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An intercomparison of procedures for the determination of total mercury in seawater and recommendations regarding mercury speciation during GEOTRACES cruises

Carl H. Lamborg[1], Chad R. Hammerschmidt[2], Gary A. Gill[3], Robert P. Mason[4], and Susan Gichuki[4]*
[1]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA USA
[2]Department of Earth and Environmental Sciences, Wright State University, Dayton, OH USA
[3]Marine Sciences Laboratory, Pacific Northwest National Laboratory, Sequim, WA USA
[4]Department of Marine Sciences, University of Connecticut, Groton, CT USA

Abstract

We conducted a laboratory intercomparison of total mercury (Hg) determination in seawater collected during U.S. GEOTRACES Intercalibration cruises in 2008 and 2009 to the NW Atlantic and NE Pacific Oceans. Results indicated substantial disagreement between the participating laboratories, which appeared to be affected most strongly by bottle cleanliness and preservation procedures. In addition, we examined the effectiveness of various collection and sample preparation procedures that may be used on future GEOTRACES cruises. The type of sampling system and filtration medium appeared to make little difference to results. Finally, and in light of results from experiments that considered sample bottle material effect and the development of new methods for CH_3Hg^+ extraction from seawater, we propose a recommended procedure for determining all four of the major Hg species in seawater (elemental, dimethyl-, monomethyl-, and total Hg).

Concentrations of mercury (Hg) species in seawater are very low (fM to low pM), but are sufficient to drive bio-accumulation and -magnification of this toxic metal to levels in fish that can pose human and ecological health risks (e.g., Mergler et al. 2007; Scheuhammer et al. 2007). A central component of the study of Hg biogeochemistry and bioaccumulation in the ocean is accurate determination of Hg species concentrations in "dissolved" and particulate phases of seawater. The current GEOTRACES program (www.geotraces.org) seeks to dramatically increase the available data concerning trace elements and isotopes in the ocean, and thus far Hg has been represented in these efforts. As GEOTRACES is a multi-national, multi-investigator endeavor, proper and consistent procedures are needed for the collection, handling, and analysis of samples that can ensure both intercomparability of the resulting data and that the results are of the highest quality.

We recently conducted a laboratory intercomparison of protocols to identify the optimal procedures for both at-sea and on-shore analysis of Hg species on samples collected during GEOTRACES expeditions, and describe the findings in this article. In a related paper (Hammerschmidt et al. 2011), practices for sample bottle cleaning and storage are examined, particularly in light of the high sampling frequency that is characteristic of GEOTRACES cruises. In this article, we examine the importance of filter type and laboratory environmental conditions on determinations of total Hg in filtered seawater. We also report the results of an international interlaboratory comparison of total Hg analyses on stored seawater samples, which has lead to several recommended protocols for improved data quality. Finally, we combine our findings to formulate a recommended workflow for the determination of all four major dissolved Hg species [total, elemental Hg, CH_3Hg^+, and dimethylmercury $(CH_3)_2Hg$] from a 2.25 L sample of seawater.

Materials and procedures

Cruises

We participated in two intercalibration/intercomparison oceanographic cruises sponsored by the U.S. National Science

*Corresponding author: E-mail: clamborg@whoi.edu

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Foundation. The first occupied the Bermuda Atlantic Time Series (8–27 Jun 2008; BATS, 31°40′N 64°10′W; e.g., Steinberg et al. 2001), whereas the second was at the Sampling and Analysis of Iron (SAFe) station (6–30 May 2009; 30°N 140°W; Johnson et al. 2007). During the BATS cruise, we focused on intercomparison of methods for total Hg, as well as testing the intercomparability of sampling and filtering methods. During the SAFe cruise, we continued the total Hg intercomparison while examining the efficacy of large-volume, shipboard determinations of CH_3Hg^+ (Bowman and Hammerschmidt 2011).

Sampling

Seawater analyzed as part of this study came from three sampling systems. The first two, used for generating vertical profiles of Hg species, were the GEOTRACES Clean Rosette (Sea-Bird Electronics custom 24-place rosette deployed with 24 10-L General Oceanics GO-Flo bottles) and the CLIVAR Clean Rosette (Sea-Bird SBE 32, with 12 10-L GO-Flo bottles; Measures et al. 2008). The third sampling system consisted of two large polyethylene plastic tanks, developed for use in the SAFe program, that allowed large numbers of relatively homogenous subsamples to be prepared and distributed among laboratories for intercomparison. One tank was filled by use of a surface-sampling towed "fish" that was capable of pumping surface water on board. The second tank was filled with deeper water (1000 m) collected with 30-L GO-Flo bottles deployed from a Kevlar line. Water for both tanks was filtered to 0.2 µm during filling, and the pH adjusted to 1.8 using trace-metal grade HCl (concentrated, 12 M).

Processing of both fish/tank and GO-Flo samples from rosettes deployed on Kevlar line was performed inside shipboard clean laboratories, while observing trace metal handling protocols (acid-cleaned plasticware, gloves, double-bagged bottles; e.g., Patterson and Settle 1976).

Receiving bottles

Sample storage bottles used for the first laboratory intercomparison were cleaned by the participating individual laboratories (we do not document those here). As noted in a companion paper (Hammerschmidt et al. 2011), a variety of cleaning protocols, when applied to either Teflon, polycarbonate, or borosilicate glass can result in bottles that are sufficiently clean for seawater having low-pM levels. All bottles received from participating labs were Teflon. All bottles used for the baseline profiles (Teflon) and in the second intercomparison (borosilicate glass) were rigorously acid-cleaned with a protocol developed at Wright State University (modified from methods developed at UC Santa Cruz and WHOI). The cleaning approach included (Hammerschmidt et al. 2011): 2 × rinse with reagent grade water (RGW, >18 MΩ-cm resistivity with reverse osmosis pretreatment), 6 d filled with ~1% Citranox in RGW followed by copious rinsing, 6 d filled with 1.2 M HCl (Instra-analyzed, J.T. Baker or equivalent) followed by 5 × rinse with RGW, 1 d filled with 0.5% BrCl (Bloom and Crecelius 1983) followed by 3 × rinse with 0.01 M HCl and 5 × rinse with RGW. The BrCl solution was made as per U.S. EPA Standard Method 1631, and is a solution consisting of 90.5 mM of both KBr and $KBrO_3$ in concentrated (12 M) HCl.

