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ORIGINAL PAPER

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Certification of three mussel tissue standard reference materials (SRM) for methylmercury and total mercury content

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Abstract SRM 1974a, Organics in Mussel Tissue (*Mytilus edulis*); SRM 2974, Organics in Mussel Tissue (freeze-dried); and SRM 2976, Mussel Tissue (trace elements and methylmercury) have been recently certified for methylmercury and total mercury content. Three independent analytical procedures were used to determine the certified methylmercury concentrations. Four independent procedures combined with data from two intercomparison exercises were used to determine the certified total mercury concentrations. These materials are the first certified metal speciation environmental SRMs issued by the National Institute of Standards and Technology.

Introduction

Recently a new Standard Reference Material, SRM 1974a, Organics in Mussel Tissue (*Mytilus edulis*), was issued by the National Institute of Standards and Technology (NIST). This SRM is a frozen tissue homogenate and was collected from Dorchester Bay within the Boston Harbor, MA. A portion of the frozen homogenate was freeze-dried, and has been issued as a separate Standard Reference Material, SRM 2974, Organics in Mussel Tissue (freeze-dried). Both mussel tissue materials are primarily intended for use as trace organic analyte SRMs, having been certified for a variety of organic constituents such as chlorinated biphenyl congeners, polycyclic aromatic hydrocarbons, and chlorinated pesticides [1, 2]. As with the previous issue of SRM 1974, SRM 1974a has only noncertified values for selected inorganic analytes which were determined using a single analytical method.

As part of a recent effort by NIST to investigate metal speciation in the marine environment, SRM 1974a and SRM 2974 were analyzed for methylmercury (MeHg) content using a new gas chromatography-atomic emission detection (GC-AED) method developed at NIST [3]. Both materials contained MeHg at levels typical of those in mollusks and other life forms at the bottom of the marine food web [4, 5]. Considering these environmentally relevant MeHg concentrations and the need for new certified reference materials (CRMs) for use in method validation and data quality assurance, the materials were chosen to be the first metal speciated environmental CRMs generated at NIST. SRM 1974a and SRM 2974 are the first mussel tissue matrix CRMs certified for MeHg content, and the preparation of the two materials provided a study of the effects of freeze-drying on MeHg and total mercury (Hg) concentrations in biological tissues. A third mussel tissue reference material collected in the Mediterranean Sea, SRM 2976 Mussel Tissue, was also included in the MeHg certification efforts due to its similarity in matrix composition and its differing sample collection site.

Certification of natural matrix reference materials at NIST typically requires the use of at least two independent analytical methods for analyte concentration measurements. Complete method independence requires the development of unique extraction, cleanup, and quantification procedures. Although these

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individual methods are usually developed and implemented at NIST for the certification of SRMs, the utilization of data from other laboratories using different methods greatly facilitated the certified value assignment process. Multiple bottles of each of the three SRMs were provided to the Marine Environment Laboratory, International Atomic Energy Agency (IAEA, Monaco) and the Institute for Applied Physical Chemistry, Research Centre of Jülich (Germany) for MeHg and total Hg determinations. The three materials also were analyzed at NIST for total Hg content using two independent methods. The MeHg content was determined at NIST using the previously mentioned GC-AED method. Lastly, SRMs 2974 and 2976 were analyzed for total Hg content during two separate intercomparison exercises coordinated by the Institute for National Measurement Standards, National Research Council of Canada (NRC) in conjunction with the U.S. National Oceanic and Atmospheric Administration (NOAA). Certified values for MeHg and total Hg content in all three materials were then determined using the combined laboratory data.

Experimental

Sample collection and preparation

Approximately 6400 mussels (*Mytilus edulis*) were collected from a mud flat in Dorchester Bay, Boston Harbor (MA) at low tide in October, 1992. The mussels were rinsed with harbor water before placing them in wooden crates. The mussels then were cleaned with tap water, placed in polyethylene bags, and shipped to NIST packed in dry ice. At NIST, the mussels were warmed to 0 °C and shucked using titanium knives to prevent trace element contamination. The mussel tissue was placed in Teflon bags and stored in a liquid nitrogen freezer. The mussel tissue was cryogenically ball milled and homogenized in 700 g batches using a Teflon disk and ring configuration [6]. Approximately 80 kg of the homogenized frozen tissue were blended for 2 h in an aluminum mixing drum in batches of approximately 20 to 30 kg each. Approximately 60 kg of this frozen material were bottled in 15 g units for distribution as SRM 1974a. The remaining 21 kg of homogenized tissue were freeze-dried in batches of approximately 1 kg (starting settings of 1 Pa, − 20 °C shelf temperature, and − 50 °C condenser temperature; shelf temperature gradually raised over the drying time to a final temperature of 5 °C), blended, and radiation sterilized (^{60}Co). The material was then bottled in 8 g units for distribution as SRM 2974.

