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

IMEP-107: Total and inorganic As in rice

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Heavy Metals
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Total and inorganic As in rice

IMEP®

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1 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 seventh ILC of the EU-RL-HM (former CRL-HM) which focused on the determination of total and inorganic As in rice. The test item used in this exercise is rice purchased in a local supermarket and was provided by the University of Aberdeen. The test item was processed, bottled and labelled at IRMM and dispatched to the participants the first half of December 2009. Each participant received one bottle containing approximately 20 g of test material. 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 rice. One hundred and three laboratories from 35 countries registered to the exercise, of which 98 reported results for total As and 32 for inorganic As. Twenty-nine of the participants were NRLs of the EU-RL-HM network, 8 out of which reported values for inorganic As.

The assigned values for IMEP-107 were provided by a group of laboratories expert in the field: seven for total As and six for inorganic As.

The uncertainties of the respective assigned values, u_{ref}, were derived from the standard deviation of the means provided by the experts, propagated with a 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[1]. The standard deviation for proficiency assessment (also called target standard deviation) was fixed to 15 % by the advisory board of this ILC, on the basis of the outcome of previous ILCs organised by the EU-RL-HM and on the state-of-the-art in this field of analysis.

Around 75 % of the participants performed satisfactory for total and inorganic As.

2 Introduction

The main anthropogenic sources of arsenic into the environment are man's use of pesticides, non-ferrous smelters and coal-fired and geothermal power plants. The longterm consequences of exposure to inorganic forms of As are important because these compounds are recognised as carcinogens affecting especially the lungs and the skin. According to MacKenzie et al.[2] anthropogenic release of arsenic into the environment from mining, industry and burning of fossil fuels is as high as 124000 tons per year, compared to continental and volcanic dust fluxes of 2800 tons. Arsenic is an element that has been used for therapeutic purposes since more than 2000 years, for instance to stimulate the production of haemoglobin and to treat rheumatism, arthritis, asthma and some infectious deseases such as malaria and tuberculosis[3].

Most food contains little arsenic and consumption of seafood represents the main source of the daily arsenic ingestion. In aquatic species, however, arsenic is found in the form of stable, non-toxic organic compounds such as arsenosugars, and arsenobetaine. High levels of arsenic in natural waters may be transferred to plants (such as rice), invertebrates and finally fish through the food chain. In terrestrial organisms arsenic is mainly found in the form of dimethylarsinic acid, methylarsonic acid and inorganic As(III) and As(V) compounds.

When discussing arsenic, speciation plays an especially important role: hydrides, halogenides, oxides, sulfides, arsenites, arsenates and organic arsenic compounds have very different properties. From the toxicity point of view inorganic As species are more toxic than the organic ones. Arsenic accumulates in liver, kidney, lung, spleen, the wall of the gastrointestinal tract, bones, hair, nails and skin[4]. Absorption of As is affected by the type of arsenic compounds. As (V) is more readily absorbed than As (III), and inorganic more that organic. Arsenic is excreted mainly through urine. As (V) is excreted faster than As (III) compounds and organic As compounds are excreted faster than the inorganic ones.

According to the Scientific Opinion on As in food of the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain[5] the following food sub classes were identified as largely contributing to the **inorganic arsenic** 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.

The highest **total arsenic** 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, in some of these food groups the levels of inorganic arsenic were low (e.g. fish and seafood). To draw conclusions on the human exposure to total arsenic from these commodities the amounts consumed must also be taken into account.

The report also indicates that *"the national inorganic arsenic exposures from food and water across 19 European countries are estimated to be in the range 0.13-0.56 µg kg^{-1} body weight (b.w.) per day for average consumers. Children under three years of age are the most exposed to inorganic arsenic. Inorganic arsenic intake in children ranges from 0.50 to 2.66 µg kg^{-1} b.w. per day, which is 2 to 3 fold that of adults"*.

In 2008, Meharg et al.[6] published a paper indicating that the intake of inorganic As via rice and rice-based products consumption in babies from 4 to 12 months could be higher than the maximum exposures from drinking water predicted for adults and so it could negatively affect the health of the babies.

At European level only one standard method has been published and it deals with the termination of inorganic As in seaweed[7]. In China a standard for determination of total arsenic and abio-arsenic in foods exists since 2003[8]

The Directorate General for Health and Consumers (DG SANCO) of the European Commission requested the EU-RL-HM to evaluate the performance of European laboratories with regard to total and inorganic arsenic with a view to future discussions on the need for possible regulatory measures.

With that scope the EU-RL-HM organised a PT on the determination of total and inorganic As in rice which was open to all laboratories around the world with analytical capabilities in that field.

3 The International Measurement Evaluation Programme support

The organisation of IMEP-107 follows the administrative and logistics procedures of the International Measurement Evaluation Programme (IMEP), which is accredited according to ISO Guide 43.

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

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

IMEP helps laboratories to assess their estimate of measurement uncertainty. The participants are invited to report the uncertainty on their measurement 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-107, it was realised in the context of the European legislation on contaminants in food[9-10] and in support to the activities of the EU-RL-HM[11].

IMEP-107 provided support to the European Co-operation for Accreditation (EA) in the frame of a Memorandum of Understanding (MoU), and to the Asia Pacific Laboratory Accreditation Cooperation (APLAC). National accreditation bodies of the two networks mentioned above were informed about IMEP-107 via an e-mail sent to the chairmen of the EA/LC Working Group for ILCs in the field of testing and of the Proficiency Testing Committee of APLAC, respectively.

4 Scope

As stated in Regulation (EC) 882/2004 of the European Parliament and of the Council[11], two of the core duties of the EU-RL-HM are to provide advice on scientific matters to DG SANCO and to organise ILCs for the benefit of staff from National Reference Laboratories. The scope of this PT is to test the competence of the appointed NRLs to determine the total content of arsenic in rice and to evaluate the state-of-the-art of the determination of inorganic As in rice worldwide, with the aim to provide support to the EU policy makers in performing risk assessment studies and eventually in fixing maximum levels for inorganic As in legislation.

5 Time frame

This PT was agreed upon by the NRL network at the third EU-RL-HM workshop held on 25-26 September 2008, for inclusion in the work program of 2009. Invitation letters were sent to the participants on 29th October 2009 (cf Annex 1). On the same day the exercise was announced to EA and to APLAC via an e-mail sent to the chairmen of the EA Working group on ILCs and of the Proficiency Testing Committee of APLAC, respectively (cf Annex 2).

6 Material

6.1 Preparation

Twenty kilograms of locally purchased rice was purchased by the University of Aberdeen (UK) that pre-screened the rice with respect to the As speciation and total concentration of As. At IRMM 10 kg of the rice was divided over three stainless steel drums placed in liquid nitrogen for pre-cooling. A cryogenic vibrating mill (Palla VM-KT, Humboldt-Wedag, Köln, Germany) was cooled down to -196 °C and the pre-cooled rice grains were then slowly fed into the cryogenic mill using a vibrating feeder. Each milling cycle lasted about 15 minutes from -196 °C to about -100 °C. Milling was then interrupted and the mill was cooled again. As a first test a 5 g portion of the resulting powder was passed through a small 250 µm sieve. No material was retained so the feeding speed resulted in very efficient milling. Therefore, no sieving was needed to obtain a top particle size of 250 µm. The material from combined milling cycles was finally homogenised in a three-dimensional mixer (WAB, Dynamix CM-200, Basel, Switzerland) for 30 minutes. The homogenised product was tested for its particle size distribution using laser diffraction where it was confirmed that the top-particle size was below 250 µm, X_{50} was about 35 µm and X_{90} approximately 100 µm. The water content was determined by volumetric Karl Fischer titration and it was 14.4 % (m/m). Such a high value is expected for materials like wheat and rice flours. Thereafter, 20 g powder portions were filled in 60 ml bottles with a PE-insert and screw-cap. A total of 228 bottles were produced of which 31 units were randomly selected for homogeneity and short term stability tests.

6.2 Homogeneity and stability

The measurements for the homogeneity and stability studies were performed by the University of Aberdeen. Homogeneity was evaluated according to ISO 13528[1] and to the method proposed by Fearn and Thompson[12] (one of the approaches recommended by the IUPAC International Harmonised Protocol[13]). The material proved to be homogeneous for total and inorganic As according to the two mentioned approaches. The contribution from homogeneity, u_{bb}, to the uncertainty of the reference value, u_{ref}, was calculated using SoftCRM[14], Table 1.

The stability study of the test item was conducted following the isochronous approach[15]. The evaluation of the stability of the test item was made using the software SoftCRM[16]. The material proved to be stable, even at 60 °C for the six weeks that elapsed between the dispatch of the samples and the deadline for submission of results, for both total and inorganic As.

The analytical results and statistical evaluation of the homogeneity and stability studies are provided in Annex 3.

6.3 Distribution

The samples were dispatched to the participants by IRMM during the first week of December 2009. Each participant received 1) one bottle containing approximately 20 g of test material, 2) an accompanying letter with instructions on sample handling and reporting (cf. Annex 4) and 3) a form that had to be sent back after receipt of the test item to confirm its arrival (cf. Annex 5).

7 Instructions to participants

Details on this intercomparison were discussed with the NRLs and DG SANCO at the third workshop organised by the EU-RL-HM, held in Geel on 25-26 September 2008. Concrete instructions were given to all participants in a letter accompanying the test material (cf. Annex 4). The measurands and matrix were defined as *"total and inorganic As in rice"*.

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, cf. Annex 4) 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.

8 Reference values and their uncertainties

The assigned values for IMEP-107 were provided by a group of laboratories expert in the field: seven for total As and six for inorganic As. The expert laboratories involved in the establishment of the assigned values are:

- Institute of Agricultural Chemistry and Food Technology (CSIC)
- Institute of Chemistry, Karl-Franzens University Graz
- The Food and Environment Research Agency (FERA)
- New Technical University of Denmark (DTU)
- Department of Analytical Chemistry, University of Barcelona
- TESLA (Trace Element Speciation Laboratory), University of Aberdeen
- Center of Studies for Nuclear Energy (SCK-CEN)*

** SCK-CEN used neutron activation analysis (NAA) and provided values only for total As since NAA cannot be used for speciation.*

The experts were asked to use the method of their choice and no further requirements were imposed regarding methodology. The experts were also asked to report their results together with the measurement result uncertainty and 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 As, was used as assigned values, X_{ref}, for this PT according to ISO Guide 35[17]. The standard uncertainties, u_{ref}, associated to the assigned values were calculated using the calculations in Eq. 1 and Eq. 2.

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

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

u_{char} is calculated according to ISO Guide 35[17]

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

Where:

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

The means reported by the expert laboratories and their associated uncertainties, u_{char}, for total and inorganic As are shown in Table 1, which also contains the assigned values and their respective estimated uncertainties, expanded and not expanded.

Table 1: Assigned vales for total and inorganic As and their associated expanded uncertainties as calculated from the values reported by the expert laboratories. All values are expressed in mg kg^{-1}.

Certifier	**Total As**		**Inorganic As**	
	X_n	**u_n**	**X_n**	**u_n**
Certifier 1	0.164	0.009	0.105	0.005
Certifier 2	0.190	0.013	0.105	0.007
Certifier 3	0.176	0.007	0.099	0.003
Certifier 4	0.139	0.003	0.116	0.002
Certifier 5	0.172	0.018	0.097	0.007
Certifier 6	0.190	0.010	0.120	0.010
Certifier 7	0.171	0.004	n.a.	n.a.
X_{ref}	**0.172**		**0.107**	
u_{char}		0.007		0.004
u_{bb}		0.003		0.002
u_{st}		0.005		0.005
u_{ref}		**0.009**		**0.007**
U_{ref} (k = 2)*		**0.018**		**0.014**
$X_{ref} \pm U_{ref}$	**0.172 ± 0.018**		**0.107 ± 0.014**	

** 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 8.

Figure 1 shows the results reported by the expert laboratories both for total and inorganic As. For years a debate has taken place within the scientific community on whether the inorganic As 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 value for the rice sample used in IMEP-107, used various method of analysis (with the exception of cert. 2 and cert. 5 which applied the same approach for the sample pre-treatment, although with a different instrumental set up, for the determination of inorganic As) as summarised in Table 2. All the results agree within a range of about 9 % (95 % confidence interval), Figure 1, which would indicates that the concentration of inorganic As is not method dependent in rice. Interestingly, the expert laboratories found a better agreement on the concentration of inorganic As than on the total concentration on As for which a wider dispersion of results was observed.

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

Cert. ID	Sample treatment	Detection
Total Arsenic		
1	0.5 g of sample + 5 mL concentrated HNO_3 + 8 mL H_2O_2, digestion in a microwave (max temperature 190 °C). Cool down to room temperature, filter through Whatman 40 and dilute with water up to 20 mL.	ICP-MS
2	0.5 g + 2.5 mL (20 % w/v $MgNO_3$ + 2 % w/v MgO) + 5 mL 7 mol L^{-1} HNO_3. Evaporate the mixture to dryness in a sand bath and place in a muffle for 12 hours (max. temperature 450 °C). Repeat the mineralisation till the sample is completely incinerated. White ash dissolved in 5 mL of 6 mol L^{-1} HCl + 5 mL (5% w/v KI and 5 % w/v ascorbic acid). Let reduce for 30 min, filter the solution through Whatman no 1 and dilute up to 25 mL with 6 mol L^{-1} HCl.	FI-HG-AAS
3	0.1 g sample + 1 mL HNO_3 + 2 mL H_2O_2, let stand overnight. Digest in a microwave (max temperature 95 °C). Allow to cool down at room temperature.	ICP-MS
4	0.5 g of sample + 4 mL concentrated HNO_3. Digest in a microwave oven and dilute with milli-Q water up to approximately 20 g.	ICP-MS
5	Aliquot of test sample + 5 mL HNO_3. Digest in microwave and dilute up to 10 mL with pure water. Dilute ten times with diluted HNO_3 containing rhodium.	ICP-MS
6	0.25 g + 1 mL HNO_3 + 4 mL water. Digest in a microwave (max. temperature 250 °C). Dilute after mineralisation up to 10 mL with water	ICP-MS

Cert. ID	Sample treatment	Detection
7	0.7 g were were irradiated for 7 hours together with IRMM-530 (Al-0.1%Au alloy) neutron flux monitors in order to determine the neutron flux during irradiation, a parameter required by the k_0-NAA formalism. For each sample one spectrum was collected on a k_0-calibrated HPGe detector under intermediate precision conditions after a cooling time of about 1 day. Measurement times were 8 hours. As was determined using the γ-ray peak at 559 keV.	k_0-NAA
Inorganic As		
1	**Method A:** Extraction with 1 % (v/v) HNO_3 and 1 % H_2O_2 in a microwave (max. temperature 95 °C). Centrifugation at 3000 rpm. Supernatant filtered (0.45 µm). **Method B:** Addition of 1 % (v/v) HNO_3 overnight at room temperature and digestion in a microwave (max. temperature 95 °C). Centrifugation at 3000 rpm. Supernatant filtered (0.45 µm).	HPLC-ICP-MS
2	1 g of sample + 4.1 mL of H_2O + 18.4 mL of HCl agitated for 15 hours, let stand overnight. Add a reducing agent (2 mL HBr + 1 mL of hydrazine sulphate). Add 10 mL of chloroform and shake for 3 min. Separate the two phases centrifuging at 2000 rpm for 5 min. Repeat the extraction another two times. Eliminate remnants of organic As with a Whatman GD/X syringe filters with 25 mm PTFE membrane. Back extract into 10 mL of 1 mol L^{-1} HCl. Add 2.5 mL of 20 % w/v $Mg(NO_3).6H_2O$ and 2 % w/v MgO) + 10 mL of 14 mol L^{-1} HNO_3. Evaporate to dryness at 425 °C for 12 h. Dissolve the ash in 5 mL of 6 mol L^{-1} HCl reduce with 5 mL reducing solution (5 % w/v KI + 5 % w/v ascorbic acid). After 30 min, filter the solution through Whatman No. 1 filter paper and dilute with 6 mol L^{-1} HCl.	FI-HG-AAS
3	0.5 g + 10 mL 1 % (v/v) HNO_3 in a microwave (max. temp. 80 °C), centrifuged.	HPLC-ICP-MS
4	0.2 g rice + 10 mL 0.07 mol L^{-1} HCl in 3 % H_2O_2 in a microwave (max. temp. 90 °C). Centrifuged at 4000 rpm for 10 min and filtered (0.45 µm).	ICP-MS
5	Same approach than certifier 2 with some modifications, namely: No filtration through Whatman GD/X syringe filters was done before extracting into 1 mol L^{-1} HCl and no ashing step was applied; the 1 mol L^{-1} HCl was directly introduced in the HR-ICP-MS tuned to a resolution of at least 12,000.	HR-ICP-MS
6	0.250 g + 5 mL 1 mol L^{-1} trifluoracetic acid. Sonicate for 10 min and let stand overnight. Microwave in an argon atmosphere (max temp. 95 °C)	HPLC-ICP-MS

The information in this table appears as given by the expert laboratories in their respective reports.