Filters

A number of different filtering approaches were investigated during the first intercalibration cruise and their results compared. These included two kinds of capsule filters, the GE Osmonics Teflon (0.2 µm), which was used for samples received from the GEOTRACES Clean Rosette as well as water in the tanks used in the intercomparison, and the Pall Acropak polyethersulfone (0.2 µm), which was used to filter water from the CLIVAR Clean Rosette. Membrane filters were used to filter water from the surface fish. All membranes were 25 mm diameter, 0.2 µm pore size, and made of different polymers by multiple vendors, including Nuclepore (Whatman; polycarbonate), Nylaflo (Pall; hydrophilic nylon), Versapor (Pall; acrylic on nylon), Supor (Pall, polyethersulfone) and Tuffyrn (Pall; polysulfone). All membranes were cleaned by first rinsing with RGW, followed by a 2-week soak in 1.2 M HCl (Optima Grade), and then rinsing in RGW and allowing to dry.

Distribution of intercomparison samples and analysis

Subsamples of filtered seawater from the two tanks (surface and deep) used as part of the intercomparison were distributed among 12 participating labs within 1 month of the completion of each cruise. No deadline was imposed for completion of the analyses and individual labs followed their own protocols regarding handling and analysis of the samples. As previously noted, the first intercomparison included the analysis of seawater collected into sample bottles prepared and provided by each laboratory, followed by preservation at sea following each laboratory's protocol. For analysis, all but two of the laboratories used a version of the U.S. EPA Method 1631, which includes an oxidation/digestion step with 0.5% BrCl (v/v), pre-reduction of free halogens with 0.2% (v/v) of 4.3 M NH_2OH and purge-and-trap of Hg° generated from the sample by reduction with 0.4 mmol $SnCl_2$ (0.5 mL of 0.8 M) and trapping on a gold surface with subsequent detection by cold vapor atomic fluorescence spectrometry (CVAFS; e.g., Bloom and Crecelius 1983; Gill and Fitzgerald 1987; U.S. EPA 2002). The two other labs employed Hg determination by 1) isotope dilution-inductively coupled plasma mass spectrometry (ICP-MS; e.g., Hintelmann and Ogrinc 2003) and 2) purge and trap, cold vapor atomic absorbance spectrometry (CVAAS; e.g., U.S. EPA 1998).

Assessment

Comparison of sampling systems and filters

A comparison of results from the GEOTRACES Clean Rosette and CLIVAR Clean Rosette at BATS is shown in Fig. 1. This comparison incorporates all of the various sources of variability that might occur between different cruises, including differences of sampling systems, sample bottle cleaning methods, handling approaches, and in-line filtering. As can be seen for the upper 1000 m at BATS, there were no substantial and systematic differences between either UConn and WHOI lab-

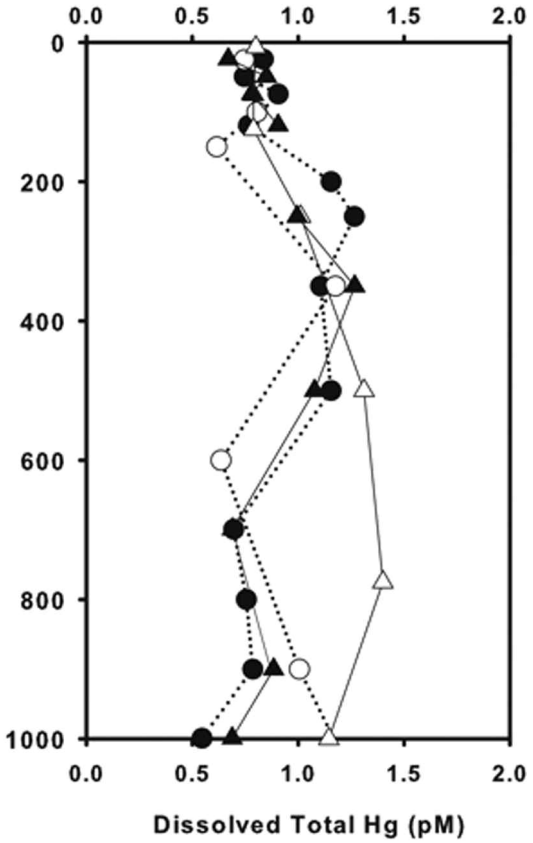

Fig. 1. Total Hg in filtered water from the upper 1000 m at BATS. Water from the GEOTRACES rosette (open symbols) was filtered with an Osmonics 0.2 µm capsule, whereas the CLIVAR samples (closed symbols) were filtered with a 0.2 µm Acropak capsule. UConn results are shown as circles and WHOI as triangles.

