

Techniques for the Collection and Species-Specific Analysis of Low Levels of Mercury in Water, Sediment, and Biota

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ABSTRACT

The U.S. Geological Survey has established a laboratory to develop and implement methods for the analysis of low levels of specific species of mercury. This paper outlines sample collection and analysis techniques used to determine species specific mercury concentrations in environmental samples. The laboratory has the capability of determining the concentration of total, methyl, reactive, and dissolved gaseous mercury in surface water, and total and methylmercury in ground water, porewater, sediment, and biota. Until the analytical methods used by the Wisconsin District Mercury Laboratory are approved by the U.S. Geological Survey's Office of Water Quality, the data produced are classified as provisional. The analytical method for total mercury is scheduled for approval by the U.S. Environmental Protection Agency in the spring of 1999. The Wisconsin District Mercury Laboratory will seek approval of the analytical method for methylmercury through the U.S. Geological Survey Office of Water Quality. Currently, results are validated using matrix spikes, blanks, laboratory duplicates, quality-control check samples, and certified reference materials.

INTRODUCTION

Mercury (Hg) has been recognized as an environmental pollutant for several decades. Until recently, scientists have been unable to accurately measure Hg in the parts-per-trillion range, primarily due to sample contamination and lack of instrument sensitivity. The collection of mercury samples requires the use of ultra-clean sampling techniques first published by Patterson and others (1977) for lead (Pb) research, and refined by Gill and Fitzgerald (1985) for Hg. Ultra-clean techniques prevent direct contact between sample media and sampling equipment, field personnel, and any other potential contaminant sources during collection and analysis.

The presence of very high Hg concentrations (≥ 1.5 micrograms per gram ($\mu\text{g/g}$) muscle tissue) found in game fish in the Florida Everglades (Ware and others, 1990) initiated the Aquatic Cycling of Mercury in the Everglades (ACME) project in 1995. To provide the analytical support for this project, the U.S. Geological Survey (USGS) office in Middleton,

Wisconsin established the Wisconsin District Mercury Laboratory (WDML) to develop and implement methods for the analysis of specific Hg species. Analyses performed by the WDML include total, methyl, reactive, and dissolved gaseous mercury in surface water, and total and methylmercury in ground water, porewater, sediment, and biota.

METHODS AND TECHNIQUES

Equipment Preparation and Sample Collection

Contamination of sampling equipment and sample containers is the largest source of error associated with low-level mercury analysis. To decrease the effect of contamination from equipment and containers, Teflon (any use of trade, product, or firm names is for descriptive purposes and does not imply endorsement by the

U.S. Government) is used whenever possible and is subjected to a rigorous cleaning protocol. Teflon containers minimize Hg adsorption to container walls and eliminate gaseous mercury exchange into or out of the container (Horvat and others, 1993). New Teflon equipment is rinsed with tap water, and immersed in an acid bath containing 4 N trace pure hydrochloric acid (HCl), then heated to 65°C for 48 hours. Immediately following removal from the bath, equipment is immersed in fresh reagent-grade water followed by a minimum of three rinses with fresh reagent-grade water. After rinsing, containers are partly filled (25 percent) with 1 percent Omni Trace HCl and capped tightly. The surface of all equipment is then allowed to air dry under a Hg-free class 100 laminar flow-hood. Sampling line and pump-head tubing interior surfaces are dried by purging with Hg-free nitrogen (N₂). Pump-head tubing is cleaned by filling with 50 percent Omni Trace nitric acid (HNO₃) and soaking in a 10 percent HCl bath for a minimum of 7 days. Dry equipment is double bagged in new zip-type plastic bags with the unique identifier and date cleaned written on the outer bag. Subsequent cleaning of containers and equipment requires only a 24 hour period in the acid bath followed by the rinsing procedure outlined above. A minimum of 10 percent of the equipment is tested for total mercury to assure cleanliness. If the equipment is suspect, the batch must be recleaned and retested.

