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Methods for the Preparation and Analysis of Solids and Suspended Solids for Methylmercury

Chapter 7 of
Book 5, Laboratory Analysis
Section A, Water Analysis

Techniques and Methods 5 A–7

U.S. Department of the Interior
U.S. Geological Survey

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By John F. DeWild, Shane D. Olund, Mark L. Olson, and Michael T. Tate

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Conversion Factors

Multiply	By	To obtain
	Length	
centimeter (cm)	3.94×10^{-1}	inch (in)
micrometer (μm)	3.94×10^{-5}	inch (in)
millimeter (mm)	3.94×10^{-2}	inch (in)
	Mass	
gram (g)	3.53×10^{-2}	ounce, avoirdupois (oz)
nanogram (ng)	3.53×10^{-11}	ounce, avoirdupois (oz)
milligram (mg)	3.53×10^{-5}	ounce, avoirdupois (oz)
	Volume	
liter (L)	2.64×10^{-1}	gallon (gal)
liter (L)	3.38×10^{-1}	ounce, fluid (oz)
microliter (μL)	2.64×10^{-7}	gallon (gal)
milliliter (mL)	2.64×10^{-4}	gallon (gal)
	Flow	
milliliters per minute (mL/min)	6.10×10^{-2}	cubic inch per minute (in^3/min)
	Resistivity	
megohm centimeter (MΩ-cm)	3.94×10^{-1}	megohm inch (MΩ-in)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Concentrations for suspended solids samples are in nanograms per liter (ng/L).
Concentrations for solids samples are in nanograms per grams (ng/g).

Abbreviated Water-Quality Units

g	gram
mg	milligram (10^{-3} grams)
ng	nanograms (10^{-9} grams)
pq	picograms (10^{-12} grams)
L	liters
mL	milliliters (10^{-3} liters)
µL	microliters (10^{-6} liters)
ng/L	nanograms per liter (parts per trillion)
ng/g	nanograms per gram (parts per billion)
cm	centimeters (10^{-2} meters)
mm	millimeters (10^{-3} meters)
µm	micron (10^{-6} meters)
MΩ-cm	megohm centimeter
mL/min	milliliters per minute
M	molar (mole per liter)

Abbreviations and Acronyms

Ar	argon
CH_2Cl2	methylene chloride
CRM	certified reference material
$CuSO_4$	copper sulfate
CVAFS	cold vapor atomic fluorescence spectroscopy
DDL	daily detection limit
DQO	data quality objective
GC	gas chromatography
HCl	hydrochloric acid
Hg	mercury
HPLC	high pressure luquid chromatography
IAEA	International Atomic Energy Agency
I.D.	inside diameter
KCl	potassium chloride
KOH	potassium hydroxide
MDL	method detection limit
MeHg	methylmercury
N_2	nitrogen
N_2SO_4	sulfuric acid
$NaBEt_4$	sodium tetraethyl borate
NH_2OH*HCl	hydroxylamine hydrochloride
NIST	National Institute of Standards and Technology
O.D.	outside diameter
QA	quality assurance
QFF	quartz fiber filter
RPM	revolutions per minute
RSD	relative standard deviation
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WDML	Wisconsin District Mercury Lab

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Abstract

This report presents the methods and method performance data for the determination of methylmercury concentrations in solids and suspended solids. Using the methods outlined here, the U.S. Geological Survey's Wisconsin District Mercury Laboratory can consistently detect methylmercury in solids and suspended solids at environmentally relevant concentrations. Solids can be analyzed wet or freeze dried with a minimum detection limit of 0.08 ng/g (as-processed). Suspended solids must first be isolated from aqueous matrices by filtration. The minimum detection limit for suspended solids is 0.01 ng per filter resulting in a minimum reporting limit ranging from 0.2 ng/L for a 0.05 L filtered volume to 0.01 ng/L for a 1.0 L filtered volume. Maximum concentrations for both matrices can be extended to cover nearly any amount of methylmercury by limiting sample size.

Introduction

Since the industrial revolution, pronounced increases (approximately three-fold) in atmospheric mercury emissions, transport, and subsequent deposition have yielded what is now considered a global contamination issue. Although most surface waters and sediments are now (2004) enriched with mercury (Hg) relative to historic times, environmental Hg concentrations remain a substantial analytical challenge to quantify accurately. These difficulties are primarily due to sample contamination, artifact formation of methylmercury (MeHg) during distillation, matrix interferences, and natural heterogeneity of samples.

Naturally occurring microbial processes increase Hg toxicity by the conversion of inorganic mercury to MeHg. Methylmercury, the most toxic and bioaccumulative form of mercury in food webs (Wiener and Spry, 1996; Brumbaugh and others 2001; Wiener and others 2003), is generally about 0.1 to 5 percent of the total mercury pool in most waters and sediments (Krabbenhoft and others, 1999; Wiener and others, 2003). Although MeHg represents greater than 95 percent of the mercury in consumable game fish tissues and commonly reaches concentrations at the low part per million level (Brumbaugh and others, 2001; Wiener and others, 2003), MeHg concentrations in water from the aquatic ecosystems these fish inhabit range from about 0.04 to 0.8 nanograms per liter (ng/L) (St. Louis and others, 1994; Hurley and others, 1995; Gilmour and others 1998; Babiarz and others, 1998; Bodaly and others, 1998; Krabbenhoft and others, 1999; Waldron and others, 2000). Concentrations of MeHg in sediment range from about 0.1 to 10 nanograms per gram (ng/g) dry weight (Gilmour and others 1998; Krabbenhoft and others, 1999). Methylmercury concentrations in anoxic waters or waters affected by industrial pollution (for example, chloralkali plants) can reach levels near 10 ng/L (Bloom and Effler, 1990; Krabbenhoft and others 1998; Brigham and others, 2002) and sediment MeHg concentrations can reach approximately 100 ng/g dry weight (Benoit and others, 2003).

