seen in Table 3. The table also lists the number and percentages of participants having reported "less than" values. These were not included in further data evaluations.

Table 3 – Number of reported results per measurand and matrix

	Wheat			Vegetable food			Algae		
	N°	"less than"		N°	"less than"		N°	"less than"	
totAs	62	2	3 %	47	2	4 %	51	-	-
iAs	40	3	7 %	30	11	27 %	38	2	5 %

N° - number of participants having reported evaluable results

Fig 1 – Distribution per country of the participants in IMEP-112

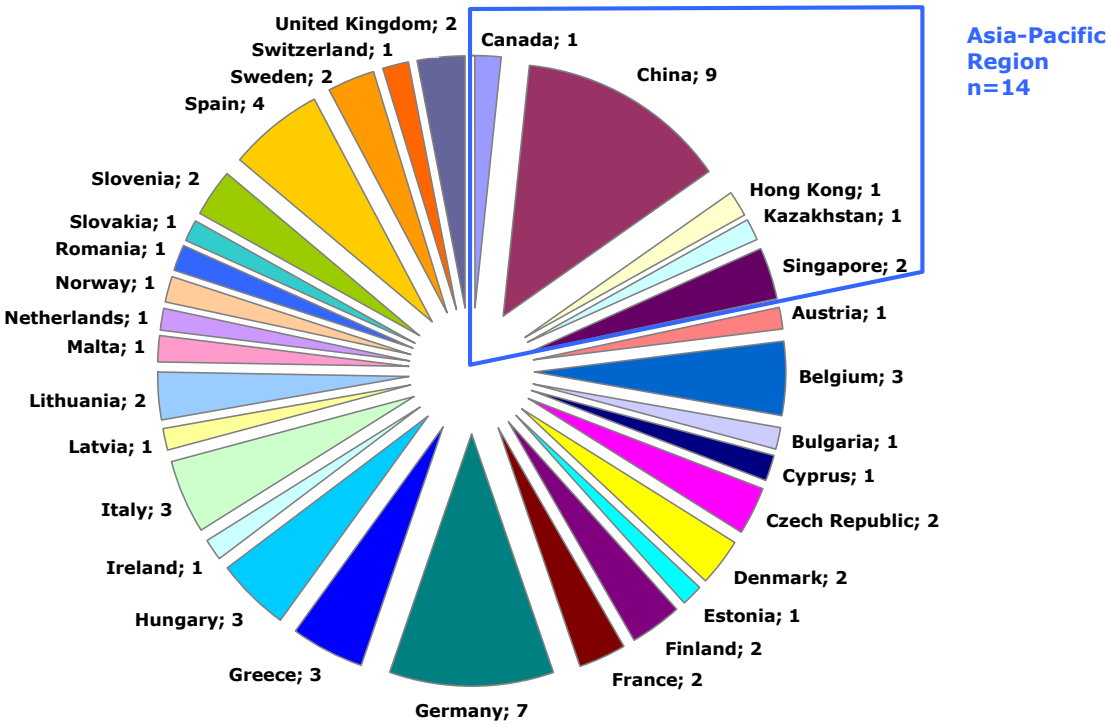


*Table 4 – Methods used by the expert laboratories for sample pre-treatment in the determination of **totAs**.*

ID	Sample treatment	Technique
1	0.25-1 g of sample were weighed into a tall heat-resistant glass beaker (250 mL), treated with 2.5 ml of ashing aid suspension (20% w/v $MgNO_3$ + 2% w/v MgO) and 5 mL of nitric acid (7 mol/L). The mixture was evaporated to dryness in a sand bath and placed in the muffle furnace at an initial temperature not higher than 150 °C. The temperature was increased to (425 ± 25) °C at a maximum rate of 50 °C/h and maintained for 12 h. The mineralization procedure was repeated until the sample was completely incinerated. For this purpose 5 ml nitric acid (7 mol L^{-1}) was added, the mixture was evaporated in the sand bath and the ashes were again placed in the muffle furnace, i.e. the ashes had to be white/grey or slightly coloured. The white ash obtained was dissolved in 6 mol L^{-1} HCl and reduced with pre-reducing solution (5% w/v KI and 5% w/v ascorbic acid). After 30 min, this solution was filtered through Whatman No. 1 filter paper into a volumetric flask and diluted to volume with 6 mol L^{-1} HCl. The arsenic was quantified by FI-HG-AAS using the following instrumental conditions: loop sample 0.5 mL; reducing agent, 0.2% (w/v) $NaBH_4$ 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; wavelength 193.7 nm; spectral band-pass 0.7 nm; electrodeless discharge lamp system 2, lamp current setting 400 mA; cell temperature 900 °C.	FI-HG-AAS
2	0.35 g of sample (0.2 g for the algae) were placed in the digestion vessels with a mixture of 2.5 mL of concentrated HNO_3 and 0.5 mL of H_2O_2, covered and let stand overnight in a clean air hood at ambient temperature (predigestion). The next day the samples were placed in the digestion system, the temperature was raised to 180 °C within 37 min and held for 15 min. The digested samples were cooled to room temperature, transferred to polypropylene test tubes and diluted to 10000 g with water. The total As concentration was determined by ICP-MS in the DRC mode Using H_2 as reaction gas (10 % in Ar, flow 0.35 mL min^{-1}, RPq 0.4) and ^{75}As as the analytical mass. It enabled to overcome any bias arising from the $^{40}Ar^{35}Cl$ interference. Quantification was performed by the method of standard additions using rhodium (1 µg L^{-1}) as internal standard.	ICP-MS
3	Aliquots of test sample and certified reference material were digested in 5 mL nitric acid using quartz high pressure vessels and microwave heating, then the resulting solution was diluted to 10 mL with pure water. A further tenfold dilution with dilute nitric acid containing rhodium was completed just prior to measurement by ICP-MS using collision cell technology (helium mode).	ICP-MS
4	All the samples were digested with HNO_3 solution (diluted 1:1 with doubly deionised water) and H_2O_2, under microwaves closed system. For the digestion, 0.5 g aliquots of the wheat and vegetable samples (0.25 of algae samples) were weighed in the digestion vessels, and 8 mL of 1:1 nitric acid solution and 2 mL of hydrogen peroxide were added. Mixtures were digested according to the following programme: 10 min from room temperature to 90 °C, maintained for 5 min at 90 °C, 10 min from 90 °C to 120 °C, 10 min from 120 °C to 190 °C and 20 min maintained at 190 °C. After cooling to room temperature, the digested samples were filtered through ash-free filter papers 7 (Whatman 40) and diluted in water up to 20 mL. For the final measurements further dilution was carried out if it necessary. Measurements were carried out by ICP-MS with He as the gas in the collision cell to remove interferences. ^{103}Rh was used as the internal standard.	ICP-MS
5	Approximately 0.1 g of sample is weighed out into a 50 ml polypropylene digest tube and 2 mL of conc. HNO_3 is added; the mixture is left to steep overnight. 2 mL conc. hydrogenperoxide are added before the samples are digested in a microwave oven. The temperature program is: first to 55 °C (and held for five minutes) then to 75 °C (and held for five minutes). Finally the digest is taken up to 95 °C and maintained for 30 minutes. Samples are cooled to room temperature and diluted to a mass of 20 g (for wheat and vegetable matter) or 50 g (for algae, further diluted 1:10) with ultrapure deionised water. Quality controls of CRM and blanks are run with each digest set. Samples from the extraction are treated the same way, with the difference that 1 mL extract is mixed with 1 mL conc. HNO_3 and the sample is filled up to 5 g (wheat and vegetable matter, algae: 0.5 mL to 15 g).	ICP-MS
6	0.500 g were weighed into high-pressure (70 bar) quartz containers and 5 mL of concentrated nitric acid was added. The solutions were then digested in a microwave oven, left to cool and diluted with ultrapure milli-Q water to approximately 20 g. The density of the solution was calculated by weighing of 1 mL solution. The solution was further diluted with milli-Q-water prior to analysis (dilution factor 1.6) by ICP-MS. To all samples and blanks Rhodium (^{103}Rh) was added as an internal standard (at 1 µg/L).	ICP-MS
7	0.250 g of powder (wheat and vegetable food) were weighed with a precision of 0.1 mg and mineralised in an ultraclave microwave digestion system. The powders were transferred to 12 mL quartz tubes and were mineralised with 2 mL nitric acid and 2 mL H_2O. The tubes were transferred to a Teflon® rack, covered with Teflon® caps and then the rack was mounted in the microwave system. An Ar pressure of 4 x 10^6 Pa was applied and the mixture was heated to 250 °C for 30 min. After mineralisation, the samples were diluted with water to 9.5 mL (based on weight) in polypropylene tubes. For the determination of total arsenic in algae samples the procedure was similar with the exception that 0.250 mG of algae were digested with 5 mL nitric acid. The digested solutions were diluted with water to 47.5 mL (based on weight). To avoid enhancement of the signal due to the presence of carbon, 500 µL methanol were added to all digested samples.	ICP-MS

*Table 5 – Methods used by the expert laboratories for sample pre-treatment in the determination of **iAs**.*

ID	Sample treatment	Technique
1	Lyophilized sample (0.5-1 g) was weighed into a screw-top centrifuge tube. Then 4.1 mL of water was added and agitated until the sample was completely moistened. After that, 18.4 mL of concentrated HCl was added and the sample was agitated again for 5 min. It was left to stand for 12-15 h (overnight). The reducing agent (2 mL of HBr and 1 mL of hydrazine sulphate) was added and the sample was agitated for 30 s. Then 10 mL of $CHCl_3$ was added and the sample was agitated for 5 min. The phases were separated by centrifuging at 2000 rpm for 5 min. The chloroform phase was separated by aspiration and then poured into another tube. The extraction process was repeated two more times. The chloroform phases were combined and centrifuged again. The remnants of the acid phase were eliminated by aspiration. Possible remnants of organic material in the chloroform phase were eliminated by passing it through Whatman GD/X syringe filters with 25 mm PTFE membrane. The inorganic arsenic in the chloroform phase was back-extracted by agitating for 5 min with 10 mL of HCl (1 mol/L). The phases were separated by centrifuging (2000 rpm for 5 min), and the aqueous phase was aspirated and poured into a beaker. This step was repeated once more and the back-extraction phases obtained were combined. For the determination of inorganic arsenic, 2.5 mL of ashing aid suspension (20% w/v $Mg(NO_3).6H_2O$ and 2% w/v MgO) and 10 mL of nitric acid (14 mol/L) were added to the combined back-extraction phases. This was evaporated to dryness in a sand bath and placed in the muffle furnace at an initial temperature not higher than 150 °C. The temperature was increased to (425 ± 25) °C at a maximum rate of 50 °C/h and maintained for 12 h. The white ash obtained was dissolved in 6 mol/L HCl and reduced with pre-reducing solution (5% w/v KI and 5% w/v ascorbic acid). After 30 min, this solution was filtered through Whatman No. 1 filter paper into a volumetric flask and diluted to volume with 6 mol/L HCl.	FI-HG-AAS
2	0.35 g of sample was added with 10 mL of 1 % (v/v) HNO_3 and 1% H_2O_2 and left to stand overnight. Microwave irradiation was performed 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 75 C, 2 min ramp to 95 C, 30 min at 95 C. The extracts were centrifuged (10 min, 8000 rpm, 7 C) and the supernatants filtered through a 0.22 mm filter.	HPLC-ICP-MS
3	Aliquots of test sample and "in-house" certified reference material were solubilised overnight with concentrated HCl then HBr and hydrazine sulphate added prior to chloroform extraction. The chloroform extract was back extracted into 1 mol/L HCl and this solution was directly measured by ICP-MS	ICP-MS
4	For speciation analysis, 0.4 g aliquots of the wheat and vegetable food samples and 0.25g of the algae sample were weighed in the digestion vessels and were extracted adding10 mL of 0.2 % (w/v) nitric acid and 1% (w/v) hydrogen peroxide solution using 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 3000 rpm for 12 min. The supernatant was filtered through PET filters (pore size 0.45 µm).	HPLC-ICP-MS
5	Milled sub-samples (0.5 g, 0.25 g for algae) are weight into polypropylene vials and mixed with 10 mL 1 % HNO_3. The mixture is allowed to stand overnight. The mixture is heated 10 min at 50°C, 10 min at 75°C and 20 min at 95°C in a temperature-controlled microwave. After cooling the mixture will be centrifuged, 1 % (v/v) hydrogen peroxide will be added and the sample stored at -20°C before analysis for speciation or total arsenic content.	HPLC-ICP-MS
6	For the determination of inorganic arsenic subsamples of approximate 0.25 g were weighed into microwave quartz containers and 10.00 mL of 0.07 M hydrochloric acid (Merck) in 3% hydrogenperoxide was added. The solutions were placed in the microwave oven and the power was programmed to keep the solutions at 90°C for 20 min. By this procedure the inorganic arsenic is extracted from the sample matrix and furthermore As(III) is oxidized to As(V), thus allowing for the determination of total inorganic arsenic as As(V). Then the solutions were allowed to cool to room temperature and the supernatant transferred to 15 mL plastic tubes and centrifuged at approximately 4000 rpm for 10 min and subsequently filtered (0.45 µm) prior to analysis.	HPLC-ICP-MS
7	For the extraction about 250 mg of powder (50, 100 and 200 mg for micro-homogeneity studies) were weighed with a precision of 0.1 mg into 12 mL quartz tubes, 5 mL of 0.02mol·L-1 trifluoracetic acid containing 1 % (v/v) of a 30 % H2O2 solution were added and the suspension was sonicated for 15 minutes. Samples were microwave-extracted with an ultraclave microwave digestion system. The tubes were transferred to a Teflon® rack, covered with Teflon® caps and then the rack was mounted in the microwave system. After closing the system, an argon pressure of 4 x 106 Pa was applied. Extraction was done using a one stage temperature ramping program ramping to 95 °C over 10 minutes and maintaining the temperature for 60 minutes. After cooling to room temperature the extracts were transferred to polypropylene tubes and centrifuged for 15 min at 8900 rcf.	HPLC-ICP-MS

7.2 Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z- and ζ-scores in accordance with ISO 13528 [8].

$$z = \frac{x_{lab} - X_{ref}}{\hat{\sigma}} \qquad \text{and} \qquad \zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}}$$

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

The assigned reference values (X_{ref}), and their respective uncertainties are summarised in Table 1 and Table 2. The interpretation of the z- and ζ-score is done as follows:

|score| ≤ 2	satisfactory result	(green in the tables of Annexes 8-13)
2 < |score| ≤ 3	questionable result	(orange in the tables of Annexes 8-13)
|score| > 3	unsatisfactory result	(red in the tables of Annexes 8-13)

The ζ-score states if the laboratory result agrees with the assigned value within the respective uncertainty. The denominator 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 or both.

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_{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 √3, as recommended by Eurachem and CITAC [14].

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 $u_{lab} > u_{max}$, 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. Values for $\hat{\sigma}$ in IMEP-112 were set to:

- 15 % for total and inorganic arsenic in **wheat.** Fifteen percent proved to be a sound target standard deviation in IMEP-107 on Total and inorganic arsenic in rice, a matrix similar to wheat.
- 22% for totAs and 25 % for iAs in **vegetable food.** The uncertainty associated to the certified value (totAs) as provided by NIST was of 17%. The standard deviation of the means provided by the experts *(u_{char})* was 23 % (iAs). Such high $\hat{\sigma}$ reflects the difficulty analysing relatively low concentrations of totAs and iAs.
- 15 % for totAs and 22 % for iAs in **algae**, to account for the high complexity of the determination of iAs in this type of samples due to the complex distribution of species in marine matrices.

