The GEOTRACES Hg Cookbook

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Introduction

The intent of this document is to summarize the results of an NSF-sponsored international intercalibration/comparison exercise into the accurate and efficacious collection and analysis of open ocean seawater samples for total mercury (Hg) determinations as well as Hg speciation within the context of a GEOTRACES cruise. This report is not meant to be a standalone description of all aspects of on board collection activity during a GEOTRACES cruise, but rather those aspects that we have come to view as the "recommended practice" with regard to Hg determinations. These activities include bottle selection and cleaning, sample collection and handling on board, sample filtration, the recommended analytical procedures for both on board or on-shore analyses and the latest view of optimal storage/preservation approaches if immediate analysis is not possible.

Bottle Selection and Cleaning

As part of this Intercomparison exercise, we revisited some of the most fundamental analytical considerations regarding bottle selection and cleaning (Hammerschmidt *et al.*, 2011). Particular care was taken to examine the susceptibility of sample bottles to the diffusion of elemental Hg (Hg^0) through the walls. Consideration of this potential contamination pathway is unique to mercury and is particularly important because many GEOTRACES cruises are likely to have large amounts of Hg^0 on board for electrochemical-based speciation analyses of Zn, Co, Pb and Fe. In addition, Hg is often used to preserve biological samples and there may be legacy Hg^0 in the ships laboratories from broken Hg thermometers. The potential for significantly elevated Hg^0 levels in shipboard laboratory spaces may result in airborne Hg concentrations that are highly elevated with respect to ambient air (ca. 1.5 ng m^{-3}). For example, on the two US GEOTRACES Intercalibration

cruises, we found Hg^0 concentrations in the Hg Group work spaces that ranged from 20 to 50 ng m^{-3}. Given this range in ship-board air mercury concentrations, capturing Hg^0 from the shipboard laboratory air in a half-filled 500 mL sample bottle would result in a contamination increase ranging from 0.1-0.25 pM. Since the range of total Hg anticipated in open ocean seawater is around 0.25 to 2.5 pM, the potential impact from airborne contamination is quite significant. While there are methods to fix this contamination (see below), every effort should be made to minimize work space Hg^o concentrations, including the use of activated charcoal scrubbers in laminar flow benches and the requisition of a separate laboratory van so that analyses may be performed outside of ship's lab spaces.

With Hg^0 concentrations present in work spaces a potential problem, gas impermeability is an important consideration when selecting bottles to receive samples, especially for long term storage aboard ship. We found that glass and impermeable plastics (like polycarbonate) are the best for long-term (months) storage of seawater for Hg analysis.

Our recommended bottle cleaning procedure is shown below, and was found to be effective for the very low-level seawater concentrations, and resulted in low blanks for bottles made of almost any material. The key ingredient seemed to be BrCl, which is the commonly used wet chemical oxidant for digesting aqueous samples prior to total Hg analyses. The BrCl concentration used during cleaning should be greater than that used in subsequent sample digestion to ensure best results. Bottles used for minority species analyses (Hg^o, $(CH_3)_2Hg$ and $CH_3Hg(I)$) should be thoroughly cleaned of BrCl prior to use, to avoid destruction of these forms. For example, a rinse with low Hg NH_2OH (see below) following the BrCl cleaning could be useful; however, we have found that copious rinses with high-purity water are equally effective. In our recommended workflow described below, we also segregate the analysis of total Hg (which uses BrCl) and the minority species into different bottles, to avoid accidental oxidation.

6 day Citranox soak
>6 day 10% HCl
1 day 0.5% BrCl
pH 2 water rinse

Table 1. Recommended cleaning procedure for new bottles for Hg species in seawater.

We recommend that GEOTRACES samples for Hg be collected into those bottles that best fit the individual workflow of the cruise. For example, Teflon is recommended for short-term storage when samples will be analyzed within a few days as they are unquestionably clean, highly durable and less gas permeable than polyethylene. If longer term storage is intended, then collection in either polycarbonate or glass are recommended to provide the best protection against Hg^o diffusion. It should be noted that polycarbonate does not fair well when exposed to strong oxidizing acid (>4N HNO_3) or strong base for extended periods. Thus, if the cleaning regimen includes either of these solutions, polycarbonate is not recommended.