The common Mediterranean mussels (*Mytillus galloprovincialis*) used for the preparation of SRM 2976 were collected in Etang de Thau, a semi-enclosed bay approximately 100 km west of Marseilles, France. The mussels were obtained from commercial producers by the IAEA International Laboratory of Marine Radioactivity in Monaco, and the tissue was freeze-dried by IAEA prior to shipment to NIST. Approximately 69 kg of freeze-dried material was blended in a food-processor, jet milled, blended, and radiation sterilized. The material then was bottled in 25 g units for distribution as SRM 2976.

SRM 1974a and SRM 2974 were intended to be identical materials on a dry weight basis. Therefore, the preparation of SRM 2974 was designed to minimize any differences other than freeze-drying. The mussel tissues for both materials were from the same cryogenically ground homogenate and the freeze-dried tissue was not sieved to remove any fibrous material. The only difference in the processing of the two materials besides freeze-drying was the radiation sterilization of SRM 2974 to minimize the effects of biological activity, thus allowing it to be stored at room temperature. Conversely, SRM 2976 was jet milled after freeze-drying to reduce the amount of fibrous particles. When compared to SRM 2974, SRM 2976 exhibits a more homogeneous appearance.

Conversion to dry mass basis

The moisture content of SRMs 1974a, 2974, and 2976 were determined at NIST by measuring the mass loss from freeze-drying. The entire content of each of 20 bottles of SRM 1974a was freeze-dried, resulting in a mean moisture content for this material of 88.61 ± 0.08% (uncertainty expressed as a 95% confidence interval). Analytical results collected at NIST for MeHg and total Hg in SRM 1974a were determined on a wet mass basis (except for INAA analyses which were performed on freeze-dried material) and then converted to a dry mass basis by dividing by the conversion factor of 0.1139 kg dry mass/kg wet mass.

SRM 2974 is very hygroscopic and contained between 7% and 14% residual moisture at the time of certification measurements. A separate conversion factor was determined for each bottle of SRM 2974 analyzed at NIST for MeHg and total Hg content. These six bottles ranged in moisture content from 8.4% to 13.2%. Lastly, the percent water loss upon freeze-drying for SRM 2976 was determined in December 1994 and July 1995 as 3.21% and 3.26%, respectively, on two separate bottles. An average of these two values was used to calculate the conversion factor of 0.968 kg dry mass/kg wet weight for use in converting the NIST data to a dry mass basis.

The moisture content of the three mussel tissue materials were determined by IAEA by drying subsamples of the materials for 48 h in an oven at 150 °C. A separate moisture content was determined for each bottle of SRM 2974 and SRM 2976 and for only a single bottle of SRM 1974a for use in the conversion of all IAEA data for these materials to a dry mass basis. The Research Centre of Jülich determined the moisture content of each bottle of SRM 2976 and SRM 1974a and only a single bottle of SRM 2974 for conversion of data to a dry mass basis. The Research Centre of Jülich used oven drying to determine the moisture content of the materials.

Determination of methylmercury

The three mussel tissue materials were analyzed for MeHg content using the following three independent extraction and quantification procedures: solid-liquid extraction under acidic conditions and gel permeation chromatography cleanup followed by GC-AED (GC-AED, NIST); saponification at 70 °C followed by ethylation-room temperature precollection-GC-pyrolysis-cold vapor atomic fluorescence spectrometric detection (GC-CV-AFS, IAEA); and water steam distillation under acid conditions and further anion exchange chromatographic separation of inorganic Hg and organomercury followed by cold vapor atomic absorption spectrometric detection before and after ultraviolet radiation (IEC-CV-AAS, Jülich). Brief summaries of these methods are provided; however, more detailed descriptions can be found in the references indicated.