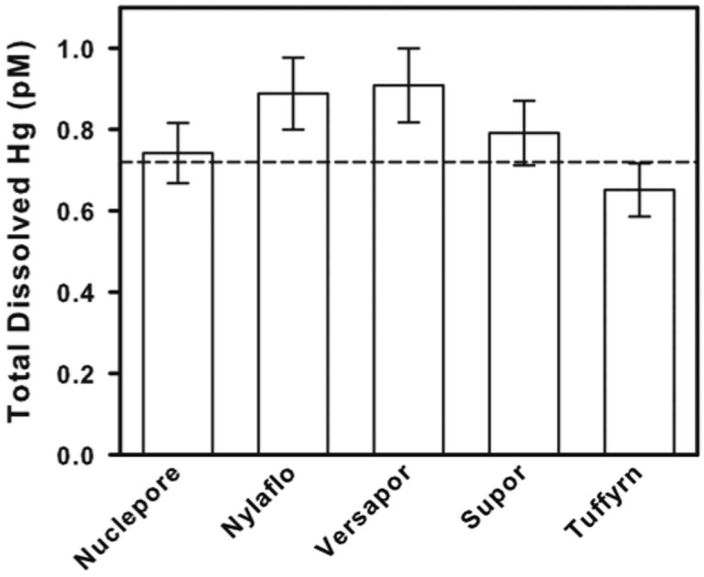

Fig. 2. Total Hg results from vacuum, membrane-filtered surface water at BATS. The dotted line indicates the value obtained from Osmonics-filtered surface water drawn from a SAFe tank that was filled via a towed "fish." The membrane-filtered samples were also drawn from the "fish."

oratories or rosette and filter types with the possible exception of the 800 m WHOI sample from the CLIVAR Clean Rosette. Differences between sampling systems were about the same as difference between the UConn and WHOI groups (~10% relative standard deviation, RSD). This suggests that the various types of water collection bottles (GO-Flo, X-Niskin, or the equivalent) that are frequently used in oceanographic settings can be suitably cleaned for sampling seawater for total Hg analysis. The similar scale of variability also suggests that precision is likely determined by differences between individual laboratories and their careful handling, preparation, and analysis of samples. In addition to the two different capsule filtration approaches that were implicitly tested in the GEOTRACES/CLIVAR Clean Rosette comparison, we also examined several vacuum-assisted membrane filtering strategies (all 25 mm diameter, held in a Pall polyethersulfone filter funnel #4203). Fig. 2 shows that the comparability of these membranes with the Osmonics capsule was generally good for filtering surface water from BATS, with only water filtered through Nylaflo and Versapor membranes having significantly greater concentrations. The most commonly used membranes (0.2 µm pore size Nuclepore and Supor) compare well with the Osmonics capsule, suggesting that the filtering medium is not critical as long as it has been tested to ensure a low blank. Results from a different cruise to the Sargasso Sea (Bergquist and Lamborg unpubl. data) suggest that there is essentially no "colloidal" total Hg or CH_3Hg^+ present in open ocean seawater, where colloidal was defined as particles between 0.02–0.45 µm effective size. Thus, we should not be surprised that different filtering media, assuming that they do not contribute a blank or absorb Hg, provide similar "dissolved" Hg results. Colloidal Hg is significant in coastal ocean environments, however, so 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).

Interlaboratory comparison of full depth profiles and consensus results

Another example of inter-laboratory comparison is shown in Fig. 3 in the form of two full depth profiles from the SAFe site. Here again, the agreement is excellent even at the surface where total Hg concentrations were exceptionally low due to scavenging. This comparison was performed on samples collected into cleaned FEP Teflon bottles, with both laboratories analyzing the samples about 2 months after sampling. This demonstrates that high quality data may be obtained for total Hg from acidified samples stored in Teflon for as long as 2 months, although we've found longer storage periods may impart a positive artifact to seawater in Teflon (Hammerschmidt et al. in press).

Consensus profiles for total Hg at BATS and SAFe are shown in Fig. 4 and Table 1, and represent averaged or combined data

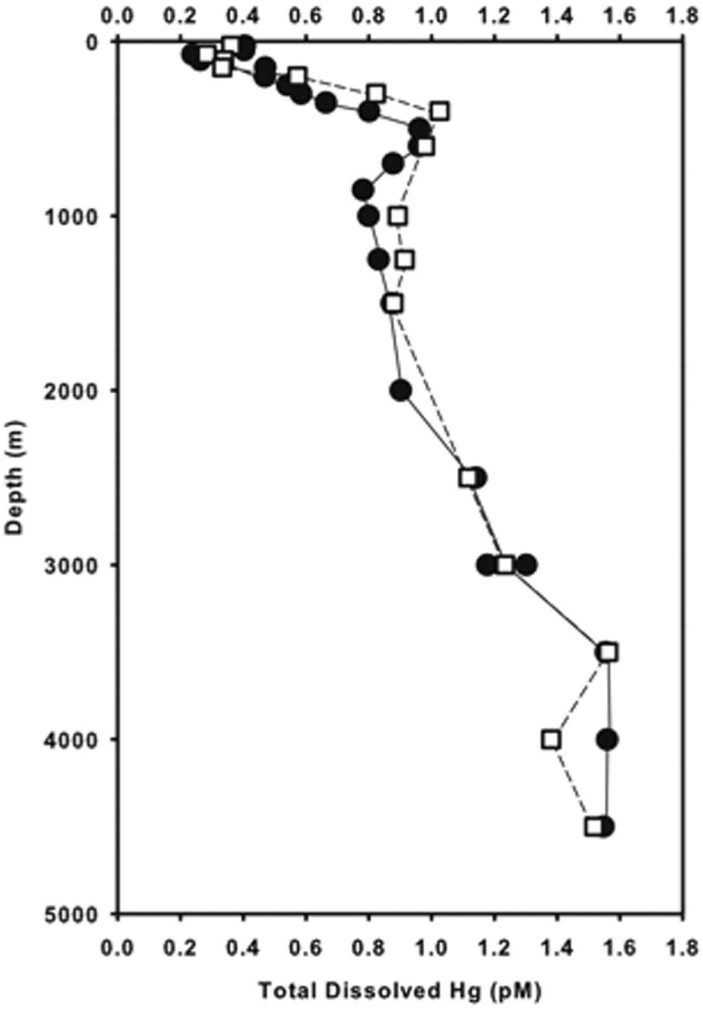

Fig. 3. Comparison of total Hg in filtered seawater determined by Wright State University (filled circles) and WHOI (open squares) on a full depth profile from the SAFe station.