Sample Collection and Handling

We found that the collection of Hg is relatively insensitive to the sampling platform used (e.g., CLIVAR clean rosette, GEOTRACES rosette or GO-Flo bottle hung sequentially on a non-metallic hydrographic line, such as Kevlar). Thus, as long as the collection bottle (GO-Flo, X-Niskin or the equivalent) has been shown to be appropriately cleaned for other

metals (e.g. Zn and Pb), it should be suitable for the collection of total Hg and Hg species. Furthermore, a number of different filtering strategies were tested, including the use of pressurized GO-Flos and in-line capsule filters (Osmonics 0.2 μm Teflon and the Acropak 0.2 μm Polyethersulfone) and as well as vacuum-assisted membrane filtration. The most commonly used membrane (0.45 μm pore size Nuclepore) and the capsule filters all seemed to compare well, suggesting that the particular filtering medium used is not critical, as long it has been previously tested to ensure a low blank. The conventional filter used during US GEOTRACES thus far has been 0.2 μm Acropak.

Results from the highly oligotrophic Sargasso Sea (Bergquist and Lamborg, unpublished) suggested that there is essentially no "colloidal" Hg or $CH_3Hg(I)$ present in open ocean seawater, where colloidal was defined as particles between 0.02 – 0.45 μm effective size. Thus, under most circumstances, we should not be surprised that different filtering media, assuming that they do not contribute a Hg blank or absorb Hg, should provide similar "dissolved" Hg results. Colloidal Hg is significant in coastal ocean environments and may be important in the Arctic Ocean and by extension in other shallower seas, however, so that near-shore sampling should include a pore size-dependent definition of "dissolved" (e.g., Stordal *et al.*, 1996; Choe and Gill, 2003; Choe *et al.*, 2003; Bowman and Fitzsimmons, unpublished).

Sample Analysis

A major advancement in the determination of $CH_3Hg(I)$ in seawater was made during this project, which has lowered the detection limit, increased accuracy and facilitated a further streamlining of Hg species determinations (Bowman and Hammerschmidt, 2011; Munson *et al.*, 2014). We now recommend this method and describe it below, as well as its integration into the general workflow.

During the Intercalibration/comparison exercise, all but two of the participating laboratories used cold vapor atomic fluorescence spectroscopic (CVAFS) determination of Hg (as Hg^0). The other two laboratories employed the other commonly used analytical approaches, inductively coupled plasma-mass spectrometry (ICP-MS) (with isotope dilution) and cold vapor atomic absorption spectrometry (CVAAS). Both CVAFS and ICP-MS compared well, while the CVAAS did not exhibit adequate sensitivity to detect total Hg on the intercomparison samples (250 mL). Thus, we recommend CVAFS or ICP-MS for Hg determinations. The CVAFS approach has the distinct advantage of being field employable allowing rapid determination of Hg^0 and $(CH_3)_2Hg$ at sea. ICP-MS, especially when employed with isotope dilution, has the potential for a lower absolute detection limit. Thus, we recommend CVAFS for at-sea determinations, but feel that either approach is appropriate for on-shore analyses.

Our recommended workflow is illustrated in Figure 1 The details of instrument use are documented elsewhere (e.g., Fitzgerald and Gill, 1979; Gill and Fitzgerald, 1985; 1987; Horvat, 1991; Hintelmann and Wilken, 1993; Horvat *et al.*, 1993; Hintelmann *et al.*, 1997; Hintelmann, 1998; Hintelmann and Simmons, 2003; Bowman and Hammerschmidt, 2011; Munson *et al.*, 2014). The workflow presented is oriented toward at-sea, multi-species