7.3 Laboratory results and scorings

The results as reported by the participants for total and inorganic arsenic in wheat, vegetable food and algae are summarised in Annexes 8 to 13, together with the z- and ζ-scores. These annexes also include figures showing the individual mean values, associated expanded uncertainties and the Kernel distribution plots, obtained using a software tool developed by AMC [15]. NRLs are marked with an * in Annexes 8 to 13.

The uncertainty values reported by laboratory L84 seem to be abnormally high; it looks as if that laboratory had reported the uncertainty in percentage and not in mg kg^{-1}.

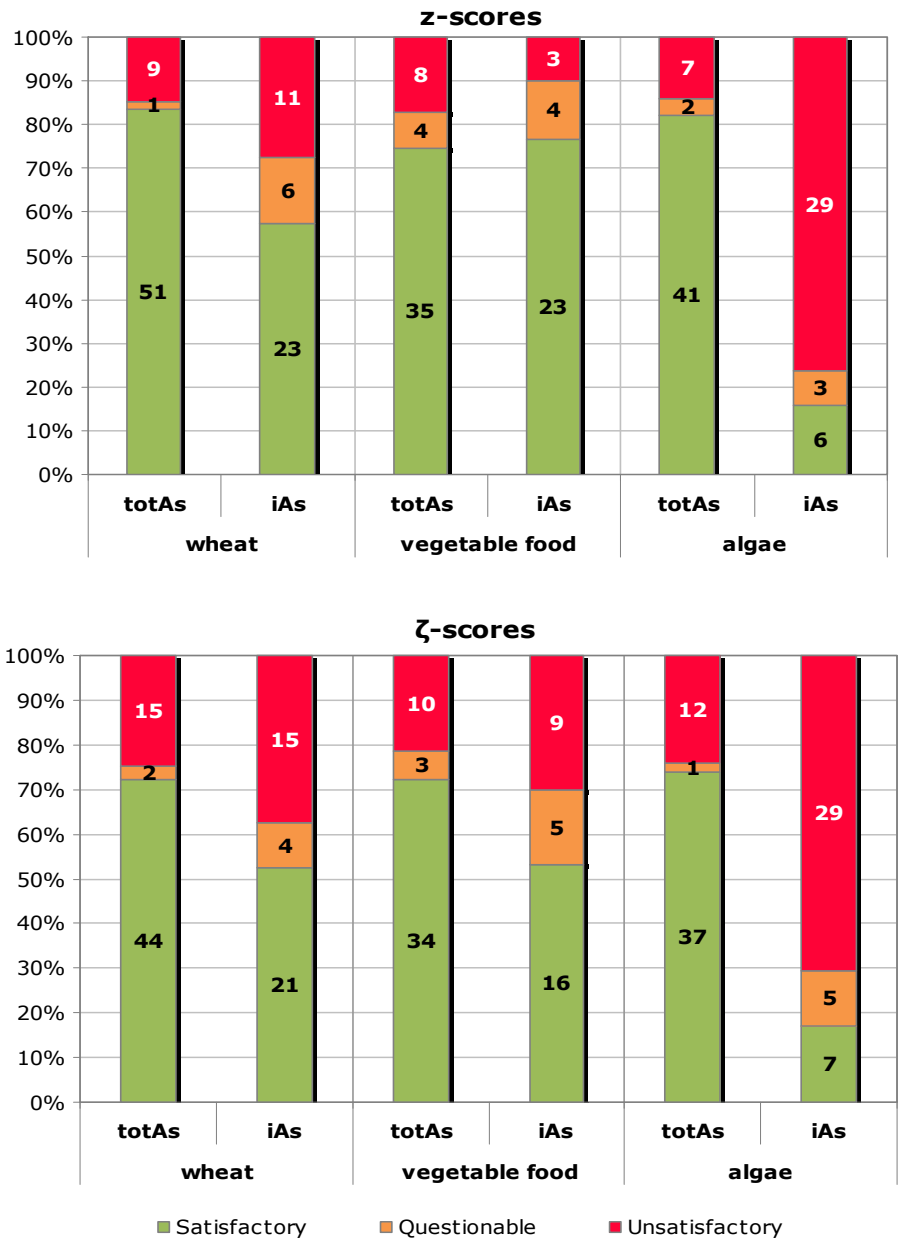
L42 has reported values for totAs in wheat which match the assigned values for totAs in algae and vice versa. No scores were attributed to that laboratory for those two matrices.

Some laboratories reported "less than" values which are not correct because the actual assigned values were higher than those indicated by them.

Regarding the z- and ζ-scores, the results for total and inorganic arsenic in wheat, vegetable food and algae are summarised in Fig 2. Considering the z-score, between 75 and 85 % of the participants performed satisfactorily for totAs. As for iAs, about 60 % and 75 % of the participants reported satisfactory results for wheat and vegetable food, respectively, despite the relatively low concentration of iAs in the vegetable food. Less than 20 % of the participants scored satisfactorily for iAs in algae. The distribution of satisfactory results reported for the three test materials included in this exercise could reflect the difficulty introduced by the different matrices.

The percentage of satisfactory ζ-scores is even lower than for the z-scores, indicating which points at the fact that laboratories have problems in estimating the uncertainty of their results.

Fig 2 – Overview of scores



7.3.1 Wheat

Although it could be thought that wheat would behave in a similar way than rice, this exercise provided evidence that the determination of iAs in this matrix requires some extra care in the digestion step. For instance H_2O_2 needs to be added before the microwave digestion of the sample to improve the efficiency of the digestion, while for rice H_2O_2 is only needed to oxidise As (III) to As (V). Several of the laboratories that underestimated iAs in wheat did not add H_2O_2 during the digestion of the matrix (see L35, L38, L39, L50, L73 and L88 in Annex 14). Of course, this is not the only parameter that

plays a role in the quantitative determination of iAs and some laboratories that did not add H_2O_2 obtained satisfactory results. Other parameters such as digestion reagent concentration and digestion temperature may contribute to improve the efficiency of the digestion even in the absence of H_2O_2.

7.3.2 Vegetable food

It seems that the determination of iAs in vegetable food (spinach) presents less difficulties than in the other two matrices, despite the low concentration of iAs in the test material. However it must be kept in mind that $\hat{\sigma}$ for this matrix was 25 % (as opposed to 15 % for wheat and 22 %) and this could explain to a certain level the higher percentage of satisfactory results reported for iAs in this test material, certainly when compared to those reported for wheat.

7.3.3 Algae

As expected algae seems to be a particularly difficult matrix, very likely due to the variety of organic arsenic species and the relatively large concentration in which they are present when compared to the concentration of iAs. Concerning totAs, a number of laboratories have significantly **underestimated** the mass fraction in this test material probably because digestion of some organic compound of arsenic, such as arsenobetaine, is difficult and requires temperatures of at least 280 °C when microwave digestion is used.

On the other hand, for iAs there is a tendency to **overestimate the concentration**, since 26 of the participants reported results for this measurand above the accepted range (X_{ref} + $2\hat{\sigma}$) even when taking into consideration their associated uncertainties. Only three participants (L3, L47 and L63) have underestimated the concentration of iAs in algae. A thorough discussion about the problems associated to the determination of iAs in marine samples is presented in the IMEP-30 report for participants [16] dealing with the determination of iAs in seafood:

> *"… "less than" values could be explained by an insufficient amount of oxidant added, H_2O_2 to oxidize As(III) to As(V), which is the species of As measured when using HPLC-based methods. Furthermore, the use of MeOH/water and diluted HCl as extracting reagents might not have provided quantitative extraction of iAs. On the other hand, when applying extraction of iAs with chloroform and concentrated HCl, a cleaning step of the chloroform phase should be carried out to eliminate all*

traces of HCl and with it the there present arsenobetaine. Remains of the concentrated HCl in the chloroform phase might introduce a high contamination of the sample in organic species. Finally, it appears that when analysing complex matrices by HPLC-ICP-MS the retention time of the iAs shifts and it cannot be detected because of possible co-elution with minor organic species. This can be remedied by introducing an extra step of hydride generation between the HPLC and the ICP-MS."

In IMEP-112 three laboratories (L24, L28 and L29) used the EN 15517:2008 standard [2] and six participants (L44, L51, L59, L63, L68 and L69) used the standard GB/T 5009.11-2003 [3]. None of them obtained satisfactory scores for iAs. With the exception of L24 who reported <0.025 mg kg^{-1}, the remaining laboratories having used the mentioned standards reported largely overestimated values ranging from 5 to 46 mg kg^{-1}. The results obtained with GB/T 5009.11-2003 range from 5.0 to 11 mg kg^{-1} with the exception of the result reported by L63 (0.076 ± 0.005 mg kg^{-1}).

However IMEP-112 has shown that when the analytical methods are properly optimised it is possible to agree on a value for iAs in algae. This has been proven by 5 of the expert laboratories and by 20 % of the participants. The methods summarised in Table 5 for certifiers 1, 2, 3, 4 and 7 can serve as a basis for laboratories that want to develop a method for the determination of iAs in this type of matrix.

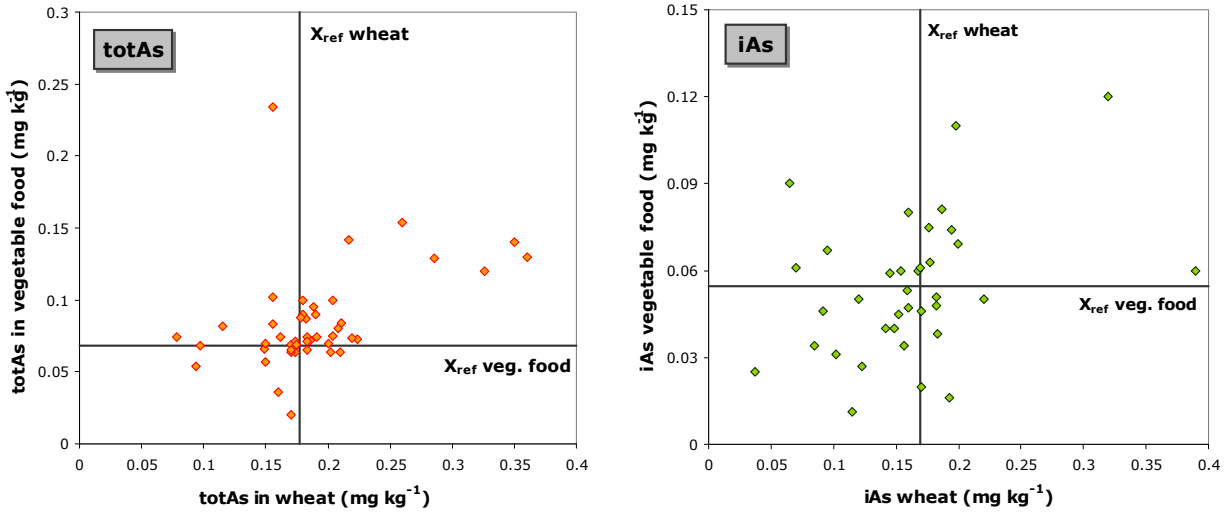
An important outcome of IMEP-112 is that neither for total nor for iAs a clustering of results has been observed for any of the test items on the basis of the method of analysis used.

7.4 Youden plots

The reported results for wheat and vegetable food have been represented in Youden plots (Fig 3), which is a mean to display systematic and random errors in a result set. For both measurands, most of the points are scattered around the crossing of the respective X_{ref} values, which means that no pattern can be observed. Systematic over- or underestimation for one measurand / matrix or the other is therefore unlikely.

Results for algae have not been included in this graphical evaluation, as the mass fractions for both measurands are very different and the matrix particular.

Fig 3 – Youden plots for reported totAs and iAs results in wheat and vegetable food



8 Additional information extracted from the questionnaire

Additional information was gathered from the questionnaire filled by most of the participants (Annex 6).

Twenty-six laboratories have corrected their results for recovery. The recoveries reported range from 72 to 110 %. The approach used for the determination of recovery is given in Table 6. The reasons provided by the participants for not having corrected for recovery are listed in Table 7.

Table 6 – Approach used for the determination of the recovery factors

Recovery factor R determined by:	Number of participants
1. adding a known amount of the same analyte to the sample	10
2. using a certified reference material	13
3. Other	2*
1. and 2.	2
	*Recovery = total extracted/total mineralised

Table 7 – Reasons for not having corrected the results for recovery

Lab ID	Reason
L01	Recovery = total extracted/total mineralised
L06	Because recovery factor for total As determined by using a CRM were practically 100%. For inorg. As no CRM was used.
L13	Complete recovery of As from reference material.
L15	-1.0%
L16	in routine analysis we don't apply a recovery factor. The recovery was in the range 90-110 %, control material was used IMEP 107 rice

Lab ID	Reason
L18	The matrix is diluted
L19	Total digestion for element
L23	Because in routine work we do not use the recovery factor
L24	A reference material is used before and after an analysis sequense
L25	-1.0%
L26	Results of CRM showed no problem. Internal standard used.
L27	-1.0%
L29	use of IMEP-109 and IMEP-110
L32	The recovery checks on IQC materials yield good recoveies between 95% to 115%.
L33	for total As no recovery factor is used in general
L39	-1.0%
L47	The recovery is covered by uncertainty.
L50	For inorganoc As recovery can not be determined due to unknown binding and possible presence of (unextractable) unknown compounds. Stronger extractants might extract 100% of As but might as well decompose organoarsenic molecules.
L54	agreeing to CE 333/2007 if our result considering our uncertainty matches the certified value of a reference material, it is not needed to correct by the recovery factors.
L55	-1.0%
L57	Not a practise for routine samples
L75	Use of CRM
L85	Total amount must not be corrected, extraction conditions are quantitative within uncertainty
L86	-1.0%
L88	Because validation of the methods gave a quantitative recovery

Fifty-four laboratories corrected their results for moisture content and five did not. The reported moisture contents range from 0.41 to 12.84 % for wheat, from 0.16 to 11.26 % for vegetable food and from 0 to 2.89 % for algae. The reason given by some of the laboratories for not correcting for moisture content are summarised in Table 8.

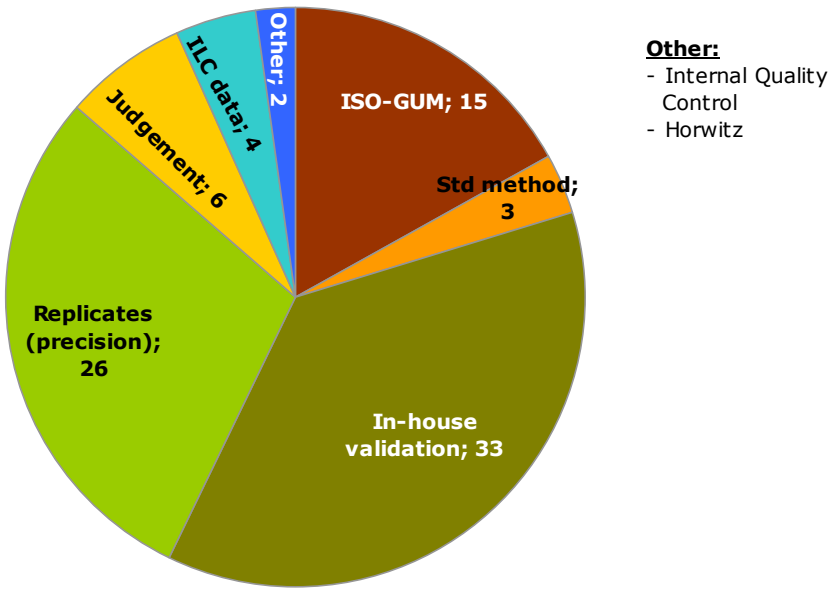
For uncertainty estimates, various combinations of one or more options were given. Two laboratories gave an additional method to base their uncertainty on. Details are shown in Fig 4.