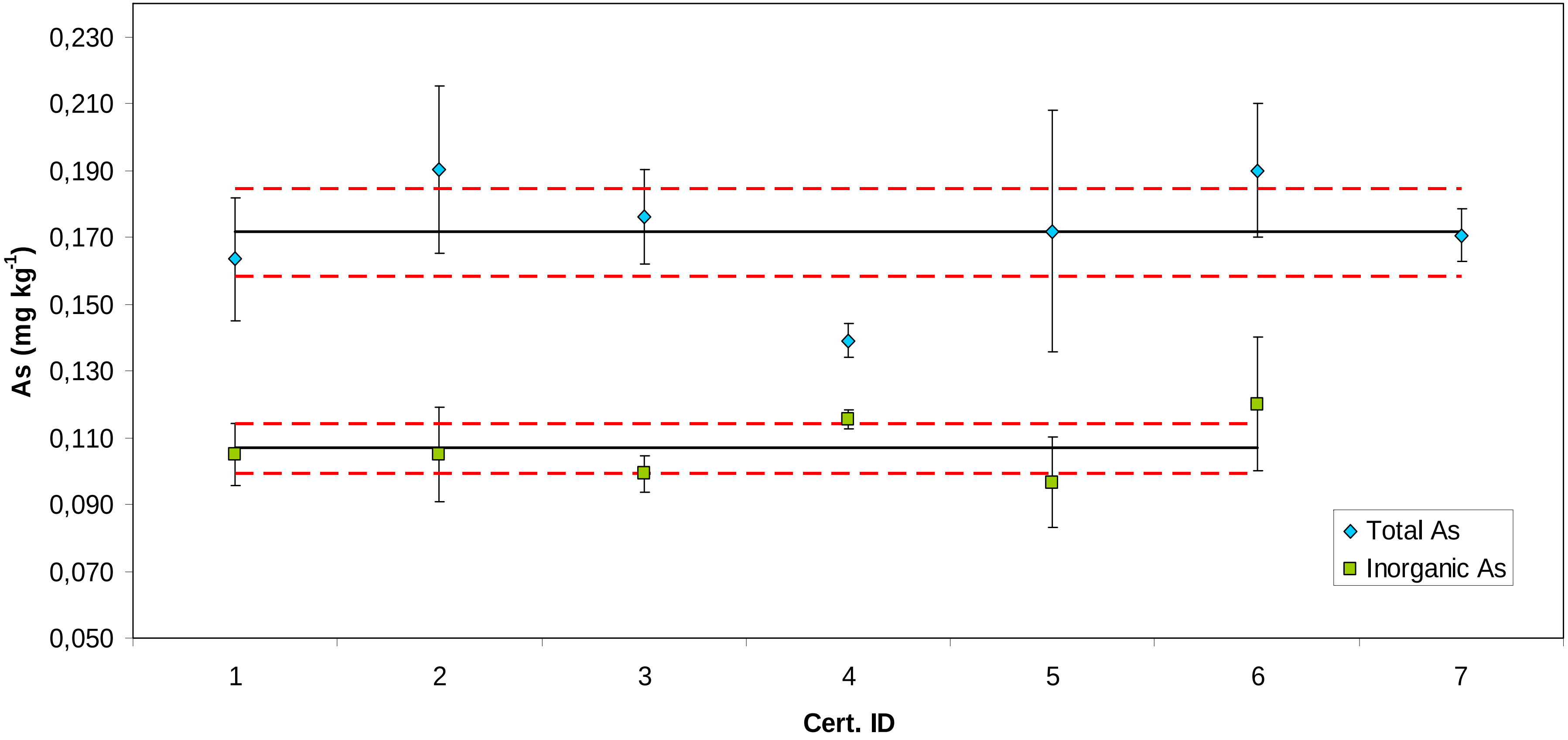

***Figure 1**: Results reported by the expert laboratories for total and inorganic As. The thick black lines correspond to X_{ref} for total and inorganic As respectively, the red lines mark the expanded standard uncertainties of the characterisation by the expert laboratories, $2u_{car}$.*

9 Evaluation of results

9.1 General observations

One hundred and three laboratories from 35 countries registered to the exercise. Ninety-eight laboratories (22 from the Asia-Pacific region) reported results for total As (6 out of the 98 reported "les than" values) and 32 participants reported results for inorganic As (3 out of the 32 reported "less than", 1 reported Non Detected). One laboratory submitted the result form without filling in any value neither for total nor for inorganic As. The distribution of participants per country is shown in Figure 2.

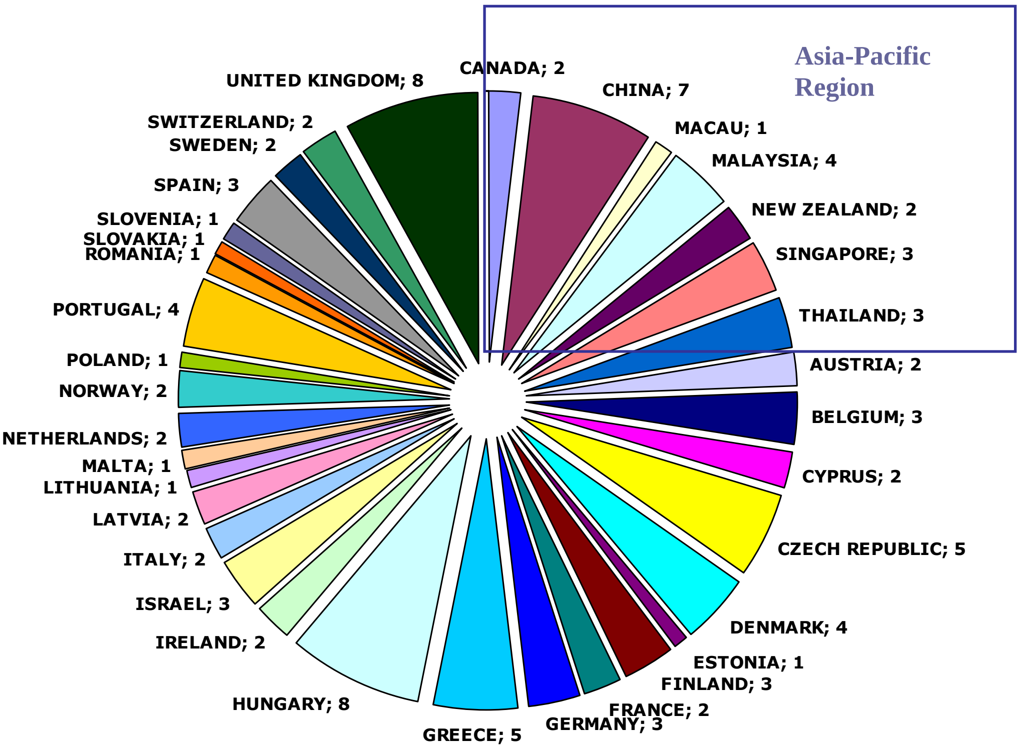

Figure 2: *Distribution per country of the participants in IMEP-107.*

9.2 Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and ζ-scores in accordance with ISO 13528[1] and the International Harmonised Protocol[13].

$$z = \frac{x_{lab} - X_{ref}}{\sigma} \qquad \text{Eq. 3}$$

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u_{ref}^{2} + u_{lab}^{2}}} \qquad \text{Eq. 4}$$

where:

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

The z-score compares the participant's deviation from the reference value with the standard deviation accepted for the proficiency test, $\hat{\sigma}$. For z-scores, the standard deviation $\hat{\sigma}$ is used as common quality criterion. Metrologically speaking, $\hat{\sigma}$, is the maximum acceptable standard uncertainty as defined by the organiser of the proficiency test. Based on feedback from experts on the state-of-the-art and on discussions among the PT organisers, values for $\hat{\sigma}$ were set as 15 % of the assigned value for all measurands.

If $\hat{\sigma}$ is regarded as satisfactory, the z-score can be interpreted as:

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

Should participants feel that the $\hat{\sigma}$ values used in this exercise are not fit for their purpose they can recalculate their scorings with a standard deviation matching their requirements.

The ζ-score states if the laboratory result agrees with the assigned value within the respective uncertainties. The denominator of equation 2 describes 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, its uncertainty and the unit of the result as well as the uncertainty of the assigned values. An unsatisfactory ζ-score can either be caused by a wrong estimation of the expected value (the value before the "±") or of its uncertainty.

Accordingly, the interpretation of the ζ-score is similar to the interpretation of the z -score:

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

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 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 (but possible) that a laboratory carrying out the analysis on a routine basis could measure the measurand with a smaller uncertainty than a reference laboratory. u_{max} is set to the target standard deviation accepted for the PT, $\hat{\sigma}$. If the standard uncertainty from the laboratory, u_{lab}, is smaller than u_{min}, the laboratory probably underestimated its uncertainty. Such a statement has to be taken with care as each laboratory will report only the uncertainty of its measurement, 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 also plausible. If $u_{lab} > u_{max}$, the laboratory possibly overestimated the uncertainty. An evaluation of this statement can be made when looking at the comparison of the reported value and the assigned value: if the difference is small but the uncertainty is large, then overestimation is likely. If, however, the deviation is large but is covered by the uncertainty, then the uncertainty was assessed correctly but is large. Naturally, this assessment will gain from pooling data from various intercomparisons. It should be pointed out that u_{max} is not a normative criterion: Which uncertainty is acceptable for a certain measurement is the decision of the customer of the respective result.

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[18].

9.3 Laboratory results and scorings

The results as reported by the participants for total and inorganic As are summarised in Annex 7 and Annex 8, respectively, together with the z and ζ-scores. The laboratory codes shown in Annexes 7 and 8 were given randomly and communicated to the participants in a confidential letter that was sent to each participant together with the report. Annexes 7 and 8 also include figures in which the individual mean values and associated expanded uncertainties are shown. NRLs are marked with an * in annexes 7 and 8. The Kernel distribution plots for total and inorganic As, obtained using a software tool developed by AMC[19] are shown in Annex 9.

Laboratory 65 reported two sets of results obtained with two different techniques (HG-ICPOES and ICP-AES). The two sets were scored independently and referred to as 65(a) and 65(b), respectively.

The uncertainty values reported by laboratories 68 and 79 for total As and by laboratory 103 for total and inorganic As seem also to be subject of a mistake being abnormally high; it looks as if those laboratories have reported the uncertainty in percentage and not in mg kg^{-1}.

Laboratory 31 reported < 0.10 mg kg^{-1} for total As which is not correct because the concentration of total As in the test item is 0.172 mg kg^{-1}.

Regarding the z and ζ-scores, the results for total and inorganic As are summarised in Table 3. Taking into consideration the z-score, around 75 % of the participants performed satisfactory for total and inorganic As. When the associated uncertainties are taken into account the share of laboratories that scored satisfactorily decreased to 60 %, which means that many laboratories encountered difficulties to provide a reasonable uncertainty estimate. The issue of associated uncertainties have been discussed in detail in chapter 9.2.

Table 3: Number and percentage of laboratories reporting results not "less than" with satisfactory, questionable and unsatisfactory scores.

	Total As		Inorganic As	
	N°	**%**	**N°**	**%**
z				
S	71	77	21	75
Q	11	12	3	11
U	10	11	4	14
ζ				
S	54	59	16	57
Q	15	16	2	7
U	23	25	10	36

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

A univariant analysis was performed to evaluate the influence of the technique used to perform the measurements. No significant difference was observed among the results obtained with the different techniques.

9.4 Additional information extracted from the questionnaire

Additional information was gathered from the questionnaire that participants were asked to fill in (Annex 6) and it is summarised hereafter in different chapters corresponding to the different issues covered. Laboratories 38, 70 and 77 did not fill in the questionnaire.

9.4.1 Sample treatment related questions

Forty laboratories analysed total As following an official method. The information provided by the remaining laboratories about their method of analysis is summarised in Annex 10. Five participants used an official method for the determination of inorganic As. The information provided by the remaining laboratories about their method of analysis is summarised in Annex 11. Different approaches have been used by the participants but no cluster of results could be observed depending on the method used. This supports the conclusion already extracted from the results reported by the expert laboratories which participated in the establishment of the assigned value that the fraction of inorganic As in rice is not method dependent.

Sixty-seven laboratories did not correct their results for total As for recovery; twenty-eight did using one of the following options or a combination of them: fourteen participants added a known amount of the same analyte to the sample, sixteen used a certified reference material and one use a different approach although they did not specify which one. Regarding inorganic As only nine laboratories corrected their results for recovery.

A high share of the laboratories that did not correct for recovery obtained satisfactory scorings. Nevertheless, even if satisfactory, most of the scorings were negative (for total As results) which reflects a tendency to underestimate the total concentration of As. Such a tendency was not observed in the results reported for inorganic As.

Different justifications were given by those that did not apply a recovery correction factor: Most of them indicated that it was proved during the validation of their method and by introducing CRMs and spiked samples as controls during the analysis, that the results were not biased. One participant said that they have taken part in other proficiency tests for the same matrix and they know that their method is not biased. Several laboratories indicated that correction for recovery is not included in their procedures. One participant said that the method used is not fully validated and they do not know yet the recovery.

Nine laboratories did not correct their results for the moisture content of the sample. Three out of them obtained negative questionable z-scores for total As and one for inorganic As. Laboratory 67, would have obtained a satisfactory z-score for total As if moisture correction would have been applied. The remaining six laboratories got z-scores around -1, would they have corrected for moisture they would have obtained results much closer to the assigned value. Control laboratories are requested by the European legislation to report their results on the samples as received (not in dry mass). However it would be difficult to judge the performance of laboratories from all around the world if the results would not be reported as dry mass. The moisture content reported by the laboratories that apply a correction factor for it ranged from 0.5 % up to 14 %. The way how the moisture content of the test material was to be determined was described in detail in the accompanying letter (Annex 4).

9.4.2 Uncertainty related questions

Various approaches were used to scrutinise the measurement uncertainty, Figure 3. Guesstimate corresponds to "estimation based on judgement" as defined in the Eurachem/CITAC Guide on Quantifying Uncertainty in Analytical Measurements[18]. Four participants indicated that they used "other" approaches to estimate the uncertainty of their measurements. One out of the four calculated the uncertainty according to ISO 5725. The remaining three reported options which when analysed in detail they were considered to fall in one of the other offered options.

Forty laboratories usually report uncertainty to their customers.

When asked about the level of confidence covered by the reported coverage factor (k), most of the participants reported 95 %, 1 reported 90 % and another one 99 %. Some participants seem not to have understood the question and have provided answers which did not fit to that question and some did not answer the question at all. The following information regarding coverage factors can be found in the web page of the National Institute of Standards and Technology (NIST): "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 %[20]". For a deeper insight into this issue participants are encouraged to read ISO GUM[21] and/or Eurachem/CITAC Guide on Quantifying Uncertainty in Analytical Measurements[18].

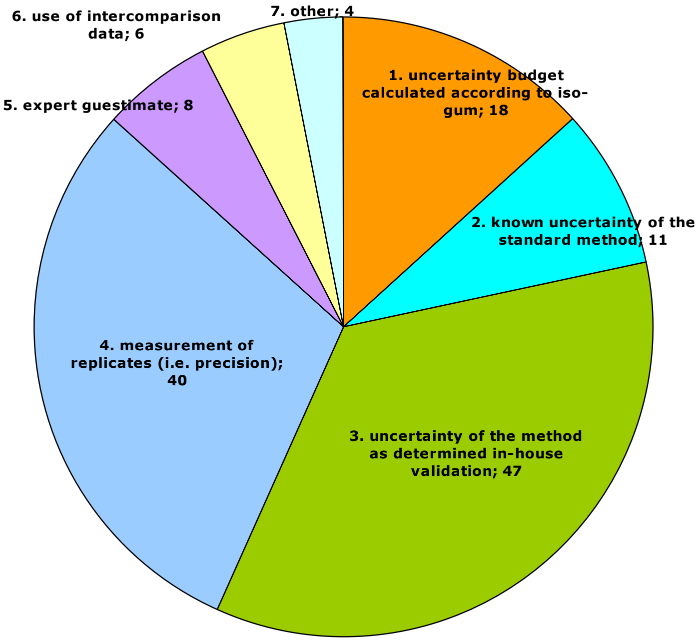

Figure 3: *Different approaches used by the participants in IMEP-107 to estimate the uncertainty of their measurements.*

9.4.3 Quality assurance related questions

Seventy participants regularly take part in proficiency tests for the determination of total As, and eleven participate in proficiency test (Fapas, APLAC and ARL) for determination of inorganic As

Participants were asked whether they use of CRMs in their laboratories and for which pourpose. The answers to those questions are summarised in Figure 4.

