# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA MX009: Trace Elements in Freeze-Dried Prawn Tissue

## Certified values

| Element | Mass Fraction Dry Mass Basis mg/kg | Coverage Factor Dry Mass Basis | Mass Fraction Wet Mass Basis (Undried) mg/kg | Coverage Factor Wet Mass Basis (Undried) |
|---|---|---|---|---|
| As | 4.06 ± 0.15 | 2.16 | 3.69 ± 0.27 | 2.45 |
| Co | 0.0932 ± 0.0068 | 2.07 | 0.0846 ± 0.0075 | 2.09 |
| Cr | 1.06 ± 0.18 | 2.02 | 0.96 ± 0.16 | 2.02 |
| Cu | 17.1 ± 1.2 | 2.01 | 15.5 ± 1.3 | 2.09 |
| Fe | 116 ± 10 | 2.06 | 105 ± 10 | 2.06 |
| Hg | 0.1483 ± 0.0051 | 1.98 | 0.1345 ± 0.0090 | 2.36 |
| Mn | 5.55 ± 0.33 | 2.05 | 5.04 ± 0.40 | 2.12 |
| Ni | 0.515 ± 0.070 | 2.11 | 0.467 ± 0.067 | 2.09 |
| Se | 2.78 ± 0.11 | 2.10 | 2.53 ± 0.19 | 2.36 |
| V | 0.281 ± 0.025 | 2.10 | 0.255 ± 0.025 | 2.09 |
| Zn | 74.0 ± 4.8 | 2.07 | 67.1 ± 5.5 | 2.12 |

*Uncertainties are expanded to provide a level of confidence of 95%.*

Certified values for a range of analytes in MX009 are given above. The mass fraction of each analyte is given on a dry mass basis and on a wet mass basis (undried). Dry mass basis measurements give a more accurate characterisation of MX009 as the analyte mass fractions are not affected by moisture content variation over time. Instructions for use of dry mass basis and wet mass basis (undried) certified values are described in this certificate. Certified values for Hg were determined by isotope dilution ICP-MS analysis, the certified values for all other elements were determined by standard addition analysis. All certified reference values are traceable to SI units. Details of the analytical methodology and measurement uncertainty estimation are provided in the following sections.

**Expiry:** 27 August 2030 **Batch No.:** 2008.01

### Description

Certified reference material MX009 is a freeze-dried prawn tissue powder bottled in units of approximately 10 g. Certified values are provided for the mass fractions of As, Co, Cr, Cu, Fe, Hg, Mn, Ni, Se, V and Zn in MX009. Information values are also given for the mass fractions of Ag, B, Ba, Bi, Ca, Cd, Cs, K, Li, Mg, Na, P, Pb, Rb, S, Sn, Sr, U and for the mass fraction of moisture.

### Intended use

Certified reference material MX009 is intended to be used to verify and/or validate analytical methods for elemental analysis in prawn tissue, marine biota and similar sample types.

## Instructions for use

**Subsampling:** The contents of the MX009 bottle should be mixed thoroughly shortly before subsampling. For trace element analysis, a minimum sample size of 0.5 g (dry mass basis) should be used for MX009 analysis. Trace element certified values may not be valid for sample sizes less than 0.5 g.

**Contamination minimisation:** MX009 should only be opened to the atmosphere in a clean environment such as a ULPA-filtered laminar flow hood. For trace element analysis, MX009 should be subsampled and handled in such a way as to avoid contamination of both the bulk material and the subsample.

**Use of dry mass basis certified values:** To remove the effect of moisture content variation on trace element mass fraction measurements, correction for moisture content should be applied. Gravimetric moisture content determination should be performed on a separate portion of MX009 to that used for trace element analysis to avoid contamination and/or loss of analytes through vaporisation. A sample size not less than 0.5 g (dry mass basis) should be used for moisture content measurement. The drying protocol for MX009 is oven-drying at 100°C for 24 hours. Longer oven drying should not be used as mass loss will continue but does not correspond to loss of moisture from the reference material. The moisture content measured at time of analysis should then be used to calculate the moisture content correction factor to convert wet mass basis (undried) measurements to dry mass basis measurements.

**Use of wet mass basis (undried) certified values:** Where correction for MX009 moisture content is not applied to analytical results, comparison can be made to the wet mass basis (undried) certified values. In this case, possible moisture content variation over time is accounted for in the measurement uncertainty estimate of the certified value. Consequently, the relative expanded uncertainty is larger for the wet mass basis (undried) basis certified values than for the dry mass basis certified values.

**Storage:** MX009 should be stored tightly sealed in the original bottle at 20°C ± 5°C. Dry mass basis measurements are not affected by the humidity of storage conditions, whereas wet mass basis (undried) measurements rely on avoiding extremes of humidity. Storage in a desiccator is therefore not recommended if wet mass basis (undried) results are to be used. MX009 should not be exposed to direct sunlight or intense sources of radiation.