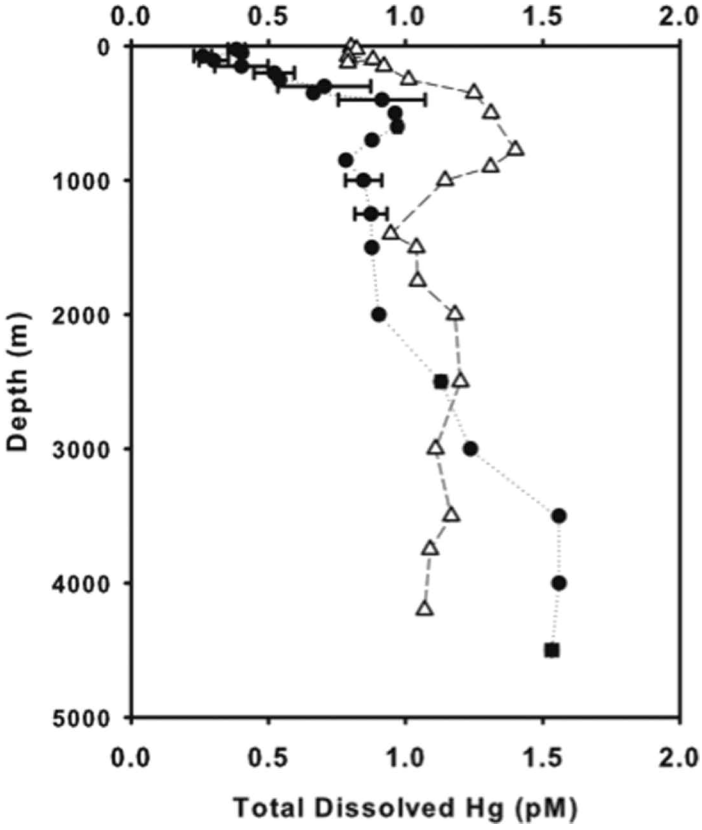

Fig. 4. Consensus Value full depth profiles of total Hg in filtered seawater at BATS (open triangles; UConn and WHOI) and SAFe (WSU and WHOI). Numerical values are shown in Table 1.

from two laboratories for each site. These stations have been selected as benchmarks for the U.S. GEOTRACES program and will be re-occupied by GEOTRACES cruises in the future to allow an additional form of inter-cruise comparison.

Note the differences and major features of these two profiles and the oceanographic implications they represent. First, the profile in the oligotrophic Sargasso Sea shows greater total Hg concentrations in the surface ocean than in the NE Pacific. The profile at BATS is consistent with its generally low rate of primary production and associated low power to scavenge trace components of seawater. In contrast, the subsurface minimum of dissolved total Hg at SAFe is evidence of scavenging, and the depth of the maximum corresponds to a location of redissolution of scavenged Hg as organic detrital material is degraded. Finally, the deepest waters of the NE Pacific appear to be enhanced in Hg relative to the corresponding deep waters of the NW Atlantic. This is consistent, in the broadest sense, with the slow enrichment in a number of chemical constituents that occurs as deep waters travel from Atlantic to Pacific via thermohaline circulation, picking up material from particles received during this approximately 1000 y journey.

International multi-laboratory intercomparison

As noted above, samples were collected for a multi-laboratory intercomparison on each of the two cruises, with the water used for these samples drawn from the two large shipboard tanks. On the first cruise, sample bottles and preservation protocols of each laboratory were used to store filtered seawater that was subsequently returned to them. On the second cruise, seawater was stored in glass bottles cleaned at WHOI (Hammerschmidt et al. in press) and with no acid added above what was added to the tank (i.e., HCl to pH 1.8). The results for these two tests are shown in Fig. 5.

Intra-laboratory agreement (analysis of replicates), expressed as the average relative standard deviation of the individual analyses was 149% RSD (48% RSD if results from Lab 3 are excluded) at BATS and 9.4% RSD at SAFe, showing a marked improvement in the second comparison. We take these differences of intra-laboratory precision to demonstrate the importance of bottle cleaning, as the bottles for the second intercomparison were all prepared in one laboratory. Furthermore, and as demonstrated in a companion paper (Hammerschmidt et al. in press), glass bottles tend to perform better for

Table 1. Consensus values for total Hg (pM) in filtered seawater at the baseline stations.

BATS		SAFe	
Depth	Total Hg	Depth	Total Hg
7	0.80	25	0.38
25	0.82	50	0.40
75	0.79	75	0.26
100	0.88	107	0.30
125	0.79	150	0.40
150	0.92	200	0.52
250	1.01	250	0.54
350	1.25	300	0.70
500	1.31	350	0.66
775	1.40	400	0.91
900	1.31	500	0.96
1000	1.14	600	0.97
1400	0.94	700	0.88
1500	1.04	850	0.78
1750	1.04	1000	0.85
2000	1.18	1250	0.87
2500	1.20	1500	0.88
3000	1.11	2000	0.90
3500	1.17	2500	1.13
3750	1.09	3000	1.24
4200	1.07	3500	1.56
		4000	1.47
		4500	1.53

long-term storage than do Teflon, presumably due to the inherent gas permeability of Teflon.

Agreement among laboratories was not good during the first comparison (62% RSD), but improved modestly by the second (43% RSD). Part of the improvement, again, was likely due to the uniformity of bottle preparation and preservation. However, and as demonstrated in Fig. 6, there were substantial differences reported by the laboratories that we infer may result from either inadequate calibration, blank correction, or contamination. For example, the results from analysis of surface and 1000-m water samples collected at SAFe from Labs 4, 5, 6, and 9 all fell on a 1:1 line when results from the two depths were plotted against each other, normalized to the Consensus values. These labs report differences in the two samples that appear proportionally appropriate, assuming the Consensus values are accurate. Any deviation between laboratories on this line, therefore, would be due only to issues relating to calibration. In this test, Labs 4, 6, and 9 appeared to agree well with one another, while Lab 5 was slightly higher, suggesting a calibration bias.

