Revised Mercury Speciation Soil Investigation Work Plan
Revision 02

Olin North Parcels I and II
AOC Index No. R9-4171-94-08
NYSDEC Site No. 932051A
Niagara Falls, New York

Prepared for:

OLIN CORPORATION

Prepared by:

Wood Environment & Infrastructure Solutions, Inc.
1075 Big Shanty Road NW, Suite 100
Kennesaw, Georgia 30144

March 22, 2019

RECORD OF PLAN REVISIONS

Revision No.	Revision Date	Revision Description/Reason
00	1/9/2015	Original version.
01	7/13/2018	Revision 01 prepared to address NYSDEC and NYDoH comments provided during a June 8, 2018 conference call. • Additional SOP and quality assurance information for the proposed analytical method added. • Data evaluation section updated to specify preparation of Data Usability Summary Report (DUSR) based on NYSDEC guidance. • Schedule updated.
02	3/22/2019	Revision 02 prepared to address NYSDEC questions and comments provided via email on December 3, 2018. • Number of samples increased from 3 to 10. • Figure 3.1 updated to reflect additional sample locations. • Sample depth increased from 0-2” to 0-6”. • Schedule updated. • Updated analytical method SOPs included.

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ACRONYMS AND ABBREVIATIONS

DD	Day
DI	De-ionized
DUSR	Data Usability Summary Report
Eurofins	Eurofins Frontier Global Services Laboratory
FSTM	Flue-Gas Sorbent Total Mercury
GPS	Global Positioning System
HASP	Health and Safety Plan
HAZWOPER	Hazardous Waste Operations and Emergency Response
Hg	mercury
mg/kg	milligram per kilogram
MM	Month
NYSDEC	New York State Department of Environmental Conservation
PPE	Personal Protective Equipment
QC	Quality Control
SCO	Soil Cleanup Objective
SOP	Standard Operating Procedure
SS	Stainless Steel
USEPA	United States Environmental Protection Agency
YYYY	Year

1.0 INTRODUCTION

Olin Parcels I and II (North Parcels) are located north of Buffalo Avenue, across from Olin's Niagara Falls, Chlor-Alkali Plant (Figure 1.1). Results from previous sampling and analysis showed sporadic mercury detections in the North Parcels surface soil. The mercury is thought to be associated with brine muds generated in the chlor-alkali process that were used to repair potholes at the North Parcels in the 1950s or 1960s.

In 2014, Olin conducted a soil investigation in which representative surface soils from the North Parcels were collected and analyzed for total mercury (Hg) to characterize the surface soils (AMEC, 2014). The investigation report provided analytical results for 50 soil samples collected at the North Parcels. At thirteen sample locations mercury was detected at concentrations above the NYSDEC Industrial Soil Cleanup Objective (SCO) of 5.7 mg/kg for elemental mercury. No results exceeded the Industrial SCO of 220 mg/kg for inorganic salts of mercury. A literature review of mercury in the chlor-alkali processes indicated dissolved mercury in the brine circuit is likely to be an inorganic salt; therefore a presumption in the 2014 report was that the primary form of mercury in the brine muds was an inorganic salt.

After reviewing the 2014 AMEC report, the NYSDEC submitted a December 4, 2014 letter to Olin which indicated that NYSDEC must assume that the mercury in the soil samples is in the elemental form unless shown otherwise by analytical data. Following a series of teleconferences with NYSDEC and the NYS Department of Health (NYSDoH), Olin offered to prepare this work plan for submittal, review, and approval by NYSDEC which includes an analytical method that can reliably and quantitatively differentiate various mercury species. This work plan was originally submitted to NYSDEC in January 2015. The Record of Plan Revisions (page ii) summarizes the revisions to the plan since the original submission.

Olin proposes to collect samples at ten locations, distributed spatially across the parking lot, at locations where mercury concentrations were detected during the 2014 sampling event. The surface soil samples will then be analyzed for total and elemental mercury using the speciation techniques described in this plan. The resulting elemental mercury data can then be compared to the industrial SCOs for elemental mercury (5.7 mg/kg). These data would then be used to develop an average ratio of elemental to total mercury that could be compared to the remaining original samples for evaluating SCOs. This work plan describes the objectives, sample collection and analysis, data evaluation, reporting, and schedule for this work.

2.0 OBJECTIVES

The characterization objectives are to:

- Collect surface soil samples from the North Parcels at ten separate locations.
- Analyze the samples for total mercury and elemental mercury to obtain speciation data.

3.0 SAMPLE COLLECTION AND ANALYSIS

This section describes the sample collection procedures, decontamination process, analytical method, and health and safety requirements.

3.1 SAMPLE COLLECTION

Ten surface soil borings will be advanced at the North Parcels at the locations are shown on Figure 3.1. These locations coincide with previously collected 2014 samples to allow correlation of results obtained by using different analytical methods. The latitude and longitude of the initial borings were surveyed with a handheld global positioning system (GPS), and a GPS will again be used to identify these locations and collect the samples.

The borings will be advanced by hand to 6-inches below the existing vegetation or cover material (gravel, etc.) as specified for surface soil investigations of volatile compounds in NYSDEC Technical Guidance Document DER-10 (NYSDEC, 2010a). The hand borings will be advanced and samples collected using pre-cleaned, dedicated, stainless steel (SS) hand augers or other SS hand-operated tools. Soil will be collected from the boring and placed in a clean, laboratory-supplied, 4-ounce glass jar.

3.2 FIELD QUALITY CONTROL SAMPLES

A Field Quality Control (QC) sample will consist of one duplicate sample collected at the PLS-SS-19 location where the highest mercury concentration was previously detected.

3.3 SAMPLE LABELS

The samples to be submitted to the laboratory will be identified and labeled as follows:

1. PLS-SS-X-MMDDYYY (X = 1 – 50 – corresponding to boring location)
2. DUP01-SS-MMDDYYYY (for the duplicate sample)

3.4 DECONTAMINATION

Sample collection tools, utensils, and bowls will be decontaminated prior to sampling, with individually dedicated sets of tools to be used at each of the locations. The tools brought to the site will be decontaminated prior to mobilization and will be wrapped in aluminum foil during transportation to the site. Decontamination of the tools will be as follows:

1. Liquinox and water wash
2. DI water rinse
3. Nitric Acid rinse
4. DI water rinse

After the samples are collected, any excess solids will be placed back into the individual excavations. Field personnel will wear nitrile gloves when handling the samples or sample tools. New gloves will be used at each sample location. Nitrile gloves and other personal protective equipment (PPE) will be disposed of with Olin's general waste.

3.5 HEALTH AND SAFETY REQUIREMENTS

Safety requirements will be outlined in a separate Health and Safety Plan (HASP). The HASP will be provided to field personnel for review before the investigation, and personnel performing the on-site investigation work will be required to sign an acknowledgement that they are familiar with the HASP.

Personnel engaged in field activities with potential for exposure to contaminants are required to have completed 40 hours of initial Hazardous Waste Operations and Emergency Response (HAZWOPER) training and annual 8-hour refreshers. Site personnel will be required to wear the PPE specified in the HASP while engaged in field activities or while onsite during field activities. Level D PPE (hard hat, safety shoes, safety glasses) will be required at a minimum for personnel collecting the soil samples. Level D PPE is anticipated to offer sufficient protection to personnel working onsite.

3.6 SAMPLE PRESERVATION AND ANALYSIS

The surficial soil samples will be preserved and shipped to Eurofins Frontier Global Services Laboratory (Eurofins) for analyses. Eurofins has been selected to perform the metals analyses based on their experience and qualifications in the field of trace metals analyses. Eurofins has over 25 years of experience in the development and validation of many trace metals and metals speciation analysis methods that today are used around the world. Eurofins helped developed the art of low-level mercury and mercury speciation analytical methods. They served as the United States Environmental Protection Agency (USEPA) Reference and Validation Laboratory for the USEPA 1600 Series Trace Metal and Metals Speciation Analysis Methods. These methods include USEPA 1630 (Method Hg), EPA 1631 (Low-level Hg) and a host of additional

trace metals methods as applied to waters, biological tissues, sediments and soils. Eurofins holds accreditation certificates for ISO/IEC 17025-2005, FDA, DOD, NELAC/NELAP in 3 states and 8 additional state accreditations.

Upon collection, samples will be preserved by being placed immediately on dry ice to freeze the soil. Headspace in the glass jar will be provided for soil expansion so that the glass jars do not break. Each sample will consist of two jars to insure against breakage.

The samples will be analyzed for total mercury by USEPA Method 1631E, Eurofins SOP "Determination of Total Mercury in Various Matrices by Flow Injection - Atomic Fluorescence Spectrometry (FI-AFS)" (EFAFS-T-AFS-SOP2822) after sample preparation by Eurofins Standard Operating Procedure (SOP) "Preparation of Solid Samples for Total Mercury by Modified Cold Aqua-Regia Digestion" (EFAFS-T-AFS-SOP2807). The soils will also be analyzed for elemental mercury by USEPA Method 1631E, after preparation by Eurofins SOP "Selective Sequential Extraction of Geological Samples for the Determination of Biogeochemically Relevant Inorganic Mercury Fractionation" (EFAFS-T-AFS-SOP2813). For these methods, the typical reporting level for Total Mercury is 1.0 ng/g and for elemental mercury it is 2.0 ng/g, which is well below the NYSDEC SCO of 5.7 mg/kg for elemental mercury. The Eurofins SOPs referenced above represent the laboratory procedures for the USEPA methods and are included as Appendix B.

The analytical procedures will provide results for total mercury and elemental mercury in the surficial soils. The total mercury digestion and analytical procedures are described in Eurofins SOPs previously referenced. The sample preparation and analysis for total mercury is designed to detect all forms of mercury present in the soils, including elemental and total. The sample preparation is a strong acid, modified aqua regia digestion that has the oxidizing action of concentrated nitric acid or hydrochloric acid resulting in the formation of nitrosyl chloride and free chlorine, which are particularly strong oxidizers. After sample digestion is complete, an aliquot is pipetted into a vial with bromide monochloride and the final volume is neutralized with hydroxylamine-hydrochloride. The sample is now ready for instrumental analysis. The prepared sample is introduced into the Flow Injection Atomic Fluorescence Spectrometry system and is mixed with stannous chloride prior to entering the phase separator. As the sample travels through the phase separator it is exposed to a flow of argon gas which transports the mercury through a soda-lime acid vapor wash and amalgamates onto it. The trap is then heated, and mercury is released back into the argon stream and amalgamates onto a second trap, which is subsequently heated, releasing the mercury into the AFS detector for quantitation.

The elemental mercury preparation and analytical procedures are also described in Eurofins SOPs previously referenced. Elemental mercury concentrations are determined by preparing the soil samples as described in the SOP Section 5.2 F0, which is based on the known volatility of elemental mercury. The sample is placed in a Teflon bomb vessel which is purged with nitrogen gas transporting the elemental mercury onto a Flue Gas Sorbent Total Mercury (FSTM) trap where it is collected while other forms of mercury remain behind since they do not volatilize. The elemental mercury is now captured on the FSTM trap. The trap is then digested according to EFAFS-T-AFS-SOP2985 and is ready for instrumental analysis by Method 1631E.

The method performance criteria for the total mercury and elemental mercury analyses of soil samples are detailed in following tables:

Total Mercury Analysis Method Performance Criteria Table

Quality Control Parameter	Acceptance Criteria
Initial Calibration Verification (ICV)	77-123% Recovery
Continuing Calibration Verification (CCV)	77-123% Recovery
Ongoing Precision and Recovery (OPR)	77-123% Recovery
Initial Calibration Blank (ICB) / Continuing Calibration Blank (CCB)	Individually, IBL and CCB < 0.50 ng/L, but the mean of all the IBLs shall be < 0.25 ng/L with a standard deviation of 0.10 ng/L.
Laboratory Control Standard (LCS) (1) or Quality Control Standard (QCS) (1)	80-120% Recovery RSD <24%
Calibration Curve (minimum 5 standards) RSD	RSD of Calibration Response Factor < 15%
Lowest Calibration Point	75-125%
1% BrCl Method Blank (BLK)	Less than 0.50 ng/L
Matrix Duplicate (MD) and Analytical Duplicate (AD)	< 24% RPD
Matrix Spike and Matrix Spike Duplicate; Analytical Spike (AS) and Analytical Spike Duplicate (ASD)	71-125% Recovery < 24% RPD
Method Detection Limit (MDL) (2)	0.11 ng/g
Method Reporting Limit (MRL) (3)	1.0 ng/g

(1) LCS or QCS in a known concentration of mercury that is used to monitor the complete method Performance and is a matrix matched Certified Reference Material (CRM) whenever available.

(2) MDL is determined by analyzing ten standard replicates (9 degrees of freedom) spiked 3 – 10 times the expected MDL. The standard deviation (s) is taken from the resulting data and the MDL calculated as follows: MDL = 2.821*s.

(3) MRL is lowest calibration curve point and is determined by running ten samples with a concentration that will produce a recovery 70-130%.

Elemental Mercury Analysis Method Performance Criteria Table

Quality Control Parameter	Acceptance Criteria
Initial Calibration Verification (ICV)	77-123% Recovery
Continuing Calibration Verification (CCV)	77-123% Recovery
Ongoing Precision and Recovery (OPR)	77-123% Recovery
Initial Calibration Blank (ICB) / Continuing Calibration Blank (CCB)	Individually, IBL and CCB < 0.50 ng/L, but the mean of all the IBLs shall be < 0.25 ng/L with a standard deviation of 0.10 ng/L.
Laboratory Control Standard (LCS) (4) or Quality Control Standard (QCS) (4)	80-120% Recovery RSD <24%
Calibration Curve (minimum 5 standards) RSD	RSD of Calibration Response Factor < 15%
Lowest Calibration Point	75-125%
Matrix Duplicate (MD) and Analytical Duplicate (AD)	< 24% RPD
Matrix Spike and Matrix Spike Duplicate; Analytical Spike (AS) and Analytical Spike Duplicate (ASD)	71-125% Recovery < 25% RPD
Method Detection Limit (MDL) (5)	0.344 ng/g
Method Reporting Limit (MRL) (6)	2.00 ng/g

(4) LCS or QCS in a known concentration of mercury that is used to monitor the complete method Performance and is a matrix matched Certified Reference Material (CRM) whenever available.

(5) MDL is determined by analyzing ten standard replicates (9 degrees of freedom) spiked 3 – 10 times the expected MDL. The standard deviation (s) is taken from the resulting data and the MDL calculated as follows: MDL = 2.821*s.

(6) MRL is lowest calibration curve point and is determined by running ten samples with a concentration that will produce a recovery 70-130%.

4.0 DATA EVALUATION AND REPORTING

A Data Usability Summary Report (DUSR) will be prepared from the laboratory results generated from the soil samples collected to meet the Work Plan objectives. The data evaluation will include the checks identified in the NYSDEC DUSR guidance (NYSDEC, 2010b) and USEPA Stage IV element review:

- Sample integrity (Chain of Custody)
- Sample completeness
- Sample holding times
- Laboratory methods for extraction and analysis
- Method Detection Limits (MDL) and Method Reporting Limits (MRL)
- Method blanks, initial and continuing calibration blanks, instrument blanks/blubber blanks, reagent blanks
- Instrument initial and continuing calibration (OPRs)
- Laboratory control spikes /Quality control sample
- Matrix spike/Matrix spike duplicate
- Equipment, field and bottle blanks, ambient blanks
- Field and lab duplicates
- Raw data calculations
- EDD vs. hardcopy check

After the Stage IV element review has been performed, and the data validation has been completed, the sample results will be compared with the NYSDEC Industrial SCOs for elemental and inorganic mercury. A report will be prepared that will detail the sampling effort, results and conclusions.

5.0 SCHEDULE

Olin proposes to complete the field work during Second Quarter 2019. A report of the field investigation results, evaluation of the sampling data, and speciation conclusions will be submitted to NYSDEC within 60 days of the final lab report data validation.

6.0 REFERENCES

Amec, 2014. North Parking Lot Soil Investigation, Niagara Falls, New York. Prepared for Olin Corporation, August 15, 2014.

NYSDEC, 2010a. DER 10 / Technical Guidance for Site Investigation and Remediation. Albany, NY: NYSDEC.

NYSDEC, 2010b. "Technical Guidance for Site Investigation and Remediation-Appendix 2B"; DER-10; Division of Environmental Remediation; May 2010.

USEPA, 2002. Method 1361, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. August 2002.

FIGURES

OLIN Parking Lot: Parcel I

OLIN Parking Lot: Parcel II

OLIN Plant 1

OLIN Plant 2

Legend

- Gill Creek
- Approximate Olin Boundary
- Olin Parking Lot: Parcel I (Niagra County Parcel 97)
- Olin Parking Lot: Parcel II (Niagra County Parcel 111)
- Industrial Soil Cleanup Objective - 5.7 mg/kg

0 200 400 Feet

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Mercury Speciation Soil Investigation Work Plan Rev 02

Olin North Parcels I and II, Niagara Falls, NY

Site Layout

Prepared By-Date: JRM - 3/8/2019	
Checked By-Date: AWE - 3/8/2019	wood.
Project Number: 6107180002	**Figure: 1.1**

PLS-SS-1 0.65
PLS-SS-2 0.93
PLS-SS-3 5.55
PLS-SS-4 7.28
PLS-SS-5 1.95
PLS-SS-6 1.94
PLS-SS-7 6.11
PLS-SS-8 1.07
PLS-SS-9 0.54
PLS-SS-10 20.8
PLS-SS-11 0.43
PLS-SS-12 0.79
PLS-SS-13 2.5
PLS-SS-14 2.57
PLS-SS-15 15.2
PLS-SS-16 17.9
PLS-SS-17 3.67
PLS-SS-18 19.7
PLS-SS-19 53.5
PLS-SS-20 2.48
PLS-SS-21 5.5
PLS-SS-22 14.6
PLS-SS-23 3.32
PLS-SS-24 12.4
PLS-SS-25 3.88
PLS-SS-26 2.19
PLS-SS-27 1.32
PLS-SS-28 3.81
PLS-SS-29 3.51
PLS-SS-30 1.6
PLS-SS-31 2.22
PLS-SS-32 2.58
PLS-SS-33 1.21
PLS-SS-34 1.95
PLS-SS-35 0.46
PLS-SS-36 2.54
PLS-SS-37 1.3
PLS-SS-38 3.73
PLS-SS-39 2.41
PLS-SS-40 5.26
PLS-SS-41 3.29
PLS-SS-42 25.9
PLS-SS-43 0.62
PLS-SS-44 4.56
PLS-SS-45 2.89
PLS-SS-46 5.53
PLS-SS-47 1.78
PLS-SS-48 8.44
PLS-SS-49 10
PLS-SS-50 7.72

OLIN Plant 1

OLIN Plant 2

Legend

Mercury Results (mg/kg) from May 2014 Soil Investigation

- <47
- 47 - 220
- >220
- Gill Creek
- Approximate Olin Boundary
- Approximate DuPont Boundary
- Olin Parking Lot: Parcel I (Niagra County Parcel 97)
- Olin Parking Lot: Parcel II (Niagra County Parcel 111)
- Highlighted Locations are proposed for Hg Speciation Sampling

0 200 400 Feet

Path: G:\olin_niagra\mxds\Investigation Workplan\Mercury_Speciation_Results.mxd

Mercury Speciation Soil Investigation Work Plan Rev 02

Olin North Parcels I and II, Niagara Falls, NY

Surface Soil Sample Locations

Prepared By-Date: THP - 1/7/2019	
Checked By-Date: AWE - 1/7/2019	wood.
Project Number: 6107190002	**Figure: 3.1**

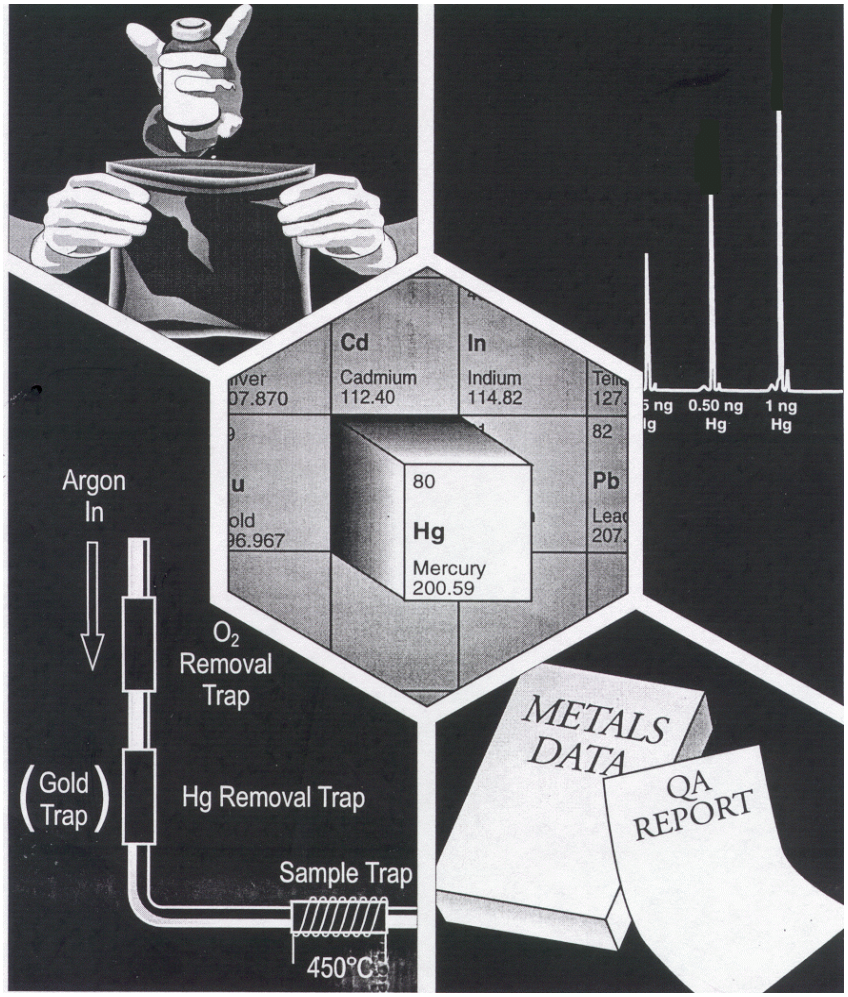
APPENDIX A

USEPA METHOD 1631, REVISION E

United States
Environmental Protection
Agency

Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

August 2002



United States Environmental Protection Agency
Office of Water 4303
EPA-821-R-02-019 August 2002

Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

Acknowledgments

This Method was developed under the direction of William A. Telliard and Maria Gomez-Taylor of the Engineering and Analysis Division (EAD) within the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST). EPA acknowledges contributions to this method by Frontier Geosciences, Inc., Albion Environmental, Battelle Marine Sciences Laboratory, STL-Canton, and Tekran Inc. Additional assistance in preparing the Method was provided by DynCorp Environmental and Interface, Inc.

Disclaimer

This Method has been reviewed and approved for publication by the Statistics and Analytical Support Branch within EPA's Engineering and Analysis Division. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this Method or its application should be addressed to:

W.A. Telliard
Statistics and Analytical Support Branch (4303T)
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
Phone: 202/566–1061
Fax: 202/566–1053

Table of Contents

Introduction

Method 1631 (the "Method") supports technology-based and water quality-based monitoring programs authorized under the Clean Water Act (CWA; the "Act").

CWA Sections 301 and 306 require EPA to publish effluent standards that restrict the direct discharge of pollutants to the nations waters, and CWA Sections 307(b) and (c) require EPA to promulgate nationally applicable pretreatement standards which restrict pollutant discharges into sewers flowing to publicly owned treatment works (POTWs). The effluent limitations guidelines are published at CFR parts 401-503.

CWA Section 303 requires each State to set a water quality standard for each body of water within its boundaries. A State water quality standard consists of a designated use or uses of a water body or a segment of a water body, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. CWA Section 304(a) requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific water body, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by CWA Sections 301(b) and 306.

In 1987, amendments to the CWA required States to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses. Method 1631 was specifically developed to provide reliable measurements of mercury at EPA WQC levels.

In developing methods for determination of trace metals, EPA found that one of the greatest difficulties was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. Method 1631 is designed to preclude contamination in nearly all situations. It also contains procedures necessary to produce reliable results at the lowest WQC levels published by EPA. In recognition of the variety of situations to which this Method may be applied, and in recognition of continuing technological advances, Method 1631 is performance based. Alternative procedures may be used so long as those procedures are demonstrated to yield reliable results.

Requests for additional copies of this draft Method should be directed to:

U.S. EPA Sample Control Center
6101 Stevenson Avenue
Alexandria, VA 22304-3540
703/461–2100

Note: This Method is performance based. The laboratory is permitted to omit steps or modify procedures provided that all performance requirements in this Method are met. The laboratory must not omit or modify any procedure defined by the term "shall" or "must" and must perform all quality control tests.

Method 1631, Revision E

Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

1.0 Scope and Application

1.1 Method 1631, Revision E (the "Method") is for determination of mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry (CVAFS). This Method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The Method is based on a contractor-developed procedure (Reference 16.1) and on peer-reviewed, published procedures for the determination of mercury in aqueous samples, ranging from sea water to sewage effluent (References 16.2–16.5).

1.2 This Method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (Sampling Method). The Sampling Method guidance document is recommended to preclude contamination during the sampling process.

1.3 This Method is for determination of Hg in the range of 0.5–100 ng/L. Application may be extended to higher levels by selection of a smaller sample size or by calibration of the analytical system across a higher range. For measurement of blank samples, the Method may be extended to a lower level by calibration to a lower calibration point. Section 10.4 gives requirements for extension of the calibration range.

1.4 The ease of contaminating ambient water samples with mercury and interfering substances cannot be overemphasized. This Method includes suggestions for improvements in facilities and analytical techniques that should minimize contamination and maximize the ability of the laboratory to make reliable trace metals determinations. Certain sections of this Method contain suggestions and other sections contain requirements to minimize contamination.

1.5 The detection limit and minimum level of quantitation in this Method usually are dependent on the level of interferences rather than instrument limitations. The method detection limit (MDL; 40 CFR 136, Appendix B) for Hg has been determined to be 0.2 ng/L when no interferences are present. The minimum level of quantitation (ML) has been established as 0.5 ng/L. An MDL as low as 0.05 ng/L can be achieved for low Hg samples by using a larger sample volume, a lower BrCl level (0.2%), and extra caution in sample handling.

1.6 Clean and ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this Method because they lack an exact definition. However, the information provided in this Method is consistent with the summary guidance on clean and ultraclean techniques (References 16.6-16.7).

1.7 This Method follows the EPA Environmental Methods Management Council's "Guidelines and Format for Methods to Be Proposed at 40 CFR, part 136 or part 141."

1.8 This Method is "performance based." The laboratory is permitted to modify the Method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2.1 gives the requirements for establishing method equivalency.

1.9 Any modification of this Method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.10 This Method should be used only by analysts experienced in the use of CVAFS techniques and who are trained thoroughly in the sample handling and instrument techniques described in this Method. Each laboratory that uses this Method must demonstrate the ability to generate acceptable results using the procedures in Section 9.2.

1.11 This Method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring* (Reference 16.8), that can be used for verification and validation of the data obtained.

1.12 This Method uses either a bubbler or flow-injection system for determination of mercury in water. Separate calibration, analysis, and calculation procedures are provided for a bubbler system (Sections 10.2, 11.2.1, and 12.2) and for a flow-injection system (Sections 10.3, 11.2.2, and 12.3).

2.0 Summary of Method

2.1 A 100- to 2000-mL sample is collected directly into a cleaned, pretested, fluoropolymer or glass bottle using sample handling techniques designed for collection of mercury at trace levels (Reference 16.9).

2.2 For dissolved Hg, the sample is filtered through a 0.45-µm capsule filter prior to preservation.

2.3 The sample is preserved by adding either pretested 12N hydrochloric acid (HCl) or bromine monochloride (BrCl) solution. If a sample will also be used for the determination of methyl mercury, it should be preserved according to procedures in the method that will be used for determination of methylmercury.

2.4 Prior to analysis, all Hg in a 100-mL sample aliquot is oxidized to Hg(II) with BrCl.

2.5 After oxidation, the sample is sequentially reduced with $NH_2OH{\cdot}HCl$ to destroy the free halogens, then reduced with stannous chloride ($SnCl_2$) to convert Hg(II) to volatile Hg(0).

2.6 The Hg(0) is separated from solution either by purging with nitrogen, helium, or argon, or by vapor/liquid separation. The Hg(0) is collected onto a gold trap (Figures 1, 2, and 3).

2.7 The Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg(0) to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas stream that carries the Hg into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection (Figures 2 and 3).

2.8 Quality is assured through calibration and testing of the oxidation, purging, and detection systems.

3.0 Definitions

3.1 Total mercury—all BrCl-oxidizable mercury forms and species found in an unfiltered aqueous solution. This includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organo-mercurials (e.g., CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of Hg bound within microbial cells may require the additional step of UV photo-oxidation. In this Method, total mercury and total recoverable mercury are synonymous.

3.2 Dissolved mercury—all BrCl-oxidizable mercury forms and species found in the filtrate of an aqueous solution that has been filtered through a 0.45-µm filter.

3.3 Apparatus—Throughout this Method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis that come in contact with the sample and therefore require careful cleaning will be referred to collectively as the Apparatus.

3.4 Definitions of other terms used in this Method are given in the glossary (Section 17.0).

4.0 Contamination and Interferences

4.1 Preventing samples from becoming contaminated during the sampling and analysis process constitutes one of the greatest difficulties encountered in trace metals determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing samples for trace metals.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metals contamination during sampling include: metallic or metal-containing labware (e.g., talc gloves that contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned or stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination. For example, it has been demonstrated that dental work (e.g., mercury amalgam fillings) in the mouths of laboratory personnel can contaminate samples directly exposed to exhalation (Reference 16.9).

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is metal free and free from any material that may contain mercury.

4.3.1.1 The integrity of the results produced cannot be compromised by contamination of samples. This Method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metals measurements. This Method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5 of this Method give suggestions and requirements for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to work being done. Therefore, it is imperative that the procedures described in this Method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class-100 clean room. If a clean room is not available, all sample preparation should be performed in a class-100 clean bench or a nonmetal glove box fed by mercury-and particle-free air or nitrogen. Digestion should be performed in a nonmetal fume hood equipped with HEPA filtration and ideally situated in a clean room.

