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CERTIFICATION REPORT

The certification of the mass fractions of total arsenic, dimethylarsinic acid and the sum of arsenite and arsenate in rice

Certified Reference Material ERM®-BC211

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European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information
Reference materials sales
Retieseweg 111
B-2440 Geel, Belgium
E-mail: jrc-irmm-rm-sales@ec.europa.eu
Tel.: +32 (0)14 571 705
Fax: +32 (0)14 590 406

http://irmm.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

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The certification of the mass fractions of total arsenic, dimethylarsinic acid and the sum of arsenite and arsenate in rice

Certified Reference Material ERM®-BC211

J. Boertz, H. Emteborg, J. Charoud-Got, J. Snell, A. Held, H. Emons

European Commission, Joint Research Centre,
Institute for Reference Materials and Measurements (IRMM), Geel, Belgium

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Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM-BC211, a powdered rice material certified for the mass fractions of total arsenic, dimethylarsinic acid and the sum of arsenite and arsenate. The material has been produced following ISO Guide 34:2009 [1].

The starting material, which had been checked for its arsenic species was purchased and supplied by the University of Aberdeen. The rice was milled, sieved, dried, homogenised, filled in vials and sterilised.

Between unit-inhomogeneity was quantified and stability during dispatch and storage was assessed in accordance with ISO Guide 35:2006 [2]. Within-unit inhomogeneity was quantified to determine the minimum sample intake.

The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were estimated in compliance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM) [3] and they include contributions from possible between-unit inhomogeneity, instability and characterisation.

The material is intended for quality control and assessment of method performance. Moreover, it can be used for validation purposes and trueness determination. The CRM is available in glass bottles containing 10 g of dried rice powder closed under argon atmosphere. The minimum amount of sample to be used for total arsenic and dimethylarsinic acid is 50 mg. The minimum amount of sample to be used for the sum of arsenite and arsenate is 100 mg.

The CRM has been accepted as European Reference Material (ERM) after peer evaluation by the partners of the European Reference Materials consortium.

The following values were assigned:

RICE		
	Mass fraction based on dry mass	
	Certified value [3] [µg/kg]	Uncertainty [4] [µg/kg]
Total arsenic	260	13
Dimethylarsinic acid [1,2]	119	13
The sum of arsenite and arsenate [1]	124	11

1) Expressed as arsenic
2) As obtained by extraction and subsequent quantification *via* HPLC-ICP-MS
3) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified values are traceable to the SI.
4) The certified uncertainty is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

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Glossary

AAS	Atomic absorption spectrometry
AEX	Anion exchange
AFS	Atomic fluorescence spectrometry
ANOVA	Analysis of variance
Arsenite/arsenate	The sum of arsenite and arsenate
c	Mass concentration $c = m / V$ (mass / volume)
CEN	European Committee for Standardization
CRM	Certified Reference Material
DMA	Dimethylarsinic acid
EC	European Commission
ERM	Trademark of European Reference Materials
EU	European Union
GUM	Guide to the Expression of Uncertainty in Measurements *[ISO/IEC Guide 98-3:2008]*
HG-AFS	Hydride generation-atomic fluorescence spectrometry
HPLC	High performance liquid chromatography
IAEA	International Atomic Energy Agency
ICP	Inductively Coupled Plasma
ICP-QMS	ICP-Quadrupole mass spectrometry
ICP-SFMS	ICP-Sector field mass spectrometry
IRMM	Institute for Reference Materials and Measurements
IC	Ion chromatography
ISO	International Organization for Standardization
JRC	Joint Research Centre
k	Coverage factor
k_0 NAA	k_0 Neutron Activation Analysis
m	Mass
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
n.a.	Not applicable
n.c.	Not calculated
NIST	National Institute of Standards and Technology (USA)
NMIJ	National Metrology Institute of Japan
p	Number of technically valid datasets
PTFE	Polytetrafluorethylene
QC	Quality control
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference Material
RSD	Relative standard deviation
s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added as appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SCK	Studiecentrum voor Kernenergie
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
s_{ns}	Standard deviation of results of normal stock samples
SRM®	Trademark of NIST CRMs (Standard Reference Material)
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation; an additional index "rel" is added as

	appropriate
T	Temperature
T	Time
TFA	Trifluor acetic acid
t_i	Time point for each replicate
t_R	Retention time
t_{sl}	Proposed shelf life
U	standard uncertainty
U	expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_{Δ}	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_m	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability
UV	Ultraviolet
V	Volume
$\overline{x}$	Arithmetic mean
Δm	Absolute difference between mean measured value and the certified value
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}
$\overline{y}$	Average of all results of the homogeneity study

1 Introduction

1.1 Background

Arsenic is a very wide-spread trace element in the natural environment. The inorganic forms of arsenic are highly toxic and carcinogenic; therefore there is a need to keep human diets as free from these forms as reasonably achievable. Rice is known to concentrate arsenic within the plant compartments [4,5]. Moreover, a substantial quantity of rice is grown in regions where the arsenic concentration in the ground water is high [6]. In contrast to fish and seafood, rice grains and rice-based products are the main sources of inorganic arsenic to the general European population [7,8]. Rice contributes significantly to the arsenic burden of ethnic groups with a high daily rice intake [9]. Arsenic occurs in different molecular forms in rice, i.e. inorganic forms (arsenite and arsenate) and the organic species (mainly dimethylarsinic acid (DMA) and methylarsonic acid. Hence, regulatory bodies have changed regulations from total arsenic concentration in foodstuff to the concentration of inorganic arsenic [10]. At this time the only rice certified reference materials available in Europe are certified for total arsenic content. The addition of a rice reference material with certified arsenic speciation data for use as a quality control for the arsenic species determination will better address the salient health risks of arsenic in rice.

ERM®-BC211 is certified for mass fractions of total arsenic, the sum of arsenite and arsenate (arsenite/arsenate) and dimethylarsinic acid using an inter-laboratory comparison approach. The material was chosen after a feasibility study investigating ten different rice samples [11]. The selected rice showed an increased arsenic level and elevated level of DMA. The rice was milled, mixed and freeze-dried in order to ensure homogeneity and stability of the material.

The use of ERM-BC211 is mainly intended for method development as well as instrument performance checks, and quality assurance, namely determination of analytical trueness and precision related to arsenic speciation in rice and similar matrices.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.2 Sourcing and processing

University of Aberdeen, Trace Element Speciation Laboratory, Aberdeen, UK

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

Isotron Nederland B.V., Etten-Leur, NL

2.3 Homogeneity study

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

Karl-Franzens University Graz, Institute of Chemistry, Analytical Chemistry, Graz, AT

2.4 Stability study

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

Karl-Franzens University Graz, Institute of Chemistry, Analytical Chemistry, Graz, AT

University of Aberdeen, Trace Element Speciation Laboratory, Aberdeen, UK

2.5 Characterisation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

Bavarian Health and Food Safety Authority (LGL), Erlangen, DE
(measurements under the scope of ISO/IEC 17025 accreditation, SAL No. SAL-BY-L20-04-03)

Bavarian Health and Food Safety Authority (LGL), Oberschleissheim, DE
(measurements under the scope of ISO/IEC 17025 accreditation, SAL No. SAL-BY-L20-04-03)

Danish Veterinary and Food Administration (DVFA), Region West, Lystrup, DK
(measurements under the scope of ISO/IEC 17025 accreditation, DANAK 424)

Fraunhofer Institute for Molecular Biology and Applied Ecology (Fraunhofer IME), Department Environmental Specimen Bank and Elemental Analysis, Schmallenberg, DE
(measurements under the scope of ISO/IEC 17025 accreditation, DACH No. DAC-PL-0029-97-20)

French Agency for Food, Environmental and Occupational Health & Safety, Laboratory of Food Safety, Maisons-Alfort, FR
(measurements under the scope of ISO/IEC 17025 accreditation, COFRAC No. 1-0245)

GALAB Technologies GmbH, Geesthacht, DE
(measurements under the scope of ISO/IEC 17025 accreditation, DACH No. DAC-PL-0114-00-00)

Institute of Agrochemistry and Food Technology (IATA-CSIC), Paterna (Valencia), ES

Karl-Franzens University Graz, Institute of Chemistry, Analytical Chemistry, Graz, AT

Lab Salud Publica Vizcaya, Bilbao, ES
(measurements under the scope of ISO/IEC 17025 accreditation, ENAC No. 132/LE326)

Laboratory of the Public Health Agency of Barcelona (ASPB), Barcelona, ES
(measurements under the scope of ISO/IEC 17025 accreditation, ENAC No. 227/LE459
and No. 227/LE1338)

University of Aberdeen, Trace Element Speciation Laboratory, Aberdeen, UK

University of Barcelona, Analytical Chemistry Department, Barcelona, ES

University of Corvinus, Analytical Laboratories, Corvinus, HU
(measurements under the scope of ISO/IEC 17025 accreditation, Hungarian Accreditation Board NAT-1-1462/2006)

Studiecentrum voor Kernenergie, SCK, Mol, BE
(measurements performed under ISO/IEC 17025 accreditation; BELAC, 015-TEST)

Veterinary and Agrochemical Research Centre (CODA-CERVA), Tervuren, BE
(measurements under the scope of ISO/IEC 17025 accreditation, BELAC No. 172-TEST)

3 Material processing and process control

3.1 Origin and processing of the material

The rice selected for the production of ERM-BC211 was purchased, analysed for total arsenic as well as arsenic species and supplied by the University of Aberdeen. After receipt at IRMM the rice was transferred into plastic drums and stored at -20 °C. Thereafter, about 25 kg of rice was milled, sieved and mixed. The milling procedure was carried out under liquid nitrogen by using a cryo-grinding vibrating mill (Palla mill, KHD, Humboldt-Wedag, Köln, DE) which was previously cooled to about -190 °C. All parts in contact with the material were of high purity titanium. Afterwards, the material was sieved at 125 µm. The coarse fraction was milled and sieved again. The <125 µm fraction corresponded to about 84% (21 kg) of the total mass. The material was homogenised for several hours by using a DynaMix CM200 3-dimensional mixer (WAB, Basel, CH).

3.2 Process control

Particle size distribution was checked on randomly selected samples by using a Laser Light Diffraction Sympatech Helos Analyser as well as *via* microscopic pictures by means of a Zeiss microscope Stemi 2000-C (Figure 1).

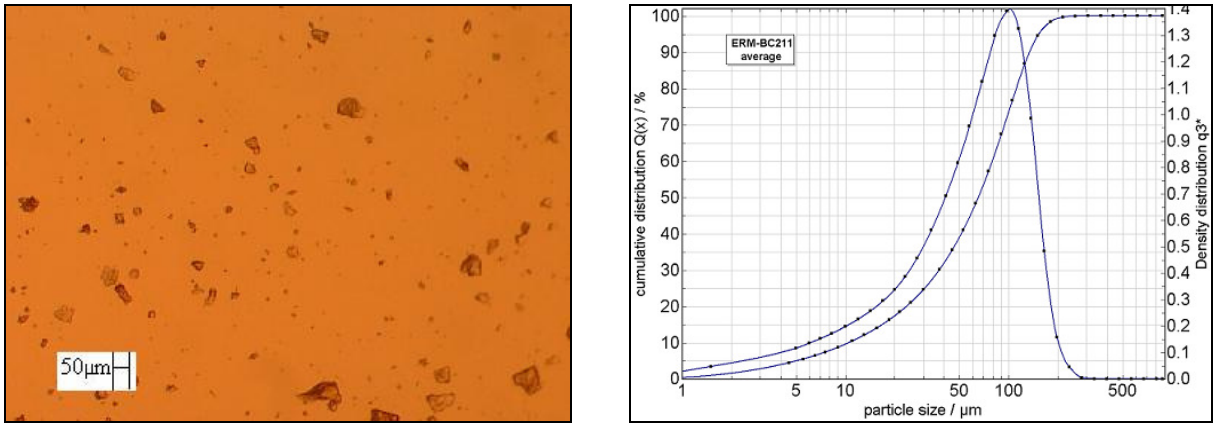

Figure 1: Typical micrograph for milled rice (left) and average particle size distribution obtained using 2-propanol as dispersant (5 replicates, right)

The moisture content of the milled rice was checked throughout the entire process and decreased to a final value below 5 % (m/m) by vacuum drying before bottling. A batch of 1558 units each filled with 10 g of rice was produced. The units were sterilised by gamma irradiation (Isotron B.V., NL) with an average radiation dose of 25 kGy, in order to minimise any remaining bacterial activity.

4 Assessment of homogeneity

A key requirement for any reference material is the equivalence between the various units. In this respect, it is not relevant whether the variation between units is significant compared to the analytical variation, but whether this variation is significant to the certified uncertainty. Consequently, ISO Guide 34 requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit inhomogeneity is therefore necessary to determine the minimum sample intake.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all units of the material within the stated uncertainty.