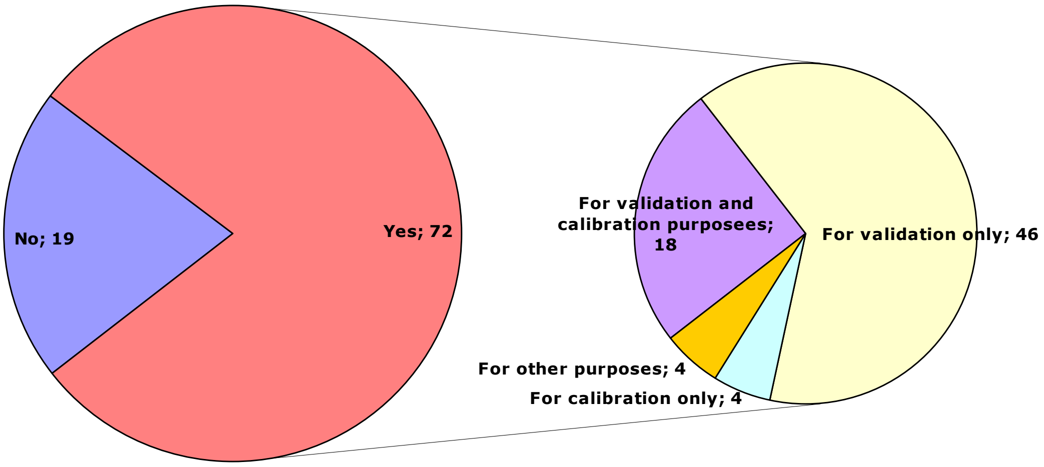

Figure 4: *Information gathered in the questionnaire about the use of CRMs*

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

Eighty-six laboratories carry out this type of analysis for total As determination on a routine basis, 9 do not. Seventy participants do not analyse inorganic As in rice routinely and 8 do. The distribution in terms of number of analysis per year is shown in Figure 5.

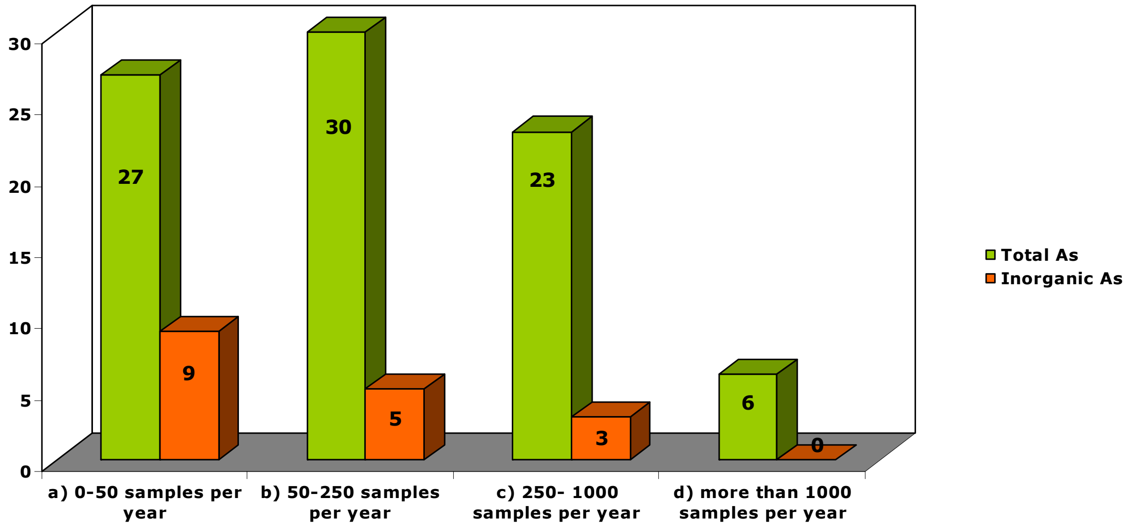

Figure 5: *Participant's experience in this type of analyses expressed as number of analyses/year*

9.4.5 Quality system related questions

Ninety-two participants have a quality system in place and 3 do not. One laboratory has a quality system based on the ISO 9000 series, 2 on both the ISO 9000 series and ISO 17025 and the rest on ISO 17025. Eighty-four participants are accredited and 9 are not.

9.4.6 Other

The comments made by the participants are summarised in Table 4.

Table 4: Comments related to IMEP-107 submitted by the participants.

Lab ID	**Do you have any comments? Please let us know:**
1	This laboratory does not have the instrumentation for determining inorganic Arsenic.
5	We are interested in cooperation in development of method for inorg.As determination
9	We are very pleased with the organisation of an ILC on inorganic As
10	More effective would be analyse sample with total content of arsenic more than 15 mg/kg and for example total inorganic arsenic lower than 2 mg/kg as it is in legislation.
12	Result uncertainty of As determination: 12% k=2.04
16	Meaning of coverage factor in result page is not clear
27	According to IUPAC the term "heavy metal" must be avoided in a scientific communication (Pure Appl. Chem., Vol. 74, No. 5, pp. 793-807, 2002.)
33	We would welcome further rounds for total and inorganic arsenic, in particular in marine samples (eg. shellfish or kelp).
42	Using as part of our method development and validation
43	Don't know what is meant by coverage factor k so left it blank.
55	We can only test total Arsenic not inorganic hence only 1 set of results

Lab ID	Do you have any comments? Please let us know:
57	An indication about the expected concentration range (like the guidelines provided by PT providers) would greatly help in optimising analysis procedure.
62	Low level of total arsenic thus low level of inorganic just above detection limit
73	The instruction is not clear
75	This is our 2nd time to take part in IMEP studies and I find the whole process (registration, sample receipt, submitting, report) very easy to follow. Thank you for the opportunity to take part in this exercise.
83	Results submitted are mg per kg dry matter. Your report scheme indicates mg/kg. This gives a hazard of reporting faulty results if one does not very accurately read the instructions following the samples. And the
85	Our Laboratory does not measure inorganic As from food samples
91	Result of inorganic As is 0.039mg/kg by using ASU 26.06-1 (extraction 2h 37°C)
96	Concentration is spelled incorrectly in question 6.
100	We are not provide the result of inorganic since we not validate this.
103	Sample inhomogeneity detected - assessed through 9 replicates (total As)

10 Conclusions

The main conclusion that can be derived from this exercise is that the concentration of inorganic As in rice is not dependent of the analytical method applied, as has been proved by the results submitted by six expert laboratories and by the participants in IMEP-107.

Along the years a debate among experts in the field of arsenic speciation has taken place according to which several extraction protocols would define a measurand which should be correctly named *"by method XXX extractable As"*. Results from different extraction protocols would be by definition not comparable, as they could possibly measure different things. In the frame of the same discussion it was said that unless a thorough speciation analysis was done, agreement between different methods could be a pure coincidence.

In IMEP-107 a wide range of sample pre-treatment methods (extraction into water, acid extraction with different acids, basic extraction, 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 value for inorganic As. Despite that, no cluster of results related to the analytical approach was observed. It is quite unlikely that the agreement among results obtained with so many and different analytical methods would be reached just by chance.

No particular problem related to the determination of inorganic As in rice has been detected in this proficiency test and the performance of the participating laboratories was satisfactory. The performance of laboratories was similar for total arsenic and inorganic arsenic. However, the number of laboratories who determined inorganic arsenic was considerably less than the number of laboratories who determined total arsenic. 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 inorganic arsenic in further discussions on risk management.

Once more it became evident that an extra effort is needed in the evaluation of uncertainties associated to the results, since the number of unsatisfactory ζ -scores is systematically higher than those of z-scores for the two measurands. The uncertainty associated to a certain result is of paramount importance in cases of litigation and so it is fundamental to be able to report a sound uncertainty statement.

11 Acknowledgements

C. Contreras and P. Connely from the Reference Materials Unit are acknowledged for their support in the processing of the test material and in checking the frying method against Karl-Fisher titration, respectively. F. Cordeiro, I. Baer and P. Robouch are thanked for the fruitful discussions about the organisation of the IMEP-107 exercise and the thorough revision of this report. Anne-Mette Jensen is acknowledged for revising the manuscript.

I. Mann (EA) and D. Tholen (APLAC) are thanked for announcing IMEP-107 in their respective networks.

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

Organisation	Country
AGES Zentrum Analytik und Mikrobiologie	Austria
AGES Kompetenzzentrum Elemente	Austria
Scientific Institute of Public Health	Belgium
Laboratorium ECCA NV	Belgium
CODA-CERVA	Belgium
Alberta Agriculture and Rural Development	Canada
Canadian Food Inspection Agency	Canada
COFCO East Ocean Oils & Grains Industries (Zhangjiagang) Co.,Ltd.	China
Agricultural Products quality & Safety Supervision & Testing Center of Huizhou	China
Test Center for Quality of Chengdu Storage Science Research Institute of the State Administration of Grain Reserves	China
Sino Analytica (Qingdao)	China
Dalian Institute of Product Quality Supervision and Inspection	China
Food & Food Machinery Testing Center, Chinese Academy of Agricultural Mechanization Sciences	China
Sino Analytica (Ningbo)	China
Panchris Animal Premix Ltd	Cyprus
Aristos Loucaides Chemical Laboratory Ltd.	Cyprus
CISTA	Czech Republic
Ekocentrum Ovalab,S.R.O.	Czech Republic
State Veterinary Institute Olomouc	Czech Republic
Institut pro testovani a certifikaci, a.s.	Czech Republic

Organisation	Country
Czech Agriculture and Food Inspection Authority	Czech Republic
Danish Veterinary and Food Administration	Denmark
Eurofins Miljø A/S	Denmark
DTU Food	Denmark
The Danish Plant Directorate	Denmark
Agricultural Research Centre	Estonia
Finnish Customs Laboratory	Finland
MetropoliLab	Finland
Evira	Finland
AFSSA	France
Laboratoire SCL de Bordeaux	France
Bayer. Landesamt f. Gesundheit + Lebensmittelsicherheit	Germany
Federal Office of Consumer Protection and Food Safety (BVL)	Germany
Institut Kirchhoff Berlin GmbH	Germany
General Chemical State Laboratory	Greece
General Chemical State Laboratory	Greece
University of Athens	Greece
Regional Center Of Plant Protection And Quality Control Of Magnisia	Greece
Central Agricultural Office, Food and Feed Safety Directorate	Hungary
Corvinus University of Budapest	Hungary
Veszprém County Central Agriculture Office	Hungary
Central Agricultural Office Of Hajdu-Bihar County	Hungary
Central Agricultural Office Food and Feed Safety Directorate	Hungary
Central Agricultural Office Food and Feed Safety Directorate	Hungary
Fejér County Agricultural Office Foodchain Safety and Animal Directorate	Hungary
B.A.Z. county Agricultural Office	Hungary
HEALTH SERVICE EXECUTIVE	Ireland
Health Service Executive	Ireland
The standards institution of Israel	Israel
Bactochem	Israel
Milouda	Israel
Istituto Superiore di Sanità	Italy
Istituto Zooprofilattico Sperimentale Del Piemonte, Liguria E Valle D'aosta	Italy
National Diagnostic Centre	Latvia
State Ltd. "Latvian Certification Centre"	Latvia
National Food and Veterinary Risk Assessment Institute	Lithuania
Macau Institute for Applied Research in Medicine and Health	Macau
Permulab Sdn Bhd	Malaysia
National Public Health Laboratory, Ministry Of Health	Malaysia
ALS Technichem (M) Sdn BHd	Malaysia
Allied Chemists Laboratory Sdn. Bhd.	Malaysia
Public Health Laboratory	Malta
Food and Consumer Product Safety Authority (VWA)	Netherlands
RIKILT	Netherlands
AsureQuality Auckland Laboratory	New Zealand
Cawthron institute	New Zealand
Trondheim kommune	Norway
National Institute of Nutrition and Seafood Research	Norway
National Institute of Public Health-National Institute of Hygiene	Poland
ISQ	Portugal
a.LOGOS	Portugal
Silliker Portugal, SA	Portugal
Sagilab - Laboratório de Análises Técnicas S.A.	Portugal
Hygiene and Veterinary Public Health Institute	Romania
Agri-Food & Veterinary Authority of Singapore	Singapore
Pacific Lab Services	Singapore

Organisation	Country
Mechem COnsultancy Services Pte Ltd	Singapore
State veterinary and food institute - Kosice	Slovakia
National Veterinary Institute	Slovenia
Laboratorio Agroalimentario y de Sanidad Animal	Spain
Laboratorio Arbitral Agroalimentario	Spain
Laboratorio Normativo De Salud Publica	Spain
National Food Administration	Sweden
ALS Scandinavia AB	Sweden
Kantonales Labor Zürich	Switzerland
Nestlé Research Center	Switzerland
Ministry of Science and Technology	Thailand
Central laboratory (Thailand) Co., Ltd. Bangkok branch	Thailand
Overseas Merchandise Inspection Co., Ltd.	Thailand
Aberdeen City Council	United Kingdom
Hampshire Scientific Service	United Kingdom
Kent County Council	United Kingdom
Premier Analytical Services	United Kingdom
Durham County Council	United Kingdom
Tayside Scientific Services	United Kingdom
Minton Treharne & Davies	United Kingdom
Reading Scientific Services Limited	United Kingdom

12 References

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[1] ISO 13528:2005; Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons.

[2] F.T. MacKenzie, R.J. Lantzy, V. Paterson, *J. Int. Assoc. Math. Geol.*, (1979), **11**, 99-142.

[3] J. Emsley, *New Sci.*, (1985), **19-26**, 10-13.

[4] A. Léonard, "Arsenic", in: Metals and their Compounds in the Environment. Occurrence, Analysis and Biological Relevance. Ed: E. Merial, VCH, (1991)

[5] *EFSA Journal*, (2009), **7(10)**, 1351-1549.

[6] A.A. Meharg,G. Sun, P.N. Williams, E. Adomako, C. Deacon, Y.Z. Zhu, J. Feldman, A. Raab, *Evironmental Pollution*, (2008), **152**, 746-749.

[7] EN 15517: 2008 Determination of trace elements - Determination of inorganic As in seaweed by hydride generation atomic absorption spectrometry (HG-AAS) after acid digestion.

[8] GB/T5009.11-2003. Determination of total arsenic and abio-arsenic in foods.

[9] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

[10] Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

[11] Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

[12] T. Fearn, M. Thompson, *Analyst*, (2001), **126**, 1414-1416.

[13] M. Thompson, S.L.R. Ellison, R. Wood, *Pure Appl. Chem.*, (2006), **78(1),** 145-196.

[14] See www.softCRM.com

[15] A. Lamberty, H. Schimmel, J. Pawels, *Fresenius J. Anal. Chem.*, (1998), **360**, 359-361.

[16] T.P.J. Linsinger, J. Pawels, A. Lamberty, H.G. Schimmel, A.M.H. van der Veen, L. Siekmann, *Anal. Bioanal. Chem.*, (2001), **370**, 183-188.

[17] ISO Guide 35:2006; Reference materials - General and statistical principles for certification

[18] Eurachem/CITAC guide; Quantifying Uncertainty in Analytical Measurements, 2000 (www.eurachem.ul.pt).