Table 8 – Reasons for not having corrected the results for moisture content

Lab ID	Reasons
L30	Not in our routine
L59	We had dried the samples before we test them, as the direction require.
L66	We think that with the sample pre-treatment ,we can provide the contents of the metal in the samples, and the results can reflect the Ideal sample.

Fig 4 – Approaches used to estimate the uncertainty associated to the results

Q3. *What is the basis of your uncertainty estimate?*



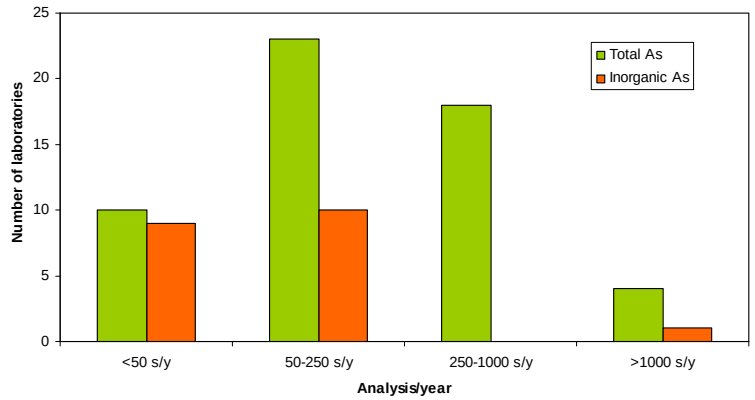
When asked about the level of confidence covered by the reported coverage factor (*k*), most of the participants reported 95 %. Some participants did not understand the question and provided irrelevant answers or did not answer. Some participants reported the recovery factor and not the coverage factor for the uncertainty. The following information regarding coverage factors can be found on the NIST webpage [17]:

> *"In general, the value of the coverage factor k is chosen on the basis of the desired level of confidence to be associated with the interval defined by* $U = ku_c$. *Typically, k is in the range 2 to 3. When the normal distribution applies and* u_c *is a reliable estimate of the standard deviation of a measurement,* $U = 2\ u_c$ *(i.e., k = 2) defines an interval having a level of confidence of approximately 95 %, and* $U = 3\ u_c$ *(i.e., k = 3) defines an interval having a level of confidence greater than 99 %".*

For a deeper insight into this issue participants are encouraged to read ISO GUM [18] and/or Eurachem/CITAC Guide on Quantifying Uncertainty in Analytical Measurements [14].

Fifty-seven laboratories analyse totAs on a routine basis while only 20 do for iAs. The distribution of laboratories on the basis of the number of analysis performed/year is given in Fig 5.

Fig 5 – Number of samples analysed per year by the participants in IMEP-112



Fifty-eight participants have a quality system in place, one has not and 55 are accredited according to ISO 17025 and / or ISO 9000 series (3 participants). Ninety-seven percent of the participants take regularly part in PTs for totAs and 87 % do in PTs for iAs .

Fifty-five laboratories use CRMs and 5 do not. Fifty-one use the CRM for validation purposes and 11 for calibration purposes. Four do not use the CRMs for any of the two mentioned purposes. The reference materials used by the participants are listed in Table 9.

Finally, comments by the participants are summarised in Table 10.

Table 9 – CRMs used by the participants.

Lab ID	CRM
L01	NIST-1568a
L02	CRM NIST 1515
L03	samples remained from PTs
L04	Merck standard, Fapas T0775
L06	Total As: Rice Flour 1568A, Speciation of As: BCR-628-7 Tuna Fish Tissue
L08	IMEP 107
L09	previous runs PT material
L10	CRM 2976
L11	LGC 7162 (STRAWBERRY LEAVES)
L12	DORM-3 (NRC-CNRC)
L13	Nist 1547
L14	NIST1586a rice powder
L15	NIST 1547
L16	IMEP-107
L19	BCR 627, BCR278R, NIST1568a
L21	LICHEN BCR CRM 482
L22	IRMM-804, IMEP-107 test material, NCS ZC73009
L23	BCR, NIST
L26	NIST 1567a Wheat Flour

Lab ID	CRM
L27	IMEP 107 rice
L28	BCR-610
L29	IMEP-109 and IMEP-110
L33	NIST 1568a
L34	CRM 1568a - Rice
L35	BCR 281 (Rye Grass)
L38	IMEP-107, DORM-3
L40	NIST Standard Reference Materials Gaithersburg USA, Analytical Reference Materials Environment Canada, NCS Certified Reference Materials, China National Analzsis Center Reference Materials
L44	GBW(E)080117
L47	GBW 7602, GBW 7603, GBW 7604, CZ 9003(1N)
L48	IMEP 107 rice
L49	IMEP 102
L50	NIST-1547 Peach Leaves
L66	GBW10010,GBW10014
L69	GBW08611 (Standard Solution of As)
L74	Dolt-4
L75	Rice Flour
L84	NIST 1568a, old interlaboratory controls
L86	PT Material from FAPAS T08110 (Seaweed)
L88	Rice SRM 1568a

Table 10 – Comments made by the participants.

Lab ID	Comment
L04	No
L08	In the algae sample the concentration of Asi is very small compare to the total arsenic concentration. So that the quantification is not very accurate
L11	There is some confusion about the number of samples in this PT Round (1,2 or 3). This laboratory received only one (Wheat).
L16	No comments
L18	The samples were not stored at cool temperature but at room temperature
L22	We wonder about the untypical high inorganic arsenic content especially in wheat but also in the vegetable
L27	We did not determine inorganic arsenic because we had technical problems with our measurement equipment (FIAS-AAS).
L38	1/ According to IUPAC the term "heavy metal" should be avoided in a scientific communication (Pure Appl. Chem., 2002, Vol. 74, No. 5, pp/ 793-807). 2/ The water content of the wheat RM was too high to be a stable RM, it should be under 10%, or even 5%.
L48	We used spiked samples on wheat
L50	In on-line submission it was possible only to save Technique used for one sample code. In our case I leaved it for Algae. Of course, the same technique was used for total As and inorganic As for Vegetable food and Wheat.
L51	the quantity of sample is small.
L55	No
L57	A larger sample size could have been provided
L59	No
L66	We think that it is a good chance for us to participate in this project. It can improve the communication of the laboratories.
L68	No
L69	No
L74	NIL
L86	When using a lower extraction temperature (37°C instead of 95°C) you get lower results. We submitted the higher results for wheat and vegetable. We also determined the inorganic arsenic with the extraction temperature 37°C. We can provide these results if it is in your interest.

9 Conclusions

The main conclusion derived from this exercise is that the concentration of iAs determined in any of the matrices covered does not depend on the analytical method applied, as has been proven by the results submitted by the seven expert laboratories and by the participants in IMEP-112.

In IMEP-112 a wide range of sample pre-treatment methods (extraction into water, acid extraction with different acids, enzymatic digestion etc), and instrumental set-ups (HG-AAS, HPLC-ICP-MS, ETAAS) have been applied by participants and by the expert laboratories that provided the assigned values for iAs. Despite the use of these different methods, clustering of results related to the analytical approach was not observed.

No particular problem related to the determination of iAs was detected in this PT and the performance of the participating laboratories was, in general, satisfactory for the determination of iAs in wheat and vegetable food. Laboratories should remember that not all cereals behave analytically in the same way for the determination of iAs and that methods which perform satisfactory in rice can provide biased results when applied to wheat. The low number of laboratories obtaining a satisfactory score for iAs in algae indicates that this matrix poses special problems for this type of analysis and that the methods need to be carefully validated for different matrices. Unfortunately, two existing standards for the determination of iAs (EN 15517:2008 and GB/T 5009.11-2003) did provide biased results when applied to algae. The number of laboratories that used these standards in this exercise is rather limited and so further studies should be carried out before making more definitive statements.

The results show that, purely from the analytical point of view, there is no reason not to consider the option of introducing possible maximum levels for iAs in wheat, vegetable food and algae in further discussions on risk management.

Furthermore, attention should also be paid to the determination of totAs in algae, since underestimations due to incomplete digestion of some organic compounds of arsenic can occur.

10 Acknowledgements

C. Contreras and P. Connely from the Reference Materials Unit at IRMM are acknowledged for their support for setting-up the short-term stability study. of the test material and in checking the drying method against Karl-Fisher titration. A. Santoro is acknowledged for the useful discussion about the characteristics of the algae material. F. Cordeiro is thanked for the fruitful discussions about the organisation of the IMEP-112 exercise and the thorough revision of this report. K. Jebjerg Andersen (EA) and D. Tholen (APLAC) are thanked for serving as interface in their respective networks and for announcing IMEP-112.

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

Organisation	Country
AGES GmbH	Austria
Scientific Institute of Public Health	Belgium
CODA CERVA	Belgium
CODA-CERVA	Belgium
Central Laboratory for Chemical Testing and Control	Bulgaria
Canadian Food Inspection Agency	Canada
Xiangfan Entry-Exit Inspection and Quarantine Bureau	China
Daqing Entry-Exit Inspection And Quarantine Bureau Of The Peoples Republic Of China	China
Centre Testing International (Shen Zhen) Corporation ShangHai Branch Company	China
Zhoushan Branch of Zhejiang Academy of Science and Technology for Inspection and Quarantine	China
Quality Testing Center for Non-wood Forest Products of State Forestry Administration (HangZhou)	China
Foshan Haitian Flavoring & Food Co., Ltd. Inspection Center	China
Centre Testing International(Qingdao) Limited	China
Centre Testing International (shenzhen)Corporation	China
Qingdao Supervision & Testing Center of Product Quality	China
State General Laboratory	Cyprus
CISTA (ÚKZÚZ)	Czech Republic
State Veterinary Institute Olomouc	Czech Republic
Danish Veterinary and Food Administration, Region West	Denmark
DTU Food	Denmark
AGRICULTURAL RESEARCH CENTRE	Estonia
Finnish Customs Laboratory	Finland
Evira	Finland
Laboratoire SCL de Bordeaux-Pessac	France
ANSES - Laboratoire de sécurité des aliments	France
LAVES	Germany
Bay. Landesamt f. Gesundheit u. Lebensmittelsicherheit	Germany
Federal Office of Consumer Protection and Food Safety (BVL)	Germany
Kreis Mettmann	Germany
Landeslabor-Berlin-Brandenburg	Germany
Institut Kirchhoff Berlin GmbH	Germany
Intertek Food Services GmbH	Germany

Organisation	Country
General Chemical State Laboratory	Greece
General Chemical State Laboratory	Greece
University of Athens	Greece
Centre for Food Safety	Hong Kong
Central Agricultural Office, Food and Feed Safety Directorate	Hungary
Corvinus University of Budapest	Hungary
Agricultural Office Food and Feed Safety Directorate	Hungary
HEALTH SERVICE EXECUTIVE	Ireland
Chemical Control srl	Italy
ARPA.FVG	Italy
Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta	Italy
Astana Center for Sanitary and Epidemiological Expert Examination (Republican State Budget-Supported Enterprise) of the Committee for Sanitary Surveillance of the RK Ministry of Healthed	Kazakhstan
National food and veterinary risk assessment institute	Lithuania
National Food And Veterinary Risk Assessment Institute	Lithuania
Public Health Laboratory Malta	Malta
RIKILT	Netherlands
NIFES- National Institute of Nutrition and Seafood Research	Norway
Hygiene Institute of Veterinary Public Health	Romania
Agri-Food & Veterinary Authority	Singapore
HEALTH SCIENCES AUTHORITY	Singapore
State veterinary and food institute - Kosice	Slovakia
Jozef Stefan Institute	Slovenia
National Veterinary Institute	Slovenia
Laboratory of the Public Health Agency of Barcelona	Spain
Laboratorio Arbitral Agroalimentario	Spain
Sanidad y Consumo Gobierno Vasco	Spain
UCM	Spain
National Food Administration	Sweden
ALS Scandinavia AB	Sweden
SQTS	Switzerland
Kent County Council	United Kingdom
Minton Treharne & Davies Limited	United Kingdom

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Annexes

Annex 1 : Invitation letter to NRLs

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

European Union Reference Laboratory
Heavy Metals in Feed and Food

Geel, March 2011
JRC.DDG.D6/BCa/ive/ARES(2011)305478

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

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

Dear Madam / Sir,

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-112] for the determination of "**total and inorganic As in wheat, vegetable food and algae**".

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 EU-RL 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=660

As discussed during the 5th workshop organised by the EU-RL-HM in Brussels on 24/09/2010, participation in this exercise is not limited to the network of NRLs. If you know laboratories that could be interested in taking part in IMEP-112 please inform them. Due to a limited amount of test material available only laboratories with capabilities to perform inorganic arsenic analysis should register to this exercise. This does not apply to NRLs, so all NRLs with a mandate for this type of matrix should register to the exercise even if they can only report results for total arsenic.

Due to the special nature of this proficiency test participation is **free of charge** for all participants.

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

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 26th April 2011**. Samples will be sent to participants during the first week of May. The deadline for submission of results is 10th June 2011.

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

Yours sincerely

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

Cc: Franz Ulberth

Annex 2 : Invitation letter to EA

Ref. Ares(2011)349010 - 30/03/2011

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements

IMEP

Geel, 25 March 2011
JRC.DG.D6/DLCB/mdr/ARES(2011)

Mrs Kirsten Andersen
Dyregaardsvej 5 B,
DK-2740 Skovlunde,
DENMARK

IMEP-112 Proficiency test for total and inorganic arsenic in wheat, vegetable food and algae

Dear Kirsten,

The Institute for Reference Materials and Measurements (IRMM) organises IMEP-112, an interlaboratory comparison for the **"Determination of total and inorganic arsenic in wheat, vegetable food and algae".**

In the frame of the EA-IRMM collaboration agreement, IRMM kindly invites EA to nominate laboratories for free participation. They should hold (or be in the process of obtaining) an accreditation for this type of measurement.

I suggest that you forward this invitation to the national EA accreditation bodies for their consideration.

Due to the special nature of this exercise, participation in IMEP-112 is free of charge for all participants under the condition though that **only laboratories with capacities for inorganic arsenic determination should register.**

Confidentiality of the participants and their results towards third parties is guaranteed. However, IMEP will disclose details of the participants that have been nominated by EA to the EA working group for ILCs in Testing. Please inform the nominees of this disclosure.

The **registration page is open until 26 April 2011**. Distribution of the samples is foreseen for beginning of May 2011. Deadline for **submission of results is 10 June 2011**.