For the GC-AED method, 1–8 g subsamples of each material were mixed with 4 g copper powder, 1 mL 1 mol/L copper sulfate, 8 mL water (only added for standard solutions and SRM 2976), 4 mL acidic potassium bromide solution, and 2 mL toluene in individual 50 mL centrifuge tubes for 1 h. Double these reagent volumes were used for SRM 2976. The toluene layers were then separated by centrifugation, and the extraction was repeated with a second aliquot of toluene. The combined toluene extracts for each sample were spiked with internal standard solution and concentrated to 0.5 mL. Ethylmercury chloride (EtHgCl) was used as the internal

standard for SRM 1974a and SRM 2974, and propylmercury chloride (PrHgCl) was used for SRM 2976; EtHgCl was not used as the internal standard for SRM 2976 because a small amount of ethylmercury was found to be present naturally in the material. High molecular weight pigments and lipids were removed from the SRM 1974a and SRM 2974 extracts using preparative gel permeation chromatography (GPC); the GPC cleanup step was not performed on the SRM 2976 extracts to prevent analyte losses during concentration of the large GPC fraction volume required when PrHgCl is used as the internal standard instead of EtHgCl. The extracts were analyzed using gas chromatography (OV-1701 column, 0.53 mm × 15 m, 3.0 μm film thickness, Quadrex, New Haven, CT) with Hg-specific microwave-induced plasma atomic emission detection using the emission line at 254 nm [3].

For the GC-CV-AFS method, samples were saponified in closed Teflon vials overnight at 70 °C with 10 mL 25% (w/v) potassium hydroxide in methanol and diluted to 25.8 mL with methanol. MeHg in the saponified samples was derivatized with sodium tetraethyl borate at pH 4.9 and collected on a Tenax trap. Separation of the individual organomercury species was achieved using isothermal gas chromatography (glass capillary SPB-5 column, 0.75 mm × 30 m, 1.0 μm film thickness, Supelco, Bellefonte, PA) followed by pyrolysis and cold vapor atomic fluorescence spectrometric detection [7]. All analyses were done during the same day.

For the IEC-CV-AAS method, samples were distilled by water steam distillation in a mixture of sulfuric acid and sodium chloride. MeHg and inorganic Hg in the distillate were further separated by anion exchange chromatography (Dowex 1 × 8 resin, Cl-form, 74 to 149 μm) using minimal light conditions. The MeHg in the collected fraction then was decomposed to inorganic Hg with ultraviolet light, reduced to Hg(0) with tin chloride and sulfuric acid, and preaccumulated on a gold wire. The collected Hg was thermally desorbed from the wire by heating the wire to 600 °C, and transported to the glass cuvette for absorption measurement at 253.5 nm (slit width 2.0 nm, cuvette temperature 50 °C) [8–10].

Determination of total mercury

The three mussel tissue materials were analyzed for total Hg content using the following four independent analytical procedures: microwave digestion followed by flow injection cold-vapor atomic absorption spectrometry (FI-CV-AAS, NIST); instrumental neutron activation analysis (INAA, NIST); acid digestion at 70 °C followed by double gold trap amalgamation cold-vapor atomic fluorescence spectrometry (CV-AFS, IAEA); and acid digestion at 150 °C followed by gold wire amalgamation CV-AAS (GA-CV-AAS, Research Centre of Jülich). Brief summaries of these methods are provided, however more detailed descriptions can be found in the references indicated.

For the FI-CV-AAS method, samples were mixed with 5 mL concentrated nitric acid and microwave digested for 10 min to 20 min at a maximum temperature of 228 °C and a maximum pressure of 1242 kPa. After digestion was complete, the contents of the vessels were transferred to 100-mL volumetric flasks containing 0.75 mL 1% (w/v) potassium dichromate solution and diluted to volume with 1% (v/v) sulfuric acid. Samples were then analyzed by cold-vapor generation using a commercial flow injection system and atomic absorption spectrometer. A 3% (v/v) hydrochloric acid solution containing 1% (w/v) tin chloride was used as the carrier stream in the FIA system. Sample was swept from the injection loop to the mixing coil where it reacted with the tin chloride reductant. The resulting mercury vapor was separated from the solution and swept into a heated (200 °C) quartz absorption cell mounted in the light path of the electrodeless discharge lamp (Hg) and detected by AAS. Peak height measurement was used for quantification [11].