[19] The software to calculate Kernel densities is provided by the Statistical Subcommittee of the Analytical Methods Committee (AMC) of the Royal Society of Chemistry and described in the AMC Technical Brief "Representing data distributions with Kernel density estimates" (2006), see www.rsc.org/amc

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[20] http://physics.nist.gov/cuu/Uncertainty/coverage.html

[21] Evaluation of measurement data — Guide to the expression of uncertainty in measurement. JCGM 100, (2008)

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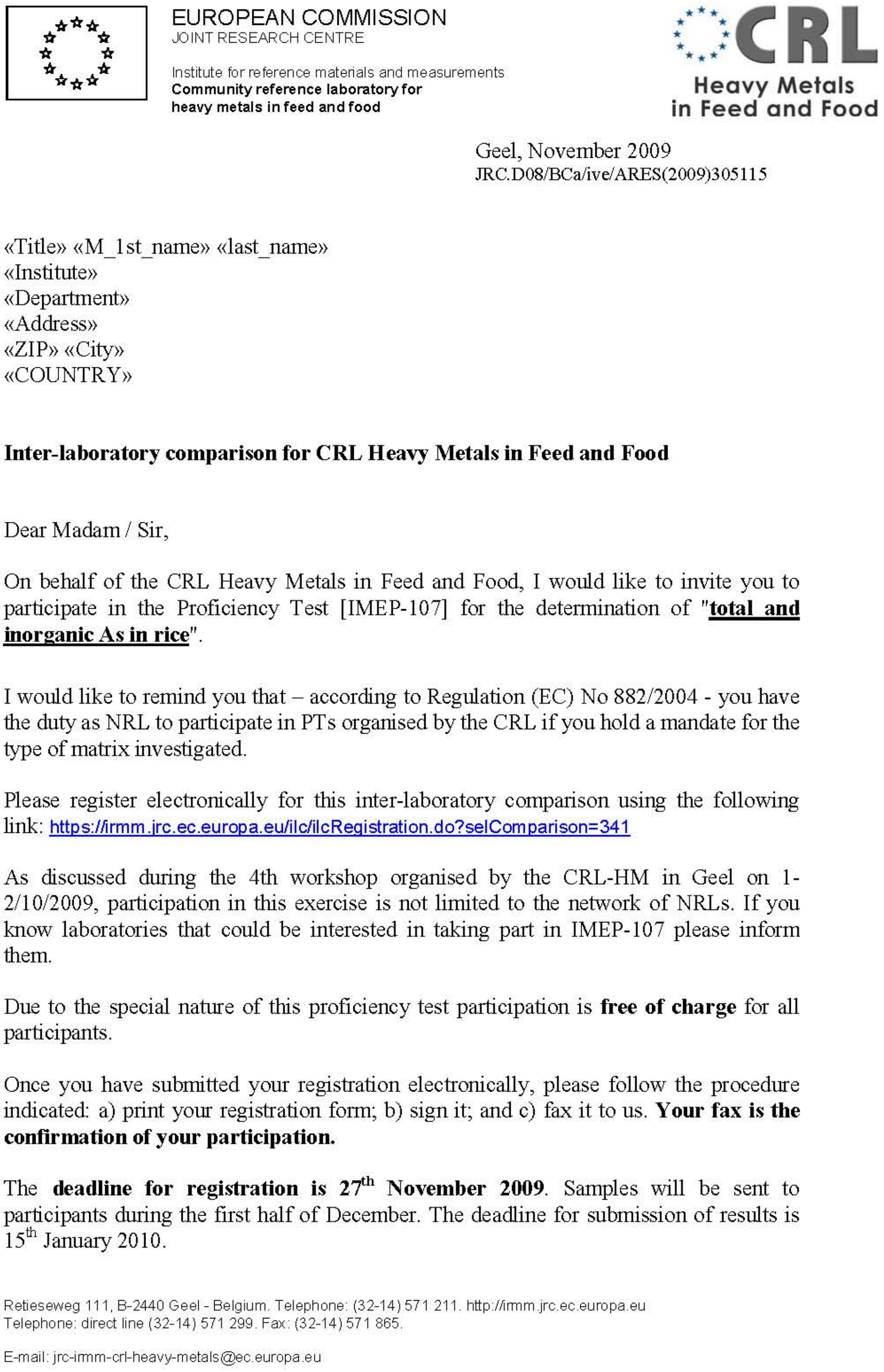
Annex 1: Invitation letter



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

CRL
Heavy Metals
in Feed and Food

Geel, November 2009
JRC.D08/BCa/ive/ARES(2009)305115

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

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

Dear Madam / Sir,

On behalf of the CRL Heavy Metals in Feed and Food, I would like to invite you to participate in the Proficiency Test [IMEP-107] for the determination of "**total and inorganic As in rice**".

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

Please register electronically for this inter-laboratory comparison using the following link: https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=341

As discussed during the 4th workshop organised by the CRL-HM in Geel on 1-2/10/2009, 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-107 please inform them.

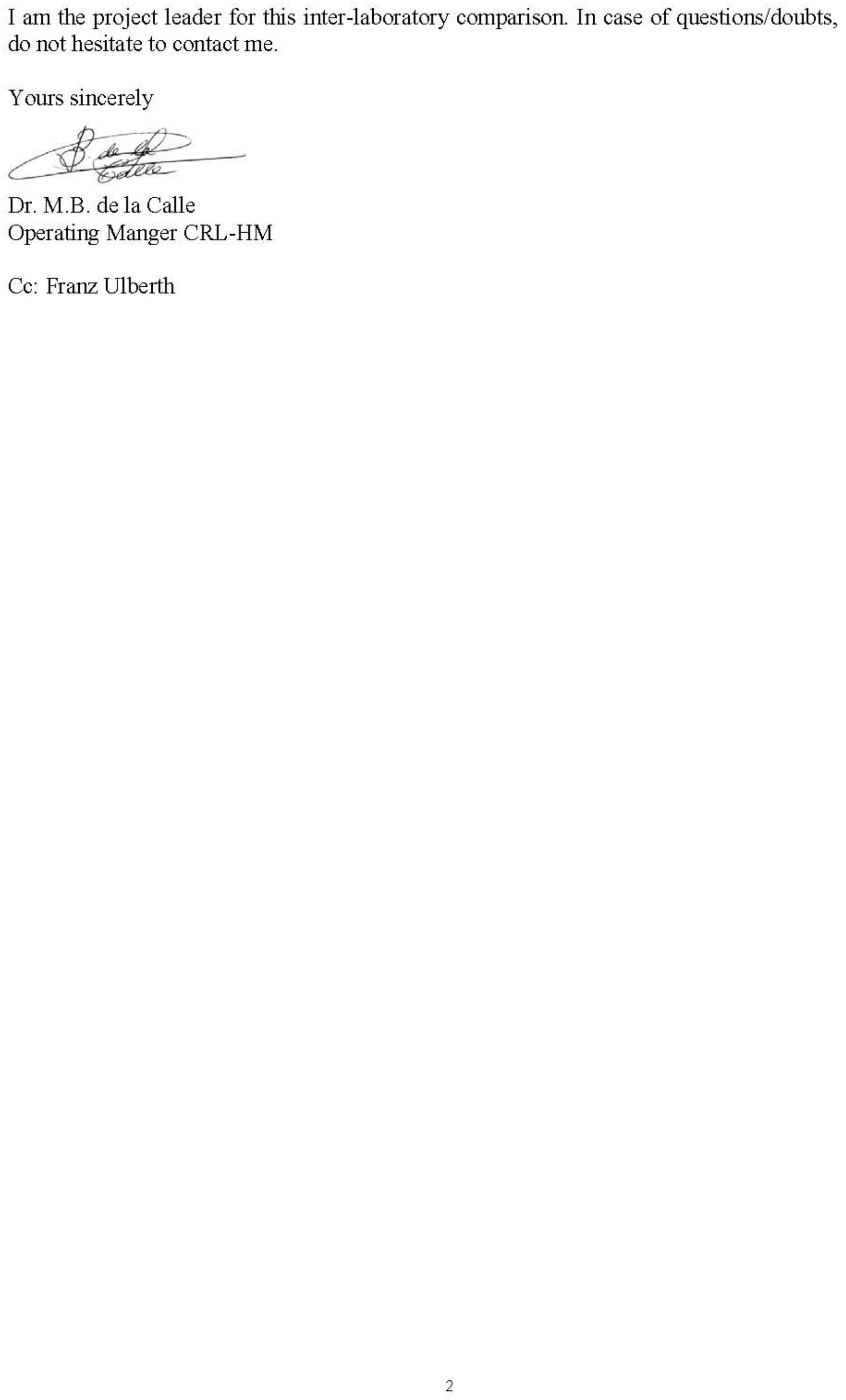
Due to the special nature of this proficiency test participation is **free of charge** for all participants.

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 27th November 2009**. Samples will be sent to participants during the first half of December. The deadline for submission of results is 15th January 2010.

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

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



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

Yours sincerely

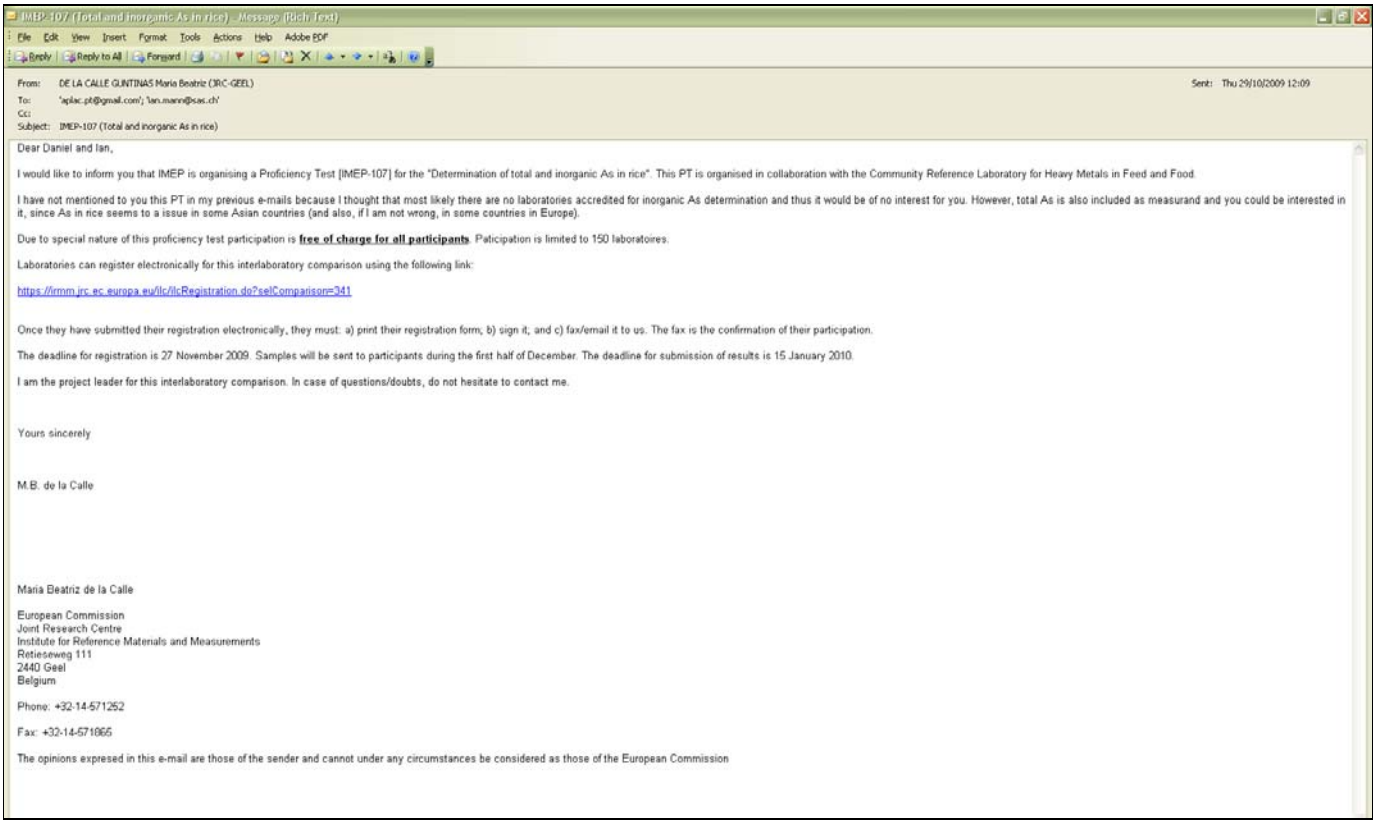
B. de la Calle

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

Cc: Franz Ulberth

2

Annex 2: Announcement e-mail to EA and APLAC



From: DE LA CALLE GUNTINAS Maria Beatriz (JRC-GEEL)
Sent: Thu 29/10/2009 12:09
To: 'aplac.pt@gmail.com'; 'ian.mann@sas.ch'
Cc:
Subject: IMEP-107 (Total and inorganic As in rice)

Dear Daniel and Ian,

I would like to inform you that IMEP is organising a Proficiency Test [IMEP-107] for the "Determination of total and inorganic As in rice". This PT is organised in collaboration with the Community Reference Laboratory for Heavy Metals in Feed and Food.

I have not mentioned to you this PT in my previous e-mails because I thought that most likely there are no laboratories accredited for inorganic As determination and thus it would be of no interest for you. However, total As is also included as measurand and you could be interested in it, since As in rice seems to a issue in some Asian countries (and also, if I am not wrong, in some countries in Europe).

Due to special nature of this proficiency test participation is **free of charge for all participants**. Paticipation is limited to 150 laboratoires.

Laboratories can register electronically for this interlaboratory comparison using the following link:

https://irmm.jrc.ec.europa.eu/ilc/ilcRegistration.do?selComparison=341

Once they have submitted their registration electronically, they must: a) print their registration form; b) sign it; and c) fax/email it to us. The fax is the confirmation of their participation.

The deadline for registration is 27 November 2009. Samples will be sent to participants during the first half of December. The deadline for submission of results is 15 January 2010.

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

Yours sincerely

M.B. de la Calle

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

Annex 3: Results of the homogeneity and stability studies

1a. Homogeneity study for total arsenic

	Total arsenic (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
19	0,168	0,162
33	0,166	0,162
43	0,159	0,167
95	0,161	0,167
112	0,166	0,161
151	0,159	0,159
166	0,158	0,157
181	0,157	0,170
202	0,162	0,158
226	0,153	0,168
Mean of 20 results	0,162	
$\hat{\sigma}$	15 %	
Homogeneity test according to the IUPAC Harmonised Protocol[11]		
S_{an}^2	2,94E-05	
S_{sam}^2	-8,25556E-06 (MSB < MSW)	
Test result	Passed	
Homogeneity test according to ISO 13528[1]		
$0.3\hat{\sigma}$	0,00729	
S_x	0,002538591	
S_w	0,005422177 (S_x<S_w)	
Test result	Passed	

1b. Stability data for total arsenic in rice

As computed by the SoftCRM

Stability Study - Total As

TEMPERATURE = 18°C

Meas.Unit: mg kg^{-1}

		Time in Weeks			
samples		0	3	5	8
	1	160	161	166	148
	2	168	158	157	166

CALCULATION OF U_{st} for given X_{shelf}

Given $X_{shelf\ life}$ = 6 Weeks

Ust[%] = 2,9%

REGRESSION LINE PARAMETERS

Slope = -0,765
SE Slope = 0,786
Intercept = 163,559
SE Intercept = 3,892
Correlation Coefficient =0,136

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

1a. Homogeneity study for inorganic arsenic

	Total arsenic (mg kg^{-1})	
Bottle ID	**Replicate 1**	**Replicate 2**
19	0,087	0,086
33	0,096	0,083
43	0,09	0,084
95	0,079	0,069
112	0,067	0,089
151	0,088	0,092
166	0,077	0,08
181	0,082	0,09
202	0,087	0,078
226	0,089	0,091
Mean of 20 results	0,0842	
$\hat{\sigma}$	15 %	
Homogeneity test according to the IUPAC Harmonised Protocol[11]		
S_{an}^2	0,0000482	
S_{sam}^2	8,07778E-06	
σ_{all}^2	1,43565E-05	
Critical	7,56723E-05	
Test result (S_{sam}^2<critical?)	Passed	
Homogeneity test according to ISO 13528[1]		
$0.3\hat{\sigma}$	0,003789	
S_x	0,005672546	
S_w	0,006942622	
S_s	0,002842143	
Test result ($S_s \leq 0.3\sigma$)	Passed	