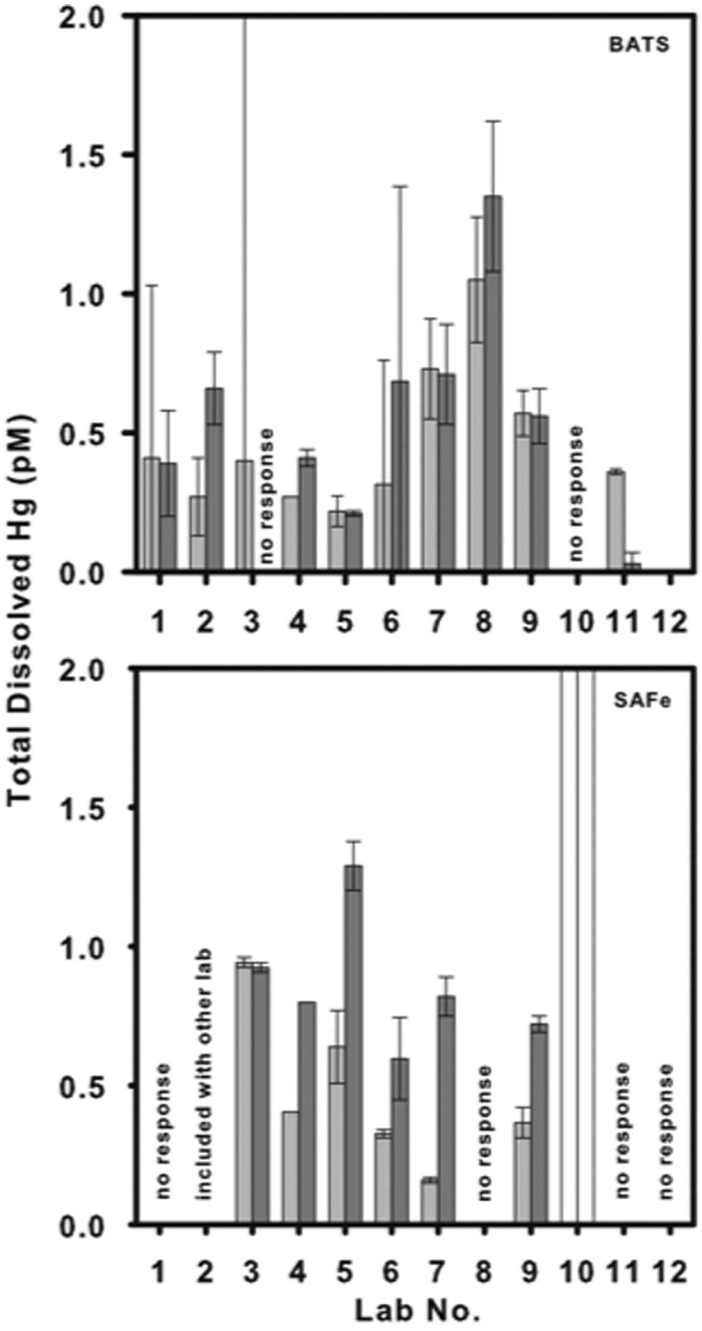

Fig. 5. Results from the two intercalibrations/comparisons for total Hg in seawater. Open bars indicate that the laboratory could not determine the concentration and the height of the bar was placed at the limit of detection.

Results from the other two labs that reported concentrations for both samples (3 and 7) are not near the 1:1, one is to the right and other to the left. Their agreement with Labs 4, 6, and 9 for the 1000-m sample ("Deep") is good, whereas their results for the surface sample did not agree as well, giving rise to their deviation from the 1:1 line. It is perhaps not surprising that the agreement is better for the deeper sample, as the concentration at depth (as indicated by the Consensus Value) is greater, while

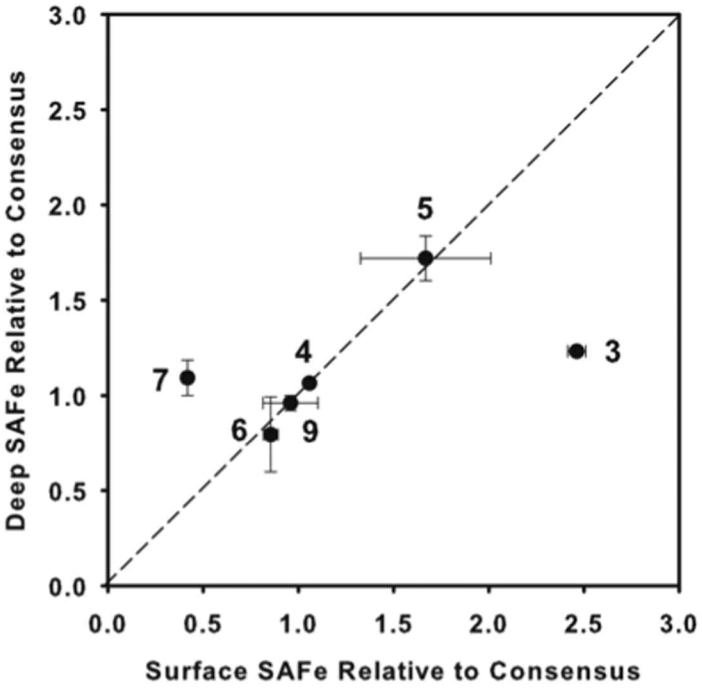

Fig. 6. Results of laboratory intercomparison at SAFe, with values from the surface and deep samples for each laboratory normalized to the Consensus Value (Table 1) and plotted against each other. Assigned lab numbers are next to symbols.

the surface water was depleted of Hg. Deviation from the 1:1 line suggests that issues related to blank correction or analyte recovery could be to blame, but not merely calibration.