Researchers from the U.S. Geological Survey (USGS) Wisconsin District Mercury Laboratory (WDML) published a method for the determination of MeHg concentrations in natural waters (DeWild and others, 2002). The current report describes the methods used by the WDML to analyze solids (bed sediments from aquatic ecosystems and soil from terrestrial ecosystems) and suspended solids (solids isolated from natural waters by filtration) for MeHg concentration, and documents the method detection limit (MDL) for these sample media using the described techniques. Because the WDML employs different procedures for the preparation of solids and suspended solids, the procedures are presented separately in this report.

Methylmercury can be a difficult parameter to measure in solids because of matrix interferences and the possibility of unintentionally producing MeHg during

distillation (Bloom and others, 1997; Hintelmann and others, 1997; Hammerschmidt and Fitzgerald, 2001). Researchers at the WDML have adopted a previously published technique for extracting MeHg from solids that eliminates formation of MeHg in samples with high inorganic mercury levels (Hintelmann, 1999).

The WDML gratefully acknowledges support for this study from the USGS Toxic Substances Hydrology Program.

Part 1. Method for the Extraction and Analysis of Methylmercury from Solids

Scope and Application

The method presented here is suitable for the detection of MeHg in solid samples collected from terrestrial and aquatic ecosystems. Material consists of bed sediment samples and soil samples. Samples are processed and analyzed as they are received, which may be as wet sediment or freeze-dried. Method performance therefore is evaluated on an as-processed basis to eliminate factors not related to sample processing and analysis. Minimum detectable concentration is 0.08 ng/g as-processed, with the maximum concentration dependent on mass of sample extracted and volume of extractant analyzed. Results are reported on a dry weight basis by dividing the concentration as-processed by the percent dry weight.

Method Summary

Solids (0.5 to 1.0 g) are placed into a centrifuge tube. Potassium bromide (KBr), copper sulfate ($CuSO_4$), and methylene chloride (CH_2Cl_2) are sequentially added. The mixture is allowed to react for an hour and then is shaken for an hour to ensure complete extraction of the MeHg. Following the shaking, the samples are centrifuged to break any emulsion that has formed. An aliquot of the CH_2Cl_2 is cleanly transferred to a vial containing reagent water. These vials are placed in a heating block until all CH_2Cl_2 has been evaporated and the MeHg has been back-extracted into the reagent water. The pH of the extractant is adjusted to 4.9 (to maximize ethylation potential) using acetate buffer. The extractant then is ethylated using sodium tetraethyl borate ($NaBEt_4$) and allowed to react for 15 minutes. After reaction with $NaBEt_4$, the extractant is purged with nitrogen gas (N_2) for 20 minutes and the ethylated Hg species are collected on a sample trap containing Carbotrap. These ethylated Hg species are desorbed thermally from the sample trap, separated using a gas chromatographic (GC) column, reduced using a pyrolytic column, and detected using cold vapor atomic fluorescence spectrometry (CVAFS). This extraction procedure (Bloom and others, 1997) eliminates all known interfereneces from organic matter, particulates, and sulfides in addition to greatly reducing potential positive artifact generated from interaction of ambient inorganic Hg with organic compounds.

Sample Collection and Preservation

Methylmercury analysis is extremely sensitive to contamination; therefore, care must be taken to avoid contamination in sample collection and analysis. Sample collection should be conducted using clean hands/dirty hands protocol (Olson and DeWild, 1999). Solids samples are collected and placed into precleaned vials. Collection and analysis equipment is cleaned according to the procedures outlined in DeWild and others (2002). Vials can consist of Teflon, cleaned according to DeWild and others (2002); baked glass vials (prepared by heating to 550°C for four hours); or acid rinsed polycarbonate vials. Samples are frozen as soon as possible after collection, shipped to the lab on dry ice by overnight mail, and held at a temperature of -15°C or less until processing. The WDML has not performed a holding time study; however, a frozen reference material (CRM) certified for Hg is available through the National Institute of Standards and Technology (NIST), and is stable for nine years.

Method Detection Limit

MDL of 0.08 ng/g (as-processed) was determined according to U.S. Environmental Protection Agency (USEPA) protocol (U.S. Environmental Protection Agency, 1990) from multiple analyses of a bottom sediment sample (table 1) collected from Sleepers River in New Hampshire. Because samples are received with varying water content (including freeze-dried), MeHg per gram of sediment as-processed is the most useful way to evaluate the MDL. The sample was homogenized with a Teflon policeman and seven aliquots, ranging from 0.696 to 1.22 g, were transferred from the sample container to individual centrifuge tubes that were then refrozen until processed. The subsamples were processed and analyzed over a period of several days.

