National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2976

Trace Elements and Methylmercury in Mussel Tissue (Freeze-Dried)

Standard Reference Material (SRM) 2976 is intended for use in evaluating analytical methods for the determination of selected trace elements and methylmercury in marine bivalve mollusk tissue and similar matrices. All of the constituents for which certified and reference values are provided in SRM 2976 are naturally present in the freeze-dried mussel tissue. A unit of SRM 2976 consists of one bottle containing approximately 25 g of freeze-dried mussel tissue.

The development of this material was in response to the recommendations of the Group of Experts on Standards and Reference Materials (GESREM) established by the Intergovernmental Oceanographic Commission (IOC), United Nations Environment Program (UNEP), and the International Atomic Energy Agency (IAEA) [1]. The collection, preparation, and value assignment of SRM 2976 was a collaboration among the National Institute of Standards and Technology (NIST), the National Research Council (NRC) Canada, and the International Atomic Energy Agency (IAEA), Marine Environment Laboratory, Monaco.

Certified Concentration Values: Certified values are provided in Tables 1 and 2 for the concentrations, expressed as mass fractions, for eight trace elements and methylmercury. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST. The certified values for these constituents are based on the agreement of results obtained at NIST and other collaborating laboratories using two or more independent analytical techniques. The measurand is the total mass fraction of each constituent listed in Tables 1 and 2. The certified value is metrologically traceable to the SI-derived unit for mass fraction, expressed as percent.

Reference Concentration Values: Reference values are provided in Tables 3 and 4 for the concentrations, expressed as mass fractions, for 20 additional trace elements. Reference values are noncertified values that represent best estimates of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The measurand is the concentration of each constituent listed in Tables 3 and 4, and the certified value is metrologically traceable to the SI-derived unit for mass fraction, expressed as percent, as realized by the methods used.

Information Concentration Values: Information values are provided in Table 5 for the concentrations, expressed as mass fractions, for three additional major and trace elements. An information value is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 2976** is valid, within the measurement uncertainty specified, until **01 October 2027,** provided the SRM is handled in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements leading to certification was under the direction of M.M. Schantz, G.C Turk, and S.A. Wise of the NIST Chemical Sciences Division.

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Certificate Issue Date: 26 January 2018
Certificate Revision History on Last Page

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Consultation on the statistical design of the experimental work and evaluation of the data were provided by M.S. Levenson and S.D. Leigh of the NIST Statistical Engineering Division.

Analytical measurements were performed by D.A. Becker, W.C. Davis, R. Demiralp, M.K. Donais, E.A. Mackey, R. Saraswati, and L.L. Yu of NIST.

Analytical measurements for total mercury and methylmercury were also performed at the Institute of Applied Physical Chemistry, Research Centre of Jülich (Jülich, Germany) by K. May and H. Emons and at the Marine Environment Laboratory, International Atomic Energy Agency (Monaco) by V. Mandic, S. Azemard, and M. Horvat. Results for selected trace elements were also used from eight laboratories that participated in an intercomparison exercise coordinated by S. Berman and S. Willie of the Institute for National Measurement Standards, National Research Council (NRC) Canada. The eight laboratories from the NRC interlaboratory comparison exercise providing data for selected trace elements were: NOAA Northwest Fisheries Science Center (Seattle, WA); Department of Chemistry, Texas A&M University (College Station, TX); Battelle Pacific Northwest (Sequim, WA); Pennsylvania Department of Environmental Services (Harrisburg, PA); Queensland Department of Primary Industries (Queensland, Australia); Water Pollution Control Laboratory, California Department of Fish and Game (Rancho Cordova, CA); California Department of Fish and Game (Moss Landing, CA); and School of Pharmacy, University of Mississippi (University, MS).

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USERS

Storage: SRM 2976 is provided as a freeze-dried tissue homogenate in glass bottles. The tissue material should be stored at room temperature (21 °C to 25 °C) or below.

Handling: Normal biohazard safety precautions for the handling of biological tissues should be exercised.

Instructions for Use: The freeze-dried mussel tissue homogenate is hygroscopic and care should be exercised during weighing. At the time of certification this material contained 3 % - 4 % (mass fraction expressed as percent) residual moisture. The mussel tissue sample should be dried to a constant weight before weighing for analysis. Alternatively, a separate subsample of the mussel tissue can be removed from the bottle at the time of analysis and dried to determine the residual moisture, allowing the concentration to be converted to a dry-mass basis.

PREPARATION AND ANALYSIS[1]

Sample Collection and Preparation: The mussels (*Mytilus galloproincialis*) used for SRM 2976 were obtained by the International Atomic Energy Agency (IAEA) from commercial producers in the Etang de Thau on the Mediterranean coast of France (a semi-enclosed bay about 100 km to the west of Marseille). The mussel tissue was freeze-dried and 72 kg of dry material was shipped to NIST for further processing. At NIST the dry mussel tissue was first ground in a commercial blender with titanium blades, then jet milled, and finally mixed in a cone blender. The blended material was radiation sterilized (^{60}Co) and then aliquoted into bottles (25 g each).