For the INAA method, samples were weighed into individual quartz vials and flash frozen in liquid nitrogen prior to sealing the vials. The vials were encapsulated in polyethylene film followed by packaging in sets of eight in polyethylene irradiation vessels. Each vessel was irradiated for 3–4 h at a reactor power of 20 MW. The samples were removed from the vessel approximately 1–2 months after irradiation, the polyethylene films removed, and the outer vial surface cleaned with a mixture of hydrochloric and nitric acids. Each vial then was encapsulated in clean polyethylene films and gamma radiations were collected for ⩾ 8 h using a germanium detector and associated electronics. Quantification of Hg was based on comparison with standards using the 279 keV line from ^{203}Hg, corrected for the interference from ^{75}Se.

For the CV-AFS method, samples were digested in closed Teflon vials with 4 mL concentrated nitric acid and 2 mL concentrated sulfuric acid for 3 h at 70 °C and diluted to 25.8 mL with deionized water and 500 μL of a preservative. The digested samples were added to individual reaction vessels containing water, hydrochloric acid, and tin chloride to reduce the Hg(II) to Hg(0). The Hg vapor produced from this reaction was swept from the solution with nitrogen through a soda-lime trap to remove bubbler solution droplets and onto a gold-coated sand (sampling) trap. The Hg collected on the sampling trap was transferred via thermal desorption at 450 °C to the second (analytical) gold trap, then finally thermally desorbed into the detector cell. Hg quantification was based on atomic fluorescence spectrometric detection (Brooks Rand, Seattle, WA) [12]. Each sample was analyzed twice and all analyses were done during the same day.

For the GA-CV-AAS method, samples were mixed with 10 mL nitric acid and digested at 150 °C for 10 h. The digested samples were diluted to 20 mL with distilled water prior to analysis by cold vapor atomic absorption spectrometry. A portion of each sample solution was mixed with tin chloride and sulfuric acid to reduce any Hg(II) to Hg(0), and the resulting Hg vapor was collected by amalgamation on a gold wire. The collected Hg was thermally desorbed from the wire and analyzed by CV-AAS as described for the MeHg method [13].

SRM 2974 and SRM 2976 also were distributed to approximately 50 laboratories each during the NRC/NOAA intercomparison exercises NOAA/9 and NOAA/7 in 1995 and 1993, respectively. Results for laboratories that had participated in the intercomparison exercises for a number of years and performed well were selected by NRC and combined with results from two NRC methods (i.e., a total of 7 results for SRM 2974 and 6 results for SRM 2976). These selected intercomparison exercise results will be referred to as RR/9 and RR/7 for the remainder of the manuscript. The mean of each of the RR/9 and RR/7 data sets was treated as a separate "method" for determination of the certified total Hg values for both mussel tissue materials.

The RR/9 and RR/7 data were treated as a group and not from individual methods for the determination of the certified total Hg values for two reasons. First, the RR/9 and RR/7 laboratories analyzed only single bottles of each material as compared with the multiple-bottle studies performed by NIST, IAEA, and the Research Centre of Jülich. Between-bottle variation in analyte concentration is not taken into account and, therefore, a bias may exist in the analyte concentration determined through analyses of a single bottle of material. Second, the sample preparation and detection procedures used by the RR/9 and RR/7 laboratories are not all completely independent from the NIST, IAEA, and Research Centre of Jülich methods. Nevertheless, these individual laboratory results represent values determined from several additional detection methods, including inductively-coupled plasma mass spectrometry (ICP-MS), graphite furnace atomic absorption spectrometry, and isotope dilution ICP-MS, and, therefore, are valuable additions to the multiple-bottle studies. Brief summaries of the analytical methods used by each RR/9 and RR/7 laboratory can be found elsewhere [14, 15].

Control materials

Control materials were analyzed by NIST, IAEA, and the Research Centre of Jülich during the analyses of the three mussel tissue