Table 1. Methylmercury concentrations from multiple analyses of bottom sediment for the determination of the method detection limit

[All concentrations in nanograms per gram (ng/g)]

Sleepers River s-1	0.132
Sleepers River s-1	0.144
Sleepers River s-1	0.171
Sleepers River s-1	0.108
Sleepers River s-1	0.146
Sleepers River s-1	0.097
Sleepers River s-1	0.123
Average	**0.132**
Standard deviation	**0.025**
Detection limit (standard deviation x 3.143*)	**0.078**

*students T-value at the 99 percent confidence interval for n=7

Sample Preparation

As practiced at the WDML, an extraction batch consists of 22 environmental samples, four method blanks, two triplicate environmental sample sets, and two CRMs. All reagent additions, sample transfers, and the back-extraction are carried out inside a certified fume hood.

Reagents:

A. Reagent water. Ultra-pure reagent water containing less than 0.1 ng/L Hg with a resistance greater than 18 MΩ-cm starting from a prepurified source (distilled, reverse osmosis, and others). The water is delivered through a 0.2 μm filter, as obtained from a Millipore Academic water-purification system or equivalent.

B. Potassium bromide extraction solution. Dissolve 180 g of reagent grade KBr in reagent water, add 50 mL of concentrated sulfuric acid (H_2SO_4) and dilute to 1 liter. Adding 0.2 g of hydroxylamine hydrochloride (NH_2OH*HCl) stabilizes this solution. This solution should prepared in either glass or Teflon, be stored refrigerated and in the dark, and made fresh monthly.

C. 1M Copper sulfate. Dissolve 125 g of reagent grade $CuSO_4$ in 500 mL of reagent water. This solution is stored in a mercury clean Teflon bottle for up to six months.

D. Methylene chloride. HPLC grade CH_2Cl_2 that has been blank tested and found to be low in methylmercury.

E. Nitrogen. Prepurified or reagent grade N_2 passed through a gold bead trap attached to the outlet of the tank to remove any Hg.

F. Acetate buffer. 11.8 mL of glacial acetic acid and 27.2 g reagent grade sodium acetate trihydrate diluted to 100 mL with reagent water.

G. Ethylating Reagent. 1 g of Sodium Tetraethyl Borate ($NaBEt_4$; Strem 11-0575) dissolved in 100 mL of 2 percent Potassium Hydroxide (KOH), weight to weight (w/w), solution that has been chilled to form slush. The $NaBEt_4$ solution is divided equally among 10 clean 15 mL Teflon vials that then are capped and frozen. This solution should be kept frozen and made fresh every two weeks. Never use $NaBEt_4$ solid or solutions that are yellow in color. *Note: $NaBEt_4$ is toxic, gives off toxic gases (triethylboron) and is spontaneously combustible. Any $NaBEt_4$ use should take place in a high-volume fume hood. To discard unused portions of ethylating reagent, empty bottles into a large beaker of 6N hydrochloric acid (HCl) inside a high-volume fume hood. Place beaker on a hotplate and boil down to half-volume, then discard the remaining solution as an acid waste. Triethylboron will boil off into the air where it is oxidized to harmless boric acid.*

H. Argon (Ar). Ultra high purity grade 5.0 Ar passed through a gold bead trap attached to the outlet of the tank to remove any Hg.

I. Nitrogen. Ultra high purity grade 5.0 N_2 passed through a gold bead trap attached to the outlet of the tank to remove any Hg.

Extraction Equipment:

A. An analytical balance capable of weighing sediment samples to the nearest mg.

B. Pneumatic fixed-volume and variable pipettes ranging from 5 μL to 5 mL. Pipettes need to be calibrated monthly to ensure accuracy and precision of +/- 3 percent.

C. Repipet capable of consistent (+/- 3 percent) delivery of 10 mL of organic solvent.

D. Teflon centrifuge tubes (35 mL). Oak-Ridge type or equivalent.

E. Laboratory shaker capable of sustaining vigorous agitation for one hour.

F. Centrifuge capable of maintaining 3000 RPM for 20 minutes.

G. Teflon distillation tubes (60 mL). Savillex, Inc. part # 0202 or equivalent.

H. Midget impingers for distillation tubes. Savillex, Inc. part # 338 or equivalent.

I. Flow meters capable of maintaining N_2 flow at 100 +/- 20 mL per minute.

J. A custom-fabricated aluminum block heated with a Thermolyne type 2200 (or equivalent) hot plate is used during the back-extraction step. A probe placed in the center of the block monitors block temperature.

Extraction Procedure:

A. Homogenize the sample with a mercury-clean Teflon policeman in the original sample collection container. Weigh approximately 0.5 to 1.0 g of material into each centrifuge tube.

B. Pipette 5 mL of the KBr extraction solution, 1 mL of $CuSO_4$, and 10 mL of CH_2Cl_2 into each tube.

C. Allow the tubes to sit at room temperature for one hour.

D. Place the tubes on a lab shaker and shake vigorously for 1 hour.

E. Place the tubes in a centrifuge and spin at 3000 RPM for 20 minutes to break any emulsion that may have formed.

F. Prepare back-extraction vials by adding 40.0 mL of reagent water to each 60 mL Teflon vial with a repipettor.

G. Carefully pipette 2 mL of the CH_2Cl_2 (lower layer) into a back-extraction vial. Care must be taken to pipette only clean, clear CH_2Cl_2 from the centrifuge tube and a new pipette tip should be used for each sample. Pasteur-type pipette tips are recommended for pipetting the CH_2Cl_2 due to its low surface tension.

H. Place the back-extraction vials into the heating block and attach N_2 lines to inlets of impingers. Ensure block temperature is at 45°C and that flow is at 100 mL per minute.