4.3.4 Minimize exposure—The Apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not being used, the Apparatus should be covered with clean plastic wrap, stored in the clean bench or in a plastic box or glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, non-talc gloves during all operations involving handling of the Apparatus, samples, and blanks. Only clean gloves may touch the Apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the Apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free Apparatus—All Apparatus used for determination of mercury at ambient water quality criteria levels must be nonmetallic, free of material that may contain metals, or both.

4.3.7.1 Construction materials—Only fluoropolymer or glass containers must be used for collection of samples that will be analyzed for mercury because mercury vapors can diffuse in or out of other materials, leading to results that are biased low or high. Polyethylene and/or polypropylene labware may be used for digestion and other purposes because the time of sample exposure to these materials is relatively short. All materials, regardless of construction, that will directly or

indirectly contact the sample, must be known to be clean and free of Hg at the levels specified in this Method before proceeding.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of reusable Apparatus so that contamination can be traced, and logbooks should be maintained to track the sample from the container through the labware to introduction into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters vs. effluents. However, the Apparatus used for processing blanks and standards must be mixed with the Apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the Apparatus used by the sampling team. If there are any indications that the Apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the Apparatus is contaminated. If the Apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before any sampling activity resumes.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing a low concentration of mercury is processed immediately after a sample containing a relatively high concentration of mercury. The Hg concentration at which the analytical system (purge, traps, detector) will carry greater than 0.5 ng/L of Hg into a succeeding bubbler or system blank must be determined by analyzing calibration solutions containing successively larger concentrations of Hg. This test must be run prior to first use of the analytical system and whenever a change is made that would increase the amount of carryover. When a sample contains ½ or greater of this determined Hg concentration, a bubbler blank (bubbler system) or system blank (flow injection system) must be analyzed to demonstrate no carryover at the blank criteria level. For the bubbler system, the blank must be run using the same bubbler and sample trap used to run the high concentration sample. Samples analyzed following a sample that has been determined to result in carryover must be reanalyzed. Samples that are known or suspected to contain the lowest concentration of mercury should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other undiluted samples containing concentrations of mercury greater than 100 ng/L are processed and analyzed. Samples known or suspected to contain Hg concentrations greater than 100 ng/L should be diluted prior to bringing them into the clean room or laboratory dedicated for processing trace metals samples.

4.3.8.3 Contamination by indirect contact—Apparatus that may not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and subsequently transfer the contamination to the sample. It is imperative that every piece of the Apparatus that is directly or indirectly used in the collection, processing, and analysis of water samples be thoroughly cleaned (Section 6.1.2).

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.3.8.5 Contamination from reagents— Contamination can be introduced into samples from method reagents used during processing and analysis. Reagent blanks must be analyzed for contamination prior to use (see Section 9.4.3). If reagent blanks are contaminated, a new batch of reagents must be prepared (see Section 9.4.3.2).

4.4 Interferences

4.4.1 At the time of promulgation of this Method, gold and iodide were known interferences. At a mercury concentration of 2.5 ng/L and at increasing iodide concentrations from 30 to 100 mg/L, test data have shown that mercury recovery will be reduced from 100 to 0 percent. At iodide concentrations greater than 3 mg/L, the sample should be pre-reduced with $SnCl_2$ (to remove the brown color) and additional or more concentrated $SnCl_2$ should be added. To preclude loss of Hg, the additional $SnCl_2$ should be added in a closed vessel or analysis should proceed immediately. If samples containing iodide concentrations greater than 30 mg/L are analyzed, it may be necessary to clean the analytical system with 4N HCl after the analysis (Reference 16.10).

4.4.2 The potential exists for destruction of the gold traps if free halogens are purged onto them, or if they are overheated (>500 °C). When the instructions in this Method are followed, neither of these outcomes is likely.

4.4.3 Water vapor may collect in the gold traps and subsequently condense in the fluorescence cell upon desorption, giving a false peak due to scattering of the excitation radiation. Condensation can be avoided by predrying the gold trap. Traps that tend to absorb large quantities of water vapor should not be used.

4.4.4 The fluorescent intensity is strongly dependent upon the presence of molecular species in the carrier gas that can cause "quenching" of the excited atoms. The dual amalgamation technique eliminates quenching due to trace gases, but it remains the laboratory's responsibility to ensure high purity inert carrier gas and a leak-free analytical train.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this Method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability and nervousness. Organo-mercurials may cause permanent brain damage. Because of the toxicological and physical properties of Hg, pure standards should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase a dilute standard solution of the Hg in this Method. If primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn.

5.2 This Method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current file of OSHA regulations for safe handling of the chemicals specified in this Method. OSHA rules require that a reference file of material safety data sheets (MSDSs) must be made available to all personnel involved in these analyses (29 CFR 1917.28, Appendix E). It also is suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this Method and that the results of this monitoring be made available to the analyst. Personal hygiene monitoring should be performed using OSHA or NIOSH approved personal hygiene monitoring methods. Additional information on laboratory safety can be found in References 16.11-16.14. The references and bibliography included in Reference 16.14 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain concentrations of Hg at µg/L or higher levels are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a safety program for handling Hg.

5.3.1 Facility—When samples known or suspected of containing high concentrations of mercury are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak-tight or in a fume hood demonstrated to have adequate airflow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent from the CVAFS should pass through either a column of activated charcoal or a trap containing gold or sulfur to amalgamate or react mercury vapors.

5.3.7 Waste handling—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Sulfur powder will react with Hg to produce mercuric sulfide, thereby eliminating the possible volatilization of Hg. Satisfactory cleaning may be accomplished by dusting a surface lightly with sulfur powder, then washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washer without contact. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests—A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by this Method can achieve a limit of detection of less than 1 ng per wipe. Less than 0.1 µg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

6.0 Apparatus and Materials

Disclaimer: The mention of trade names or commercial products in this Method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

6.1 Sampling equipment

6.1.1 Sample collection bottles-fluoropolymer or glass, 125- to 1000-mL, with fluoropolymer or fluoropolymer-lined cap.

6.1.2 Cleaning

6.1.2.1 New bottles are cleaned by heating to 65–75 °C in 4 N HCl or concentrated HNO_3 for at least 48 h. The bottles are cooled, rinsed three times with reagent water, and filled with reagent water containing 1% HCl. These bottles are capped and placed in a clean oven at 60-70°C overnight. After cooling, they are rinsed three more times with reagent water, filled with reagent water containing 0.4% (v/v) HCl, and placed in a mercury-free Class-100 clean bench until the outside surfaces are dry. The bottles are tightly capped (with a wrench), double-bagged in new polyethylene zip-type bags until needed, and stored in wooden or plastic boxes until use. The bottles may be shipped to the sampling site containing dilute HCl solution (e.g., 0.04%), containing reagent water, or empty.

6.1.2.2 Used bottles known not to have contained mercury at high (>100 ng/L) levels are cleaned as above, except for only 6–12 h in hot 4 N HCl.

6.1.2.3 Bottle blanks must be analyzed as described in Section 9.4.7. To verify the effectiveness of the cleaning procedures, bottle blanks must be demonstrated to be free of mercury at the ML of this Method.

6.1.2.4 As an alternative to cleaning by the laboratory, bottles may be purchased from a commercial supplier and each lot certified to be clean. Bottles from the lot must be tested as bottle blanks (Section 9.4.7) and demonstrated to be free of mercury at the ML of this Method. If mercury is present above this level in any bottle, either the lot must be rejected or the bottles must be re-cleaned.

6.1.3 Filtration Apparatus

6.1.3.1 Filter—0.45-µm, 15-mm diameter capsule filter (Gelman Supor 12175, or equivalent)

6.1.3.2 Peristaltic pump—115-V a.c., 12-V d.c., internal battery, variable-speed, single-head (Cole-Parmer, portable, "Masterflex L/S," Catalog No. 07570-10 drive with Quick Load pump head, Catalog No. 07021-24, or equivalent).

6.1.3.3 Tubing—styrene/ethylene/butylene/silicone (SEBS) resin for use with peristaltic pump, approx 3/8-in ID by approximately 3 ft (Cole-Parmer size 18, Catalog No. 06424-18, or approximately 1/4-in OD, Cole-Parmer size 17, Catalog No. 06424-17, or equivalent). Tubing is cleaned by soaking in 5–10% HCl solution for 8–24 h, rinsing with reagent water in a clean bench in a clean room, and drying in the clean bench by purging with metal-free air or nitrogen. After drying, the tubing is double-bagged in clear polyethylene bags, serialized with a unique number, and stored until use.

6.2 Equipment for bottle and glassware cleaning

6.2.1 Vat, 100–200 L, high-density polyethylene (HDPE), half filled with 4 N HCl in reagent water.

6.2.2 Panel immersion heater, 500-W, all-fluoropolymer coated, 120 vac (Cole-Parmer H-03053-04, or equivalent)

WARNING: *Read instructions carefully!! The heater will maintain steady state, without temperature feedback control, of 60–75°C in a vat of the size described. However, the equilibrium temperature will be higher (up to boiling) in a smaller vat. Also, the heater plate MUST be maintained in a vertical position, completely submerged and away from the vat walls to avoid melting the vat or burning out!*

6.2.3 Laboratory sink—in Class-100 clean area, with high-flow reagent water (Section 7.1) for rinsing.

6.2.4 Clean bench—Class-100, for drying rinsed bottles.

6.2.5 Oven—stainless steel, in Class-100 clean area, capable of maintaining ± 5°C in the 60–70°C temperature range.

6.3 Cold vapor atomic fluorescence spectrometer (CVAFS): The CVAFS system used may either be purchased from a supplier, or built in the laboratory from commercially available components.

6.3.1 Commercially available CVAFS—Tekran (Toronto, ON) Series 2600 CVAFS, Brooks-Rand (Seattle, WA) Model III CVAFS, Leeman Labs Hydra AF Gold*plus* CVAFS, or equivalent

6.3.2 Custom-built CVAFS (Reference 16.15). Figure 2 shows the schematic diagram. The system consists of the following:

6.3.2.1 Low-pressure 4-W mercury vapor lamp

6.3.2.2 Far UV quartz flow-through fluorescence cell—12 mm x 12 mm x 45 mm, with a 10-mm path length (NSG Cells, or equivalent).

6.3.2.3 UV-visible photomultiplier (PMT)—sensitive to < 230 nm. This PMT is isolated from outside light with a 253.7-nm interference filter (Oriel Corp., Stamford, CT, or equivalent).

6.3.2.4 Photometer and PMT power supply (Oriel Corp. or equivalent), to convert PMT output (nanoamp) to millivolts

6.3.2.5 Black anodized aluminum optical block—holds fluorescence cell, PMT, and light source at perpendicular angles, and provides collimation of incident and fluorescent beams (Frontier Geosciences Inc., Seattle, WA, or equivalent).

6.3.2.6 Flowmeter—with needle valve capable of reproducibly keeping the carrier gas flow rate at 30 mL/min

6.4 Hg purging system—Figure 2 shows the schematic diagram for the purging system. The system consists of the following:

6.4.1 Flow meter/needle valve—capable of controlling and measuring gas flow rate to the purge vessel at 350 ± 50 mL/min.

6.4.2 Fluoropolymer fittings—connections between components and columns are made using 6.4-mm OD fluoropolymer tubing and fluoropolymer friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2-mm OD fluoropolymer tubing because of its greater flexibility.

6.4.3 Acid fume pretrap—10-cm long x 0.9-cm ID fluoropolymer tube containing 2–3 g of reagent grade, nonindicating, 8–14 mesh soda lime chunks, packed between wads of silanized glass wool. This trap is cleaned of Hg by placing on the output of a clean cold vapor generator (bubbler) and purging for 1 h with N_2 at 350 mL/min.

6.4.4 Cold vapor generator (bubbler)—200-mL borosilicate glass (15 cm high x 5.0 cm diameter) with standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the bubbler bottom (Frontier Geosciences, Inc. or equivalent).

6.5 The dual-trap Hg(0) preconcentrating system

6.5.1 Figures 2 and 3 show the dual-trap amalgamation system (Reference 16.5).

6.5.2 Gold-coated sand traps—10-cm long x 6.5-mm OD x 4-mm ID quartz tubing. The tube is filled with 3.4 cm of gold-coated 45/60 mesh quartz sand (Frontier Geosciences Inc., Seattle, WA, or equivalent). The ends are plugged with quartz wool.

6.5.2.1 Traps are fitted with 6.5-mm ID fluoropolymer friction-fit sleeves for making connection to the system. When traps are not in use, fluoropolymer end plugs are inserted in trap ends to eliminate contamination.

6.5.2.2 At least six traps are needed for efficient operation, one as the "analytical" trap, and the others to sequentially collect samples.

6.5.3 Heating of gold-coated sand traps—To desorb Hg collected on a trap, heat for 3.0 min to 450–500 °C (a barely visible red glow when the room is darkened) with a coil consisting of 75 cm of 24-gauge Nichrome wire at a potential of 10-14 vac. Potential is applied and finely adjusted with an autotransformer.

6.5.4 Timers—The heating interval is controlled by a timer-activated 120-V outlet (Gralab, or equivalent), into which the heating coil autotransformer is plugged. Two timers are required, one each for the "sample" trap and the "analytical" trap.

6.5.5 Air blowers—After heating, traps are cooled by blowing air from a small squirrel-cage blower positioned immediately above the trap. Two blowers are required, one each for the "sample" trap and the "analytical" trap.

6.6 Recorder—Any multi-range millivolt chart recorder or integrator with a range compatible with the CVAFS is acceptable. By using a two-pen recorder with pen sensitivity offset by a factor of 10, the dynamic range of the system is extended to 10^3.

6.7 Pipettors—All-plastic pneumatic fixed-volume and variable pipettors in the range of 10 µL to 5.0 mL.

6.8 Analytical balance capable of weighing to the nearest 0.01 g

7.0 Reagents and Standards

Note: The quantities of reagents and the preparation procedures in this section are for illustrative purposes. Equivalent performance may be achievable using quantities of reagents and procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

7.1 Reagent water—18-MΩ minimum, ultrapure deionized water starting from a prepurified (distilled, reverse osmosis, etc.) source. Water should be monitored for Hg, especially after ion exchange beds are changed.

7.2 Air—It is very important that the laboratory air be low in both particulate and gaseous mercury. Ideally, mercury work should be conducted in a new laboratory with mercury-free paint on the walls. A source of air that is very low in Hg should be brought directly into the Class-100 clean bench air intake. If this is not possible, air coming into the clean bench can be cleaned for mercury by placing a gold-coated cloth prefilter over the intake. Gold-coated cloth filter: Soak 2 m^2 of cotton gauze in 500 mL of 2% gold chloride solution at pH 7. In a hood, add 100 mL of 30% $NH_2OH{\cdot}HCl$ solution, and homogenize into the cloth with gloved hands. The material will turn black as colloidal gold is precipitated. Allow the mixture to set for several hours, then rinse

with copious amounts of deionized water. Squeeze-dry the rinsed cloth, and spread flat on newspapers to air-dry. When dry, fold and place over the intake prefilter of the laminar flow hood.

CAUTION: Great care should be taken to avoid contaminating the laboratory with gold dust. This could cause interferences with the analysis if gold becomes incorporated into the samples or equipment. The gilding procedure should be done in a remote laboratory if at all possible.

7.3 Hydrochloric acid—trace-metal purified reagent-grade HCl containing less than 5 pg/mL Hg. The HCl should be analyzed for Hg before use.

7.4 Hydroxylamine hydrochloride—Dissolve 300 g of $NH_2OH \cdot HCl$ in reagent water and bring to 1.0 L. This solution may be purified by the addition of 1.0 mL of $SnCl_2$ solution and purging overnight at 500 mL/min with Hg-free N_2. Flow injection systems may require the use of less $SnCl_2$ for purification of this solution.

7.5 Stannous chloride—Bring 200 g of $SnCl_2 \cdot 2H_2O$ and 100 mL concentrated HCl to 1.0 L with reagent water. Purge overnight with mercury-free N_2 at 500 mL/min to remove all traces of Hg. Store tightly capped.

7.6 Bromine monochloride (BrCl)—In a fume hood, dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Place a clean magnetic stir bar in the bottle and stir for approximately 1 h in the fume hood. Slowly add 38 g reagent grade $KBrO_3$ to the acid while stirring. When all of the $KBrO_3$ has been added, the solution color should change from yellow to red to orange. Loosely cap the bottle, and allow to stir another hour before tightening the lid.

WARNING: *This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl), which are released from the bottle. Add the $KBrO_3$ slowly in a fume hood!*

7.7 Stock mercury standard—NIST-certified 10,000-ppm aqueous Hg solution (NIST-3133). This solution is stable at least until the NIST expiration date.

7.8 Secondary Hg standard—Add approx 0.5 L of reagent water and 5 mL of BrCl solution (Section 7.6) to a 1.00-L Class A volumetric flask. Add 0.100 mL of the stock mercury standard (Section 7.7) to the flask and dilute to 1.00 L with reagent water. This solution contains 1.00 µg/mL (1.00 ppm) Hg. Transfer the solution to a fluoropolymer bottle and cap tightly. This solution is considered stable until the NIST expiration date.

7.9 Working Hg Standard A—Dilute 1.00 mL of the secondary Hg standard (Section 7.8) to 100 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 10.0 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.10 Working Hg Standard B—Dilute 0.10 mL of the secondary Hg standard (Section 7.8) to 1000 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 0.10 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.11 Initial Precision and Recovery (IPR) and Ongoing Precision and Recovery (OPR) solutions—Using the working Hg standard A (Section 7.9), prepare IPR and OPR solutions at a

concentration of 5 ng/L Hg in reagent water. IPR/OPR solutions are prepared using the same amounts of reagents used for preparation of the calibration standards.

7.12 Nitrogen—Grade 4.5 (standard laboratory grade) nitrogen that has been further purified by the removal of Hg using a gold-coated sand trap.

7.13 Argon—Grade 5.0 (ultra high-purity, GC grade) argon that has been further purified by the removal of Hg using a gold-coated sand trap.

8.0 Sample Collection, Preservation, and Storage

8.1 Before samples are collected, consideration should be given to the type of data required (i.e., dissolved or total), so that appropriate preservation and pretreatment steps can be taken. An excess of BrCl should be confirmed either visually (presence of a yellow color) or with starch iodide indicating paper, using a separate sample aliquot, prior to sample processing or direct analysis to ensure the sample has been properly preserved.

8.2 Samples are collected into rigorously cleaned fluoropolymer bottles with fluoropolymer or fluoropolymer-lined caps. Glass bottles may be used if Hg is the only target analyte. It is critical that the bottles have tightly sealing caps to avoid diffusion of atmospheric Hg through the threads (Reference 16.4). Polyethylene sample bottles must not be used (Reference 16.15).

8.3 Collect samples using guidance provided in the Sampling Method (Reference 16.9). Procedures in the Sampling Method are based on rigorous protocols for collection of samples for mercury (References 16.4 and 16.15).

NOTE: *Discrete samplers have been found to contaminate samples with Hg at the ng/L level. Therefore, great care should be exercised if this type of sampler is used. It may be necessary for the sampling team to use other means of sample collection if samples are found to be contaminated using the discrete sampler.*

8.4 Sample filtration—For dissolved Hg, a sample is filtered through a 0.45-µm capsule filter (Section 6.1.3.1) in a mercury-free clean area prior to preservation. If the sample is filtered, it must be accompanied by a blank that has been filtered under the same conditions. The Sampling Method describes sample filtration procedures.

8.5 Preservation—Samples are preserved by adding either 5 mL/L of pretested 12N HCl or 5 mL/L BrCl solution to the sample bottle. If a sample will be used also for the determination of methyl mercury, it should be collected and preserved according to procedures in the method that will be used for determination of methyl mercury (e.g., HCl or H_2SO_4 solution). Preserved samples are stable for up to 90 days of the date of collection.

8.5.1 Samples to be analyzed for total or dissolved Hg only may be shipped to the laboratory unpreserved and unrefrigerated if they are collected in fluoropolymer or glass bottles and capped tightly. Samples must be either preserved or analyzed within 48 hours of collection. If a sample is oxidized in the sample bottle, the time to preservation can be extended to 28 days.

8.5.2 Samples that are acid-preserved may lose Hg to coagulated organic materials in the water or condensed on the walls (Reference 16.16). The best approach is to add BrCl directly to the sample bottle at least 24 hours before analysis. If other Hg species are to be analyzed, these aliquots must be removed prior to the addition of BrCl. If BrCl

cannot be added directly to the sample bottle, the bottle must be shaken vigorously prior to sub-sampling.

8.5.3 Handling of the samples in the laboratory should be undertaken in a mercury-free clean bench, after rinsing the outside of the bottles with reagent water and drying in the clean air hood.

NOTE: *Because of the potential for contamination, it is recommended that filtration and preservation of samples be performed in the clean room in the laboratory. However, if circumstances prevent overnight shipment of samples, samples should be filtered and preserved in a designated clean area in the field in accordance with the procedures given in Method 1669 (Reference 16.9). If filtered in the field, samples ideally should be filtered into the sample bottle.*

8.6 Storage—Sample bottles should be stored in clean (new) polyethylene bags until sample analysis.

8.7 Sample preservation, storage, and holding time requirements also are given at 40 CFR part 136.3(e) Table II.

9.0 Quality Control

9.1 Each laboratory that uses this Method is required to operate a formal quality assurance program (Reference 16.17). The minimum requirements of this program consist of an initial demonstration of laboratory capability, ongoing analysis of standards and blanks as a test of continued performance, and the analysis of matrix spikes (MS) and matrix spike duplicates (MSD) to assess precision and recovery. Laboratory performance is compared to established performance criteria to determine that the results of analyses meet the performance characteristics of the Method.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable accuracy and precision. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted certain options to improve results or lower the cost of measurements. These options include automation of the dual-amalgamation system, single-trap amalgamation (Reference 16.18), direct electronic data acquisition, calibration using gas-phase elemental Hg standards, use of the bubbler or flow-injection systems, or changes in the detector (i.e., CVAAS) when less sensitivity is acceptable or desired. Changes in the determinative technique, such as the use of colorimetry, are not allowed. If an analytical technique other than the CVAFS technique specified in this Method is used, that technique must have a specificity for mercury equal to or better than the specificity of the technique in this Method.

9.1.2.1 Each time this Method is modified, the laboratory is required to repeat the procedure in Section 9.2 to demonstrate that an MDL (40 CFR part 136, Appendix B) less than or equal to one-third the regulatory compliance limit or less than or equal to the MDL of this Method (Table 1), whichever is greater, can be achieved. If the change will affect calibration, the instrument must be recalibrated according to Section 10.

Note: If the compliance limit is greater than the concentration of Hg in the OPR/OPR (5 ng/L), the acceptance criteria for blanks and the concentrations of mercury spiked into quality control samples may be increased to support measurements at the compliance limit. For example, if the compliance limit is 12

ng/L (National Toxics Rule, 40 CFR 131.36), the MDL must be less than or equal to 4 ng/L; concentrations of the calibration standards may be 5, 10, 20, 50 , and 100 ng/L; concentrations of the IPR/OPR samples may be 10 ng/L; spike concentrations and acceptance criteria for MS/MSD samples would remain as specified in Section 9.3; and an appropriate blank acceptance criterion would be 5 ng/L.

9.1.2.2 The laboratory is required to maintain records of modifications made to this Method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and the quality control officer who witnessed and will verify the analyses and modification

9.1.2.2.2 A narrative stating the reason(s) for the modification(s)

9.1.2.2.3 Results from all quality control (QC) tests demonstrating the performance of the modified method, including the following:

(a) Calibration (Section 10)
(b) Initial precision and recovery (Section 9.2.2)
(c) Analysis of blanks (Section 9.4)
(d) Matrix spike/matrix spike duplicate analysis (Section 9.3)
(e) Ongoing precision and recovery (Section 9.5)
(f) Quality control sample (Section 9.6)
(g) Method detection limit (Section 9.2.1)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracking the instrument output to the final result. These data are to include the following:

(a) Sample numbers and other identifiers
(b) Processing dates
(c) Analysis dates
(d) Analysis sequence/run chronology
(e) Sample weight or volume
(f) Copies of logbooks, chart recorder, or other raw data output
(g) Calculations linking raw data to the results reported

9.1.3 Analyses of MS and MSD samples are required to demonstrate the accuracy and precision and to monitor matrix interferences. Section 9.3 describes the procedure and QC criteria for spiking.

9.1.4 Analyses of blanks are required to demonstrate acceptable levels of contamination. Section 9.4 describes the procedures and criteria for analyzing blanks.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample and the quality control sample (QCS) that the system is in control. Sections 9.5 and 9.6 describe these procedures, respectively.

9.1.6 The laboratory shall maintain records to define the quality of the data that are generated. Sections 9.3.7 and 9.5.3 describe the development of accuracy statements.

9.1.7 Quality of the analyses is controlled by an analytical batch. An analytical batch is a set of samples oxidized with the same batch of reagents, and analyzed during the same 12-hour shift. A batch may be from 1 to as many as 20 samples. Each batch must be accompanied by 3 system blanks (Section 9.4.2 for the flow-injection system), a

minimum of 3 bubbler blanks (Section 9.4.1 for the bubbler system), 1 OPR sample at the beginning and end of the batch (Section 9.5), a QCS (Section 9.6), and at least 3 method blanks (Section 9.4.4). In addition, there must be 1 MS and 1 MSD sample for every 10 samples (a frequency of 10%). A typical analytical sequence would be:

(a) Three system blanks (Section 9.4.2) or a minimum of 3 bubbler blanks (Section 9.4.1)
(b) A minimum of five, non-zero calibration standards (Section 10.2.2.1)
(c) On-going precision and recovery (Section 9.5)
(d) Quality control sample (Section 9.6)
(e) Method blank (Section 9.4.4)
(f) Seven samples
(g) Method blank (Section 9.4.4)
(h) Three samples
(i) Matrix spike (Section 9.3)
(j) Matrix spike duplicate (Section 9.3)
(k) Four samples
(l) Method blank (Section 9.4.4)
(m) Six samples
(n) Matrix spike (Section 9.3)
(o) Matrix spike duplicate (Section 9.3)
(p) Ongoing precision and recovery (Section 9.5)

The above sequence includes calibration. If system performance is verified at the end of the sequence using the OPR, analysis of samples and blanks may proceed without recalibration (i.e., the analytical sequence would be entered at Step (d) above), unless more than 12 hours has elapsed since verification of system performance. If more than 12 hours has elapsed, the sequence would be initiated at Step (c) above.

9.2 Initial demonstration of laboratory capability

9.2.1 Method detection limit—To establish the ability to detect Hg, the laboratory shall achieve an MDL that is less than or equal to the MDL listed in Section 1.5 or one-third the regulatory compliance limit, whichever is greater. The MDL shall be determined according to the procedure at 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this Method. This MDL shall be used for determination of laboratory capability only, and should be determined when a new operator begins work or whenever, in the judgment of the laboratory, a change in instrument hardware or operating conditions would dictate reevaluation of capability.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.2.2.1 Analyze four replicates of the IPR solution (5 ng/L, Section 7.11) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (X), and the standard deviation of the percent recovery (s) for Hg.

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the

precision limit or X falls outside the acceptance range, system performance is unacceptable. Correct the problem and repeat the test (Section 9.2.2.1).

9.3 Matrix spike (MS) and matrix spike duplicate (MSD)—To assess the performance of the Method on a given sample matrix, the laboratory must spike, in duplicate, a minimum of 10% (1 sample in 10) from a given sampling site or, if for compliance monitoring, from a given discharge. Therefore, an analytical batch of 20 samples would require two pairs of MS/MSD samples (four spiked samples total).

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of Hg in the sample is being checked against a regulatory compliance limit, the spiking level shall be at that limit or at 1–5 times the background concentration of the sample (as determined in Section 9.3.2), whichever is greater.

9.3.1.2 If the concentration of Hg in a sample is not being checked against a limit, the spike shall be at 1–5 times the background concentration or at 1-5 times the ML in Table 1, whichever is greater.

9.3.2 To determine the background concentration (B), analyze one sample aliquot from each set of 10 samples from each site or discharge according to the procedure in Section 11. If the expected background concentration is known from previous experience or other knowledge, the spiking level may be established *a priori*.

9.3.2.1 If necessary, prepare a standard solution to produce an appropriate level in the sample (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with identical amounts of the spiking solution and analyze these aliquots as described in Section 11.1.2 to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery (R) in each aliquot using the following equation:

$$\%\ R = 100\ \frac{(A-B)}{T}$$

where:

A = *Measured concentration of analyte after spiking*
B = *Measured concentration of analyte before spiking*
T = *True concentration of the spike*

9.3.4 Compare percent recovery (R) with the QC acceptance criteria in Table 2.

9.3.4.1 If results of the MS/MSD are similar and fail the acceptance criteria, and recovery for the OPR standard (Section 9.5) for the analytical batch is within the acceptance criteria in Table 2, an interference is present and the results may not be reported or otherwise used for permitting or regulatory compliance purposes. If the interference can be attributed to sampling, the site or discharge should be resampled. If the interference can be attributed to a method deficiency, the laboratory must modify the method, repeat the test required in Section 9.1.2, and repeat analysis of the sample and MS/MSD. However, during the development

of Method 1631, very few interferences have been noted in the determination of Hg using this Method. (See Section 4.4 for information on interferences.)

9.3.4.2 If the results of both the spike and the OPR test fall outside the acceptance criteria, the analytical system is judged to be not in control, and the results may not be reported or used for permitting or regulatory compliance purposes. The laboratory must identify and correct the problem and reanalyze all samples in the sample batch.

9.3.5 Relative percent difference (RPD)—Compute the RPD between the MS and MSD results according to the following equation using the concentrations found in the MS and MSD. Do not use the recoveries calculated in Section 9.3.3 for this calculation because the RPD is inflated when the background concentration is near the spike concentration.

$$RPD = 200 \times \frac{(|D1-D2|)}{(D1+D2)}$$

Where:
D1 = concentration of Hg in the MS sample
D2 = concentration of Hg in the MSD sample

9.3.6 The RPD for the MS/MSD pair must not exceed the acceptance criterion in Table 2. If the criterion is not met, the system is judged to be out of control. The problem must be identified and corrected, and the MS/MSD and corresponding samples reanalyzed.