## Metrological traceability

Certified values are traceable to the SI units for mass (kilogram) and amount of substance (mole). Gravimetric preparation is traceable to the SI kilogram through balance calibrations. Isotope ratio measurements are traceable to the mole through double isotope dilution (a primary ratio method). Isotope dilution mass fraction quantification is traceable to SI units through NIST single element calibration solutions. Elements quantified via ICP-MS Standard Addition are traceable to the SI unit for mass (kg) through the primary calibration standards certified by NIST (USA) and the SI unit for amount of substance (mole) through gravimetric measurement and data for isotopic composition and relative atomic mass. Isotopic composition is traceable to IUPAC published data. For dry mass basis certified values, moisture content correction is traceable to the method described in the “use of dry mass basis certified values” section above.

## Stability

The main source of instability for MX009 is variation of moisture content in transport and/or storage which will directly affect elemental mass fraction values. Applying a moisture content correction and using dry mass basis certified values is recommended to negate the effect of moisture content variation. Wet mass basis (undried) certified values include a measurement uncertainty contribution for variation in the moisture content of MX009 under recommended storage conditions.

The long-term stability of certified values has been demonstrated through periodic analysis of MX009 both at NMIA and by inter-laboratory testing. Statistical assessment followed ISO Guide 35[2]. The long term stability of MX009 was reassessed in 2019. Based on this long term stability testing this certificate has been re-issued with an extended expiry date.

## Homogeneity

Homogeneity assessment for MX009 certified values was conducted according to ISO Guide 35[2]. A representative number of sample bottles of MX009 were randomly selected to assess the homogeneity of the reference material batch. Measurements were made on duplicate subsamples taken from these bottles. Analysis of variance (ANOVA) was used to estimate the measurement uncertainty due to method precision and reference material inhomogeneity.

## Production

MX009 was prepared from fresh prawns (approximately 300 kg) collected from the Central Coast, Queensland, Australia. The prawns were cooked and shelled then freeze-dried. The prawn tissue was then ground in an industrial blender fitted with stainless steel blades. The ground prawn tissue was passed through a 212 µm sieve. The sieved powder was thoroughly homogenised in a drum-hoop mixer and then bottled into 10 g portions using a rotary sample divider. The entire production batch was gamma irradiated (25 kGy minimum) to prevent microbial activity. Each unit of MX009 is labelled with a unique bottle number.



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## Analytical method

The certified trace element mass fraction of Hg in MX009 was measured by double isotope dilution ICP-SF-MS[1]. $^{201}$Hg-enriched mercury was used as the isotope dilution internal standard added prior to sample digestion and the $^{202}$Hg/$^{201}$Hg isotope ratio was used for isotope dilution quantification. The mass fractions for the remaining certified elements was measured by the method of standard additions using ICP-SF-MS. Internal standardisation for the standard addition measurememnts was made using $^{44}$Ca, $^{69}$Ga, $^{89}$Y and $^{115}$In isotopes. NIST 3100 series primary calibration materials were used for all certified elements, see table below for details, these were diluted gravimetrically to working concentrations. ICP-SF-MS measurements were made using a sample size of ≈ 0.5 g and the samples prepared via microwave digestion using various combinations of nitric acid and hydrogen peroxide with and without Hydrochloric acid in sealed high-pressure vessels. Following digestion, samples were diluted with ultra high purity water.

| Element | Standard Name | Lot No. |
|---|---|---|
| As | NIST 3103a | 100818 |
| Co | NIST 3113 | 190630 |
| Cr | NIST 3112a | 170630 |
| Cu | NIST 3114 | 121207 |
| Fe | NIST 3126a | 140812 |
| Hg | NIST 3133 | 061204 |
| Mn | NIST 3132 | 050429 |
| Ni | NIST 3136 | 120619 |
| Se | NIST 3149 | 100901 |
| V | NIST 3165 | 160906 |
| Zn | NIST 3168a | 120629 |

## Measurement uncertainty

Measurement uncertainty was estimated according to international standards[2, 3] and NMIA standard procedures. All factors that could reasonably be expected to affect the measurement result were identified and the standard uncertainty of each was estimated from experimental data. The standard uncertainty estimates were combined and expanded to a 95% coverage interval using the coverage factors given in the certified values table.

For MX009 certified values, the measurement uncertainty contributors examined were the calibration standard, gravimetric preparation, isotopic composition, moisture content, isotopic equilibration, isotope ratios measurements, method precision, method bias, between-bottle homogeneity and long-term stability.