I. After all of the CH_2Cl_2 has been purged (approximately one hour) the samples can be removed from the block and stored in the dark at 4°C for up to 48 hours prior to analysis.

Sample Analysis

After the samples have been back-extracted, they are ready for analysis and should be analyzed within 48 hours. The analysis is a two-step process consisting of purging the mercury species from the sample and detecting the mercury species with a cold vapor atomic fluorescence detector (DeWild and others 2002). All chemical additions to the reaction vessels are carried out in a fume hood and then the vessels are transferred to a clean bench below a laminar-flow hood equipped with a HEPA filter which is 99.99 percent efficient on particles less than 0.3 microns in diameter.

A. Create a standard curve by adding varying amounts of working standard (typically 100, 50, 25, and 10 pg, but the range needs to cover the expected concentrations in the analytical batch) to approximately 100 mL of reagent water in each of the reaction vessels. Pipette 200 µL of acetate buffer and 100 µL of $NaBEt_4$ into each of the reaction vessels. The $NaBEt_4$ reagent serves to derivatize the two remaining ionic Hg species after the extraction step (inorganic Hg(II) and MeHg) to their ethylated forms (diethyl Hg and methylethyl Hg, respectively). Elemental Hg does not react with the $NaBEt_4$. *Note: The $NaBEt_4$ needs to remain near 0°C. It should be removed from the freezer approximately 3 minutes before being added to the reaction vessels and placed in a dark place to partially thaw. A new vial of $NaBEt_4$ should be used each day.*

B. Tighten the sparging stoppers, ensure the four-way valve is in the closed position, gently swirl the reaction vessels, and allow the reaction to proceed

for 15 minutes. After the reaction time has elapsed, remove the plugs from the ends of the sample traps. Place the sample traps onto the outlet of the reaction vessels, with the identification number downstream, turn the four-way valve to the open position, and allow grade 5 N_2 to purge the vessel at a rate of 250 mL/min for 20 minutes.

C. After the samples have been purged, turn the four-way valve to the closed position and remove the sample trap from the reaction vessel outlet. Remove the N_2 line from the inlet of the four-way valve and place the sample trap on the end of the N_2 line. Allow the N_2 to flow through the sample traps at 250 mL/min for 7 minutes to remove any water vapor that has collected on the sample trap.

D. Four ethylation blanks are prepared by adding approximately 100 mL of reagent grade water, 200 µL of acetate buffer, and 100 µL of $NaBEt_4$ to separate reaction vessels. Then proceed as in step B.

E. While one set of reaction vessels and sample traps are being used to collect the purged sample, the other set can be desorbed and analyzed. Remove the sample traps from the N_2 lines, attach the N_2 lines to the inlets of the four-way valves, and cap both ends of the sample traps.

F. To desorb and analyze the traps, remove the plugs from the ends of the first trap and place it into the analytical train by threading it, with the identification number upstream, through the center of the nichrome wire coil. Center the nichrome wire over the Carbotrap, allow the flow to stabilize for approximately 30 seconds, and start the system. The nichrome wire will heat to 250°C with a ramp time of 30 seconds to desorb the Hg species from the sample trap. As the Hg species are desorbed from the sample trap they are carried by the Ar carrier gas at a flow of 25 mL/min into the GC column where the elemental Hg, methylethyl Hg and the diethyl Hg are separated. Following separation, the individual Hg species are carried into the pyrolytic column where the methylethyl and diethyl Hg species are reduced thermally to elemental Hg. The CVAFS detector can only detect elemental mercury. The detector then outputs a millivolt signal to the peak integration software resulting in three distinct peaks (the center peak represents the MeHg).

G. After the standard curve and the ethylation blanks have been analyzed, and found to meet the data-quality objectives (DQO), the extractants from the batch can be analyzed. The procedure for analyzing the method blanks, environmental samples, and CRM samples are identical to the procedure used for the standards and ethylation blanks. Simply dispense an appropriate amount of extractant into the reaction vessel, add reagent water to bring volume in reaction vessel to approximately 100 mL, add the acetate buffer and the $NaBEt_4$, and proceed as in step B. An appropriate amount of sample would be an amount that produces a MeHg peak with an area that falls within the calibration range (for most samples this amount is the entire extractant volume).

Data Analysis

Peak areas obtained from the CVAFS detector are used in the following series of calculations to determine the concentration of MeHg in the original sediment sample.

MASS OF MERCURY IN ALIQUOT ANALYZED

$$M_A = PA/S, \quad (1)$$

where

M_A = mass per aliquot (ng)
PA = peak area
S = slope of calibration line

MASS OF MERCURY IN BACK-EXTRACTION VIAL

$$M_V = (M_A * (40/(W_B - W_A))) - MB_{AVE}, \quad (2)$$

where

M_V = mass in back-extraction vial (ng)
M_A = mass per aliquot (ng)
The factor of 40 represents the total volume of water in the back-extraction vial
W_B = weight of receiving vessel before pouring off aliquot to be ethylated (g)
W_A = weight of receiving vessel after pouring off aliquot to be ethylated (g) *Note: Because water has a specific gravity of 1, 1 gram of water is assumed to equal to 1 mL of water.*
MB_{AVE} = average mass in method blanks (ng)

CONCENTRATION OF METHYLMERCURY IN ORIGINAL SAMPLE

$$C = (M_V * 5) / W_S, \quad (3)$$

where

C = concentration (ng/g)

M_V = mass of MeHg in back-extraction vial (ng)

W_S = weight of sample added to centrifuge tube (g)

The factor of 5 represents the correction for taking 2 mL from the total of 10 mL of CH_2Cl_2 for the back-extraction

Acceptance Criteria

Included with each batch of environmental samples are method blanks, replicate analyses, and CRM samples. Each of these samples provides quality-control information used to evaluate the acceptability of the analytical run.