9.3.7 As part of the QC program for the laboratory, method precision and recovery for samples should be assessed and records maintained. After analyzing five samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy assessment as a percent recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 90\%$ and $s_r = 10\%$ for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment regularly (e.g., after every five to ten new accuracy measurements).

9.4 Blanks—Blanks are critical to the reliable determination of Hg at low levels. The sections below give the minimum requirements for analysis of blanks. Analysis of additional blanks is recommended as necessary to pinpoint sources of contamination in, and external to, the laboratory.

9.4.1 Bubbler blanks—Bubbler blanks are analyzed to demonstrate that bubbler systems are free from contamination at levels that could affect data quality. At least three bubbler blanks must be run during calibration and with each analytical batch.

9.4.1.1 To analyze a bubbler blank, place a clean gold trap on the bubbler. Purge and analyze previously purged water using the procedure in Section 11, and determine the amount of Hg remaining in the system.

9.4.1.2 If the bubbler blank is found to contain more than 50 pg Hg, the system is out of control. The problem must be investigated and remedied, and the samples run on that bubbler must be reanalyzed. If the blanks from other bubblers contain less than 50 pg Hg, the data associated with those bubblers remain valid, provided that all other criteria in Section 9 also are met.

9.4.1.3 The mean result for all bubbler blanks (from bubblers passing the specification in Section 9.4.1.2) must be < 25 pg (0.25 ng/L) Hg with a standard deviation (n-1) of <10 pg (0.10 ng/L). If the mean is < 25 pg, the average peak area or height is subtracted from all raw data before results are calculated (Section 12.2).

9.4.1.4 If Hg in the bubbler blank exceeds the acceptance criteria in Section 9.4.1.3, the system is out of control. The problem must be resolved and the system recalibrated. Usually, the bubbler blank is too high for one of the following reasons:

(a) Bubblers need rigorous cleaning;
(b) Soda-lime is contaminated; or
(c) Carrier gas is contaminated.

9.4.2 System blanks— System blanks are analyzed to demonstrate that flow injection systems are free from contamination at levels that could affect data quality. Three system blanks must be run during calibration and with each analytical batch.

9.4.2.1 To analyze a system blank, analyze reagent water containing the same amount of reagents used to prepare the calibration standards.

9.4.2.2 If a system blank is found to contain ≥ 0.50 ng/L Hg, the system is out of control. The problem must be investigated and remedied, and the system recalibrated. If the blanks contain < 0.50 ng/L Hg, the data associated with the blanks remain valid, provided that all other criteria in Section 9 also are met.

9.4.2.3 The mean result for the three system blanks must be <0.5 ng/L Hg with a standard deviation (n-1) <0.1 ng/L. If the mean exceeds these criteria, the system is out of control, and the problem must be resolved and the system recalibrated. If the mean is <0.5 ng/L, the average peak height or area is subtracted from all raw data before results are calculated (Section 12.3).

9.4.3 Reagent blanks—Reagent blanks are used to demonstrate that the reagents used to prepare samples for Hg analyses are free from contamination. The Hg concentration in reagent blanks is determined by analyzing the reagent solutions using either the bubbler or flow-injection system. For the bubbler system, reagent may be added directly to previously purged water in the bubbler.

9.4.3.1 Reagent blanks are required when the batch of reagents (bromine monochloride plus hydroxylamine hydrochloride) are prepared. The amount of Hg in a reagent blank containing 0.5% (v/v) BrCl solution (Section 7.6) and 0.2% (v/v) hydroxylamine hydrochloride solution (Section 7.4) must be < 20 pg (0.2 ng/L).

9.4.3.2 The presence of more than 20 pg (0.2 ng/L) of Hg indicates a problem with the reagent solution. The purging of certain reagent solutions, such as $SnCl_2$ or NH_2OH, with mercury-free nitrogen or argon can reduce Hg to acceptable levels. Because BrCl cannot be purified, a new batch must be prepared and tested if the BrCl is contaminated.

9.4.4 Method blanks— Method blanks are used to demonstrate that the analytical system is free from contamination that could otherwise compromise sample results. Method blanks are prepared and analyzed using sample containers, labware, reagents, and analytical procedures identical to those used to prepare and analyze the samples.

9.4.4.1 A minimum of three method blanks per analytical batch are required for both the bubbler and flow-injection systems.

9.4.4.2 If the result for any method blank containing the nominal amount of reagent used to prepare a sample (Section 11.1.1) is found to contain ≥ 0.50 ng/L (50 pg) Hg, the system is out of control. Mercury in the analytical system must be reduced until a method blank is free from contamination at the 0.50 ng/L level. Samples associated with a contaminated method blank must be reanalyzed.

9.4.4.3 Because method blanks are analyzed using procedures identical to those used to analyze samples, any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

9.4.5 Field blanks–Field blanks are used to demonstrate that samples have not been contaminated by the sample collection and transport activities.

9.4.5.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). Analyze the blank immediately before analyzing the samples in the batch.

9.4.5.2 If Hg or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.3 Alternatively, if sufficient multiple field blanks (a minimum of three) are collected, and the average concentration (of the multiple field blanks) plus two standard deviations is equal to or greater than the regulatory compliance limit or equal to or greater than one-half of the level in the associated sample, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.4.6 Equipment blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks on all sampling equipment that will be used to demonstrate that the sampling equipment is free from contamination.

9.4.6.1 Equipment blanks are generated in the laboratory or at the equipment cleaning facility by processing reagent water through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility for low level mercury measurements. If grab samples are to be collected using any ancillary equipment, e.g., an extension pole or a dipper, an equipment blank

is generated by submersing this equipment into the reagent water and analyzing the resulting reagent water collected.

9.4.6.2 The equipment blank must be analyzed using the procedures in this Method. If mercury or any potentially interfering substance is detected in the blank at or above the level specified for the field blank (Section 9.4.5), the source of contamination or interference must be identified, and the problem corrected. The equipment must be demonstrated to be free from mercury and interferences before the equipment may be used in the field.

9.4.7 Bottle blanks— Bottles must be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles (Section 6.1.2) should be filled with reagent water acidified to pH <2 and allowed to stand for a minimum of 24 h. At least 5% of the bottles from a given lot should be tested, and the time that the bottles are allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water must be analyzed for any signs of contamination. If a bottle shows contamination at or above the level specified for the field blank (Section 9.4.5), the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles re-cleaned.

9.5 Ongoing precision and recovery (OPR)—To demonstrate that the analytical system is within the performance criteria of this Method and that acceptable precision and recovery is being maintained within each analytical batch, the laboratory shall perform the following operations:

9.5.1 Analyze the OPR solution (5 ng/L, Section 7.11) prior to the analysis of each analytical batch according to the procedure beginning in Section 11. An OPR also must be analyzed at the end of an analytical sequence or at the end of each 12-hour shift.

9.5.2 Compare the recovery with the limits for ongoing precision and recovery in Table 2. If the recovery is in the range specified, the analytical system is in control and analysis of samples and blanks may proceed. If, however, the concentration is not in the specified range, the analytical process is not in control. Correct the problem and repeat the ongoing precision and recovery test. All reported results must be associated with an OPR that meets the Table 2 performance criteria at the beginning and end of each batch.

9.5.3 The laboratory should add results that pass the specification in Section 9.5.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory also should develop a statement of laboratory data quality by calculating the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 95\%$ and $s_r = 5\%$, the accuracy is 85–105%.

9.6 Quality control sample (QCS) – The laboratory must obtain a QCS from a source different from the Hg used to produce the standards used routinely in this Method (Sections 7.7–7.10). The QCS should be analyzed as an independent check of system performance.

9.7 Depending on specific program requirements, the laboratory may be required to analyze field duplicates and field spikes collected to assess the precision and accuracy of the sampling, sample transportation, and storage techniques. The relative percent difference (RPD) between field duplicates should be less than 20%. If the RPD of the field duplicates exceeds 20%, the laboratory should communicate this to the sampling team so that the source of error can be identified and corrective measures taken before the next sampling event.

10.0 Calibration and Standardization

10.1 Calibration and standardization— Separate calibration procedures are provided for a bubbler system (Section 10.2) and flow-injection system (Section 10.3). Both systems are calibrated using standards traceable to NIST Standard Reference Materials. If system performance is verified at the end of an analytical batch using the OPR, analysis of samples and blanks may proceed without recalibration, unless more than 12 hours has elapsed since verification of system performance.

10.2 Bubbler system calibration

10.2.1 Establish the operating conditions necessary to purge Hg from the bubbler and to desorb Hg from the traps in a sharp peak. Further details for operation of the purge-and-trap, desorption, and analysis systems are given in Sections 11.2.1 and 11.2.2.

10.2.2 The calibration must contain a minimum of five non-zero points and the results of analysis of three bubbler blanks. The lowest calibration point must be at the Minimum Level (ML).

NOTE: *The purge efficiency of the bubbler system is 100% and is independent of volume at the volumes used in this Method. Calibration of this system is typically performed using units of mass. For purposes of working in concentration, the volume is assumed to be 100 mL.*

10.2.2.1 Standards are analyzed by the addition of aliquots of Hg working standard A (Section 7.9) and Hg working standard B (Section 7.10) directly into the bubblers. Add 0.50 mL of working standard B and 0.5 mL $SnCl_2$ to the bubbler. Swirl to produce a standard containing 50 pg of Hg (0.5 ng/L). Purge under the optimum operating conditions (Section 10.2.1). Sequentially follow with the addition of aliquots of 0.05, 0.25, 0.50 and 1.0 mL of working standard A to produce standards of 500, 2500, 5000, and 10,000 pg Hg (5.0, 25.0, 50.0 and 100.0 ng/L).

NOTE: *If calibration to the higher levels results in carryover (Section 4.3.8.1), calibrate the system across a narrower range (Section 10.4)*

10.2.2.2 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for each peak.

10.2.2.3 Prepare and analyze a minimum of 3 bubbler blanks. If multiple bubblers are used, there must be 1 bubbler blank per bubbler (to a maximum of 4 bubblers). Calculate the mean peak area or height for the bubbler blanks.

10.2.2.4 For each calibration point, subtract the mean peak height or area of the bubbler blanks from the peak height or area for each standard. Calculate the calibration factor (CF_x) for Hg in each of the five standards using the mean bubbler-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\overline{A}_{BB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*

$\overline{A}_{BB}$ = *mean peak height or area for Hg in bubbler blank*

C_x = *mass in standard analyzed (ng)*

10.2.2.5 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.2.2.6 If RSD ≤ 15%, calculate the recovery for the lowest standard using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.2.2.7 Calculate the concentration of Hg in the bubbler blanks (Section 10.2.2.1) using CF_m. The bubbler blanks must meet the criteria in Section 9.4.1; otherwise, mercury in the system must be reduced and the calibration repeated until the bubbler blanks meet the criteria.

10.3 Flow-injection system calibration

10.3.1 Establish the operating conditions necessary to purge Hg from the gas-liquid separator and dryer tube and desorb Hg from the traps in a sharp peak. Further details for operating the analytical system are given in Section 11.2.1.

10.3.2 The calibration must contain a minimum of 5 non-zero points and the results of analysis of 3 system blanks. The lowest calibration point must be at the minimum level (ML).

10.3.2.1 Place 25-30 mL of reagent water and 250 µL of concentrated BrCl solution (Section 7.6) in each of 5 calibrated 50-mL autosampler vials. Prepare the 0.5 ng/L calibration standard by adding 250 µL of working standard B (Section 7.10) to the vial. Dilute to the mark with reagent water. Sequentially follow with the addition of aliquots of 25, 125, 250 and 500 µL of working standard A (Section 7.9) to produce standards of 5.0, 25.0, 50.0 and 100.0 ng/L, respectively. Cap the vials and invert once to mix.

10.3.2.2 Immediately prior to analysis, remove the caps and add 125 µL of NH_2OH solution (Section 7.4). Re-cap, invert once to mix, and allow to stand until the yellow color disappears. Remove all caps and place vials into the analysis rack.

10.3.2.3 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for the Hg peak.

10.3.2.4 Prepare and analyze a minimum of 3 system blanks and tabulate the peak heights or areas. Calculate the mean peak area or height for the system blanks.

10.3.2.5 For each calibration point, subtract the mean peak height or area of the system blanks (Section 9.4.2) from the peak height or area for each standard. Calculate

the calibration factor (CF_x) for Hg in each of the five standards using the mean reagent-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\bar{A}_{SB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\bar{A}_{SB}$ = *mean peak height or area for Hg in calibration blanks*
C_x = *concentration of standard analyzed (ng/L)*

10.3.2.6 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.3.2.7 If RSD ≤ 15%, calculate the recovery for the lowest standard (0.5 ng/L) using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples, blanks, and OPRs. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.3.2.8 Calculate the concentration of Hg in the system blanks (Section 9.4.2) using CF_m. The system blanks must meet the criteria in Section 9.4.2; otherwise, mercury in the system must be reduced and the calibration repeated until the system blanks meet the criteria.

10.4 Calibration to a range other than 0.5 to 100 ng/L—This Method may be calibrated to a range other than 0.5 to 100 ng/L, provided that the following requirements are met:

(a) There must be a minimum of five non-zero calibration points.
(b) The difference between successive calibration points must be no greater than a factor of 10 and no less than a factor of 2 and should be approximately evenly spaced on a logarithmic scale over the calibration range.
(c) The relative standard deviation (RSD) of the calibration factors for all calibration points must be less than 15%.
(d) The calibration factor for any calibration point at a concentration greater than 100 ng/L must be within ±15% of the average calibration factor for the points at or below 100 ng/L.
(e) The calibration factor for any point <0.5 ng/L must be within 25% of the average calibration factor for all points.
(f) If calibration is to a higher range and this Method is used for regulatory compliance, the ML must be less than one-third the regulatory compliance limit

11.0 Procedure

NOTE: *The following procedures for analysis of samples are provided as guidelines. Laboratories may find it necessary to optimize the procedures, such as drying time or potential applied to the Nichrome wires, for the laboratory's specific instrument set-up.*

11.1 Sample Preparation

11.1.1 Pour a 100-mL aliquot from a thoroughly shaken, acidified sample, into a 125-mL fluoropolymer bottle. If BrCl was not added as a preservative (Section 8.5), add the amount of BrCl solution (Section 7.6) given below, cap the bottle, and digest at room temperature for a 12 h minimum.

11.1.1.1 For clear water and filtered samples, add 0.5 mL of BrCl; for brown water and turbid samples, add 1.0 mL of BrCl. If the yellow color disappears because of consumption by organic matter or sulfides, more BrCl should be added until a permanent (12-h) yellow color is obtained.

11.1.1.2 Some highly organic matrices, such as sewage effluent, will require high levels of BrCl (e.g., 5 mL/100 mL of sample) and longer oxidation times, or elevated temperatures (e.g., place sealed bottles in oven at 50 °C for 6 h). The oxidation must be continued until it is complete. Complete oxidation can be determined by either observation of a permanent yellow color remaining in the sample or the use of starch iodide indicating paper to test for residual free oxidizer. The sample also may be diluted to reduce the amount of BrCl required, provided that the resulting level of mercury is sufficient for reliable determination.

11.1.2 Matrix spikes and matrix spike duplicates—For every 10 or fewer samples, pour 2 additional 100-mL aliquots from a selected sample (see Section 9.3), spike at the level specified in Section 9.3, and process in the same manner as the samples. There must be a minimum of 2 MS/MSD pairs for each analytical batch of 20 samples.

11.2 Hg reduction and purging—Separate procedures are provided for the bubbler system (Section 11.2.1) and flow-injection (Section 11.2.2).

11.2.1 Hg reduction and purging for the bubbler system

11.2.1.1 Add 0.2-0.25 mL of NH_2OH solution to the BrCl-oxidized sample in the 125-mL sample bottle. Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 min with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.1.2 Connect a fresh trap to the bubbler, pour the reduced sample into the bubbler, add 0.5 mL of $SnCl_2$ solution, and purge the sample onto a gold trap with N_2 at 350 ± 50 mL/min for 20 min.

11.2.1.3 When analyzing Hg samples, the recovery is quantitative, and organic interferents are destroyed. Thus, standards, bubbler blanks, and small amounts of high-level samples may be run directly in previously purged water. After very high samples (Section 4.3.8.1), a small degree of carryover (<0.01%) may occur. Bubblers that contain such samples must be demonstrated to be clean prior to proceeding with low level samples. Samples run immediately following a sample that has been determined to result in carryover must be reanalyzed using a bubbler that is demonstrated to be clean as per Section 4.3.8.1.

11.2.2 Hg reduction and purging for the flow-injection system

11.2.2.1 Add 0.2-0.25 mL of NH_2OH solution (Section 7.4) to the BrCl-oxidized sample in the 125-mL sample bottle or in the autosampler tube (the amount of NH_2OH required will be approximately 30 percent of the BrCl volume). Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 minutes with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.2.2 Pour the sample solution into an autosampler vial and place the vial in the rack.

11.2.2.3 Carryover may occur after analysis of a sample containing a high level of mercury. Samples run immediately following a sample that has been determined to result in carryover (Section 4.3.8.1) must be reanalyzed using a system demonstrated to be clean as per Section 4.3.8.1.

11.3 Desorption of Hg from the gold trap

11.3.1 Remove the sample trap from the bubbler, place the Nichrome wire coil around the trap and connect the trap into the analyzer train between the incoming Hg-free argon and the second gold-coated (analytical) sand trap (Figure 2).

11.3.2 Pass argon through the sample and analytical traps at a flow rate of approximately 30 mL/min for approximately 2 min to drive off condensed water vapor.

11.3.3 Apply power to the coil around the sample trap for 3 minutes to thermally desorb the Hg (as Hg(0)) from the sample trap onto the analytical trap.

11.3.4 After the 3-min desorption time, turn off the power to the Nichrome coil, and cool the sample trap using the cooling fan.

11.3.5 Turn on the chart recorder or other data acquisition device to start data collection, and apply power to the Nichrome wire coil around the analytical trap. Heat the analytical trap for 3 min (1 min beyond the point at which the peak returns to baseline).

11.3.6 Stop data collection, turn off the power to the Nichrome coil, and cool the analytical trap to room temperature using the cooling fan.

11.3.7 Place the next sample trap in line and proceed with analysis of the next sample.

NOTE: *Do not heat a sample trap while the analytical trap is still warm; otherwise, the analyte may be lost by passing through the analytical trap.*

11.4 Peaks generated using this technique should be very sharp and almost symmetrical. Mercury elutes at approximately 1 minute and has a width at half-height of about 5 seconds.

11.4.1 Broad or asymmetrical peaks indicate a problem with the desorption train, such as improper gas flow rate, water vapor on the trap(s), or an analytical trap damaged by chemical fumes or overheating.

11.4.2 Damage to an analytical trap is also indicated by a sharp peak, followed by a small, broad peak.

11.4.3 If the analytical trap has been damaged, the trap and the fluoropolymer tubing downstream from it should be discarded because of the possibility of gold migration onto downstream surfaces.

11.4.4 Gold-coated sand traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded.

12.0 Data Analysis and Calculations

12.1 Separate procedures are provided for calculation of sample results using the bubbler system (Section 12.2) and the flow-injection system (Section 12.3), and for method blanks (Section 12.4).

12.2 Calculations for the bubbler system

12.2.1 Calculate the mean peak height or area for Hg in the bubbler blanks measured during system calibration or with the analytical batch (A_{BB}; n = 3 minimum).

12.2.2 Calculate the concentration of Hg in ng/L (parts-per-trillion; ppt) in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{A_s - \overline{A}_{BB}}{CF_m \times V}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{BB}$ = *peak height (or area) for Hg in bubbler blank*
CF_m = *mean calibration factor (Section* 10.2.2.5)
V = *Volume of sample (L)*

12.3 Calculations for the flow-injection system

12.3.1 Calculate the mean peak height or area for Hg in the system blanks measured during system calibration or with each analytical batch (A_{SB}; n = 3)

12.3.2 Calculate the concentration of Hg in ng/L in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{(A_s - \overline{A}_{SB})}{CF_m} \times \frac{V_{std}}{V_{sample}}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{SB}$ = *mean peak height (or area) for Hg in system blanks*
CF_m = *mean calibration factor (Section* 10.3.2.6)
V_{std} = *volume (mL) used for standards – volume (mL) reagent used in standards*
V_{sample} = *volume (mL) of sample – volume (mL) reagent used in sample*

12.4 Calculations for concentration of Hg in method blanks, field blanks, and reagent blanks.

12.4.1 Calculate the concentration of Hg in the method blanks (C_{MB}), field blanks (C_{FB}), or reagent blanks (C_{RB}) in ng/L, using the equation in Section 12.2.2 (if bubbler system is used) or Section 12.3.2 (if flow injection system is used) and substituting the peak height or area resulting from the method blank, field blank, or reagent blank for A_s.

12.4.2 Determine the mean concentration of Hg in the method blanks associated with the analytical batch (a minimum of three). If a sample requires additional reagent(s) (e.g., BrCl), a corresponding method blank containing an identical amount of reagent must be analyzed (Section 9.4.4.3). The concentration of Hg in the corresponding method blank may be subtracted from the concentration of Hg in the sample per Section 12.5.2.

12.5 Reporting

12.5.1 Report results for Hg at or above the ML, in ng/L, to three significant figures. Report results for Hg in samples below the ML as <0.5 ng/L, or as required by the regulatory authority or in the permit. Report results for Hg in reagent blanks and field blanks at or above the ML, in ng/L, to three significant figures. Report results for Hg in reagent blanks, method blanks, or field blanks below the ML but at or above the MDL to two significant figures. Report results for Hg not detected in reagent blanks, method blanks, or field blanks as <0.2 ng/L, or as required by the regulatory authority or in the permit.

12.5.2 Report results for Hg in samples, method blanks and field blanks separately. In addition to reporting results for the samples and blank(s) separately, the concentration of Hg in the method blanks or field blanks associated with the sample may be subtracted from the results for that sample, or must be subtracted if requested or required by a regulatory authority or in a permit.

12.5.3 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results.

13.0 Method Performance

13.1 This Method was tested in 12 laboratories using reagent water, freshwater, marine water and effluent (Reference 16.19). The quality control acceptance criteria listed in Table 2 were verified by data gathered in the interlaboratory study, and the method detection limit (MDL) given in Section 1.5 was verified in all 12 laboratories. In addition, the techniques in this Method have been compared with other techniques for low-level mercury determination in water in a variety of studies, including ICES-5 (Reference 16.20) and the International Mercury Speciation Intercomparison Exercise (Reference 16.21).

13.2 Precision and recovery data for reagent water, freshwater, marine water, and secondary effluent are given in Table 3.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When it is not feasible to reduce wastes at the source, the Agency recommends recycling as the next best option. The acids used in this Method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this Method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

15.2 Acids, samples at pH <2, and BrCl solutions must be neutralized before being disposed of, or must be handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

16.1 Bloom, Nicolas, Draft "Total Mercury in Aqueous Media," Frontier Geosciences, Inc., September 7, 1994.

16.2 Fitzgerald, W.F.; Gill, G.A. "Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis," *Anal. Chem.* 1979, *15*, 1714.

16.3 Bloom, N.S; Crecelius, E.A. "Determination of Mercury in Sea water at Subnanogram per Liter Levels," *Mar. Chem.* 1983, *14*, 49.

16.4 Gill, G.A.; Fitzgerald, W.F. "Mercury Sampling of Open Ocean Waters at the Picogram Level," *Deep Sea Res* 1985, *32*, 287.

16.5 Bloom, N.S.; Fitzgerald, W.F. "Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection," *Anal. Chim. Acta.* 1988, *208*, 151.

16.6 Guidance on Establishing Trace Metal Clean Rooms in Existing Facilities, U.S. EPA, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, January 1996, EPA 821-B-96-001.

16.7 Trace Metal Cleanroom, prepared by Research Triangle Institute for U.S. Environmental Protection Agency, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, RTI/6302/04-02 F.

16.8 Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring, U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, July 1996, EPA 821-B-96-004.

16.9 Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, April 1995 with January 1996 revisions.

16.10 Correspondence from Nicolas Bloom, Frontier Geosciences, Inc. to Dale Rushneck, Interface, Inc., December 31, 1998.

16.11 "Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service. Centers for Disease Control. NIOSH Publication 77-206, Aug. 1977, NTIS PB-277256.

16.12 "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910.

16.13 "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

16.14 "Standard Methods for the Examination of Water and Wastewater," 18th ed. and later revisions, American Public Health Association, 1015 15th Street NW, Washington, DC 20005. 1-35: Section 1090 (Safety), 1992.

16.15 Bloom, N.S. "Trace Metals & Ultra-Clean Sample Handling," *Environ. Lab.* 1995, *7*, 20.

16.16 Bloom, N.S. "Influence of Analytical Conditions on the Observed 'Reactive Mercury,' Concentrations in Natural Fresh Waters," In *Mercury as a Global Pollutant*; Huckabee, J. and Watras, C.J., Eds.; Lewis Publishers, Ann Arbor, MI: 1994.

16.17 "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," U.S. Environmental Protection Agency. Environmental Monitoring Systems Laboratory, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

16.18 Liang, L.; Bloom, N.S. "Determination of Total Mercury by Single-Stage Gold Amalgamation with Cold Vapor Atom Spectrometric Detection," *J. Anal. Atomic Spectrom.* 1993, *8*, 591.

16.19 "Results of the EPA Method 1631 Validation Study," February, 1998. Available from the EPA Sample Control Center, 6101 Stevenson Avenue, Alexandria, VA, 22304; 703/461-2100.

16.20 Cossa, D.; Couran, P. "An International Intercomparison Exercise for Total Mercury in Sea Water," *App. Organomet. Chem.* 1990, *4*, 49.

16.21 Bloom, N.S.; Horvat, M.; Watras, C.J. "Results of the International Mercury Speciation Intercomparison Exercise," *Wat. Air. Soil Pollut.*, 1995, 80, 1257.

17.0 Glossary

The definitions and purposes below are specific to this Method, but have been conformed to common usage as much as possible.

17.1 **Ambient Water**—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

17.2 **Analytical Batch**—A batch of up to 20 samples that are oxidized with the same batch of reagents and analyzed during the same 12-hour shift. Each analytical batch must also include at least three bubbler blanks, an OPR, and a QCS. In addition, MS/MSD samples must be prepared at a frequency of 10% per analytical batch (one MS/MSD for every 10 samples).

17.3 **Bottle Blank**—The bottle blank is used to demonstrate that the bottle is free from contamination prior to use. Reagent water known to be free of mercury at the MDL of this Method is added to a bottle, acidified to pH <2 with BrCl or HCl, and allowed to stand for a minimum of 24 hours. The time that the bottle is allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water is analyzed.

17.4 **Bubbler Blank**—For this Method, the bubbler blank is specific to the bubbler system and is used to determine that the analytical system is free from contamination. After analysis of a standard, blank, or sample, the solution in the bubbler is purged and analyzed. A minimum of three bubbler blanks is required for system calibration.

17.5 **Equipment Blank**—Reagent water that has been processed through the sampling device at a laboratory or other equipment cleaning facility prior to shipment of the sampling equipment to the sampling site. The equipment blank is used to demonstrate that the sampling equipment is free from contamination prior to use. Where appropriate, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility.

17.6 **Field Blank**—Reagent water that has been transported to the sampling site and exposed to the same equipment and operations as a sample at the sampling site. The field blank is used to demonstrate that the sample has not been contaminated by the sampling and sample transport systems.

17.7 **Intercomparison Study**—An exercise in which samples are prepared and split by a reference laboratory, then analyzed by one or more testing laboratories and the reference laboratory. The intercomparison, with a reputable laboratory as the reference laboratory, serves as the best test of the precision and accuracy of the analyses at natural environmental levels.

17.8 **Matrix Spike (MS) and Matrix Spike Duplicate (MSD)**—Aliquots of an environmental sample to which known quantities of the analyte(s) of interest is added in the laboratory. The MS and MSD are analyzed exactly like a sample. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for these background concentrations.

17.9 **May**—This action, activity, or procedural step is allowed but not required.

17.10 **May not**—This action, activity, or procedural step is prohibited.

17.11 **Method blank**— Method blanks are used to determine the concentration of mercury in the analytical system during sample preparation and analysis, and consist of a volume of reagent water that is carried through the entire sample preparation and analysis. Method blanks are prepared by placing reagent water in a sample bottle and analyzing the water using reagents and procedures identical to those used to prepare and analyze the corresponding samples. A minimum of three method blanks is required with each analytical batch.

17.12 **Minimum Level (ML)**—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to (1, 2, or 5) x 10^n, where n is an integer (See Section 1.5).

17.13 **Must**—This action, activity, or procedural step is required.

17.14 **Quality Control Sample (QCS)**—A sample containing Hg at known concentrations. The QCS is obtained from a source external to the laboratory, or is prepared from a source of standards different from the source of calibration standards. It is used as an independent check of instrument calibration.

17.15 **Reagent blank**—Reagent blanks are used to determine the concentration of mercury in the reagents (BrCl, $NH_2OH{\cdot}HCl$, and $SnCl_2$) that are used to prepare and analyze the samples. In this Method, reagent blanks are required when each new batch of reagents is prepared.

17.16 **Reagent Water**—Water demonstrated to be free of mercury at the MDL of this Method. It is prepared from 18 MΩ ultrapure deionized water starting from a prepurified source. Reagent water is used to wash bottles, as trip and field blanks, and in the preparation of standards and reagents.

17.17 **Regulatory Compliance Limit**—A limit on the concentration or amount of a pollutant or contaminant specified in a nationwide standard, in a permit, or otherwise established by a regulatory authority.

17.18 **Shall**—This action, activity, or procedure is required.

17.19 **Should**—This action, activity, or procedure is suggested, but not required.

17.20 **Stock Solution**— A solution containing an analyte that is prepared from a reference material traceable to NIST, or a source that will attest to the purity and authenticity of the reference material.

17.21 **System Blank**— For this Method, the system blank is specific for the flow-injection system and is used to determine contamination in the analytical system and in the reagents used to prepare the calibration standards. A minimum of three system blanks is required during system calibration.