Capsule filters used for field filtering are cleaned by filling with Omni Trace HNO₃ and soaking for 4 days. After four days, the filters are rinsed with 20 filter volumes of fresh reagent-grade water, and refilled with Omni Trace HCl, immersed in a 10 percent HCl bath at room temperature, and allowed to soak for 3 days. Finally, the filters are emptied of the HCl, rinsed with 20 filter volumes of fresh reagent-grade water, filled with reagent-grade water, capped, and double bagged until use. If lab filtering is desired, the unfiltered water samples are shipped on ice via overnight courier to the WDML for vacuum filtering in a clean environment.

Aqueous samples are collected either by means of a peristaltic pump or by grab technique. During sample collection, care is taken to prevent dust or other contaminants (for example, breath from a person with dental amalgam) from entering the sample containers. To help prevent

contamination during sample collection, field personnel are required to wear Tyvek suits and arm-length polypropylene gloves.

Aqueous samples collected for total mercury analysis are preserved by acidification to 1 percent (v/v) with Omni Trace HCl. All other samples are preserved by freezing.

Clean Areas

All unbagged equipment is handled with gloved hands and in clean areas. Clean areas are countertops covered by a Teflon overlay within a Hg-free laminar flow-hood. The laminar flow-hoods meet or exceed Federal Standard 209 for class 100 conditions. The laminar flow-hood intakes are covered with gold-coated cheesecloth to remove any mercury vapor prior to particle removal.

TOTAL MERCURY ANALYSIS

Water Samples

Total mercury (Hg_T) analysis is performed by U.S. Environmental Protection Agency (USEPA) Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) with modifications. USEPA Method 1631 is a draft method and should be approved in the spring of 1999. The method consists of three essential parts: Oxidation of Hg species to reactive mercury (Hg^{II}), reduction of Hg^{II} to volatile mercury (Hg⁰), and detection of Hg⁰ by CVAFS.

Aqueous samples are treated to 1–2 percent (v/v) with 0.2 N Bromine monochloride (BrCl) to oxidize all of the forms of Hg to Hg^{II}. Samples are placed in an oven at 50°C for a minimum of 12 hours to accelerate the oxidation reaction. Oxidation is considered complete if excess BrCl is present after 12 hours as determined by a yellow tint in the sample; therefore, samples must be colorless before BrCl is added. If the BrCl has been consumed, additional BrCl must be added and allowed to react for another 12 hours. Samples with high concentrations of dissolved organic carbon (DOC) are commonly stained a yellow or brown color. These samples are exposed

to UV light until all DOC has been oxidized and the sample becomes colorless (Olson and others, 1997).

Prior to analysis, the excess BrCl needs to be reduced with hydroxylamine hydrochloride (NH₂OH-HCl). Failure to reduce the BrCl will result in destruction of the gold traps by the free halogens in the remaining BrCl. Approximately 10 minutes after BrCl reduction, an aliquot is poured into a bubbling flask and 0.5 ml of stannous chloride (SnCl₂) is added to reduce the Hg^{II} to volatile mercury or Hg⁰. The aliquot is then purged with Hg-free N₂ for 20 minutes. Water vapor and free halogens are removed from the gas-stream by an inline soda-lime trap and the volatile mercury amalgamates onto a gold-coated glass bead trap.

The analytical train consists of the sample trap, an analytical trap, and the detector. The volatile mercury is thermally desorbed from the sample trap to an analytical trap to provide consistent chromatograms. After thermal desorption from the analytical trap, the volatile mercury is carried to a CVAFS with Hg-free Argon (Ar). Peak area is measured and compared to a standard curve for that day, and concentration is determined by the size of the aliquot that was purged.

Data quality objectives (DQO) for precision, accuracy, system control, and background contamination have been established to evaluate analytical results. A summary of DQO for Hg_T analysis is outlined in table 1.