15 samples were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into 15 groups (with similar number of units) and one unit was randomly selected from each group. The number of selected units corresponds to approximately the cubic root of the total number of the produced units. Three independent samples were taken from each unit and analysed by ICP-QMS (total arsenic) and HPLC-ICP-QMS (arsenite/arsenate and DMA). The measurements were performed under repeatability conditions and in a randomised sequence in order to separate a potential analytical drift from a trend in the filling sequence. The results were corrected for the water content determined three times in each unit as follows: a minimum mass of 0.5 g of the material was dried for at least 24 h in a ventilated oven at 100 ± 2 °C until constant mass was attained (maximum of two weighings).The measured water content was in the range from 25.6 to 28.1 g/kg.

The relative between-unit variation of the total arsenic content was 1.3 % whereas between-unit variations of arsenite/arsenate and DMA were all below 3 %.The results are also shown graphically in Annex A.

Regression analyses were performed to evaluate potential trends in the analytical sequence or trends in the filling sequence. No trends in neither the filling sequence nor the analytical sequence were observed for total arsenic and DMA (95 % and 99 % confidence level). However a significant (95 % and 99 % confidence level) trend in the analytical sequence was observed for arsenite/arsenate, which was caused by an instability of the analytical system on the first day of measurements. As the effect of this instability is very small, no correction was applied.

The dataset was tested for consistency using Grubbs outlier tests on a confidence level of 99 % on the individual results and the unit means. Some outlying individual results and outlying unit means were detected. Since no technical reason for the outliers could be found, all data were retained for statistical analysis.

Quantification of between-unit inhomogeneity is most easily done by analysis of variance (ANOVA), which can separate the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples are representative for the whole unit.

Evaluation by ANOVA requires unit means to follow at least a unimodal distribution and results for each unit to follow unimodal distributions with approximately the same standard deviation. Distribution of the unit averages was tested using histograms and normal probability plots. Too few unit averages were available to make a clear statement of the distribution of the averages. Therefore, it was checked whether all individual data followed a unimodal distribution using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not grossly affect the estimate of between-unit standard deviations. The results of the statistical evaluations are given in Table 1.

Table 1: Results of the statistical evaluation

	Trends		Outliers		Distribution	
	Analytical sequence	**Filling sequence**	**Individual results**	**Unit means**	**Individual results**	**Unit means**
Total arsenic	no	no	1–statistical reason (retained)	none	normal	normal
DMA [1)]	no	no	2–statistical reason (retained)	1	normal	normal
Arsenite/ arsenate [1)]	yes	no	1–statistical reason (retained)	2	normal	normal

1) expressed as arsenic

One has to bear in mind that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [12]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between–unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\overline{y}}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\overline{y}}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}}\sqrt[4]{\frac{2}{v_{MSwithin}}}}{\overline{y}}$$

MS_{within}: mean square within a unit from an ANOVA

$MS_{between}$: mean squares between-unit from an ANOVA

$\overline{y}$:
average of all results of the homogeneity study

n: average number of replicates per unit

$v_{MSwithin}$: degrees of freedom of MS_{within}

However, a different approach was adopted for the sum of arsenite and arsenate and DMA for which at least one outlying unit average was detected. In this case between-unit

inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit average, and the rectangular standard uncertainty of homogeneity was estimated as given by

$$u_{rec} = \frac{\left| outlier - \bar{\bar{y}} \right|}{\sqrt{3} \cdot \bar{\bar{y}}}$$

where:

$\bar{\bar{y}}$

: average of all results of the homogeneity study

It should be mentioned that the outlying unit averages are a result of the presence of outlying individual values and do not necessarily reflect the real distribution of these elements in the material.

The results of the evaluation of the between-unit variation are summarised in Table 2.

Table 2: Results of the homogeneity study
n.c.: cannot be calculated as $MS_{between} < MS_{within}$; n.a.: not applicable

	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rec,rel}$ [%]	$u_{bb,rel}$ [%]
Total arsenic	4.453	1.141	1.306	n.a.	1.306
DMA [1)]	3.676	n.c.	1.078	2.857	2.857
Arsenite/ arsenate [1)]	4.256	n.c.	1.249	2.596	2.596

1) expressed as arsenic

The homogeneity study showed no trend in the filling sequence for any of analytes and no outlying unit mean for total arsenic. Thus, the between-unit standard deviation for total arsenic can be used as an estimate of u_{bb}. As u^*_{bb} sets the detection limit of the homogeneity study, the larger values of s_{bb} and u^*_{bb} are adopted as uncertainty contributions to account for potential inhomogeneity.
In case of the sum of arsenite and arsenate and DMA, for which outlying unit means were found, u_{rec} was used as a conservative estimate of u_{bb}.

4.2 Within-unit homogeneity and minimum sample intake

The within-unit inhomogeneity is closely correlated to the minimum sample intake. Due to the intrinsic inhomogeneity, individual aliquots of a material will not contain the same amount of analyte. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the certified value within its stated uncertainty.

4.2.1 Study of decreasing sample intakes

To estimate the minimum sample intake, a series of measurements with decreasing amounts of sample for one randomly selected unit were performed. The following sample intakes were tested: 50, 100 and 200 mg. For each sample intake 5 samples were measured in triplicate by ICP-QMS (total arsenic) and HPLC-ICP-MS (arsenite/arsenate and DMA) under repeatability conditions, and in a randomised manner. The measurement method was robust over the whole range of the sample intake tested and its repeatability was in the same range or better than the repeatability achieved during the material characterisation (see Section 6).

The obtained data sets (all sample intakes taken together) were first tested for normal or at least unimodal distribution. This was done by visual inspection of normal probability plots and histograms (if the data do not follow at least a unimodal distribution, the calculation of standard deviations is doubtful or impossible). All results were normally and unimodally distributed.

Furthermore, the results (all sample intakes taken together) were scrutinised for outliers using the single Grubbs-test. No outliers were found for total arsenic and DMA. One outlier was found for arsenite/arsenate (unit 1553, 50 mg sample intake). Since no technical reason for the outlier could be found, the result was retained. In any case, its removal would not affect the results of the minimum sample intake determination.

The minimum sample intake was established by comparison of variances obtained for 50, 100 and 200 mg sample intakes with the variance obtained for 250 mg sample intake during the assessment of between-unit homogeneity. It was done using the F-test for equality of two samples for variances with 14 degrees of freedom and a confidence level of 95 %. The obtained results are presented in Annex B and the minimum sample intakes are summarised in Table 3.

Table 3: Minimum sample intakes of ERM-BC211

	Minimum sample intake [mg]
Total arsenic	50
DMA [1)]	50
Arsenite/arsenate [1)]	100

1) expressed as arsenic

The minimum amount of sample to be used for total arsenic and dimethylarsinic acid is 50 mg. The minimum amount of sample to be used for the sum of arsenite and arsenate is 100 mg.

5 Stability

Time, temperature and radiation were regarded as the most relevant influencing factors on stability of the materials. The influence of ultraviolet or visible radiation was minimised by the choice of the containment, which eliminates most of the incoming light. In addition, materials are stored and dispatched in the dark, thus practically eliminating the possibility of radiative degradation. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as conditions for dispatch to the customers (short-term stability).

The stability studies have been carried out using an isochronous design [13]. In that approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible ("reference conditions"), effectively "freezing" the degradation status of the materials. At the end of the isochronous storage, the samples are analysed simultaneously under repeatability conditions.

5.1 Short-term stability study

Samples were stored at -20, 18 and 60 °C for 0, 1, 2 and 4 weeks (at each temperature) for the short-term stability study. The reference temperature was set to -70 °C. Two samples per storage time were selected using a random stratified sampling scheme. From each unit,

three samples were measured by ICP-SFMS (total arsenic) and HPLC-ICP-SFMS (arsenite/arsenate and DMA). The measurements were performed under repeatability conditions and in a randomised manner to be able to separate a potential analytical drift from a trend over storage time. The results were corrected for the water content determined in each unit in triplicate as followed: a minimum mass of 1 g of the material was dried at least for 8 h in a ventilated oven at 75 ± 2 °C. Then weighing and repeated drying until constant mass is attained, to a maximum of 3 days.

The obtained data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test. Two outliers were detected in the dataset of -20 °C for arsenite and arsenate, while for DMA two outlying values were found in the dataset of 60 °C. These were all individual results (see Table 4). As no technical reason for the outliers could be found all data were retained for statistical analysis leading to a conservative estimation of the short-term stability uncertainty.

Furthermore, the data were plotted against storage time and regression lines of mass fraction versus time were calculated. The slope of the regression lines was then tested for statistical significance (loss/increase due to shipping conditions). For total arsenic and arsenite/arsenate, the slopes of the regression lines were not significantly different from 0 (on 99 % confidence level) for -20, 18 and 60 °C. For DMA, the slope of the regression line was not significantly different from 0 (on 99 % confidence level) at -20 and 18 °C. The slope of the regression line was significantly different from 0 (on 99 % confidence level) at 60 °C. The regression lines were also calculated without the observed outliers in order to avoid masking of material degradation by outliers. No changes of the results were observed.

The results of the measurements are shown in Annex C. The results of the statistical evaluation of the short-term stability are summarised in Table 4.

Table 4: Results of the short-term stability tests

	Number of individual outlying results (statistical, retained)			**Significance of the trend on a 99% confidence level**		
	-20 °C	**18 °C**	**60 °C**	**-20 °C**	**18 °C**	**60 °C**
Total arsenic	none	none	none	no	no	no
DMA [1)]	none	none	2	no	no	yes
Arsenite/arsenate [1)]	2	none	none	no	no	no

1) expressed as arsenic

Since a significant slope was observed for DMA at 60 °C, the transport of the material will occur under cooled conditions.

5.2 Long-term stability study

Data from two isochronous stability studies have been combined to assess the stability of the CRM. One study was performed after one year of storage and one was performed after two years of storage.

For the first isochronous study, vials of the CRM have been stored at -20 and 4 °C for 0, 4, 8 and 12 months. The reference temperature was set to -70 °C. Two vials per storage time were selected using a random stratified sampling scheme. For the second isochronous study, vials of the CRM have been stored at -20 and 4 °C for 0, 8 and 16 and 24 months. The reference temperature was set to -70 °C. Two vials per storage time were selected using a random stratified sampling scheme. From each vial within each study, three samples were measured by ICP-QMS (total arsenic) and HPLC-ICP-QMS (DMA and arsenite/arsenate),

respectively. This design allows separation of a potential analytical drift from a trend over storage time. The measurements were performed under repeatability conditions.

The obtained data were evaluated individually for each study. The results were screened for outliers using the single and double Grubbs test. Some outliers were found (99 % confidence level). As no technical reason for this outlier could be found all data were retained for statistical analysis.

Furthermore, the data were plotted against storage time and regression lines of mass fraction versus time were calculated. The slope of the regression lines was tested for statistical significance (loss/increase due to storage conditions). For both studies, the slopes of the regression lines were not significantly different from zero (99 % confidence level) for total arsenic and arsenite/arsenate at each temperature. The slopes of the regression lines for DMA were not significant different from zero (99 % confidence level) at -20 °C in both studies. At 4 °C the slopes of the regression lines were significant different from zero (95 % confidence level) for the two years study and significant different from zero (99 % confidence level) for the one year study.

Afterwards the results of the two isochronous studies were combined as described in [14]. A measurement bias between the two studies was found and corrected using the correction factor *d* as calculated in the following equation:

$$d = \overline{x_2} / \overline{x_1}$$

$\overline{x_1}$ mean measurement result of study 1 at -70 °C

$\overline{x_2}$ mean measurement result of study 2 at -70 °C

The relative uncertainty of this correction $u_{d,\,rel}$ was calculated as:

$$u_{d,rel} = \sqrt{\frac{1}{n_1} RSD_1^2 + \frac{1}{n_2} RSD_2^2}$$

RSD_1: relative standard deviation of all results in study 1

RSD_2: relative standard deviation of all results in study 2

n_1: number of data points is study 1

n_2: number of data points in study 2

The combined data were plotted against storage time and regression lines of mass fractions versus time were calculated. The slope of the regression lines was tested for statistical significance. For total arsenic and arsenite/arsenate the slopes of the regression lines were not significantly different from zero (99 % confidence level) at both temperatures. The regression line for DMA showed a significant trend (99 % confidence level) different from zero at 4 °C and no significant trend (99 % confidence level) at -20 °C.

The results of the measurements are shown in Annex D. The results of the statistical evaluation of the long-term stability study are summarised in Table 5.

Table 5: Results of the long-term stability tests

	Number of individual outlying results (statistical/retained)		Significance of the trend on a 99% confidence level	
	-20 °C	4 ºC	-20 ºC	4 ºC
Total arsenic	1	1	no	no
DMA [1)]	1	none	no	yes
Arsenite/arsenate [1)]	1	1	no	no

1) expressed as arsenic

No technically unexplained outliers were observed and the trend for DMA was statistically significant on a 99 % confidence level at 4 °C. No trend was observed at -20 °C (99 % confidence level) for all analytes. The material can therefore be stored at -20 °C.