Discussion

Recommended workflow

A new method for the determination of CH_3Hg^+ in seawater was published recently (acid extraction and direct ethylation), which has lowered the detection limit and facilitated a further streamlining of Hg species determinations (Bowman and Hammerschmidt 2011). An example of the results of this method from an open-ocean profile at SAFe in 2009 can be found elsewhere (Hammerschmidt and Bowman in press). The promise of this new scheme for the extraction and determination of CH_3Hg^+ from a 2-L sample of seawater, along with results from our bottle cleaning and storage tests (Hammerschmidt et al. 2011), and from verification of the appropriateness of GEOTRACES-style sampling and handling described in this report has lead us to a workflow for analysis of all four major Hg species in open ocean water samples that is described below (Fig. 7). However, there are other approaches for CH_3Hg^+ analysis in seawater that are in current use including solvent:solvent extraction (e.g., Bloom 1989) or distillation (e.g., Horvat et al. 1993) to eliminate matrix interferences to further analysis as well as direct derivitization by hydride generation and cryofocusing (e.g., Tseng et al. 2000; Stoichev

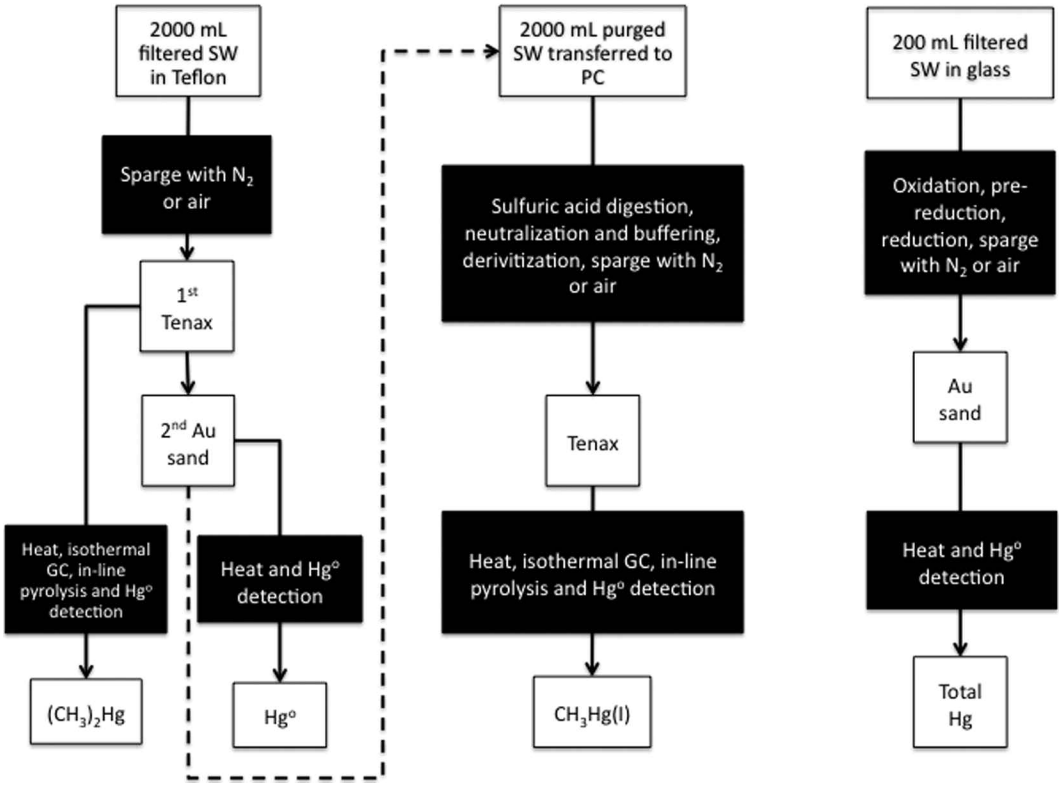

Fig. 7. Recommended workflow for shipboard analysis of Hg°, $(CH_3)_2Hg$, CH_3Hg^+, and total Hg. For at-sea measurements, we recommend two separate aliquots be collected: one 250-mL sample for total Hg and one 2-L sample for Hg°, $(CH_3)_2Hg$, and CH_3Hg^+.

et al. 2004). With some modification of the workflow, these methods also could be employed for CH_3Hg^+ determination within the GEOTRACES context. The most obvious and immediate approach would be to store N_2-purged samples acidified until analysis after the cruise (Samples at pH = 2 can be stored at 4°C for up to 6 months; Parker and Bloom 2005). Analysis of samples at sea following solvent extraction or distillation is likely impractical due to the substantial additional workload these approaches require. But the two direct derivitization methods are comparably well suited to application at sea. Our currently recommended approach, however, is that of direct ethylation, and the remainder of our discussion below adopts that as the method of choice.

As noted, during the Intercalibration/comparison exercise, all but two of the participating laboratories used cold vapor atomic fluorescence spectroscopic (CVAFS) determination of Hg (as Hg°). The other two laboratories employed the other commonly used analytical approaches, ICP-MS (with isotope dilution) and CVAAS. Results from ICP-MS compared well with CVAFS, while the CVAAS did not exhibit adequate sensitivity to detect total Hg on the intercomparison samples (250 mL). Thus, we recommend either CVAFS or ICP-MS for seawater Hg determinations. The CVAFS approach has the distinct advantage of being used at sea and permitting rapid determination of Hg° and $(CH_3)_2Hg$. 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 either approach is appropriate for on shore analyses.

Details of Hg analysis by either ICP-MS or CVAFS 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). The workflow presented is oriented toward at-sea, multi-species determinations by CVAFS, but could be easily adapted for use with ICP-MS on shore. A ready supply of high quality water (>18 MΩ-cm resistivity) is needed for cleaning and preparation of reagents and standards. 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, researchers also need to conduct careful determinations of analytical, bottle, and reagent blanks to assure that they are at levels appropriate for the measurement of Hg in open-ocean seawater (~0.2–2 pM; Fitzgerald et al. 2007). These checks should be done on shore and during the cruise. Replicate analyses of several samples also is highly desirable when adequate seawater is available. Measurements of known addition recoveries from sample matrixes, especially for the CH_3Hg^+ determination, also should be performed routinely, with all sample analyses calibrated against certified or traceable standards. Quality control results should be reported along with Hg results to demonstrate capability, precision, and bias.