17.22 **Ultraclean Handling**— A series of established procedures designed to ensure that samples are not contaminated during sample collection, storage, or analysis.

18.0 Tables and Figures

Table 1

Lowest Ambient Water Quality Criterion for Mercury and the Method Detection Limit and Minimum Level of Quantitation for EPA Method 1631

Metal	Lowest Ambient Water Quality Criterion[1]	Method Detection Limit (MDL) and Minimum Level (ML)	
		MDL[2]	ML[3]
Mercury (Hg)	1.3 ng/L	0.2 ng/L	0.5 ng/L

1. Lowest water quality criterion for the Great Lakes System (Table 4, 40 CFR 132.6). The lowest Nationwide criterion is 12 ng/L (40 CFR 131.36).
2. Method detection limit (40 CFR 136, Appendix B)
3. Minimum level of quantitation (see Glossary)

Table 2

Quality Control Acceptance Criteria for Performance Tests in EPA Method 1631

Acceptance Criteria	Section	Limit (%)
Initial Precision and Recovery (IPR)	9.2.2	
Precision (RSD)	9.2.2.3	21
Recovery (X)	9.2.2.3	79-121
Ongoing Precision and Recovery (OPR)	9.5.2	77-123
Matrix Spike/Matrix Spike Duplicate (MS/MSD)	9.3	
Recovery	9.3.4	71-125
Relative Percent Difference (RPD)	9.3.5	24

Table 3

Precision and Recovery for Reagent Water, Fresh Water, Marine Water, and Effluent Water Using Method 1631

Matrix	*Mean Recovery (%)	*Precision (% RSD)
Reagent Water	98.0	5.6
Fresh Water (Filtered)	90.4	8.3
Marine Water (Filtered)	92.3	4.7
Marine Water (Unfiltered)	88.9	5.0
Secondary Effluent (Filtered)	90.7	3.0
Secondary Effluent (Unfiltered)	92.8	4.5

*Mean percent recoveries and RSDs are based on expected Hg concentrations.

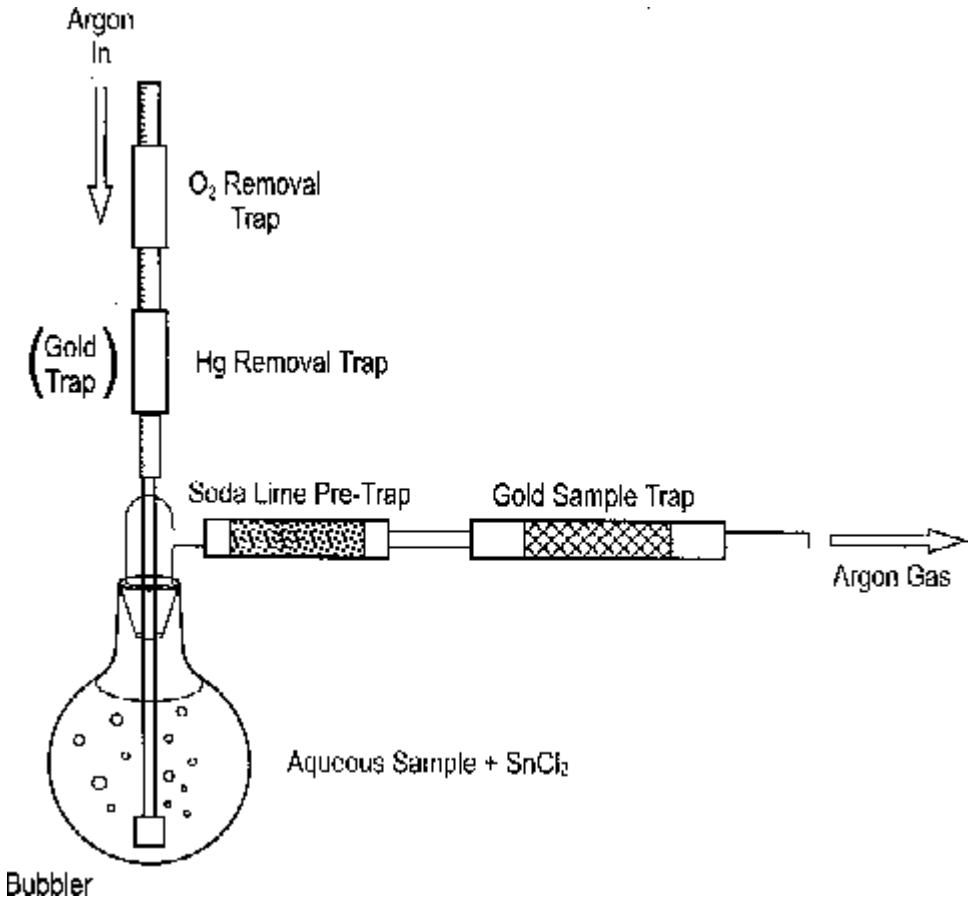


Figure 1. Schematic Diagram of Bubbler Setup

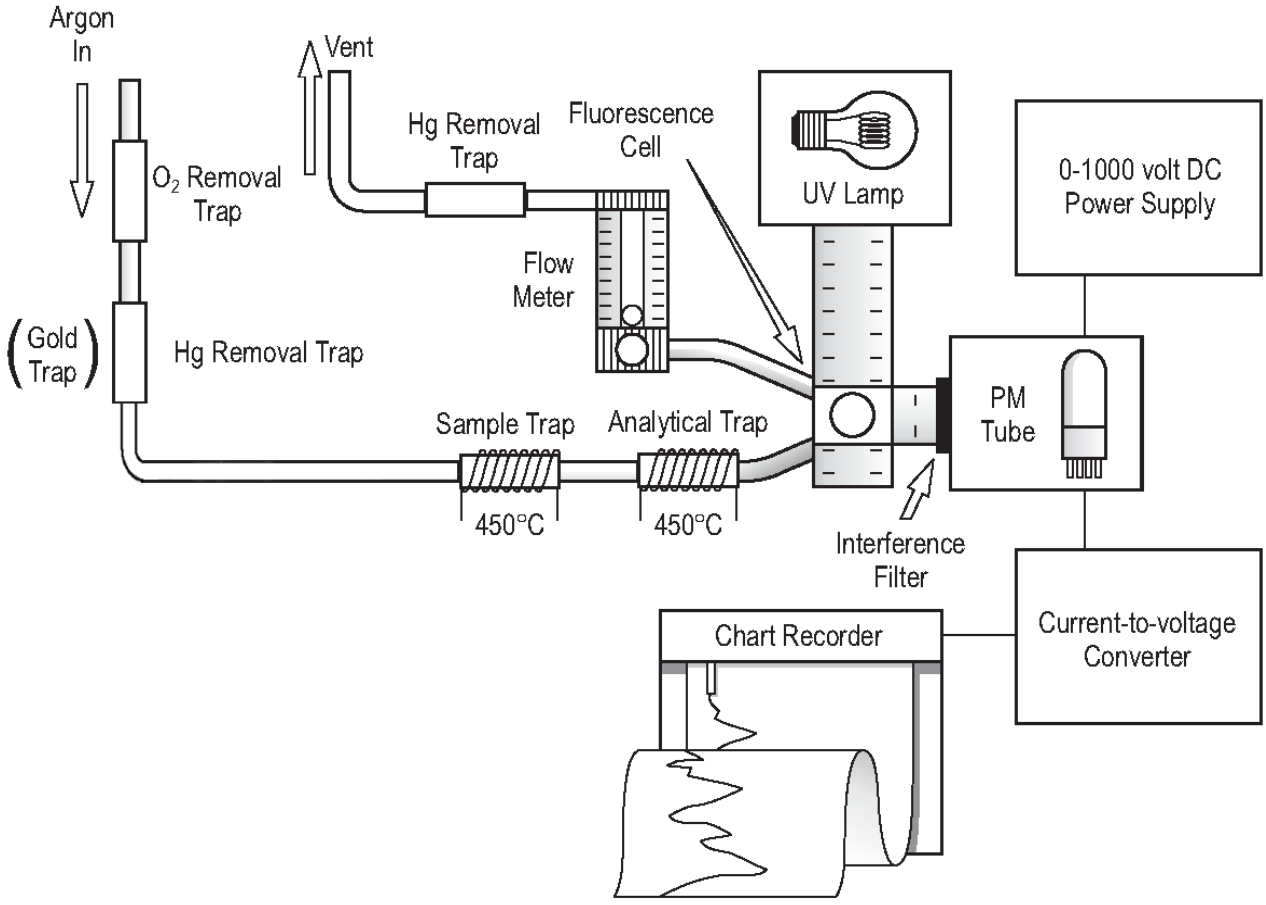


Figure 2. Schematic Diagram of the Bubbler, Purge and Trap, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

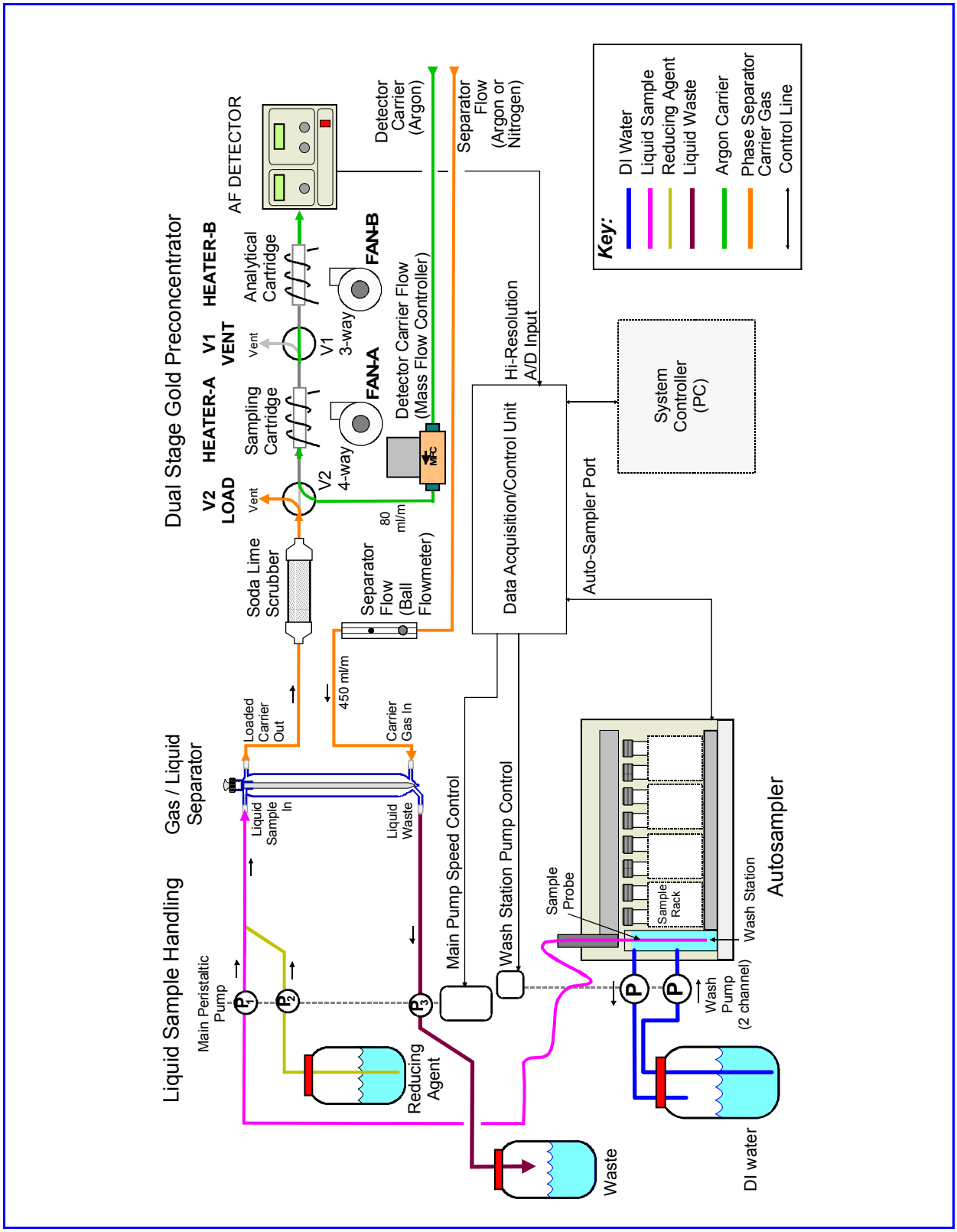


Figure 3. Schematic Diagram of the Flow-Injection, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

APPENDIX B

ANALYTICAL LABORATORY SOPS FOR PERFORMING METHOD 1631, REVISION E

1) Revision Log:

Revision: 11	**Effective Date:** This version	
Section	**Justification**	**Changes**
2.4, 2.5	Required	Updated references
6.5	Reword and added reference to total solids SOP	Reword and added reference to total solids SOP
8.1	Removed	Removed Matrix Duplicate requirement
8.8	Removed	Removed 8.8, then re-numbered 8.9 to 8.13
10.5	Delete	Deleted repetitive statement "May cause severe digestive tract irritation with possible burn"
11.2	Corrected	Changed analysis to analyst
11.6	Update	Added "training" after hazardous goods
12.4	Removed	Removed comment about samples containing elemental mercury
13.7	Update	Replaced disposable spatula with Tongue depressors and/or paper scoops
13.9	Update	Replaced centrifuging with centrifuge
15.2.1	Removed	Removed Matrix Duplicate requirement
17.1	Required	Referenced new MDL requirements.
18.5	Removed	Removed 18.5, then re-numbered 18.6 to 18.8

2) Reference:

2.1 Chemical Hygiene Plan, Eurofins Frontier Global Sciences, current version.

2.2 EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, 2002.

2.3 Appendix to Method 1631 - Total Mercury in Tissue, Sludge, Sediment and Soil by Acid Digestion and BrCl Oxidation, 2001.

2.4 TNI Environmental Laboratory Sector, Vol 1, Management and Technical Requirements, ELV1-2016.

2.5 Department of Defense / Department of Energy Consolidated Quality Systems Manual for Environmental Laboratories, prepared by DoD Environmental Data Quality Workgroup (EDQW) and DOE Consolidate Audit Program (DOECAP) Operations Team, Version 5.1.1, February 2018.

3) Cross Reference:

Document	Document Title
EFQA-Q-QM-QM5805	Quality Manual
EFQA-Q-QD-SOP10098	Procedures for Determining IDLs, MDLs, LODs, LOQs, OPRs, IQs, OQs, and PQs
EFQA-R-EQ-SOP2711	Pipette and volumetric dispenser Verification, Calibration and Maintenance
EFSR-S-CS-SOP2794	Ultra Clean Aqueous Sample Collection
EFQA-P-DR-SOP2801	Data Review and Validation
EFHS-S-HS-SOP2991	Waste Disposal Procedure for Client Sample Waste
EFAFS-T-AFS-SOP2821	HF/HNO_3 /HCl Bomb Digestion of Sediments, Soils, Rocks, and Other Solids for Mercury, followed by Repeated HNO_3 Evaporation for other Metals
EFAFS-T-AFS-SOP2822	Determination of Total Mercury in Various Matrices by Flow Injection Atomic Fluorescence Spectrometry (EPA Method 1631E)

4) Purpose:

4.1 The purpose of this Standard Operating Procedure (SOP) is to describe the method for digesting geological samples (sediments and soils) and carbon (coal) samples prior to analysis for total mercury.

5) Scope:

5.1 This is a strong acid digestion procedure for the preparation of sediments, soils, and other types of solid materials prior to analysis for total mercury. If other metals besides Hg are to be analyzed as well, the preferred digestion is the HF/HNO_3 bomb digestion method (SOP *EFAFS-T-AFS-SOP2821*, "HF/HNO_3 /HCl Bomb Digestion of Sediments, Soils, Rocks, and Other Solids for Mercury, followed by Repeated HNO_3 Evaporation for other Metals"). Modified aqua regia is particularly capable of solubilizing cinnabar (HgS).

6) Basic Principles:

6.1 Modified aqua regia is not simply a mixture of the two acids. Rather, the oxidizing action of concentrated HNO_3 on HCl results in the formation of nitrosyl chloride (NOCl) and free Cl_2, which are particularly strong oxidizers for noble metals and metal-sulfide minerals. Modified aqua regia only exists as a concentrated acid species—dilution of modified aqua regia with water destroys its unique oxidizing capability, rendering it a simple mixture of HNO_3 and HCl. Furthermore, modified aqua regia loses its strength rapidly after preparation because of the loss of Cl_2 to the atmosphere. Modified aqua regia must therefore always be prepared fresh at the time of use.

6.2 This method involves leaching the sample overnight with modified aqua regia (4:1 HCl/HNO_3) at room temperature.

6.3 This procedure must be performed in a fume hood due to the copious quantities of noxious fumes, including, but not limited to, Cl_2, which are generated during this process.

6.4 Prior to digestion, samples must be homogenized as thoroughly as possible.

6.5 If dry weight correction is required, a separate aliquot of the samples are accurately weighed into small aluminum foil weighing dishes, dried for at least 12 hours at 103-105°C, and then weighed again according to *EFAFS-T-AFS-SOP5133*.

7) Reference Modifications:

7.1 No significant modifications were made to this method.

8) Definitions:

8.1 Batch – no more than 20 client samples grouped for preparation. 3 Preparation Blanks, 1 CRM or 1 LCS/LCSD (or BS/BSD) set are prepared per every 20 samples; 1 MS/MSD set is prepared for every 10 samples.

8.2 Celsius (C), conversion of Celsius to Fahrenheit: (C * 1.8) + 32.

8.3 Fahrenheit (F), conversion of Fahrenheit to Celsius: (F - 32) * 5/9

8.4 Method Detection Limit (MDL) – the limit derived from an exercise as described in 40 CFR, Part 136, Appendix B. The exercise produces a defined value that is the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero from a given matrix.

8.5 Certified Reference Material (CRM) – a standard of known composition that is certified by a recognized authority and representing a sample matrix. It is used to verify the accuracy of a method.

8.6 Blank Spike (BS) and Blank Spike Duplicate (BSD), is a QC sample containing known concentrations of the analytes of interest that is taken through the entire preparation and analysis process in the same manner as the samples to monitor complete method performance.

8.7 Method or Preparation Blank (BLK) – Method blanks consist of the same reagents used to digest the samples, in the same volume or proportion, and are carried through the complete sample preparation and analytical procedure.

8.8 Matrix Spike (MS) and Matrix Spike Duplicate (MSD) – a representative sample is selected and should be spiked with a secondary source at *two* to five times the ambient concentration or at five times the MRL, whichever is greater These QC samples will indicate sample matrix effects on the analytes of interest.

8.9 May: This action, activity, or procedural step is optional.

8.10 May Not: This action, activity, or procedural step is prohibited.

8.11 Shall: This action, activity, or procedure is required.

8.12 Should: This action, activity, or procedure is suggested, but not required.

9) Interferences:

9.1 All free halogens in a sample must be reduced prior to purging onto gold traps, or the traps will be destroyed. For small aliquots of digest, this is accomplished by adding $SnCl_2$.

9.2 Low recoveries will result for carbon materials such as coal and iodated carbon traps unless the final solution contains > 40% acid, it remains oxidizing, and all carbon particles are settled out of the aliquot to be analyzed. Low recoveries result from re-adsorption of metals to the carbon particles, which can occur in the digest, after dilution. Following this SOP accurately will avoid this source of error.

9.3 Modified aqua regia is a leaching method and as such does not dissolve silicate minerals. Thus, crustal elements such as Fe, Al, Cr, Ba, and Si will not be quantitatively recovered in most media using this procedure.

10) Safety Precautions, Pollution Prevention and Waste Handling:

10.1 Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan (*EFHS-S-HS-QP12066*). This includes, but is not limited to, laboratory coat, eye protection, and protective gloves.

10.2 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the SDS (Safety Data Sheets) for each chemical they are working with.

10.2.1 Note: Use particular caution when preparing and using BrCl, as it releases extremely irritating, corrosive fumes similar in effect to free chlorine. Always handle this reagent in an approved fume hood.

10.2.2 Note: Modified aqua regia is very hazardous! Always work in fume hood wearing safety goggles and gloves while using this chemical.

CAUTION: THIS MIXTURE GETS HOT AND EMITS CAUSTIC FUMES.

10.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease-causative agents. Frontier will reimburse the expense of Hepatitis A and B immunizations for any laboratory staff member who desires this protection.

10.4 Hydrochloric acid: Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion. Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. For more information see SDS.

10.5 Nitric acid (HNO_3): Corrosive. Strong oxidizer. Contact with other material may cause a fire. Causes eye and skin burns. May cause severe respiratory tract irritation with possible burns. For more information see SDS.

10.6 See Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP) for general information regarding employee safety, waste management, and pollution prevention.

10.7 Pollution prevention information can be found in the current Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP), which details and tracks various waste streams and disposal procedures.

10.8 All laboratory waste is accumulated, managed, and disposed of in accordance with all federal, state, and local laws and regulations. Any waste generated by this procedure should be disposed of according to SOP *EFHS-S-HS-SOP2991* "Waste Disposal Procedures for Client Sample Waste," which provides instruction on dealing with laboratory and client waste.

11) Personnel Training and Qualifications:

11.1 An analyst and technician must perform an initial demonstration of capability (IDOC) that includes four replicates of a secondary source spiked at 8 ng/g before being qualified to analyze samples without supervision. Recoveries for the IDOC need to be 79-121% with an RSD of < 20%. Continuing DOC will be maintained and monitored via performance on CRMs and other QC samples, as well as obtaining acceptable results on proficiency testing exercises.

11.2 The analyst/laboratory technician must have read this SOP and other relevant SOPs and have the training documented on the applicable form(s). The analyst may be questioned on SOP by supervisor(s) and/or trainers.

11.3 Training is documented by the employee and supervisor, and is kept on file in the QA Office. The employee must read, understand, and by signing the training document, agree to perform the procedures as stated in all Standard Operating Procedures (SOPs) related to this method.

11.4 All employees must read the Quality Manual (QM) and complete annual Ethics training.

11.5 All training documents including IDOCs, CDOCs, Initial QA orientation, and Ethics training are stored by the Quality Assurance Manager in the employees training file for ten years after the employee is no longer working for Frontier Global Sciences.

11.6 Chemical Safety Training, Compressed Gas Training, Chemical Hygiene Plan documentation, and Shipping of Hazardous goods training, are stored by the Health and Safety Officer for ten years after the employee is no longer working for Frontier Global Sciences.

12) Sample Collection, Preservation, and Handling:

12.1 Sediment, soil, and other geological samples must be collected in accordance with established ultraclean sampling techniques (see e.g. *EFSR-S-CS-SOP2794* "Ultra Clean Aqueous Sample Collection"). Samples may be placed in commercially available clean glass containers with Teflon-lined caps (i.e., I-Chem glass jars), or 125-mL or 250-mL HDPE jars.

12.2 Soil and sediment samples must be frozen at < –11 °C. Maximum holding time is 180 days at < –11 °C.

12.3 For Wisconsin, sediment samples will be refrigerated upon receipt and then homogenized, prepared and analyzed within 28 days of collection.

13) Apparatus and Equipment:

13.1 Digestion Vials: In most cases, this digestion is performed in tested-cleaned (i.e., I-Chem series 300 or equivalent) 40.0-mL borosilicate glass vials with Teflon lined caps.

13.2 Pipettors: Hydrochloric and nitric acids are conveniently dispensed separately from all glass or glass and Teflon bottle-top repetitive pipettors (10-mL size Re-Pipette or equivalent). Pipettes are to be calibrated according to SOPs *EFQA-R-EQ-SOP2711*.

13.3 Clean hood.

13.4 Analytical Balance: A laboratory analytical balance capable of weighing to ± 1 mg, with documented calibration.

13.5 Sample Digestion Log (LOG-HG-013) logbook.

13.6 Plastic or glass tools for homogenization.

13.7 Tongue Depressors and/or paper scoops.

13.8 Teflon boiling chips.

13.9 Centrifuge operating at 2000 RPM.

13.10 0.45-μm disposable filters.

14) Reagents and Standards:

14.1 Reagent Water: 18 MΩ ultra-pure deionized water starting from a pre-purified (distilled, R.O., etc.) source. As a final mercury and organic removal step, the activated carbon cartridge on the 18-MΩ system is placed between the final ion exchange bed and the 0.2 μm filter.

14.2 Nitric Acid (HNO_3): Several brands (Baker, Fisher, Omnitrace) have been found to have lots with acceptably low levels of trace metals. This reagent should be from a lot number that has been previously tested to be low for the analytes of interest. This reagent shall be entered into LIMS and the expiration date is set to the same as the manufacturer's expiration date.

14.3 Hydrochloric Acid (HCl): Trace metal purified reagent-grade HCl is pre-analyzed to < 50 ng/L Hg and lot sequestered and before purchase. This solution shall be entered into LIMS and is considered stable until the expiration date on the bottle, 6 months from receipt.

14.4 Potassium Bromide (KBr), neat: This reagent is pre-certified by the vendor to be low in mercury. Reagent shall be entered into LIMS with a five year expiration date.

14.5 Potassium Bromate ($KBrO_3$), neat: This reagent is pre-certified by the vendor to be low in mercury. Reagent shall be entered into LIMS with a five year expiration date.

14.6 0.2N Bromine Monochloride (BrCl):

14.6.1 37.5 g of KBr is added to a 2.5-L bottle of concentrated HCl (pre-analyzed and found to be below 50 ng/L Hg). The bottle is then inverted in a fume hood to mix the acid and KBr. The solution then sits overnight allowing for the KBr to be dissolved.

14.6.2 27.5 g of $KBrO_3$, certified to be low in Hg, is slowly added to the acid. When all of the $KBrO_3$ has been added, the solution should have gone from yellow to red to orange.

14.6.3 Loosely cap the bottle, and allow sitting for 30 minutes in a fume hood before tightening the lid. Once capped invert bottle to make sure all of the solids goes into solution.

CAUTION: This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl) which are released from the bottle. Add the $KBrO_3$ SLOWLY and in a well operating fume hood.

14.6.4 The BrCL solution must be tested by analyzing a prepared 0.5% solution. The result must be ≤ 0.20ng/L.

14.6.5 Reagent shall be entered into LIMS with a six months expiration date.

14.7 0.07 N (35%) Bromine Monochloride. Dilute 330 ± 40 mL of the 0.2 N BrCl solution to 1.0 L with reagent water in a 1000-mL Teflon bottle.

14.7.1 Reagent shall be entered into LIMS with a six months expiration date.

14.8 5% (v/v) of 0.2N BrCl: 125 ± 40 mL of 0.2N BrCl is diluted up to 2.5 L with reagent water in a clean, empty HCl bottle. This bottle is fitted with a 50 mL repipettor.

14.8.1 Reagent shall be entered into LIMS with a six months expiration date.

15) Procedure:

15.1 Obtain samples from the designated refrigerator or freezer. All samples must be completely thawed before homogenizing and weighing out. All tools used for homogenization and weighing must be new, unused disposable tools or cleaned thoroughly between samples with one 10% HCl acid bath and one reagent bath.

15.2 Weigh a 0.5g to 1.0g aliquot for common, low-level or large-grain samples. Place the aliquot directly into a 40-mL digestion vial.

15.2.1 Batch requirements for this digestion limit the number of samples to 20. In each batch, there must be three method blanks (BLKs), a Blank Spike and Blank Spike Duplicate (BS/BSD, liquid spike, prepared at 8 ng/g), and a Matrix Spike and Matrix Spike Duplicate (MS/MSD). The customary spiking volume used for the MS is 200 uL of 1000 ng/mL. A certified reference material should also be added to the batch if available and representative of the sample matrix.

15.3 Add 8 mL of concentrated HCl and swirl the sample to wet all particles. Next, add 2 mL of concentrated HNO_3, swirl, and LOOSELY cap the vials.

15.4 Allow the loosely capped samples to digest in the fume hood at room temperature for at least 4 hours, or preferably overnight. Tightening the caps or heating the samples can cause the vials to explode. At temperatures cooler than 18 °C the reaction is less vigorous so there may be a longer digestion time. At temperatures higher than 25°C, the reaction can generate much free chlorine, causing some samples to foam over.

15.5 After digestion is complete, dilute soil or sediment digestions by adding 30 mL of 5% solution of 0.2 N BrCl. Shake vigorously and allow settling until the supernatant is clear prior to analysis.

15.6 For coal and other carbon materials, dilute the samples with 0.07 N BrCl solution (35% v/v). This ensures that of Hg will not re-adsorb to the carbon particles, producing low recoveries. Be sure that all finely grained particles are completely settled prior to analysis. This settling can be hastened by centrifuging for 20 minutes at 2000 RPM or by pre-filtering the sample through 0.45-μm disposable filters.

Caution: when adding BrCl to modified aqua regia, be aware of possible rapid bubble formation and foaming out of the vial. This is particularly a problem with carbon media, warm digests, and samples that have only been digested for a few hours

15.7 Analysis for total mercury is according to Eurofins Frontier SOP *EFAFS-T-AFS-SOP2822*.

16) Calculations:

16.1 This preparation procedure does not involve calculations.

17) Statistical Information/Method Performance:

17.1 Method Detections Limits (MDL) are determined during method development and then annually thereafter according to 40 CFR Part 136, Section B and and *EFQA-Q-QD-SOP10098*.

17.2 The Practical Quantitation Limit (PQL) is the reporting limit for this method and is included as the lowest calibration point (2003 NELAC regulation 5.5.5.2.2.1.h.3). The PQL is determined by running ten replicate samples with a concentration the will produce a recovery of 70-130% for most analytes, but the recovery requirements are analyte dependent. The PQL is referred to as the Method Reporting Limit (MRL) in LIMS.

17.3 The current values for THg in sediments prepared by a Cold Modified Aqua Regia Digestion are 0.11 ng/g for the MDL and 1.00 ng/g for the PQL.

17.4 Current MDLs and PQLs are stored at: \General and Admin\Quality Assurance\MDLs & PQLs.

18) Quality Assurance/Quality Control:

18.1 Maximum Sample Batch Size: 20 samples.

18.2 Preparation Blanks: Minimum of three per batch. The preparation blanks are prepared with a similar mass of Teflon boiling chips as the samples, with the same reagents, and put through the same preparation process as the samples. The LIMS ID or lot # of the Teflon boiling chips is documented in the digestion logbook. Each preparation blank must be less than one-half the PQL for the method.