5.3 Estimation of uncertainties associated with stability studies

Due to the intrinsic variation of measurement results no study can rule out degradation of materials completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time".

Uncertainties of stability during dispatch and storage were estimated as described in [15] for each analyte. For this approach, the uncertainty of the linear regression line with a slope of zero is calculated. The uncertainty contribution (u_{lts}) is then calculated as the product of the chosen shelf life and the uncertainty of the regression lines as

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{sl}$$

RSD: relative standard deviation of all results of the stability study

x_i: time point for each replicate

$\bar{x}$: mean results for all time points

t_{sl}: proposed shelf life (24 months at -20 ºC in this case)

$$u_{lts,\mathrm{comb},rel} = \sqrt{u_{d,rel}^2 + u_{lts,rel}^2}$$

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated for the max. dispatch temperature of 18 °C and for a time of 0.25 months (1 week).
- $u_{lts,rel}$, the uncertainty of stability during storage. This uncertainty contribution was estimated for -20 °C and for a time of 24 months.

The results of these evaluations are summarised in Table 6.

Table 6: Uncertainties of stability during storage and dispatch. $u_{sts,rel}$ was calculated for a temperature of 18 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of -20 °C and 24 months.

	$u_{sts,\ rel}$ [%]	$u_{d,\ rel}$ [%]	$u_{lts,\ rel}$ [%]	$u_{lts,\ comb,\ rel}$ [%]
Total arsenic	0.414	0.26	1.029	1.06
DMA [1)]	0.532	0.37	0.963	1.03
Arsenite/arsenate [1)]	0.420	0.44	1.207	1.28

1) expressed as arsenic

The material showed statistically significant degradation at 60 °C but the uncertainty of stability for transport below 18 °C is negligible. Cooled shipment is therefore necessary.

After the certification campaign, the material will be subjected to IRMM's regular stability monitoring programme to control its further stability.

6 Characterisation

The material characterisation was based on an intercomparison of expert laboratories. The mass fraction of total arsenic, arsenite/arsenate and DMA in ERM-BC211 was determined in different laboratories, which applied different measurement procedures to avoid the introduction of a measurement bias. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

6.1 Selection of participants

26 laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the field of trace element analysis and/or elemental speciation measurements in relevant matrices by submitting results for intercomparison exercises or method validation reports. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.2 Study setup

Each laboratory received 2 units of ERM-BC211 and was requested to provide 6 independent results, 3 per unit. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations and measurements had to be spread over at least two days to ensure intermediate precision conditions. An independent calibration was performed for each result. The water content had to be determined in each unit and results are reported based on dry mass.

Each participant received a sample of NIST SRM 1568a (rice flour) or NMIJ 7503-a (arsenic compounds and trace elements in white rice flour) as an anonymised blinded quality control (QC) sample. Correctness of calibration was confirmed by agreement of results with a CRM used as a quality control sample. Traceability of the NAA measurements was guaranteed by using IRMM-530 R (Al-Au alloy).

Laboratories were requested to give estimations of the expanded uncertainties of the mean value of the six results as well. No approach for the estimation was prescribed, i.e. top-down and bottom-up approaches were regarded as equally valid procedures.

6.3 Methods used

A variety of acid digestion procedures, mainly employing HNO_3 or a mixture of HNO_3 and H_2O_2, followed by different quantification steps (ICP-QMS, ICP-SFMS, HG-AFS) were used to characterise mass fraction of total arsenic. Two labs used a dry-ashing protocol and subsequent quantification *via* HG-AAS and one lab characterised the material without sample preparation (k_0 NAA).

For the characterisation of arsenite/arsenate a variety of extraction procedures with different solvents (e.g. HNO_3/H_2O_2, TFA, HNO_3, HCl, HCl/H_2O_2) was applied. One lab used an enzymatic digestion protocol. Thereafter, quantification was carried out *via* different analytical techniques (HPLC-ICP-QMS, HPLC-ICP-SFMS, IC-ICP-QMS, HPLC-AFS, HG-AAS). In the case of HG-AAS two different reduction protocols (HBr/hydrazine sulphate and KI/ascorbic acid) were applied.

The combination of results from methods based on completely different principles virtually rules out undetected method bias for the certified parameters total arsenic and arsenite/arsenate.

DMA was extracted by different procedures using a variety of solvents (e.g. HNO_3/H_2O_2, TFA, HNO_3, H_2O). One lab carried out a protocol *via* enzymatic digestion. Quantification was accomplished by HPLC-ICP-QMS or HPLC-ICP-SFMS in all cases.
All methods used during the characterisation study are summarised in Annex E. The laboratory code (e.g. L1) is a random number and does not correspond to the order of laboratories in section 2. The lab-method code consists of a number assigned to each laboratory (e.g. L1) and abbreviation of the measurement method used (e.g. ICP-QMS).

All measurement results were provided dry mass corrected and the water content was determined as explained in section 6.4.

6.4 Dry mass determination

For all measurements carried out during the characterisation study the following protocol for dry mass determination was applied:

The water content had to be measured for each vial at the time of sample preparation. The method to be used is (atmospheric pressure) oven drying of a portion of at least 0.2 g at 103 ± 2 °C until constant mass is attained.

The water content determined by the laboratories was in the range of 5 g/kg to 40 g/kg and reflect the different conditions in terms of humidity and storage (dessicator or not) in the labs of all participants. However, results within each laboratory were consistent.

6.5 Evaluation of results

The characterisation campaign resulted in 13 datasets for total arsenic, 13 datasets for arsenite/arsenate and 8 datasets for DMA. All individual results of the participants, grouped per analyte are displayed in tabular and graphical form in Annex F.

6.6 Technical evaluation

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- compliance with the analysis protocol: sample preparations and measurements performed on two days and water content determination as requested (see. 6.4)
- correctness of the measurements based on knowledge of the method
- absence of values given as below limit of detection or below limit of quantification

- method performance, i.e. agreement of the measurement results with the assigned value of the QC sample

Two datasets were rejected because they were not technically valid. For arsenite/arsenate, the dataset of lab 25 was not used for the certification. For DMA the dataset of lab 21 was rejected. The labs failed to measure the certified value of the QC sample for the property measured.

6.7 Statistical evaluation

The datasets accepted on technical grounds were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations, (both at a 99 % confidence level). Standard deviation within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 7.

Table 7: Statistical evaluation of the technically accepted datasets for total arsenic, arsenite/arsenate and DMA. p: number of technically valid datasets

	p	Outliers		Normal distribution	Statistical parameters			
		Means	Variances		Means [µg/kg]	*s* [µg/kg]	$s_{between}$ [µg/kg]	s_{within} [µg/kg]
Total arsenic	13	none	none	yes	260.182	16.347	25.351	17.834
DMA [1)]	8	none	none	yes	118.867	14.235	23.827	8.965
Arsenite/ arsenate [1)]	13	none	none	yes	123.759	12.838	21.724	6.712

1) expressed as arsenic

The laboratory averages follow normal distributions. None of the data contains outlying means and variances. The datasets are therefore consistent and the mean of laboratory means is a good estimate of the true value. Standard deviations between laboratories are considerably larger than the standard deviation within laboratories, showing that confidence intervals of replicate measurements are unsuitable as estimate of measurement uncertainty. The relative uncertainty of the characterisation study ($u_{char,rel}$) was estimated as follows:

$$u_{char,rel} = \frac{RSD}{\sqrt{n}}$$

RSD:

relative standard deviation of technically valid results of the characterisation study

n:

number of technically valid datasets

It should be borne in mind that the methods used in the characterisation study are methods routinely applied for measuring total arsenic, arsenite/arsenate and DMA in rice samples. The agreement of results from different methods demonstrates that the processing did not

affect any properties relevant for these methods and that ERM-BC211 behaves like a real sample.

Table 8: Relative uncertainties of the characterisation study

	$u_{char,\ rel}$ [%]
Total arsenic	1.743
DMA [1)]	4.234
Arsenite/ arsenate [1)]	2.877

7 Value Assignment

Certified values are values that fulfil the highest standards of accuracy. Procedures at IRMM require generally pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets in accordance with the Guide to the expression of uncertainty in measurement [3] must be established.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 7 was assigned as certified value for the element.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (see Section 6), potential between-unit inhomogeneity, u_{bb} (see Section 4) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (see Section 5). These different contributions were combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM,\ rel}$) with a coverage factor k as

$$U_{CRM,rel} = k \cdot \sqrt{u^2_{char,rel} + u^2_{bb,rel} + u^2_{sts,rel} + u^2_{lts,comb,rel}}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.
- u_{sts} was estimated as described in section 5. As can be seen in Table 6, the uncertainty of degradation during dispatch is negligible compared to the other uncertainty contributions.
- $u_{lts,\ comb}$ was estimated as described in Section 5.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor k of 2 was applied, to obtain the expanded uncertainties.

The certified values and their uncertainties are summarised in Table 9.

Table 9: Certified values and their uncertainties for ERM-BC211

	Certified value [2] [µg/kg]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{lts, comb, rel}$ [%]	$U_{CRM, rel}$ [3] [%]	U_{CRM} [3] [µg/kg]
Total arsenic	260	1.743	1.306	1.06	4.84	13
DMA [1]	119	4.234	2.857	1.03	10.42	13
Arsenite/ arsenate [1]	124	2.877	2.596	1.28	8.16	11

1) expressed as arsenic
2) reported on dry mass basis (see Section 6.4)
3) $U=u \cdot k$, where $k=2$

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

Total arsenic and arsenite/arsenate are chemically clearly defined analytes. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias. The measurand is therefore structurally defined and independent of the measurement method.

DMA is a well defined molecule, but an extraction step is employed to liberate it from the matrix. As it cannot be proven that this step releases all DMA molecules, the measureable DMA content is a method defined measurand and can only be obtained by following the procedures specified in the section 6.3. Adherence to these procedures was confirmed by agreement of the laboratories' results with the assigned value for the CRM that was used as quality control sample. The certified measurand is therefore operationally defined by HPLC-ICP-MS.

Quantity value

Total arsenic and arsenite/arsenate: Only validated methods were used for the determination of the assigned values. Different calibrants of (known purity and) specified traceability of their assigned values were used and all relevant input parameters were calibrated. The individual results are therefore traceable to the SI, as it is also confirmed by the agreement among the technically accepted datasets. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

DMA: Traceability of the obtained results is based on the traceability of all relevant input factors. Instruments in individual laboratories were verified and calibrated with tools ensuring traceability to the SI. Consistency in the inter-laboratory comparison demonstrates that all relevant input factors were covered. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is nowadays summarised in a concept called 'commutability of a reference material'. There are various definitions expressing this concept. For instance, the CSLI Guideline C-53A [16] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and, thus, is a crucial characteristic in case of the application of different measurement methods.

ERM-BC211 was produced from a naturally grown rice material by milling and mixing. The analytical behaviour will be the same as for a routine sample of rice flour. Nevertheless, one has to bear in mind that the extractability of the arsenic species from this CRM can be different to the extractability from a sample as milled in the user's laboratory due to a different particle size. For samples other than rice flour the commutability has to be assessed.

9 Instructions for use

9.1 Storage conditions

The material shall be stored at -20 °C ± 5 °C in the dark. Care shall be taken to avoid change of the moisture content once the vials are open.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

9.2 Safety and protection for the environment

The usual laboratory safety measures apply.

9.3 Preparation and use of the material

The unit shall be thawed before use. Prior to use, invert and shake for at least two minutes.

9.3.1 Minimum sample intake

The minimum amount of sample to be used for total arsenic and dimethylarsinic acid is 50 mg. The minimum amount of sample to be used for the sum of arsenite and arsenate is 100 mg.

9.3.2 Dry mass correction

Dry mass determination shall be carried out on a separate portion of at least 0.2 g, by drying in an oven at 103 ± 2 °C until constant mass is attained (separate weighings should not differ by more than 5 mg). Weighing of the samples for dry mass determination and weighing for the analysis shall be done at the same time to avoid differences due to possible take up of moisture by the material.

9.4 Use of the certified value

The main purpose of the material is to assess method performance, i.e. for checking accuracy of analytical results/calibration. Moreover, it can be used for validation purposes and trueness determination.

Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, www.erm-crm.org) [17].

For assessing the method performance, the measured values of the CRM are compared with the certified values. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_m) with the uncertainty of the certified value (u_{CRM}): $u_\Delta = \sqrt{u_m^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty ($u_{\Delta,}$) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_m \leq U_\Delta$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95 %.

Use as a calibrant

It is not recommended to use this matrix material as calibrant. If used nevertheless, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty.

Use in quality control charts

The material can be used for quality control charts. Different CRM-units will give the same result as inhomogeneity was included in the uncertainties of the certified values.

