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Ref. No.: CML-HRM-1032A/01
Date of Issue: 17 Jun 2026

Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM – 1032A

Organic Contaminants in Black Soldier Fly Larvae Powder

Batch Number
STY-0146-001

Description
The certified reference material (CRM) was produced by the Chemical Metrology Laboratory (CML). The CRM consists of about 12 g of freeze-dried black soldier fly larvae powder, prepared from naturally incurred material. The larvae were supplied by a local insect farmer and raised on fortified feeds during their growth to produce the naturally incurred material.

The larvae were harvested at maturity on Day 15, or upon emergence of the first prepupa. Following harvest, the larvae were processed and frozen at the farm before their transfer to CML. The frozen larvae were ground, freeze-dried, ground again and then sieved. The powder was homogenised and bottled into amber glass bottles, crimp-sealed with rubber septa and packaged under argon.

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO 33405:2024 [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 aflatoxin B1 and zearalenone listed in the Table below were determined by liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS) and were corrected for dry mass.

Analyte	CAS No	Certified Mass Fraction	Unit
Aflatoxin B1	1162-65-8	5.4 ± 1.7	µg/kg
Zearalenone	17924-92-4	204 ± 39	µg/kg

The mass fraction value is expressed as the certified value ± the expanded uncertainty.

The uncertainty listed with the certified mass fraction 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 mass fraction of calibration solution (which included the purity of the standard), weighing, bias using different ion pairs, bias using different solid phase extraction cartridges, bias using different columns, bias using different IDMS approaches, method recovery and moisture content, in accordance with ISO/IEC Guide 98-3:2008 [4].

Homogeneity

Homogeneity testing on the analytes in the material was performed on ten bottles with two sub-samples taken from each bottle. LC-MS/MS was employed for the determination of both 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 aflatoxin B1 using one-way ANOVA at 95 % confidence level [3]. The between-bottle standard deviation for zearalenone was sufficiently small compared to the standard uncertainty of the certified mass fraction value [3]. Thus, the analytes in the material were regarded to be sufficiently homogeneous. The u_{bb} was evaluated from the uncertainty due to between-bottle inhomogeneity.

Bottling trend was observed for aflatoxin B1 and zearalenone in the homogeneity study. To account for the observed trend, an additional uncertainty on trend analysis (u_{trend}) was evaluated from the standard error of the slope and included in their overall measurement uncertainties.

Stability

The long-term stability of aflatoxin B1 and zearalenone in HRM-1032A stored at below -60 °C (storage temperature) was evaluated on three occasions over a period of up to about 8 months. The results showed that the analytes were stable when stored at below -60 °C over the study period. The u_{stab} was evaluated from the standard error of the slope from the long-term stability study.

Validity of Certified Mass Fraction Values

The certified mass fraction values are valid within their respective measurement uncertainties until **17 June 2027**, 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, its user will be advised or an updated CoA may be issued when the property value of the CRM is found to have changed.

Analytical Methods

The certified mass fractions of aflatoxin B1 and zearalenone in the material were determined by exact-matching LC-IDMS/MS. CRMs of aflatoxin B1 in acetonitrile (CRM 0012) and zearalenone in acetonitrile (CRM 0005) from the National Metrology Institute of South Africa (NMISA) were used as calibration standards. Isotope-labelled $^{13}C_{17}$-aflatoxin B1 in acetonitrile and $^{13}C_{18}$-zearalenone in acetonitrile from Romer Laboratory 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 gravimetrically prepared by mixing of about 1 g of the material and internal standard solutions. Quality control blends were also prepared and analysed concurrently. All the blends were equilibrated to room temperature before subjected to extraction and clean-up procedures. Each sample and quality control blends were bracketed with a calibration blend and analysed at least three times.

Metrological Traceability

The certified mass fraction values for aflatoxin B1 and zearalenone are traceable to the International System of Units (SI) through the use of CRMs from NMISA.

Intended Use

HRM-1032A is intended for the use in the validation of methods or as quality controls used in the determination of the mass fraction of aflatoxin B1 and zearalenone in food products with high protein content, such as insect and similar matrices.

Instructions for Use

1) Prior to use, the material should be equilibrated to room temperature and **thoroughly** mixed by swirling and inverting the bottle.
2) The minimum sample size for the analysis should no less than 0.5 g.
3) After use, the bottle should be resealed immediately and stored at below - 60 °C. To prevent the material from absorbing moisture, do not leave the bottle opened for a long period of time. *(Note: The certified values may not be valid after two cycles of freeze-thaw as the stability of the analytes subjected to more than two freeze-thaw cycles has not been investigated.)*
4) Dry mass correction:
    a. A correction for dry mass should be made by taking about 1 g of material and drying in a desiccator containing anhydrous calcium sulphate as the desiccant at room temperature for at least 20 days until a constant weight is obtained.
    b. Before weighing, the empty container should be dried in an oven at 70 °C or above for overnight and cooled down to the room temperature in a desiccator with anhydrous calcium sulphate.
    c. Do not re-use the material for analysis of the analytes.
5) The moisture content at the time of certification was approximately 3.8 %.
6) Dry mass correction and sample analysis should be carried out at the same time on separate test portions. The exposure period of the material in a certain humidity level during the weighing process for both dry mass correction and sample analysis should be similar.
7) If results differ from certified value in subsequent sampling, customers are advised to purchase a new CRM.

Transport and Storage

HRM-1032A is transported in frozen state (in dry ice). Upon receipt, the CRM should be stored at below - 60 °C in its original bottle. Exposure to direct intense light and ultraviolet radiation should be avoided. Storage of the material at room temperature or in the refrigerator may result in changes in the mass fraction of the analytes.

Safety Precautions for Users

Treat the material as hazardous substance. Use appropriate work practices when handling the material, in order 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 33405:2024 Reference materials – Approaches for characterisation and assessment for homogeneity and stability.
4. ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).

Certificate Revision Record

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-1032A/01	17 Jun 2026	Issuance of first certificate

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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