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 715. Fax: (32-14) 571 865.

E-mail: jrc-irmm-imep@ec.europa.eu

In order to register, laboratories must

1. **Enter** their details online:

https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=660

2. **Print** the completed form when the system asks to do so and clearly indicate on the printed form that they have been appointed by the European Cooperation for Accreditation to take part in this exercise.

3. **Send** the printout to both the IMEP-112 and the EA-IMEP-112 coordinators:

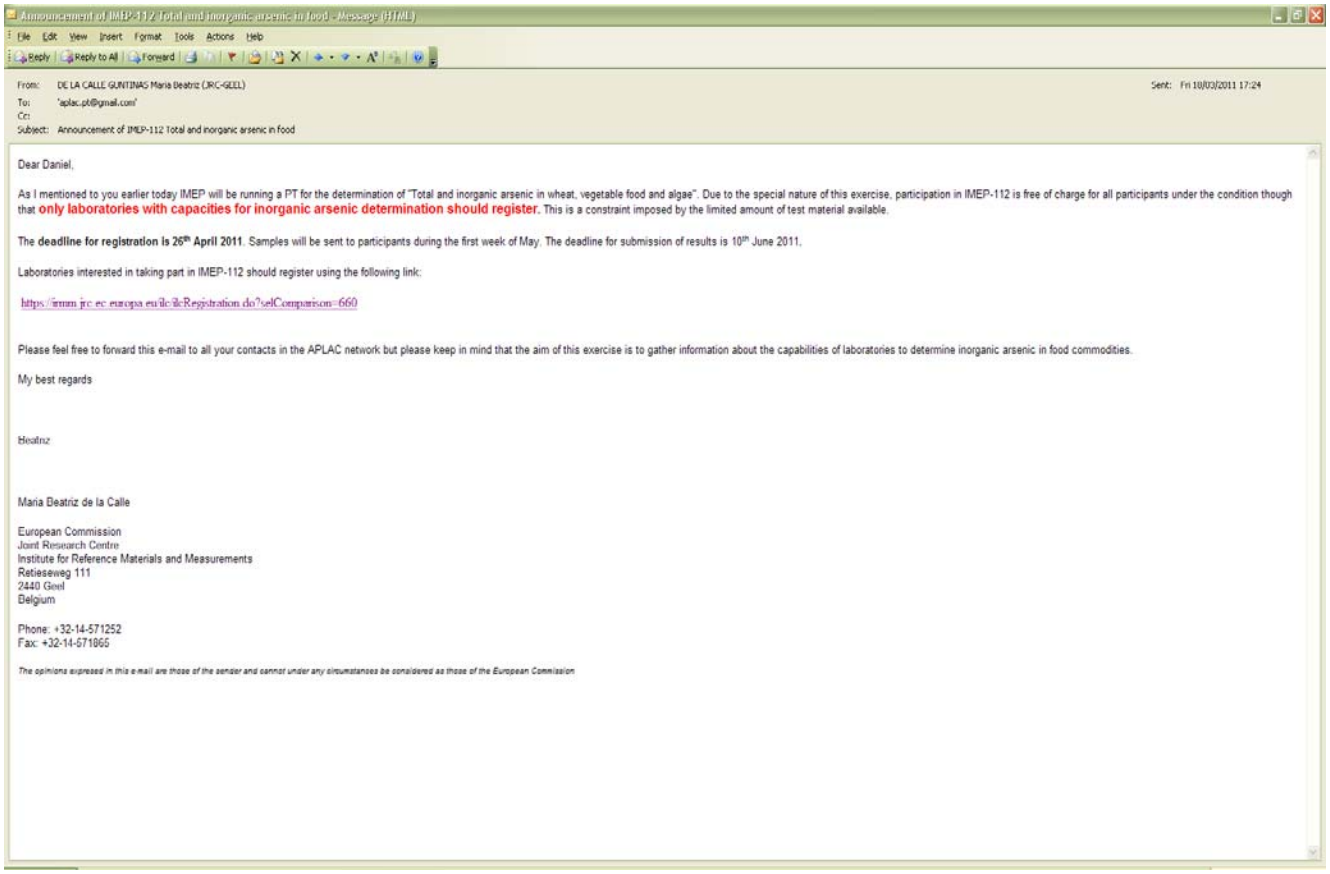
IMEP-112 coordinator	**EA-IMEP-112 coordinator**
Mrs Beatriz de la Calle	Mrs Kirsten Andersen
Fax +32 14 571 865	Fax + 45 77 33 95 01
E-mail: jrc-irmm-imep@ec.europa.eu	E.Mail: kja@danak.dk

Please contact me you have any questions or comments. We are looking forward to our cooperation!

With kind regards

Beatriz de la Calle
IMEP-112 Coordinator

Annex 3 : Announcement e-mail to APLAC



Announcement of IMEP-112 Total and inorganic arsenic in food - Message (HTML)

File Edit View Insert Format Tools Actions Help

Reply | Reply to All | Forward

From: DE LA CALLE GUNTINAS Maria Beatriz (JRC-GEEL) Sent: Fri 18/03/2011 17:24

To: 'aplac.pt@gmail.com'

Cc:

Subject: Announcement of IMEP-112 Total and inorganic arsenic in food

Dear Daniel,

As I mentioned to you earlier today IMEP will be running a PT for the determination of "Total and inorganic arsenic in wheat, vegetable food and algae". Due to the special nature of this exercise, participation in IMEP-112 is free of charge for all participants under the condition though that **only laboratories with capacities for inorganic arsenic determination should register.** This is a constraint imposed by the limited amount of test material available.

The **deadline for registration is 26th April 2011**. Samples will be sent to participants during the first week of May. The deadline for submission of results is 10th June 2011.

Laboratories interested in taking part in IMEP-112 should register using the following link:

https://imm.jrc.ec.europa.eu/ilc/ileRegistration.do?selComparison=660

Please feel free to forward this e-mail to all your contacts in the APLAC network but please keep in mind that the aim of this exercise is to gather information about the capabilities of laboratories to determine inorganic arsenic in food commodities.

My best regards

Beatriz

Maria Beatriz de la Calle

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements
Retieseweg 111
2440 Geel
Belgium

Phone: +32-14-571252
Fax: +32-14-571865

The opinions expressed in this e-mail are those of the sender and cannot under any circumstances be considered as those of the European Commission

Annex 4 : Accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

European Union Reference Laboratory
Heavy Metals in Feed and Food

Geel, 16 May 2011
JRC.DDG.D6/BCa/ive/ARES(2011)/

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

Participation in IMEP-112, a proficiency test exercise for the determination of total and inorganic As in wheat, vegetable food and algae.

Dear «TITLE» «SURNAME»,

Thank you for participating in the IMEP-112 intercomparison for the determination of **total and inorganic As in wheat, vegetable food and algae**. This exercise takes place in the frame of the EU-RL Heavy Metals in Feed and Food.

This parcel contains:
a) One bottle containing approximately 20 g of wheat
b) One bottle containing approximately 15 g of vegetable food
c) One bottle containing approximately 5 g of algae
d) A "Confirmation of Receipt" form
e) This accompanying letter

Please check whether the bottles containing the test material remained undamaged during transport. Then fax (at +32-14-571865) or e-mail the "Confirmation of receipt" form. You should store the samples in a dark and cool place (not more than 4 °C) until analysis.

The measurands are: **total and inorganic As in wheat, vegetable food and algae**. The procedure used for the analyses should resemble as closely as possible the one that you use in routine sample analysis.

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

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

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

Wheat and vegetable food

1. *Weigh accurately 1 g of test material in a glass container of 5-7 cm diameter. Preferably with a lid because when the prescribed drying time has passed, the glass container must cool down about 30 minutes in a desiccator before weighing.*
2. *Place it in an oven for 60 ± 5 min at 120 ± 2 ºC.*
3. *Place the glass container covered with a lid in a desiccator and wait 30 min before weighing the test material again.*

Algae

1. *Weigh accurately 0.5 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 50 ± 5 min at 90 ± 2 ºC.*
3. *Place the glass container covered with a lid in a desiccator and wait 30 min before weighing the test material again.*

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

You can find the reporting website at 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 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 15/06/2011.

The result reporting website is not available due to maintenance on 3 and 4 June 2011.

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

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

With kind regards

Dr. M.B. de la Calle
IMEP-112 Co-ordinator

Enclosures: a) One bottle containing approximately 20 g of wheat, b) one bottle containing approximately 15 g of vegetable food, c) one bottle containing approximately 5 g of algae, d) a "Confirmation of Receipt" form and e) this accompanying letter

Cc: F. Ulberth

Annex 5 : Confirmation of receipt form

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

European Union Reference Laboratory
Heavy Metals in Feed and Food

Annex to JRC.DDG.D6/BCD/ive/ARES(2011)/

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

EU-RL-HM-12 / IMEP-112

total and inorganic arsenic in wheat, vegetable food and algae

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-112 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) 571 299. Fax: (32-14) 571 865.

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

IMEP

Annex 6 : Questionnaire

Submission Form

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

- ○ no
- ○ yes

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

1.1.1. for total As (in %)

1.1.2. for inorganic As (in %)

1.2. If Yes, did you determine R by:

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

1.3. If no, please state why?

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

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

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

3.1. If other, please specify

4. **Do you usually provide an uncertainty statement to your customers 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 were the water contents (in % of the sample mass)?

5.1.1. in wheat?

5.1.2. in vegetable food?

5.1.3. in algae?

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

6. **Did you determine the cocentration of total As applying an official method?**

- ○ no
- ○ yes

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

6.1.1. sample pre-treatment

6.1.2. digestion step

6.1.3. extraction / separation step

6.1.4. instrument calibration step/instrumental set-up

6.2. If Yes, which:

7. **Did you determine the concentration of inorganic As applying an official method?**

- ○ no
- ○ yes

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

7.1.1. sample pre-treatment

7.1.2. digestion step

7.1.3. extraction / separation step

7.1.4. instrument calibration step/instrumental set-up

7.2. If Yes, which:

8. **Does your laboratory analyse total As on a routine basis?**

- ○ no
- ○ yes

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

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

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

- ○ no
- ○ yes

10.1. If Yes, which:

- ☐ a) ISO 17025
- ☐ b) ISO 9000 series
- ☐ c) Other

10.1.1. If other, please specify

10.2. If yes, are you accredited?

- ○ No
- ○ Yes

10.2.1. If yes, by which Accreditation Body have you been accredited?

11. **Does your laboratory take part in an interlaboratory comparison for the determination of total As on a regular basis?**

- ○ no
- ○ yes

11.1. If yes, which one(s)

12. **Does your laboratory take part in an interlaboratory comparison for the determination of inorganic As on a regular basis?**

- ○ no
- ○ yes

12.1. If yes, which one(s)

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

- ○ no
- ○ yes

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

- ○ no
- ○ yes

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

- ○ no
- ○ yes

13.3. If yes, which one(s)

14. **How did you get to know about this proficiency test?**

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

Annex 7 : Homogeneity and stability studies for wheat

1a. Homogeneity study for total arsenic

	Total arsenic (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
3	0.185	0.194
111	0.187	0.189
201	0.182	0.186
330	0.188	0.206
405	0.191	0.181
481	0.188	0.180
599	0.187	0.196
704	0.177	0.186
766	0.179	0.187
858	0.188	0.196
Mean of 20 results	0.188	
$\hat{\sigma}$	15 %	
Homogeneity test according to ISO 13528 [8]		
$0.3\,\hat{\sigma}$	0.00844425	
S_x	0.004870832	
S_w	0.00662948 (S_x<S_w)	
Test result	Passed	

1b. Stability study for total arsenic

Stability Study - Total arsenic in wheat

TEMPERATURE = 18°C

Meas.Unit: mg kg^{-1}

	Time in Weeks			
samples	0	3	5	8
1	0.194	0.187	0.198	0.195
2	0.183	0.192	0.193	0.19

CALCULATION OF U_{lt} for given $X_{shel\ life}$

Given $X_{shell\ life}$ = 4 Weeks

U_{lt} = 0.002

Ul_t[%] = 1.2%

REGRESSION LINE PARAMETERS

Slope = 0.001
SE Slope = 0.001
Intercept = 0.189
SE Intercept = 0.003
Correlation Coefficient =0.180

Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

2a. Homogeneity study for inorganic arsenic

	Inorganic arsenic (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
3	0.177	0.175
111	0.190	0.158
201	0.164	0.184
330	0.176	0.186
405	0.160	0.197
481	0.172	0.153
599	0.168	0.176
704	0.162	0.174
766	0.180	0.173
858	0.173	0.173
Mean of 20 results	0.174	
$\hat{\sigma}$	15 %	
Homogeneity test according to ISO 13528 [8]		
$0.3\,\hat{\sigma}$	0.00780975	
S_x	0.005278099	
S_w	0.013257074 (S_x<S_w)	
Test result	Passed	

2b. Stability study for inorganic arsenic

Stability Study - Inorganic arsenic in wheat

TEMPERATURE = 18°C

Meas.Unit: mg kg^{-1}

	Time in Weeks			
samples	0	3	5	8
1	0.15	0.146	0.164	0.163
2	0.181	0.165	0.126	0.157

CALCULATION OF U_{st} for given $X_{shel\ life}$

Given $X_{shel\ life}$ = 4 Weeks

U_{st} = 0.008

U_{st}[%] = 5.0%

REGRESSION LINE PARAMETERS

Slope = -0.001
SE Slope = 0.002
Intercept = 0.160
SE Intercept = 0.010
Correlation Coefficient =0.034