Table 1. Summary of data quality objectives for total mercury analysis for water samples. [ng/L, nanograms per liter; pg, picograms; avg, average; σ , standard deviation; n, number of observations].

Type	DQO	avg	σ	n
QCCS (ng/L)	5.0 ±10%	5.05	0.22	99
% Recovery	90-110%	99.5	7.6	88
DDL (pg)	10	8.7	8.4	95

Precision is evaluated by duplicate analysis of all samples. The analysis is acceptable if the DQO is less than or equal to 10 percent difference. If the percent difference is greater than 10 percent, the sample is analyzed a third time or until a relative standard deviation (RSD) of less than 10 percent between the replicates is achieved.

A minimum of one in every 10 samples is spiked to assess accuracy. Spike recoveries ranging from 90 to 110 percent are acceptable (fig. 1). If the spike recovery fails to meet the DQO, another sample is spiked. If the second spike recovery fails to meet the DQO, all of the samples in that batch will be flagged indicating potential matrix interference.

A quality-control check sample (QCCS) is used to determine statistical control of the system. The QCCS needs to be within 10 percent of the theoretical value to proceed with sample analysis (fig. 2). A QCCS is analyzed at the beginning of the run, at least every tenth sample, and at the end of the run. A National Institute of Standards and Technology (NIST) 3133 certified standard originating from a different source than the calibration standard is used to prepare the QCCS.

Bubbler blanks are used to correct for background contamination and calculate detection limits. A bubbler blank is a pre-purged aliquot of either sample or reagent-grade water and 0.5 ml of SnCl₂. A set of bubbler blanks is analyzed at the beginning of the run, at least every ten samples, and at the end of the run. Background contamination is corrected by subtracting the average bubbler-blank peak area from each standard or sample-peak area. An initial detection limit (IDL) is calculated from bubbler blanks analyzed at the beginning of the run. The IDL must be below 5 picograms (pg) (0.04 ng/L based on an aliquot of 125 ml) before proceeding with sample analysis. The daily detection limit (DDL) is determined from the results of all bubbler blanks for the run (fig. 3). Detection limits are 3 times the standard deviation (SD) of the bubbler blanks, expressed as a mass. If a bubbler blank is found to contain more than 25 pg of Hg, the system is out of specified control and data produced on that bubbling flask should be reanalyzed.

A method detection limit of 0.04 ng/L was determined for Hg_T by the WDML according to MDL; 40 CFR 136, Appendix B. When the DDL is less than the MDL, samples that fall at concentrations below the MDL will be reported as less than 0.04 ng/L. When the DDL exceeds the MDL, all sample results that fall below the DDL will be reported as less than the runs DDL.

Presently there are no certified reference materials (CRM) for low-level total mercury analysis of water samples.

Sediment and Biota Samples

Total mercury in solid material is analyzed by placing a homogenized subsample of the material into a Teflon pressure vessel and weighing. Seven milliliters of a 7:3 mixture of HNO₃ and sulfuric acid (H₂SO₄) is then added to the vessel. The vessel is wrench tightened and digested at 125°C for a minimum of 2 hours. The sample is cooled for 1 hour and diluted to 30 ml with a 5% BrCl solution and allowed to oxidize at 50°C for a minimum of 12 hours. The BrCl oxidation is critical because the HNO₃/H₂SO₄ digestion itself is not rigorous enough to convert all the methylmercury to Hg^{II} (N.S. Bloom, Frontier Geosciences Inc. written commun., 1998). An aliquot from the vessel is then reduced with SnCl₂ in a bubbling flask and analyzed as described above for Hg_T.

A minimum of 3 digestion blanks are analyzed and used to correct for background contamination and calculate detection limits. The average mass of Hg from the blanks is subtracted from each sample and the daily detection limit is defined as three times the SD of the digestion blanks. If a sample is determined to be less than the detection limit, a larger subsample of the solid must be digested.