1b. Stability data for inorganic arsenic in rice

As computed by the SoftCRM

Stability Study - inorganic As

TEMPERATURE = 18°C

Meas.Unit: mg kg^{-1}

Time in Weeks

samples		0	3	5	8
	1	84	79	83	81
	2	81	77	82	67

CALCULATION OF U_{st} for given X_{shelf}

Given $X_{shelf\ life}$ = 6 Weeks

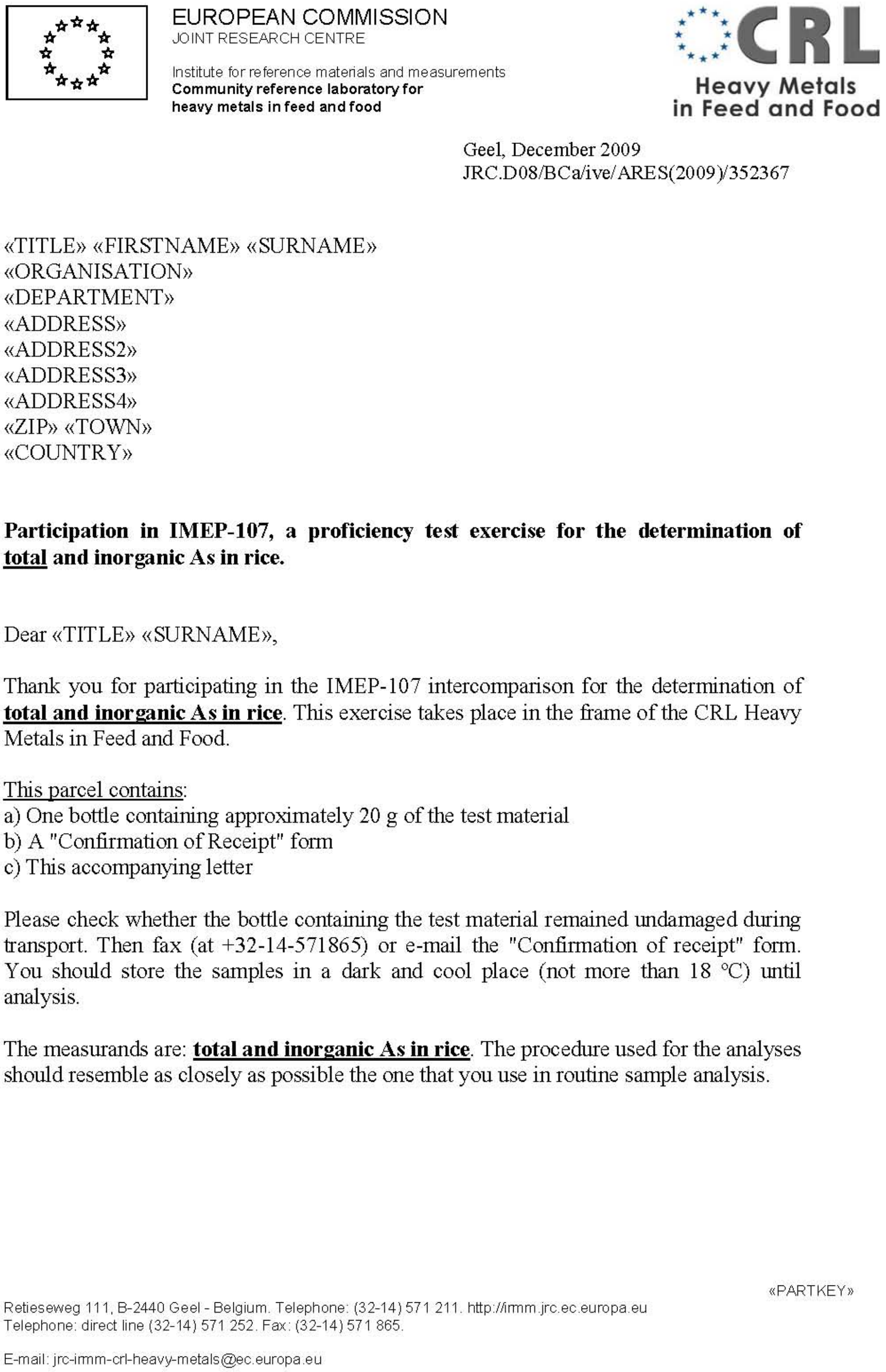
U_{st}[%] = 5,0%

REGRESSION LINE PARAMETERS

Slope = -0,868
SE Slope = 0,615
Intercept = 82,721
SE Intercept = 3,044
Correlation Coefficient =0,249

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

Annex 4: Accompanying letter



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for reference materials and measurements
Community reference laboratory for
heavy metals in feed and food

CRL
Heavy Metals
in Feed and Food

Geel, December 2009
JRC.D08/BCa/ive/ARES(2009)/352367

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

Participation in IMEP-107, a proficiency test exercise for the determination of total and inorganic As in rice.

Dear «TITLE» «SURNAME»,

Thank you for participating in the IMEP-107 intercomparison for the determination of **total and inorganic As in rice**. This exercise takes place in the frame of the CRL Heavy Metals in Feed and Food.

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

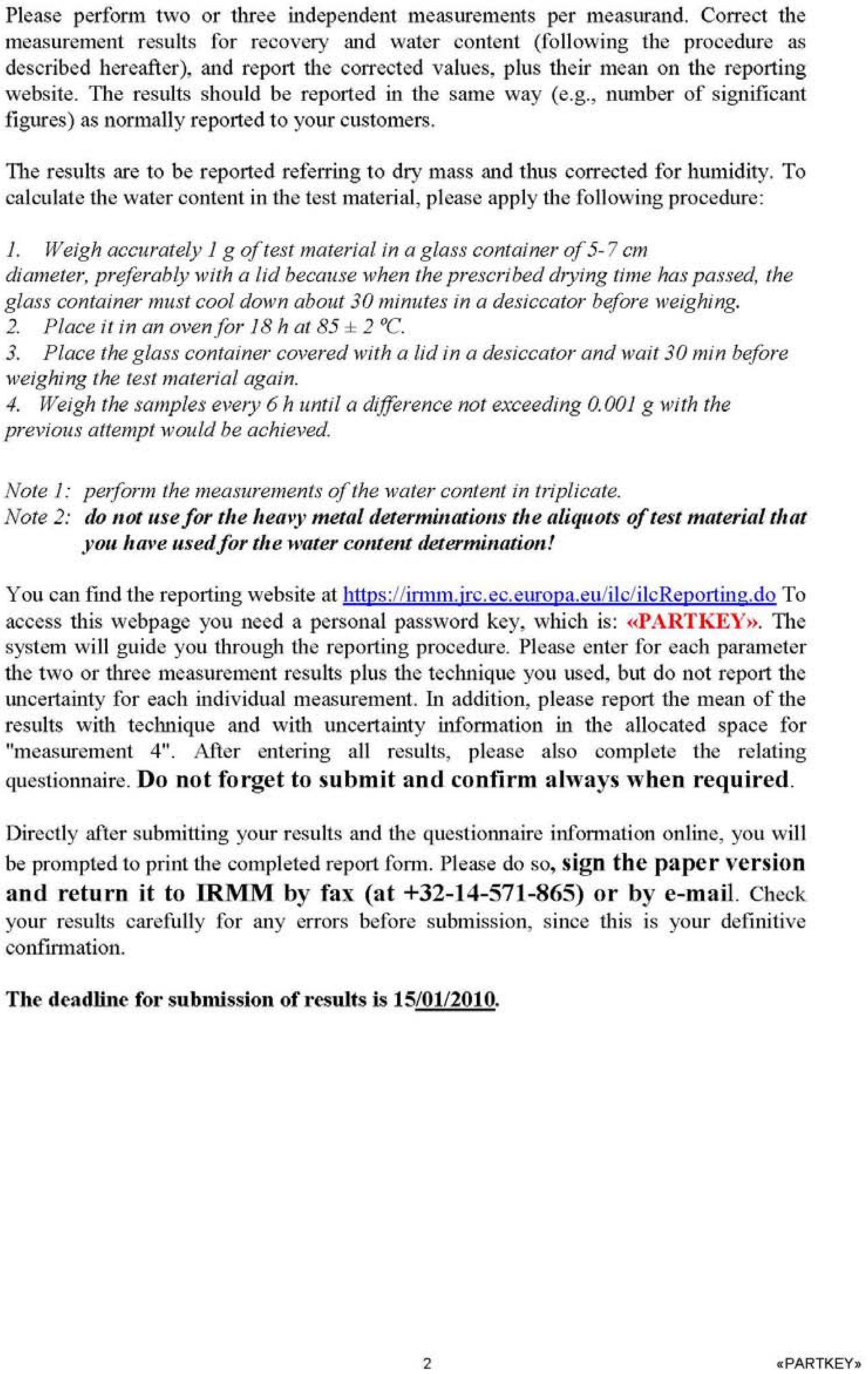
Please check whether the bottle containing the test material remained undamaged during transport. Then fax (at +32-14-571865) or e-mail the "Confirmation of receipt" form. You should store the samples in a dark and cool place (not more than 18 °C) until analysis.

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

«PARTKEY»

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

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



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

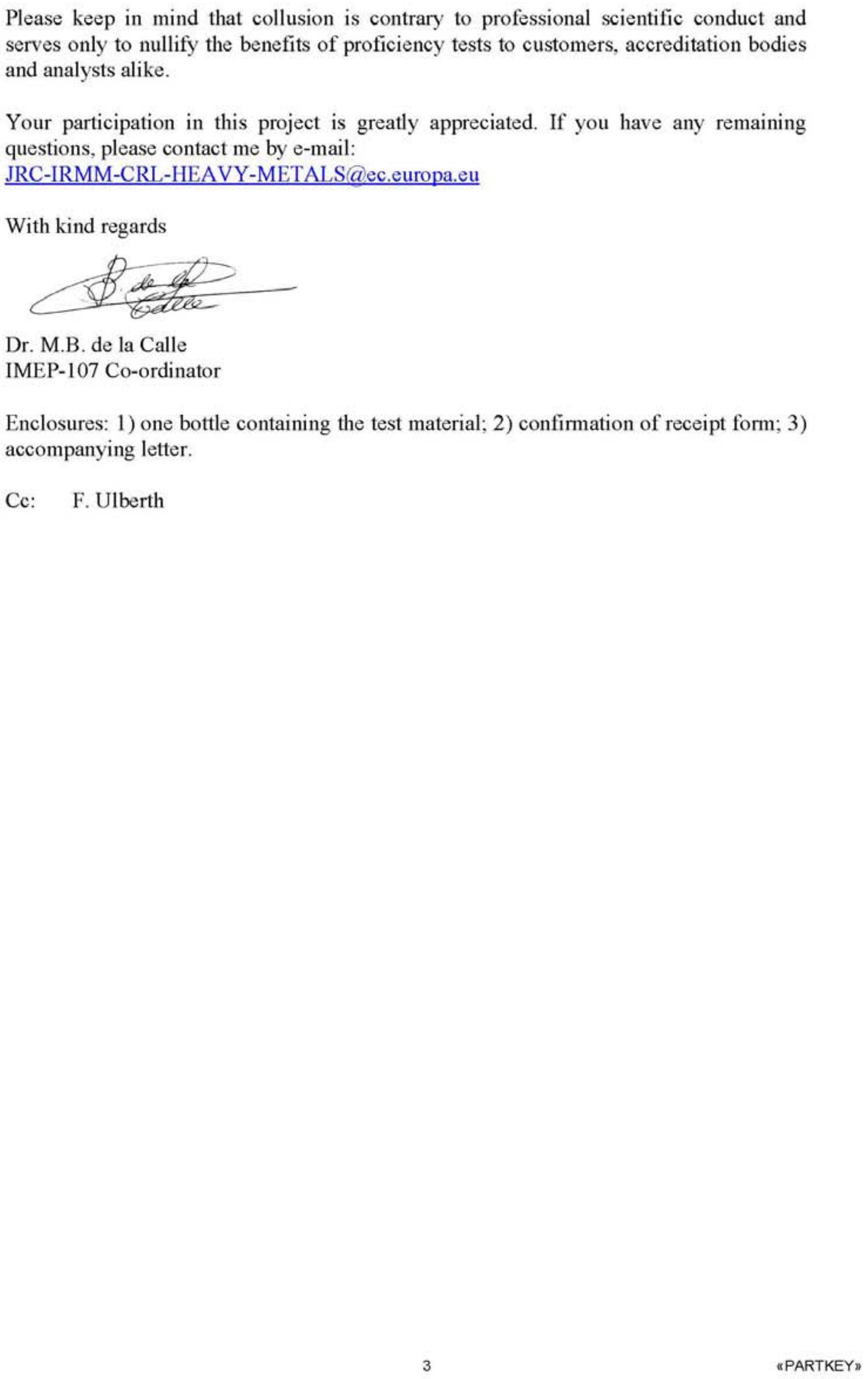
1. Weigh accurately 1 g of test material in a glass container of 5-7 cm diameter, preferably with a lid because when the prescribed drying time has passed, the glass container must cool down about 30 minutes in a desiccator before weighing.
2. Place it in an oven for 18 h at 85 ± 2 ºC.
3. Place the glass container covered with a lid in a desiccator and wait 30 min before weighing the test material again.
4. Weigh the samples every 6 h until a difference not exceeding 0.001 g with the previous attempt would be achieved.

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 parameter the two or three measurement results plus the technique you used, but do not report the uncertainty for each individual measurement. In addition, please report the mean of the results with technique and with uncertainty information in the allocated space for "measurement 4". After entering all results, please also complete the relating questionnaire. **Do not forget to submit and confirm always when required**.

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

The deadline for submission of results is 15/01/2010.



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

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

With kind regards

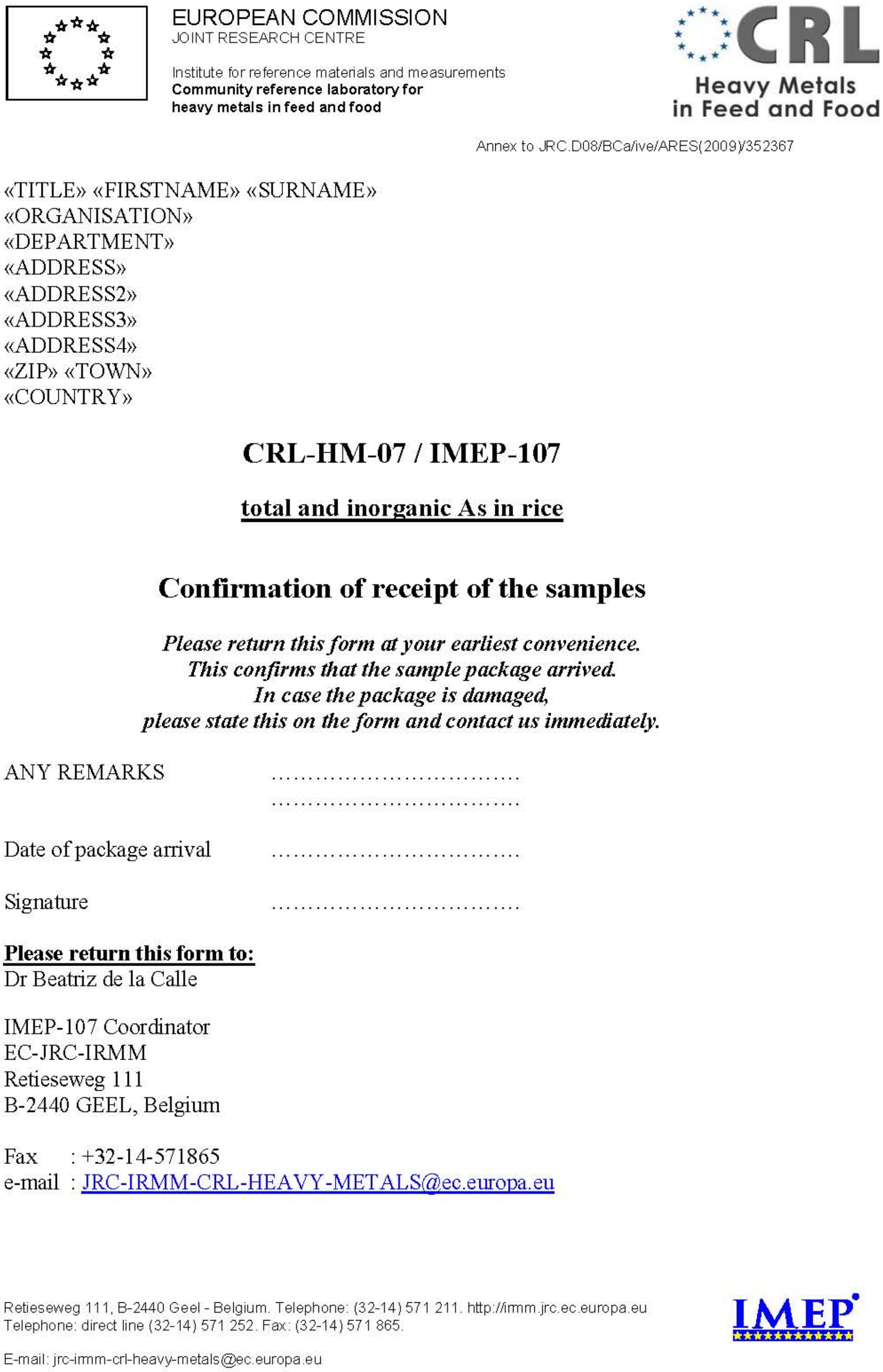
B. de la Calle

Dr. M.B. de la Calle
IMEP-107 Co-ordinator

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

Cc: F. Ulberth

Annex 5: Confirmation of receipt form



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

CRL
Heavy Metals
in Feed and Food

Annex to JRC.D08/BCa/ive/ARES(2009)/352367

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

CRL-HM-07 / IMEP-107

total and inorganic As in rice

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-107 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