10 Acknowledgments

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Annexes

Annex A: Results of the homogeneity measurements

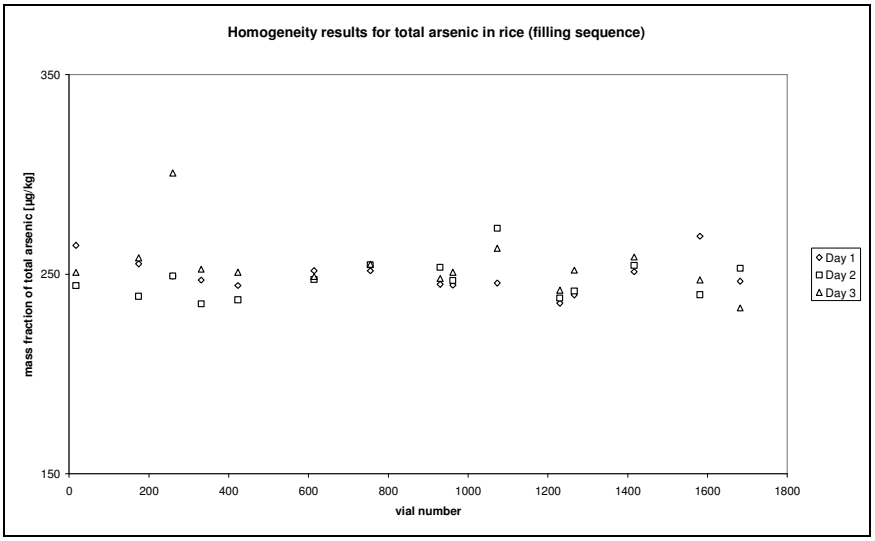

Figure 1: Homogeneity results for the mass fraction of total arsenic in rice (filling sequence)

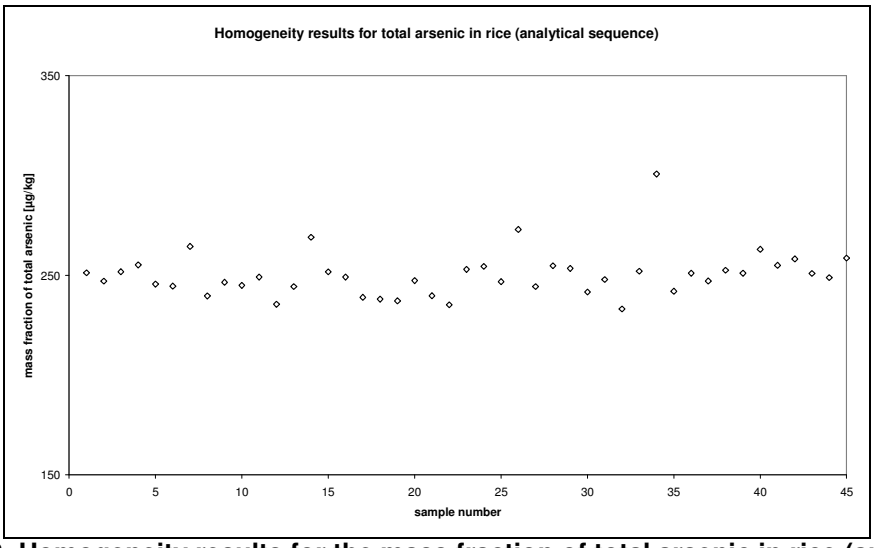

Figure 2: Homogeneity results for the mass fraction of total arsenic in rice (analytical sequence)

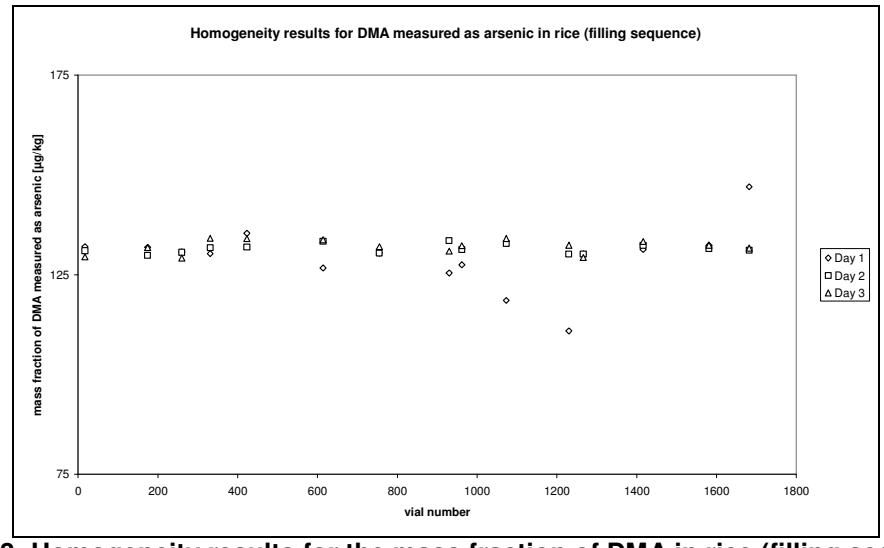

Figure 3: Homogeneity results for the mass fraction of DMA in rice (filling sequence)

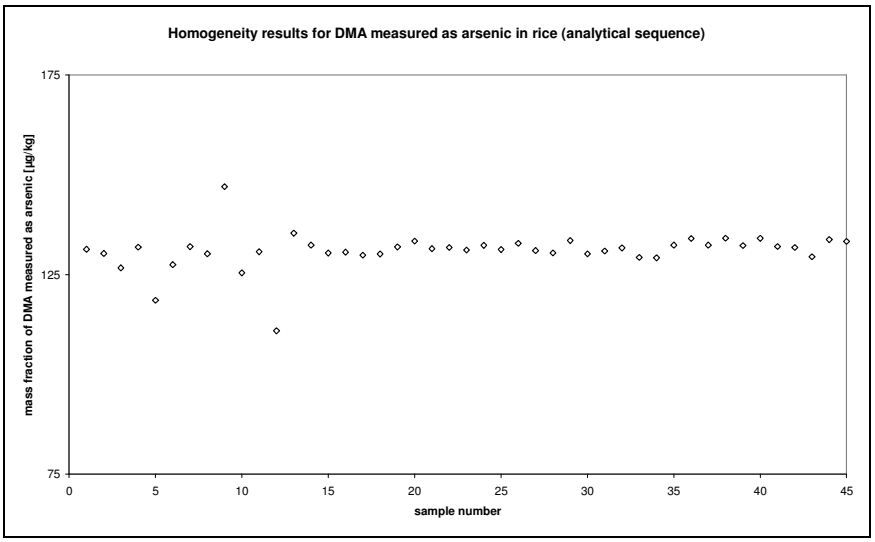

Figure 4: Homogeneity results for the mass fraction of DMA in rice (analytical sequence)

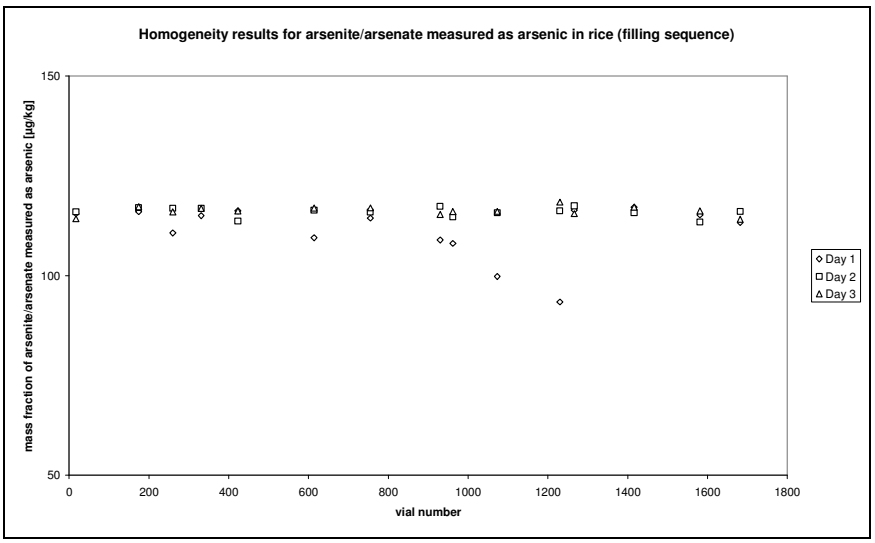

Figure 5: Homogeneity results for the mass fraction of arsenite/arsenate in rice (filling sequence)

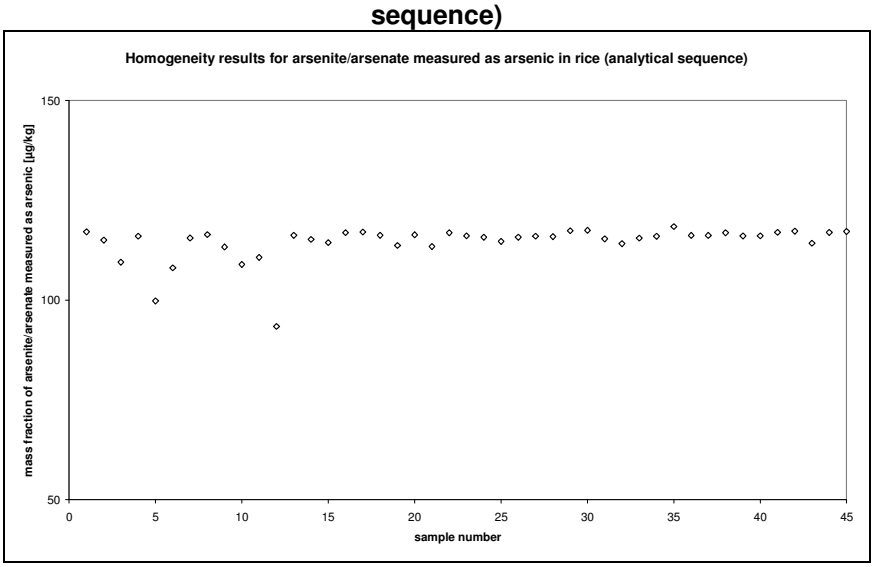

Figure 6: Homogeneity results for the mass fraction of arsenite/arsenate in rice (analytical sequence)

Annex B: Results of the minimum sample intake measurements

Table 1: Mass fractions of total arsenic in rice of the minimum sample intake measurements

Unit (sample intake)	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]
289 (50 mg)	230.2	242.9	239.9
727 (50 mg)	230.6	242.8	264.3
1146 (50 mg)	242.3	250.4	279.8
1553 (50 mg)	282.7	218.5	242.6
1828 (50 mg)	276.9	231.2	274.4
289 (100 mg)	247.4	266.3	268.9
727 (100 mg)	250.8	268.7	266.4
1146 (100 mg)	256.0	265.7	262.7
1553 (100 mg)	261.1	265.6	243.0
1828 (100 mg)	244.8	255.3	273.1
289 (200 mg)	246.2	255.7	261.9
727 (200 mg)	262.0	269.9	268.8
1146 (200 mg)	250.6	243.4	244.7
1553 (200 mg)	261.9	230.3	260.4
1828 (200 mg)	246.0	266.3	261.5

Table 2: Mass fractions of DMA measured as arsenic in rice for minimum sample intake measurements

Unit (sample intake)	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]
289 (50 mg)	130.0	140.2	133.4
727 (50 mg)	128.5	135.0	133.3
1146 (50 mg)	133.5	140.2	134.9
1553 (50 mg)	132.2	139.3	138.7
1828 (50 mg)	128.6	141.0	133.9
289 (100 mg)	132.2	129.9	131.3
727 (100 mg)	135.5	131.9	132.1
1146 (100 mg)	124.5	133.7	132.3
1553 (100 mg)	124.2	136.4	136.7
1828 (100 mg)	126.9	134.0	134.8
289 (200 mg)	127.8	132.2	132.0
727 (200 mg)	126.2	128.8	130.7
1146 (200 mg)	128.6	133.4	133.8
1553 (200 mg)	129.3	130.6	133.0
1828 (200 mg)	126.0	133.0	133.1

Table 3: Mass fractions of arsenite/arsenate measured as arsenic in rice of minimum sample intake measurements

Unit (sample intake)	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]
289 (50 mg)	118.1	113.8	119.1
727 (50 mg)	124.2	116.0	117.2
1146 (50 mg)	123.7	118.8	117.4
1553 (50 mg)	138.3	115.5	120.0
1828 (50 mg)	114.7	117.2	122.4
289 (100 mg)	112.6	111.5	113.0
727 (100 mg)	117.0	109.9	116.3
1146 (100 mg)	116.9	114.4	113.1
1553 (100 mg)	117.0	118.3	117.2
1828 (100 mg)	107.6	117.3	120.6
289 (200 mg)	110.1	115.8	113.9
727 (200 mg)	114.8	115.9	115.3
1146 (200 mg)	117.0	116.4	118.3
1553 (200 mg)	114.0	114.4	115.9
1828 (200 mg)	112.7	115.2	117.6