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 for samples containing as little as 0.1 pM total Hg. As typical CVAFS arrangements have absolute detection limits on the order of 10 fmol, sample volumes of ca. 200 mL are recommended to ensure a resolved signal (e.g., 2 × the detection limit).

The analysis workflow for total Hg is depicted on the right side of Fig. 7. We have found that borosilicate glass bottles work well as sample storage vessels. As noted earlier, they are also one of the more attractive options for long-term storage if total Hg will not be determined at sea. If a relatively small number of bottles are to be reused continuously during a single cruise, cleaning procedures at sea should include a base or detergent wash and simulate the full cleaning cycle used in the laboratory. Furthermore, rinsing residual sample and reagent from the bottles following analysis and before refilling should be done with either surface seawater or high quality water.

Filtered aliquots of seawater should be pretreated before analysis as follows: oxidize the sample with 0.05–0.1% (v/v) bromine monochloride (BrCl) solution (90.5 mM) or equivalent for at least 1 h in the original sample bottle, remove excess halogens with 0.05% v/v hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) solution (4.3 M) for at least 5 min, and reduce Hg with 0.05% v/v stannous chloride ($SnCl_2$) solution (0.8 M) followed by purging of Hg° and trapping on either Au or Au-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}).

The sparging step should be conducted in a manner that minimizes introduction of laboratory air to the bubbler system. This is especially important on GEOTRACES cruises because electrochemists, using hanging mercury drop electrodes, are likely to be members of the shipboard science party. A closed sample introduction system is ideal for Hg analysis. For samples less than about 300 mL, we recommend either a customized blown-glass UConn Bubbler (diagram in Fig. 8), 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 sample bottles. Expelling room air from the headspace of the UConn Bubbler is accomplished by having the purge gas flowing through the headspace—off-line of the collection Au trap—for enough time to affect at least 5 volume exchanges. Alternatively, laboratory air in the system can be minimized with a procedure that flushes the headspace above the sample with Hg°-free air (achieved using a Au trap column on the air inlet) before sample sparging. Entrainment of room-air bubbles in the sample also should be avoided by decanting samples slowly from sam-

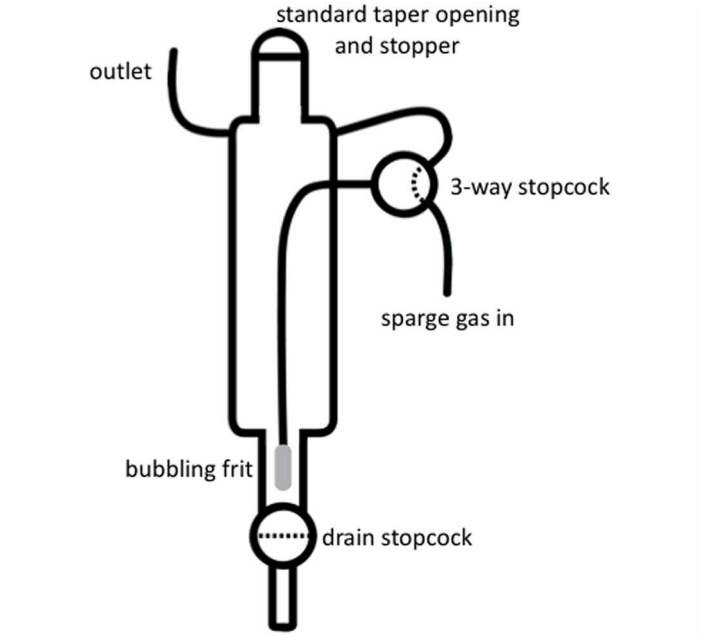

Fig. 8. The UConn (University of Connecticut) Bubbler. It allows samples to be poured in at the top through the standard taper joint, while simultaneously allowing zero-Hg gas to vent the headspace. Emptying of the bubbler in preparation for another sample is achieve through the stopcock at the bottom, which will allow the bubbler to again fill with clean gas instead of room air. The three-way stopcock on the side allows gas to be directed through either the headspace or sparging frit near the bottom.

ple bottle to bubbler and by avoiding turbulent mixing after reagents have been added.

Hg° and $(CH_3)_2Hg$.

Although the two dissolved gaseous mercury species [Hg° and $(CH_3)_2Hg$] are minor components (typically sub-pM concentrations) of total Hg present in seawater, they are nonetheless important to measure as they are involved in air-sea exchange of Hg and possibly in the formation of CH_3Hg^+. Given the extremely low concentrations of these species, we recommend using 2-L sample sizes for analysis, with determination of Hg°, $(CH_3)_2Hg$, and CH_3Hg^+ all performed on the same seawater aliquot. Procedurally, Hg° and $(CH_3)_2Hg$ are the easiest of the species to extract, requiring only that a volume of stripping gas of at least 15× the volume of seawater be sparged through the liquid without any chemical amendment. For these species, we use 2-L Teflon bottles to receive samples, and the bottle-top sparging adaptors mentioned above, and carefully purge the headspace of the bottle before attaching sorbent columns. We have successfully used two sorption media in series to preconcentrate and discriminate between these two gaseous species. Gas exiting the sparger should pass first through a moisture trap (e.g., soda lime), then either Tenax or Carbotrap (or the equivalent) for $(CH_3)_2Hg$ collection, followed by Au or Au-coated sand for Hg° concentration (e.g., Bloom and Fitzgerald 1988; Tseng et al. 2004; Conaway et al. 2009; Lamborg unpubl. data). Following sparging, the traps are analyzed separately using a CVAFS system that is equipped with a gas flow train. The Hg° collected on Au traps is liberated for detection by heating (600-800°C) in an argon gas-flow train connected to the CVAFS detector. The $(CH_3)_2Hg$ retained on the other trap is liberated under low heat (90-250°C), passed through a packed separation column (~0.5 cm diameter; ~60 cm length) of 15% OV-3 on Chromosorb WAW-DMCS, at 60-105°C, and last through a high temperature (600-800°C) column packed with quartz wool to pyrolyze the $(CH_3)_2Hg$ to Hg° and make it available for detection by CVAFS (Bloom and Fitzgerald 1988). Tenax and Carbotrap columns should be rigorously conditioned with multiple loadings and subsequent desorption of alkylHg prior to use. Furthermore, they should be tested to ensure that they do not retain Hg° to a large degree. We recommend use of Tenax rather than Carbotrap because it appears to retain less moisture and Hg° than Carbotrap, but we have recent anecdotal evidence that, after many uses, Tenax may begin to retain some fraction of the Hg° liberated from samples. Fresh soda lime drying agent should be used for each sample, which can be prepared ahead of the cruise without accumulation of a blank.