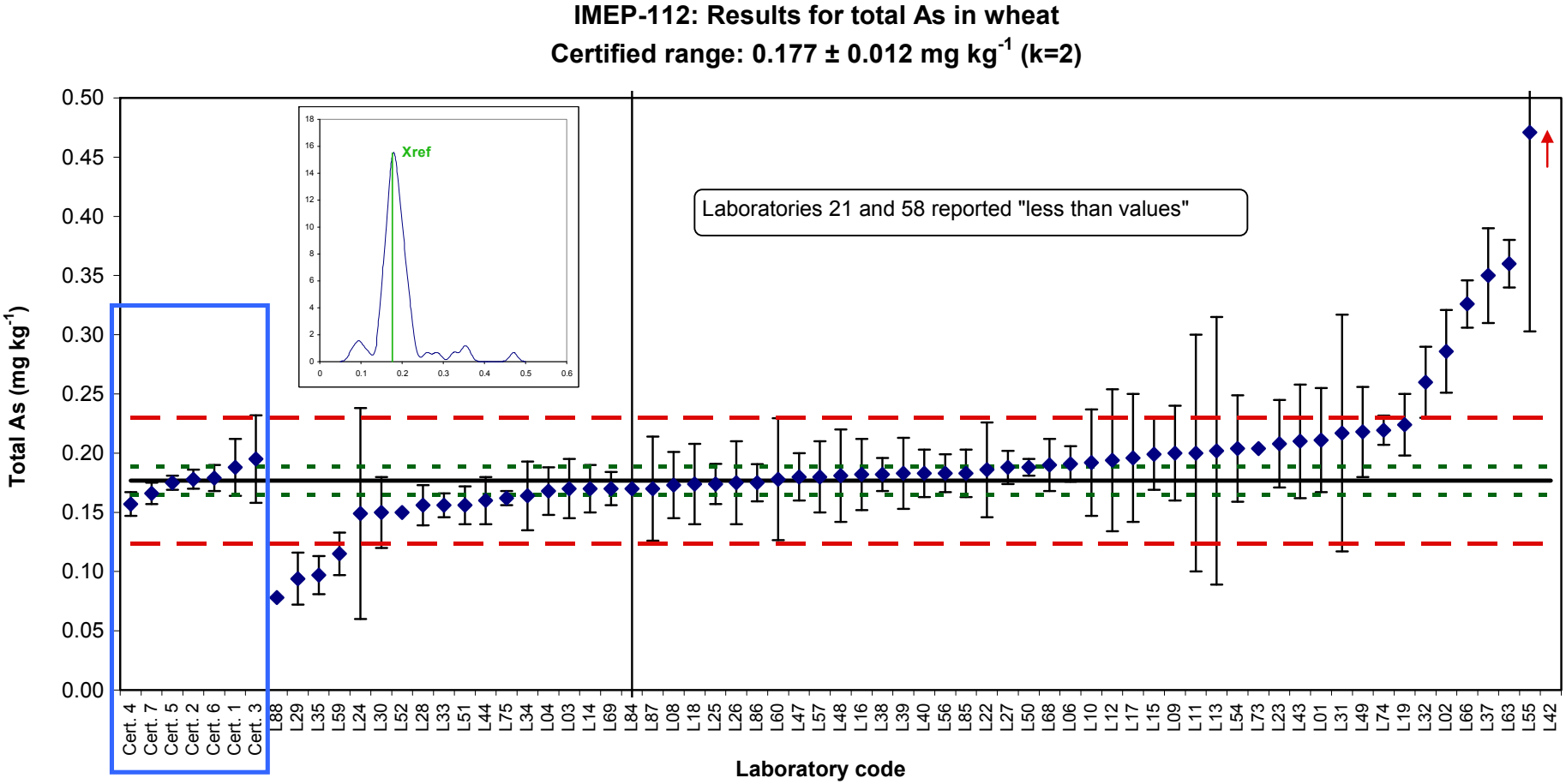
Slope of the linear regression significantly <> 0 (95%) :No
Slope of the linear regression significantly <> 0 (99%) :No

Annex 8 : Total arsenic in wheat

Certified range: 0.177 ± 0.012 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	$Qual_u$
L01*	0.211	0.044	2	0.022	ICP-MS	1.3	1.5	a
L02	0.286	0.035	2	0.018	HG-AAS	4.1	5.9	a
L03*	0.17	0.025	2	0.013	HG-AAS	-0.3	-0.5	a
L04*	0.168	0.02	2	0.01	HG-AAS	-0.3	-0.8	a
L06*	0.191	0.015	√3	0.009	ICP-MS	0.5	1.3	a
L08*	0.173	0.028	√3	0.016	ICP-MS	-0.1	-0.2	a
L09*	0.20	0.04	2	0.02	HG-AAS	0.9	1.1	a
L10*	0.192	0.045	2	0.023	HG-AAS	0.6	0.7	a
L11*	0.20	0.10	2.0	0.05	ICP-MS	0.9	0.5	c
L12*	0.194	0.0600	2	0.0300	ICP-MS	0.6	0.6	c
L13	0.202	0.113	2	0.057	ICP-MS	0.9	0.4	c
L14*	0.17	0.02	2	0.01	ICP-MS	-0.3	-0.6	a
L15*	0.199	0.030	2	0.015	ICP-MS	0.8	1.4	a
L16*	0.182	0.030	2	0.015	ICP-MS	0.2	0.3	a
L17*	0.196	0.054	2	0.027	ICP-MS	0.7	0.7	c
L18*	0.174	0.034	2	0.017	ICP-MS	-0.1	-0.2	a
L19*	0.224	0.026	2	0.013	ICP-MS	1.8	3.3	a
L21*	<0.85				ZETA-AAS			
L22*	0.186	0.040	2	0.020	ICP-MS	0.3	0.4	a
L23*	0.208	0.037	2	0.019	ICP-MS	1.2	1.6	a
L24*	0.149	0.089	2	0.045	ETAAS	-1.1	-0.6	c
L25	0.174	0.017	2	0.009		-0.1	-0.3	a
L26*	0.175	0.035	2	0.018	ICP-MS	-0.1	-0.1	a
L27*	0.188	0.014	2	0.007	ICP-MS	0.4	1.2	a
L28*	0.156	0.017	2	0.009	ETAAS	-0.8	-2.0	a
L29*	0.094	0.022	2	0.011	ETAAS	-3.1	-6.6	a
L30*	0.15	0.03	√3	0.02	ICP-MS	-1.0	-1.5	a
L31	0.217	0.1	2	0.1	HG-AAS	1.5	0.8	c
L32	0.260	0.030	2	0.015	HG-AAS	3.1	5.1	a
L33	0.156	0.01	3	0.00	ICP-MS	-0.8	-3.0	b
L34*	0.164	0.029	2	0.015	HG-AAS	-0.5	-0.8	a
L35	0.097	0.016	1.96	0.008	ETAAS	-3.0	-7.9	a
L37	0.35	0.04	√3	0.02	ICP-MS	6.5	7.3	a
L38	0.182	0.014	2	0.007	ICP-MS	0.2	0.6	a
L39	0.183	0.03	0.06	0.50	ICP-MS	0.2	0.0	c
L40*	0.183	0.0201	√3	0.0116	ICP-MS	0.2	0.5	a
L42	56.987							
L43	0.21	0.048	0.963	0.050	ICP-AES	1.2	0.7	c
L44	0.16	0.02	0.01	2.00	HG-AFS	-0.6	0.0	c
L47*	0.18	0.02	2	0.01	ICP-MS	0.1	0.3	a
L48*	0.181	0.039	2	0.020	ICP-MS	0.2	0.2	a
L49*	0.218	0.038	2	0.019	ICP-MS	1.6	2.1	a
L50	0.188	0.007	1	0.007		0.4	1.2	a
L51	0.156	0.016	2	0.008		-0.8	-2.1	a
L52	0.15	0	√3	0	HG-AAS	-1.0	-4.5	b
L54	0.204	0.045	2	0.023	ICP-MS	1.0	1.2	a
L55	0.471	0.168	2	0.084	ETAAS	11.1	3.5	c
L56	0.183	0.016	2	0.008	AFS	0.2	0.6	a
L57	0.18	0.03	2.26	0.01	ICP-MS	0.1	0.2	a
L58	<0.168913							
L59	0.115	0.018	2	0.009	AFS	-2.3	-5.7	a
L60	0.178	0.051442	2	0.025721	ICP-MS	0.0	0.0	a
L63	0.36	0.02	2	0.01		6.9	15.7	a
L66	0.326	0.020	2	0.010		5.6	12.8	a
L68	0.190	0.022	2	0.011	AFS	0.5	1.0	a
L69	0.17	0.014	2	0.007		-0.3	-0.7	a
L73	0.204	0	√3	0	HG-AAS	1.0	4.5	b
L74	0.2193	0.0123	2	0.0062	HR-ICP-MS	1.6	4.9	a
L75	0.162	0.006	1	0.006	ICP-MS	-0.6	-1.7	b
L84	0.17	6.0	2	3.0	ICP-MS	-0.3	0.0	c
L85	0.183	0.02	2	0.01	HR-ICP-MS	0.2	0.5	a
L86	0.175	0.0156	2	0.0078	ICP-MS	-0.1	-0.2	a
L87*	0.17	0.044	2	0.022	ETAAS	-0.3	-0.3	a
L88	0.078	0.0004	2	0.0002	ICP-MS	-3.7	-16.4	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 7.2.



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 Xref, the green dotted lines delimit the reference interval (**X_{ref} ± 2u_{ref}: 0.177 ± 0.012 mg kg^{-1}**), the red dashed lines delimit the target interval (**X_{ref} ± 2σ: 0.177 ± 0.053 mg kg^{-1}**)

Annex 9 : Inorganic arsenic in wheat

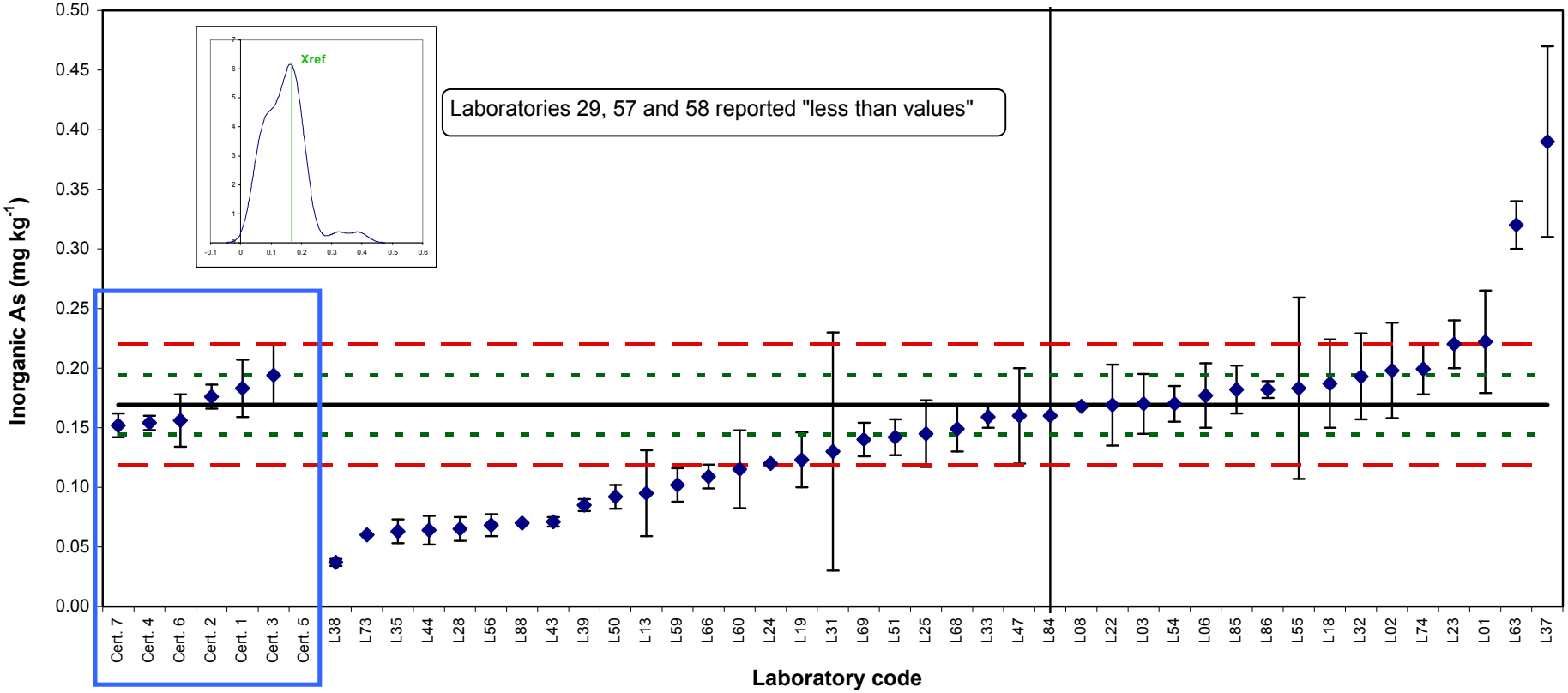
Certified range: 0.169 ± 0.025 mg kg^{-1} (k=2)

Lab ID	X_{lab}(mg kg^{-1})	U_{lab} (mg kg^{-1})	*k*	u_{lab}(mg kg^{-1})	Technique	z	ζ	$Qual_u$
L01*	0.222	0.043	2	0.022	LC-ICP-MS	2.1	2.1	a
L02	0.198	0.040	2	0.020	HG-AAS	1.1	1.2	a
L03*	0.17	0.025	2	0.013	HG-AAS	0.0	0.0	a
L06*	0.177	0.027	√3	0.016	LC-ICP-MS	0.3	0.4	a
L08*	0.168	0	√3	0	LC-ICP-MS	0.0	-0.1	b
L13	0.095	0.036	2	0.018	LC-ICP-MS	-2.9	-3.4	a
L18*	0.187	0.037	2	0.019	HG-ICP-AES	0.7	0.8	a
L19*	0.123	0.023	2	0.012	LC-ICP-MS	-1.8	-2.7	b
L22*	0.169	0.034	2	0.017	LC-ICP-MS	0.0	0.0	a
L23*	0.22	0.02	2	0.01	LC-ICP-MS	2.0	3.2	b
L24*	0.12	0	√3	0	HG-AAS	-1.9	-4.0	b
L25	0.145	0.028	2	0.014		-1.0	-1.3	a
L28*	0.065	0.010	2	0.005	ETAAS	-4.1	-7.8	b
L29*	<0.025				ETAAS			
L31	0.130	0.1	2	0.1	HG-AAS	-1.5	-0.8	c
L32	0.193	0.036	2	0.018	HG-AAS	0.9	1.1	a
L33	0.159	0.0091	3	0.0030	LC-ICP-MS	-0.4	-0.8	b
L35	0.063	0.010	1.96	0.005	ETAAS	-4.2	-8.0	b
L37	0.39	0.08	√3	0.05	ICP-MS	8.7	4.6	c
L38	0.037	0.003	2	0.002	LC-ICP-MS	-5.2	-10.6	b
L39	0.085	0.005	0.01	0.500	ICP-MS	-3.3	-0.2	c
L43	0.071	0.004	1.133	0.004	HG-AAS	-3.9	-7.7	b
L44	0.064	0.012	0.04	0.300	HG-QFS	-4.1	-0.4	c
L47*	0.16	0.04	2	0.02	LC-ICP-MS	-0.4	-0.4	a
L50	0.092	0.010	1	0.010		-3.0	-4.9	b
L51	0.142	0.015	2	0.008		-1.1	-1.9	b
L54	0.170	0.015	2	0.008	LC-ICP-MS	0.0	0.1	b
L55	0.183	0.076	2	0.038	HG-ICP-MS	0.5	0.3	c
L56	0.0682	0.0092	2	0.0046	AFS	-4.0	-7.7	b
L57	<0.2				HG-AAS			
L58	<0.168913							
L59	0.102	0.014	2	0.007	AFS	-2.6	-4.7	b
L60	0.115	0.03266	2	0.01633	LC-ICP-MS	-2.1	-2.6	a
L63	0.32	0.02	2	0.01		5.9	9.5	b
L66	0.109	0.010	2	0.005		-2.4	-4.5	b
L68	0.149	0.019	2	0.010	AFS	-0.8	-1.3	b
L69	0.14	0.014	2	0.007		-1.1	-2.1	b
L73	0.060	0	√3	0	HG-AAS	-4.3	-8.9	b
L74	0.1993	0.0212	2	0.0106	HG-ICP-MS	1.2	1.9	b
L84	0.16	8.0	2	4.0	LC-ICP-MS	-0.4	0.0	c
L85	0.182	0.02	2	0.01	LC-ICP-MS	0.5	0.8	b
L86	0.182	0.0070	2	0.0035	HG-AAS	0.5	1.0	b
L88	0.070	0.001	2	0.001	LC-ICP-MS	-3.9	-8.0	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 7.2.