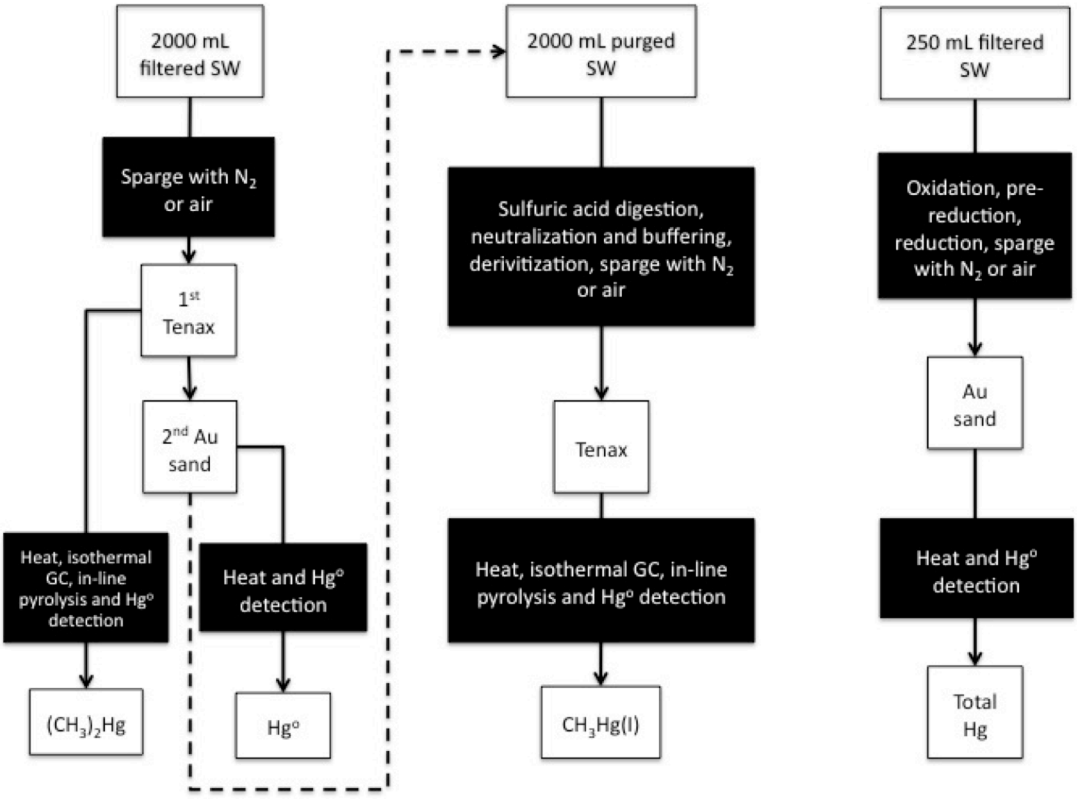

Figure 1. Our recommended workflow. All four analyses could be performed on one 2-L sample, but the reagents associated with analysis of $CH_3Hg(I)$ have a larger blank than those associated with total Hg determination. Therefore, for at-sea measurements, we recommend two separate aliquots be collected: one 250-mL sample for total Hg and one 2-L sample for Hg^0, $(CH_3)_2Hg$ and $CH_3Hg(I)$.

determinations by CVAFS, but could be easily adapted for use with ICP-MS back on shore. A ready supply of high quality water (18 MΩ-cm resistivity) will be necessary for at-sea or on-shore cleaning, standard and reagent making. Most commercially available "ultrapure" water systems are adequate for Hg analyses, but a check of the ship's system should be done immediately, and it may be prudent to bring a back up system. Though not shown in the workflow, laboratories need to also do a very careful determination of analytical, bottle, and reagent blanks to assure that they are working at levels appropriate to the determination of open ocean seawater. If possible, this should be done on-shore prior to a cruise as well as during the cruise. Replicate analyses on several samples to demonstrate precision is also a highly desirable when adequate sample is available. Standard spikes recoveries, especially for the $CH_3Hg(I)$ determination, should also be performed. These QA results should be reported along with the Hg results to demonstrate capability, reproducibility and accuracy.

Total Hg. During recent cruises, we have documented concentrations of total Hg in surface waters that are often highly depleted due to biological uptake and particle scavenging. Thus, GEOTRACES analysts should be prepared to deal with samples containing as little as 0.1 pM total Hg. As typical CVAFS arrangements have absolute detection limits on the order of 10 fmole, analyses performed on sample volumes of ca. 250 mL is recommended to ensure a resolvable signal.