A. Method blanks. Four method blanks are included in each sample batch and are used to evaluate potential contamination during the extraction and analytical steps. The daily detection limit (DDL) is defined as three standard deviations of all method blanks. The WDML DQO for DDL is 0.08 ng.

B. Replicate analyses. Two samples from each batch are set up in triplicate to evaluate the precision of the method. DQOs for replicate analyses are a relative standard deviation of no more than 25 percent.

C. Certified reference material. There are two CRM samples included in each batch that are used to evaluate the accuracy of the analytical run. DQOs for CRMs are 55 to 95 percent recovery because the certified value was established using a distillation technique known to produce positively biased results (Bloom, 1997; Hintelmann, 1997, 1999; Hammerschmidt, 2001).

Method Performance

Precision and accuracy for this method were determined by multiple analyses of a CRM, a bed material sample from Sleepers River, Vermont, and an upland sandy soil. The CRM used to evaluate this method was IAEA-405 (a polluted marine sediment obtained from the International Atomic Energy Agency) that has a certified value of 5.49 ng/g and a range of 4.96–6.02 ng/g. The certified value was determined using a distillation procedure that has been shown to cause significant amounts of artifact MeHg formation when the MeHg fraction is less than 1 percent of the inorganic pool (Hintelmann, 1999), which is the case with IAEA-405. Using the method described in this report, multiple analyses (19) by the WDML resulted in a 76 percent recovery of the CRM as compared to the literature value. Because the potential exists for the certified value to be biased high, and multiple analyses from this lab produced a consistently lower value, the accepted range of recovery for the WDML has been established at 55 to 95 percent. Method precision was evaluated by determining the percent relative standard deviation of the concentrations obtained from all analyses of the CRM and the Sleepers River sample (table 2). The upland sandy soil sample was not used to evaluate precision because the MeHg concentration was below detection limit. Accuracy was evaluated by calculating the percent recovery from analyses of the CRM and spiked environmental samples (table 3). The environmental samples were spiked with standard by weighing out the aliquots and adding 2.547 ng

Table 2. Methylmercury concentrations for multiple analyses of IAEA-405 (certified reference material) and Sleepers River sediment.

[all concentrations in nanograms per gram (ng/g)]

	Concentration in IAEA-405	Concentration in Sleepers River sediment
	4.50	
	4.84	
	4.21	
	4.01	
	4.46	
	4.30	
	4.09	
	3.95	
	4.00	
	3.84	
	3.78	
	4.70	
	4.89	0.132
	3.68	0.144
	3.82	0.171
	3.98	0.108
	3.95	0.146
	4.18	0.097
	3.95	0.123
Average	**4.16**	**0.132**
Standard deviation	**0.359**	**0.025**
Percent relative standard deviation	**8.63**	**19.0**

of MeHg to each aliquot before adding reagents. Percent recovery was calculated using equation 4 for the CRM and equation 5 for the spiked environmental samples.

PERCENT RECOVERY FOR CRM ANALYSIS

$$\%R = (C_a / C_c) * 100, \quad (4)$$

where

%R = percent recovery

C_a = analytically determined concentration (ng/g)

C_c = certified concentration of CRM (ng/g)

PERCENT RECOVERY FOR SPIKED SAMPLES

$$\%R = ((MM_s - (C_a * M_s)) / S_m) * 100, \quad (5)$$

where

%R = percent recovery

MM_s = analytically determined methylmercury mass in spiked aliquot (ng)

C_a = average concentration of unspiked sample (ng/g)

M_s = mass of sample aliquot (g)

S_m = mass of spike added (ng)

Table 3. Methylmercury concentration and percent recovery data for IAEA-405, Sleepers River sediment and upland soil samples

[all concentrations in nanograms per gram (ng/g)]

	Concentration in IAEA-405	Percent recovery	Concentration in spiked Sleepers River sediment	Percent recovery	Concentration in spiked upland soil	Percent recovery
	4.50	82.0				
	4.84	88.3				
	4.21	76.7				
	4.01	73.0				
	4.46	81.3				
	4.30	78.3				
	4.09	74.5				
	3.95	72.0				
	4.00	72.8				
	3.84	69.9				
	3.78	68.9				
	4.70	85.7				
	4.89	89.1			2.05	80.4
	3.68	67.0	2.32	100.6	2.08	81.6
	3.82	69.6	2.16	96.9	1.69	66.2
	3.98	72.5	3.97	98.1	2.26	88.8
	3.95	72.0	3.04	89.0	2.20	86.2
	4.18	76.1	2.28	84.9	2.26	88.8
	3.95	72.0	3.22	89.4	2.09	82.2
Average	**4.16**	**75.9**		**93.2**		**82.0**
Standard deviation	**0.359**	**6.6**		**6.2**		**7.8**

Part 2. Method for the Extraction and Analysis of Methylmercury from Suspended Solids

Scope and Application

The method presented in this report is suitable for suspended solids samples isolated from aqueous samples by filtration. Samples are collected onto baked (prepared by firing to 550°C) quartz fiber filters (QFF) and submitted to the laboratory frozen. Performance tests on this method show it can be used to determine MeHg concentrations for filter-collected suspended solids with a MDL of 0.01 ng of MeHg on a filter. Because suspended particulate loads vary considerably within hydrologic settings, varying amounts of sample water need to be filtered; therefore, mass of MeHg per filter is the most useful way to evaluate the MDL. A direct comparison of mass detected on the filter to the MDL eliminates the need for a volume-predicated MDL. Results are reported on a ng/L basis by dividing the mass of MeHg on a filter by the volume filtered; therefore, the minimum reporting limit ranges from 0.2 ng/L for a 0.05 L filtered volume to 0.01 ng/L for a 1.0 L filtered volume.

