

National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 7402-a

No. +++



## Trace Elements, Arsenobetaine and Methylmercury in Cod Fish Tissue

This certified reference material (CRM) is produced in accordance with the NMIJ's management system and is in compliance with ISO 17034 and ISO/IEC 17025. This CRM is intended for use in evaluating or validating analytical methods and instruments used for the determination of the elements listed below, arsenobetaine, and methylmercury in fish tissue and similar matrices.

**Certified Values**

The certified values for 13 elements and two compounds in this CRM are given in the tables below. The values are expressed as mass fractions based on dry mass (the drying procedure is given in this certificate). The uncertainty of the certified value is the half-width of the expanded uncertainty interval calculated using a coverage factor ( $k$ ) of 2, which gives a level of confidence of approximately 95 %.

Element	Certified value, Mass fraction (mg/kg)	Expanded uncertainty, Mass fraction (mg/kg)	Analytical methods (see below)
Cr	0.72	0.09	1, 2, 3, 5
Mn	0.41	0.03	2, 3, 5
Fe	11.2	0.9	1, 2, 5
Ni	0.38	0.05	1, 2, 3, 5
Cu	1.25	0.07	1, 2, 5
Zn	21.3	1.5	1, 2, 4
As	36.7	1.8	2, 3, 4, 5
Se	1.8	0.2	1, 2, 5
Hg	0.61	0.02	1, 2, 8

Element	Certified value, Mass fraction (g/kg)	Expanded uncertainty, Mass fraction (g/kg)	Analytical methods (see below)
Na	3.6	0.2	4, 6, 7
Mg	1.34	0.03	2, 4, 6
K	22.3	1.0	4, 6, 7
Ca	0.52	0.05	4, 6, 7

## Analytical methods:

- 1) Isotope dilution-inductively coupled plasma mass spectrometry (ID-ICP-MS)
- 2) ICP-MS
- 3) High-resolution ICP-MS
- 4) Inductively coupled plasma atomic emission spectrometry (ICP-AES)
- 5) Graphite furnace atomic absorption spectrometry
- 6) Flame atomic absorption spectrometry

- 7) Flame photometry  
 8) Thermal decomposition Au–amalgam trap cold vapor atomic absorption spectroscopy  
 [1) – 7): with microwave acid digestion, 8): without digestion]

Compound	Certified value, Mass fraction (mg/kg)	Expanded uncertainty Mass fraction (mg/kg)	Analytical methods (see below)
Arsenobetaine (as As)	35.5	1.8	9, 10
Methylmercury (as Hg)	0.58	0.02	11, 12, 13

Analytical methods:

- 9) Ultrasonic extraction/high-performance liquid chromatography-ICP-MS (HPLC-ICP-MS)  
 10) Ultrasonic extraction/isotope dilution-liquid chromatography-electrospray mass spectrometry (ID-LC-MS)  
 11) Ultrasonic alkali extraction/phenyl-derivatization/isotope dilution-gas chromatography-ICP-MS (ID-GC-ICP-MS)  
 12) Ultrasonic alkali extraction/ethyl-derivatization/isotope dilution-GC-ICP-MS (ID-GC-ICP-MS)  
 13) Ultrasonic acid extraction/phenyl-derivatization/isotope dilution-GC-ICP-MS (ID-GC-ICP-MS)

### Analysis

The certified values are the weighted means of results from two or more analytical methods at NMIJ:

- (1) Single primary method with one or more reference methods.  
 (2) Three or more reference methods.

The expanded uncertainty in each certified value is equal to  $U = k u_c$ , where  $u_c$  is the combined standard uncertainty derived from the analytical results, the method-to-method variance, the standard solution, and the sample homogeneity. The expanded uncertainties were calculated using a coverage factor ( $k$ ) of 2, which gives a level of confidence of approximately 95 %.

### Metrological Traceability

For the trace elements, the certified values were determined by ID-ICP-MS with using Japan Calibration Service System (JCSS) standard solutions and an NIST SRM 3149 Se standard solution, and the certified values are traceable to the International System of Units (SI). For the arsenobetaine, the certified value was determined by ID-LC-MS with NMIJ CRM 7901-a, and the certified value is traceable to SI. For the methylmercury, the certified value was determined by ID-GC-ICP-MS with a methylmercury standard solution, which was calibrated by using a JCSS standard solution, and the certified value is traceable to SI. All sample preparation was carried out by the gravimetric method using a balance calibrated by JCSS.

### Mutual Recognition Arrangement under Metre Convention

The certified values (Element) of this CRM are recognized for international equivalence based on the Mutual Recognition Arrangement under the Metre Convention (CIPM MRA). The calibration measurement capabilities (CMC) of NMIJ related to this CRM are registered in the Key Comparison Database (KCDB) (see <https://www.bipm.org/kcdb/>) of the International Bureau of Weights and Measures (BIPM).

### Expiration of Certification

This certificate is valid for one year from the date of shipment, provided that this CRM is stored in accordance with the instructions given in this certificate.