Filtered aliquots of seawater should be pre-treated prior to analysis as follows: oxidize the sample with 0.05% (w/v) bromine monochloride (BrCl) solution or equivalent for at least 1 hour, removal of excess halogens with 0.05% v/v hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) solution for at least 5 minutes, and final reduction with 0.05% v/v stannous chloride ($SnCl_2$) solution followed by purging of Hg^o and trapping on gold or gold-coated sand (or the equivalent). Purging should progress until a volume of gas of at least 15 times the volume of liquid has been sparged, and at a volumetric flow rate of no more than 1 L min^{-1} (we recommend 0.5 L min^{-1}).

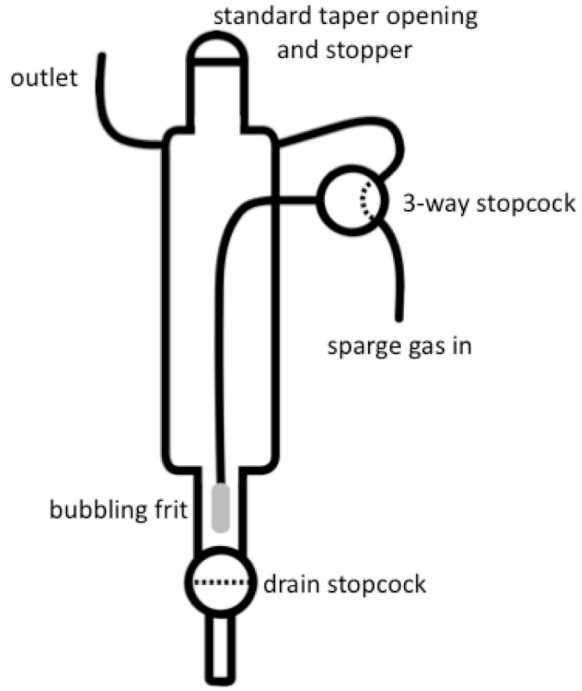

Figure 2. The sparging design developed at the University of Connecticut. It allows samples to be poured in at the top through the standard taper joint, while simultaneously allowing clean gas to vent the headspace. Emptying of the bubbler in preparation for another sample is achieve through the stopcock at the bottom, which will allow the bubbler to again fill with clean gas instead of room air. The three-way stopcock allows for the direction of sparging gas either through the headspace or the sparging frit at the bottom.

The sparging step should be conducted in a manner that minimizes introduction of shipboard laboratory air to the bubbler system. A closed sample introduction system is ideal, or a procedure that allows complete flushing of the headspace above the sample with Hg^0-free air (achieved using a Au trap column on the air inlet) prior to initiation of sample sparging. For samples less than about 300 mL in size, we recommend either a custom Fitzgerald Bubbler (diagram in Figure 2), or a 3 port bottle top sparging adaptor (e.g., Bio-Chem Omnfit #00945Q-3; fits any glass bottle with a GL45 thread) that can be fitted with a simple three-way manual valve (e.g. Cole-Parmer EW-30600-23) and attached to the sample bottle. Expelling the room air from the headspace of the Fitzgerald Bubbler is accomplished by having the purge gas flowing through the headspace and off-line with the collection gold trap for enough time to affect at least 5 volume exchanges. Entrainment of room air bubbles in the sample should also be avoided by decanting samples slowly and avoiding turbulent mixing after reagents have been added.