In addition to the DQO established for the analysis of water samples, a laboratory split and a CRM is digested and analyzed for every ten samples. The CRM chosen for a given batch is selected to best represent the matrix of interest. The WDML utilizes the following CRMs from the National Research Council (NRC) Canada: DORM-2 or the DOLT-2 for fish tissues, the TORT-2 or the LUTS-1 for biota other than fish, and the BEST-1 for sediments. The WDML DQO for CRM is plus or minus 10 percent of the theoretical value or the reported 95 percent confidence interval whichever is greater. Theoretical values and tolerance limits established by the NRC are outlined in table 2.

Mercury concentrations for sediments are reported on a dry weight basis. Percent dry weight is determined by drying a subsample of material at

105°C overnight. The percent dry weight is calculated by dividing the dry weight by the wet weight and multiplying by 100.

Table 2. Theoretical values and tolerance limits for certified reference materials used for total mercury in sediment and biota analysis. Units are ng/Kg, nanograms per kilogram.

CRM	Theoretical	Tolerance
DORM-2	4.64	±0.26
DOLT-2	2.14	±0.28
TORT-2	0.27	±0.06
LUTS-1	0.0167	±0.0022
BEST-1	0.092	±0.009

METHYLMERCURY ANALYSIS

Water Samples

Methylmercury (MeHg) analysis is performed according to USEPA Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS with minor modifications. USEPA 1630 is a draft method yet to be validated or published, therefore, the WDML will seek method approval through the USGS Office of Water Quality.

Direct ethylation of natural waters has been shown to release only “reactive” MeHg, which represents only 5–60 percent of the total MeHg (Horvat and others, 1993). Therefore, samples must first be distilled to eliminate potential interferences. Approximately 100 ml of sample is dispensed into a Teflon reaction vessel. A mixture of potassium chloride (KCl), H₂SO₄, and copper sulfate (CuSO₄) is added (Olson and others, 1997). The reaction vessels are then placed in an aluminum block maintained at 121°C until 80–95 percent of the sample has been distilled. The distillates are collected in Teflon receiving vessels housed in a refrigerator.

Ethylation is achieved by transferring the distillate to the bubbling flask, adjusting the pH to 4.7, adding sodium tetraethyl borate (NaTEB) and allowing the mixture to react for 15 minutes. The sample is then purged for 20 minutes with Hg-free N₂. The mercury species are collected onto a Carbotrap. The Carbotrap is placed in an analytical train consisting of a gas

chromatography (GC) column, a pyrolytic column and a CVAFS detector using Hg free Ar as the carrier gas. The mercury species are thermally desorbed from the Carbotrap and separated in the GC column. Following separation, the species are reduced in a pyrolytic column to Hg⁰. Volatile mercury is then detected by CVAFS where peak area is measured. The peak area is compared to a standard curve and concentration is determined by the size of aliquot that was purged and the percent of sample that was distilled. A summary of DQO for MeHg analysis is outlined in table 3.

Table 3. Summary of data quality objectives for methylmercury analysis for water samples. [pg, picograms; avg, average; σ , standard deviation; n, number of observations].

Type	DQO	avg	σ	n
% Recovery	80-120%	94.7	21.4	67
DDL (pg)	<5	2.2	2.7	77

The distillation block is designed to hold 16 samples. A batch consists of 11 environmental samples, 1 replicate, 1 matrix spike, and 3 distillation blanks.

The replicate is used to evaluate precision. An acceptable percent difference between the two replicates is 20 percent. If the percent difference exceeds 20 percent, samples from that batch are evaluated and potentially reanalyzed based on additional DQO.

Spike recoveries assess accuracy and matrix effects. The DQO for accuracy is a spike recovery between 80 and 120 percent (fig. 4). If the spike recovery fails to meet the DQO, another sample from that batch is spiked and the original spiked sample is reanalyzed. If the second spike recovery fails to meet the DQO, all of the samples in that batch will be reanalyzed or flagged indicating potential matrix interference.