Method Summary

Filters containing the suspended solids are placed in distillation bottles, reagents are added, and the samples are distilled. The distillation procedure extracts MeHg from the solid matter into the dissolved phase, converts MeHg into methyl mercury chloride, and removes potential interferences. Analysis of the distillate follows the method described in DeWild and others (2002) and summarized above, with minor modifications to the data calculations.

Sample Collection and Preservation

To provide reliable concentrations for MeHg in suspended solids, the WDML has developed a method to concentrate suspended solids from unfiltered water by either in-line filtration or by vacuum filtration. In either case, samples should be collected using clean hands/dirty hands sampling protocols (Olson and DeWild, 1999) to ensure sample integrity. Sample size is dependent on suspended solids load and MeHg concentration and can range from 0.05 to 1 L. The suspended solids are retained on baked QFFs. To provide MeHg concentrations for suspended solid samples in mass per unit volume (for example, ng/L), field crews must measure the volume of water filtered. Individual filters are placed into stackable Teflon petri dishes, double bagged in sealable plastic bags, and frozen. Samples are shipped to the lab on dry ice by overnight mail, and held frozen at a temperature of -15°C or less until processing. The WDML has not performed a holding time study; however, a frozen CRM for Hg, available through NIST is stable for 9 years.

Method Detection Limit

To document the WDML's ability to provide quality data at commonly observed natural levels of MeHg in suspended solids (about 0.04 to 25 ng MeHg/L), an artificial whole-water sample was created by adding 0.20814 g of a CRM to 2.0 L of reagent water. A polluted marine sediment (IAEA-405) was used as the surrogate for suspended solids. The artificial water sample was mixed thoroughly, and filtered using vacuum filtration. The suspended solids retained on the filters were analyzed using the method presented in this report, and unfiltered and filtered water samples were analyzed using the methods described by DeWild and others (2002). The suspended solids samples were processed in five separate distillation batches and analyzed over five days. Unfiltered and filtered water samples were collected to determine the concentrations of the slurry and the filtrate. The water samples were distilled during a single distillation.

Because suspended particulate loads vary considerably within hydrologic settings, varying amounts of sample water need to be filtered; therefore, mass of MeHg per filter is the most useful way to evaluate the MDL. A direct comparison of mass detected on the filter to the MDL eliminates the need for a volume-predicated MDL. For this study, the target volume per filter was 100.0 mL. This volume was not accurately achieved for each filter; therefore, the analytically determined mass per filter was converted to a 100.0 mL equivalency to accurately determine a mass-based MDL. The MDL was determined to be 0.01 ng per filter from multiple analyses of the particulate filters (table 4).

Table 4. Mass of methylmercury per filter from artificial water sample, after conversion to 100.0 mL equivalency, for detection limit assay

[all concentrations in nanograms (ng)]

	Particulate CRM
	0.054
	0.056
	0.046
	0.047
	0.050
	0.045
	0.048
	0.049
	0.050
Average	**0.049**
Standard deviation	**0.004**
Percent relative standard deviation	**7.300**
Detection limit (standard deviation x 2.896*)	**0.01**

*students T-value at the 99 percent confidence interval for n=9

Sample Preparation

Samples must be distilled prior to analysis (see details in DeWild and others, 2002) to extract the MeHg from the suspended solids into the dissolved phase, to convert MeHg to methyl mercury chloride, and to remove potential interferences.

Reagents:

A. Reagent water. Ultra-pure reagent water containing less than 0.1 ng/L Hg with a resistance greater than 18 MΩ-cm starting from a prepurified source (distilled, reverse osmosis, and others). The water is delivered through a 0.2 µm filter, as obtained from a Millipore Academic water-purification system or equivalent.

B. 8M Sulfuric acid. Equal volumes of reagent water and trace pure H_2SO_4. This reagent should be prepared in a glass or Teflon container as need to prepare the combined reagent.

C. 20 percent Potassium chloride. 20 g reagent-grade KCl diluted to 100 mL total volume with reagent water. This reagent should be prepared in a glass or Teflon container as need to prepare the combined reagent.

D. 1M Copper sulfate. Dissolve 125 g of reagent grade CuSO4 in 500 mL of reagent water. This solution is stored in a mercury clean Teflon bottle for up to six months.

E. Combined reagent. Combine 200 mL of 8M H_2SO_4, 100 mL of 20 percent KCl, and 200 mL of $CuSO_4$. This solution is stored in a mercury clean Teflon bottle for up to six months.

Distillation Equipment

The distillation system (DeWild and others, 2002) consists of a solid aluminum heating block, a hot plate, a small refrigerator, Teflon distillation and receiving vessels, and Teflon transfer lines.