Hg^0 and $(CH_3)_2Hg$. Although these two dissolved gaseous mercury species are minor components (typically sub-pM concentrations) of the total mercury present in seawater, they are nonetheless highly important to measure as they are involved in air-sea exchange of Hg and probably in the formation of $CH_3Hg(I)$. Given the extremely low concentrations of these species, we recommend using 2 L sample sizes for analysis, with determination of Hg^0, $(CH_3)_2Hg$ and $CH_3Hg(I)$ all performed on the same aliquot. Procedurally, Hg^0 and $(CH_3)_2Hg$ are the easiest of the species to measure, requiring only that a volume of stripping gas of at least 15x the volume of liquid be sparged through the fluid without further amendment. We have successfully used two sorption media in series to discriminate between these two gaseous mercury species. The gas exiting the sparger should pass first through a moisture trap (e.g., soda lime), then either Tenax, Carbotrap or Bond-Elut (preferred) for $(CH_3)_2Hg$ collection, followed by Au or Au-coated sand for Hg^o collection (e.g., Bloom and Fitzgerald, 1988; Tseng *et al.*, 2004; Conaway *et al.*, 2009; Baya *et al.*, 2013). Following sparging, the traps are analyzed separately using a CVAFS system that is equipped with a gas flow train. The Hg^0 collected on the gold trap is liberated for detection by simply heating (600-800 °C) in an argon gas-flow train connected to the CVAFS detector. The $(CH_3)_2Hg$ retained on the chromatography material trap is liberated under low heat (90-250 °C, depending on the sorbent) and is passed first through a low temperature, isothermal chromatographic column (see in $CH_3Hg(I)$ section below) and then through a high temperature (600-800 °C) column packed with quartz wool to pyrolyze the $(CH_3)_2Hg$ to Hg^0 and make it available for detection by CVAFS (Bloom and Fitzgerald, 1988). Tenax, Carbotrap and Bond-Elut columns should be rigorously preconditioned prior to use by sparging and heating them several times. Furthermore, they should be tested to ensure that they do not retain Hg^0 to a large degree. We recommend the use of Bond-Elut and Tenax over Carbotrap as they retain much less moisture and Hg^0. Bond-Elut has been demonstrated to be the most effective at collecting $(CH_3)_2Hg$ of the three sorbents, and all three have some temperature dependence in their efficiency (lower temperature is better; Baya *et al.*, 2013). Fresh soda lime drying agent should be used on each sample, and can be recycled through baking.

$CH_3Hg(I)$. Following the sparging of Hg^0 and $(CH_3)_2Hg$, the 2 L sample can be processed for $CH_3Hg(I)$ determination. The sample must first be "digested" for > 12 h, through addition of 40 mL of conc. H_2SO_4. Following digestion, the sample is first neutralized with ca. 60 mL of 50% KOH, and then buffered to ca. pH=5 with 30 mL of 2 M K-Acetate/Acetic Acid buffer. The acetate buffer should be remade frequently and kept in the dark to avoid the formation of artifact $CH_3Hg(I)$. The pH should be checked and adjusted as necessary with small additions of strong acid (H_2SO_4) or strong base (KOH). Over titration to the point of precipitation of $Mg(OH)_2$ should be avoided. Citrate buffer can also be used, but results in a lower overall $CH_3Hg(I)$ yield due to stronger complexation.

To sparge the $CH_3Hg(I)$ from solution, it must first be derivatized or converted into a more volatile compound. Both alkylation (ethylation or propylation) and hydride generation have been used for this purpose (e.g., Monperrus *et al.*, 2005; Cossa *et al.*, 2011). The new method described here, and in more detail elsewhere (Bowman and Hammerschmidt,

2011; Munson *et al.*, 2014), makes use of a direct ethylation reaction applied to the seawater matrix. They have found that with the digestion step, close attention to pH and the use of fresh and cold ethylating agent (Na-tetraethylborate; NaTEB), and use of a small quantity of ascorbic acid, quantitative ethylation in seawater can be achieved. This new method eliminates the common practice currently employed of including a sample distillation step in the analysis to isolate the $CH_3Hg(I)$ from the matrix prior to the ethylation step. Prior to ethylation, 3.33 mL of 2.5% (w:v) ascorbic acid should be added. Much like the acetate buffer, the ascorbic acid can go bad and should be remade frequently. Though it does not add much acidity, the ascorbic acid can be added before buffering and pH adjustment.