### Description of the material

This CRM was prepared from fish tissue that was powdered by freeze-pulverization after freeze-drying. This CRM is in the form of a pale yellow powder in an amber glass bottle (10 g each).

### Instructions for Storage

This CRM should be stored at temperatures of 15 °C to 35 °C in a clean place and shielded from light.

**Instructions for Use**

- 1) Be careful of contamination when opening. Seal the bottle for storage after opening.
- 2) After opening, be careful of contamination. It is desirable to use this CRM promptly once the bottle is opened.
- 3) Dry mass correction is required when the CRM is analyzed. The correction factor is obtained by the following procedure:
  - ① Weigh approximately 0.3 g of the CRM in a weighing glass vessel and then heat it at  $102\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  for 6 h in a drying oven.
  - ② Weigh the CRM with the vessel after cooling in a desiccator with silica gel.

The difference in the mass before and after drying is assumed to be the moisture content. The dry mass correction factor at the time of the certification was ca. 9.5 % (mass fraction).

Weighing for dry mass correction has to be done simultaneously with weighing for analysis.
- 4) From the viewpoint of homogeneity for the determination of each element except for Hg and methylmercury, more than 300 mg of this CRM should be used. More than 20 mg of this CRM should be used for the determination of Hg, and more than 500 mg of the CRM should be used for the determination of arsenobetaine.

**Precautions for Handling**

This CRM is for laboratory use only and is not edible. Wear a protective mask and gloves for safety when this CRM is used. All relevant laws regarding waste handling and management must be obeyed when disposing of this CRM. Refer to the safety data sheet (SDS) on this CRM before use.

**Preparation**

The cod fish for this CRM was caught in the northern part of the Sea of Japan. The fish were filleted and only muscle tissue was collected. The fillets were freeze-dried, freeze-pulverized, sieved (250  $\mu\text{m}$ ), and mixed for homogenization. The homogenized sample was packaged into amber glass bottles (10 g each) and sterilized by  $^{60}\text{Co}$   $\gamma$  radiation (20 kGy). The bottles were sealed individually in aluminum-coated nylon packages. These preparation procedures were carried out by Japan Chemical Analysis Center (Japan), Funtai Giken Co., Ltd. (Japan), Radiation Application Development Association, Takasaki Establishment (Japan), and Asahi-rika seisakusho Co., Ltd. (Japan).

**Technical Information**

The concentrations of the following elements, expressed as mass fractions on a dry-mass basis, are provided as information.

Element	Mass fraction	Analytical methods*	Element	Mass fraction	Analytical methods*
Al	5 mg/kg	1, 2	Cd	0.009 mg/kg	1, 2
Co	0.04 mg/kg	1, 2	Sb	0.02 mg/kg	1, 2
Sr	2 mg/kg	1, 2	Pb	0.04 mg/kg	1, 2
Mo	0.01 mg/kg	1, 2	P	12 g/kg	3

\*Analytical methods

- 1) Inductively coupled plasma mass spectrometry
- 2) High-resolution inductively coupled plasma mass spectrometry
- 3) Inductively coupled plasma atomic emission spectrometry

At the time of certification, tetramethylarsonium ions (TeMA), arsenocholine (AsC), dimethylarsinic acid (DMAA), and trimethylarsine oxide (TMAO) were identified by speciation analysis with high-performance liquid chromatography-inductively coupled plasma mass spectrometry, and their rounded concentrations were 0.75, 0.5, 0.25, and 0.25 % of the extracted total arsenic, respectively. Each value is relative to the peak area of the chromatogram for each species for the total identified peak area of the chromatogram that monitored As.

**NMIJ Analysts**

The technical manager for this CRM is CHIBA K., the production manager is KUROIWA T., and the analysts are KUROIWA T., INAGAKI K., NARUKAWA T., EYAMA S., NARUSHIMA I., and JIMBO Y.

Date of Shipment: Xxxxx xx, 20xx

7402a00-090804-220324

### Information

If substantive technical changes occur that affect the certification before the expiration of this certificate, NMIJ will notify the registered customers. Customer registration on the NMIJ Website (given below) will facilitate notification. Technical reports regarding this CRM can be obtained from the contact details given below.

### Reproduction of Certificate

In reproducing this certificate, it should be clearly indicated that the document is a copy.

April 1, 2020

ISHIMURA Kazuhiko  
President

National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact:  
National Institute of Advanced Industrial Science and Technology,  
National Metrology Institute of Japan,  
Center for Quality Management of Metrology, Reference Materials Office,  
1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan  
Phone: +81-29-861-4059; Fax: +81-29-861-4009, <https://unit.aist.go.jp/nmij/english/refmate/>

#### Revision history

August 4, 2009: Revised and re-certificated the certified value for arsenobetaine based on the results that characterized arsenobetaine concentration.

March 30, 2010: The expiration date was extended from March 31, 2011 to March 31, 2016.

October 1, 2014: The description in "Expiration of Certification" was changed to "one year after the date of shipment."

April 1, 2015: "Metrology Management Center" was renamed to "Center for Quality Management of Metrology."