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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM – 2020A

Inorganic Elements and Arsenic Species in Cricket Powder

Batch Number
STY-0149-001

Description
The certified reference material (CRM) was produced by the Chemical Metrology Laboratory (CML). The material comprised ground crickets supplied by a local insect farmer. Fortified chicken broiler grains were fed to the crickets during their growth phase to produce the naturally incurred material.

Matured crickets with a size of around one inch were harvested and processed. The crickets were dried at 100°C for 6-12 hours, ground and sieved with a 500 µm vibratory sieve shaker to achieve a uniform particle size. The resulting cricket powder was freeze-dried and then homogenised using a drum mixer for at least 7 days. The material was then packed into pre-cleaned amber glass bottles, flushed with argon inside a glovebox before capping. Following that, the bottled samples were subjected to gamma irradiation at 22.1 – 24.9 kGy[1] to prolong their shelf-life. A unit of the CRM consists of one amber glass bottle containing approximately 10 g of material.

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO Guide 35:2017 [3].

Certified Mass Fraction Values
A certified value is a value for which a laboratory has the highest confidence in its accuracy, in that all known or suspected sources of biases have been investigated and accounted for. The certified mass fraction values for eight analytes in the CRM are listed below. The certified mass fraction values for cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and selenium (Se) were determined by inductively coupled plasma mass spectrometer using isotope dilution mass spectrometry (ICP-IDMS) [4]. The certified mass fraction value for arsenic (As) was determined by inductively coupled plasma high resolution mass spectrometer (ICP-HR-MS) using standard addition method. The certified mass fraction values for As species [dimethylarsinic acid (DMA) and inorganic arsenic compounds (iAs)] were

[1] The irradiation work was performed by a subcontractor.

determined by high performance liquid chromatography inductively coupled plasma mass spectrometer (HPLC-ICP-MS) using standard addition method [5].

Analyte	Mass fraction	Units
Arsenic	7.84 ± 0.74	mg/kg
Cadmium	1.871 ± 0.088	mg/kg
Chromium	12.4 ± 1.9	mg/kg
Lead	10.69 ± 0.71	mg/kg
Mercury	3.38 ± 0.24	mg/kg
Selenium	20.5 ± 1.3	mg/kg

Analyte	Mass fraction as As	Units
Dimethylarsinic Acid	4.46 ± 0.43	mg/kg
Inorganic arsenic compounds (arsenite + arsenate)	2.10 ± 0.17	mg/kg

Each mass fraction value is expressed as the certified value ± the expanded uncertainty. The values are reported on a dry-mass basis.

The uncertainty listed with the certified value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence). The certified value has an associated measurement uncertainty attributed to uncertainty contribution from characterisation of the material (u_{char}), uncertainty in the homogeneity of the material (u_{bb}) and uncertainty in the stability of the material (u_{stab}). The u_{char} was evaluated by combining uncertainties from method precision, the concentration of calibration solution, weighing, different ion pairs used (for Cd, Pb, Hg and Se), isotope ratios (for Cd, Cr, Pb, Hg and Se), the relative atomic mass (for Pb only), method recovery (As) and linear regression (As species), in accordance with ISO/IEC Guide 98-3:2008 [6].

Homogeneity

Homogeneity testing on the analytes in the material was performed on at least ten bottles with two sub-samples taken from each bottle. ICP-MS was employed for the determination of the analytes. The sample size taken for homogeneity testing was about 0.5 g. No significant differences in the between- and within-bottle variances were found for As, Cr, Se, DMA and iAs using one-way ANOVA at 95 % confidence level [3]. For Cd, Hg and Pb significant differences in the between- and within-bottle variances were observed using one-way ANOVA at 95 % confidence level. However, the between-bottle standard deviations were sufficiently small compared to the standard uncertainties of the certified mass fraction values. Thus, the analytes in the material were assessed to be sufficiently homogeneous. The u_{bb} was evaluated from the uncertainty due to between-bottle inhomogeneity.

Stability

Short-term stability testing on the analytes in the material at 50 °C (maximum allowable transportation temperature) showed that they were stable up to 21 days.

The long-term stability of the analytes at storage temperature (18 °C to 25 °C) was evaluated on three occasions over a period of up to 5.8 months for As, Cd, Cr, Pb, Hg and Se, and up to 3 months for iAs and DMA. The results showed that the analytes were stable over the study period. The u_{stab} was evaluated from the standard error of the slope.

Validity of Certified Mass Fraction Values

The certified mass fraction values are valid within its measurement uncertainty until **8 Aug 2026**, provided that the CRM is subjected to the same handling and storage conditions as stated in this Certificate of Analysis (COA).