CH_3Hg^+

Following the sparging of Hg° and $(CH_3)_2Hg$, the 2-L sample can be processed for CH_3Hg^+ determination (Bowman and Hammerschmidt 2011). The sample must first be "digested" for > 12 h, through reaction with 20 mL of 18 M H_2SO_4 (~1% sample volume). This and subsequent steps need not be performed in Teflon bottles, and we have found that transferring the pre-purged seawater to polycarbonate vessels is equally effective. Following digestion, sample pH is neutralized with KOH and buffered to about pH = 5 with 4 M acetic acid/sodium acetate. The pH should be checked and adjusted as necessary with small additions of strong acid (H_2SO_4) or strong base (KOH). The CH_3Hg^+ in solution is converted into a more volatile compound by either alkylation (ethylation or propylation) or hydride generation. The new method (Bowman and Hammerschmidt 2011) makes use of a direct ethylation reaction applied to the seawater matrix, and the authors have found that with close attention to pH and use of fresh ethylating agent (Na-tetraethylborate; NaTEB), reproducible ethylation of CH_3Hg^+ in seawater can be achieved after acid digestion. This newly proposed method eliminates the common practice of aqueous distillation in the analysis to isolate CH_3Hg^+ from the matrix prior to derivatization. It also precludes the need to store samples for extended periods before analysis.

As noted below, the ethylating agent is made up in small batches, but often are not completely consumed within 1 week. After a week, even when kept frozen, the ethylating agent loses efficacy and should be discarded. Thawed aliquots of NaTEB also will unavoidably lose efficacy during the course of an analytical batch, which can be slowed by storing the solution cold. We recommend analyzing samples in batches of

Table 2. Compilation of various marine relevant reference materials for total Hg and CH_3Hg^+. All concentrations are mg/kg unless otherwise noted. CH_3Hg^+ concentrations are as mass of Hg.

Agency	Item	Description	Certified for	Amount
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 pg/g
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-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

T=total Hg, T/M=total, and CH_3Hg^+.
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.

four, by adding 0.3–2 mL of 1% (m/v) NaTEB directly to the buffered 2-L sample, allowing 15 min for derivatization reaction, and then sparging the methylethylmercury ($CH_3CH_2HgCH_3$) derivative from solution with a bottle top sparging adaptor described above. Purge gas exiting the bottle should first pass through a soda lime trap to remove moisture and then the $CH_3CH_2HgCH_3$ is concentrated on a Tenax column. Determination of $CH_3CH_2HgCH_3$ is conducted in an analogous way to $(CH_3)_2Hg$.

Standardization of the detector for CH_3Hg^+ is accomplished through standard additions to deionized water in purging apparatus similar to that used for total Hg, or from a 2-L bottle. It is essential that the recovery of standard spikes onto a seawater matrix be checked as well, and this is accomplished through a set of standard additions to previously analyzed samples. With these standard additions, fresh NaTEB is also added. As noted in the original description of this method (Bowman and Hammerschmidt 2011), spike recovery is generally excellent (>90%).

Calibration and comparability

One of the key findings of this intercomparison was that interlaboratory comparability was on the order of about 40% (Fig. 5). This lack of interlaboratory agreement is unacceptable, as basin-to-basin variation in Hg concentrations (when comparing regions of similar productivity) can be expected to be of this order or less. If datasets from cruises where different research groups were involved are to be comparable, then overall accuracy must be improved. To obtain accurate results, we recommend analysts use a combination of both saturated vapor (Gill and Fitzgerald 1987) 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.

Furthermore, we recommend frequent analysis of traceable Standard Reference Materials. Table 2 is a list of Certified and Standard Reference Materials relevant to marine Hg research. BCR-579 (Hg in coastal seawater) is the material most relevant to determination of total Hg in seawater. It is supplied by IRMM (European Commission-Joint Research Centre-Institute for Reference Materials and Measurements) and has a certified (±95% CI) concentration of 9.5 ± 2.5 pM. Thus, and as open-ocean seawater typically has 0.2–2 pM total Hg (Fitzgerald et al. 2007), analysis of 10–15 mL of BCR-579 should be compa-

rable to the analytical challenge posed by analyzing about 200 mL of 0.5 pM seawater. The BCR-579 material is supplied in 1-L glass bottles, which permits many analyses to be performed. It should be noted, however, that the shelf life of this material may not be very long. We have observed recently that the CRM was recovered well during a cruise (95 ± 5%) yet the measured concentration increased to 14.2 pM (when calibrated against self-consistent vapor and aqueous standards) within just a few months following the expedition, likely due to inclusion of Hg from the considerable headspace in the bottle. As noted, such contamination could be especially aggravated during lengthy GEOTRACES cruises when electrochemists may increase Hg° in the air of shipboard laboratories.

A seawater reference material for CH_3Hg^+ is not currently available to our knowledge. One of us (CHL) is currently in the process of developing a solution that may work as Consensus Reference Material that could be shared between laboratories/cruises. Without a reference material, analysts are encouraged to perform known-addition assessments of the accuracy of their methods and calibrate with aqueous CH_3Hg^+ solutions that, after digestion with BrCl, are standardized versus traceable solutions of total Hg.

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