In addition to daily calibration of the instrument, standards are analyzed throughout the analysis run to monitor instrument stability. If a standard differs by more than 10 percent from the original response, the system is recalibrated and all samples analyzed after the last acceptable standard are reanalyzed.

The distillation blanks are used to correct for background contamination and calculate detection limits. Distillation blanks are reagent-grade water, and distillation reagents. If a

distillation blank is found to contain more than 15 pg of Hg, the system is considered out of specified control and the sample batch must be redistilled. The DDL is calculated as 3 times the SD of the distillation blanks and may not exceed 5 pg for any batch (fig. 5).

A method detection limit of 0.025 ng/L was determined for MeHg by the WDML according to MDL 40 CFR 136, Appendix B. When the DDL is less than the MDL, samples that fall at concentrations below the MDL will be reported as less than 0.025 ng/L. When the DDL exceeds the MDL, all sample results that fall below the DDL will be reported as less than the runs DDL.

Currently there are no certified standards or CRM for methylmercury analysis in water. The MeHg standard used by the WDML is calibrated against NIST 3133 Hg_T standard to determine a true titer.

Sediment and Biota Samples

A homogenized sediment or biota subsample is weighed into a Teflon reaction vessel. Approximately 50 ml of reagent-grade water and the KCl, H₂SO₄, and CuSO₄ mixture is added and the distillation proceeds as detailed above.

In addition to the DQO established for MeHg analysis of water samples, a CRM is distilled and analyzed every batch. The WDML utilizes NRC DORM-2 or the DOLT-2 for fish tissues, the TORT-2 or the LUTS-1 for biota other than fish, and the BEST-1 for sediments. The WDML DQO for CRM is plus or minus 10 percent of the theoretical value or the reported 95 percent confidence interval, whichever is greater. Theoretical values and tolerance limits established by the NRC are outlined in table 4.

Mercury concentrations for sediments are reported on a dry weight basis. Percent dry weight is determined by drying a subsample of material at 105°C overnight. The percent dry weight is calculated by dividing the dry weight by the wet weight and multiplying by 100

Table 4. Theoretical values and tolerance limits for certified reference materials used for methylmercury in sediment and biota analysis. Units are ng/Kg, nanograms per liter.

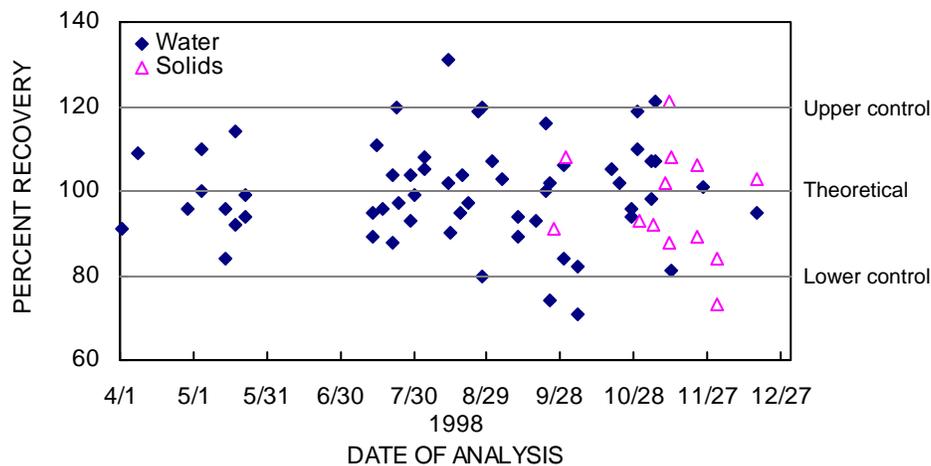


Figure 4. Percent recoveries for analyses of methylmercury.