The CRM will be continuously monitored during the validity period to determine if any substantive change to the certified values has occurred. If necessary, the user will be advised or an updated COA will be issued when the property value of the CRM is found to have changed.

Analytical Methods

The certified mass fractions of Cd, Cr, Pb, Hg and Se in the material were determined by exact-matching ICP-IDMS. Standard reference materials (SRMs) from the National Institute of Standards and Technology (NIST, USA) (SRM 3108 for Cd, SRM 3112a for Cr, SRM 3128 for Pb, SRM 3133 for Hg and SRM 3149 for Se) were used as calibration standards for IDMS measurements. Enriched isotopes ^{111}Cd, ^{53}Cr, ^{206}Pb, ^{201}Hg and ^{77}Se from Oak Ridge National Laboratory (USA) were used as the internal standards. The calibration blends were prepared gravimetrically by mixing appropriate amount of calibration standard solutions and internal standard solutions. The sample blends were prepared by spiking appropriate amount of internal standard into the material.

The certified mass fraction of As was determined by ICP-HR-MS using standard addition method. Gallium SRM from NIST (SRM 3119a) was added to the sample digest as internal standard. Different amounts of As SRM from NIST (SRM 3103a) were then spiked into the sample digest to produce sample blends.

The certified mass fraction values of As species were determined by HPLC-ICP-MS using standard addition method. Different amounts of As species certified reference material from NIST [SRM3036 for As(V) and SRM3031 for DMA] were then spiked into the diluted sample to produce sample blends.

Both sample and quality control blends were also prepared and analysed concurrently. For As, Cd, Cr, Pb, Hg and Se, microwave acid digestion was employed using 5 mL nitric acid (HNO_3), 0.2 mL hydrofluoric acid (HF) and 2 mL hydrogen peroxide (H_2O_2) to give clear digests. Meanwhile, As species were treated differently, undergoing microwave-assisted extraction with 35 mL of 1% HNO_3 and 1 mL of H_2O_2. After extraction, all solutions were centrifuged at 4,000 rpm for 10 minutes to separate any solid residues. The resulting supernatants, which contained the extracted analytes, were then collected for standard addition and subsequent analysis [7].

Metrological Traceability

The certified mass fraction values are traceable to the International System of Units (SI) through the use of SRMs from NIST.

Intended Use

For the validation of methods or as quality controls used to determine the mass fraction of As, Cd, Cr, Pb, Hg, Se, iAs and DMA in food products with high protein content, such as insect and similar matrices.

Instruction for Use

After use, the amber glass bottle should be re-capped, sealed with Parafilm and stored properly under the recommended storage conditions. If results differ from certified value in subsequent sampling, customers are advised to purchase a new CRM. To reduce moisture absorption, weighing needs to be performed as quickly as possible and the amber glass bottle should not be left open after sampling. The minimum sample size for each use should be about 0.5 g. A correction for dry mass must be made by taking 1.0 g of sample and drying in an oven for 1.5 hours at 102°C. Before weighing, the closed container should be cooled down in a desiccator with silica gel for at least 30 min. Dry mass correction and sample analysis should be carried out at the same time on separate test portion. The moisture content at the time of certification was approximately 4.5 %.

Storage

The material should be stored at 18 °C to 25 °C in its original amber glass bottle. Exposure to direct intense light and ultraviolet radiation should be avoided.

Safety Precautions for Users

Treat the material as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact or ingestion.

Further Information

Please direct all enquiries regarding this CRM to the contact provided in this COA.

References

1. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
2. ISO 17034:2016 General requirements for the competence of reference material producers.
3. ISO Guide 35:2017 Reference materials – Guidance for characterisation and assessment for homogeneity and stability.
4. Sargent, M.; Harrington, C.; Harte, R.; *Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry*, RSC Publishing, 2002.
5. Abbyad, P.; Tromp, J.; Lam, J.; Salin, E.; *Optimization of the technique of standard additions for inductively coupled plasma mass spectrometry*, J. Anal. At. Spectrom. (2001) 16: 464 – 469.
6. ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).
7. Reyes L.H, Mar J.L.G, Rahman G.M.M, Seybert B, Fahrenholz T, Kingston H.M.S, *Simultaneous determination of arsenic and selenium species in fish tissue using microwave-assisted enzymatic extraction and ion chromatography-inductively coupled plasma mass spectrometry*. Talanta (2009) 78: 983-990.

Certificate Revision Record

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-2020A/01	8 Aug 2024	Issuance of first certificate
CML-HRM-2020A/02	31 Jul 2025	Extension of expiry date

Note

HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the reference material by the customer.

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