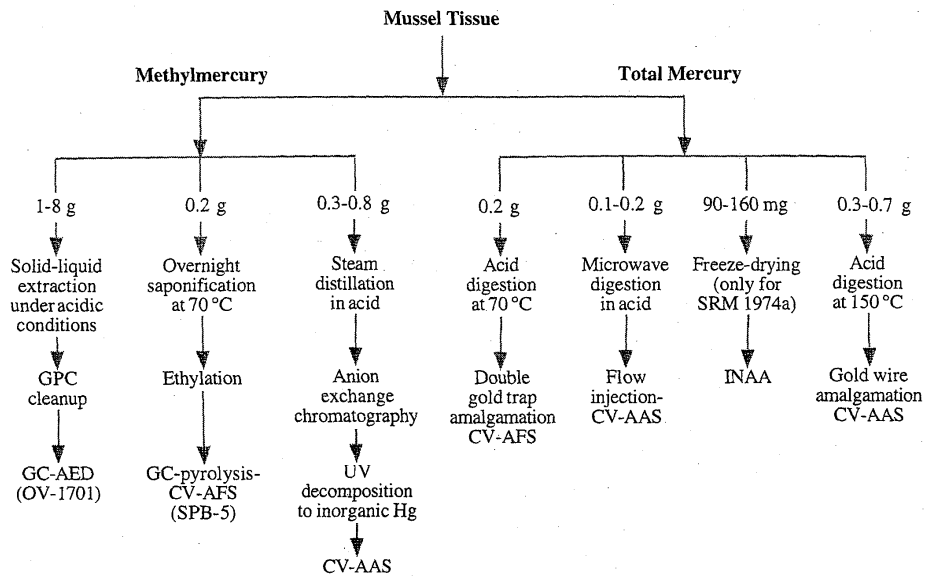

Fig. 1 Analytical approach for the certification of methylmercury and total mercury contents in SRM 1974a, SRM2974, and SRM 2976

materials to provide support for the validity of the MeHg and total Hg data. For the MeHg measurements in SRM 1974a and SRM 2974 at NIST, SRM 2976 was used as the control material. At that time SRM 2976 had been analyzed for MeHg content by NIST and IAEA using two independent methods, and the values from the two laboratories were in good agreement. SRM 2976 was chosen as the control material for the MeHg measurements in SRM 1974a and SRM 2974 due to the similarity in matrix composition between the two mussel species. No control material was used for the SRM 2976 MeHg measurements at NIST. The MeHg data from IAEA and the Research Centre of Jülich had been collected at that time, however, so comparison of the data among these independent methods provides validity to the NIST data for this material. SRM 1566a Oyster Tissue and SRM 2710 Montana I Soil were used as the control materials for the total Hg measurements conducted at NIST by INAA, and SRM 1566a Oyster Tissue was used for the FI-CV-AAS measurements.

TORT-1 Lobster Hepatopancreas (National Research Council, Canada) was used by IAEA and SRM 1566a Oyster Tissue was used by the Research Centre of Jülich as the control materials for the MeHg and total Hg measurements in SRM 1974a, SRM 2974, and SRM 2976. TORT-1 is certified for both MeHg and total Hg content. SRM 1566a is certified only for total Hg content, but has been analyzed by the Research Centre of Jülich for MeHg content using independent methods to establish a reference value of 17.6 ng/g MeHg as Hg (dry weight). SRM 1566a was used as the control material for total Hg by the RR/9 and RR/7 laboratories.

Results and discussion

The analytical scheme for the measurement of MeHg and total Hg in the three mussel tissue reference materials is shown in Figure 1. MeHg concentrations were determined using three independent analytical procedures, and total Hg concentrations were determined using four independent procedures and two intercomparison exercises. The mean concentrations determined using each analytical method for the three materials are given in Table 1. Uncertainties for the method mean concentrations are expressed as 95% confidence intervals with the degrees of freedom calculated from the number of subsamples analyzed using each method. SRM 1974a was not analyzed for total Hg by CV-AFS. The mean concentrations determined by the RR/9 and RR/7 laboratories are summarized in Table 2. Uncertainties are expressed as 95% confidence intervals with the degrees of freedom calculated from the number of subsamples analyzed by each laboratory.

Table 1 Summary of results for methylmercury and total mercury (dry mass basis)

		SRM 1974a	SRM 2974	SRM 2976
MeHg (ng/g as Hg)	GC-AED:	74.3 ± 15	80.4 ± 8.4	28.3 ± 2.2
	IEC-CV-AAS:	80.4 ± 1.7	71.7 ± 1.5	28.1 ± 0.8
	GC-CV-AFS:	76.5 ± 6.5	79.8 ± 3.2	26.8 ± 1.5
Mean:		77.1 ± 7.7	77.3 ± 12.1	27.7 ± 2.0
Total Hg (ng/g)	FI-CV-AAS:	184 ± 17	186 ± 18	61.6 ± 1.2
	INAA:	203 ± 18	155 ± 20	59.3 ± 4.6
	GA-CV-AAS:	170 ± 3	182 ± 2	57.0 ± 1.3
	CV-AFS:		175 ± 6	63.8 ± 4.1
	RR/9:		157 ± 20	
	RR/7:			63.2 ± 4.1
Mean:		186 ± 41	171 ± 18	61.0 ± 3.5

The MeHg and total Hg concentrations measured in the control materials used by NIST, IAEA, the Research Centre of Jülich, and the RR/9 and RR/7 laboratories were in agreement with the certified or reference values for the CRMs. The analytical systems used to perform the measurements on the candidate mussel tissue reference materials can therefore be considered in control.