IMEP-112: Results for inorganic As in wheat

Certified range: 0.169 ± 0.025 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 Xref, the green dotted lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$: 0.169 ± 0.025 mg kg^{-1}) the red dashed lines delimit the target interval ($X_{ref} \pm 2\sigma$: 0.169±0.051 mg kg^{-1})

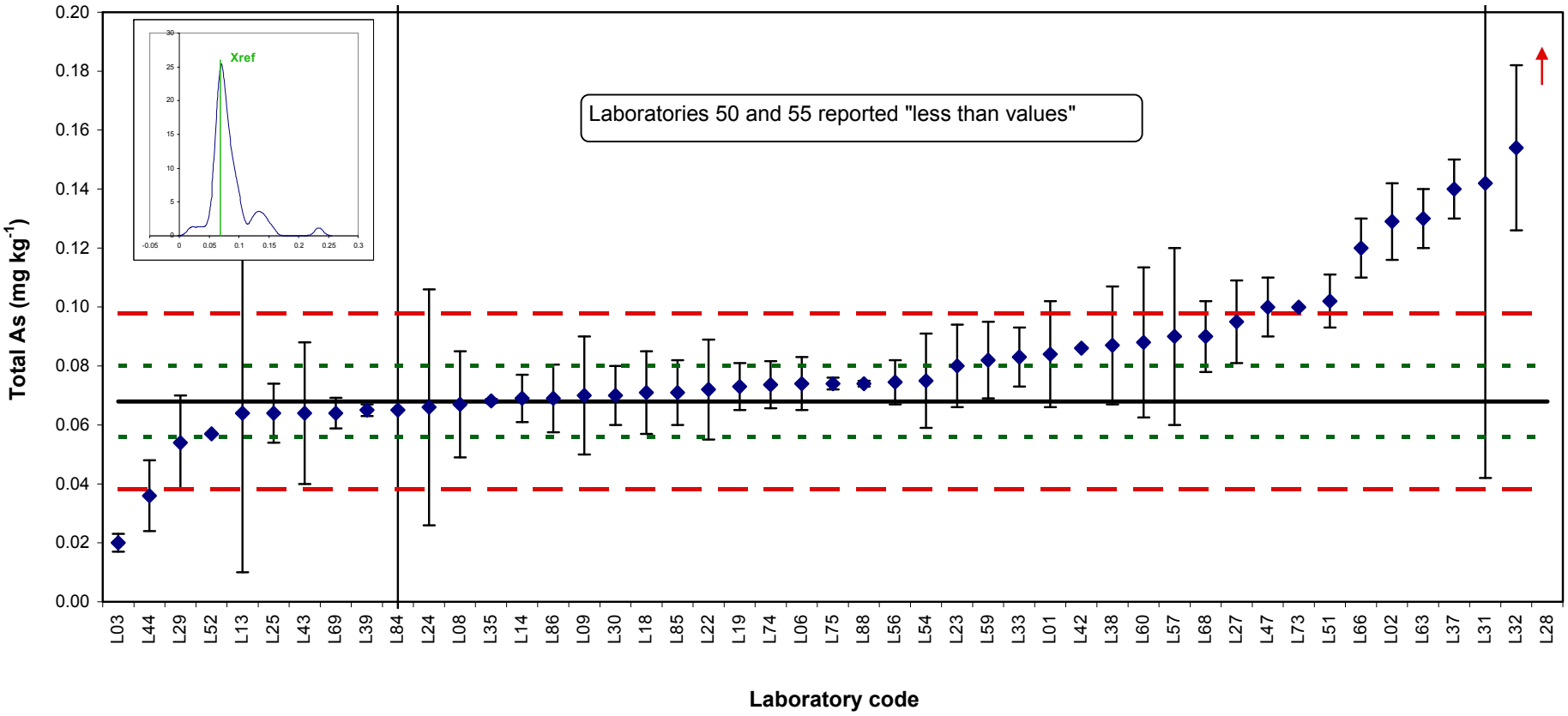
Annex 10 : Total arsenic in vegetable food

Certified range: 0.068 ± 0.012 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	$Qual_u$
L01*	0.084	0.018	2	0.009	ICP-MS	1.1	1.5	a
L02	0.129	0.013	2	0.007	HG-AAS	4.1	6.9	a
L03*	0.02	0.003	2	0.002	HG-AAS	-3.2	-7.8	b
L06*	0.074	0.009	2	0.005	ICP-MS	0.4	0.8	b
L08*	0.067	0.018	2	0.009	ICP-MS	-0.1	-0.1	a
L09*	0.07	0.02	2	0.01	HG-AAS	0.1	0.2	a
L13	0.064	0.054	2	0.027	ICP-MS	-0.3	-0.1	c
L14*	0.069	0.008	2	0.004	ICP-MS	0.1	0.1	b
L18*	0.071	0.014	2	0.007	ICP-MS	0.2	0.3	a
L19*	0.073	0.008	2	0.004	ICP-MS	0.3	0.7	b
L22*	0.072	0.017	2	0.009	ICP-MS	0.3	0.4	a
L23*	0.080	0.014	2	0.007	ICP-MS	0.8	1.3	a
L24*	0.066	0.04	2	0.02	ETAAS	-0.1	-0.1	c
L25	0.064	0.010	2	0.005	HG-AAS	-0.3	-0.5	b
L27*	0.095	0.014	2	0.007	ICP-MS	1.8	2.9	a
L28*	0.234	0.024	2	0.012	ETAAS	11.1	12.4	a
L29*	0.054	0.016	2	0.008	ETAAS	-0.9	-1.4	a
L30*	0.07	0.01	√3	0.01	ICP-MS	0.1	0.2	b
L31	0.142	0.10	2	0.05	HG-AAS	4.9	1.5	c
L32	0.154	0.028	2	0.014	HG-AAS	5.7	5.6	a
L33	0.083	0.01	2	0.01	ICP-MS	1.0	1.9	b
L35	0.0681	0.0007	1.96	0.0004	ETAAS	0.0	0.0	b
L37	0.14	0.01	√3	0.01	ICP-MS	4.8	8.6	b
L38	0.087	0.02	2	0.01	ICP-MS	1.3	1.6	a
L39	0.065	0.002	0.004	0.500	ICP-MS	-0.2	0.0	c
L42	0.086	0	√3	0		1.2	3.0	b
L43	0.064	0.024	0.963	0.025	ICP-AES	-0.3	-0.2	c
L44	0.036	0.012	0.01	1.200		-2.1	0.0	c
L47*	0.10	0.01	2	0.01	ICP-MS	2.1	4.1	b
L50	<0.14							
L51	0.102	0.009	2	0.005		2.3	4.5	b
L52	0.057	0	√3	0	HG-AAS	-0.7	-1.8	b
L54	0.075	0.016	2	0.008	ICP-MS	0.5	0.7	a
L55	<0.5				ETAAS			
L56	0.0745	0.0075	2	0.0038		0.4	0.9	b
L57	0.09	0.03	2.26	0.013	ICP-MS	1.5	1.5	a
L59	0.082	0.013	2	0.007		0.9	1.6	a
L60	0.0880	0.025432	2	0.012716	ICP-MS	1.3	1.4	a
L63	0.13	0.01	2	0.01		4.1	7.9	b
L66	0.120	0.010	2	0.005	AFS	3.5	6.7	b
L68	0.090	0.012	2	0.006		1.5	2.6	a
L69	0.064	0.0052	2	0.0026		-0.3	-0.6	b
L73	0.100	0	√3	0	HG-AAS	2.1	5.3	b
L74	0.07363	0.00794	2	0.00397	HR-ICP-MS	0.4	0.8	b
L75	0.074	0.002	1	0.002	ICP-MS	0.4	0.9	b
L84	0.065	6.0	2	3.0	ICP-MS	-0.2	0.0	c
L85	0.071	0.011	2	0.006	HR-ICP-MS	0.2	0.4	b
L86	0.069	0.0115	2	0.0058	ICP-MS	0.1	0.1	b
L88	0.074	0.001	2	0.001	ICP-MS	0.4	1.0	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 7.2.

IMEP-112: Results for total As in vegetable food
Certified range: 0.068 ± 0.012 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 Xref, the green dotted lines delimit the reference interval (**X_{ref} ± 2u_{ref}: 0.068 ± 0.012 mg kg^{-1}**), the red dashed lines delimit the target interval (**X_{ref} ± 2σ: 0.068 ± 0.030 mg kg^{-1}**)

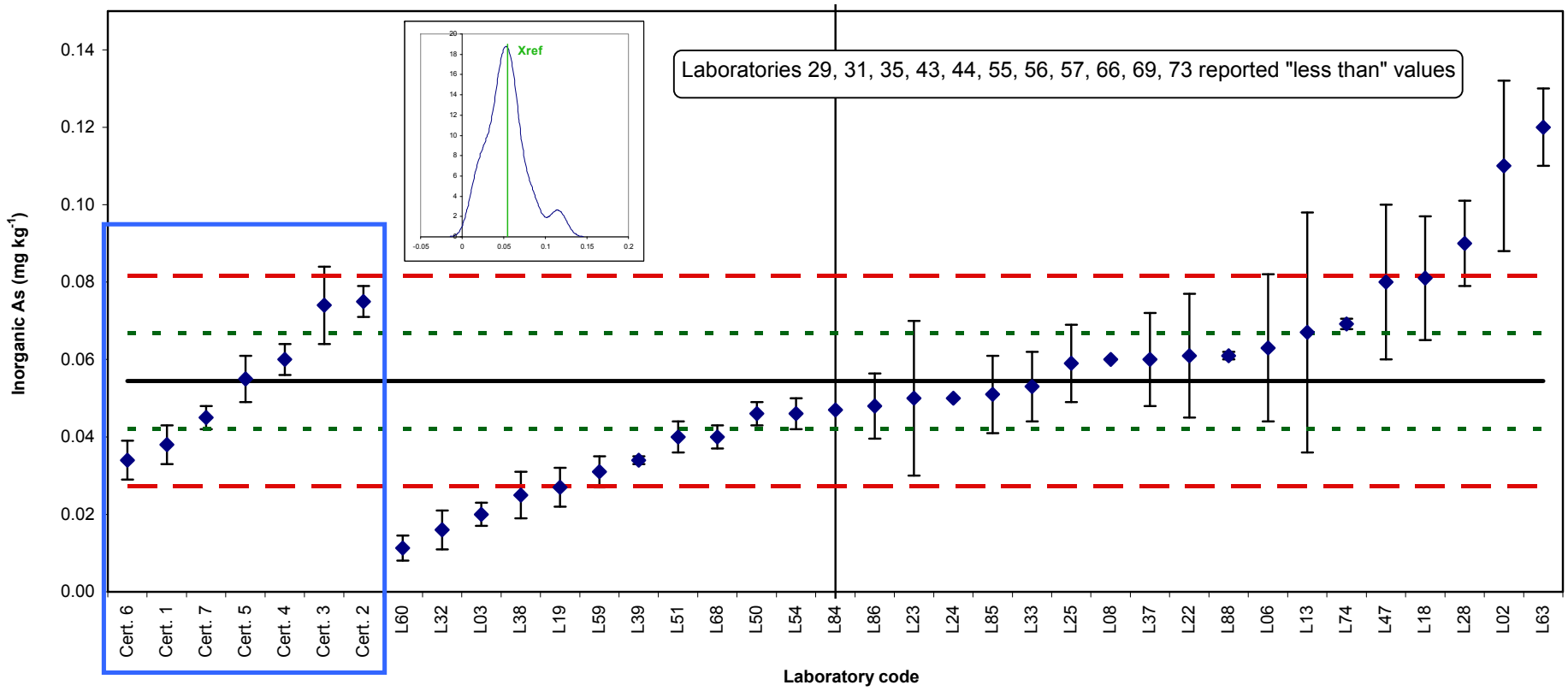
Annex 11 : Inorganic arsenic in vegetable food

Certified range: 0.054 ± 0.012 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	$Qual_u$
L02	0.110	0.022	2	0.011	HG-AAS	4.1	4.4	a
L03*	0.02	0.003	2	0.002	HG-AAS	-2.5	-5.4	b
L06*	0.063	0.019	2	0.010	LC-ICP-MS	0.6	0.8	a
L08*	0.060	0	√3	0	LC-ICP-MS	0.4	0.9	b
L13	0.067	0.031	2	0.016	LC-ICP-MS	0.9	0.8	c
L18*	0.081	0.016	2	0.008		2.0	2.6	a
L19*	0.027	0.005	2	0.003	LC-ICP-MS	-2.0	-4.1	b
L22*	0.061	0.016	2	0.008	LC-ICP-MS	0.5	0.6	a
L23*	0.05	0.02	2	0.01	LC-ICP-MS	-0.3	-0.4	a
L24*	0.05	0	√3	0	HG-AAS	-0.3	-0.7	b
L25	0.059	0.010	2	0.005	ETAAS	0.3	0.6	b
L28*	0.090	0.011	2	0.006	ETAAS	2.6	4.3	b
L29*	<0.075				ETAAS			
L31	<0.1				HG-AAS			
L32	0.016	0.005	2	0.003	HG-AAS	-2.8	-5.7	b
L33	0.053	0.009	2	0.005	LC-ICP-MS	-0.1	-0.2	b
L35	<0.05				ETAAS			
L37	0.06	0.012	√3	0.007	ICP-MS	0.4	0.6	a
L38	0.025	0.006	2	0.003	LC-ICP-MS	-2.2	-4.3	b
L39	0.034	0.001	0.002	0.500	ICP-MS	-1.5	0.0	c
L43	<0.05				HG-AAS			
L44	<0.04							
L47*	0.08	0.02	2	0.01	LC-ICP-MS	1.9	2.2	a
L50	0.046	0.003	1	0.003		-0.6	-1.2	b
L51	0.040	0.004	2	0.002		-1.1	-2.2	b
L54	0.046	0.004	2	0.002	LC-ICP-MS	-0.6	-1.3	b
L55	<0.05				HG-ICP-MS			
L56	<0.04							
L57	<0.2				HG-AAS			
L59	0.031	0.004	2	0.002		-1.7	-3.6	b
L60	0.0113	0.0032092	2	0.0016046	LC-ICP-MS	-3.2	-6.7	b
L63	0.12	0.01	2	0.01		4.8	8.2	b
L66	<0.04				AFS			
L68	0.040	0.003	2	0.002		-1.1	-2.3	b
L69	<0.04							
L73	<0.02				HG-AAS			
L74	0.06916	0.00134	2	0.00067	HG-ICP-MS	1.1	2.4	b
L84	0.047	16	2	8	LC-ICP-MS	-0.5	0.0	c
L85	0.051	0.010	2	0.005	LC-ICP-MS	-0.3	-0.4	b
L86	0.048	0.0084	2	0.0042	HG-AAS	-0.5	-0.9	b
L88	0.061	0.001	2	0.001	LC-ICP-MS	0.5	1.1	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 7.2.