A. A custom-fabricated aluminum block with 40 positions is heated with a hotplate capable of maintaining 125 +/- 5°C. Two probes are used to monitor block temperature.

B. A small commercially available refrigerator is used to hold the receiving vials, aid in condensation, maintain distillate at 4°C, and protect the distillate from exposure to light. Small holes are drilled in the side of the refrigerator to accommodate the transfer lines.

C. The distillation and receiving vessels are 125 mL Teflon bottles (Nalgene catalog number 1630-0004 or equivalent). Distillation vessel caps are custom-made Teflon caps with two ports integrated into the cap itself. One port is a ¼-in compression fitting, into which a ¼-in outside diameter (O.D.) Teflon tube is inserted so that it will extend to within 2 mm of the bottom of the distillation vessel to insure complete sample purging. The other port is a ⅛-in compression fitting, which the transfer line connects to. The receiving vessel caps consist of a Teflon insert (Savillex part number 0738-4-2 or equivalent) molded integrally with two transfer ports equipped with compression fittings for ¼-in O.D. tubing. A length of ¼-in O.D. tubing is inserted into one of the ports so that it will extend to within 2 mm of the bottom of the receiving vessel to insure complete sample recondensation. Teflon transfer lines of ⅛-in O.D. are connected by friction fit from the outlet tubing of the distillation vessels to the inlet tubing of the receiving vessels.

D. Flowmeters capable of maintaining a flow of 60 mL/min of N_2 are placed immediately upstream of the distillation vials to maintain constant and equal flow to all distillation vials. Gas is supplied through ⅛-in O.D. Teflon line inserted into the inlet tubing of the distillation vessel.

Distillation Procedure

A WDML suspended solids distillation batch consists of 34 environmental samples, 4 filter blanks, and 2 CRMs. A filter blank consists of a baked QFF and the CRM consists of a pre-weighed mass into the distillation vessel.

A. Using clean Teflon tweezers, transfer the QFF sample from the petri dish into a distillation vessel. Use reagent water to rinse the petri dishes three times back into the distillation vessel. A total volume of 50.0 mL of reagent water is added to each vessel. Add 2.0 mL combined reagent to each bottle and cap tightly with the distillation cap corresponding to the block position to be occupied by that vial.

B. Dispense 50 mL of reagent water to each receiving vessel. Record the bottle identifier of each. Cap each vessel with the receiving cap corresponding to the block position occupied by the matching distillation vessel.

C. Place the distillation vials in their respective positions in the distillation block and thread the transfer lines through the numbered holes in the refrigerator.

D. Turn on the N_2 flow to the flowmeters and connect the gas lines to the inlet ports of the distillation caps.

E. Place the receiving vial tray in the refrigerator and begin placing the receiving vials into the tray. As the receiving vials are placed into the tray, connect the transfer lines to the inlet ports of the receiving caps. Check for bubbling in the reagent water to verify unrestricted flow.

F. Adjust the flow on the flowmeters to 60 mL/min. Adjust the hot plate temperature to maintain a block temperature of 125 +/- 5°C. This temperature should result in a distillation rate of 6–8 mL per hour but adjustments may be needed for individual systems.

G. Periodically throughout the distillation, check the receiving vials to ensure unrestricted flow, the distillation vials to ensure no leakage, and the block temperature for stability.

H. Remove the transfer lines from the receiving vessels and the distillation vessels from the block when approximately 25 percent of the volume in the distillation vessel remains. The distillation caps and the inside of the transfer lines should be rinsed thoroughly with reagent water.

I. Weigh the receiving vessels and record the weight for later determination of the percent of the original sample that was distilled. Cap the bottles and place in a refrigerator at 4°C until analysis (distillates should be analyzed within 48 hours).

Sample Analysis

Sample analysis is performed following the method described in DeWild and others (2002) and outlined previously.

Data Analysis

Peak areas obtained from the CVAFS detector are used in the following series of calculations to determine the concentration of MeHg in the original sediment sample.

FRACTION DISTILLED:

$$D = V_S/(W_{F2} - W_W), \quad (6)$$

where

D = fraction distilled

V_S = sample volume (mL). Equal to 50 mL as dispensed from repipettor.

W_{F2} = weight of receiving vessel after distillation (g)

W_W = weight of receiving vessel with reagent water before distillation (g). *Note: Because water has a specific gravity of 1, 1 gram of water is assumed to equal to 1 mL of water.*

MASS OF METHYLMERCURY IN ALIQUOT ANALYZED

$$M_A = (P_A/S), \quad (7)$$

where

M_A = mass per aliquot (ng)

P_A = peak area

S = slope of calibration line

MASS OF METHYLMERCURY IN FILTER BLANK

$$MB = (M_A/D) * ((W_{F2} - W_{T2})/(W_{F2} - W_A)), \quad (8)$$

where

MB = mass in filter blank (ng)
M_A = mass per aliquot (ng)
D = fraction distilled
W_{F2} = weight of receiving vessel after distillation (g)
W_{T2} = weight of receiving vessel (g)
W_A = weight of receiving vessel after pouring off aliquot to be ethylated (g)

MASS OF METHYLMERCURY IN ORIGINAL SAMPLE

$$M_S = (M_A/D) * ((W_{F2} - W_{T2})/(W_{F2} - W_A)) - MB_{AVE}, \quad (9)$$

where

M_S = mass in original sample (ng)
M_A = mass per aliquot (ng)
D = fraction distilled
W_{F2} = weight of receiving vessel after distillation (g)
W_{T2} = weight of receiving vessel (g)
W_A = weight of receiving vessel after pouring off aliquot to be ethylated (g)
MB_{AVE} = average mass found in filter blanks (ng)

FINAL METHYLMERCURY CONCENTRATION

$$C = M_S / V_F, \quad (10)$$

where

C = concentration (ng/L)
M_S = mass in original sample (ng)
V_F = volume filtered during filtration process (L)

Acceptance Criteria

Included with each batch of environmental samples are filter blanks and CRM samples. Each of these samples provides quality-control information used to evaluate the acceptability of the analytical run.