Annex C: Results of the short-term stability measurements

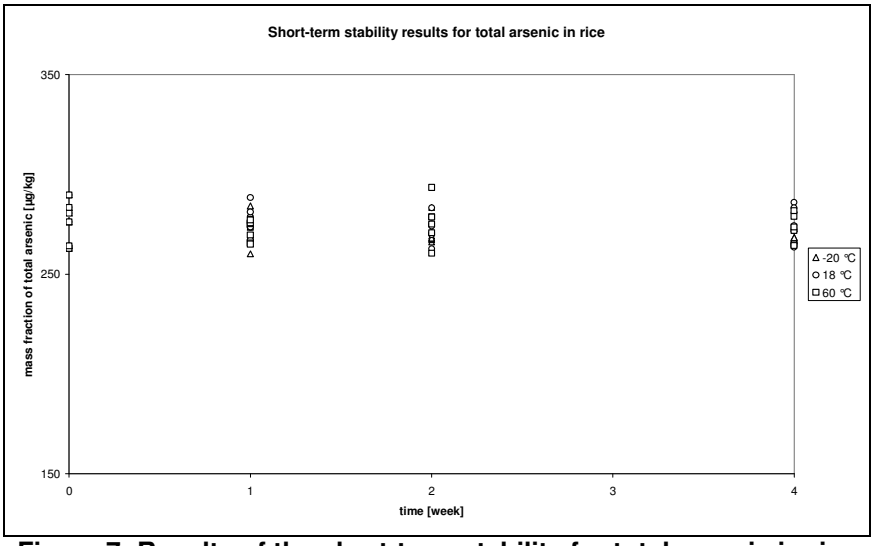

Figure 7: Results of the short-term stability for total arsenic in rice

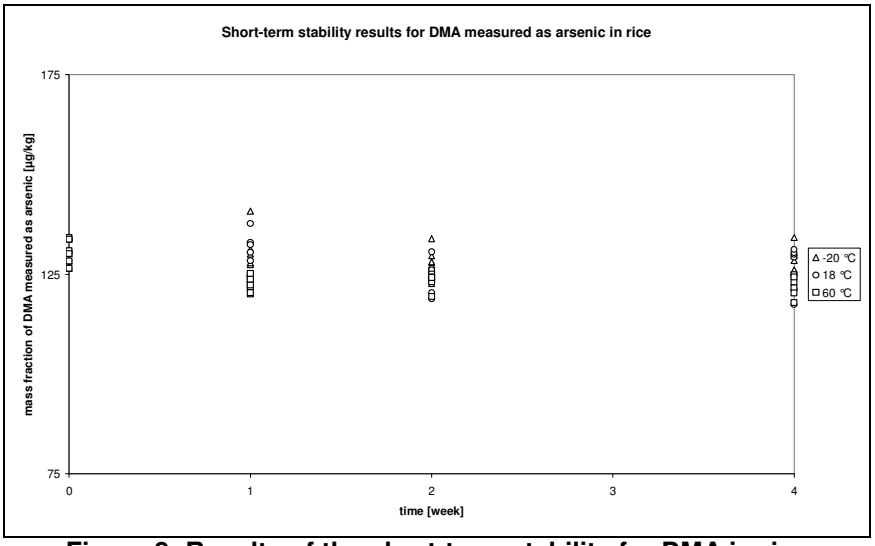

Figure 8: Results of the short-term stability for DMA in rice

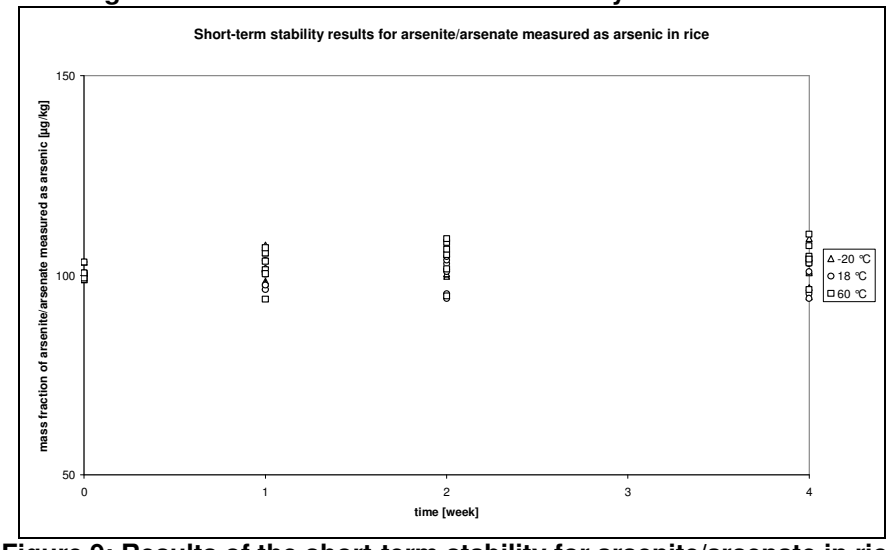

Figure 9: Results of the short-term stability for arsenite/arsenate in rice

Annex D: Results of the long-term stability measurements

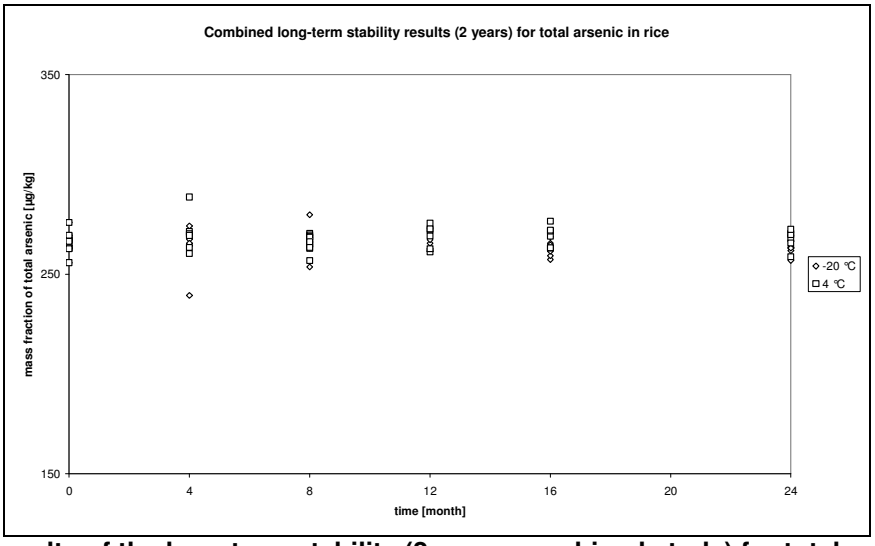

Figure 10: Results of the long-term stability (2 years, combined study) for total arsenic in rice

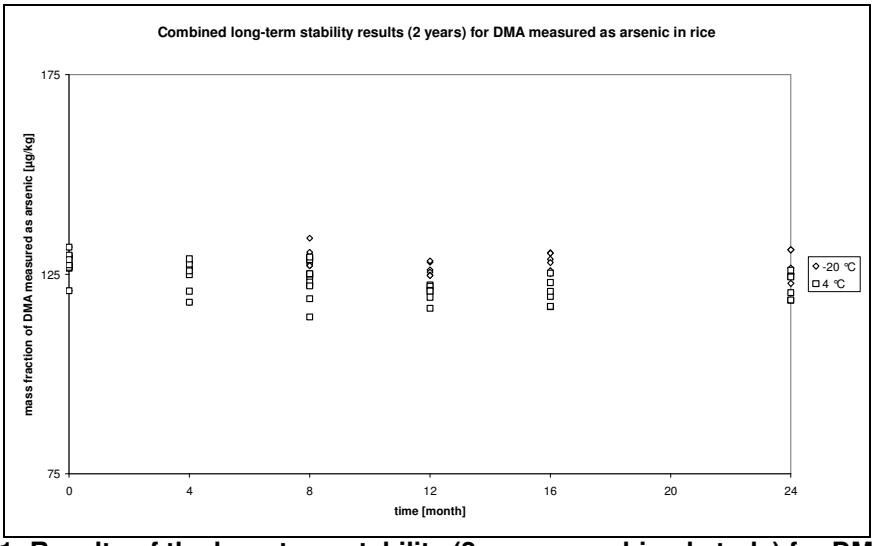

Figure 11: Results of the long-term stability (2 years, combined study) for DMA in rice

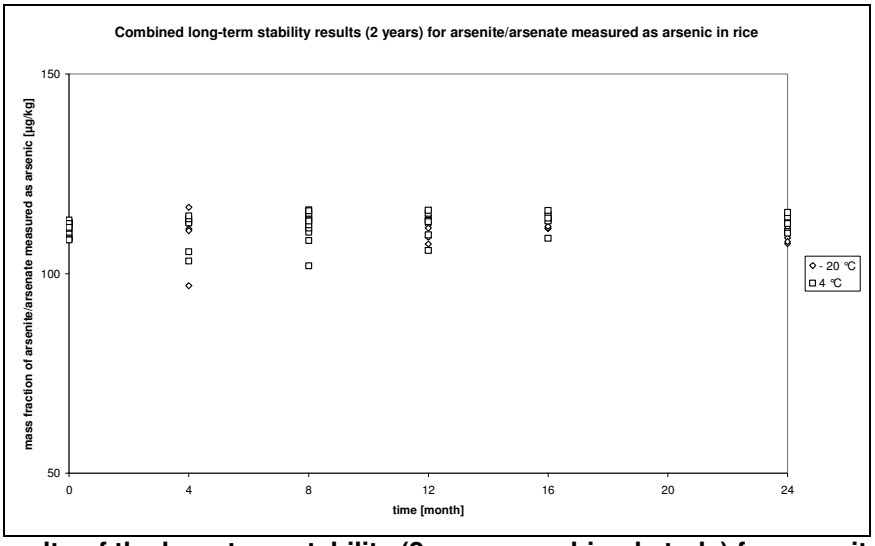

Figure 12: Results of the long-term stability (2 years, combined study) for arsenite/arsenate in rice

Annex E: Summary of methods used in the characterisation

Table 4: Methods used in the characterisation study for the quantification of total arsenic as reported by participants

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L0-k_0 NAA	No specific sample pre-treatment before irradiation; samples of about 500 mg were irradiated at a nominal fluence rate of $4 \cdot 10^{11}$ neutrons $cm^{-2}s^{-1}$. After a decay time of 1 day samples were counted on k_0 efficiency calibrated 40% HP Ge detectors. Traceability in k_0 NAA is guaranteed through the use of IRMM-530R Al-Au fluence rate monitors. Total As was determined using the 559 keV peak of ^{76}As.	CRM used: IRMM-530R
L1-ICP-QMS	Microwave digestion (T_{max} = 250 °C) of 0.25 g sample with 2 mL HNO_3 and 2 mL H_2O_2 using ^{74}Ge and ^{115}In as internal standards; 7-point external calibration; ICP-QMS (Agilent 7500ce) measurement using He as collision gas; measured m/z = 74, 75, 77 and 117; 1 % CO_2 in Argon	Calibrant: As in 2% nitric acid, 1000 ± 3 µg $As \cdot mL^{-1}$ (CPI International), traceable to NIST SRM 3103a; CRMs used: NIST SRM 1568a, NIST SRM 1643e, NMIJ 7503-a
L2-HG-AAS	Dry ashing of 0.25-1 g sample according to protocol 14546 CEN (European Committee for Standardization, 2004); no internal standard; 6-point external calibration; quantification by HG-AAS (AAS 3300, Perkin Elmer); sample loop = 0.5 mL; reducing agent = 0.2 % (w/v) $NaBH_4$ in 0.05 % (w/v) NaOH; flow rate = 5 mL/min; HCl solution = 10 % (v/v), flow rate = 10 mL/min; argon as carrier gas, flow rate = 100 mL/min; measured wavelength = 193.7 nm; spectral band-pass = 0.7 nm; electrodeless discharge lamp system 2, lamp current setting 400 mA; cell temperature 900 °C; blank correction	Calibrant: As CertiPUR® (Merck), H_3AsO_4 in 0.5 mol/L nitric acid, 1002 ± 3 mg/L As; CRMs used: NIST SRM 1568a, NMIJ 7503-a
L3-HG-AFS	Weighing of approx. 0.2 g sample; addition of 2 mL HNO_3 (65%); the mixture is left to stand overnight; Microwave-assisted digestion (T_1 = 55 °C, 10 min; T_2 = 75 °C, 10 min, T_3 = 75 °C, 30 min); Dilution to 10.000 g with ultrapure water; no internal standard; 6-point external calibration; UV-HG-AFS (Millennium Excalibur, PS Analytical); arsenic lamp (Superlamp 803S, Photron Pty. Ltd), PSA 10.570 UV oxidation system; flow injection system: Rheodyne's Type 70 valves, high pressure switching valves, model 7010; sample loop = 100 µL; argon as carrier gas, flow rate = ~ 250 mL/min; reducing agent = 1.5 % (w/v) $NaBH_4$ in 0.1 mol/L NaOH; flow rate = ~ 2.7 mL/min; HCl = 3 mol/L, flow rate = ~ 4.1 mL/min; baseline correction and no blank correction	Calibrant: As CertiPUR® (Merck), H_3AsO_4 in 0.5 mol/L nitric acid, 1002 ± 3 mg/L As; CRM used: NMIJ 7503-a
L4-ICP-QMS	Microwave-assisted digestion (T_{max} = 200 °C) of 0.5 g sample with 8 mL HNO_3 (200 g/kg) and 1 mL H_2O_2 (300 g/kg) using ^{72}Ge as internal standard, dissolved in 10 g/kg aqueous 2-propanol in order to minimise carbon effects; 6-point external calibration; ICP-QMS (Agilent 7500cx) measurement using He as collision gas; measured m/z = 72, 75	Calibrant: Multi analyte trace metal plasma standard (J.T. Baker), 100 mg/L As; CRM used: NMIJ 7503-a