IMEP-112: Results for inorganic As in vegetable food
Certified range: 0.054 ± 0.012 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 Xref, the green dotted lines delimit the reference interval (**$X_{ref} \pm 2u_{ref}$: 0.054 ± 0.012 mg kg^{-1}**), the red dashed lines delimit the target interval (**$X_{ref} \pm 2\sigma$: 0.054 ± 0.027 mg kg^{-1}**)

Annex 12 : Total arsenic in algae

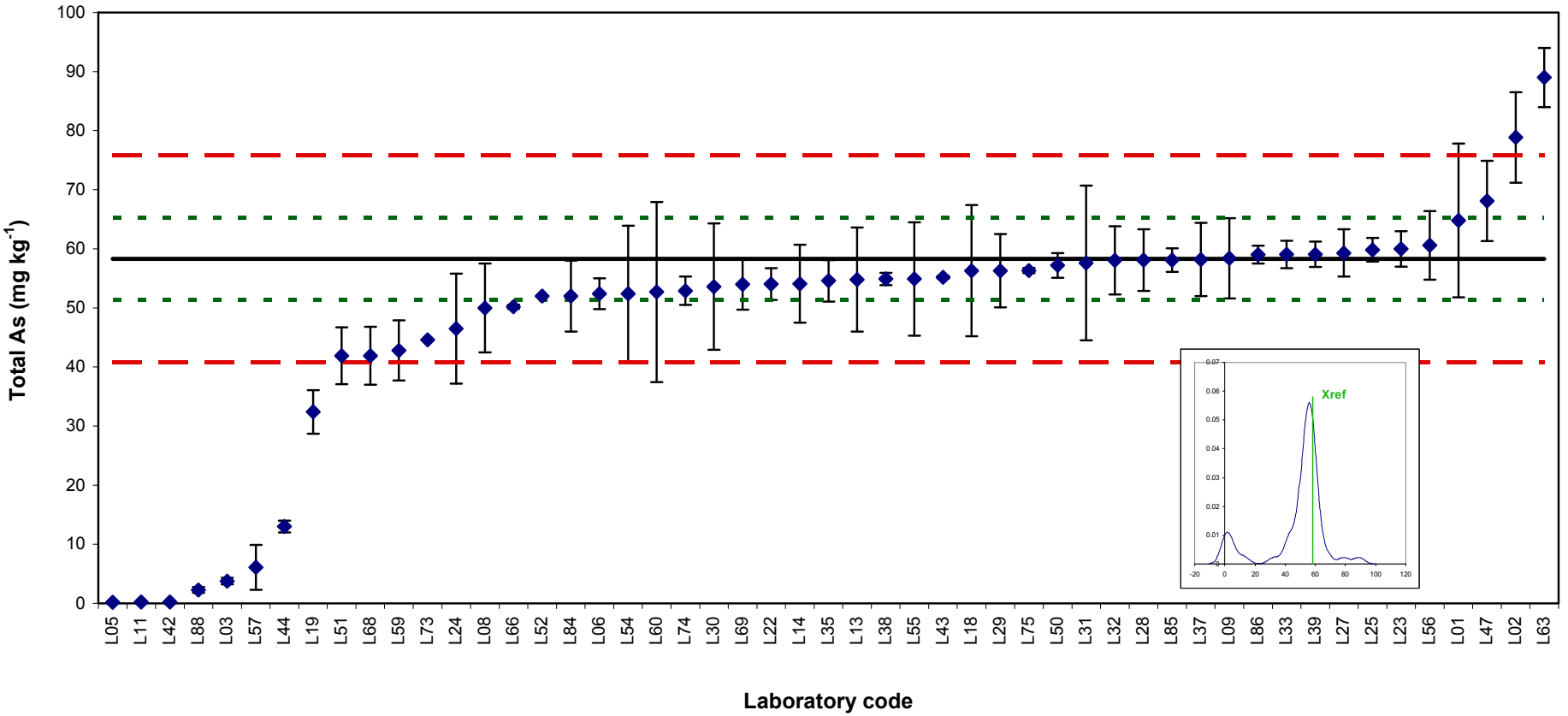
Certified range: 58.3 ± 7.0 mg kg^{-1} (k=2)

Lab ID	X_{lab} (mg kg^{-1})	U_{lab} (mg kg^{-1})	*k*	u_{lab} (mg kg^{-1})	Technique	z	ζ	$Qual_u$
L01*	64.8	13.0	2	6.5	ICP-MS	0.7	0.9	a
L02	78.85	7.67	2	3.84	HG-AAS	2.3	4.0	a
L03*	3.77	0.56	2	0.28	HG-AAS	-6.2	-15.5	b
L05*	0.197	0.049	0.94	0.052	HG-AAS	-6.6	-16.6	b
L06*	52.4	2.62	2	1.31	ICP-MS	-0.7	-1.6	b
L08*	50.0	7.5	2	3.8	ICP-MS	-0.9	-1.6	a
L09*	58.4	6.8	2	3.4	HG-AAS	0.0	0.0	b
L11*	0.2	0.1	2	0.1	ICP-MS	-6.6	-16.6	b
L13	54.8	8.8	2	4.4	ICP-MS	-0.4	-0.6	a
L14*	54.1	6.6	2	3.3	ICP-MS	-0.5	-0.9	b
L18*	56.3	11.1	2	5.6	ICP-MS	-0.2	-0.3	a
L19*	32.4	3.7	2	1.9	ICP-MS	-3.0	-6.5	b
L22*	54.03	2.70	2	1.35	ICP-MS	-0.5	-1.1	b
L23*	60	3	2	2	ICP-MS	0.2	0.4	b
L24*	46.5	9.3	2	4.7	ICP-AES	-1.3	-2.0	a
L25	59.836	2.018	2	1.009	HG-AAS	0.2	0.4	b
L27*	59.3	4.0	2	2.0	ICP-MS	0.1	0.2	b
L28*	58.1	5.2	2	2.6	ETAAS	0.0	0.0	b
L29*	56.3	6.2	2	3.1	ETAAS	-0.2	-0.4	b
L30*	53.6	10.7	√3	6.2	ICP-MS	-0.5	-0.7	a
L31	57.60	13.09	2	6.55	HG-AAS	-0.1	-0.1	a
L32	58.07	5.78	2	2.89	ICP-MS	0.0	-0.1	b
L33	59.06	2.32	3	0.77	ICP-MS	0.1	0.2	b
L35	54.6	3.5	1.96	1.8	ETAAS	-0.4	-0.9	b
L37	58.2	6.2	√3	3.6	ICP-MS	0.0	0.0	a
L38	54.9	1.04	2	0.52	ICP-MS	-0.4	-1.0	b
L39	59.083	2.129	4.258	0.500	ICP-MS	0.1	0.2	b
L42	0.203							
L43	55.2	0.25	0.998	0.25	ICP-AES	-0.4	-0.9	b
L44	13	1	0.01	100		-5.2	-0.5	c
L47*	68.1	6.8	2	3.4	ICP-MS	1.1	2.0	b
L50	57.2	2.1	1	2.1	k0-INAA	-0.1	-0.3	b
L51	41.9	4.8	2	2.4	AFS	-1.9	-3.9	b
L52	52	0	√3	0	HG-AAS	-0.7	-1.8	b
L54	52.4	11.5	2	5.8	ICP-MS	-0.7	-0.9	a
L55	54.9	9.6	2	4.8	ETAAS	-0.4	-0.6	a
L56	60.6	5.8	2	2.9		0.3	0.5	b
L57	6.1	3.8	2.26	1.7	ICP-MS	-6.0	-13.4	b
L59	42.8	5.1	2	2.6		-1.8	-3.6	b
L60	52.70	15.2303	2	7.6152	ICP-MS	-0.6	-0.7	a
L63	89	5	2	3	AFS	3.5	7.1	b
L66	50.2	0.35	2	0.18		-0.9	-2.3	b
L68	41.9	4.9	2	2.5		-1.9	-3.8	b
L69	54	4.3	2	2.2	HG-AFS	-0.5	-1.0	b
L73	44.6	0	√3	0	HG-AAS	-1.6	-3.9	b
L74	52.91	2.41	2	1.21	HR-ICP-MS	-0.6	-1.5	b
L75	56.32	0.45	1	0.45	ICP-MS	-0.2	-0.6	b
L84	52	6.0	2	3.0	ICP-MS	-0.7	-1.4	b
L85	58.1	2.0	2.0	1.0	HR-ICP-MS	0.0	-0.1	b
L86	59.02	1.516	2	0.758	ICP-MS	0.1	0.2	b
L88	2.27	0.52	2	0.26	ICP-MS	-6.4	-16.0	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 7.2.

IMEP-112: Results for total As in algae

Certified range: 58.3 ± 7.0 mg kg-1 (k=2)



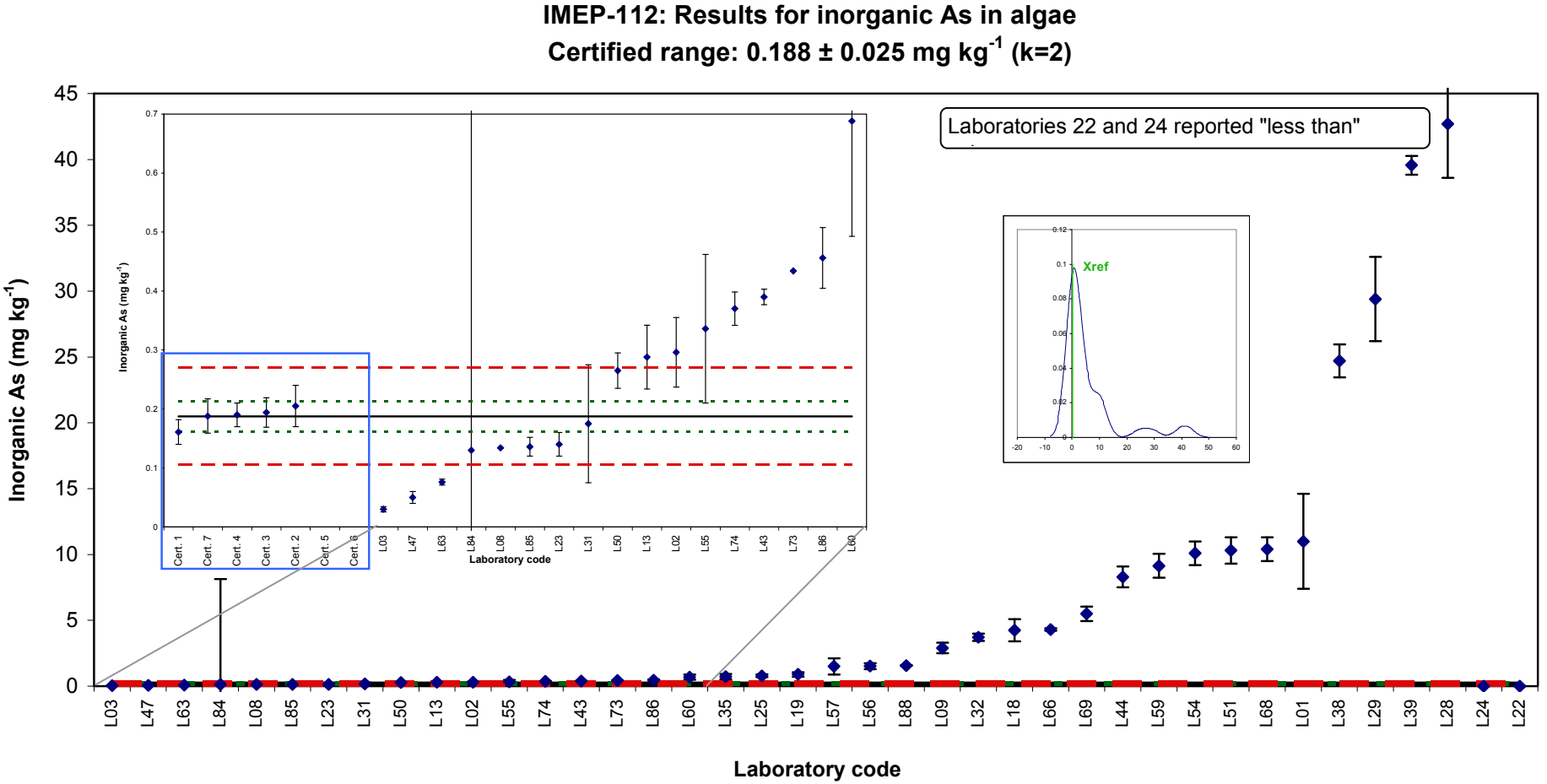
This graph displays all measurements results and their associated uncertainties.
The uncertainties are shown as reported, with various expansion factors and levels of confidence.
The black line represents Xref, the green dotted lines delimit the reference interval **($X_{ref} \pm 2u_{ref}$: 58.3± 7.0 mg kg^{-1})** the red dashed lines delimit the target interval **($X_{ref} \pm 2\sigma$: 58.3 ± 17.49 mg kg^{-1})**

Annex 13 : Inorganic arsenic in algae

Certified range: 0.188 ± 0.025 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	$Qual_u$
L01*	11.0	3.6	2	1.8	LC-ICP-MS	262.0	3.0	c
L02	0.296	0.059	2	0.030	HG-AAS	2.6	1.8	a
L03*	0.03	0.0045	2	0.0023	HG-AAS	-3.8	-11.7	b
L08*	0.134	0	√3	0	LC-ICP-MS	-1.3	-4.2	b
L09*	2.9	0.4	2	0.2	HG-AAS	65.7	6.8	c
L13	0.288	0.054	2	0.027	LC-ICP-MS	2.4	1.8	a
L18*	4.25	0.84	2	0.42		98.4	4.8	c
L19*	0.898	0.166	2	0.083	LC-ICP-MS	17.2	4.3	c
L22*	<0.1				LC-ICP-MS			
L23*	0.14	0.02	2	0.01	LC-ICP-MS	-1.2	-2.0	b
L24*	<0.025				HG-AAS			
L25	0.787	0.110	2	0.055	HG-AAS	14.5	5.4	c
L28*	42.7	4.1	2	2.1	ETAAS	1030.1	10.4	c
L29*	29.4	3.2	2	1.6	ETAAS	707.8	9.1	c
L31	0.175	0.10	2	0.05	HG-AAS	-0.3	-0.1	c
L32	3.71	0.27	2	0.14	HG-AAS	85.3	13.0	c
L35	0.72	0.19	1.96	0.10	ETAAS	12.9	2.8	c
L38	24.7	1.26	2	0.63	LC-ICP-MS	593.9	19.5	c
L39	39.56	0.717	1.434	0.500	ICP-MS	954.0	54.9	c
L43	0.39	0.013	1.133	0.011	HG-AAS	4.9	11.1	b
L44	8.3	0.8	0.04	20.0		196.6	10.1	c
L47*	0.05	0.01	2	0.01	LC-ICP-MS	-3.3	-8.5	b
L50	0.265	0.030	1	0.030	HPLC-HG AFS	1.9	2.4	a
L51	10.3	1.0	2	0.5	AFS	245.0	10.1	c
L54	10.1	0.9	2	0.5	LC-ICP-MS	240.2	11.0	c
L55	0.336	0.126	2	0.063	HG-ICP-MS	3.6	1.2	c
L56	1.52	0.23	2	0.12		32.3	5.8	c
L57	1.5	0.62	2.26	0.27	HG-AAS	31.8	2.1	c
L59	9.14	0.91	2	0.46		216.9	9.8	c
L60	0.688	0.195392	2	0.0977	LC-ICP-MS	12.1	2.6	c
L63	0.076	0.005	2	0.003	AFS	-2.7	-8.2	b
L66	4.30	0.10	2	0.05		99.6	40.8	c
L68	10.4	0.9	2	0.5		247.4	11.3	c
L69	5.5	0.55	2	0.28	HG-AFS	128.7	9.7	c
L73	0.434	0	√3	0	HG-AAS	6.0	19.4	b
L74	0.3702	0.0283	2	0.0142	HG-ICP-MS	4.4	5.9	a
L84	0.13	8.0	2	4.0	LC-ICP-MS	-1.4	0.0	c
L85	0.136	0.016	2.0	0.008	LC-ICP-MS	-1.3	-2.5	b
L86	0.456	0.0515	2	0.0258	HG-AAS	6.5	5.1	a
L88	1.57	0.03	2	0.02	LC-ICP-MS	33.5	42.4	a

$Qual_u$: 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 7.2.