As noted below, the ethylating agent is made up in small batches, but which often are not completely consumed within one week. After a week, even when kept frozen, the ethylating agent loses its potency and should be discarded. The thawed, working aliquot of 1% (wt:vol) NaTEB will also unavoidably lose potency throughout the course of the day, which can be slowed by keeping the solution cold. We recommend working samples in batches of four, by adding 1.5 mL of NaTEB directly to the buffered 2 L sample, allowing each sample to react for at least 15 minutes, and then sparging the methylethyl mercury ($CH_3CH_2HgCH_3$) from the sample using a bottle top sparging adaptor as mentioned above. The NaTEB can also be made up under inert atmosphere (N_2) and the solution stored in sealed vials with rubber septa that allow the withdrawal of the necessary volume.

The purge gas should first pass through a soda lime trap to remove moisture and then the $CH_3CH_2HgCH_3$ is collected on a Tenax or Bond-Elut trap column. Determination of $CH_3CH_2HgCH_3$ is conducted in an analogous way to $(CH_3)_2Hg$. The chromatographic separation is accomplished with a packed column (~0.5 cm diameter; ~60 cm length) of OV-3 on Chromasorb, held at about 60 °C.

Particle-phase Hg and $CH_3Hg(I)$. If particle subsamples are available, we highly recommend that this phase be analyzed for Hg species as well. On U.S. GEOTRACES, we have been receiving filter "punches" 13-25 mm in diameter from quartz fiber filters deployed on battery-operated in-situ pumps. This filter material, as gauged by blanks, is adequately clean for Hg following decontamination procedures (e.g., Lam *et al.*, 2015). It is convenient to extract marine particles in such a way as to facilitate both total and $CH_3Hg(I)$ analyses. Our preferred method is that of Hammerschmidt and Fitzgerald (2006) using 2 M trace-metal grade HNO_3. Aliquots of this solution can then be neutralized and derivatized as for $CH_3Hg(I)$ analyses mentioned above, and can be digested with BrCl as above for total Hg analysis as well.

Calibration and Comparability

One of the findings of the Intercomparison was that interlaboratory comparability was on the order of 50%. This lack of interlaboratory accuracy is unacceptable, as basin-to-basin variation in Hg concentrations (when comparing regions of similar productivity) can be expected to be considerably less. If datasets from cruises where different groups were

involved are to be comparable, then overall accuracy must be improved. We therefore recommend that traceable Standard Reference Materials be included at numerous times during analyses. A list of Certified and Standard Reference Materials relevant to marine research is included below in the Appendix. However, reasonably sized seawater Reference Materials are not readily available for Hg determinations in the range that analysts will face in the open ocean.

In order to achieve the most accurate results, we recommend analysts use the combination of both saturated vapor standard and aqueous standard calibrations. The combination of two working standards will aid in identification of gas leaks, column inefficiencies, standard degradation and low process yields. These processes can result in both random and systematic errors for individual samples as well as high- and low-biased calibrations.

Recipes

Hydroxylamine hydrochloride – dissolve 300 g of $NH_2OH{\cdot}HCl$ in 18 MW-cm water and bring to 1.0 L.

Stannous chloride – Bring 200 g of $SnCl_2{\cdot}2H_2O$ and 100 mL conc. HCl to 1.0 L with 18 MW-cm water. Purge with N_2 to lower blank. Store cold and tightly capped.

Bromine monochloride – In a fume hood, dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Stir on stir plate if available. Slowly add 38 g $KBrO_3$ to the acid while stirring.

Acetate Buffer – Add 11.8 mL of glacial acetic acid and 2.2 g reagent grade sodium acetate trihydrate to ca. 50 mL 18 MW-cm water and shake until dissolved. Test pH, and adjust with acetic acid or sodium acetate to equal 5.5. Add more water to make up to 100 mL.

Sodium tetraethylborate – add 1 g of NaTEB (Strem 11-0575 or equivalent) to 100 mL of reagent-grade water. Divide the solution equally among plastic vials that then are capped and frozen. This solution should be kept frozen until used and made fresh every week or earlier.

Ascorbic acid – add 35.2 g of ascorbic acid to 100 mL of reagent-grade water. Store cool and discard if it begins to yellow.