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L5-ICP-QMS	Microwave-assisted mineralisation (T_{max} = 180 °C) of approx. 0.25 g sample with 10 mL HNO_3 (50 %) without internal standard; 4-point external calibration; ICP-QMS (VARIAN 820) measurement using H_2 as collision gas; measured m/z = 75; blank correction	Calibrant: Arsenic standard for ICP, As_2O_3 diluted in 2 % nitric acid, 999 ± 2 mg/L, traceable to NIST SRM 83; CRM used: NIST SRM 1568a
L6-ICP-SFMS	Weighing of approx. 0.2 g sample; addition of 1 mL HNO_3 (65 %); the mixture is left to steep overnight; addition of 2 mL H_2O_2; microwave-assisted digestion (T_{max} = 95 °C) using ^{74}Ge as internal standard; dilution to 10 g with ultrapure water; 6-point external calibration; ICP-SFMS (Thermo Scientific Element 2) measurement using high resolution mode; measured m/z = 74, 75; no blank correction	Calibrant: ICP standard (AccuTrace), 10 mg/kg As, traceable to NIST SRM 3103a; CRMs used: NIST SRM 1568a, NMIJ 7503-a
L7-ICP-QMS	Microwave-assisted digestion (T_{max} = 180 °C) of 0.5 g sample with 4 mL HNO_3 (65 %, subboiled) and 1 mL H_2O_2 using ^{103}Rh as internal standard; 3-point external calibration; ICP-QMS (Agilent 7500x) measurement using He as collision gas; measured m/z = 75, 103	Calibrant: As in 2 % nitric acid, 1000 ± 3 µg As·mL^{-1} (CPI International), traceable to NIST SRM 3103a; CRMs used: NIST SRM 1568a, NMIJ 7503-a
L8-ICP-QMS	Microwave digestion ((T_{max} = 200 °C) of 0.3 g sample with 5 mL HNO_3 (65 %) using ^{72}Ge as internal standard; 4-point external calibration; ICP-QMS (Agilent 7500cx) measurement using He as collision gas; measured m/z = 72, 75; blank correction	Calibrant: PlasmaCal As standard (SCP science), 996 ± 6 µg/mL, CRMs used: NIST SRM 1568a, IRMM 804, NMIJ 7503-a
L9-HG-ICP-QMS	Microwave-assisted digestion (T_{max} = 290 °C) of approx. 0.2 g sample with 5 mL HNO_3; dilution with ultrapure water; pre-reduction of As(V) was performed with a solution of 50 g/L KI and 50 g/L ascorbic acid; 2.5 mL sample/2.5 mL reduction solution/2.5 mL 30 % HCl left to stand for two 2 h; dilution with H_2O; no internal standard; 6-point external calibration; hydride generation with 5 g/L $NaBH_4$ in 1 g/L NaOH; ICP-QMS (Agilent 7500ce) measurement using no gas mode; measured m/z = 75	Calibrant: As single standard (Merck), 995 ± 5 mg/L, traceable to NIST SRM 3103a; CRM used: NIST 1568a
L10-ICP-SFMS	Microwave-assisted digestion (T_{max} = 218 °C) of approx. 0.4 g sample with 5 mL HNO_3 (65 %, subboiled) and 1.5 mL H_2O_2 (30 %) using ^{115}In as internal standard; 4-point external calibration, I CP-SFMS (Thermo Finnigan Element 2) measurement using high resolution mode (R =10000); measured m/z = 75, 115	Calibrant: ICP Multistandard solution VI, CertiPUR® (Merck); CRMs used: NIST SRM 1568a, NMIJ 7503-a
L11-ICP-QMS	Weighing of approx. 0.5 g sample; addition of 5 mL HNO_3 (65 %); the mixture is left to steep overnight; addition of 3 mL H_2O_2; microwave-assisted digestion (p_{max} = 18 bar) using ^{103}Rh as internal standard; dilution to 25 g with ultrapure water; 4-point external calibration; ICP-QMS (Agilent 7500ce) measurement using He as collision gas; measured m/z = 75, 103; blank correction	Calibrant: CertiPUR® (Merck) arsenic ICP standard, 983 ± 7 mg/L As, H_3AsO_4 solution; traceable to NIST SRM 3103a; CRM used: NIST 1568a

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L12-HG-AAS	Dry ashing of 0.5 g sample with 6 mL of ashing aid suspension (20 % w/v $MgNO_3$); no internal standard; 5-point external calibration; quantification by HG-AAS (AA 1100, Perkin Elmer); sample loop = 0.5 mL; reducing agent = 0.2 % (w/v) $NaBH_4$ in 0.05 % (w/v) NaOH; flow rate = 5 mL/min; HCl solution = 10 % (v/v), flow rate = 10 mL/min; argon as carrier gas, flow rate = 100 mL/min; measured wavelength = 193.7 nm; spectral band-pass = 0.7 nm; electrodeless discharge lamp system 2, lamp current setting = 400 mA; cell temperature = 900 °C; blank correction	Calibrant: Arsenic AAS standard (Inorganic Ventures), 1000 ± 10 µg/mL, traceable to NIST SRM 3103a; CRM used: NIST SRM 1568a

Table 5: Methods used in the characterisation study for the quantification of arsenic species as reported by participants

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L13-HPLC-ICP-QMS	Microwave-assisted extraction (T_1 = 55 °C, 10 min; T_2 = 75 °C, 10 min, T_3 = 75 °C, 30 min) of 0.25 g sample with 10 mL of 2 g/L nitric acid and 10 g/L H_2O_2; centrifugation (1200 g) and filtration (pore size 0.45 µm); AEX-chromatography (Hamilton PRP-X100 (250 mm x 4.1 mm, 10 µm particle size); injection volume = 100 µL; mobile phase = 20 mmol/L $NH_4H_2PO_4$; pH = 5.8 (adjusted with aqueous ammonia); isocratic at 25 °C; flow rate = 1.5 mL/min, no internal standard, 6-point external calibration; ICP-QMS (Agilent 7500ce) measurement using no gas modus; measured m/z = ^{75}As, ^{77}Se; retention times: $t_{R, DMA}$ = 2.3 min and $t_{R, arsenate}$ = 6.9 min; blank correction	Calibrants: $Na_2HAsO_4 \cdot 7H_2O$ (Carlo Erba) and $(CH_3)_2AsNaO \cdot H_2O$ (Fluka) were standardised against As_2O_3 (NIST Oxidimetric Primary Standard 83d); CRMs used: NIST SRM 1568a, NMIJ 7503-a
L14-HPLC-ICP-QMS	Water bath extraction (T = 95 °C) of 0.5 g sample with 10 mL of 0.02 mol/L TFA; centrifugation; AEX-chromatography (Hamilton PRP X-100, 150x4.6 mm, 5 µm particle size); injection volume = 20 µL; mobile phase = 5 mmol/L maleic acid, pH = 5.6; isocratic at 40 °C; flow rate = 1 mL/min; no internal standard; 6-point external calibration; ICP-QMS (Agilent 7500ce) measurement using no gas modus; measured m/z = 75, 77; 1 % CO_2 in Argon; blank correction for 0.02 mol/L TFA	Calibrants: $Na_2HAsO_4 \cdot 7H_2O$ (Merck), sodium dimethylarsinate (Fluka); CRMs used: NIST SRM 1568a, NIST SRM 1643e, NMIJ 7503-a
L15-HPLC-ICP-SFMS	Extraction (T_{max} = 95 °C) of 1 g sample with 10 mL extraction solvent (0.28 mol/L nitric acid); centrifugation (2900 g) and filtration (pore size = 0.45 µm); AEX-chromatography (Hamilton PRP X-100, 250x4.1 mm, 10 µm particle size); injection volume = 20 µL; mobile phase = 20 mmol/L $NH_4H_2PO_4$; pH = 5.6 (adjusted with aqueous ammonia); isocratic at 22 °C; flow rate = 1.2 mL/min; no internal standard; 6-point external calibration; ICP-SFMS (Thermo Finnigan Element 2) measurement using high resolution mode (R = 10000); measured m/z = 74.914-74.929; retention times: $t_{R, arsenite}$ = 2.1 min, $t_{R, DMA}$ = 2.6 min and $t_{R, arsenate}$ = 9.2 min; no blank correction	Calibrants: Arsenite and arsenate solution (SPEX Certiprep) and sodium cacodylate (powder, Sigma-Aldrich), both traceable to NIST SRM 3103a, CRMs used: NIST SRM 1568a, NMIJ 7503-a

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L16-HG-AAS	Analysis was performed by acid digestion, solvent extraction and hydride generation-atomic absorption spectrometry (HG-AAS) quantification (Muñoz et al., 1999). Lyophilized sample (0.5-1 g) was weighed into a screw-top centrifuge tube; addition of 4.1 mL water; agitation until the sample was completely moistened; addition of 18.4 mL conc. HCl; agitation for 5 min; left to stand for 12-15 h (overnight); addition of the reducing agent (2 mL of HBr and 1 mL of hydrazine sulphate); agitation for 30 s; addition of 10 mL of $CHCl_3$; agitation for 5 min; centrifugation at 2000 rpm for 5 min; separation of the chloroform and pouring into another tube; extraction process done in triplicate; combination of chloroform phases and again centrifugation; elimination of the remnants of the acid phase by aspiration; filtering through Whatman GD/X syringe filters with 25 µm PTFE membrane; back-extracting by agitation for 5 min with 10 mL of HCl (1 mol/L); phase separation by centrifugation (2000 rpm for 5 min); aspirating of the aqueous phase pouring into a beaker; repeating of the back-extraction and combining of aqueous phases; addition of 2.5 mL of ashing aid suspension (20 % w/v $Mg(NO_3)_2 \cdot 6H_2O$ and 2 % w/v MgO) and 10 mL of nitric acid (14 mol/L) to the combined back-extraction phases; evaporation to dryness in a sand bath; placing in a muffle furnace at an initial temperature not higher than 150 °C; increasing of temperature to (425 ± 25) °C; maximum temperature rate = 50 °C/h and maintaining for 12 h; dissolution of white ash obtained in 6 mol/L HCl and reducing with pre-reducing solution (5 % w/v KI and 5 % w/v ascorbic acid); no internal standard; 6-point external calibration; quantification by HG-AAS (AAS 3300, Perkin Elmer); sample loop = 0.5 mL; reducing agent = 0.2 % (w/v) $NaBH_4$ in 0.05 % (w/v) NaOH; flow rate = 5 mL/min; HCl solution = 10 % (v/v), flow rate = 10 mL/min; argon as carrier gas, flow rate = 100 mL/min; measured wavelength = 193.7 nm; spectral band-pass = 0.7 nm; electrodeless discharge lamp system 2, lamp current setting = 400 mA; cell temperature = 900 °C; blank correction	Calibrant: As CertiPUR® (Merck), H_3AsO_4 in 0.5 mol/L nitric acid, 1002 ± 3 mg/L As; CRMs used: NIST SRM 1568a, NMIJ 7503-a
L17-HPLC-ICP-QMS	Microwave-assisted extraction (90 °C; t = 20 min) of 0.2 g sample with 10 mL extraction solvent (0.07 mol/L HCl in 3 % H_2O_2); centrifugation (3660 g) for 10 min; filtration (pore size = 0.45 µm); AEX-chromatography (Transgenomic ICSep ION-120, 120x4.6 mm, 100 µeqv/g), injection volume = 20 µL; mobile phase = 30 mmol/L NH_4CO_3 in 3 % methanol; pH = 10.3 (adjusted with 25% aqueous ammonia); isocratic at ambient temperature; flow rate = 1 mL/min; 4-point external calibration; ICP-QMS (Agilent 7500i) measurement using no gas modus; measured m/z = 75; retention time: $t_{R,\ arsenate}$ = 7.0 min, blank correction	Calibrant: As standard (Spectrascan), 997 ± 5 µg/mL); CRMs used: NIST SRM 1568a, NMIJ 7503-a
L18-HG-AAS	Extraction (T = 90 °C; t = 90 min) of 0.5 g sample with 10 mL extraction solvent (2 mL H_2O_2 (30 %) + 8 mL 0.28 mol/L HNO_3) on a heated magnetic stirrer; after cooling down centrifugation (41000 g) and filtration (pore size = 0.45 µm); Reducing agent = 0.3 % (w/v) $NaBH_4$ in 0.1 % (w/v) NaOH; HCl solution = 1.5 % (v/v); 3-point external calibration; HG-AAS measurement	Calibrant: As CertiPUR® (Merck), H_3AsO_4 in 0.5 mol/L nitric acid, 1002 ± 3 mg/L As; CRM used: NIST SRM 1568a