Analytical Approach: Value assignment of the concentrations of selected trace elements and methylmercury was accomplished by combining results of the analyses of SRM 2976 at NIST, NRC Canada, IAEA, Research Centre Jülich, and eight selected laboratories that participated in an interlaboratory comparison exercise coordinated by the NRC Canada [2]. The analytical methods used for the determination of each element in SRM 2976 are summarized in Table 6.

For the certified concentration values listed in Table 1, results were combined from analyses at NIST using inductively coupled plasma mass spectrometry (ICP-MS) or instrumental neutron activation analysis (INAA), analyses at NRC Canada using isotope dilution ICP-MS and graphite furnace atomic absorption spectrometry (GFAAS), and the mean of the results from eight laboratories that participated in the NRC Canada interlaboratory comparison exercise.

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[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

For the certified values for total mercury and methylmercury in Table 2, results of analyses of SRM 2976 were combined from NIST, using two techniques for total mercury and three techniques for methylmercury, and from two other laboratories: IAEA Marine Environment Laboratory (Monaco) and the Institute of Applied Physical Chemistry, Research Centre of Jülich, (Jülich, Germany). For the certified total mercury values, results were also included from the NRC Canada interlaboratory comparison exercise.

For the reference values provided in Table 3, results were combined from NIST, NRC Canada, and/or the NRC Canada interlaboratory comparison exercise. The reference values in Table 4 are based on NIST results using INAA. The information values in Table 5 are based on results of analyses at Research Centre of Jülich using inductively coupled plasma atomic emission spectroscopy (ICP-AES) for phosphorus and sulfur and ICP-MS for thallium.

NIST Trace Element Analyses: For the ICP-MS analyses, duplicate subsamples (0.5 g) from each of four bottles of SRM 2976 were digested in 5 mL of concentrated HNO_3 in a microwave oven. The tissue digest was then analyzed by ICP-MS using added yttrium as an internal standard. For the INAA analyses for elements in Tables 1 and 3, duplicate subsamples (200 mg - 300 mg) from each of six bottles of SRM 2976 were pelletized and then analyzed by INAA as described previously [3]. For the elements in Table 4, INAA analyses were performed as above on six subsamples from a single bottle.

Methylmercury and Total Mercury: The certified value for methylmercury is based on results of analyses of SRM 2976 at NIST and two other laboratories: Institute of Applied Physical Chemistry, Research Centre of Jülich, (Jülich, Germany) and the IAEA Marine Environment Laboratory (Monaco). For the determination of methylmercury, SRM 2976 was analyzed at NIST using three different analytical techniques. The first method consisted of liquid-solid extraction under acidic conditions and size exclusion chromatography followed by GC with atomic emission detection (GC-AED) as described in detail by Donais et al. [4,5]. The GC-AED analyses were performed using a 0.53 mm x 15 m fused silica capillary column with a 14 % (mole fraction expressed as percent) cyanopropyl-substituted methylpolysiloxane phase (3.0 µm film thickness)(OV-1701, Quadrex, New Haven, CT).The second analytical procedure [6] at NIST consisted of the distillation of the organomercury compounds from a mixture of the powdered sample, sulfuric acid, and cupric sulfate followed by binding of the organomercury compounds to cysteine fixed onto filter paper. The filter paper was then subjected to neutron activation analysis for mercury determination. The third analytical procedure at NIST used speciated isotope dilution GC/inductively coupled plasma mass spectrometry (GC/ICPMS) [7]. For the speciated isotope dilution GC/ICPMS analyses, approximately 0.25 g subsamples were spiked with an appropriately diluted sample of IRMM-670 ^{202}Hg enriched methylmercury isotopic CRM and subjected to an alkaline microwave extraction (using 25 % volume fraction tetraammoniumhydroxide in water). Sodium tetraethylborate was used for ethylation. The derivatized methylmercury was back-extracted into isooctane and injected into a GC/ICPMS. The GC analysis used a 30 m × 0.32 mm column with a 100 % dimethylpolysiloxane phase (0.17 µm film thickness) (HP-1, Agilent Technolgies). The method used at IAEA consisted of saponification at 70 EC followed by ethylation, room temperature precollection, GC pyrolysis, and cold vapor atomic fluorescence spectrometric detection [8]. At the Research Centre of Jülich the analytical procedure for methylmercury consisted of water steam distillation under acid conditions, anion exchange chromatographic separation of inorganic and methylmercury, followed by cold vapor atomic absorption spectrometric detection before and after ultraviolet radiation [9-11]. Subsamples from six bottles of SRM 2976 were analyzed by each of the three laboratories.