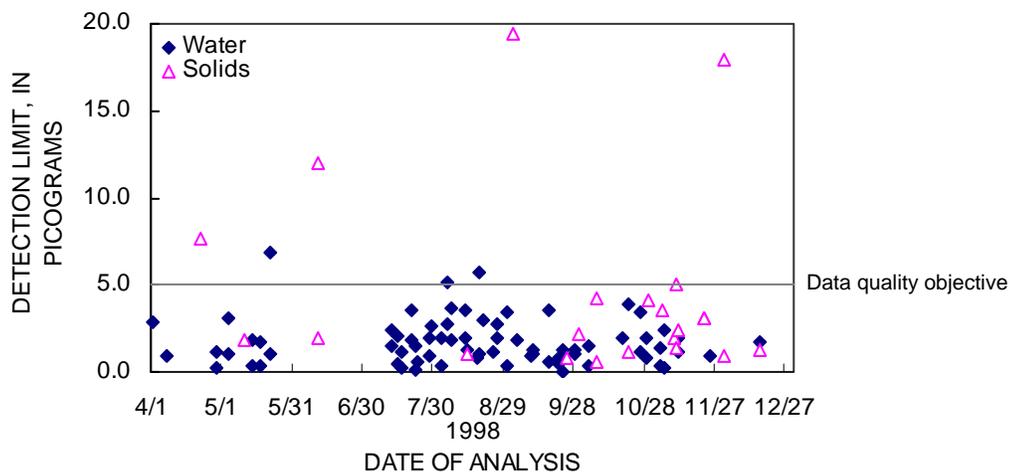


Figure 5. Daily detection limits for analyses of methylmercury.

CRM	Theoretical	Tolerance
DORM-2	4.47	±0.32
DOLT-2	0.693	±0.053
TORT-2	0.152	±0.013
LUTS-1	0.0094	±0.0006
BEST-1	0.000162	±0.000052

DISSOLVED GASEOUS AND REACTIVE MERCURY

To increase understanding of the fate and transport of Hg in the environment, the WDML refined techniques for the analysis of dissolved gaseous mercury (DGM) and reactive mercury (RHg). DGM is the measurement of volatile Hg species and RHg is the measurement of easily reducible Hg^{II} in the water column.

Because concentrations of DGM and RHg are generally less than 50 pg/L, a sample volume of 1,700 ml is required to assure sufficient Hg for detection. Unfiltered water is collected into a 2 L Teflon purging vessel. DGM is collected from the sample simply by purging with Hg-free N_2 , while the RHg requires the addition of 5 ml of $SnCl_2$ prior to purging, in order to reduce Hg^{II} to Hg^0 . The 2 L sample vessel is capped tightly and purged with Hg-free N_2 at a flow rate of 500 ml/min for 60 minutes. The Hg^0 is collected on a gold-coated glass bead trap connected to the outlet of the vessel. The gold trap is sealed and shipped to the WDML for analysis.

Quality assurance (QA) samples are primarily the responsibility of the field personnel. QA samples for DGM and RHg include bubbler blanks, duplicate samples, backup traps and trip blanks.

Bubbler blanks for both DGM and RHg are performed by adding reagents to a prepurged sample and purging for another 60 minutes onto a new gold trap. The DQO for bubbler blanks collected for DGM and RHg are less than 5 pg and less than 10 pg respectively.

A duplicate sample is collected in a separate vessel and purged at the same time as the primary sample. The samples are treated identically and the DQO for precision is a difference of plus or minus 10 pg for each parameter.

A backup trap (a gold trap connected to the outlet of the sample trap) is used to trap any Hg not amalgamated to the sample trap. Mercury detected on backup traps should not exceed 5 pg.

Several gold traps not used in sampling, will be shipped from the WDML. These traps are analyzed as trip blanks and evaluated to assess potential contamination of the traps during shipping. Trip blanks should not exceed 5 pg.