18.3 Certified Reference Material (CRM, representing the sample matrix when commercially available); One per batch if available.

18.4 Blank Spike (BS) and Blank Spike Duplicate (BSD): For every set of 20 samples or less, a blank spike and blank spike duplicate (BS1/BSD1) - made from spiking a liquid standard onto Teflon boiling chips - are digested. The LIMS ID or lot # of the Teflon boiling chips is documented in the digestion logbook.

18.5 Matrix Spike/Matrix Spike Duplicate (MS/MSD) Samples: One set per 10 samples.

18.6 Follow the flow charts in SOP *EFQA-P-DR-SOP2801* "Data Review and Validation" to determine if any QC falling outside the established control limits can be qualified.

18.7 All of the quality control limits for the analysis method are included on the "Data Review Checklist."

18.7.1 The data review checklists are located at: \\cuprum\General and Admin\Quality Assurance\Data Review\Current Data Review Checklists.

19) Corrective Action:

19.1 A failing QC point does not necessary fail the entire dataset. If upon analysis a QC sample is out of control, some investigation must be performed to assess if the difficulties are related to matrix effects. The cause and method of determining the set's failure

must be documented on the checklist and in the MMO notes, and the Group Supervisor shall be informed. See SOP *EFQA-P-DR-SOP2801* "Data Review and Validation" for flow charts regarding analytical issues.

19.2 If there is any question as to the completeness of the digestion procedure, the samples must be allowed to react for an additional amount of time. If there is no change in the sample, the digestion is considered complete and can be diluted.

EFAFS-T-AFS-SOP2822 Determination of Total Mercury in Various Matrices by FI-AFS
EFHS-S-HS-12066 Chemical Hygiene Plan
EFHS-S-HS-SOP2991 Waste Disposal Procedures for Client Sample Waste
EFQA-P-DR-SOP2801 Data Review and Validation and Monthly Logbook Reviews
EFQA-Q-QD-SOP10098 Procedures for Determining IDLs, MDLs, LODs, PQLs, LOQs, OPRs, IQs, OQs and PQs
EFQA-Q-QM-QM5805 Quality Manual
EFQA-R-EQ-SOP2711 Pipette and Dispenser: Operation, Calibration & Maintenance
EFSR-S-CS-SOP2794 Ultra-Clean Aqueous Sample Collection
EFTM-T-TM-SOP2821 HF/HNO3/ HCl Bomb Digestion of Solids for Total Mercury Followed by Repeated HNO3 Evaporation for Other Metals

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End of document

Version history

Version	Approval	Revision information
9	19.MAY.2016	
10	09.NOV.2016	
11	08.MAR.2018	

1) Revision Log:

Revision: 06 **Effective Date:** This version

Section	Justification	Changes
All	Formatting	All font in Verdana, normal, 10 and indentation controlled by D4
5	Clarity	Reword F1 summary. Added Parentheses to F5 summary "(if present"
5.2	Correction	Changed F5 to F6
6.3	Correction	Changed Table 2 reference to Appendix A
8.2	Clarification	BS/BSDs are not able to be performed for F1 through F4 preparations due to the nature of the digestions.
9.2	Correction	Deleted section on Hg0 loss
9.3	Correction	deleted word "even"
9.3.1	Correction	"N" instead of "?", font updated.
9.5	Correction	Added "the" in front of final
10.2, 10.5 and 10.6	Correction	Changed MSDS to SDS.
10.3.3	Correction	Added "4%"
10.3.4	Correction	Updated to reference CHP.
10.3.7.1	Correction	Deleted section
11.4	Correction	Reworded section
11.5	Correction	employee's instead of employees
12.1	Correction	updated wording
13.9	updated	rotator not shaker
13.10	Correction	updated rpm
14.2	Deletion	Reagent not used. renumbered section.
14.8 (new 14.7)	Deletion	previously analyzed
14.9 (new 14.8)	Deletion	previously analyzed
14.13	Addition	Add HgS after cinnabar in title
14.14	Correction	Reference material has been changed to Hg_2Cl_2 since we exhausted the $HgCl_2$.
14.15	Deletion	random period in sentence
15.1	Deletion	KCl scrubber
15.5	Correction	Updated wording
15.5, 15.10, 15.16, 15.22	Correction	Shaker box changed to rotator.

2) Reference:

2.1 Bloom, N.S., Katon, J., and Turner, R.R. 1999. "Can Selective Extractions Provide useful Information about Mercury Speciation in Sediments and Soils," presentation at the American Chemical Society National Meeting, New Orleans, LA, August 22, 1999.

2.2 Bloom, N.S. 1999. Method 1631*:* Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, US EPA 821-R-95-027, Office of Water, Engineering and Analysis Division (4303) Washington DC 20460.

2.3 Bloom, N.S. 1994. Sampling and Analysis for Mercury in Environmental Media of Importance to the Natural Gas Industry, Gas Research Institute Topical Report #GRI-94/0033, Chicago, IL.

2.4 Davis, A., Bloom, N.S., and Hee, S.Q. 1997. "The Environmental Geochemistry and Bioaccessability of Inorganic Mercury in Soils and Sediments--A Review." Risk Anal 17: 557-569.

2.5 Revis, N. W., Osborne, T.R., Holdsworth, G., and Hadden, C. (1990) "Mercury in Soil: A Method for Assessing Acceptable Limits," *Arch.* Environ. Contam. Toxicol., 19: 221-226.

2.6 Revis, N. W., Osborne, T.R., Holdsworth, G., and Hadden, C. (1989) "Distribution of Mercury Species in Soil from a Mercury-Contaminated Site," Wat. Air. Soil Pollut. 45: 105-113.

2.7 Revis, N. W., Osborne, T.R., Sedgely, D., and King, A. (1989) "Quantitative Method for Determining the Concentration of Mercury (II) Sulphide in Soils and Sediments," *Analyst*, 114: 823-826.

2.8 Willett K.L., Turner R.R., and Beauchamp J.J. 1992. "Effect of Chemical Form of Mercury on the Performance of Dosed Soils in Standard Leaching Protocols: EP and TCLP," Haz. Waste Haz. Mat. 9(3): 275-288.

3) Cross Reference:

Document	Document Title
EFQA-R-MT-SOP2710	Balance Verification, Calibration and Maintenance
EFQA-R-EQ-SOP2711	Pipette and Volumetric Dispenser Verification, Calibration and Maintenance
EFSR-S-CS-SOP2794	Ultra Clean Aqueous Sample Collection
EFSR-P-SP-SOP2796	Oxidation of Aqueous Samples for Total Mercury Analysis
EFAFS-T-AFS-SOP2985	Digestion for Gas/Air Samples Collected on Fluegas Sorbent for Total Mercury Traps
EFSR-P-SP-SOP2798	Ultra-Clean Sample Filtration
EFAFS-T-AFS-SOP5134	Preparation of Sediments by Acidic KBr Extraction Into Methylene Chloride For Determination of Methyl Mercury
EFHS-S-HS-SOP2991	Waste Disposal Procedure for Client Sample Waste
EFAFS-T-AFS-SOP2821	HF/HNO3/HCl Bomb Digestion of Sediments, Soils, Rocks, and Bayer Process Solids and Slurries for Mercury, followed by Repeated HNO_3 Evaporation
EFHS-S-HS-SOP5670	Health and Safety Evaluation and Auditing
EFAFS-T-AFS-SOP2992	Mercury in Water by Oxidation, Purge and trap CV-AFS (EPA Method 1631E)

4) Purpose:

4.1 The purpose of this standard operating procedure is to describe the selective extraction procedure used for geological samples (soils, sediments, ores, mine tailings, etc.) using hydrofluoric, nitric, and hydrochloric acids ($HF/HNO_3/HCl$) for mercury analysis.

5) Scope:

5.1 This method is for the selective extraction of geological samples (soils, sediments, ores, mine tailings, etc.), with the goal of determining the biogeochemically relevant associations of inorganic Hg within, and leachability of inorganic Hg from, the solid phase.

5.2 When applied exactly as written, this method defines the following extraction fractions (F-0 through F-6, and F-S). The representativeness of each fraction varies from sample to sample, depending upon ancillary parameters such as TOC, soil pH, co-leached substances (i.e., Cl^-, $SO_4^=$, etc.) and actual solid phase speciation of the analyte. Additional ancillary chemistry measurements or kinetic studies may be required to fully interpret the extraction pattern for each sample.

Volatile Elemental Mercury (Hg^o): This test is performed by placing sample in a teflon bomb vessel with a inlet and outlet and letting nitrogen purge the system collecting all exhaust on a FSTM trap. The trap is then digested using *EFAFS-T-AFS-SOP2985*. The mass of the sample is used as the initial mass in the LIMS database.

F-1 Water Soluble Mercury: Mercury extracted in this test is useful in assessing the potential leaching of soils by rain or groundwater, and is a reasonably good (±50%) predictor of the performance of the sample on an official TCLP or EP-toxicity leaching test. At high solid phase concentrations (100s-1000s of µg/g), the water soluble salts such as $HgCl_2$, $Hg(NO_3)_2$, etc., will appear largely in this fraction, but as total Hg concentrations decrease, the percentage found in this fraction decreases dramatically, due to adsorption of the free Hg on the soil particles. This fraction is extremely dependent upon the co-leached soil components such as Cl-, I-, DOC, and pH. Increases in any of these co-leached Hg complexing agents can greatly increase the solubility of water-soluble mercury compounds. High values for this parameter (greater than either 1 µg/g or 5% of the total Hg) may warrant further investigations into the kinetics of leaching, such as column percolation studies.

F-2 pH 2 Soluble Mercury. Mercury extracted in this fraction is a surrogate for what might be extracted by the human stomach upon ingestion, or of leachability under the conditions of acid mine drainage. In cases where the sample contains high TOC, this fraction is usually the lowest in Hg, because of readsorption of Hg(II) by coagulated humic matter at this pH. High concentrations of pH 2 leachable Hg might warrant additional testing that more accurately models the human digestive tract in terms of pH regime and contact time, or acid mine drainage conditions present at the contaminated site.

F-3 1N KOH Extractable Mercury. Under the conditions of this extraction, most of the Hg associated with humic organic matter appears to be solubilized, while none of the HgS is co-solubilized. 1N KOH soluble Hg dominates marine and freshwater sediments, as well as the soil humus layer. Not only does most of the CH_3Hg in the sample also leach out in this fraction, but also this fraction has been found to strongly correlate with *in situ* CH_3Hg concentrations, and the potential methylatability of the sample (Ref 10.1). The contribution of the CH_3Hg content to the total Hg extracted is usually small, but if high concentrations of methyl Hg (greater than 1% of total) are measured in the samples (*EFAFS-T-AFS-SOP5134*), a correction might be appropriate. The most appropriate way to correct this data is to also measure CH_3Hg directly on the 1N KOH extract, and subtract it from the measured total Hg value on the same extract.

F-4 12N HNO_3 Soluble Mercury. This fraction serves largely to separate out all remaining non-HgS, so that the final measured fraction may safely be taken to represent the HgS content of the sample. In cases where F-0 detected a saturation level of Hg^0, and the fractions F-1 through F-3 are small by comparison to F-4, the latter fraction may be interpreted as representing essentially the total Hg^0 content of the sample. At lower Hg concentrations in natural samples, much of the non-humic bound Hg(II) is found in this fraction, because it is strongly adsorbed to the particle surfaces, and so not leachable by the weak extractants F-1 and F-2.

F-5 Aqua Regia Soluble Mercury (Residue). If the previous steps of the extraction scheme have been carried out accurately, this fraction consists of the cinnabar and meta-cinnabar (HgS) content of the samples. Also included in this fraction, (if present in the sample) would be HgSe, and amalgams of Hg with noble metals such as gold and platinum. Hg is leached from the surface of these amalgams, but the bulk concentrations require the dissolution of the noble metal particles, which is accomplished readily by aqua regia.

F-6 Mineral-Bound Mercury. For hard mineral samples, such as bauxite, the F5 (aqua regia) step is not vigorous enough to release all mercury from the crystal lattice. In samples of this type, a HF/HNO_3/HCl Bomb digest is necessary to recover all the mercury in the sample (see SOP *EFAFS-T-AFS-SOP2821*).

F-S Total Mercury by the Sum of Species. The sum of all of the fractions, F-0 through F-6 is the total Hg in the sample. It is ***inadvisable*** to try to measure total Hg (*EFAFS-T-AFS-SOP2992*) on a separate aliquot of the sample, unless this is being done only for the purpose of assessing sample homogeneity. For real-world samples, heterogeneity is often so great that direct comparison of selective extraction on one aliquot and total Hg on a separate aliquot will produce misleading conclusions (such as that there is a "missing" Hg species, in cases where the total is much greater than the sum of species). For very fine, homogeneous samples such as CRMs, F-S should compare to the independently measured total to within ± 20%.

5.3 This leaching is optimized for and only applicable to Hg analysis. Other leaching procedures are necessary to obtain reliable and biogeochemically meaningful results for other trace metals.

5.4 This method is a protocol for the extraction only. All recovered aqueous fractions are then analyzed by an appropriate Hg quantification technique. Because of its low detection limits and high tolerance for complex matrices, EPA Method 1631 (ref 10.2), with preparation described in Frontier SOP *EFSR-P-SP-SOP2796* (Total Hg in aqueous media) and analysis in *EFAFS-T-AFS-SOP2992* (Total Hg analysis) are recommended, as indicated in the text below.

6) Basic Principles:

6.1 Prior to digestion, the sample should be sieved through a 2-mm plastic mesh screen to remove large chunks, and as an aid in homogenization. Inherently fine-grained samples do not need to be sieved prior to extraction.

6.2 Fresh samples should be extracted in a form as close to their natural state as possible. Under no circumstances should samples be dried or pulverized prior to extraction, as this may lead to dramatic changes in leachability.

6.3 This method involves the sequential extraction of the same sample aliquot through a sequence of different extractants of increasing chemical strength. Recovery in a wide range of geological materials, as the sum of the selective extraction fractions was found to typically be 100 ± 15% (Appendix A).

6.4 This procedure contains many steps, and must be conducted on four consecutive days (no more, no less). Selective extractions of this type must be started on Monday or Tuesday, unless the analyst is planning to continue work on the weekend. Please read this SOP over several times, and plan the extraction timing, use of an analyzer for Hg^0 on the second day, and quantity of filters, bottles, etc. carefully before beginning. Be sure to make a big enough volume of extraction reagents for all samples in a set (every 20 samples plus 8 QC samples requires 28 times 80 mL, or at least 2,300 mL of each extractant).

7) Reference Modifications:

7.1 No significant modifications were made to this method.

8) Definitions:

8.1 Certified Reference Material (CRM) – a standard of known composition that is certified by a recognized authority and representing a sample matrix. It is used to verify the accuracy of a method.

8.2 Laboratory Control Sample (LCS) and Laboratory Control Sample Duplicate (LCSD), is a sample containing known concentrations of the analytes of interest that is taken through the entire preparation and analysis process in the same manner as the samples to monitor complete method performance. A Certified Reference Material (CRM) is preferred as the LCS, but a blank spike sample also meets the requirement. Due to the nature of the preparation it is not possible to spike a true BS/BSD on fractions 1 through 4 as the digestion is not a complete digestion for all species of mercury.

8.3 Limit of Detection (LOD) – equal to MDL and verified on an annual basis by spiking within three times the established LOD for Hg and within four times the established LOD for all other applicable metals showing a positive result on the instrument.

8.4 Limit of Quantitation (LOQ) – equal to PQL and verified on an annual basis by spiking within 2 times the established LOQ for Hg and 3 times for all other applicable analytes.

8.5 LIMS – Laboratory Information Management System.

8.6 Matrix Duplicate (MD) – a representative sample is selected and digested in the same manner. This QC sample will indicate sample homogeneity on the analytes of interest.

8.7 Matrix Spike (MS) and Matrix Spike Duplicate (MSD) – a representative sample is selected and spiked with a secondary source at *two* to five times the ambient concentration or at *two* to five times the MRL, whichever is greater. These QC samples will indicate sample matrix effects on the analytes of interest.

8.8 May: This action, activity or procedure is optional.

8.9 May Not: This action, activity or procedure is prohibited.

8.10 Method Detection Limit (MDL) – the limit derived from an exercise as described in 40 CFR, Part 136, Appendix B. The exercise produces a defined value that is the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater

than zero from a given matrix.

8.11 Preparation Blank (BLK) – Method blanks consist of the same reagents used to digest the samples, in the same volume or proportion, and are carried through the complete sample preparation and analytical procedure. Teflon boiling chips are added to the preparation blanks.

8.12 Shall: This action, activity or procedure is required.

8.13 Should: This action, activity or procedure is suggested, but is not required.

9) Interferences:

9.1 As an operationally defined analytical method, sequential selective extractions generate data which may range from somewhat to extremely ambiguous, depending upon the nature of the matrix, and the concentrations and species of the mercury compounds present. In general, this method provides an excellent resolution between "HgS" and "non-HgS," as well as a good assay for organically associated inorganic Hg, which has been found to be strongly linked to potential for methylation.

9.2 Samples may contain significant free elemental Hg, if indicated by one of the following: (a) the presence of visible balls of liquid Hg, (b) saturation of Hg^0 in the water extraction step of this method ([Hg^0] >40 mg/L at room temperature), or (c) saturation of the Hg^0 in headspace above the sample in a closed container (i.e., [Hg^0] > 20 ng/mL at room temperature). In these cases, the interpretation of all weak leachate fractions is confounded by the fact that significant Hg^0 can be dissolved into each aqueous fraction (approximately 40-60 mg/L of extractant, or 5.0 ppm by weight under the conditions used in these extractions). However, these values will be truly reflective of the potential leachability of total Hg from such samples, and may still be successfully interpreted that way. If the Hg found in the 12 N HNO_3 extract of such samples is much greater than that found in the previous extractions, then the 12 N HNO_3 fraction can be relatively comfortably assigned to be elemental Hg.

9.3 Successful resolution of HgS in the last extraction step is critically dependent upon the absence of chloride in the sample during the 12 N HNO_3 step. If chloride residue were in the sediment at this time, it would be converted to free halogens, which quickly and easily dissolve HgS, and so void the selectivity of the method. As described, the various dilute extraction steps prior to the 12 N HNO_3 step are sufficient ~~even~~ to rinse out the chloride from marine sediments.

9.3.1 However, if an abbreviated extraction involving only "non-HgS" (12N ~~2~~HNO_3) and "HgS" (aqua regia) were being employed, it is critical that any chloride possibly present in the samples be rinsed out with at least two consecutive water rinses, prior to the addition of the 12N HNO_3. The water rinses and 12 N HNO_3 rinses can then be combined to minimize analytical steps. If it is known that the sample does not contain chloride (i.e., <100ppm solids basis), then this step may be omitted.

9.4 Selective extractions must be performed sequentially on the same sample aliquot, exactly in the sequence described, and for the time periods described to produce meaningful results.

9.5 Although empirical evidence shows that the final aqua regia step effectively extracts all remaining mercury from virtually all sample types, there remains a possibility that Hg contained in large silicate mineral grains will not be fully extracted by this technique. If this becomes a question, the residue from fraction F-5 can be completely solubilized using hydrofluoric acid plus aqua regia in Teflon bombs (SOP *EFAFS-T-AFS-SOP2821*).

10) Safety Precautions and Waste Handling:

10.1 Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan. This includes, but is not limited to, laboratory coat, safety goggles, and nitrile gloves under clean gloves.

10.2 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the SDS (Safety Data Sheets) for each chemical they are working with.

10.3 Hydrofluoric Acid (HF): HF is used for many purposes including mineral digestion, surface cleaning, etching, and biological staining. HF's unique properties make it significantly more hazardous than many of the other acids used on site.

10.3.1 Always wear HF apron, face shield, tyvex sleeves and double gloves when working with HF.

10.3.2 Check PPE after you have finished working for broken PPE.

10.3.3 For spills, briefly spray with 4% Boric acid and then neutralize. Make sure the acids have fully reacted before disposing of the neutralized waste.

10.3.4 If HF is spilled on you follow instructions in CHP.

10.3.5 Ventilation:

10.3.5.1 HF should be used with adequate ventilation to minimize inhalation of vapor. Concentrations greater than 5% should always be handled inside a properly functioning chemical fume hood. The chemical fume hood needs to have a current inspection sticker (see EFHS-S-HS-SOP2834). Notify EH&S if there are any issues with the hood.

10.3.6 Eye Protection:

10.3.6.1 Always use chemical splash goggles together with a face shield when handling concentrated HF. Due to HF's highly corrosive nature, safety glasses with side shields do not provide adequate eye protection.

10.3.7 Body Protection:

10.3.7.1 Wear laboratory coat with a chemical splash apron made out of natural rubber, neoprene, or viton.

10.3.8 Gloves:

10.3.8.1 Typically, medium or heavyweight viton, nitrile, or natural rubber gloves are worn when working with HF. Always consult the manufacturer's glove selection guide when selecting a glove for HF. If you have any questions about which glove to choose, contact EH&S. A second pair of nitrile exam gloves should be worn under the gloves for protection against leaks. Gloves that have not been contaminated

with HF may be disposed of in the common trash. If gloves become contaminated with HF, remove them immediately, thoroughly wash your hands, and check your hands for any sign of contamination. Contact the EH&S Officer if your skin has been exposed to any direct HF.

10.4 Bromine Monochloride: Use particular caution when preparing and using BrCl, as it releases extremely irritating, corrosive fumes similar in effect to free chlorine. Always handle this reagent in an approved fume hood.

10.5 Nitric acid (HNO_3): Corrosive. Strong oxidizer. Contact with other material may cause a fire. Causes eye and skin burns. May cause severe respiratory tract irritation with possible burns. May cause severe digestive tract irritation with possible burns. For more information see SDS. Always work in fume hood wearing safety goggles, latex and clean gloves, apron and face shield while using this chemical.

10.6 Hydrochloric acid: Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion. Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. For more information see SDS. Always work in fume hood.

10.7 See Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP) for general information regarding employee safety, waste management, and pollution prevention.

10.8 Pollution prevention information can be found in the current Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP), which details and tracks various waste streams and disposal procedures.

10.9 All laboratory waste is accumulated, managed, and disposed of in accordance with all federal, state, and local laws and regulations. Any waste generated by this procedure should be disposed of according to SOP *EFHS-S-HS-SOP2991* "Waste Disposal Procedure for Client Sample Waste," which provides instruction on dealing with laboratory and client waste.

11) Personnel Training and Qualifications:

11.1 A lab technician/analyst must perform an initial demonstration of capability (IDOC) that includes four replicates of a secondary source before being qualified to prepare samples without supervision. Continuing DOC will be maintained and monitored via performance on CRMs and other QC samples, as well as obtaining acceptable results on proficiency testing exercises.

11.2 The analyst/laboratory technician must have read this SOP and other relevant SOPs and have the training documented on the applicable form(s). The analyst may be questioned on SOP by supervisor(s) and/or trainers.

11.3 Training is documented by the employee and supervisor, and is kept on file in the QA Office. The employee must read, understand, and by signing the training document, agree to perform the procedures as stated in all Standard Operating Procedures (SOPs) related to this method.

11.4 All employees must read the Quality Manual (QM) and complete annual Ethics training.

11.5 All training documents including IDOCs, CDOCs, Initial QA orientation, and Ethics training are stored by QA in the employee's training file for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

11.6 Chemical Safety Training, Compressed Gas Training, Chemical Hygiene Plan documentation, and Shipping of Hazardous goods, are stored by the Health and Safety Officer for ten years after the employee is no longer working for Eurofins Frontier Global Sciences

12) Sample Collection, Preservation, and Handling:

12.1 Samples should be collected into wide mouth glass or Teflon containers. Jars should be filled approximately 80% full of soil, mine tailings, etc. For sediments and other potentially anoxic samples, jars should be completely filled.

12.2 Samples should be stored cool (1-4 ^{o}C) and in the dark, for up to five days prior to processing in the laboratory. If samples must be held for more than five days, they should be frozen at less than –15 ^{o}C until processing. In this case, be sure that all samples, including sediments are packed into jars only 80% full, to avoid breakage of the jars during freezing! If the same samples are to be analyzed for methyl mercury or dimethyl mercury, these subsamples must be analyzed immediately (within one day), or a subaliquot (5-50 grams) be placed into a second (smaller) jar, and immediately frozen until analysis.

12.3 If samples are not naturally fine grained (<2 mm, i.e., sand, silt and clay), they should be quickly sieved through a 2-mm mesh screen, collected into a receiving tray, and then placed into a new wide mouth jar for storage until extraction. Quickly and thoroughly homogenize the sieved material by stirring or shaking prior to storage of the sample. The following cautions should be observed:

12.3.1 If the samples are known or suspected to contain free elemental mercury, they should be sieved as rapidly as possible, outdoors (ideally in the field) or in an exhaust hood, and the sieve should not be re-used without thorough cleaning between samples. Low cost large mesh stainless steel kitchen sieves are ideal disposable items for this purpose.

12.3.2 It may be desirable to determine the total sample mass before sieving, as well as the mass of fines and/or coarse material recovered. The client should be consulted before sieving if this is unclear. In addition, in some cases, the coarse material may need to be saved for possible future analysis. Unless otherwise stated, do not save or analyze the coarse material, however.

12.4 Under no circumstances should the raw sample or sieved material be dried, wet sieved, or stored in open air or a jar with a large headspace to sample ratio (>0.3). Such conditions will radically change some of the speciation and observed leachability.

12.5 If sample results are to be reported on a dry weight basis, a separate aliquot of the sieved sample should be taken for drying.

13) Apparatus and Equipment:

13.1 LIMS – Element, version 6 or higher; Computer – Windows XP, 7 or 8.

13.2 Extraction Vessels: off-the-shelf trace clean 40-mL borosilicate glass vials with Teflon-coated silicone rubber lined caps (I-Chem™ 200-series or equivalent).

13.3 Sample jars: off-the-shelf trace metal clean 125-mL glass jars with Teflon-lined caps (I-Chem™ 200-series or equivalent).

13.4 Dilution bottles: off-the-shelf trace metal clean 125-mL narrow mouth glass bottles with Teflon-lined caps (I-Chem™ 200-series or equivalent). NOTE: Four 125-mL bottles are needed for each sample and blank that is extracted.

13.5 Pipettors: concentrated HCl and HNO_3 are conveniently dispensed separately from all-glass or glass and Teflon bottle-top repetitive pipettors (0-10-mL size; Re-Pipette™ or equivalent). Pipettes are to be calibrated weekly according to SOP *EFQA-R-EQ-SOP2711*

13.6 Analytical Balance: any lab analytical balance capable of weighing to the nearest milligram, and taring the full mass of the extraction vials (about 100 g when filled with 12 N HNO_3). The analytical balances are verified for accuracy on a daily basis according to *EFQA-R-MT-SOP2710* "Balance Verification, Calibration and Maintenance."

13.7 2.0 mm sieve: a sieve unit, 10-30 cm diameter by 5-15 cm deep made of plastic such as nylon or polyethylene, or of a non-rusting grade of stainless steel.

13.8 Receiving tray: borosilicate glass tray or dish larger in diameter than the sieve.

13.9 Vial rotator a rotator able to rotate at approximately one rotation per 6 seconds and maintain the speed for 16 hours.

13.10 Low speed centrifuge: any centrifuge with rotor head capable of holding 40-mL I-Chem™ vials, and spinning at least 1,500 RPM. These tubes fit conveniently into a rotor designed for 35mL Oak Ridge type or 50mL conical bottom tubes.

13.10.1 HOWEVER: the tube cradle at the bottom of each rotor well must be modified to accommodate the flat-bottomed glass vials. This can be done by filling the conical or hemispherical indentation with silicone rubber caulk, and allowing to harden for at least 24 hours. Failure to take this step will result in shattering of the glass vials under the gravity of the centrifuge!

13.11 Disposable Nitrocellulose Filtration Units: disposable 0.2-m or 0.4-m pore size 150-mL polystyrene units containing a non-removable 47-mm diameter nitrocellulose membrane filter (Nalgene, or equivalent). Four filtration units are needed for each sample extracted. Because of the relatively large solids/extractant ratios, it is not critical whether 0.2-m or 0.4-m units are employed, but good laboratory practice dictates that for a given project, all of the filter units be of the same pore size, and from the same batch. *These filters do not need to be acid cleaned before use.*

13.12 Teflon boiling chips

13.13 Vacuum pump: standard laboratory vacuum pump or water aspirator capable of generating at least 0.5 atm vacuum, for the purpose of vacuum filtration.

14) Reagents and Standards:

14.1 Reagent Water: 18 MW ultra-pure deionized water starting from a pre-purified (distilled, R.O., etc.) source. As a final mercury and organic removal step, the activated carbon cartridge on the 18-MW system is placed between the final ion exchange bed and the 0.2 µm filter.

14.2 Potassium Bromide (KBr), neat: this reagent is pre-certified by the vendor to be low in mercury and is entered into the LIMS with a five year expiration date.

14.3 Potassium Bromate ($KBrO_3$), neat: this reagent is pre-certified by the vendor to be low in mercury and is entered into the LIMS with a five year expiration date.

14.4 0.2N Bromine Monochloride:

14.5.1 37.5 g of KBr is added to a 2.5 L bottle of concentrated HCl (pre-analyzed and below 5 ng/L Hg). The bottle is inverted in a fume hood to mix the acid and KBr. The solution sits overnight, allowing the KBr to dissolve.

14.5.2 27.5 g of KBrO3 (certified to be low in Hg) is slowly added to the acid. As the KBrO3 is added, the solution should go from yellow to red to orange.

CAUTION: This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl) which are released from the bottle. Add the $KBrO_3$ SLOWLY in a well operating fume hood.

14.5.3 Loosely cap the bottle and allow to sit for 30 minutes (in a fume hood) before tightening. Once tightly capped, invert bottle to make sure all of the solids go into solution.