For the determination of the certified value for total mercury, results from four different analytical procedures at three laboratories (NIST, Research Centre of Jülich, and IAEA) and from eight laboratories that participated in an interlaboratory comparison exercise coordinated by the NRC Canada, were used [2]. The four different procedures included: microwave digestion followed by flow injection cold-vapor atomic absorption spectrometry (NIST) [12]; instrumental neutron activation analysis (NIST) [3]; acid digestion at 70 EC followed by double gold trap amalgamation cold vapor atomic fluorescence spectrometry (IAEA) [13]; and acid digestion at 150 EC followed by gold wire amalgamation cold vapor atomic absorption spectrometry (Research Centre of Jülich) [14]. Subsamples from six bottles of SRM 2976 were analyzed by each of the three laboratories.

Table 1. Certified Concentrations for Selected Inorganic Constituents in SRM 2976 (Dry-Mass Basis)

Element	Mass Fraction[a] (mg/kg)		
Arsenic (As)	13.3	±	1.8
Cadmium (Cd)	0.82	±	0.16
Copper (Cu)	4.02	±	0.33
Iron (Fe)	171.0	±	4.9
Lead (Pb)	1.19	±	0.18
Selenium (Se)	1.80	±	0.15
Zinc (Zn)	137	±	13

[a] The results are expressed as the certified value ± the expanded uncertainty.   The certified value is the mean of three results: (1) the mean of NIST INAA or ICP-MS analyses, (2) the mean of two methods performed at NRC Canada, and (3) the mean of results from six or eight selected laboratories participating in the NRC Canada intercomparison exercise and results from IAEA. The expanded uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM and NIST Guides [15] and k is the coverage factor. The value of u_c is intended to represent at the level of one standard deviation the combined effect of all the uncertainties in the certified value. Here u_c is given by the standard error of the mean of the available values. The coverage factor, $k = 4.30$, is the Student's t-value for a 95 % confidence interval with two degrees of freedom.

Table 2. Certified Concentrations of Methylmercury and Total Mercury in SRM 2976 (Dry-Mass Basis)

Compound/Element	Mass Fraction[a] (µg/kg)		
Methylmercury[a,b]	28.09	±	0.31
Total Mercury (Hg)[c]	61.0	±	3.6

[a] Results for methylmercury are reported as µg mercury/kg.

[b] The certified value is a weighted mean of the results from five analytical methods [16]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance incorporating inter-method bias with a pooled, within-method variance following the JCGM and NIST Guides [15].

[c] The results are expressed as the certified value ± the expanded uncertainty. The certified value is the mean of results from four different laboratories and the mean of an interlaboratory exercise on SRM 2976. The five values were judged to be independent measurements. The uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM and NIST Guides [15] and k is the coverage factor. The value of u_c is intended to represent at the level of one standard deviation the combined effect of all the uncertainties in the certified value. Here u_c is given by the standard error of the mean of the four analyses and the interlaboratory exercise. The coverage factor, $k = 2.78$, is the Student's t-value for a 95 % confidence interval with four degrees of freedom.

Table 3.   Reference Concentrations for Selected Inorganic Constituents in SRM 2976 as Determined by Multiple Laboratories (Dry-Mass Basis)

Element	Degrees of Freedom	Mass Fraction[a,b] (mg/kg)		
Aluminum (Al)	2	134	±	34[a]
Chromium (Cr)	2	0.50	±	0.16[a]
Nickel (Ni)	7	0.93	±	0.12[b]
Silver (Ag)	2	0.011	±	0.005[b]
Tin (Sn)	2	0.096	±	0.039[a]

[a] The results are expressed as the reference value ± the expanded uncertainty. The reference value is the mean of three values: (1) the mean of NIST INAA or ICP-MS analyses, (2) the mean of two methods performed at NRC Canada, and (3) the mean of results from six or eight selected laboratories participating in the NRC Canada intercomparison exercise and results from IAEA. The expanded uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM and NIST Guides [15] and k is the coverage factor. The value of u_c is intended to represent at the level of one standard deviation the combined effect of all the uncertainties in the certified value. Here u_c is given by the standard error of the mean of the available values. The coverage factor, k, is the Student's t-value for a 95 % confidence interval with the degrees of freedom equal to the number of different results minus 1.

[b] The results are expressed as the reference value ± the expanded uncertainty. The reference value is the mean of the available results from eight laboratories participating in the NRC Canada intercomparison exercise using SRM 2976. The analyses were judged to be independent measurements with no significant errors in common. The uncertainty in the reference value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM and NIST Guides [15] and k is the coverage factor. The value of u_c is intended to represent at the level of one standard deviation the combined effect of all the uncertainties in the reference value. Here u_c is given by the standard error of the mean of the available values. The coverage factor, k, is the Student's t-value for a 95 % confidence interval with the degrees of freedom equal to the number of different results minus 1.