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# Information Values for Trace and Major Elements

| Element | Mass Fraction Dry Mass Basis mg/kg | Coverage Factor Dry Mass Basis | Mass Fraction Wet Mass Basis (Undried) mg/kg | Coverage Factor Wet Mass Basis (Undried) | *n* |
|---|---|---|---|---|---|
| Ag | 0.0165 ± 0.0030 | 2.18 | 0.0150 ± 0.0028 | 2.18 | 13 |
| B | 1.29 ± 0.30 | 2.10 | 1.17 ± 0.28 | 2.10 | 19 |
| Ba | 1.39 ± 0.17 | 2.06 | 1.26 ± 0.17 | 2.06 | 27 |
| Bi | 0.0135 ± 0.0014 | 2.26 | 0.0123 ± 0.0014 | 2.26 | 10 |
| Ca | 5800 ± 200 | 2.03 | 5300 ± 300 | 2.03 | 34 |
| Cd | 0.144 ± 0.011 | 2.01 | 0.131 ± 0.012 | 2.01 | 46 |
| Cs | 0.0224 ± 0.0048 | 2.26 | 0.0203 ± 0.0045 | 2.26 | 10 |
| K | 1578 ± 55 | 2.04 | 1431 ± 82 | 2.04 | 31 |
| Li | 0.153 ± 0.035 | 2.18 | 0.139 ± 0.033 | 2.18 | 13 |
| Mg | 1935 ± 63 | 2.03 | 1755 ± 98 | 2.03 | 35 |
| Na | 13900 ± 450 | 2.03 | 12610 ± 700 | 2.03 | 34 |
| P | 6920 ± 300 | 2.03 | 6280 ± 390 | 2.03 | 34 |
| Pb | 0.372 ± 0.040 | 2.01 | 0.338 ± 0.039 | 2.01 | 46 |
| Rb | 0.777 ± 0.078 | 2.16 | 0.705 ± 0.079 | 2.16 | 14 |
| S | 10820 ± 850 | 2.12 | 9810 ± 900 | 2.12 | 17 |
| Sn | 0.326 ± 0.044 | 2.12 | 0.296 ± 0.042 | 2.12 | 17 |
| Sr | 70.4 ± 2.5 | 2.10 | 63.9 ± 3.8 | 2.10 | 19 |
| U | 0.0074 ± 0.0012 | 2.23 | 0.0068 ± 0.0011 | 2.23 | 11 |

*Information values for 18 additional trace and major elements in MX009 are given above. Information values were obtained from multiple rounds of inter-laboratory testing in which MX009 was the test sample. Information values are given on a dry mass basis and also on an wet mass basis (undried). The number of results used to generate the information value for each element is also given. Uncertainties are expanded to provide a level of confidence of 95%.*

## Inter-laboratory testing

Data was collected from three Inter-laboratory studies coordinated by NMIA using MX009 as the test sample. All Inter-laboratory results collected were for the reference material as received (no moisture content correction was performed by participants). The dry mass basis information values table were calculated using the MX009 moisture content measured at time of certification analysis (see table for information value for moisture content below). The three sets of Inter-laboratory data were combined for determination of information values for MX009 and data was evaluated using robust statistics[4]. The robust average of participants' results has been used as the information value.

## Homogeneity assessment

With the exception of S, a partial homogeniety assement was carried out on the elements with information value in table above and the measurement uncertainty was expanded to incorporate this.

## Stability assessment

The long-term stability of MX009 information values has been monitored over the life of the material and no evidence of instability has been observed.

## Measurement uncertainty

The measurement uncertainty for information values combines estimates for measurement uncertainty due to characterisation by Inter-laboratory comparison, homogeneity and moisture content. The uncertainty of the robust average (derived from the robust standard deviation of Inter-laboratory data)[2] has been used to estimate the measurement uncertainty due to characterisation.



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## Metrological traceability

Information values are the consensus of participants' results (robust average) and are not traceable to any external reference. So although expressed in SI units, the metrological traceability of these assigned values has not been established.

# Information value for MX009 moisture content

An information value for the moisture content of MX009 at time of certification (June 2008) measured according to the procedure described above is presented in the table. Moisture content determination should be performed at the time of analysis for the most accurate moisture content corrected results.

| Property | Mass Fraction mg/g |
|---|---|
| **Moisture Content** | **92.8 ± 7** |

Raluca Iavetz
Manager Chemical Reference Values
27 August 2020
Report ID: MX009.2020.01

Accreditation No. 198
The property values specified in this report supersede any issued prior to 27 August 2020.

## References

1. L.G. Mackay, C.P. Taylor, R.B. Myors, R. Hearn and B. King. - High accuracy analysis by isotope dilution mass spectrometry using an iterative exact matching technique; Accred. Qual. Assur. (2003)
2. Reference materials — Guidance for characterization and assessment of homogeneity and stability; ISO Guide 35, 4th edition (2017)
3. JCGM, *Evaluation of measurement data — Guide to the expression of uncertainty in measurement*. JCGM100:2008
4. Statistical methods for use in proficiency testing by interlaboratory comparison; ISO 13528, 2016.



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