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

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

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

IMEP

Annex 6: Questionnaire

This questionnaire is offline

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. expert guestimate
☐ 6. use of intercomparison data
☐ 7. other

3.1. If other, please specify

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

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

6. **Did you determine 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:

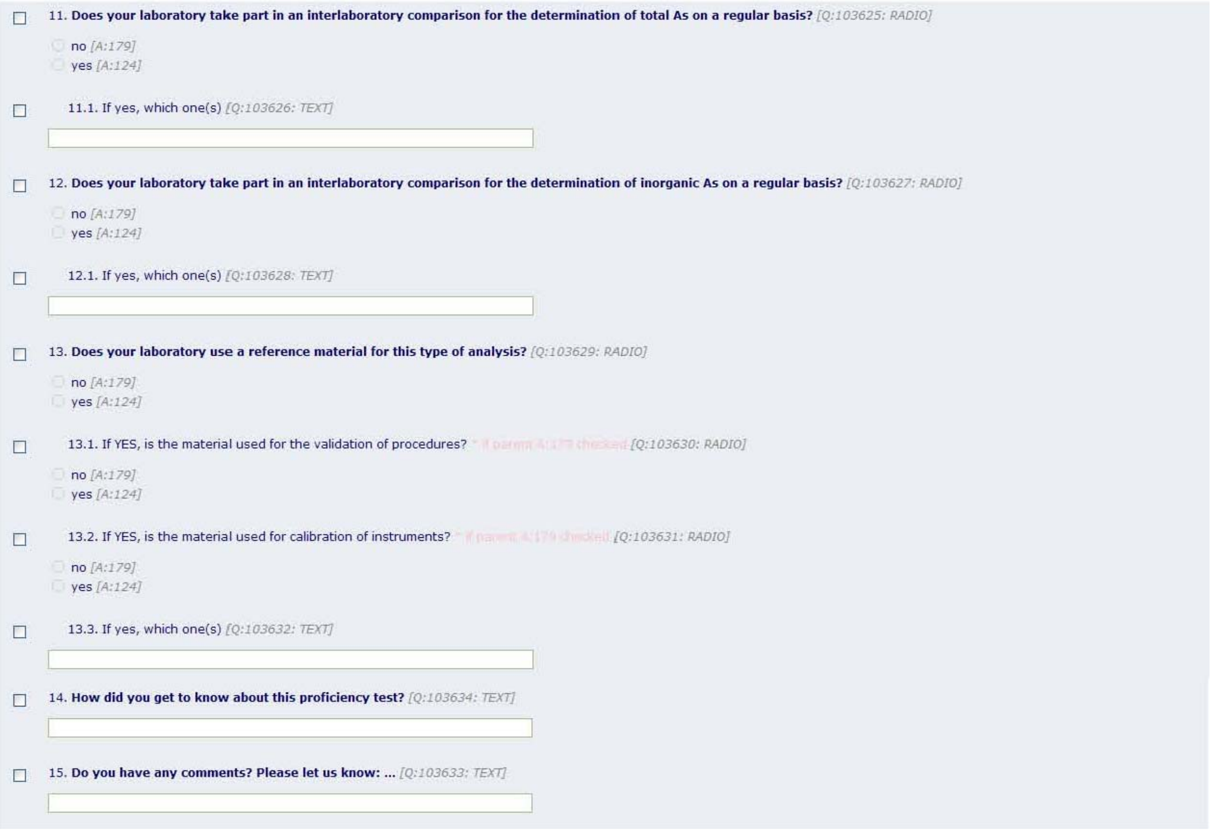
☐ a) ISO 17025
☐ b) ISO 9000 series
☐ c) Other

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?** *[Q:103625: RADIO]*

○ no *[A:179]*
○ yes *[A:124]*

☐ 11.1. If yes, which one(s) *[Q:103626: TEXT]*

☐ 12. **Does your laboratory take part in an interlaboratory comparison for the determination of inorganic As on a regular basis?** *[Q:103627: RADIO]*

○ no *[A:179]*
○ yes *[A:124]*

☐ 12.1. If yes, which one(s) *[Q:103628: TEXT]*

☐ 13. **Does your laboratory use a reference material for this type of analysis?** *[Q:103629: RADIO]*

○ no *[A:179]*
○ yes *[A:124]*

☐ 13.1. If YES, is the material used for the validation of procedures? * if parent A:179 checked *[Q:103630: RADIO]*

○ no *[A:179]*
○ yes *[A:124]*

☐ 13.2. If YES, is the material used for calibration of instruments? * if parent A:179 checked *[Q:103631: RADIO]*

○ no *[A:179]*
○ yes *[A:124]*

☐ 13.3. If yes, which one(s) *[Q:103632: TEXT]*

☐ 14. **How did you get to know about this proficiency test?** *[Q:103634: TEXT]*

☐ 15. **Do you have any comments? Please let us know: ...** *[Q:103633: TEXT]*

Annex 7: Total As in rice.

X_{ref}= 0.172 ± 0.018 mg kg^{-1} (k=2)

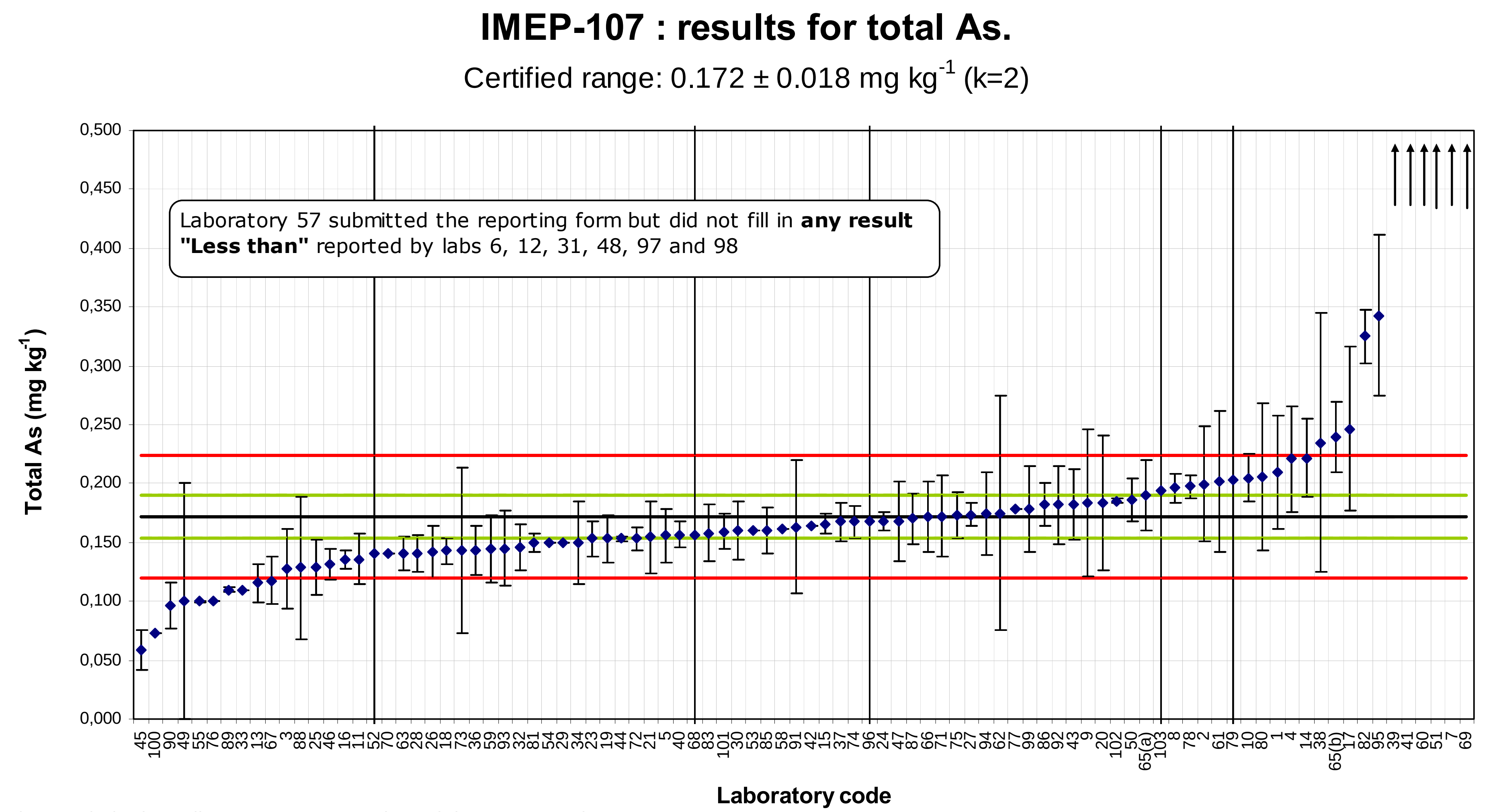
Lab ID	x_1	x_2	x_3	x_4	U_{lab}	k	u_{lab}	Mean-calc	Technique	z	ζ	$Qual_u$
1*	0,28	0,17	0,18		0,048	2	0,024	0,21	ICP-MS	1,5	1,5	a
2*	0,213	0,186			0,049	2	0,025	0,200	ICP-MS	1,1	1,1	a
3*	0,113	0,124	0,147		0,034	2	0,017	0,128	ETAAS	-1,7	-2,3	a
4*	0,227	0,227	0,207	0,223	0,045	2	0,023	0,221	ICP-MS	1,9	2,0	a
5*	0,152	0,159	0,156		0,023	2	0,012	0,156	HG-AAS	-0,6	-1,1	a
6*	<0,5	<0,5	<0,5					<0,5	ICP-AES			
7*	1,06				0,3	2	0,2	1,06	ICP-AES	34,4	5,9	c
8*	0,222	0,170			0,012	2	0,006	0,196	HG-AAS	0,9	2,2	b
9*	0,174	0,187	0,190		0,063	2	0,032	0,184	ICP-MS	0,5	0,4	c
10*	0,20	0,21			0,02	2	0,01	0,21	ICP-MS	1,3	2,4	a
11*	0,115	0,157			0,021	2	0,011	0,136	HG-AAS	-1,4	-2,6	a
12*	<0,85	<0,85	<0,85					<0,85	ZETAAS			
13*	0,107	0,123	0,116		0,016	√3	0,009	0,115	HG-AAS	-2,2	-4,4	a
14*	0,216	0,226	0,223		0,033	2	0,017	0,222	HG-AAS	1,9	2,6	a
15	0,160	0,161	0,177		0,009	2	0,005	0,166	ICP-MS	-0,2	-0,6	b
16	0,136	0,135	0,136	0,133	0,008	√3	0,005	0,135	HR-ICP-MS	-1,4	-3,6	b
17*	0,27	0,27	0,20		0,07	2	0,04	0,25	HG-AAS	2,9	2,1	c
18	0,140	0,149	0,139		0,011	2	0,006	0,143	ICP-MS	-1,1	-2,8	b
19*	0,15	0,16	0,15		0,02	2	0,01	0,15	ICP-MS	-0,7	-1,4	a
20*	0,178	0,190			0,0571	2,0	0,0286	0,184	ICP-MS	0,5	0,4	c
21*	0,167	0,143	0,153		0,031	2	0,016	0,154	HG-AAS	-0,7	-1,0	a
23	0,159	0,147			0,015	1	0,015	0,153	HG-AAS	-0,7	-1,1	a
24*	0,172	0,165	0,167		0,008	4,303	0,002	0,168	ICP-MS	-0,2	-0,4	b
25	0,141	0,121	0,125		0,023	2,49	0,009	0,129	HG-AAS	-1,7	-3,3	a
26*	0,135	0,151	0,139		0,022	2	0,011	0,142	HG-AAS	-1,2	-2,1	a
27	0,179	0,171	0,171		0,01	2	0,01	0,174	ICP-MS	0,1	0,2	b
28*	0,138	0,141	0,143		0,0151	√3	0,0087	0,1407	ICP-MS	-1,2	-2,5	b
29	0,148	0,146	0,156		0	√3	0	0,150	ICP-MS	-0,9	-2,4	b
30*	0,155	0,165			0,025	2	0,013	0,160	ETAAS	-0,5	-0,8	a
31	<0,10	<0,10	<0,10					<0,10	ICP-AES			
32	0,149	0,137	0,152		0,02	2,57	0,01	0,146	HG-AAS	-1,0	-2,2	b
33	0,11				0	√3	0	0,11	HG-AAS	-2,4	-6,9	b
34	0,15	0,15	0,15		0,035	√3	0,020	0,15	ICP-MS	-0,9	-1,0	a
36	0,143	0,145	0,143		0,021	2	0,011	0,144	HG-AAS	-1,1	-2,0	a
37	0,158	0,171	0,173		0,016	2	0,008	0,167	ICP-MS	-0,2	-0,4	b
38	0,25	0,22			0,11	2	0,10	0,24	HG-AAS	2,4	1,1	a
39	0,520	0,524	0,565		0	1,1338	0	0,536	ICP-AES	14,1	40,4	b
40	0,159	0,157	0,153		0,011	2	0,006	0,156	ICP-MS	-0,6	-1,5	b
41	0,533	0,585	0,605		0,025	2	0,013	0,574	ICP-OES	15,6	26,1	a
42	0,175	0,152			0	√3	0	0,164	HG-AAS	-0,3	-0,9	b
43	0,175	0,193	0,180		0,03	√3	0,02	0,18	HG-AAS	0,4	0,5	a
44	0,154	0,155	0,151		0,002	√3	0,001	0,153	ICP-MS	-0,7	-2,1	b
45	0,059	0,058			0,017	2	0,009	0,059	HG-AAS	-4,4	-9,2	a
46*	0,128	0,134			0,013	2	0,007	0,131	HG-AAS	-1,6	-3,7	b
47*	0,170	0,166			0,034	2	0,017	0,168	ICP-MS	-0,2	-0,2	a
48	<0,300	<0,300	<0,300	<0,300				<0,300	ETAAS			
49	0,1	0,1			0,1	2	0,1	0,1	ICP-OES	-2,8	-1,4	a
50	0,1791	0,1934			0,018	2	0,009	0,186	HG-AAS	0,6	1,1	a
51	0,90	0,84	1,03		0,26	2	0,13	0,92	ETAAS	29,1	5,8	c
52	0,14	0,14			15	√3	9	0,14	ICP-MS	-1,2	0,0	c