14.5.4 This reagent shall be entered into the LIMS with a six month expiration date.

14.5 Nitric Acid (HNO_3): concentrated reagent grade (16.0N) HNO_3, known to be low in Hg and/or other trace metals of interest. This solution should contain less than 0.01ng/mL of Hg to be suitable for selective extraction work. This reagent shall be entered into LIMS and the expiration date is set to the same as the manufacturer's expiration date.

14.6 Hydrochloric Acid (HCl): concentrated reagent grade (12.2N) HCl, known to be low in Hg and/or other trace metals of interest. This solution should contain less than 0.01ng/mL of Hg to be suitable for selective extraction work. This reagent shall be entered into LIMS and the expiration date is set to the same as the manufacturer's expiration date.

14.7 Hydrofluoric Acid (HF) (only if F6 is employed): concentrated (48-50% weight basis) low trace-metals grade hydrofluoric acid. This reagent shall be entered into LIMS and the expiration date is set to the same as the manufacturer's expiration date.

14.8 Glacial Acetic Acid: concentrated low trace-metals grade. This reagent shall be entered into LIMS and the expiration date is set to the same as the manufacturer's expiration date.

14.9 12N Nitric Acid (HNO_3): dilute 1.5 ± 0.1 L of concentrated (16.0N) HNO_3 to 2.0 L with reagent water in a 2.5-L acid bottle. This solution should contain less than 0.1ng/mL of Hg to be suitable for selective extraction work. Enter reagent into LIMS for traceability.

14.10 1N KOH Solution: dissolve 130 ± 10 grams of reagent grade KOH pellets (85% KOH assay) in 1 L of deionized water in a 2.5-L acid bottle. Dilute to 2.0 L with reagent water, and allow cooling thoroughly before tightening cap. This solution should contain less than 0.1ng/mL of Hg to be suitable for selective extraction work. Enter reagent into LIMS for traceability.

14.11 pH 2 Extraction Solution: dilute 2.0 mL of concentrated HCl and 12 mL of concentrated reagent grade glacial acetic acid to 2.0 L with reagent water in an empty reagent acid bottle. This solution should contain less than 0.01ng/mL of Hg to be suitable for selective extraction work. Enter

reagent into LIMS for traceability.

14.12 Dry suspension of cinnabar (HgS) in inert substrate (silica, boron nitride): prepare 100 grams of an approximately 45 mg/g Hg suspension of powdered red HgS in silica powder. Shake vigorously for 30 minutes in a half full jar containing two small marbles as mixing aids. Then sieve the mixture repeatedly four times to further homogenize, and store in a wide mouth jar. Accurately quantify the total Hg concentration of three replicates of this material using aqua regia digestion and CVAFS (*EFAFS-T-AFS-SOP2992*). If the RSD of the three replicates is less than or equal to 10%, then use the mean as the accepted value. If the RSD is greater than 10%, then re-homogenize the sample 5 times, and repeat the triplicate analysis until homogeneity is verified. This sample serves as a verification check for the method selectivity for HgS.

14.13 Dry suspension of Hg_2Cl_2 in inert substrate (silica, boron nitride): prepare 100 grams of an approximately 25mg/g Hg suspension of Hg_2Cl_2 in silica powder. Shake vigorously for 30 minutes in a half full jar containing two small marbles as mixing aids. Then sieve the mixture repeatedly four times to further homogenize, and store in a wide mouth jar. Accurately quantify the total Hg concentration of three replicates of this material using aqua regia digestion and CVAFS (*EFAFS-T-AFS-SOP2992*). If the RSD of the three replicates is less than or equal to 10%, then use the mean as the accepted value. If the RSD is greater than 10%, then re-homogenize the sample 5 times, and repeat the triplicate analysis until homogeneity is verified. This sample serves as a behavior comparison check for a water-soluble mercury species under the conditions of this extraction.

14.14 Dry suspension of HgO in inert substrate (silica, boron nitride) - prepare 100 grams of an approximately 1.4mg/g Hg suspension of HgO in silica powder. Shake vigorously for 30 minutes in a half full jar containing two small marbles as mixing aids. Then sieve the mixture repeatedly four times to further homogenize, and store in a wide mouth jar. Accurately quantify the total Hg concentration of three replicates of this material using aqua regia digestion and CVAFS (*EFAFS-T-AFS-SOP2992*). If the RSD of the three replicates is less than or equal to 10%, then use the mean as the accepted value. If the RSD is greater than 10%, then re-homogenize the sample 5 times, and repeat the triplicate analysis until homogeneity is verified. This sample serves as a behavior comparison check for a water-soluble mercury species under the conditions of this extraction

15) Procedure:

15.1 If F-0 is needed weigh out 2.5 g of the homogenized sample directly into a 60mL Teflon bomb vessel. Record the mass of each sample. The apparatus used for this fraction is the Teflon bomb vessel with an inlet and outlet cap, a FSTM trap and nitrogen gas line.

15.1.1 Close the vessels and setup the apparatus after each sample is weighed to minimize the loss of volatile mercury.

15.1.2 Purge the system for 3 hours at 400-500 mL/min.

15.1.3 The FSTM trap is now the F0 fraction and is digested according to *EFAFS-T-AFS-SOP2985*.

15.1.4 The solid sample in the vessel is to be used for the weigh out in the following step.

15.2 Weigh approximately 0.4 g of the homogenized sample (within 1 mg) directly into a 40 mL extraction vial. The sample masses and sample IDs must be recorded in a bound, paginated logbook and on the vial labels at the time of weighing.

15.3 Fill each vial completely to the base of the neck with mercury-free reagent water, cap the vial tightly, and shake vigorously until all solids are clearly suspended in the water.

15.4 For each complete batch of samples (8-20), three method blanks, a pure HgS in silica sample, a pure $HgCl_2$ in silica sample, a sample duplicate, and one sample of HgO (or other designated CRM) must be co-extracted. Matrix spikes are not appropriate for selective extractions, so no MS/MSD is extracted.

15.5 Place the samples in the rotator after they have been weighed and filled with reagent water. Use slow (5-10 rpm) end over end rotation for a total of 16 +/- 2 hours. Alternatively, in the absence of a rotator, shake the samples very vigorously for 1-2 minutes every half-hour until evening, and then every hour the following morning for a total elapsed extraction time of 16 ± 2 hours.

15.6 Use a 5 or 10 mL pipette to transfer 90% of the solution into a labeled 125mL bottle. Take great care in not grabbing any of the solid sample. Pipette directly into a syringe and filter (0.2 to 0.4mm) the solution into a 125 mL bottle. Add 40 more mL of solution to sample and invert 10 times. Centrifuge again and transfer as much of the solution as possible (at least 90%) without grabbing any of the solid sample again into a syringe then 125mL bottle.

15.7 To each extract and blank, add 1.25 mL of concentrated BrCl solution, swirl to mix, and bring up to volume of 125mL. Use an example bottle at 125mL for reference.

15.8 Repeat steps 15.6-15.7, adding the rinse extract to the BrCl oxidized initial reagent water extract in the 125 mL bottles. For each sample, use the same filter as was used for the initial reagent water extract to filter the second reagent water (rinse) extract. Dilute each bottle to the base of the neck with reagent water, shake thoroughly, and set aside at least one hour until analysis.

15.9 To each original sample pellet, fill the vial to the 40 mL mark with the pH 2 extracting solution, and shake vigorously to resuspend the sediment. If after vigorous shaking, the sediment pellet does not break up, use a thin, disposable glass rod (or Pasteur pipette) to dislodge the compacted sediment.

15.10 When all samples in the batch have been filled with pH 2 extracting solution, place in the rotator, and shake by slow (5-10 rpm) end-over-end rotation for a total elapsed extraction time of 16 ± 2 hours. Alternatively, in the absence of a rotator, shake the samples very vigorously for 1-2 minutes every half-hour until evening and then every hour the following morning for a total elapsed extraction time of 16 ± 2 hours.

15.11 After the extraction period, centrifuge the vials at 1500 RPM for 15 minutes.

15.12 Use a 5 or 10 mL pipette to transfer 90% of the solution into a labeled 125mL bottle. Take great care in not grabbing any of the solid sample. Pipette directly into a syringe and filter (0.2 to 0.4mm) the solution into a 125 mL bottle. Add 40 more mL of solution to sample and invert 10 times. Centrifuge again and transfer as much of the solution as possible (at least 90%) without grabbing any of the solid sample again into a syringe then 125mL bottle.

15.13 To each extract and blank, add 1.25 mL of concentrated BrCl solution, swirl to mix, and bring up to volume of 125mL. Use an example bottle at 125mL for reference.

15.14 Repeat steps 15.12-15.13, adding the filtered rinse extract to the BrCl oxidized initial pH 2 extract in the 125 mL bottles. For each sample, use the same filter as was used for the initial pH 2 extract to filter the second pH 2 (rinse) extract. Dilute each bottle to the base of the neck with reagent water, shake thoroughly, and set aside at least one hour until analysis.

15.15 To each original sample pellet, fill the vial to the 40 mL mark with the 1N KOH extracting solution, and shake vigorously to resuspend the sediment. If after vigorous shaking, the sediment pellet does not break-up, use a mechanical mixing device, such as the Thermolyne™ Mixi-Max plus to dislodge the sample. As a last resort, use a thin, disposable glass rod (or Pasture pipette) to dislodge the compacted sediment.

15.16 When all samples in the batch have been filled with 1N KOH extracting solution, place in the rotator, and shake by slow (5-10 rpm) end-over-end rotation for a total elapsed extraction time of 16 ± 2 hours. Alternatively, in the absence of a rotator, shake the samples very vigorously for 1-2 minutes every half-hour until evening and then every hour the following morning for a total elapsed extraction time of 16 ± 2 hours.

15.17 After the extraction period, centrifuge the vials at 1500 RPM for 15 minutes.

15.18 Use a 5 or 10 mL pipette to transfer 90% of the solution into a labeled 125mL bottle. Take great care in not grabbing any of the solid sample. Pipette directly into a syringe and filter (0.2 to 0.4mm) the solution into a 125 mL bottle. Add 40 more mL of solution to sample and invert 10 times. Centrifuge again and transfer as much of the solution as possible (at least 90%) without grabbing any of the solid sample again into a syringe then 125mL bottle.

15.19 To each extract and blank, add 1.25 mL of concentrated BrCl solution, swirl to mix, and bring up to volume of 125mL. Use an example bottle at 125mL for reference.

15.20 Repeat steps 15.18-15.19, adding the filtered rinse extract to the BrCl oxidized initial KOH extract in the 125 mL bottles. For each sample, use a separate filter as was used for the initial 1N KOH extract to filter the second 1N KOH (rinse) extract. Dilute each bottle to the base of the neck with reagent water, shake thoroughly, and set aside for at least 4 hours until analysis.

15.21 To each original sample pellet, fill the vial to the 40-mL mark with the 12N HNO_3 extracting solution, and shake vigorously to resuspend the sediment. If, after vigorous shaking, the sediment pellet does not break-up, use a mechanical mixing device, such as the Thermolyne™ Mixi-Max plus to dislodge the sample. As a last resort, use a thin, disposable glass rod (or Pasteur pipette) to dislodge the compacted sediment.

15.22 When all samples in the batch have been filled with 12N HNO_3 extracting solution, place in the rotator, and shake by slow (5-10 rpm) end-over-end rotation for a total elapsed extraction time of 16 ± 2 hours. Alternatively, in the absence of a rotator, shake the samples very vigorously for 1-2 minutes every half-hour until evening and then every hour the following morning for a total elapsed extraction time of 16 ± 2 hours.

15.23 After the extraction period, centrifuge the vials at 1500 RPM for 15 minutes.

15.24 After centrifugation, carefully pour the supernatant liquid of each sample into a 125 mL glass bottle labeled with the same sample ID, soil weight, and the type of leach (F-4 in this case). Be very careful to minimize resuspension and loss of the sediment during decanting. If the sediment pellet is prone to resuspension (i.e., sandy materials), use a 5.0 mL pipettor to transfer the supernatant to 125-mL glass bottle. DO NOT TRY TO FILTER THE SAMPLES, AS THE 12N HNO_3 WILL INSTANTLY DISSOLVE THE CELLULOSE NITRATE MEMBRANE! To each extract and blank, add 2.5 mL of concentrated BrCl solution, swirl to mix, and set aside until the rinse aliquot is collected.

15.25 For each sediment sample pellet in the original 40 mL vials, refill with 12N HNO_3 extracting solution (rinse step), and shake vigorously for two minutes after all sediment is resuspended into the aqueous layer. If after vigorous shaking, the sediment pellet does not break up, use a mechanical mixing device, such as the Thermolyne™ Mixi-Max plus to dislodge the sample. As a last resort, use a thin, disposable glass rod (or Pasteur pipette) to dislodge the compacted sediment.

15.26 Repeat steps 15.24-15.25, adding the decanted rinse extract to the BrCl oxidized initial 12N HNO_3 extract in the 125 mL bottles. Dilute each bottle to the base of the neck with reagent water, shake thoroughly, and set aside at least one hour until analysis.

15.27 To each original sample pellet, add 8.0 mL of concentrated HCl and 2.0 mL of concentrated HNO_3, swirling the samples between additions. Loosely replace caps to avoid pressure build up, and swirl periodically over a period of at least four hours.

15.27.1 CAUTION: Perform this operation in a fume hood as the aqua regia generated releases noxious fumes of Cl_2 and NO_2.

15.28 After digestion of 4-16 hours, dilute each sample to 40.0 mL in the original vial with a 10% BrCl solution, shake vigorously, and store at room temperature until analysis. These are the F-5 samples (unless F6 is required). If F6 is required, decant the sample into a 125 mL jar. Preserve the sample with 5 mL 0.2N BrCl. Rinse the sample by suspending the remaining sediment pellet in DI water. Then centrifuge the sample, and add the supernatant to the sample in the 125 mL jar.

15.29 If F6 is required, the sample must be transferred into a 45-mL Teflon Oak Ridge™ centrifuge vial, a 60 ml or 140 mL Teflon bomb. To ensure all of the sediment in the vial is transferred to the bomb, it may be necessary to rinse the vial up to 3 times with 5 mL of HNO_3.

15.30 To the bomb (or centrifuge vial) is added 18.75 mL HNO_3 (this includes any nitric acid used to rinse the vial), 6.25 mL HF and 3 mL HCl. The lid is screwed on tightly, and the bomb is placed in the oven at 130°C for 12hours. The bomb is then diluted up to 50 mL. A 10x dilution is taken with 5% BrCl and analyzed.

15.31 Determination of Hg^0 by Headspace analysis. As an alternative to measuring the free liquid elemental mercury in the F1 extract, it is simpler to measure mercury in the headspace air of a sample. Even a microscopic amount of elemental mercury in the sample will diffuse Hg into the air and reach a temperature-dependant equilibrium concentration. The detection of Hg in the headspace confirms the presence of elemental mercury in the sample. Essentially, a small aliquot of sediment is placed in a jar with a pre-drilled lid overnight at room temperature. An aliquot of the air overlying the sample is removed from the jar using a syringe. The headspace sample is then placed in a stream of N_2 connected to a gold trap. The trap is analyzed directly by CV-AFS.

16) Calculations:

16.1 This preparation procedure does not involve calculations.

17) Statistical Information/Method Performance:

17.1 The Method Detection Limit (MDL) is determined according to 40 CFR Part 136 Section B. Ten replicates (9 degrees of freedom) spiked 3-10 times the expected MDL are run. The standard deviation (s) is taken from the resulting data and the MDL is calculated as follows: MDL=2.821*s. This value should not be interpreted as the method reporting limit. Historical method performance data is summarized in Appendix C.

17.2 The Practical Quantitation Limit (PQL) is the reporting limit for this method and is included as one of the calibration points. The PQL is determined by running ten replicate samples with a concentration that will produce a recovery of 70-130% for most analytes, but the recovery requirements are analyte dependent. The PQL is referred to as the Method Reporting Limit (MRL) in LIMS.

17.3 Current LODs and PQLs are stored at: General and Admin\Quality Assurance\MDLs & PQLs.

18) Quality Assurance/Quality Control:

18.1 The QA/QC for the digestions follow *EFAFS-T-AFS-SOP2992* (EPA 1631) with frequency and range of recoveries and RPDs for the instrument and batch QC.

18.2 Due to the nature of the solutions used in each fraction, a true LCS and MS cannot be prepped. A minimum dilution of one BLK is spiked as the BS/BSD. An AS/ASD is also analyzed with the batch per 10 samples.

18.3 A minimum of three digestion blanks, one digestion duplicate, an HgS in clay lab standard, and an $HgCl_2$ in clay lab standard must be prepared for each discrete sample set (8-20 samples). For smaller sample sets, under standard QA/QC, fewer QC samples may be digested, according to the project manager, or previous history with that client.

18.3.1 The digestion blanks are prepared with a similar mass of Teflon boiling chips as the samples, with the same reagents, and put through the same preparation process as the samples. The LIMS ID or lot # of the Teflon boiling chips is documented in the digestion logbook.

18.4 NOTE: the fractionation of $HgCl_2$ in clay is very strongly dependent upon its concentration (at lower concentrations, more of the Hg(II) is found adsorbed to the clay at weaker extraction levels). Thus, for best QC, it is advisable to use the "low" Hg concentration of $HgCl_2$ in clay (about 33 ppm) when doing low or moderately contaminated samples, and the "high" Hg concentration of $HgCl_2$ in clay (about 2600 ppm) when looking at heavily contaminated samples). This concentration dependence is not an issue for the HgS lab standard.

19) Corrective Action:

19.1 As there is an exceptional amount of sample handling for this method, it is very important that all samples are handled according to clean sample handling protocols (*EFSR-S-CS-SOP2794*).

19.2 Due to the significant amount of filtration in this method, any person performing the above procedure should be well versed in clean filtration. Refer to corrective actions from *EFSR-P-SP-SOP2798*.

19.3 Due to the above two concerns, very close attention is given to the blanks. As this procedure is arduous, re-preparation is very undesirable. If blanks are excessively high, the only recourse is to re-extract. The person preparing the digest needs to have good assurance that reagents and equipment are free of Hg contamination.

19.4 As the amount of QC performed in this preparation is very limited, it is critical that each extraction step is performed exactly as stated in this procedure. Any deviation could result in the failing of QC samples. This would often result in an automatic re-preparation.

20) List of Attachments:

Appendix A: Historical Method Performance Data

Appendix A: Historical Method Performance Data

The results summarized in the Table below were compiled from an August, 1999 validation study using this method. The estimated MDLs are taken as three times the standard deviation of the method blanks. An MDL study run utilizing an actual low level sample (50% NIST-1646, estuarine sediment, plus water, [Hg] = 39ng/g) confirmed these results, except that it found an MDL of 5.0 ng/g for 1N KOH. However, the concentration in the IN KOH extract was rather high, leading to the conclusion that near the blanks, the MDL associated with the variability of the blanks would be more apropos.

Method performance for the mercury selective extraction procedure (August, 1999): A total of four method blanks and three each of the HgS and $HgCl_2$ samples were run. The CRMs are the result of a single run.

	mercury concentrations, ng/g (ppb) dry weight basis					
fraction	**mean blank**	**est. MDL**	**NIST 2709**	**NIST 2710**	**kaolin + HgS**	**kaolin + $HgCl_2$**
DI water	0.07	0.1	8.7	218	0.8	2,090
pH 2	0.11	0.1	1.7	15	3.1	8,757
1N KOH	1.5	0.5	143	1,468	174	496
HNO_3	1.6	5	833	12,120	189	17,274
aqua reg	0.63	1	401	18,460	42,569	2,284
sum total			1,387	32,280	42,936	28,811
expected			1,400	31,700	45,622	33,333
% recov.			**99.1**	**102.1**	**94.1**	**86.4**

EFAFS-T-AFS-SOP5134 Preparation of Sediments for Determination of Methyl Mercury by Acidic Potassium Bromated Extraction into Methylene Chloride
EFHS-S-HS-SOP2991 Waste Disposal Procedures for Client Sample Waste
EFHS-S-HS-SOP5670 Health and Safety Evaluation and Auditing
EFQA-R-EQ-SOP2711 Pipette and Dispenser: Operation, Calibration & Maintenance
EFQA-R-MT-SOP2710 Balance Verification, Calibration & Maintenance
EFSR-P-SP-SOP2796 Oxidation of Aqueous Samples for Total Mercury Analysis
EFSR-P-SP-SOP2798 Ultra-Clean Sample Filtration
EFSR-S-CS-SOP2794 Ultra-Clean Aqueous Sample Collection
EFTM-T-TM-SOP2821 HF/HNO3/ HCl Bomb Digestion of Solids for Total Mercury Followed by Repeated HNO3 Evaporation for Other Metals

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End of document

Version history

Version	Approval	Revision information
4	30.OCT.2015	
5	20.MAY.2016	
6	16.MAY.2017	

1) Revision Log:

Revision: 06	**Effective Date:** This version	
Section	**Justification**	**Changes**
2.12, 2.13	Required	Updated references
8.5	Clarification	Add three IBL requirement / non-zero standards.
8.8, 8.15	Required	Defined terms
10.2, 10.4	Required	Changed MSDS to SDS
12.1.1	Required	Translated SOP reference to its D4 equivalent
14.8.4.1	Correction	Changed minimum pipette volume from 25 uL to 20 uL.
16.5.5	Correction	KCl minimum dilution is 20x or 2.5mL
19.8, Table 1	Required	Updated acceptance criteria for ICV
19.10.2.1, 19.11.1.2	Required	Updated acceptance criteria for WI-DNR blanks
Table 1	Required	Updated acceptance criteria for curve RSD, updated WI-DNR blank requirements and added "≤" to all areas
All	Consistency	Font and indentation changed to similar format (Verdana,normal,10) and D4 controlled indents

2) Reference:

2.1 EPA Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, 2002.

2.2 Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, April 1995 with January 1996 revisions.

2.3 Bloom, N.S.; Ultra-Clean Sample Handling, Environmental Lab 1995, March/April, 20.

2.4 Bloom, N.S.; Horvat M., and Watras C.J. Results of the International Mercury Speciation Intercomparison Exercise. Wat. Air Soil Pollut. 1995, 80, 1257.

2.5 Bloom, N.S.; Crecelius, E.A. Determination of Mercury in Seawater at Sub-nanogram per Liter Levels.Mar.Chem.1983, 14, 49.

2.6 Bloom, N.S.; Crecelius, E.A. Distribution of Silver, Lead, Mercury, Copper, and Cadmium in Central Puget Sound Sediments Mar. Chem 1987, 21, 377-390.

2.7 Bloom, N.S.; Fitzgerald, W.F. Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection. Anal. Chem. Acta. 1988, 208, 151.

2.8 Cossa, D.; Couran, P. An International Intercomparison Exercise for Total Mercury in Seawater. App.Organomet. Chem.1990, 4, 49.

2.9 Fitzgerald, W.F.; Gill, G.A. Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis. Anal. Chem. 1979, 15, 1714.

2.10 Gill, G.A.; Fitzgerald, W.F. Mercury Sampling of Open Ocean Waters at the Picogram Level Deep Sea Res.1985, 32, 287.

2.11 Chapter NR 149, Lab Certification and Registration, Wisconsin Administrative Code.

2.12 TNI Environmental Laboratory Sector, Vol 1, Management and Technical Requirements, ELV1-2016.

2.13 Department of Defense / Department of Energy Consolidated Quality Systems Manual for Environmental Laboratories, prepared by DoD Environmental Data Quality Workgroup (EDQW) and DOE Consolidate Audit Program (DOECAP) Operations Team, DOD Version 5.1, January 2017; DOE Version 3.1, January 2017.

3) Cross Reference:

Document	Document Title
EFQA-R-MT-SOP2710	Balance Verification, Calibration and Maintenance
EFQA-R-EQ-SOP2711	Pipette and Volumetric Dispenser Verification, Calibration and Maintenance
EFAFS-S-SB-SOP5132	Cleaning of Sampling Equipment and Bottles
EFSR-S-CS-SOP2794	Ultra Clean Aqueous Sample Collection
EFSR-P-SP-SOP2796	Oxidation of Aqueous Samples for Total Mercury Analysis
EFAFS-T-AFS-SOP2985	Digestion of Gas/Air Samples Collected on FSTM Traps
EFAFS-T-AFS-SOP2800	Digestion of KCL Traps for Total Mercury
EFAFS-T-AFS-SOP2795	Digestion of Tissues for Total Mercury Analysis Using Nitric and Sulfuric Acids (70:30)
EFQA-P-DR-SOP2801	Data Review and Validation and Monthly Logbooks Reviews
EFTM-T-TM-SOP2837	Total Metals Digestion for Animal or Plant Tissues
EFAFS-T-AFS-SOP2807	Preparation of Solid Samples for Total Mercury Analysis by Modified Cold Aqua-Regia Digestion
EFHS-S-HS-SOP2991	Waste Disposal Procedures for Client Sample Waste
EFAFS-T-AFS-SOP2821	HF/HNO3/HCl Bomb Digestion of Sediments, Soils, Rocks, and Bayer Process Solids and Slurries for Mercury, followed by Repeated HNO_3 Evaporation

4) Purpose:

4.1 This standard operating procedure (SOP) describes a method for the determination of total mercury (Hg) in a wide range of matrices including, but not limited to, aqueous, biological, and geological media. Total mercury is measured by Flow Injection Atomic Fluorescence Spectrometry and is calculated on a concentration (ppt) basis by comparing the instrument response of samples to the instrument response of standards.

4.2 This SOP is designed to ensure that reproducible traceable procedures are followed in the standardization of the total mercury analyzers and in the analysis of samples for total mercury, as well as to establish the limits wherein data will be considered acceptable.

5) Scope:

5.1 This method is designed for the determination of mercury in the typical range of 0.5-40ng/L (ppt). Application may be extended to higher levels by sample dilution, as long as the instrument value (intensity) remains within the calibration curve.

5.2 This SOP describes a method of the determination of mercury species in aqueous and solid matrices (biological tissue, plant tissue, nutraceutical, etc.).

6) Basic Principles:

6.1 Total mercury analyses are split into two categories: waters and solids. For analysis of aqueous samples, a dilution (up to 1x) of oxidized sample is added to a 50mL vial. If less than 30mL of sample is used, the sample is diluted to a final volume of at least 30mL with 1% BrCl. For solids, a dilution is made of the digested sample by pipetting into a 50mL vial and diluting it to 50mL with 1% BrCl. In the case of waters and solids, the final volume is neutralized with 25uL of 25% hydroxylamine-hydrochloride (NH_2OH-HCl). Approximately 25mL of sample is drawn into the system by an auto sampler. (Please refer to Figure 1. for a detailed system flow

diagram of the Tekran 2600 analysis system.) Sample is then mixed with 3% stannous chloride ($SnCl_2$) reducing Hg^{2+} to Hg^0 before entering the phase separator.

6.2 As sample travels down the phase separator Hg^0 is liberated by a counter flow of ultra pure argon (or nitrogen). Mercury then travels through a soda-lime acid vapor trap, a switching valve (V2), and amalgamates onto the fixed "sample trap". Following primary amalgamation V2 is triggered, switching on the flow of pure argon through the sample trap. At the same time the sample trap is heated, and mercury is released into the pure argon gas stream, passing through a second switching valve (V1) and amalgamating onto the "analytical trap". The analytical trap is then heated releasing mercury into the AFS detector. All event timing and peak integration is carried out via a PC running TekMDS-2 software running the EPA 1631 event timing file (ETF). NOTE: This method may also be run using 15ml vials and selecting the appropriate ETF with in the TekMDS-2 software. Sample must fill the vial up to the 15ml mark. All other procedures, reagent concentrations, and dilutions should be kept the same.

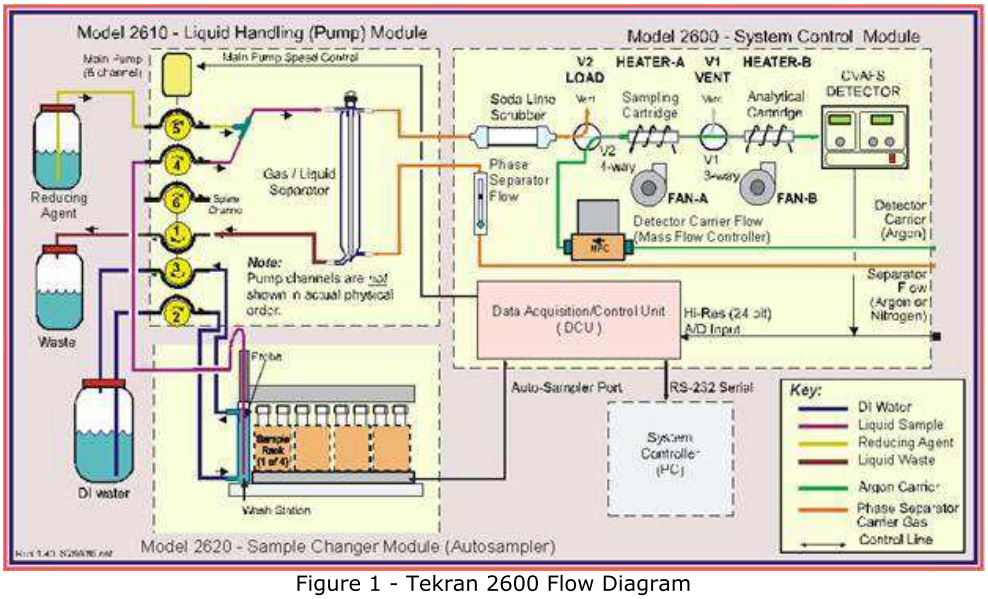


Figure 1 - Tekran 2600 Flow Diagram

7) Reference Modifications:

7.1 There were no significant modifications made to this method.

8) Definitions:

8.1 Analytical Duplicate (AD): A representative sample (that yielded a result within the calibration curve) is analyzed a second time during the analytical run. The second analysis should be at the same aliquot as the original.

8.2 Analytical Run – The continuous analysis of one or more batches during the same 12 hour-shift. Each analytical day requires 3 IBLs, a minimum five-point calibration curve, ICV, and CCV/CCB every ten runs. An analytical day must conclude with a CCV/CCB.

8.3 Analytical Spike and Analytical Spike Duplicate (AS/ASD): A representative sample is selected and spiked, with a dilution of the primary source, during the analytical run, at a target concentration of 1-5X the ambient concentration of the sample. These QC samples are used to indicate sample matrix effects on the analyte of interest. Non-detectable samples are spiked at 5x the MRL/PQL.