Table 2 RR/9 and RR/7 results for total mercury (dry mass basis)

	RR/9 (ng/g)	RR/7 (ng/g)
	167 ± 11	61 ± 2
	188 ± 6	65 ± 1
	176 ± 25	52 ± 6
	135 ± 5	63 ± 1
	130 ± 12	69 ± 2
	152 ± 20	58 ± 13
	152 ± 2	
Mean:	157 ± 20	63 ± 4

Comparison of analytical methods

The three MeHg methods are independent in their approaches to extraction, cleanup, and quantification. The GC-AED and IEC-CV-AAS methods used acidic conditions during the extraction, whereas the GC-CV-AFS method used basic conditions. The GC-AED method utilized a solid-liquid extraction procedure into toluene under mildly acidic conditions, while the IEC-CV-AAS method utilized a distillation directly into sulfuric acid and sodium chloride without an organic solvent present. The GC-AED and IEC-CV-AAS methods both used liquid chromatographic procedures for sample cleanup; however, the separation mechanisms for anion exchange chromatography and gel permeation chromatography are quite different thereby providing independence between these two approaches to sample cleanup. The GC-CV-AFS and IEC-CV-AAS methods both converted the extracted alkylmercury halide species to alternate forms prior to detection. The alkylmercury species were ethylated prior to GC-CV-AFS detection, whereas they were decomposed to inorganic mercury prior to IEC-CV-AAS detection. Lastly, the detection approaches used for the three methods are based on the three atomic spectrometric quantities of emission, absorption, and fluorescence.

The four sample preparation approaches used for the quantification of the total Hg content of the three mussel tissue materials also are independent. The CV-AFS, FI-CV-AAS, and GA-CV-AAS methods all used acid digestions but with very different conditions. The GA-CV-AAS and FI-CV-AAS methods both used only nitric acid for the sample digestions, whereas the CV-AFS method used both sulfuric and nitric acids. The FI-CV-AAS methods used microwave power to heat the samples for a short period of time. The GA-CV-AAS and CV-AFS methods both used hot plates to heat the samples for longer periods of time. The INAA method required little sample preparation other than the removal of moisture (i.e., freeze-drying) from SRM 1974a prior to irradiation. The approaches used for quantification of total mercury also varied considerably with only slight similarities in the GA-CV-AAS and FI-CV-AAS methods. Both of these methods are based on the measurement of the atomic absorption of mercury vapor, although the modes of introduction of the mercury vapor into the AAS systems were different. One method used amalgamation on a gold wire to preconcentrate the mercury vapor prior to detection, whereas the other method used a flow injection system utilized on-line with the AAS. The other two methods were based on the measurement of completely independent quantities from the CV-AAS methods, i.e., CV-AFS utilized the measurement of the atomic fluorescence of mercury vapor from the sample and INAA is based on the collection of gamma radiations.

Comparison of method results

No method bias is evident in the MeHg and total Hg data based on comparisons of the highest and lowest values for each material as determined by each analytical method. The MeHg data for SRM 1974a and SRM 2974 have a more narrow range, 71.1 ng/g to 80.4 ng/g (11%), than the total Hg data, 155 ng/g to 203 ng/g (27%), in the two similar materials. There is no obvious reason for this finding other than the large number of total Hg methods used to analyze these two materials. For SRM 2976, the data for MeHg and total Hg have narrow ranges, 5% and 11%, respectively. SRM 2976 was milled and blended more than SRM 2974, and this additional preparation appears to have resulted in greater homogeneity for SRM 2976 as compared with SRM 2974.

Determination of certified values

The mean MeHg and total Hg concentrations for each material were determined by averaging the individual method results for each analyte for each material. The uncertainties for the material mean concentrations are expressed as 95% confidence intervals with the degrees of freedom calculated from the number of methods used to analyze each material. These results are shown in Table 1.