Lab ID	x_1	x_2	x_3	x_4	U_{lab}	k	u_{lab}	Mean-calc	Technique	z	ζ	$Qual_u$
53*	0,16	0,16			0	√3	0	0,16	ICP-MS	-0,5	-1,3	c
54	0,1499				0	√3	0	0,1499	ICP-MS	-0,9	-2,4	c
55	0,1	0,1	0,1		0,00046	2	0,00023	0,1	ICP-MS	-2,8	-8,0	c
58	0,1627	0,1598	0,1615	0,1611	0	√3	0	0,1613	AFS	-0,4	-1,2	b
59	0,146	0,142	0,146		0,029	2	0,015	0,145	ICP-MS	-1,1	-1,6	a
60*	0,739	0,811	0,709	0,715	0,016	2	0,008	0,744	ICP-AES	22,2	47,4	c
61	0,202	0,206	0,199		0,06	2	0,03	0,202	ICP-TOF-MS	1,2	1,0	c
62	0,18	0,17			0,1	2	0,1	0,18	HG-AAS	0,1	0,1	c
63	0,140	0,137	0,144		0,014	2,571	0,006	0,14	HG-AAS	-1,2	-3,0	b
65(a)	0,19	0,19			0,03	2	0,02	0,19	HG-ICPOES	0,7	1,0	a
65(b)	0,19	0,29			0,03	2	0,02	0,24	ICP-AES	2,6	3,9	
66	0,171	0,170	0,174		0,03	2	0,02	0,172	ICP-MS	0,0	0,0	a
67	0,11	0,10	0,13	0,13	0,02	2	0,01	0,12	ICP-MS	-2,1	-4,0	a
68	0,15	0,16	0,16		11,7	2	5,9	0,157	ICP-MS	-0,6	0,0	c
69	28,975				0	√3	0	28,975		1116,4	3191,4	b
70	0,14				0	√3	0	0,14		-1,2	-3,5	b
71	0,154	0,191			0,035	2	0,018	0,173	HG-AAS	0,0	0,0	a
72	0,15	0,16	0,15		0,01	√3	0,01	0,15	HG-AAS	-0,7	-1,7	b
73	0,14	0,14	0,15		0,07	√3	0,04	0,14	ICP-MS	-1,1	-0,7	c
74	0,172	0,164	0,166		0,014	2	0,007	0,167	ETAAS	-0,2	-0,4	b
75	0,18	0,18	0,16		0,02	√3	0,01	0,17	ICP-OES	0,1	0,1	a
76	0,1	0,1	0,1		0	√3	0	0,1	HG-AAS	-2,8	-8,0	b
77	0,178				0	√3	0	0,178		0,2	0,7	b
78	0,197	0,195	0,200		0,01	2	0,01	0,197	ICP-MS	1,0	2,5	b
79	0,194	0,205	0,212		13,5	2	6,8	0,204	ICP-MS	1,2	0,0	c
80*	0,205	0,195	0,217		0,062	2	0,031	0,206	HG-AAS	1,3	1,0	c
81	0,150	0,148	0,151		0,008	2	0,004	0,150	ICP-MS	-0,9	-2,3	b
82	0,28	0,37			0,023	2	0,012	0,33	ICP-AES	5,9	10,5	a
83	0,180	0,161	0,125	0,166	0,024	2	0,012	0,158	HR-ICP-MS	-0,5	-0,9	a
85	0,16	0,17	0,15		0,02	2	0,01	0,16	HG-AAS	-0,5	-0,9	a
86	0,180	0,190	0,176		0,018	2	0,009	0,182	HG-AAS	0,4	0,8	a
87	0,17	0,17	0,17		0,022	2	0,011	0,17	ICP-MS	-0,1	-0,1	a
88	0,153	0,121	0,111		0,061	2,776	0,022	0,128	HR-ICP-MS	-1,7	-1,8	a
89	0,1097	0,1100	0,1102		0,0022	2	0,0011	0,11	HG-AAS	-2,4	-6,8	b
90	0,100	0,090	0,095	0,100	0,019	2	0,010	0,096	ETAAS	-2,9	-5,8	a
91	0,155	0,169	0,166		0,057	2	0,029	0,163	ICP-MS	-0,3	-0,3	c
92*	0,180	0,184	0,182		0,033	2	0,017	0,182	ICP-MS	0,4	0,5	a
93	0,150	0,140	0,145		0,032	2	0,016	0,145	HG-AAS	-1,0	-1,5	a
94	0,165	0,160	0,198		0,035	2	0,018	0,174	ICP-MS	0,1	0,1	a
95	0,343				0,068	2	0,034	0,343	ICP-AES	6,6	4,9	c
96	0,174	0,157	0,172		40,877	2	20,439	0,168	ICP-MS	-0,2	0,0	c
97	<0,215	<0,216				2			ETAAS			
98	<0,25	<0,25	<0,25					<0,25	ETAAS			
99	0,175	0,178	0,182		0,036	√3	0,021	0,178	ICP-MS	0,2	0,3	a
100	0,08	0,08	0,06		0	√3	0	0,07	HG-AAS	-3,8	-10,9	b
101*	0,159				0,015	√3	0,009	0,159	ETAAS	-0,5	-1,0	a
102	0,184	0,192	0,180		0,0016	2	0,0008	0,185	ICP-MS	0,5	1,5	b
103	0,191	0,197			10	2	5	0,194	ICP-MS	0,9	0,0	c

$Qual_u$: 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 9.2.

Laboratory 57 reported "0" for total As and "<" for . The coordinator of IMEP-107 has interpreted that as a wrong way to report results, most likely "less than", and the decision was taken not to include those values in this table. No scoring was provided for those results.

Laboratory 65 reported two sets of results obtained with two different techniques (HG-ICPOES and ICP-AES). The two sets were scored independently and referred to as 65(a) and 65(b), respectively.



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 lines delimit the reference interval ($X_{ref} \pm 2u_{ref}$: 0.172 ± 0.018 mg kg^{-1}), the red lines delimit the target interval ($X_{ref} \pm 2\sigma$: 0.172 ± 0.052 mg kg^{-1})

Annex 8: Inorganic As in rice.

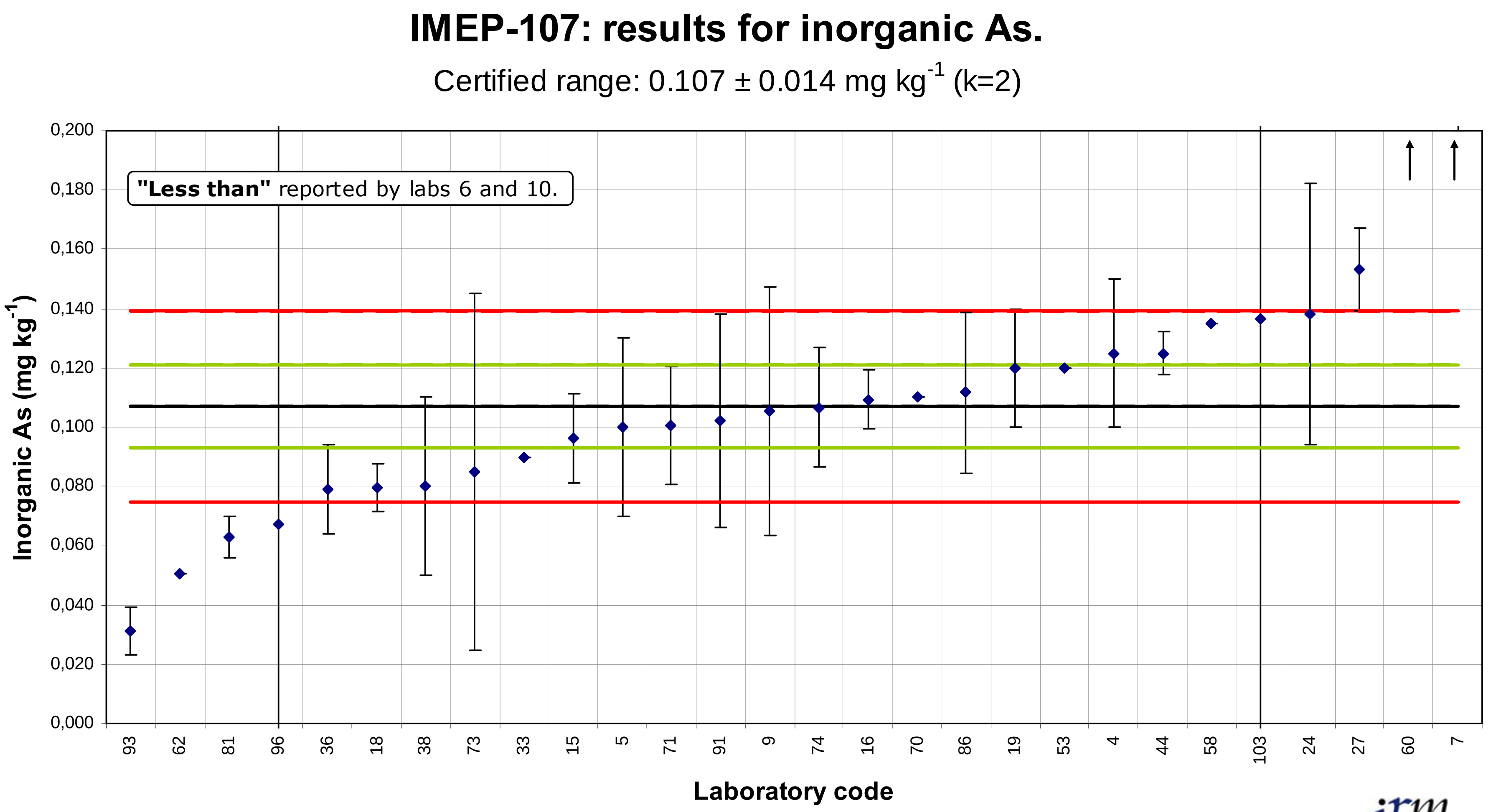
X_{ref}= 0.107 ± 0.014 mg kg^{-1} (*k*=2)

Lab ID	x_1	x_2	x_3	x_4	U_{lab}	k	u_{lab}	Mean-calc	Technique	z	ζ	$Qual_u$
4*	0,133	0,127	0,127	0,112	0,025	√3	0,014	0,125	LC-ICP-MS	1,1	1,1	**a**
5*	0,101	0,099			0,030	2	0,02	0,100	LC-ICP-MS	-0,4	-0,4	**c**
6*	<0,1	<0,1	<0,1					<0,1	HG-ICP-AES			
7*	0,29				0,09	2	0,05	0,29	ICP-AES	11,4	4,0	**c**
9*	0,109	0,110	0,097		0,042	2	0,021	0,105	LC-ICP-MS	-0,1	-0,1	**c**
10*	<0,2	<0,2						<0,2	LC-ICP-MS			
15	0,085	0,113	0,091		0,015	2	0,008	0,096	LC-ICP-MS	-0,7	-1,0	**a**
16	0,111	0,104	0,110	0,112	0,010	√3	0,006	0,109	LC-ICP-MS	0,1	0,3	**b**
18	0,080	0,079			0,008	2	0,004	0,0795	LC-ICP-MS	-1,7	-3,4	**b**
19*	0,13	0,12	0,11		0,02	2	0,01	0,12	LC-ICP-MS	0,8	1,1	**a**
24*	0,158	0,138	0,119		0,044	4,303	0,010	0,138	LC-ICP-MS	2,0	2,5	**a**
27	0,155	0,151			0,014	2	0,007	0,153	HPLC-ICP-MS	2,9	4,7	**a**
33	0,09				0	√3	0	0,09	HG-AAS	-1,1	-2,5	**b**
36	0,080	0,079	0,078		0,015	2	0,008	0,079	HG-AAS	-1,7	-2,7	**a**
38	0,08	0,07	0,09		0,03	2	0,02	0,08	HG-AAS	-1,1	-1,6	**c**
44	0,129	0,129	0,117		0,007	√3	0,004	0,125	LC-ICP-MS	1,1	2,3	**b**
53*	0,12	0,12			0	√3	0	0,12	LC-ICP-MS	0,8	1,9	**b**
58	0,1398	0,1357	0,1362	0,1284	0	√3	0	0,1350	AFS	1,7	4,1	**b**
60*	0,275	0,288	0,269	0,289	0,017	2	0,009	0,280	ICP-MS	10,8	15,8	**a**
62	0,047	0,054			0	√3	0	0,051	HG-AAS	-3,5	-8,2	**b**
70	0,11				0	√3	0	0,11		0,2	0,4	**b**
71	0,107	0,094			0,020	2	0,010	0,101	HG-AAS	-0,4	-0,5	**a**
73	0,09	0,08			0,06	√3	0,04	0,09	HG-AAS	-1,4	-0,6	**c**
74	0,108	0,104	0,108		0,020	2	0,010	0,107	ETAAS	0,0	0,0	**a**
81	0,062	0,063	0,063		0,007	2	0,004	0,063	LC-ICP-MS	-2,8	-5,7	**b**
86	0,110	0,115	0,110		0,027	2	0,014	0,112	HG-AAS	0,3	0,3	**a**
91	0,100	0,104	0,102		0,036	2	0,018	0,102	HG-AAS	-0,3	-0,3	**c**
93	0,031	0,028	0,035		0,008	2	0,004	0,031	HG-AAS	-4,7	-9,5	**b**
96	0,0683	0,0660			0,2425	2	0,1213	0,0672	LC-ICP-MS	-2,5	-0,3	**c**
101	< 0.05	0.06			0.005	√3	0.003	#	LC-ICP-MS			**b**
103	0,135	0,138			7	2	4	0,137	LC-ICP-MS	1,8	0,0	**c**

$Qual_u$: 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 9.2.

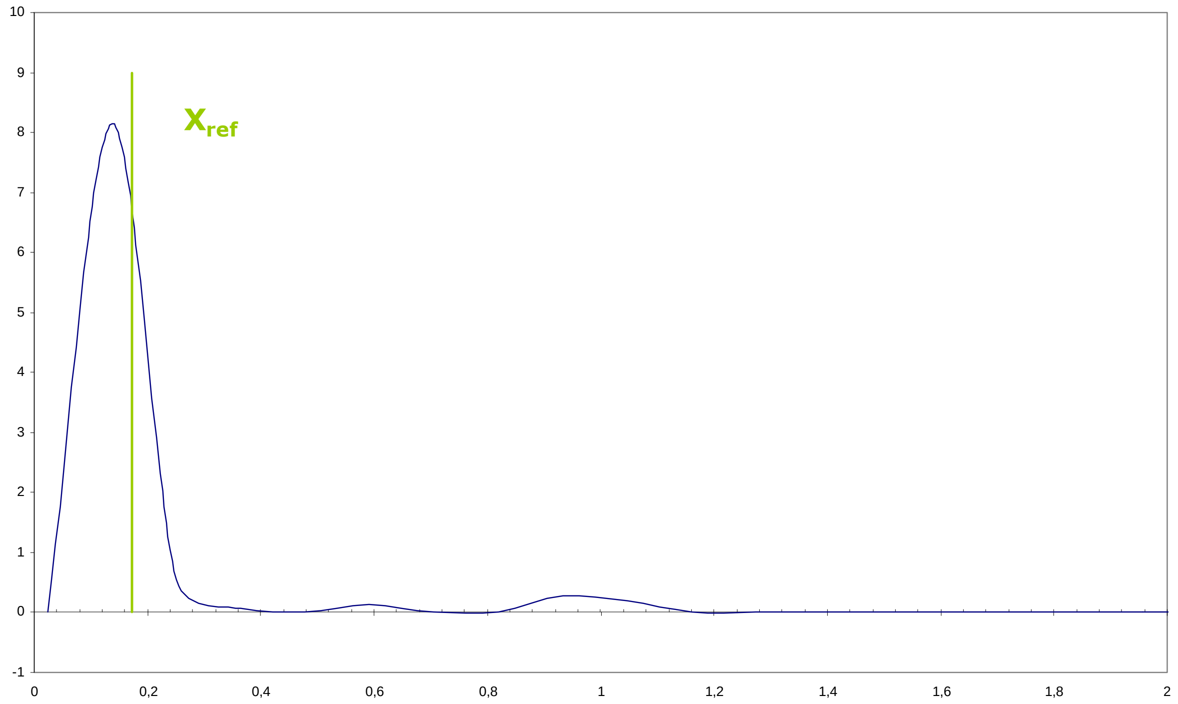
Laboratory 41 reported 0.000 ± 0.002 (k=2). The coordinator of IMEP-107 has interpreted that as a wrong way to report results, most likely "less than", and the decision was taken not to include those values in this table. No scoring was provided for those results.
Laboratory 69 reported "Not detected". Since no further numerical information was reported, this laboratory was not included in the table.

#Considering the results reported by Laboratory 101, no mean could be calculated and so no scoring was given to the results reported by this laboratory.