Working Standards –We recommend making working standards from a stock solution of CH_3HgCl (Strem 80-2250 or equivalent) and $HgNO_3$ (reference solution; Fisher Scientific SM114-100 or equivalent). For $CH_3Hg(I)$, we have found that preservation with either 1) 2% glacial acetic acid and 0.2% concentrated HCl or 0.5% HCl to be useful. For Hg(II), preservation with 0.1% BrCl (see above) is sufficient.

Nitric Acid (for sample acidification) – J.T Baker Instra-analyzed trace metal grade. The acid blank should be determined prior to use (<0.01 ng/mL).

Argon – ultra-high purity grade with in-line gold and organic vapor removal traps

Soda Lime – ACS grade, 4-8 mesh, non-indicating, Alfa Aesar (stock number 36596). Approximately 5 cm length of soda lime is packed into ~0.5 cm (ID) by ~10 cm Teflon tubing and held in place with quartz or borosilicate glass wool. The columns are purged in a bubbler system for 10-15 minutes prior to use. Prepurging of soda lime columns is not necessary for trapping of methylmercury.

Ultra-Pure Water – Obtained from a multi-column mixed-bed deionzing water system (e.g. Millipore Milli-Q Element system) that can produce 18 MW-cm water with a Hg content <0.1 ng/L.

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Agency	Item	Description	Certified for:	Amount
IAEA	IAEA-SL-1	Lake sediment	T	0.13
IRMM	BCR-060	Aquatic plant	T	0.34
IRMM	BCR-142R	Light sandy soil	T	0.067
IRMM	BCR-143R	Sludge amended soil	T	1.1
IRMM	BCR-145R	Sewage sludge	T	2.01
IRMM	BCR-145R	Sewage sludge	T	8.6
IRMM	BCR-277R	Estuarine sediment	T	0.128
IRMM	BCR-280R	Lake sediment	T	1.46
IRMM	BCR-320R	Channel sediment	T	0.85
IRMM	BCR-414	Plankton	T	0.276
IRMM	BCR-463	Tuna fish	T/M	2.85/3.04
IRMM	**BCR-579**	**Coastal sea water**	**T**	**1.9 ng/kg**
IRMM	ERM-CC580	Estuarine sediment	T/M	132/0.0755
IRMM	ERM-CE278	Mussel Tissue	T	0.196
IRMM	ERM-CE464	Tuna fish	T/M	5.24/5.50
NIST	SRM-1944	Harbor Sediment	T	3.4
NIST	SRM-1946	Lake Superior Fish Tissue	T/M	0.433/0.394 mg/kg wet
NIST	SRM-1947	Lake Michigan Fish Tissue	T/M	0.254/0.233
NIST	SRM-1974b	Mussel Tissue	T/M	167/69.6 µg/kg dry
NIST	SRM-2702	Marine sediment	T	0.4474
NIST	SRM-2703	Sediment	T	0.474
NIST	SRM-2781	Domestic sludge	T	3.64
NIST	SRM-2782	Industrial sludge	T	1.10
NIST	SRM-2976	Mussel Tissue	T/M	61.0/28.09 µg/kg
NRC-CNRC	DOLT-4	Dogfish liver	T/M	2.58/1.33
NRC-CNRC	DORM-3	Fish protein homogenate	T/M	0.382/0.355
NRC-CNRC	MESS-3	Marine sediment	T	0.091
NRC-CNRC	ORMS-4	River water	T	22.0 pg/g
NRC-CNRC	PACS-2	Marine sediment	T	3.04
NRC-CNRC	TORT-2	Lobster hepatopancreas	T/M	0.27/0.152
WHOI	WBW-1-2010	Coastal seawater	T/M	TBA /TBA

Appendix. Compilation of various marine relevant reference materials for total Hg and $CH_3Hg(I)$. All concentrations are mg/kg unless otherwise noted. $CH_3Hg(I)$ concentrations are as mass of Hg. T=total Hg, T/M=total and $CH_3Hg(I)$.

IAEA: International Atomic Energy Agency.

IRMM: European Commission-Joint Research Centre-Institute for Reference Materials and Measurements.

NIST: National Institute of Standards and Technology (USA).

NRC-CNRC: National Research Council Canada.