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L18-HG-AAS (continuation)	(Analytik Jena nova400 Hydrid AAS); measured wavelength = 193.7 nm; slit width = 0.8 nm; lamp current setting = 9 mA; concentrating the AsH_3 in an Iridium-coated graphite tube for 35 s; argon as carrier gas; atomisation at T = 2050 °C	
L19-HPLC-ICP-QMS	Microwave-assisted extraction (T = 80 °C; 27 min) of 250 mg sample with 10 mL ultrapure water; centrifugation and filtration (pore size = 0.45 µm); AEX-chromatography (Hamilton PRP-X100); injection volume = 60 µL; mobile phase = ammonium carbonate; pH, gradient at 35 °C; flow rate = 1mL/min; 4-point external calibration; ICP-QMS (VARIAN 820) measurement using H_2-mode; measured m/z = 75, retention times: $t_{R,\ arsenite}$ = 3.3 min, $t_{R,\ DMA}$ = 4.5 min and $t_{R,\ arsenate}$ = 12.0 min blank correction	Calibrants used: Arsenic trioxide solution (Fluka), arsenic (V) oxide hydrate (purity >99.99 %, Sigma-Aldrich), dimethylarsinic acid (purity 99.5 %, Chemservice); CRM used: NIST SRM 1568a
L20-HG-AAS	Analysis was performed by acid digestion, solvent extraction and hydride generation-atomic absorption spectrometry (HG-AAS) quantification (Muñoz et al., 1999). Lyophilized sample (0.5-1 g) was weighed into a screw-top centrifuge tube; addition of 4.1 mL water; agitation until the sample was completely moistened; addition of 18.4 mL conc. HCl; agitation for 5 min; left to stand for 12-15 h (overnight); addition of the reducing agent (2 mL of HBr and 1 mL of hydrazine sulphate); agitation for 30 s; addition of another tube; extraction process done in triplicate; combination of chloroform phases and again centrifugation; elimination of the remnants of the acid phase by aspiration; filtering through Whatman GD/X syringe filters with 25 µm 10 mL of $CHCl_3$; agitation for 5 min; centrifugation at 2000 rpm for 5 min; separation of the chloroform and pouring into PTFE membrane; back-extracting by agitation for 5 min with 10 mL of HCl (1 mol/L); phase separation by centrifugation (2000 rpm for 5 min); aspirating of the aqueous phase pouring into a beaker; repeating of the back-extraction and combining of aqueous phases; addition of 2.5 mL of ashing aid suspension (20 % w/v $Mg(NO_3)_2 \cdot 6H_2O$ and 2 % w/v MgO) and 10 mL of nitric acid (14 mol/L) to the combined back-extraction phases; evaporation to dryness in a sand bath; placing in a muffle furnace at an initial temperature not higher than 150 °C; increasing of temperature to (425 ± 25) °C; maximum temperature rate = 50 °C/h and maintaining for 12 h; dissolution of white ash obtained in 6 mol/L HCl and reducing with pre-reducing solution (5 % w/v KI and 5 % w/v ascorbic acid); no internal standard; 6-point external calibration; quantification by HG-AAS (AAS 3300, Perkin Elmer); sample loop = 0.5 mL; reducing agent = 0.2 % (w/v) $NaBH_4$ in 0.05% (w/v) NaOH; flow rate = 5 mL/min; HCl solution = 10 % (v/v), flow rate = 10 mL/min; argon as carrier gas, flow rate = 100 mL/min; measured wavelength = 193.7 nm; spectral band-pass = 0.7 nm; electrodeless discharge lamp system 2, lamp current setting = 400 mA; cell temperature = 900 °C; blank correction	Calibrant: Arsenic AAS standard (Inorganic Ventures), 1000 ± 10 µg/mL, traceable to NIST SRM 3103a; CRM used: NIST SRM 1568a
L21-HPLC-ICP-QMS	Microwave-assisted extraction (80 °C; 6 min) of approx. 0.15 g sample with 10 mL H_2O); centrifugation (3500 rpm; 5 min) and filtration (pore size = 0.45 µm); AEX-chromatography	Calibrants: Sodium (meta)arsenite (Fluka), sodium

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L21-HPLC-ICP-QMS (continuation)	(Dionex AS7 250x4 mm, 10 µm particle size); injection volume = 0.1 mL; mobile phase: A: 0.8 mmol/L HNO_3, 1 % MeOH, pH = 3.8; B: 500 mmol/L HNO_3, 1% MeOH pH = 1.4;gradient: 0-3 min: 99% A; 3-5 min: 10 % A; 5-12 min: 80 % A; T = 20 °C; flow rate = 1.35 mL/min; no internal standard; 6-point external calibration; ICP-QMS (X-Series II, Thermo Scientific) measurement; measured m/z = 75, 77; retention times: $t_{R, arsenite}$ = 1.1 min, $t_{R, DMA}$ = 3.2 min and $t_{R, arsenate}$ = 4.9 min; blank correction	arsenate dibasic heptahydrate (Fluka); CRMs used: BCR 627, NMIJ 7503-a
L22-HPLC-HG-AFS	Weighing of approx. 0.5 g sample; addition of 10 mL 1 % HNO_3/1 % H_2O_2; the mixture is left to stand overnight; microwave-assisted digestion (95 °C, 60 min); centrifugation; AEX-chromatography (Hamilton PRP X-100, 250x4.6 mm, 5 µm particle size); injection volume = 100 µL; mobile phase: 6.2 mmol/L ammonium nitrate and 6.5 mmol/L phosphoric acid adjusted to pH = 6 with ammonia; isocratic at room temperature; flow rate = 1.4 mL/min; no internal standard; 6-point external calibration; UV-HG-AFS (Millennium Excalibur, PS Analytical); arsenic lamp (Superlamp 803S, Photron Pty. Ltd), PSA 10.570 UV oxidation system; flow injection system: Rheodyne's Type 70 valves, high pressure switching valves, Model 7010; sample loop = 100 µL; argon as carrier gas, flow rate = ~ / 250 mL/min; reducing agent = 1.5 % (w/v) $NaBH_4$ in 0.1 mol/L NaOH; flow rate = ~ / 2.7 mL/min; HCl = 3 mol/L, flow rate = ~4.1 mL/min; retention times: $t_{R, arsenite}$ = 144 s, $t_{R, DMA}$ = 176 s and $t_{R, arsenate}$ = 337 s; baseline correction and no blank correction	Calibrant: As CertiPUR® (Merck), H_3AsO_4 in 0.5 mol/L nitric acid, 1002 ± 3 mg/L As; CRM used: NMIJ 7503-a
L23-HPLC-ICP-SFMS	Microwave-assisted extraction (95 °C; 60 min) of approx. 0.4 g sample with 10 g extraction solvent (1 % (v/v) HNO_3 and 1 % v/v H_2O_2); centrifugation (10188 g); AEX-chromatography (Dionex AS 14A, pre column 50x4 mm; 250x4 mm, 9 µm particle size); injection volume = 0.1 mL; mobile phase = 20 mmol/L $(NH_4)_2CO_3$; pH = 8.8; isocratic at 30 °C; flow rate = 1 mL/min; internal standard = ^{74}Ge; 5-point external calibration; ICP-SFMS (Thermo Scientific Element 2) measurement using high resolution mode; measured m/z = 74, 75; retention times: $t_{R, DMA}$ = 3.7 min, $t_{R, MMA}$ = 6.7 min and $t_{R, arsenate}$ = 14.5 min, no blank correction	Calibrant: Sodium cacodylate trihydrate (98 %, Sigma-Aldrich), standardised against Merck XXI; CRMs used: NIST SRM 1568a, NMIJ 7503-a
L24-HPLC-ICP-QMS	Microwave extraction (T_{max} = 95 °C) of 0.25 g sample with 10 mL extraction solvent (2 g/kg HNO_3 + 10 g/kg H_2O_2); centrifugation (3300 g) and filtration (pore size = 0.45 µm); AEX-chromatography (Hamilton PRP X-100, 150 x 4.1 mm, 5 µm particle size); injection volume = 125 µL; mobile phase = 26 mmol/L $NH_4H_2PO_4$, pH = 6.2; isocratic at 30 °C; flow rate = 1 mL/min; no internal standard; 5- or 6-point external calibration; ICP-QMS (Agilent 7500cx) measurement using He as collision gas; measured m/z = 72, 75; retention times: $t_{R, DMA}$ = 2.5 min and $t_{R, arsenate}$ = 6.5 min, no blank correction	Calibrants: Arsenic standard for ICP (As_2O_3, Fluka), 1000 mg/L, CertiPUR® (Merck) Arsenic ICP standard, 1000 mg/L, cacodylic acid (purity >99 %, Sigma-Aldrich), 1000 mg/L; CRM used: NMIJ 7503-a
L25-HPLC-ICP-QMS	Enzymatic extraction (T_{max} = 75 °C) of 0.4 g sample with 70 mg α-amylase and 20 mL ultrapure water for 24 h; filtration (0.2 µm pore size); ion-chromatography (Dionex Ion Pac AS7	Calibrants: Arsenic(III) oxide, Arsenic(V) oxide hydrate (purity

Laboratory code	Sample preparation/measurement	Calibrants/Traceability
L25-HPLC-ICP-QMS (continuation)	250x4 mm + guard column); injection volume = 200 µL; mobile phase = 0.5 - 25 mmol/L HNO_3; 0.05 mmol/L benzene-1,2-disulfonic acid dipotassium salt; gradient = HNO_3 (0.5 - 25 mmol/L); temperature = ambient temperature; flowrate = 1 mL/min; no internal standard; 3-point external calibration; ICP-QMS (Agilent 7500s); measured m/z = 75, 103; retention times: $t_{R, arsenite}$ = 1.86 min, $t_{R, DMA}$ = 3.88 min, $t_{R, arsenate}$ = 6.67 min; blank correction for arsenite/arsenate	>99,99 %), dimethylarsinic acid sodium salt trihydrate (purity >98 %, all from Sigma-Aldrich); CRM used: NMIJ 7503-a
L26-HPLC-ICP-QMS	Microwave-assisted extraction (95 °C; 90 min) of approx. 0.5 g sample with 5 mL HNO_3 (c = 0.28 mol/L); centrifugation (4100 g; 20 min) and filtration (pore size = 0.45 µm); AEX-chromatography (Hamilton PRP-X 100, 250x4.6 mm, 5 µm particle size); injection volume = 0.1 mL; mobile phase: 10 mmol/L ("A") and 50 mmol/L ("B") of ammonium carbonate, pH=8.03 in both solutions; gradient: 0-3 min: 100 % A, 3-3.5 min: up to 100% B; 3.5-15 min: 100 % B; 15-15.5 min: down to 0% B; 15.5-21 min: 100 % A; T = 22 °C; flow rate: 0-3 min: 1 mL/min; 3-3.5 min: up to 1.5 mL/min; 3.5-15 min: 1.5 mL/min; 15-15.5 min: down to 1 mL/min; 15.5-21 min: 1 mL/min; no internal standard; 4-point external calibration; ICP-QMS (Agilent 7500ce) measurement using He as collision gas; measured m/z = 75, 77, 82; retention times: $t_{R, DMA}$ = 4.8 min and $t_{R, arsenate}$ = 8.5 min; blank correction	Calibrants: CertiPUR® (Merck) Arsenic ICP standard, 983 ± 7 mg/L As, H_3AsO_4 solution; traceable to NIST SRM 3103a; sodium cacodylate x-hydrate (purity 99.9 %, 23.4 % water content, Fluka); CRM used: NIST 1568a

Annex F: Results of the characterisation measurements

Table 6: Mass fraction of total arsenic in rice as reported by each individual lab

Laboratory code	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	average [µg/kg]	Expanded uncertainty [1] [µg/kg]
L0-k_0 NAA	262	258	267	263	262	261	262.167	12
L1-ICP-QMS	259.2	256.6	254.8	255.1	254.2	271.4	258.550	9.8
L2-HG-AAS	298	293	285	283	283	274	286.000	38
L3-HG-AFS	224	266	219	244	260	314	254.500	69.7
L4-ICP-QMS	269	261	255	258	282	276	266.833	54
L5-ICP-QMS	261.9	246.7	264.3	261.1	267.3	268.5	261.630	54.9
L6-ICP-SFMS	264	280	275	275	272	268	272.333	18.3
L7-ICP-QMS	244	236	242	248	239	242	241.833	17
L8-ICP-QMS	251	257	249	255	261	254	254.500	12
L9-HG-ICP-MS	232.0	228.0	220.0	225.0	227.0	223.0	225.833	25.5
L10-ICP-SFMS	262	237	253	261	235	248	249.333	20
L11-ICP-QMS	261.0	254.1	265.1	254.7	270.7	275.9	263.583	16.0
L12-HG-AAS	274.3	272.3	261.2	289.3	295.0	319.5	285.267	42.5