8.4 Batch: 20 client samples or less grouped for preparation. See Quality Assurance Section for batch requirements.

8.5 Calibration Standards (CAL) – a series of non-zero standards that will be used to calibrate the instrument, made from a primary source stock standard. Three calibration blanks plus at least five different concentrations are required, beginning with one at PQL concentration or lower.

8.6 Certified Reference Material (CRM) – a standard of known composition that is certified by a recognized authority and representing a sample matrix. It is used to verify the accuracy of a method.

8.7 Continuing Calibration Verification (CCV): A dilution of the OPR standard resulting in an instrumental concentration of 5.0ng/L. This standard is analyzed every 10 analyses after the ICV, and determines whether the instrument is maintaining calibration.

8.8 Continuing Demonstration of Capability (CDOC): Acceptable performance on a PT sample or a new IDOC or a set of four (4) consecutive blank spike or laboratory control sample aliquots [prepared according to a method by an analyst or technician that are analyzed by the same or different analyst or technician according to a method] that meet established and documented criteria for precision and accuracy to assess the ongoing proficiency of the analysts or technicians.

8.9 Control Limit (CL) – the limit of the range of acceptability for the quality control samples

8.10 Equipment Blank (EB): Reagent water processed through the sampling devices and placed in a sample container prior to using the equipment to collect samples and used to demonstrate that the sampling equipment is free from contamination.

8.11 Field Blanks (FB): A sample of reagent water placed in a sample container in the field and used to demonstrate that samples have not been contaminated by sample collection or transport activities. EPA-1631E recommends the analysis of at least one field blank per 10 samples collected at the same site at the same time. Analyze the blank immediately before analyzing the samples in the batch.

8.12 Initial Calibration Verification (ICV) –A dilution of the OPR standard resulting in an instrumental concentration of 5.0 ng/L. This standard is run immediately following the calibration curve and verifies instrument calibration.

8.13 Initial Precision and Recovery (IPR) – A dilution or digestion of a secondary source resulting in an instrumental concentration of 5.0 ng/L mercury. (8 ng/g mercury for 70/30 and Cold AR digestions)

8.14 Instrument Blank Level and Continuing Calibration Blank (IBL and CCB) for evaluation of instrument drift, sensitivity and contamination. At least 3 IBLs must be analyzed before the first calibration standard, and the CCBs every 10 samples immediately after CCVs.

8.15 Initial Demonstration of Capability (IDOC): A set of four (4) blank spike or laboratory control sample aliquots prepared according to a method by an analyst or technician at the concentration specified by the group manager (or if unspecified, to a concentration of one (1) to four (4) times the limit of quantitation) that are analyzed by the same or a different analyst or technician according to a method. The mean and standard deviation of the four results are calculated and compared to established and documented criteria for precision and accuracy to assess the initial proficiency of the analysts or technicians before they independently process client samples.

8.16 Laboratory Control Sample (LCS and LCSD) or Quality Control Sample (QCS): A sample (and duplicate) containing a known concentration of mercury that is used to monitor complete method performance. The preferred LCS is a matrix matched Certified Reference Material (CRM), but a blank spike meets the requirement also. In LIMS, the LCS is always referred to as a Blank Spike (BS), whether it is matrix matched or not.

8.17 Limit of Detection (LOD) – equal to MDL and verified on a quarterly/annual basis, depending on the preparation, by spiking within three times the established LOD and showing a positive result on the instrument.

8.18 Limit of Quantitation (LOQ) – equal to PQL and verified on a quarterly/annual basis, depending on the preparation, by spiking within 2 times the LOQ and showing a recovery between 70 – 130%.

8.19 LIMS: Laboratory Information Management System. Computer software used for managing samples, standards, and other laboratory functions.

8.20 May: This action, activity, or procedural step is optional.

8.21 May Not: This action, activity, or procedural step is prohibited.

8.22 Matrix Spike (MS) and Matrix Spike Duplicate (MSD): A representative sample is selected and spiked with a dilution of the primary source at a known concentration. The MS and MSD are run through the entire analytical process just as the samples are. These QC samples will indicate sample matrix effects on the analyte of interest.

8.23 Method Blank (MBLK) or Preparation Blank (PB): For waters, reagent water that is prepared and analyzed in a manner identical to that of samples. For digested solids, preparations blanks consist of the same reagents used to digest the samples, in the same volume or proportion and are carried through the complete sample preparation and analytical procedure. Boiling chips are used as a blank matrix for solids. Preparation blanks are referred to as BLK in LIMS.

8.24 Method Detection Limit (MDL): A limit derived from 40 CFR, Part 136, Appendix B. This method produces a defined value that is the minimum concentration that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero from a given matrix.

8.25 Method Duplicates/Method Triplicates (MD/MT): A second or third separate sample dilution, taken from the same source sample, prepared and analyzed in the laboratory separately. An MSD may be used as a duplicate.

8.26 Reagent water: 18 MO minimum, reagent water starting from a pre-purified (distilled, Reverse Osmosis, etc.) source.

8.27 Must: This action, activity, or procedural step is required.

8.28 Ongoing Precision and Recovery (OPR): A dilution of a secondary source resulting in an instrumental concentration of 5.0 ng/L mercury.

8.29 PM: Project Manager.

8.30 Practical Quantitation Limit (PQL), Method Reporting Limit (MRL): The minimum concentration that can be reported quantitatively. The PQL is often described as 1-10 times higher than MDL. Eurofins Frontier defines the PQL as the lowest concentration that can achieve 70-130% recovery for 10 replicate sample preparations. In LIMS, the PQL is referred to as the MRL.

8.31 Primary Source: The stock standard used to make the calibration standard. Procedural Method: A method where standards and samples are run through the analytical procedure exactly the same. By NELAC definition, this SOP is a procedural method.

8.32 Secondary Source: The stock standard used to make the OPR standard

8.33 Shall: This action, activity, or procedure is required.

8.34 Should: This action, activity, or procedure is suggested, but not required.

8.35 Stock Standard Solution (SSS) – a standard of analyte that is purchased from a certified source for the preparation of working standards.

8.36 Total mercury: As defined by this method, all bromine monochloride-oxidizable mercury forms and species found in aqueous solutions. This includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg(P), and several tested covalently bound organomercurials (i.e. CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of mercury bound within microbial cells may require additional preparation steps (i.e. UV oxidation, or oven digestion).

8.37 Travel or Trip Blank (TB): A sample of reagent water placed in a sample container in the laboratory and used to demonstrate that samples have not been contaminated by transport activities.

8.38 Wash Station Blank (WSB) – a "blank" that is drawn from the wash station (0.5% BrCl). At least three WSBs are used at the beginning of analysis to assess overall cleanliness of the system/reagents. WSBs may also be used mid-run to flush the system after running a high level sample, or check the mercury levels in the wash station.

9) Interferences:

9.1 Due to high levels of acid and halogens (i.e. bromide) in digested solids, it is recommended that aliquots of no more than 5.0mL of the digestates be analyzed.

9.2 Only BrCl tested to be low in mercury should be used for the sample dilutions.

9.3 Prep blank correction is very important for this instrument.

9.4 Improperly adjusted pump tubing can cause the phase separator to fill with sample. In extreme situations, the overflow can back up into instrument causing damage to the traps and switching valves. Care must be taken to insure the pump tubing tension is properly set. DO NOT OVER TIGHTEN.

9.5 No more than 1.0mL should be analyzed if HF acid, FSTM material, or coal is present in significant concentrations. Samples prepared according to *EFAFS-T-AFS-SOP2821* "HF/HNO3/HCl Bomb Digestion of Sediments, Soils, Rocks, and Bayer Process Solids and Slurries for Mercury, followed by Repeated HNO_3 Evaporation" are diluted before analysis, therefore 5.0 mL is the maximum.

9.6 Water vapor has the potential to create recovery interferences. To prevent interference from water, ensure that soda-lime pre-traps and gold traps remain dry.

9.7 The presence of high concentrations of silver and/or gold can cause $SnCl_2$ to precipitate out of solution and adhere to the tubing and/or phase separator walls. High concentrations of these metals can sometimes be found in the matrix spike samples from the digestion sets that are shared with the trace metals group.

9.8 Analysis of samples containing high concentrations of strong acids (>2%) can cause passivation of the sample trap and lead to low bias/recoveries. When analyzing samples containing >2% strong acid (at the instrument), the analyst should verify that passivation is not an issue by running a Hg spike into MQ water containing a similar concentration of acid. If the spike recovers low (<90%) passivation may be an issue and the sample may need to be diluted.

10) Safety Precautions, Pollution Prevention and Waste Handling:

10.1 Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan. This includes, but is not limited to, laboratory coat, safety goggles and nitrile gloves under clean gloves.

10.2 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the SDS (Safety Data Sheets) for each chemical they are working with.

10.2.1 Note: Use particular caution when preparing and using BrCl, as it releases extremely irritating, corrosive fumes similar in effect to free chlorine. Always handle this reagent in an approved fume hood.

10.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease-causative agents. Eurofins Frontier will reimburse the expense of Hepatitis A and B immunizations for any laboratory staff member who desires this protection.

10.4 Hydrochloric acid: Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion. Slightly hazardous in case of inhalation (lung sensitizer). Non-corrosive for lungs. Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering. For more information see SDS.

10.5 See Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP) for general information regarding employee safety, waste management, and pollution prevention.

10.6 Pollution prevention information can be found in the current Eurofins Frontier Global Sciences Chemical Hygiene Plan (CHP), which details and tracks various waste streams and disposal procedures.

10.7 All laboratory waste is accumulated, managed, and disposed of in accordance with all federal, state, and local laws and regulations. Any waste generated by this procedure should be disposed of according to SOP *EFHS-S-HS-SOP2991* "Waste Disposal Procedure for Client Sample Waste," which provides instruction on dealing with laboratory and client waste.

11) Personnel Training and Qualifications:

11.1 An analyst must perform an initial demonstration of capability (IDOC) that includes four replicates of a secondary source before being qualified to analyze samples without supervision. Continuing DOC will be maintained and monitored via performance on CRMs and other QC samples, as well as obtaining acceptable results on proficiency testing exercises.

11.2 The analyst/laboratory technician must have read this SOP and other relevant SOPs and have the training documented on the applicable form(s). The analysis may be questioned on SOP by supervisor(s) and/or trainers.

11.3 Training is documented by the employee and supervisor, and is kept on file in the QA Office. The employee must read, understand, and by signing the training document, agree to perform the procedures as stated in all Standard Operating Procedures (SOPs) related to this method.

11.4 All employees must read the Quality Manual (QM) and complete annual Ethics training.

11.5 All training documents including IDOCs, CDOCs, Initial QA orientation, and Ethics training are stored by the Quality Assurance Manager in the employees training file for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

11.6 Chemical Safety Training, Compressed Gas Training, Chemical Hygiene Plan documentation, and Shipping of Hazardous goods, are stored by the Health and Safety Officer for ten years after the employee is no longer working for Eurofins Frontier Global Sciences.

12) Sample Collection, Preservation, and Handling:

12.1 Aqueous samples are collected in rigorously cleaned fluoropolymer (e.g. Teflon) bottles and caps (as described in FGS-007 "Cleaning of Sampling Equipment and Bottles for Mercury Analysis"). Certified clean glass bottles with fluoropolymer lids may be used if mercury is the only analyte of interest.

12.1.1 Aqueous samples are preserved upon receipt with 0.2N BrCl that has tested low in mercury. Samples are typically preserved to 1% BrCl v/v, but may require further oxidation due to high levels of organic matter or mercury. Refer to *EFSR-P-SP-SOP2796* "Oxidation of Aqueous Samples for Total Mercury Analysis".

12.2 Solid samples may be collected in glass, high density polyethylene, or fluoropolymer jars.

12.2.1 Solid samples are preserved by freezing upon receipt.

12.3 Impinger solutions preserved with nitric acid or containing potassium permangante (this category includes proficiency testing samples) need to be preserved with BrCl to insure the complete oxidation of organic species.

12.4 All samples should be collected utilizing clean techniques, so as not to cross-contaminate samples with mercury. See FGS-008 "Ultra Clean Aqueous Sample Collection" and EPA Method 1669 for aqueous sample techniques.

13) Apparatus and Equipment:

13.1 LIMS – Element, version 6 or higher; Computer – Windows XP, 7 or 8

13.2 Tekran Operating System, see instrument software log *EFQA-S-IT-WI7061*

13.3 Model 2600 System Control Module – manufactured by Tekran, this module contains dual stage pre-concentration/desorption units, a high resolution analogue to digital converter, and a high sensitivity AFS Detector (IDL<1pg).

13.4 Model 2610 Pump Unit – manufactured by Tekran, this module contains a precision 6 channel peristaltic pump with optical speed feedback. Speed may be varied manually or under computer control. The pump head has three four-roller channels to providing high pumping rates for wash station circulation and waste removal, and three eight-roller channels to providing slower/low pulse delivery of $SnCl_2$ and sample (one extra channel).

13.5 Model 2620 Auto-Sampler – a specially modified Gilson Model 223 auto-sampler, includes reticulating wash station and allows for automatic sample handling with the 2600 analysis system.

13.6 50mL Polypropylene Vials with screw caps, manufactured by Environmental Express (Item No: SC475), or equivalent pre-cleaned and/or tested vials, graduated to 50mL.

13.7 Three-stop tubing, various inner diameters of Marprene for $SnCl_2$ (1.02mm), for wash station fill (2.38mm), wash station drain (2.79mm), and phase separator drain (2.79mm) Three stop silicone tubing (2.05mm) is used for sample feed.

13.8 Teflon Fittings and FEP tubing – of various sizes and lengths. Tubing from sample probe, $SnCl_2$, rinse water, phase separator, to and from wash station, and between gold traps is 1/16 inch (inner diameter) FEP.

13.9 Soda-Lime trap – a 14cm x 1.1cm diameter glass tube containing 12-14g of reagent grade, non-indicating 8-14 mesh soda-lime ($Ca(OH)_2$+NaOH) aggregates, packed between portions of silanized glass wool. This trap is purged of mercury by placing it on the output of the phase separator and purging it to air with a 0.5% BrCl rinse solution and 3% $SnCl_2$ for approximately 20 minutes with Ar (or N_2)at 400 mL/min.

13.10 Phase separator – manufactured by Tekran, the phase separator utilizes laminar fluid flow of sample over a frosted rod counter current to a stream of argon gas at 400mL/min.

13.11 Gold Coated Quartz Sand Trap ("sample trap") – manufactured by Tekran or made in house, heated from 0% to 100%, 8 Amp maximum.

13.12 Pure Gold Bead Trap ("analytical trap") – manufactured by Tekran, heated from 0% to 100%, 8 Amp maximum.

13.13 Pipettes: Calibrated variable pipettes with a range of 5 µL – 10 mL. Used to make solutions and sample dilutions. Pipettes are to be calibrated weekly according to SOP *EFQA-R-EQ-SOP2711* and EFGS-155.

13.14 Analytical Balance – capable of accurately weighing to the nearest 0.1mg, and able to tare at least one gram. The analytical balances are verified for accuracy on a daily basis according to *EFQA-R-MT-SOP2710*, "Balance Verification, Calibration and Maintenance."

14) Reagents and Standards:

All reagents, except those made daily, must be entered into LIMS

14.1 Reagent Water: 18-MO ultra pure deionized water starting from a pre-purified (distilled, R.O., etc.) source is used. To remove any remaining trace metals and organics, an activated carbon cartridge is placed between the final ion exchange bed and the 0.2-µm filter. Reagent water used in the mercury lab is checked weekly for total mercury concentrations, and must test below 0.25ng/L.

14.2 Hydrochloric Acid (HCl): Concentrated (36-38% weight basis). Trace metal purified reagent-grade HCl. HCl is lot- tested by removing a small amount (20mL) of acid from a bottle of the new lot. To test the HCl, add 0.5 mL, using a calibrated pipette, of the HCl to a 50mL vial and dilute to 50mL with reagent water. Place into correct position in auto sampler and run like any sample. This reagent must test below 0.25ng/L. Do not prep blank correct.

14.3 0.2N Bromine Monochloride (BrCl):

14.3.1 37.5 g of KBr is added to a 2.5-L bottle of concentrated HCl (pre-analyzed and found to be below 5 ng/L Hg). The bottle is then inverted in a fume hood to mix the acid and KBr. The solution then sits overnight allowing for the KBr to be dissolved .

14.3.2 27.5 g of $KBrO_3$, certified to be low in Hg, is slowly added to the acid. When all of the $KBrO_3$ has been added, the solution should have gone from yellow to red to orange.

14.3.3 Loosely cap the bottle, and allow to sit for 30 minutes in a fume hood before tightening the lid. Once capped invert bottle to make sure all of the solids goes into solution. CAUTION: This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl) which are released from the bottle. Add the $KBrO_3$ SLOWLY and in a well operating fume hood.

14.3.3.1 To test the BrCl, add 0.5 mL, using a calibrated pipette, of the BrCl to a 50mL vial and dilute to 50mL with reagent water. Add 25 µL Hydroxylamine-HCl to the vial; and place into correct position in auto sampler. Assume a 100 mL aliquot in the Excel spreadsheet. This reagent must test below 0.25ng/L. Do not prep blank correct. Analyze one replicate per bottle.

14.3.3.2 The expiration time for this reagent is set by default to six months in LIMS. There is no suggested holding time in EPA method 1631E. Therefore the holding time can be extended, as long as the primary reagent has not expired. The mercury concentration of the BrCl is monitored through the preparation of water preparation blanks.

14.4 Hydroxylamine hydrochloride: dissolve 500g of NH_2OH-HCl in reagent water and bring the volume up to 1L. This solution may be purified by the addition of 1mL $SnCl_2$ solution and purging overnight at 500mL/min with mercury-free N_2. The working reagent is a 25% solution that is made by adding one part reagent water to one part 50% hydroxylamine hydrochloride. This reagent must test below 0.25ng/L.

14.4.1 To test the Hydroxylamine-HCl (NH_2OH-HCl), add 0.1 mL of the 50% reagent, using a calibrated pipette, to a 50mL vial and dilute to 50mL with reagent water. Place into correct position in auto sampler. Assume a 50mL aliquot in the Excel spreadsheet. This reagent must test below 0.25 ng/L. Do not prep blank correct. Analyze one replicate per bottle.

14.5 Stannous Chloride ($SnCl_2$): dissolve 500g $SnCl_2$ with three 100mL additions of concentrated HCl to original bottle and transfer (after each addition of HCl) to a 1L glass bottle. Bring this solution up to 1L of volume with reagent water and purge overnight with mercury-free N_2 at 500mL/min to remove all traces of mercury. Store tightly capped. The working reagent is a 3% solution that is made by diluting 150mL of 50% ScCl2 and 30-50mL of HCl to 2.5L with reagent water. The working reagent is continuously purged with nitrogen to prevent contamination. The 50% $SnCl_2$ reagent must test below 0.25ng/L.

14.5.1 To test the Stannous Chloride ($SnCl_2$), add 0.3 mL of the 50% reagent, using a calibrated pipette, to approximately 50 mL of purged bubbler water. Assume a 50 mL aliquot in the spreadsheet. This reagent must test below 0.25 ng/L. Do not prep blank correct. Analyze one replicate per bottle.

14.5.2 The expiration time for this reagent by default is set to six months in LIMS. There is no suggested holding time in EPA method 1631E; therefore the holding time can be extended, as long as the primary reagent has not expired.

14.6 5% HCl Shutdown Rinse Solution: 200mL concentrated low-level mercury HCl into 4,000mL of reagent water.

14.7 0.5% BrCl Rinse Solution: 30mL concentrated low-level mercury BrCl into 6,000mL of reagent water.

14.8 Preparation of Total Mercury Standard Solutions:

14.8.1 Mercury standard solutions are prepared in ultra clean volumetric glassware and gravimetrically calibrated pipettes. Resulting solutions must be stored in glass or Teflon bottles and preserved to at least 2 % BrCl. All working standards must be

tested prior to use.

14.8.2 New working standards and standard dilutions are tested prior to use. Three reps of the new standard are analyzed in the same run as three reps of the current NIST 1641D standard. Analyze 100 µl of the NIST 1641D and assume 1x dilution. The mean percent recovery of the three standards should be ±5 % (95-105 %) of the true value and also within 5 % of the average NIST 1641D recovery (e.g. If the average of NIST 1641D recovery is 97 %, the range for the standard is 95-102 %). If the standard does not test within this control limit, it is retested. If it still does not meet the control limit, it is discarded and remade, unless otherwise approved by the Quality Assurance Officer. NOTE: When making serial dilutions to create various standard levels; the lowest concentration may be used to test any of the higher concentration steps (for example: if a 10ng/mL calibration standard is created from a 1000ng/mL spiking standard, only the 10ng/mL standard requires testing. If the 10ng/mL standard passes, then both standards are considered to be passing within the control limits.)

14.8.3 Total Mercury Stock Standard Solution (Stock): Certified mercury standard purchased from High Purity Standards (1000 µg/mL (1 000 000 ng/mL) primary source) or Absolute Standards (100 µg/mL (100 000 ng/mL) secondary source), or any equivalent standard.

14.8.4 Total Mercury Spiking Standard Solutions (Spiking Standard): Spiking standards are made from either the primary or secondary sources.

14.8.4.1 To make standards, use an ultra clean volumetric flask and a calibrated pipette. Add reagent water until flask is about half full. Add 2 % 0.2N BrCl and the specific spike volume noted below (these volumes may be changed as long as ratio and resulting concentration remains the same). Bring up to the mark with reagent water and mix well prior to testing. When spiking samples, no more than 200 µL of any spiking standard is added to the sample to minimize effects on volume. It is also recommended that staff pipette no less than 20 µL. If possible, minimize headspace during standard storage. Expiration date is currently set at 6 months or when the stock standard expires, whichever is shorter.

14.8.5 100,000 ng/mL Spiking Standard: Made from the Primary Stock Standard (High Purity, or equivalent vendor). Dilute 10 mL of the stock standard to 100 mL RO water containing 2 % BrCl. (Can also be made by preserving Secondary Stock Standard to 2% BrCl).

14.8.5.1 10,000 ng/mL Spiking Standard: If made from the Primary Stock Standard (High Purity, or equivalent vendor). Dilute 1.0 mL of the stock standard to 100 mL RO water containing 2 % BrCl. If made from Secondary Stock Standard dilute 10mL of stock standard to 100mL with RO water containing 2% BrCl.

14.8.5.2 1,000 ng/mL Spiking Standard: If made from the Primary Stock Standard. Dilute 0.250 mL of the stock standard to 250 mL RO water containing 2 % BrCl. If made from Secondary Stock Standard, dilute 2.5mL of stock standard to 250mL with RO water containing 2% BrCl.

14.8.5.3 100 ng/mL Spiking Standard: Made from a stock standard or dilution of a stock standard with a concentration of 100,000 ng/mL. Dilute 0.100 mL of the 100,000 ng/mL dilution to 100 mL RO water containing 2 % BrCl. Expiration date is currently set at 3 months or when the stock standard expires, whichever is shorter.

14.8.6 Calibration Standard (10 ng/mL): Must be made from a dilution of the Primary Stock Standard. Typically made by diluting 0.5mL of a 10,000 ng/mL Primary Spiking Standard to 500 mL of RO water containing 2 % BrCl. Transfer to one 1000 mL glass or Teflon bottle. The calibration standard is considered stable for three months or until the stock standard expires.

14.8.7 Calibration Standard (1 ng/mL): Must be made from a dilution of a Primary Stock Standard. Typically made by diluting 1.0mL of a 100 ng/mL Primary Spiking Standard to 100mL with RO water containing 2% BrCl.

14.8.8 Initial Calibration Verification (ICV): ICV analysis, use 25µL of the OPR Standard (documented in LIMS as "THg ICV/OPR for 2600s"). The True value is 5.0 ng/L.

14.8.9 Continuing Calibration Verification (CCV): For CCV analysis, use 25µL of the OPR Standard (documented in LIMS as "THg ICV/OPR for 2600s"). The True value is 5.0 ng/L.

14.8.10 Certified Reference Material (CRM) for Total Mercury in Water: A 1.5679 mg/L solution (1.557 mg/kg at a density of 1.007 g/mL) is prepared by adding a 5.0 mL of CRM NIST 1641d (from ampoule) into a 1000 mL flask containing RO water. This solution is diluted to 1000 mL, and an additional 10 mL of 0.2N BrCl is added, resulting in a final volume of 1010 mL. Preparing the solution in this manner makes a 1:200 dilution of the stock CRM. This solution is considered stable for one year, or until the stock standard expires. Results are corrected for the additional 1 % BrCl in the analysis Excel spreadsheet and in LIMS.

14.8.11 OPR Standard (10 ng/mL): Must be made from a dilution of a Secondary Spiking Standard. Typically made by diluting 1.0mL of a 1000ng/mL Secondary Spiking standard to 100mL with RO water containing 2% BrCl.

14.9 Documentation of Standards:

14.9.1 Standards and Reagents are documented in LIMS upon receipt or creation. A LIMS generated label is affixed to each standard and reagent that has the name of the solution, the person who prepared or received it, the date it was prepared or received, and the expiration date.

14.9.2 Each bottle of standard must be labeled with the following: the date of receipt or creation, the initials (or name) of who entered the standard into LIMS, the concentration and analyte, the expiration date and the LIMS ID. This information must also appear on the certificate of analysis of stock standards.

14.9.3 Stock standards and CRMs are logged into LIMS upon receipt by Shipping and Receiving (S&R) or the Quality Assurance department (QA). These do not require testing, provided there is a Certificate of Analysis on file in QA. When receiving a solid CRM, QA shall generate a work order in LIMS for total solids analysis.

14.9.4 For all standards, LIMS documentation must include the following: a description of the standard, department, expiration date of the standard (not to exceed the expiration of the parent standard), the name of the person who made (or received) the standard or reagent, the date it was prepared (or received), final volume, a reference date (date entered into LIMS), concentration units (µg/mL), the vendor and vendor lot. The solvent lot is used to document the Lot Number or LIMS ID of the BrCl that was used. In the comments section, the analyst must enter the sequence and applicable results for documentation of standard testing. Other notes may be entered in here as well. The correct parent standard must be noted, as well as the amount used. Analytes are entered individually from the list. LIMS will calculate the true value of the standard based on the amount of the parent used and the final volume. Click the appropriate radio button under Standard type. A Spike Mix is a standard that is used in a bench sheet, and a Calibration standard is a standard used only in sequences. The standard must not be used until it has passed control limits and is approved by the mercury supervisor, mercury laboratory manager, or QA for use.

14.9.4.1 If the new standard is a calibration standard, a separate standard ID must be created for each calibration point based on the final concentration in the sequence (example: THg Cal Pt 0.5ppt (0.05ng) for 2600s, THg Cal Pt 20.0ppt(2.0ng) for 2600s, etc). These are given the same expiration as the standard they are made from, and will need to be generated every three months as each new working calibration standard is made and tested.

14.9.4.2 To generate new "CAL" standards in LIMS, go to the Laboratory drop down menu and select Standards. Open the current THg Cal Pt 0.5ppt (0.05ng) for 2600s standard and click "Copy". Update the appropriate information, including the Prepared Date, Expiration Date, Prepared By, and the Reference Date. For these standards, which are to be used in the sequence, the final volume is equal to the volume in the vial (50 mL). Remove the old (expired) parent standard. Choose the new parent standard, and enter the amount of standard added to the vial for that calibration point. Repeat for all "CAL" standards.

14.9.5 Neat reagents are logged into LIMS with a unique identifier upon receipt by Shipping and Receiving Department.

14.9.6 Working reagents are prepared by the analyst, logged into LIMS and assigned a unique identifier. Reagents entered into LIMS must have the information listed in section 14.9.4, in addition the parent neat reagents are added by their unique identifier and the amount of each reagent is entered. It is not necessary to enter analytes from the list for reagents. The Solvent Lot is not applicable to working reagents. The radio button must be clicked to Reagent. If the reagent requires testing, it must test clean prior to using. All reagents used during analysis and prep must be added to bench sheet.

14.9.7 Each bottle of reagent must be labeled with the following: the date of receipt or creation, the initials (or name) of who entered the reagent into LIMS, a description of the reagent including composition when applicable, the expiration date and the LIMS ID. This information must also appear on the certificate of analysis or the certificate of purity for the reagent.

14.10 Argon Grade 4.7 or better (ultra high-purity grade) – argon that has been further purified by the removal of mercury using a gold trap that is located in line between the gas output and the analyzer gas input.

14.11 Nitrogen Grade 4.5 (standard laboratory grade) – nitrogen that can be further purified of mercury using a gold trap that is located in line between the gas output and analyzer gas input.

15) Calibration:

15.1 The calibration sequence consists of three IBLs followed by a minimum 5-point calibration curve. Usual concentrations are 0.5ppt, 1.0ppt, 5.0ppt, 20.0ppt, and 40.0ppt. Standards are analyzed from lowest to highest. Immediately following the standard curve an ICV/OPR (5.0ppt) is analyzed. All standards (and samples) are added to a 50mL vial and diluted to 50mL with 1.0% BrCl.

15.1.1 Three IBLs are prepared by adding 50mL 1.0% BrCl to the sample vial.

15.1.2 Using the 1.0 ng/mL calibration standard, 25µL and 50µL are added to vials for the lowest two calibration points. Using the 10ng/mL calibration standard, 25µL, 100µL, and 200µL are added to vials for the upper three calibration points.

15.1.3 Using the OPR Standard) 25µL is diluted to 50mL with 1.0% BrCl l for the ICV/OPR (5.0ng/L).

15.2 Once the instrument is calibrated and the ICV/OPR and three IBLs are analyzed and passing, the instrument is operational. The sample concentrations must fall within the range of the calibration standards or be diluted and reanalyzed.

15.3 Additional calibration standards (linearity standards) may be added to the curve to prove instrument linearity at higher levels. This is acceptable only when there is no more client sample available, otherwise a dilution must be performed to bring the sample within calibration range.

15.4 The calibration of this system and calculation of sample results are performed using units of concentration (ppt or ng/L).