After a sample trap has been received by the WDML. The gold trap is placed in the analytical train of the Hg_T system, and treated as a Hg_T sample trap similar to Hg_T techniques. The peak areas measured are compared to calibration standards and concentration is determined by the size of the aliquot that was purged.

An MDL study has not been conducted for either DGM or RHg.

SUMMARY AND CONCLUSIONS

Method performance for Hg species is based on measurements for precision, accuracy, system control, and background contamination. Total mercury performance is based on the analysis of all samples in duplicate, a certified QCCS, spike recoveries, blanks and or DDL's, and CRM analysis for solids. Method performance for methylmercury is based on replicate samples, spike recoveries, check standards, distillation blanks, and CRM analysis for solids.

Reactive and DGM method performance is based on bubbler blanks, replicate samples, backup traps, and trip blanks which are the responsibility of the field personnel collecting the sample.

Low-level Hg methods have yet to be approved by the USGS, USEPA, American Public

Health Association, or the American Society for Testing and Materials. Results produced by the WDML are for research purposes only and can be used in any USGS reports if qualified as provisional data with references cited. The total mercury method is scheduled for approval by the USEPA in the spring of 1999. The methylmercury method is in the process of being approved by the USEPA but approval is not expected until sometime in the year 2000. The WDML will be seeking USGS approval of WDML standard operating procedures as a water-quality method in the spring of 1999.

REFERENCES CITED

- Federal Register, 1984, Definition and procedure for the determination of the method detection limit. 40 CFR Appendix B
- Gill, G.A., and Fitzgerald, W.F., 1985, Mercury sampling of open ocean waters at the picomolar level. *Deep Sea Research*, v 32, p. 287-297
- Horvat, M., Liang, L., and Bloom, N.S., 1993, Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low-level environmental samples. *Analytica Chimica Acta*. v 282, p. 153-168.
- Olson, M.L., Cleckner, L.B., Hurley, J.P., Krabbenhoft, D.P., and Heelan, T.W., 1997, Resolution of matrix effects on analysis of total and methyl mercury in aqueous samples from the Florida Everglades. *Fresenius Journal of Analytical Chemistry*. v 358, p. 392-396
- Patterson, C.C., and Settle, D.M., 1976, The reduction in orders of magnitude errors in lead analyses of biological materials and natural waters by evaluating and controlling the extent and sources of industrial lead contamination introduced during sample collection handling and analysis. In: P.D. Lefleur (Editor), *Accuracy in trace analysis: Sampling, sample handling and analysis*. U.S. National Bureau of standards special publication 422. P.321-351.
- US Environmental Protection Agency, 1996, Method 1631: Mercury in water by oxidation, purge and trap, and cold vapor atomic

fluorescence (CVAFS). Draft method EPA
821-R-96-012

——, 1997, Method 1630: Methyl mercury in
water by distillation, aqueous ethylation,
purge and trap, and cold vapor atomic
fluorescence (CVAFS). Draft method.

Ware, F.J., Royals, H., and Lange, T., 1990,
Proceedings of the annual conference of the
Southeastern association of fish and wildlife
agencies. v 44, p. 5-12.

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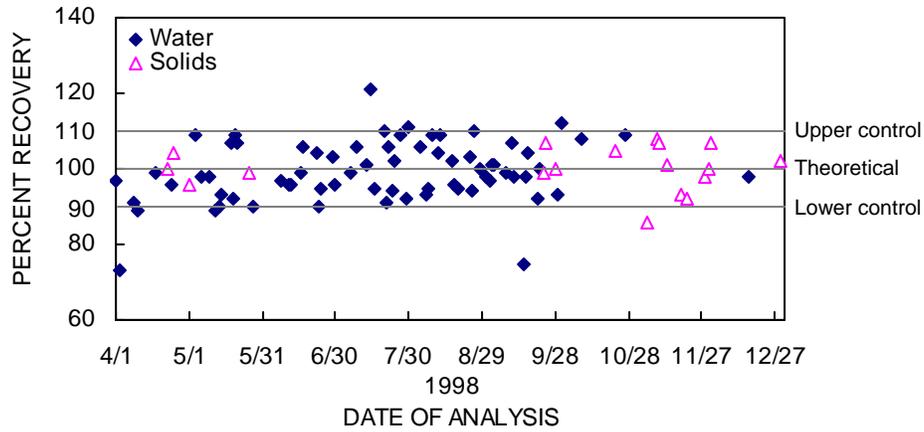