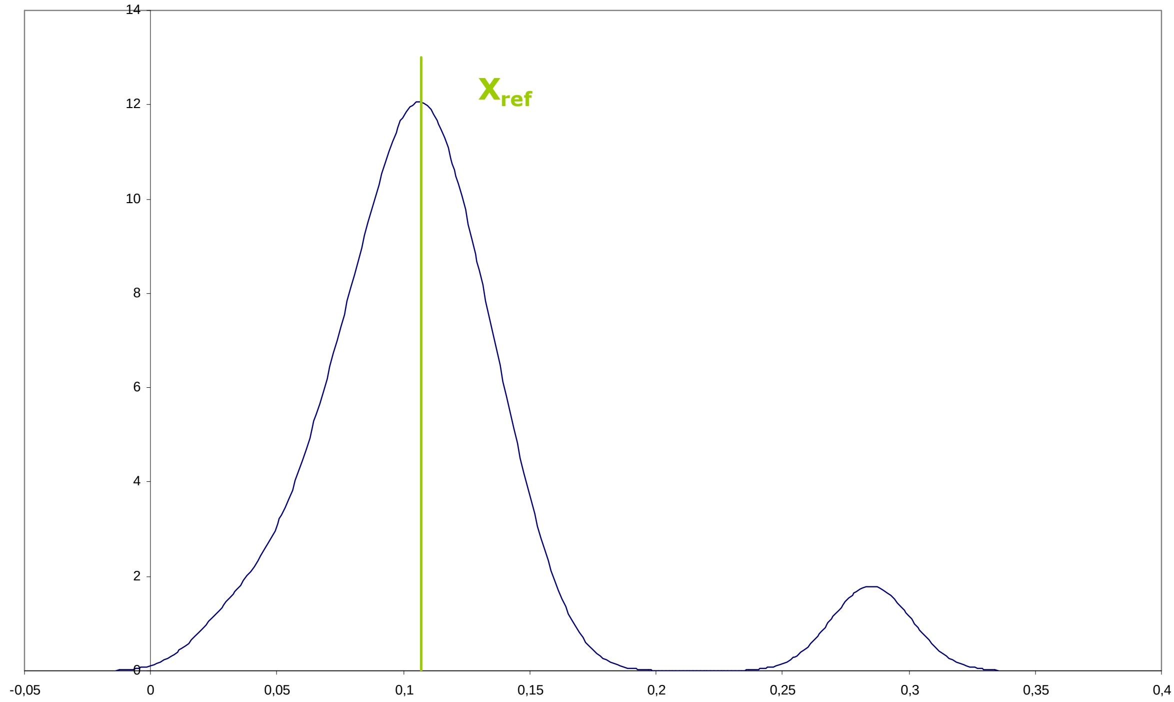


This graph displays all measurements results and their associated uncertainties.
The uncertainties are shown as reported, with various expansion factors and levels of confidence.
The black line represents Xref, the green lines delimit the reference interval (X_{ref} ± $2u_{ref}$: 0.107 ± 0.014 mg kg^{-1}), the red lines delimit the target interval (X_{ref} ± 2σ: 0.107 ± 0.032 mg kg^{-1})

Annex 9: Kernel distributions



Kernel density plot for total As (Lab 69 is not shown in the graph to avoid an unnecessarily large x axes).



Kernel density plot for inorganic As

Annex 10: Experimental details for total arsenic determination

Lab ID	SOP?	If Yes, which?	Sample pre-treatment	Digestion step	Extraction / separation step	Calibration step/instrumental set-up
1	no		None	Microwave digestion using nitric acid, hydrogen peroxide & water	None	ICP-MS instrument performance check, followed by As calibration & QC standards
2	no		Homogenise	Microwave digestion	n.a.	Calibration using certified standard solutions
3	no			Microwave and pressure digestion		
4	yes	AFSSA MET08 used as LNR method	No	Microwave digestion (3mL HNO_3 + 3mL H_2O)		External calibration
5	yes	AOAC				
6	no		No pre-treatment	Wet digestion with nitric acid 5 ml and H_2O_2 3 ml at 180°C	No extraction	External calibration
7	no			HNO_3 conc. at 90°C, 3 hours		ICP OES / 4 standards + Reference sample
8	no		Samples are grinded before ashing	Ashing of 2 g of sample with the addition of magnesium nitrate and magnesium oxide	Reduction of samples with a 20w% KI and ascorbic acid solution	HG-AAS
9	no		Shaking	Microwave assisted	With nitric acid	Yes
10	no					ICP-MS measurement with octopole reaction system
11	no					
12	no			Microwave high pressure digestion with H_20_2 (30%) and HNO_3 conc. and HF conc		Add method: std solution 20 ppb
13	no		Homogenization,	Dry	No	
14	yes	EN 14546:2005				
15	no		0.25 g of sample in 2 mL of nitric acid	High Pressure Asher (20 °C to 90 °C (20min), 90 °C to 150 °C (20min), 20 °C to 180 °C (30min), then cooled to ambient temperature)	In 10 mL of milli Q water	-1,0%
16	yes	DIN EN 13805 for decomposition		Nitric acid - closed vessel - 210°		External calibration, HR-mode of ICP-MS

Lab ID	SOP?	If Yes, which?	Sample pre-treatment	Digestion step	Extraction / separation step	Calibration step/instrumental set-up
17	no		Treated with ammonium metavanadate and nitric acid	Nitric acid digestion	Using permanganate / hydrogen peroxide and sulphuric acid	
18	no		Microwave Digestion			External Calibration plus Internal Standardisation
19	no		Shaking of sample bottle	Microwave digestion using conc HNO_3		Addition calibration with internal standardisation
20	yes	NMKL Trace Elements-As, Cd, Hg, Pb and other elements. No. 186, 2007.				
21	yes	SR EN 14546/2005				
23	yes					
24	no		cc. nitric acid	MW digestion	Only dilution with water	Fully quant (external) calibration / Q-ICP-MS
25	yes	EN 14546				
26	yes	National Feed Codex				
27	yes					
28	yes	Standard method for determination of total As by ICP-MS				
29	no			Microwave digestion		6 point calibration standard using ICP-MS
30	no			Microwave digestion with HNO_3		external standard
31	no		Dry ash 550°C	HNO_3 + HCL	No	
32	yes	MSZ EN 14627:2005				
33	no		Sample aliquot mixed with suspension of $MgNO_3$/MgO ashing aid, which was then dried at 105°C.	Ashing of dried suspension at 500°C	Ash dissolved in HCl and solution made to volume.	Analysis of solution using automated Hydride generation AAS
34	no		Sample was homogenised by stirring with a plastic spatula in a circular motion at least 5 times before weighing.	1 g sample was taken and digested at 100 degrees for an hour using nitric and hydrofluoric acid	The digested sample was made to 50 ml final volume and analysed on ICPMS.	A 5 point calibration was done for As using certified commercial standards on the ICPMS.
36	yes	EN 14546:2005				
37	no		Addition of HNO3 and H2O2, let stand half an hour	Microwave digestion	Dilution	External calibration
39	yes					
40	no		Water content removal	Nitric acid and Hydrogen peroxide		ICP MS

Lab ID	SOP?	If Yes, which?	Sample pre-treatment	Digestion step	Extraction / separation step	Calibration step/instrumental set-up
41	no			Yes, with solution of misxture HNO_3 - H_20_2 (instrument milestone) and after ICP/OES		
42	no					
43	no		Add magnesium nitrate.	Add nitric acid and leave to stand overnight.	Reflux on hotplate for 30 minutes, evaporate to dryness and heat in muffle furnace for 5 hours. Add hydrochloric acid and transfer to volumetric flask.	Hydride generation AAS. Lamp absorption optimisation and independent standard check solution analysed.
44	yes	Methods from a National Veterinary and Food Administration				
45	no		Treated with nitric acid.	Reflux for 30 minutes.	Sample dried, ashed then dissolved in hydrochloric acid.	Standards ranging from 0 - 25 ug/L As used.
46	no		$Mg(NO_3)_2$			10,30,50 ng As
47	yes	NMKL Method No Method 186, 2007				
48	no		Mixing and weighing approx. 1 g digested in 65% nitric acid	Microwave pressure digestion		Method of standard addition
49	yes	AOAC 957.22 (Modified)				
50	no			Using microwave digestion (In-house method based on AOAC 986.15)		
51	yes	EN 14627 and EN 13805				
52	yes					
53	no	External	No	Microwave with Ac. Nitric		Yes
54	no			Digestion of Sample with HNO_3 and Water 1:1		
55	no		Homogonised sample	Microwave digestion with Nitric acid	none	External calibration using commercial arsenic standard
57	no		no	Microwave digestion	no	linear calibration (0, 5, 10, 20, 40, 100ppb), R=0,99967
58	yes	GB/T5009.11-2003				
59	no		Homogenization	Digestion with aqua regia with digiprep	Filtration	internal calibration
60	yes	AOAC999.10				
61	yes	EN 13805				

Lab ID	SOP?	If Yes, which?	Sample pre-treatment	Digestion step	Extraction / separation step	Calibration step/instrumental set-up
62	yes	Based on MAFF Report FD 96/31, Determination of Arsenic in Food, November 1996 and MAFF trial 124 pretreatment 1996				
63	yes	MSZ EN 14546:2005				
65	no		-	HNO_3 digestion	-	By ICPOES or Continuous flow hydride generation using ICP/OES
66	yes	EN 13805, 2002 and ISO 17294: Part 1+2 (2005)				
67	no		Microwave digestion	Increase the digest temperature to 180°C then remain at least 3 minutes		Use ICP-MS for detection
68	no		Sample mixed thoroughly	Digested in microwave digestor using nitric and hydrochloric acid	Filtration	3 point calibration plus blank.
69	yes	GB/T 5009.11-2003				
71	No	No	No	Microwave digestion +dry mineralisation	No	Yes, calibration curve
72	yes	MSZ EN 14546:2005				
73	no		Blend and Homogenise	H_2O_2 and nitric acid	Microwave digestion	ICPMS
74	no		0.5 g digested with 5 mL c. HNO_3 + 1 mL H_2O_2	Microwave digestion	Dilution to 20 mL with ultrapure water.	Matrix matched calibration. ETAAS: SIMAA 6000-Zeeman, THGA, 1ug Pd as modifier.
75	no		Manual mixing	Microwave oven digestion with HNO_3		ICP-OES with calibration standards 0, 10, 25, 50, 100 ppb
76	yes	EN 14546				
78	no		HNO_3+H_2O_2	Microwave digestion	Microwave digestion	ICP/MS
79	no		None	Microwave assisted digestion	None	Tuning before calibration; calibration (blank + 5 calibration points)
80	yes	EN 14627				
81	no		No	Microwave digestion	no	
82	no		None	Niric acid + H_2O_2 . Microwave oven		Blank + four standards
83	yes					
85	yes	MSZ EN 14546:2005				

Lab ID	SOP?	If Yes, which?	Sample pre-treatment	Digestion step	Extraction / separation step	Calibration step/instrumental set-up
86	yes	UNE-EN 14546				
87	no		None	Microwave digestion with nitric acid	none	Multipoint calibration standard
88	no		Use honey method; CRM Rice is used as our ref. material. for this in-house method	Weight out dry rice sample, add nitric acid and do microwave digestion. Ashing at 180°C	N/A	Use Agilent 7500 ICP-MS with reaction cell. Analysis As in hydrogen mode. 6 points calibration.
89	yes	AOAC (2005) 986.15				
90	no			microwave digestion with HNO_3 and H_2O_2		GFAAS
91	yes	ASU L 00.00-19/6 (ICP-MS)				
92	no					
93	yes					
94	yes	EPA 3051 for dissolution and ISO 17294 for determination of As				
95	no		No	Microwave wet digestion	No	Yes
96	no		None.	Microwave using nitric acid and peroxide		ICP-MS in DRC mode with internal standard.
97	no		Dry sample	Microwave acid digestion		Electrothermal Atomic Absorption Spectroscopy
98	yes	Electrothermal Atomic Absortion Spectroscopy				
99	no			Microwave HNO_3 & H_2O_2		ICP-MS
100	yes	AOAC				
101	yes					
102	yes	AOAC 2005				
103	no			Closed vessel MW assisted digestion with nitric acid and hydrogen peroxide		Standard addition

Annex 11: Experimental details for inorganic arsenic determination

Lab ID	SOP?	If Yes, which:	Sample pre-treatment	Digestion step	Extraction / separation step	Instrument calibration step/instrumental set-up
04	no				Microwave assisted extraction (10mL H_2O; 7min; max 80°C)	External calibration
05	no				Trifluoracetic Acid (2 M)	HPLC-ICP-MS
07	yes					
09	no		Shaking	Microwave assisted (80°C with stirring)	With water	Yes
15	no		0.2 g in 3mL enzymatic solution (alpha-amylase 3 mg/mL + protease XIV 10mg/mL)		Ultrsonic assisted extraction (5 min; Power: 60%) then centrifugation, filtration and in 10 mL of milli Q water	-1,0%
16	no				Extraction by 0,07 m HNO_3/95°C/90 min - filtration 0,45 µm - HPLC: Hamilton PRP X-100 250mm, 20 mM $NH_4H_2PO_4$	External calibration, LR-mode of ICP-MS, 20 µl sample volume
18	no		None	None	TMAOH	External Calibration plus post-column Reference Standard
19	no		Shaking of sample bottle		Microwave extraction at 90°C using aqueous HCl/H_2O_2	External calibration with matched standards
24	no		Alpha amylase pre-treatment (shaking at room temperature) for one night	50 Met-OH extraction in ultravoice -two times	Centrifuge (4000 rpm/10 min) - two times	Fully quant (external) calibration / HPLC-ICP-MS
27	no		Self-warming to ambient temperature, hand-shake homogenisation for 2 min, settling for 3 min	Extraction with deionised water for 15 min with an ultrasonic probe	Strong anion exchange HPLC to separate As (III) and As(V), hyphenated to ICP-MS	Individual standard addition for As(III) and As(V), inorganic arsenic is counted as the sum of As(III) and As(V)
33	no		Solubilzation of As in 9 M HCl with overnight extraction. Reduction, and extraction of inorganic arsenic into chloroform. Back extraction of arsenic into 1M HCL.	Extract solution mixed with suspension of $MgNO_3$/MgO ashing aid, which was then dried at 105°C. Dried suspension ashed at 500°C, and ash dissolved in HCl		Quantification using automated Hydride generation AAS

Lab ID	SOP?	If Yes, which:	Sample pre-treatment	Digestion step	Extraction / separation step	Instrument calibration step/instrumental set-up
36	no		Hydrolysis in HCl, As(V) to As(III) reduction (HBr+hydrazine sulfate)		Selective with $CHCl_3$; back extraction with HCl	
38						
44	no				HCl/H_2O_2, 20 minutes in microwave at 90°C	
53	no	External	No		Enzimatic	Yes
58	yes	GB/T5009.11-2003				
60	no		Dry the sample for 18h at 85+/-2 C	Use 6ml concentrated HCl and 2ml concentrated HNO_3	Filtration	Standard As solutions
62	yes	The Analyst 1999 Munoz,Velez and Montoro determination of inorganic arsenic in seafood by acid digestion, solvent extraction and HG-AAS				
69	yes	GB/T5009.11-2003				
71	No		No pre-treatment	Microwave digestion	no	Yes, calibration curve
73	no		Blend and homogenesis	HCL and KI	Distillation	AA
74	no		0.5g was weighted in a 50mL tube	5 mL HNO_3 1M were added and vortexed and ultrasonicated for 15min and centrifuged 4000rpm/15min	15 mL EDTA 0.1% (w/v) were added, vortexed and centrifuged 4000rpm/15min. The supernatant were analyzed by ETAAS	Standard addition method. ETAAS: As total As
81	no		No	No	0.07mol/L HCl Microwave-assisted extraction	
86	no		Analyst 1999. Vol 124 og 601-607	Dry ashing, reduction As(V)-As (III)	Solvent extracion, chloroform, hydrocloric acid	FIAS Hydride generation AAS
91	no				Extraction with 0.07 N HCl, 1,5 h by 95°C	External calibration
93	yes					
96	no		None.	None.	25% Methanol extraction, enzymatic digestion and incubation.	LC/ICP-MS in DRC mode, anionic exchange column
103	no				Chemical extraction	Speciated standard addition

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 seventh ILC of the EU-RL-HM (former CRL-HM) which focused on the determination of total and inorganic As in rice. The test item used in this exercise is rice purchased in a local supermarket and was provided by the University of Aberdeen. The test item was processed, bottled and labelled at IRMM and dispatched to the participants the first half of December 2009. Each participant received one bottle containing approximately 20 g of test item. 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 rice. One hundred and three laboratories from 35 countries registered to the exercise, of which 98 reported results for total As and 30 for inorganic As. Twenty-nine of the participants were NRLs of the EU-RL-HM network, 8 out of which reported values for inorganic As.

The assigned values for IMEP-107 were provided by a group of laboratories expert in the field: seven for total As and six for inorganic As.

The uncertainties of the respective assigned values, u_{ref}, were derived from the standard deviation of the means provided by the experts, propagated with a 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 deviation for proficiency assessment (also called target standard deviation) was fixed to 15 % by the advisory board of this ILC, on the basis of the outcome of previous ILCs organised by the EU-RL-HM and on the state-of-the-art in this field of analysis.

Around 75 % of the participants performed satisfactory for total and inorganic As.

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

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