There is no statistically significant difference in the mean MeHg concentrations for SRM 1974a and SRM 2974, 77.1 ± 7.7 ng/g and 77.3 ± 12.1 ng/g, respectively. This is expected because the materials were from the same tissue homogenate. The uncertainties for these mean values are relatively high (10% and 16%), however, due to the low number of degrees of freedom for each data set. The six MeHg values for the two materials were averaged to produce the certified value 77.2 ± 3.8 ng/g, which is applicable to both SRMs as shown in Table 3. The uncertainty for the certified value is expressed as a 95% confidence interval with the degrees of freedom calculated from the number of

Table 3 Certified values for methylmercury and total mercury

	MeHg (ng/g as Hg)	Total Hg (ng/g)
SRM 1974a (wet mass basis)	8.82 ± 0.44	20.1 ± 1.5
SRM 1974a (dry mass basis)	77.2 ± 3.8	176 ± 13
SRM 2974 (dry mass basis)	77.2 ± 3.8	176 ± 13
SRM 2976 (dry mass basis)	27.7 ± 2.0	61.0 ± 3.5

method mean values (i.e. six) for MeHg in SRM 1974a and SRM 2974. The uncertainty in the certified value covers the range of the individual method results within the uncertainties of those results. The certified MeHg content for SRM 2976 as determined from the three method results is 27.7 ± 2.0 ng/g. The results from the three methods were in good agreement, as reflected by the low uncertainty in the certified value (7%).

As with the MeHg data, all the total Hg data collected for SRM 1974a and SRM 2974 were averaged to determine the certified value applicable to both materials, 176 ± 13 ng/g. The data for both materials were combined due to the proximity of the mean concentrations for the individual materials within the uncertainties of those means, 186 ± 41 ng/g and 171 ± 18 ng/g, respectively. Although the mean concentrations for total Hg in SRM 1974a and SRM 2974 did not agree as well as those for MeHg in the same materials, combination of the results into one certified value was done in order to treat the data in the same manner for both analytes. The certified value for SRM 2976 as determined from the results of the five methods is 61.0 ± 3.5 ng/g. The uncertainty in the certified value is expressed as the 95% confidence interval with the degrees of freedom calculated from the number of methods. The small uncertainty in the certified total Hg value for SRM 2976 (5.7%) is a result of the close agreement among the five methods for this material.

The certified values for SRM 1974a based on the dry weight of the material were converted to wet mass certified values using the material moisture content reported on the material certificate of analysis, 88.61 ± 0.08%. The wet mass certified values are shown in Table 3.

Homogeneity assessment

A statistically designed homogeneity study was performed for the MeHg content of SRM 2974. Duplicate 1 g subsamples from six randomly selected bottles of the material were analyzed for MeHg content at NIST by the GC-AED method. No statistically significant differences among the bottles were observed at the 1 g subsample size. Although a statistical homogeneity study was not performed for the MeHg content of SRM 1974a, the agreement among the MeHg data for both materials indicates SRM 1974a can be considered homogeneous at the subsample sizes used by NIST, IAEA, and the Research Centre of Jülich for the three independent MeHg methods (0.2–8 g). No conclusions can be made about the homogeneity of MeHg in SRM 2976, although the narrow range of results for MeHg in this material indicates its probable homogeneity at the 0.2 g to 8 g subsample level. No homogeneity study was performed for the total Hg content of SRM 1974a, SRM 2974, or SRM 2976.

Conclusions

The MeHg and total Hg concentrations determined using multiple independent analytical methods agreed well for each material and permitted certification of the mussel tissue SRMs for both analytes. These three materials are the first mussel tissues certified for MeHg, and SRM 2976 contains the lowest certified level of MeHg in a biological tissue CRM currently available. The relative uncertainties in the certified MeHg values are also low, 4.9% for SRM 1974a and SRM 2974 and 7.2% for SRM 2976. The relative uncertainties for SRM 1974a and SRM 2974 are the lowest of all currently available MeHg CRMs. The agreement in MeHg concentrations in SRM 1974a and SRM 2974 also supports the practice of freeze-drying mussel tissues in that no detectable amount of MeHg was lost during material preparation. Lastly, the percentages of the total Hg content in the form of MeHg in the three materials, 44% for SRM 1974a and SRM 2974 and 45% for SRM 2976, are in agreement as expected for marine species at the same level in the marine food web.

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Disclaimer 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 National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

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