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 Xref, the green dotted lines delimit the reference interval **(X_{ref} ± 2u_{ref}: 0.188 ± 0.025 mg kg^{-1})**, the red dashed lines delimit the target interval **(X_{ref} ± 2σ: 0.188 ± 0.083 mg kg^{-1})**

Annex 14 : Experimental details (Q6-7, Annex 6)

Q6 – Total Arsenic

LCode	Official method	Sample pre-treatment	Digestion	Extraction/separation	Instrument calibration
L01			Microwave assisted with HNO3		external linear
L02	EN 14546				
L03	EN 15510:2008				
L04	MSZ EN 14625:2005				
L06	AOAC				
L08			microwave 200ºC with 10 ml 20% Ac. Nitric		
L09	EN 14546:2005				
L10	SR EN 14546/2005				
L11		None	Microwave acid digestion (nitric acid, hydrogen peroxide & water)	Dilution of digestate to 100ml with D.I. water	ICP-MS, 7 Arsenic calibration standards (0 - 20ug/L)
L12	NMKL Trace Elements-As, Cd, Hg, Pb and other elements. No. 186, 2007.				
L13	EN 13805				
L14		no further pre-treatment	acid digestion using µ-wave	-	addition calibration
L15	EN 15763				
L16	LST EN 15763:2010				
L17		none	Microwave assisted acid digestion		external linear
L18		Wet digestion: HNO3 after evaporation. Then added H2O2		No	External Calibration
L19			microwave digestion		external calibration
L21	RAPPORTI ISTISAN 96/34				
L22			microwave nitric acid	dilution with water	Q-ICP-MS, external calibration
L23			microwave digestion with nitric acid and hydrogen peroxide		Measurements were carried out with direct comparison by standard solution. Rh were used as internal standard.
L24			sample : 0.5 g with 5 mL HNO3. Pre-digestion for 12 hours (ambiant temperature) and 3 hours on a hot-plate at 90°C.		GFAAS

LCode	Official method	Sample pre-treatment	Digestion	Extraction/separation	Instrument calibration
L25	UNE-EN 14546				
L26	EN 15763:2009				
L27	LST EN 15763:2010				
L28		no	microwave assisted digestion using nitric acid		ETASS, calibration, QC=BCR
L29			nitric acid, microwave digestion		external standard calibration, use of BCR-610 for calibration check and IMEP-109,IMEP-110 for digestion check
L30	NMKL procedure nr.186; 2007				
L31			cold digest overnight in nitric acid,in presence of ashing aid	evapourate digest, ash and then redissolve in acid and reduce to AsIII	external calibration to 30ppb As, hydride generation AAS @ 193.7nm
L32		Digestion with Nitric Acid in Microwave Digestor	Microwave Digestion	NA	ICP-MS was calibrated
L33		none	microwave digestion 0,3-0,4g sample + 5ml HNO3 + 1ml HCl		water soluted standards
L34	EN 14546:2005				
L35		0.2 (algae) or 0.5 (veg + wheat) g digested with 5mL c.HNO3 + 1mL H2O2	microwave digestion	dil. with ultrapure water: 20-fold for vegetable food + wheat and 1000-fold for algae	Matrix Matched Calibration / ETAAS: SIMAA6000 - Zeeman, THGA, 1 ug Pd as modifier
L37			Microwave digestion using nitric/hydrochloric acid	Filtration	4 pt calibration covering 0 - 50- ppb As. Mass 75 with chloride correction
L38		homogenization of the bottle with hand-shaking	0.1-0.5 g sample + 5.0 ml HNO3 + 3.0 ml H2O2, digestion in microwave up to 250 psi for 20 min	-	standard addition, He as collision gas, Rh as internal standard
L39	EN 15763:2009				
L40	standard method for determination of total As by ICP-MS				
L42					
L43	DIN, §64 LFGB				
L44	GB/T 5009.11-2003				
L47	EN 15763				
L48	AOAC Vol 90, No 3, 2007				
L49		Addition of nitric acid and hydrogen peroxide, let it stand for 1 hour	Microwave	Dilution	External standard

LCode	Official method	Sample pre-treatment	Digestion	Extraction/separation	Instrument calibration
L50	k0-standardization method of neutron activation analysis				
L51	GB/T5009.11-2003				
L52					
L54		homogenisation of the sample.	0,5 - 1 g of sample. Microwave digestion with HNO3 and H2O2	none	Instrumental Calibration curve with standards of As.
L55		No	Yes: HNO3 4 mL +0,5mL H202	No	20-40-80 ppb
L57		add 2ml of hydrogen peroxide and 10ml con nitric acid	microwave digestion	NA	ICP-MS
L58					
L59	GB/T 5009.11-2003				
L63	GB/T 5009.17-2003				
L66	improt and export food inspection				
L68	GB5009.11-2003				
L69	GB/T 5009.11-2003 Determination of total arsenic and abio-arenic in foods;the first method				
L73		Add to the sample Mg(NO3) and MNO	Ashing the sample at 500°C		
L74		Homogenization	Nitric acid digestion		internal standard
L75					
L84	Methods from the Danish Veterinary and Food Administration				
L85	DIN EN 15763				
L86	ASU L 00.00-19/6				
L87					
L88		Samples were weighted as stated in the instructions and placed in the Teflon reactors to perform the digestion	It has been applied by heating at 110ºC overnight in high pressure Teflon reactors. Reagents employed were HNO3 and H2O2	NO	Instrumental parameters (sensitivity and mass resolution) were calibrated as every day. Measrurement of standards were applied before samples. Validation with the reference material was applied by performing a similar digestion procedure.

Q7 – Inorganic Arsenic

LCode	Official method	Sample pre-treatment	Digestion	Extraction/separation	Instrument calibration
L01				Wheat: Microwave assisted with H2O; Algae: Microwave assisted with HNO3	External linear
L02			HCl:H2O2 MW Digestion max 90°C		
L03		no	digestion in microwave in 0.07 mol/litre HCl 90 0C 25 minutes	SPE Type STRATA	calibration line 0-10 ug/litre
L04					
L06				MW assisted extraction, 0.07M HCl + H2O2	External calibration
L08				Microwave 110ºC 0.3% Ac. Nitric	
L09	EN 15517:2008				
L11		Inorganic Arsenic was not determined - Suitable instrumentation was unavailable.			
L13				Methanol, water, HCl (0.2M) in ulta sound bath.	As-III and As V standard solutions.
L14					
L18		Wet digestion: HNO3		No	External Calibration
L19		none		µ-wave assisted extraction with water	HPLC-ICP-MS calibrated with 7 species of As (0 to 20 ppb)
L22			extraction with H2O2 and acetic acid at 120°C for 90 min in a microwave	ultrafiltration and dilution with running buffer	external calibration, gradient of (NH4)2CO3 at PRP-X100 column
L23				Microwave digestion with nitric acid in 95 degrees	Measurements were carried out with direct comparison by standard solution.
L24	NF EN 15517				
L25		solubilizatioin with HCl and subsequent extraction with chloroform	Arsenic solubilized in hydrocloric acid l m. Recuction by hydrobromic acid an hydrazine sulfate.	The inorganic arsenic was extracted into chloroform y back extracted into i mol HCL.Dry ased	Hydride generation-atomic absorption spectrometry
L28	ISO EN 15517:2008 using ETAAS for the determination of As				
L29	ISO EN 15517:2008 with graphite furnace				

LCode	Official method	Sample pre-treatment	Digestion	Extraction/separation	Instrument calibration
L31		moisten sample	shake with acid then overnight stand	chloroform extraction and centrifugation. back extraction with acid	external calibration to 30ppb As, hydride generation AAS @193.7nm
L32			Addition 50% HCl and 30%KI to sample	Distillation	Hydride AAS was calibrated
L33		none	none	1 g sample + 10 ml HNO3 (0,28m) 90min at 95°C	water soluted standards
L35		0.5 g for vegetable food and wheat - 0.2 g for algae	Vegetable + Wheat: 5 mL HNO3 1M were added, vortexed, ultrasonicated and centrifuged 4000/15min. Algae: 0.8 mL H2O + 3.6 ml HCl, left overnight.	Vegetable + Wheat: add 15mL EDTA 0.1 (w/v), vortexted, centrifuged, analyzed by ETAAS. Algae: extracted by 10 mL chloroform and back-extracted to 10 mL HCl 1M.	Standard Addition Method. / ETAAS: As total As.
L37		Defatted sample with pet ether	Hydrobromic acid/ hydrazine	Extracted with chloroform, back extracted into 1M HCl	4 pt calibration 0 - 50 ppb, analysed using mass 91 AsO
L38		homogenization of the bottle with hand-shaking	0.1-0.5 g sample + 10.0 ml 0.08 M HNO3, ultrasonication at 75 C degree for 30 min	centrifugation at 4100 g + filtration /0.45 um/	oxidation with 0.1% H2O2, standard addition of arsenate in the ranges of 0.1-2 ng/injection, SAX-HPLC-ICP-MS, no collision cell, elution with 60 mM ammonium acetate at pH 5.3, isocratic, flow 1 ml/min, injection of 100 ul, sample 1:1 diluted with the eluent
L39		dissolve the sample in 0.07 N hydrochloric acid	incubate for 2h at 37°C	centrifugate and filtrate	measure against external calibration curve
L43	§64 LFGB				
L44	GB/T 5009.11-2003				
L47				MW extraction HCl+H2O2	HPLC-ICP-MS
L50		None	None	0.2-0.3 g of sample extracted to 30 ml of 1:1 methanol/water overnight. Extraction repeated 2 more times (2 hours each), extracts pooled, dried at 45 C on rotary evaporator to dryness (cca 30 min). Taken up into Milli-Q water (3 ml), filtered (0.45 um filter) and stored frozen (- 18C) till analysis.	HPLC column: Hamilton PRP-X100, analytical column, 15 mM KH2PO4, pH 6.1, 100 ul injection loop. On-line hydride generation using HCl (3M) and NaBH4 (1.5%). Gasses swept out of gass-liquid separator by Ar, dried on-line in perma pure dryer with nitrogen and detected in atomic fluorescence spectrometer (AFS, PS Analytical, UK) using As superlamp (Photron).
L51	GB/T5009.11-2003				

LCode	Official method	Sample pre-treatment	Digestion	Extraction/separation	Instrument calibration
L54		homogenisation of the sample.	none	0,25 g of sample extracted in microwave with 10 mL of solution (HNO3 0,2 % + H2O2 1 %) up to a maximum temperature of 90 ºC	Instrumental Calibration curve with standards of As(v) (and double checking that chloride peak does not coelute with As(v)).
L55		No	Yes: HNO3 4 mL +0,5mL H202	No	5-20-100 ppb
L57		add 25ml 9.2M hydrochloric acid and 10ml 30% potassium iodide	distillation and collection of15ml distillate	repeat 7.1.1 and 7.1.2	AAS hydride
L58					
L59	GB-T 5009.11-2003				
L63	GB/T 5009.17-2003				
L66	improt and export food inspection				
L68	GB5009.11-2003				
L69	GB/T 5009.11-2003 Determination of total arsenic and abio-arenic in foods;the first method				
L73				the sample is treated with HCl	
L74		Homogenization	HCl digestion followed by reduction	chloroform extraction	external calibration
L84	Methods from the Danish Veterinary and Food Administration				
L85		no	no	wheat/vegetable: 0.28m HNO3/95°C/90min - algae: 0.07m HCl/37°C/2h	external calibration
L86	ASU L 25.06-1 (remark: for wheat and vegetables extraction temperature 95°C instead of 37°C)				

LCode	Official method	Sample pre-treatment	Digestion	Extraction/separation	Instrument calibration
L88		Samples were weighted as stated in the instructions and placed in the tubes where sonication was applied.	NO	Algae extraction: 50/50 water/isopropanol adding 5% CTBA. Focused ultrasonication for 2 minutes.Centrifu., evaporation and reconstitution with water; Vegetable and wheat extraction: enzymatic extraction with protease in water. Focused sonication for 2 min.Centrifu. The extracts were filtered (nylon filters 0.22) and injected. Species separation by using a PRP-X100. Mobile phase of phosphate buffer	Instrumental parameters (sensitivity and mass resolution) were calibrated as every day. Measurement of standards prepared on the content of As for the two inorganic As species were applied before samples. Validation with the rice reference material was applied for total and speciation analysis.

European Commission

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Abstract
The Institute for Reference Materials and Measurands (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). Two of its core tasks are to provide advice to the Directorate General for Health and Consumers (DG SANCO) on scientific matters and to organise interlaboratory comparisons (ILC) among appointed National Reference Laboratories (NRLs). This report presents the results of the twelfth ILC of the EU-RL-HM (former CRL-HM) which focused on the determination of total and inorganic As in wheat, vegetable food and algae. The test items used in this exercise are: wheat, spinach (SRM 1570a from NIST) and an algae candidate reference material. The test items were processed (in the case of wheat), bottled and labelled at IRMM and dispatched to the participants the second week of May 2011. Each participant received three bottles containing approximately 20 g of wheat, 15 g of spinach and 5 g of algae, respectively. Participation in this exercise was not limited to the NRLs but was open to laboratories from all around the world, to be able to judge the state-of-the-art of the determination of total and, more in particular, inorganic As in several food commodities. Seventy-four laboratories from 31 countries registered to the exercise, of which
– 64 reported results in wheat, 49 in spinach and 51 in algae for **total As**, and
– 43 reported results in wheat, and 40 in spinach in algae for **inorganic arsenic**.
Thirty of the participants were NRLs of the EU-RL-HM network, out of which 13 reported values for inorganic As.

The assigned values for IMEP-112 were provided by the certificates when available and otherwise by a group of seven laboratories expert in the field. The uncertainties of the respective assigned values *(u_{ref})* were derived from the standard deviation of the means provided by the experts *(u_{char})* and from the contribution for homogeneity *(u_{bb})* and stability (u_{st}).

Laboratory results were rated with z-and ζ-scores (zeta-scores) in accordance with ISO 13528. The standard deviations for proficiency assessment (also called target standard deviation) were fixed 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 to:

- 15 % for total and inorganic arsenic in **wheat**.
- 22% for total arsenic and 25 % for inorganic arsenic in **vegetable food**, to account for the difficulty introduced by the relatively low concentration of both measurands in this test material.
- 15 % for total arsenic and 22 % for inorganic arsenic in **algae**.

Most of the participants performed satisfactorily for total arsenic and for inorganic arsenic in vegetable food (75 and 85 %); 60 % did for inorganic arsenic in wheat but only 20 % of the laboratories reported satisfactory results in the algae test material.

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