Figure 1. Percent recoveries for analyses of total mercury.

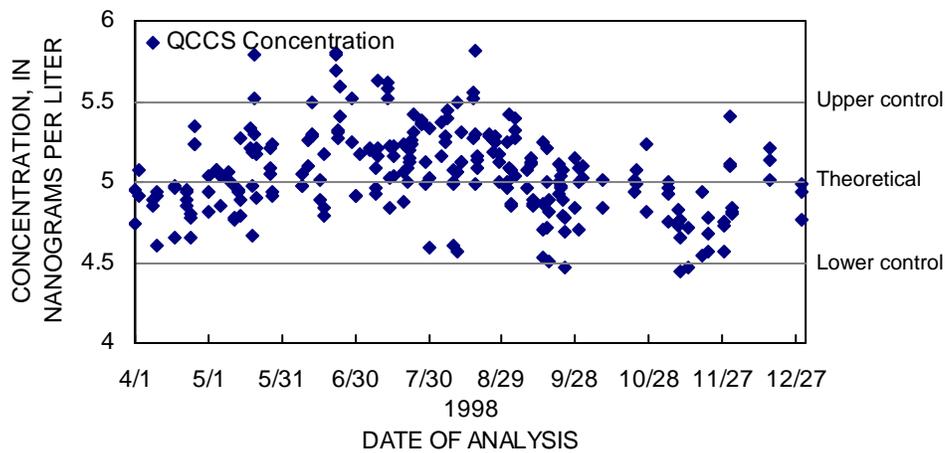


Figure 2. Quality-control check sample concentrations for analyses of total mercury.

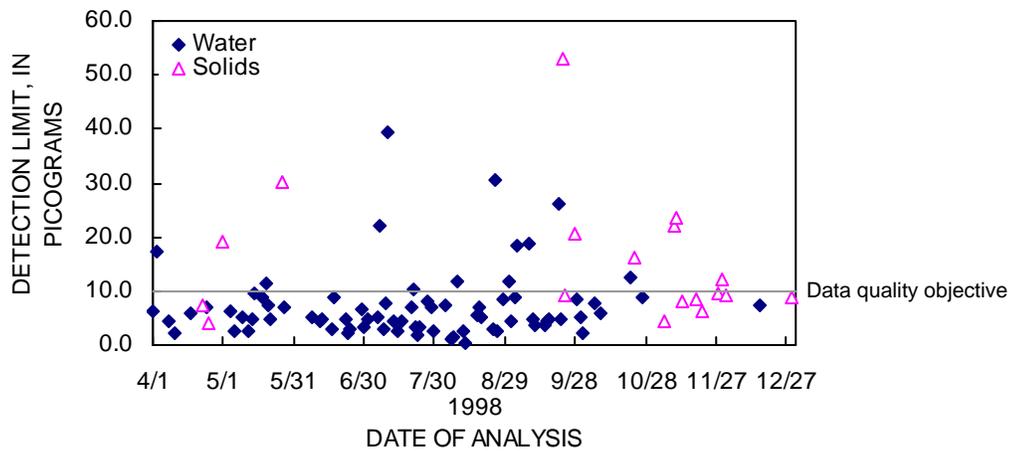


Figure 3. Daily detection limits for analyses of total mercury.