1) As reported by each individual lab (*k*=2)

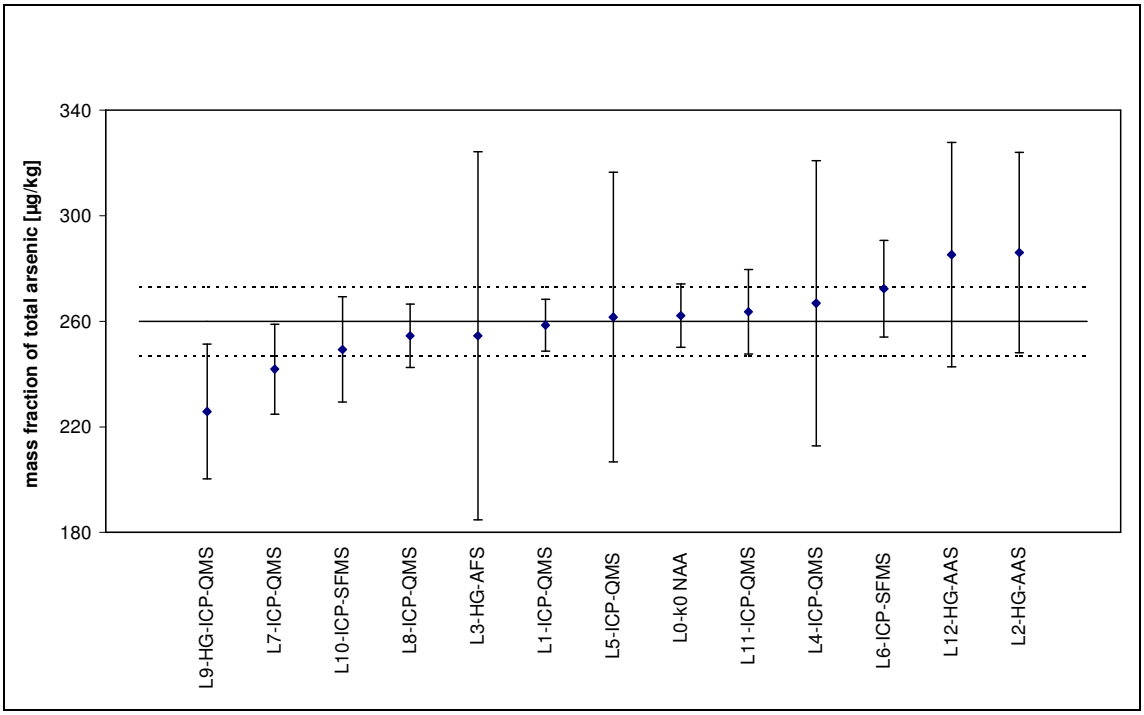

Figure 13: Results of the characterisation study for the mass fraction of total arsenic in rice
(Continuous line: certified value; dashed line: expanded uncertainty with *k*=2; results with uncertainty bars as submitted by each individual lab)

Table 7: Mass fraction of dimethylarsinic acid (DMA) measured as arsenic in rice as reported by each individual lab

Laboratory code	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	average [µg/kg]	expanded uncertainty [1] [µg/kg]
L13-HPLC-ICP-QMS	121.5	123.2	119.6	123.8	118.5	115.5	120.350	9.0
L14-HPLC-ICP-QMS	120.2	120.8	114.4	109.7	113.8	113.6	115.417	7.6
L15-HPLC-ICP-SFMS	114	119	117	117	119	118	117.333	10
L19-HPLC-ICP-QMS	109.5	116.8	109.6	98.6	106.9	108.2	108.267	29
L23-HPLC-ICP-SFMS	130	118	145	128	131	132	130.667	8
L24-HPLC-ICP-QMS	131	132	136	139	145	142	137.500	16
L25-HPLC-ICP-QMS	94.6	93.7	97.6	89.2	92.3	87.0	92.400	13.0
L26-HPLC-ICP-QMS	133	137	139	118	126	121	129.000	12
Results not used for certification								
L21-HPLC-ICP-QMS	*72*	*41*	*79*	*101*	*83*	*102*	*79.667*	*15*

1) As reported by each individual lab (*k*=2)

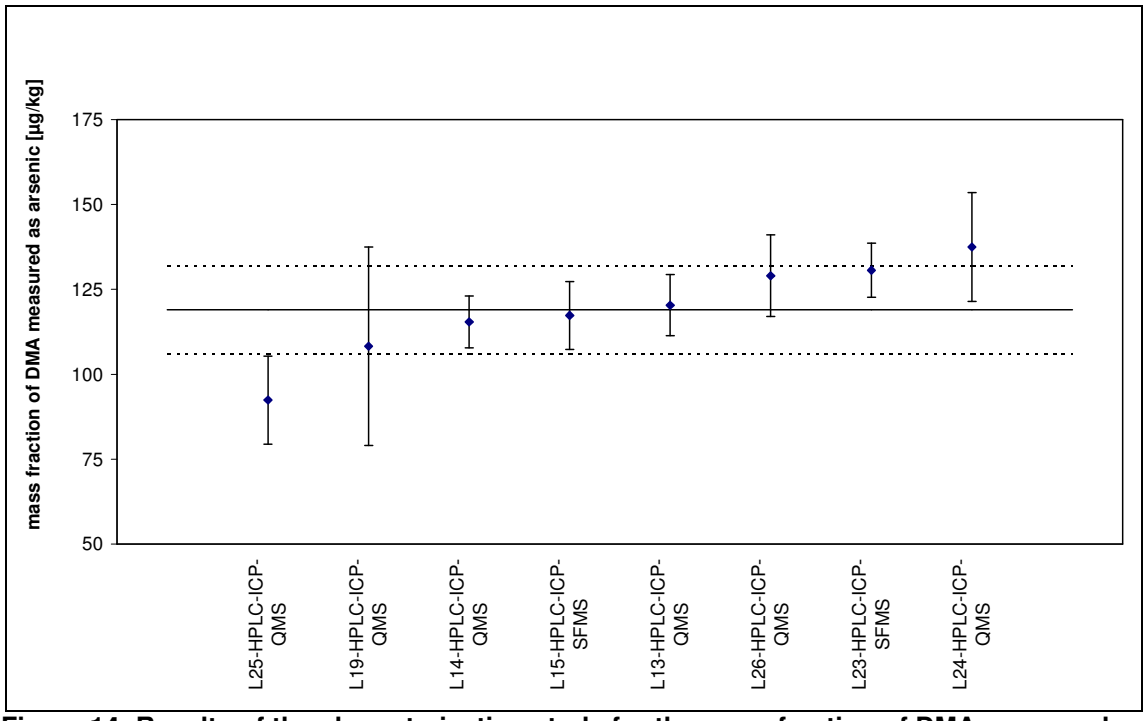

Figure 14: Results of the characterisation study for the mass fraction of DMA measured as arsenic in rice

(Continuous line: certified value; dashed line: expanded uncertainty with k=2; results with uncertainty bars as submitted by each individual lab)

Table 8: Mass fraction of arsenite/arsenate measured as arsenic in rice as reported by each individual lab

Laboratory code	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	average [µg/kg]	expanded uncertainty [1] [µg/kg]
L13-HPLC-ICP-QMS	110.1	108.2	106.7	102.2	101.4	105.5	105.683	7.8
L14-HPLC-ICP-QMS	109.7	110.1	106.8	107.3	110.4	112.2	109.417	7.2
L15-HPLC-ICP-SFMS	118	111	113	117	112	111	113.667	10
L16-HG-AAS	120	124	118	116	128	131	122.833	17
L17-HPLC-ICP-QMS	125	127	125	128	121	124	125.000	7
L18-HG-AAS	125	122	121	128	133	134	127.167	20
L19-HPLC-ICP-QMS	138.5	133.6	139.4	153.6	144.1	145.0	141.533	48.4
L20-HG-AAS	136.0	150.5	152.4	133.3	147.0	140.0	143.200	23.0
L21-HPLC-ICP-QMS	165	114	155	154	132	154	145.667	27
L22-HPLC-HG-AFS	113.0	120.5	130.8	115.8	116.5	134.6	121.867	21.9
L23-HPLC-ICP-SFMS	134	113	106	117	102	121	115.500	8
L24-HPLC-ICP-QMS	114	112	114	115	116	117	114.667	9
L26-HPLC-ICP-QMS	118	117	117	124	130	130	122.667	10.0
Results not used for certification								
L25-HPLC-ICP-QMS	*105*	*103*	*105*	*102*	*100*	*96.7*	*101.950*	*14*

1) As reported by each individual lab (k=2)

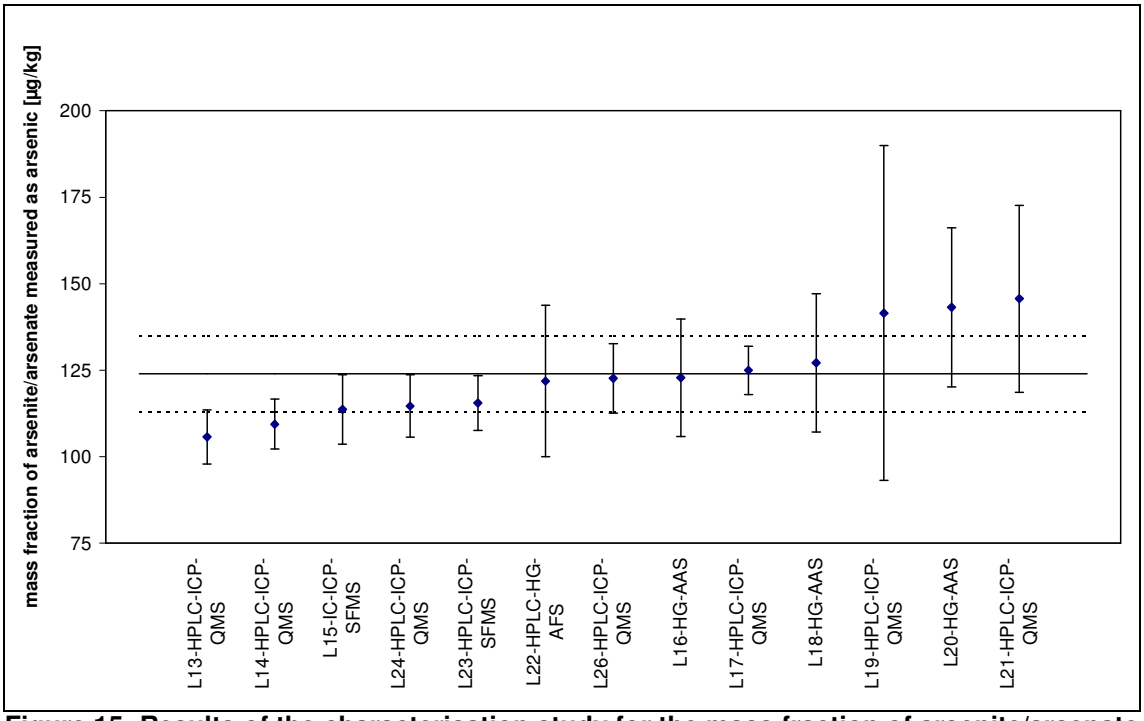

Figure 15: Results of the characterisation study for the mass fraction of arsenite/arsenate measured as arsenic in rice

(Continuous line: certified value; dashed line: expanded uncertainty with k=2; results with uncertainty bars as submitted by each individual lab)

European Commission

EUR 25366 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: The certification of the mass fractions of total arsenic, dimethylarsinic acid and the sum of arsenite and arsenate in rice: ERM-BC211

Author(s): J. Boertz, H. Emteborg, J. Charoud-Got, J. Snell, A. Held, H. Emons
European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium

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Abstract
This report describes the production of ERM-BC211, a powdered rice material certified for the mass fractions of total arsenic, dimethylarsinic acid and the sum of arsenite and arsenate. The material has been produced following ISO Guide 34:2009 [1].
The starting material, which had been checked for its arsenic species was purchased and supplied by the University of Aberdeen. The rice was milled, sieved, dried, homogenised, filled in vials and sterilised.
Between unit-inhomogeneity was quantified and stability during dispatch and storage was assessed in accordance with ISO Guide 35:2006 [1]. Within-unit inhomogeneity was quantified to determine the minimum sample intake.
The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were estimated in compliance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM) [1] and they include contributions from possible between-unit inhomogeneity, instability and characterisation.
The material is intended for quality control and assessment of method performance. Moreover, it can be used fvalidation purposes and trueness determination. The CRM is available in glass bottles containing 10 g of dried rice powder closed under argon atmosphere. The minimum amount of sample to be used for total arsenic and dimethylarsinic acid is 50 mg. The minimum amount of sample to be used for the sum of arsenite and arsenate is 100 mg.
The CRM has been accepted as European Reference Material (ERM) after peer evaluation by the partners of the European Reference Materials consortium.

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