A. Filter blanks. Four filter blanks are included in each sample batch and are used to evaluate potential contamination during the distillation and analytical steps by providing a DDL. The DDL is defined as three standard deviations of all method blanks. A DDL less than or equal to 0.01 ng/filter meets laboratory DQO.

B. Certified reference material. There are two CRM samples included in each batch that are used to evaluate the accuracy of the analytical run. DQOs for CRMs are 75 to 125 percent recovery. Values are calculated using equation 4.

Method Performance

To evaluate precision and accuracy for this method, two artificial water samples (range A and range B) were created by adding different amounts of CRM (IAEA-405) to approximately 2 L of reagent water. These artificial water samples were then filtered by vacumm filtration to create 10 suspended sediment samples from each water sample. Subsamples of the unfiltered and filtered artificial water samples were collected and analyzed according to DeWild and others (2002) to determine the concentration of MeHg in the artificial water samples. Methylmercury concentration of the suspended solids was calculated by subtracting the filtered water concentration from the unfiltered water concentration. The unfiltered concentration for range A was 0.614 ng/L, the filtered concentration was 0.101 ng/L, and the calculated suspended solids concentration was 0.513 ng/L. For range B the unfiltered concentration was 2.62 ng/L, the filtered concentration was 0.274, and the calculated suspended solids concentration was 2.35 ng/L.

Precision was evaluated by examining the percent relative standard deviation (RSD) of the concentrations obtained from all analyses of the particulate filters for each concentration range (tables 5A and 5B). The percent RSDs were 6.84 and 18.3 for range A and range B, respectively.

Accuracy was evaluated by calculating the percent recovery of the CRM from each filter (tables 5A and 5B). Percent recoveries were calculated using the formula:

PERCENT RECOVERY

$$R = (C_P/(C_U - C_F)) * 100, \quad (11)$$

where

R = percent recovery
C_P = concentration of suspended solids sample (ng/L)
C_U = concentration of unfiltered artificial water sample (ng/L)
C_F = concentration of filtered artificial water sample (ng/L)

Applying the equation above, percent recoveries ranged from 88.7 to 108.9, with an average of 95.4 percent and a standard deviation of 7.9 for range A. For range B, percent recoveries ranged from 65.2 to 117.5, with an average of 92.5 percent and a standard deviation of 17.1.

Table 5A. Methylmercury concentrations and percent recoveries from multiple analyses of range A artificial water sample

	Suspended solids concentration (ng/L)	Percent recovery
	0.536	104.6
	0.558	108.9
	0.462	90.1
	0.471	91.8
	0.499	97.4
	0.455	88.7
	0.480	93.6
	0.493	96.1
	0.499	97.3
Average	**0.495**	**95.4**
Standard deviation	**0.034**	**7.9**
Percent relative standard deviation	**6.84**	

Table 5B. Methylmercury concentrations and percent recoveries from multiple analyses of range B artificial water sample

	Suspended solids concentration (ng/L)	Percent recovery
	2.36	100.5
	2.20	93.5
	2.08	88.3
	2.08	88.5
	2.47	104.9
	2.48	105.4
	2.76	117.5
	1.63	69.1
	1.53	65.2
Average	**2.18**	**92.5**
Standard deviation	**0.40**	**17.1**
Percent relative standard deviation	**18.3**	

Summary

This document describes the methods used by the USGS Wisconsin District Mercury Laboratory to analyze solids and suspended solids for Methylmercury (MeHg) concentration. Because the procedures used to process and analyze solids and suspended solids differ, two distinct analytical performance studies were conducted and the results are presented.

The method detection limit (MDL) established for the solids procedure as outlined in this report is 0.08 ng MeHg/g (as-processed), which was considered acceptable because it is substantially below the levels commonly encountered in natural samples from a wide range of environments. The method precision, calculated as the percent relative standard deviation (RSD), ranged from 8.63 to 19.0 percent. The accuracy of the procedure, which was determined from recovery tests for spiked samples and certified reference material (CRM) replicates, ranged from 75.0 to 93.2 percent and was considered acceptable.

The second method performance test documented in this report is for the sample preparation and analysis of suspended solids on baked quartz fiber filters. An artificial raw water sample was created by suspending CRM in reagent water, which was then filtered to create suspended solid samples. The MDL was established by analyses of multiple filters, and a limit of 0.01 ng per filter was achieved. Precision was evaluated by calculating percent relative standard deviation from analyses of replicate filters. The percent RSD ranged from 6.84 to 18.3. Accuracy was evaluated from percent recovery of MeHg on the filters from their target value. Target values were determined by subtracting the analytically determined concentration of the filtrate from the raw water sample concentration. Recoveries ranged from 65.2 to 117.5 percent, with an overall mean and standard deviation of 94.5 percent and 12.7, respectively.

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