Table 4.   Reference Concentrations for Selected Inorganic Constituents in
SRM 2976 as Determined by INAA[a] (Dry-Mass basis)

Element	Degrees of Freedom	Mass Fraction (%)		
Calcium (Ca)	5	0.76	±	0.03
Chlorine(Cl)	5	5.7	±	0.5
Magnesium (Mg)	5	0.53	±	0.05
Potassium (K)	4	0.97	±	0.05
Sodium (Na)	4	3.5	±	0.1
		(mg/kg)		
Bromine (Br)	5	329	±	15
Cerium (Ce)	5	0.109	±	0.008
Cesium (Cs)	5	0.027	±	0.001
Cobalt (Co)[b]	17	0.61	±	0.02
Europium (Eu)	5	0.0024	±	0.0003
Manganese (Mn)	4	33	±	2
Rubidium (Rb)	5	4.14	±	0.09
Scandium (Sc)	5	0.0146	±	0.0003
Strontium (Sr)	5	93	±	2
Thorium (Th)	5	0.011	±	0.002

[a] The results are expressed as the reference value ± the expanded uncertainty. Each reference value is the mean of INAA analyses of multiple samples from a single bottle of SRM 2976. The expanded uncertainty in a reference value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM and NIST Guides [15] and k is the coverage factor. The value of u_c is intended to represent at the level of one standard deviation the uncertainty in the reference value determined by using INAA. No components of uncertainty due to material homogeneity or method bias are included. Here u_c is given by the standard error of the mean of the values. The coverage factor, k, is the Student's t-value for a 95 % confidence interval based on the corresponding degrees of freedom.

[b] The results are expressed as the reference value ± the expanded uncertainty. The reference value for cobalt is the mean of duplicate INAA analyses from six bottles of SRM 2976. The expanded uncertainty in a reference value is equal to $U = ku_c$ where u_c is the combined standard uncertainty, both calculated according to the JCGM and NIST Guides [15] and k is the coverage factor. The value of u_c is intended to represent at the level of one standard deviation the combined effect of all the uncertainties in the value. Here u_c accounts for both possible method biases and material inhomogeneity. The coverage factor, k, is the Student's t-value for a 95 % confidence interval based on the corresponding degrees of freedom. For this element the degrees of freedom are calculated by an approximate formula found in reference [15].

Table 5.   Information Values for the Concentrations for Selected Inorganic
Constituents in SRM 2976 (Dry-Mass basis)

Element	Mass Fraction (%)
Phosphorus (P)	0.83
Sulfur (S)	1.9
	(µg/kg)
Thallium (Tl)	1.3

Table 6. Analytical Methods Used for the Analysis of SRM 2976

Elements	Analytical Methods
Aluminum	GFAAS, ICP-AES, ICP-MS, INAA
Arsenic	GFAAS, HGAAS, ICP-MS, INAA, XRF
Bromine	INAA
Cadmium	GFAAS, ICP-MS, IDICP-MS
Calcium	INAA
Cerium	INAA
Cesium	INAA
Chlorine	INAA
Chromium	GFAAS, ICP-MS, IDICP-MS, INAA
Cobalt	INAA
Copper	FAAS, GFAAS, ICP-AES, ICP-MS, IDICP-MS, XRF
Europium	INAA
Iron	FAAS, GFAAS, ICP-AES, ICP-MS, IDICP-MS, XRF
Lead	GFAAS, ICP-MS, IDICP-MS
Magnesium	INAA
Manganese	INAA
Mercury	CVAFS, CVAAS, FIA-CVAAS, INAA
Nickel	GFAAS, ICP-MS
Phosphorus	ICP-AES
Potassium	INAA
Rubidium	INAA
Scandium	INAA
Selenium	GFAAS, HGAAS, ICP-MS, INAA, XRF
Silver	GFAAS, IDICP-MS
Sodium	INAA
Strontium	INAA
Sulfur	ICP-AES
Thallium	ICP-MS
Thorium	INAA
Tin	ICP-MS, IDICP-MS
Zinc	FAAS, GFAAS, ICP-AES, ICP-MS, IDICP-MS, XRF

Methods

CVAAS	Cold vapor atomic absorption spectrometry
CVAFS	Cold vapor atomic fluorescence spectrometry
FAAS	Flame atomic absorption spectrometry
FIA-CVAAS	Flow injection analysis cold vapor atomic absorption spectrometry
GFAAS	Graphite furnace atomic absorption spectrometry
HGAAS	Hydride generation atomic absorption spectrometry
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
IDICP-MS	Isotope dilution inductively coupled plasma mass spectrometry
INAA	Instrumental neutron activation analysis
XRF	X-ray fluorescence spectrometry

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Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.