16) Procedure:

16.1 Pre-analysis and Organization:

16.1.1 Prior to analyzing samples it is imperative to reference LIMS for all project specific information, such as QC requirements, suggested dilutions, project manager information, and specifics regarding spike levels.

16.1.2 The analyst should then locate samples and check the work order in LIMS for notes about specific project requirements.

16.1.3 The analyst should compare the sample IDs to the work order and see that the samples are accounted for, and notify the project manager of any discrepancies in analysis required, sample identification, etc.

16.1.4 All mercury analyses receive a unique dataset identifier and LIMS assigned sequence number. The dataset ID is comprised of the instrument type and number, the date and the calibration number for that day. The format is as follows: THg26001-130216-1, where "THg "refers to a total mercury analysis; "26001" refers to Tekran 2600 instrument number 1; 130216 refers to the date (February 16, 2013 in the YYMMDD format); and "1" refers to the first calibration of the day. The sequence number is assigned by LIMS when the data gets imported into LIMS. The alpha-numeric code is based on the following format: 3B02001, where the 3 refers to the year (2013), the "B" is the month (A= January, B=February…L=December), "02" is the day of the month (February 2nd) and the final 3 digits is the nth sequence created on that particular year/month/day combination.

16.1.5 In general the analyst should organize their samples in the order listed on the bench sheet. The first samples analyzed should be the preparation blanks, then the LCS if analyzing solid samples, followed by actual samples. If possible, run total and dissolved samples side by side to facilitate verification that total concentration is greater than dissolved concentration. See QA section.

16.2 Instrument Start Up:

16.2.1 If necessary, prepare the soda-lime trap. The soda-lime trap should be changed, at least, every three analytical days.

16.2.2 Start the TekMDS software and check to make sure the computer is communicating with the instrument and the autosampler. Load a new work sheet and begin to enter the sample IDs into the run. Take care to insure that the appropriate autosampler position and dilution are entered correctly into the worksheet.

16.2.3 Empty the waste carboy in accordance with *EFHS-S-HS-SOP2991* "Waste Dumping Procedure for Client Sample Waste.

16.2.4 Fill rinse basin with a fresh solution of 0.5% BrCl and refill 3% $SnCl_2$ container.

16.2.5 Check tubing for wear and re-index or replace as needed. Clamp all tubing/cassettes onto pump head and adjust tension.

16.2.5.1 Insure that there are no kinks in reagent or waste lines.

16.2.6 Turn the pump on to "local" and check flow of all reagents, including flow through the sample probe from the wash station and flow of SnCl2 into phase separator. Adjust tension on tubing, if necessary. Be sure that the waste line is flowing well and waste does not back-up into the phase separator! This can often be prevented by loosening the tension on the tubing for both the phase separator drain and the wash station drain.

16.2.7 Thoroughly wet the phase separator rod by unscrewing and moving it up and down within the housing; making sure every part of the rod gets thoroughly wet. Secure it in place by screwing the rod back into the top.

16.2.8 After wetting, turn on the phase separator gas to 400 mL/min. Nitrogen or argon can be used as the phase separator gas (Due to its lower, cost nitrogen is preferable for this application).

16.2.9 All analytical runs should begin with at least two "clean" cycle to "blank" the gold traps, followed by three wash station blanks (WSB) to assure that the system is free of mercury.

16.2.10 Set pump back to "remote" to start the run.

16.2.11 Note: It is important to check the flow, reagent levels, and waste carboy level throughout the analytical run. If a reagent is low in volume, it can be refilled mid-run. Similarly, the waste carboy should be emptied mid-run, if necessary. The SnCl2 and sample flow (tension on tubing) cannot be changed mid-run as it may affect instrument response. For this reason, it is important to verify proper sample/reagent flow prior to beginning analysis.

16.3 Batching and Analyzing Aqueous Samples:

16.3.1 All analysts will show Initial Precision and Recovery (IPR) by analyzing four secondary spikes at 5.0ng/L. The spikes need to recover between 79-121% with an RSD < 21%.

16.3.2 All aqueous samples should be preserved with BrCl according to *EFSR-P-SP-SOP2796* "Oxidation of Aqueous Samples for Total Mercury Analysis" at least 24 hours prior to analysis. In the event a sample requires further oxidation prior to analysis, additional BrCl is added and the sample is not analyzed for at least 12 additional hours. In special cases where rush turn-around-time is required and an oxidation period of less than 24 hours may be used, the analyst should consult a supervisor for approval. The supervisor must be able to confirm (based on past experience) that the particular matrix type is completely oxidized before analysis.

16.3.2.1 When setting up a batch of aqueous sample in LIMS, it's necessary to account for the amount of BrCl that was added to the samples and blanks. Adjust the final volume in the batch to account for the amount of BrCl that was added. For instance, 100 mL of sample is preserved with 3% (v/v) BrCl. The final volume should be 103 mL in the benchsheet.

16.3.3 While the standard curve is being analyzed, the analyst should prepare three 1% BrCl and any additional water preparation blanks (PBW). Add 0.5mL BrCl (for additional PBW's add an additional 0.5mL of BrCl for each percent above 1%) and 25µL hydroxylamine hydrochloride (NH_2OH-HCl) to each vial. Dilute to 50mL with reagent water. Upon request (e.g., WI DNR samples), batch QC blanks can be created at the time of preservation and will be used as the blanks for the batch; the date of preservation will be entered as the prep date in the LIMS batch sheet).

16.3.4 It is recommended that all known field, equipment, and trip blanks should be analyzed before any other sample types.

16.3.5 For all waters, select the appropriate dilution (refer to LIMS, historical data, etc.).

16.3.6 When preparing dilutions, use calibrated pipettes to dispense the aliquots into the 50mL vials. Dilute the sample to a final volume between 25mL to 50mL with 1% BrCl. Neutralize BrCl with 25µL of 25% NH_2OH-HCl. The sample should turn from a yellowish color to a clear/cloudy solution, depending on the matrix. Be sure to note any sample dilutions onto the bench sheet for cross referencing during peer review. NOTE: If using 1x dilution, water samples should be thoroughly homogenized and poured directly into 50mL vial with 25 µL of 25% NH_2OH-HCl. Sample amounts of no less than 30 mL should be analyzed provided there is adequate collected sample volume.

16.3.7 For sample aliquots greater than 10mL, you may gravimetrically weigh out the selected volume (±0.2g) into a 50mL vial. Dilute the sample to a final volume of 50mL with 1% BrCl. Neutralize BrCl with 25µL 25% NH_2OH-HCl.

16.3.8 Load sample vials into the auto sampler. Verify that the correct vial is in the proper position relative to the analysis worksheet entry in TekMDS software for that sample.

16.3.9 For each sample, the sample ID, BrCl percentage, and a dilution based on 50mL is entered into the TekMDS software. Unless otherwise stated (e.g., all WI DNR samples, PRASA), all aqueous samples should be PB corrected. Note: prep blank correction takes place after analysis in the Excel spreadsheet, not in the TekMDS software.

16.3.10 The analyst should continue preparing and adding samples to the run in the same fashion to maximize efficiency. Positions on the autosampler can be used more than one time throughout the analysis run by removing a previously analyzed sample vial and replacing it with a new sample.

16.4 Analysis of Digested Solids:

16.4.1 All analysts will show Initial Precision and Recovery (IPR) by analyzing four secondary spikes at 8.0ng/g. The spikes need to recover between 75-125% with an RSD < 20% for EFAFS-T-AFS-2795 and 2807. All other Frontier preps will be covered by the IPR performed for waters.

16.4.2 With this method it is possible to determine the concentration of total mercury in solid samples following proper digestion. The following highlight the majority of total mercury prep techniques for solids and their matrix specific SOPs.

16.4.2.1 For tissues, refer to *EFAFS-T-AFS-SOP2795* "Digestion of Tissues for Total Mercury Analysis Using Nitric and Sulfuric Acids (70:30)" for the nitric acid/sulfuric acid digestion and *EFTM-T-TM-SOP2837* "Total Metals Digestion for Animal or Plant Tissues" for the concentrated nitric acid digest.

16.4.2.2 For soils/sediments, refer to *EFAFS-T-AFS-SOP2807* "Preparation of Solid Samples for Total Mercury Analysis by Modified Cold Aqua-Regia Digestion" for the cold aqua regia digestion, *EFAFS-T-AFS-SOP2795* for the nitric acid/sulfuric acid digestion, and *EFAFS-T-AFS-SOP2821* "HF/HNO3/HCl Bomb Digestion of Sediments, Soils, Rocks, and Bayer Process Solids and Slurries for Mercury, followed by Repeated HNO3 Evaporation" for the hydrofluoric acid/nitric acid bomb digestion.

16.4.2.3 For coal and ash, refer to *EFAFS-T-AFS-SOP2807* Preparation of Solid Samples for Total Mercury Analysis by Modified Cold Aqua-Regia Digestion for the cold aqua regia digestion. For FSTM, refer to *EFAFS-T-AFS-SOP2985* "Mercury Digest for Gas/Air Samples Collected on KCl/Quartz or KCl/Lime trap" for the digestion of traps.

16.4.3 After the instrument calibration sequence, preparation blanks and the digested CRM(s) are analyzed. The analyst should use the minimum dilution allowed for each preparation blank and diluting to 50mL with reagent water preserved to 1% BrCl. Analysts are to calculate the appropriate dilution for the digested CRM, ensuring that the sample concentration stays within the calibration curve.

16.4.4 Begin analyzing samples by the order of the digestion sheet, with the exception of the sample that has separate QC digested for MD/MS/MSD. It is appropriate to analyze this sample, and any associated QC, out of bench sheet order. The analyst should review any relevant project notes, historical data, or information given by client to the PM to help determine proper dilutions that will allow samples to recover within calibration curve.

16.4.5 The procedure for analysis is similar to that of the calibration:

16.4.5.1 Selected digest samples are pipetted into a 50mL vial (one sample per vial) and diluted to 50mL with reagent water preserved to 1% BrCl.

16.4.5.2 For each sample, the sample ID and dilution based on 50mL is entered into the TekMDS software. The dilution should also be noted on the digestion bench sheet for cross referencing in peer review. Unless otherwise stated, all solid samples should be PB corrected. Note: Prep blank correction takes place after analysis in the Excel spreadsheet, not in the TekMDS software.

16.4.5.3 After loading one set of samples, the analyst should begin preparing the next round of digested solid samples in the same fashion to maximize efficiency.

16.4.6 Quality Control Procedures for Digested Solid Samples:

16.4.6.1 Please refer to the appropriate SOP for each matrix's corresponding quality control samples. All quality control samples prepared for the batch should be run with its analysis unless stated otherwise by the project manager. Please see Table 1 for acceptance criteria.

16.4.6.2 Spiking standard LIMS ID and the amount used for the MS/MSD must be noted on the digestion bench sheet for cross referencing in peer review.

16.4.6.3 The analytical day must close with a CCV(OPR) /CCB.

16.5 Analysis of Fluegas Sorbent for Total Mercury (FSTM)/KCl Traps and Particulate Filter:

16.5.1 The IPR for waters will also cover *EFAFS-T-AFS-SOP2985* and *EFAFS-T-AFS-SOP2800* digestions.

16.5.2 With a few exceptions FSTM traps and particulate filters are digested and analyzed as if they were solids.

16.5.3 FSTM traps or particulate filters should be digested according to *EFAFS-T-AFS-SOP2985* "Digestion for Gas/Air Samples Collected on Fluegas Sorbent for Total Mercury TM Traps."

16.5.4 KCl traps should be digested according to *EFAFS-T-AFS-SOP2800* "Mercury Digest for Gas/Air Samples Collected on KCl/Quartz or KCl/Lime trap."

16.5.5 The maximum amount of sample used for analysis should be 0.5mL for FSTM and 2.5 mL for KCL or less diluted to a final volume of 50mL with reagent water preserved to 1%BrCl . 5mL may be used at PM request, but is not recommended. To avoid any potential matrix interference it is recommended that the smallest reasonable aliquot sizes be used for this matrix. All blanks and low level/non-detect samples must be analyzed at the same dilution. Dilution should also be noted on the digestion bench sheet for cross referencing in peer review.

16.5.6 Quality Control Requirements for FSTM Traps and Particulate Filters:

16.5.6.1 Please refer to the appropriate SOP for each matrix's corresponding quality control samples. All quality control samples prepared for the batch should be run with its analysis unless stated otherwise by the project manager. Please see Table 1 for acceptance criteria.

16.5.6.2 The amount of spiking standard used, it's LIMS ID and dilution used for the MS/MSD as well the sample(s) used for MS/MSD/MD, must be noted on the digestion bench sheet for cross referencing in peer review.

16.6 End of analysis close-down procedure:

16.6.1 Turn off gas flow to the phase separator.

16.6.2 Move reagent lines to rinse carboy.

16.6.3 With pump set to "Local", rinse system for 5-minutes.

16.6.4 Release pump tubing for all but the "Sample" and "Waste" lines. Allow lines to drain.

16.6.5 Set pump to "Off".

16.6.6 Slowly release the "Sample" and" Waste" line. Carefully monitor the Wash station over the next 5 minutes, making sure it is not siphoning, or overflows.

16.6.7 Carryout all end of day cleaning and restocking tasks.

16.7 Analytical data is exported from the TekMDS software to an Excel file. The data is then copied and pasted into an Excel template that is LIMS compatible.

16.8 Tekran 2600 Mercury Analysis System Troubleshooting

The following is a summary of common troubleshooting techniques. This is not an all-inclusive list of what may be done to deal with analytical issues. Consultation with the group leader is preferred when performing these corrective actions. Analysts are encouraged not to rely on this guideline. Instead, this document should be used as a learning tool. Analysts should be actively striving to learn about troubleshooting as well as creative, productive ways of dealing with analytical issues.

16.8.1 ISSUE: No peaks at all

16.8.1.1 Ensure that the system is powered.

16.8.1.2 Make sure that the base line is approximately 0.1 V.

16.8.1.3 Make sure that you are running the right event table file (ETF).

16.8.1.4 Ensure that the carrier gas fitting is properly connected to the Argon source. (See System components, User guides, Section 2-3 for details).

16.8.1.5 Ensure that the phase separator gas fitting is properly connected to the Argon/Nitrogen source. (See System components, User guides, Section 2-3 for details)

16.8.1.6 Check operation of heater B. (Refer to Heater B Test)

16.8.1.7 Check operation of heater A. (Refer to Heater A Test)

16.8.1.8 Check that V2 is working properly. (Refer to V2 Test)

16.8.1.9 Check that V1 is switching properly. (Refer to V1 Tests)

16.8.2 ISSUE: Low sensitivity

16.8.2.1 Make sure that all "No peaks detected" samples are checked.

16.8.2.2 Check all connections to V2 and V1 and tighten all friction fittings up and downstream the Cartridges A and B.

16.8.2.3 Make sure that you have freshly changed soda lime in the soda lime trap, and that it is from a good source.

16.8.2.4 Check and tighten all gas Teflon lines up and downstream of the soda lime trap and P/S.

16.8.2.5 Check the sample tubing following introduction of $SnCl_2$ at the "Y" fitting for clogs. This line should also be the proper length.

16.8.2.6 Check that the frosted P/S rod is evenly wet. (Refer to P/S glass rod test).

16.8.2.7 Check that the tubing sizes in the main pump are correct. Also make sure that the tubing occlusion is set at the 12 o'clock position. Check the sample uptake (should be between 12 and 16 mL/min) and the $SnCl_2$ uptake (should be between 3 and 5 mL/min).

16.8.2.8 Do not use old calibration standards to calibrate the system.

16.8.2.9 Make sure you are running fresh $SnCl_2$ solution.

16.8.2.10 Make sure that your stock Hg standard has not expired and is from a reliable source and that it is not compromised.

16.8.2.11 Check and tighten both sides of the ¼" straight Teflon union on the cuvette.

16.8.2.12 Check the cuvette (Refer to the System Component, User Guides Cuvette Removal and Cleaning, Section 4-3 and Cuvette cleaning procedure).

16.8.2.13 Check the lamp voltage. If the voltage is low, increase to required values (refer to System Component, User Guides **Lamp voltage adjustment, Note 4-7).**

16.8.2.14 Check both cartridges for failures

16.8.2.14.1 Check if Cartridge A is having problems. (Refer to Cartridge A test procedure).

16.8.2.14.2 Check if Cartridge B is having problems. (Refer to Cartridge B test procedure

16.8.3 ISSUE: High Blanks

High blanks could be due to:

Contamination in the system (liquid and/or gas lines)
Water high in Hg
Poor quality of the reagents (KBr, $KBrO_3$, $SnCl_2$, Hydroxylamine, HCl etc).

16.8.3.1 Contamination in the system (liquid and/or gas lines), caused by:

16.8.3.1.1 System left without operating for an extended period of time (e.g. a month) and not shut down properly.

16.8.3.1.2 System not flushed and cleaned after running samples (especially high level samples).

16.8.3.1.3 Following analysis of samples high in organics and not flushing properly after the run.

Sudden contamination when an extremely high in Hg concentration sample is run through the system: From past experience this type of contamination is very dependent on the type of samples the analyst was running in the system more so than the exact concentration of Hg within the sample. There are cases when even after analysis of a very high sample (~1.0 ppb) the blanks will come back to normal very quickly. In other cases, effort will need to be made to lower the blanks. Basically the methods of cleaning are the same as those described below.

16.8.3.1.4 Not following normal and necessary maintenance procedures.

16.8.3.1.4.1 Addressing contamination in gas lines:

16.8.3.1.4.1.1 Bypass the P/S. (Refer to P/S bypassing procedure).

16.8.3.1.4.1.2 Continue by running some CLEAN cycles. If high peaks result, clean Cartridge B. (Refer to Cleaning the Cartridge B procedure).

16.8.3.1.4.1.3 Continue running dry (Sample cycle). If at this point you still get high peak areas, clean Cartridge A (Refer to Cleaning the Cartridge A procedure).

16.8.3.1.4.1.4 Once again continue running dry. If you still get high peak areas, clean V2. (Refer to cleaning V2 procedure).

16.8.3.1.4.1.5 If high blank are still present, clean/replace the gas phase Teflon lines. (Refer to cleaning of the gas phase Teflon lines).

16.8.3.1.4.2 Addressing contamination in liquid lines:

16.8.3.1.4.2.1 Check/replace the silicone sample line.

16.8.3.1.4.2.2 Check/replace the sample/$SnCl_2$ line to the P/S.

16.8.3.1.4.2.3 Check/clean the P/S. (Refer to Analytical Methods, *Tekran Guide,* Method 1631, section 5-1).

16.8.3.1.4.2.4 Clean system while running (Refer to clean while running procedure).

16.8.3.2 Poor water Quality

16.8.3.2.1 Refer to Analytical Methods, *Tekran Guide,* Method 1631, section 3-4.

16.8.3.3 Poor quality of the reagents (KBr, $KBrO_3$, $SnCl_2$, Hydroxylamine, HCl etc)

16.8.3.3.1 Refer to Analytical Methods, Tekran Guide*,* Method 1631, section 3-4, 3-5.

16.8.4 ISSUE: Nonlinearity of the calibration curve

There are a few different types of non-linearity and factors causing them:

16.8.4.1 Cal. Factors which are very low for the low calibration points (0.5 and 1 ng/L) but better for the higher standards.

16.8.4.1.1 This is probably due to high Calblank values that cause lowest points of calibration to be affected during blank correction. Stop the run and go through the procedure for investigating **High Blanks.**

16.8.4.2 Cal. Factors are very erratic (no clear increasing or decreasing tendency

16.8.4.2.1 Dry spots on the glass rod of the P/S. (Refer to P/S glass rod test).

16.8.4.2.2 Check that you are running with fresh soda lime.

16.8.4.2.3 Check the heaters (Refer to Heater B test and Heater A test).

16.8.4.2.4 Check the silicone sample tubing for any twisting, pinching, or bubbles.

16.8.4.2.5 Check and tighten all liquid and gas phase connections.

16.8.4.2.6 Make sure your calibration standards are fresh and properly prepared.

16.8.4.3 Poor results for high calibration standards

16.8.4.3.1 Previously run samples with difficult matrixes or high acid vapor content could be a potential cause for this. Such matrixes, in very rare cases, could cause temporary passivation of Cartridge A, as well as temporarily poison the liquid lines. After running such difficult samples, the system needs a very thorough cleaning (see at the procedures High blanks). Running overnight in "dry" mode with the P/S flow between 50-100mL/min can also help.

16.8.4.4 Cal. Factors decrease moving up in concentration through the calibration curve

16.8.4.4.1 Improper preparation of the standards. Check and prepare fresh calibration standards.

16.8.4.4.2 Contaminated and expired soda lime. Change soda lime.

16.8.4.4.3 Loose P/S friction fittings: tighten them.

16.8.4.4.4 Loose Soda lime trap fittings: tighten them.

16.8.4.4.5 Loose gold trap friction fittings: tighten/replace them.

16.8.4.4.6 Loose connection of the ¼" nuts on V1 and V2. Tighten them.

16.8.4.4.7 Loose connection on both sides of ¼" straight Teflon union to the cuvette. Tighten both sides.

16.8.4.4.8 Open and examine the cuvette (Refer to the System Component, User Guides Cuvette Removal and Cleaning, Section 4-3 and Cuvette cleaning procedure*)*

17) Calculations:

17.1 Average all instrument blanks (PH_X) using the peak area values from the TekMDS software. Subtract the average (IB) from the peak area for each standard and sample.

17.2 Calculate the calibration factor (CF_X) for mercury in each of the five standards using the mean instrument-blank-subtracted peak area and the following equation:

$CF_x = PA_x - IB / C_x$

Where:

17.2.1 PAx=peak area for mercury in standard

17.2.2 IB=mean peak area for mercury in instrument blank

17.2.3 C_x=mass in standard analyzed (ng/L)

17.2.4 CF_x=Response Factor of each concentration

17.2.4.1 Average the five response factors to establish mean value: CF(Avg) (units/ng/L).

17.3 Sample results are then corrected for the average peak area values of at least three preparation blanks (PBs), unless otherwise requested or for samples originating from Wisconsin *(*Wisconsin does not permit method blank subtraction*)*. This result is shown as the Initial Result on the Excel spreadsheet and in LIMS.

17.4 Total Mercury in Water:

Hg / Initial Result (ng/L) = ((((Peak Area - IB) / $CF_{(Avg)}$) x D_s)– (PB_X x D_b)) / D_s

THg Final Result (ng/L) = (THg / Initial Result) x (D_s x V_f) / V_i

Where:

17.4.1 $CF_{(avg)}$ = average response factor (in units/ng/L).

17.4.2 IB = average instrument blank peak area (in units)

17.4.3 V_f = final volume of sample analyzed in mL. The final volume should account for the added volume of BrCl needed for preservation. For instance, 100 mL of sample is preserved with 3% (v/v) BrCl. The final volume should be 103 mL.

17.4.4 V_i = initial volume of sample analyzed in mL prior to addition of BrCl.

17.4.5 D_s = final dilution factor of sample.

17.4.6 D_b = final dilution factor of corresponding blank results. All preparation blanks at the same preservation level must be analyzed at the same dilution.

17.4.7 PB_X= initial average (on instrument) of the preparation blanks in ng/L related to the preservation level of the samples (i.e., X=2 for a sample which is preserved at 2% BrCl), thus accounting for the extra contribution of mercury from the BrCl. Sample results are corrected for the average blank concentration of only the corresponding blanks preserved at the same BrCl preservation level. For example, if a sample is preserved at 2% and one PB was run at 2%, the sample is corrected using the results from that blank.

17.5 Total Mercury in Solids:

THg / Initial Result (ng/L) = ((((Peak Area - IB) / $CF_{(Avg)}$) x D_s)– (PB x D_b)) / D_s

THg Final Result (ng/g) = (THg / Initial Result) x (D_s x V_f) / (m x 1000)

Where:

17.5.1 $CF_{(avg)}$ = average response factor (in units/ng/L).

17.5.2 IB = average instrument blank peak area (in units)

17.5.3 V_f = final volume of the digested sample in mL.

17.5.4 m = initial mass of the digested sample in g.

17.5.5 D_s = final dilution factor of sample.

17.5.6 D_b = final dilution factor of corresponding blank results. All preparation blanks must be analyzed at the same dilution.

17.5.7 PB = initial average (on instrument) of the preparation blanks found in the digest in ng/L. The initial mass of the digestion blanks must be the default mass specific to the preparation.

18) Statistical Information/Method Performance:

18.1 The Method Detection Limit (MDL) is determined according to 40 CFR Part 136 Section B. Ten replicates (9 degrees of freedom) spiked 3-10 times the expected MDL are run. The standard deviation (s) is taken from the resulting data and the MDL is calculated as follows: MDL=2.821*s. This value should not be interpreted as the method reporting limit.

18.2 The Practical Quantitation Limit (PQL) is the reporting limit for this method and is included as the lowest calibration point (2003 NELAC regulation 5.5.5.2.2.1.h.3). The PQL is determined by running ten samples with a concentration that will produce a recovery of 70-130 % for most analytes, but the recovery requirements are analyte dependent. The PQL is referred to as the Method Reporting Limit (MRL) in LIMS.

18.3 Current LODs, LOQs, MDLs and PQLs are stored at: Cuprum\General and Admin\Quality Assurance\MDLs & PQLs.

19) Quality Assurance/Quality Control:

19.1 A minimum of three preparation blanks and one LCS/LCSD (preferably NIST 1641d), must be analyzed per preparation batch. The upper control limit for each preparation blank is equal to the PQL.

19.2 Initial Precision and Recovery test for water must be four spikes (at 5.0 ng/L) recovering between 79-121% with an RSD < 21%; the IPR for soil must be four spikes (at 8.0 ng/g) recovering between 75-125% with an RSD < 20%.

19.3 Matrix Spikes: One Matrix Spike/Matrix Spike Duplicate (MS/MSD) must be performed for every 10 samples. The recovery of the MS/MSD must be between 71%-125% recovery, and the Relative Percent Difference (RPD) below 24%. If an MS/MSD is out of control, the analyst should investigate to identify the source of the failure. The MS and MSD may be used as duplicates. Some failures may be qualified using QA Qualification Flow Charts (Appendix A).

19.3.1 For aqueous samples, the MS/MSD is spiked at 1 to 5 times the ambient concentration, with 0.25 ng being the minimum spiking level. Sample dilutions for the MS/MSD should be the same as the ambient sample dilution, if sufficient sample volume exists. NEVER ADD SPIKE DIRECTLY TO THE ORIGINAL SAMPLE CONTAINER UNLESS OTHERWISE INSTRUCTED.

19.4 Matrix Duplicates – One Matrix Duplicate (MD) may be analyzed for every batch of 20 samples. Upon request, a Matrix Triplicate (MT) may be performed. The MSD may serve as the MD if necessary. The Relative Percent Difference (RPD) and the Relative Standard Deviation (RSD) of duplicate samples must be less than 24%. Some failures may be qualified using QA Qualification Flow Charts.

19.4.1 For aqueous samples, analyze the parent, duplicate and triplicate at the same dilution.

19.5 Laboratory Control Standard (LCS) or Quality Control Sample (QCS): For every batch of samples, at least one LCS is processed and analyzed. The recovery of the LCS must be within 80-120% for the aqueous NIST 1641d. An LCS Duplicate (LCSD) should accompany the LCS.

19.5.1 A Certified Reference Material (CRM) is the preferred LCS, but a Blank Spike may serve as an LCS if an appropriate CRM does not exist. The spiking level is based on client request, historical data, or a default of mid-curve. A duplicate blank spike must also be prepared as an LCSD. Recoveries need to be 75-125% with an RPD < 24%.

19.6 Ongoing Precision and Recovery (OPR): An OPR must be analyzed at the beginning and end of each analytical batch, or at the end of each 12-hour shift. The recovery of the OPR must be within 77-123% to be considered in control.

19.7 All calibration standards must be traceable to the original standard source. The calibration curve must be established at the beginning of the analytical run. It must include at least five different concentrations, with the lowest concentration equal to the PQL. The average response factor of each calibration standard is used to calculate the sample values. The RSD of the response factors must be less than 15% of the mean or the calibration fails.

19.8 ICV control limit is 79-121%, while the CCV control limit is 77-123%. The CCV is analyzed every 10 analyses, and at the end of an analytical run. CCBs are always analyzed after the CCVs.

19.9 Field Blanks: To be compliant with EPA 1631, clients must submit a field blank for each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples).

19.9.1 If no field blanks are submitted by the client, their data will be flagged with "FB-1631." "Required equipment/field/filter blank not submitted by the client. The sample has been analyzed according to 1631E, but does not meet 1631E criteria."

19.10 Method or Preparation Blanks (BLK): Method blanks are used to demonstrate that the analytical system is free from contamination that could otherwise compromise sample results. Method blanks are prepared and analyzed using sample containers, labware, reagents, and analytical procedures identical to those used to prepare and analyze the samples.

19.10.1 A minimum of three 1 % BrCl method blanks per analytical batch are required. Any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

19.10.2 If the result for any 1 % BrCl method blank is found to contain =0.50 ng/L Hg (0.25 ng/L for DOD), the system is out of control. Mercury in the analytical system must be reduced until a method blank is free of contamination at the 0.50 ng/L level.

19.10.2.1 For WI DNR samples, the method blank results need to be assessed to 0.15 ng/L or ten percent of the measured concentration in the sample. The results must be qualified if the method blank exceeds the highest of either of these values.

19.10.3 For method blanks containing more than 1% BrCl, the control limit is equal to 0.50 ng/L multiplied by the final preservation percentage of BrCl. For example, for a method blank preserved to 2 % BrCl, the control limit for the blank is 0.50 ng/L * (102/101), or 0.50 ng/L. For 3% BrCl the control limit is (103/101)*0.50ng/L, or 0.51ng/L.

19.11 Instrument Blanks (IBL): A minimum of three instrument blanks must be analyzed with each analytical batch (before the first calibration standard). To analyze an instrument blank, analyze a sample of reagent water preserved to 1% BrCl.

19.11.1 If the instrument blank is found to contain more than 0.50ng/L, the system is out of control. The problem must be investigated and remedied before proceeding, and any samples run must be reanalyzed.

19.11.1.1 The mean result for all instrument blanks must be ≤ 0.25ng/L with a standard deviation of 0.10 ng/L.

19.11.1.2 For WI DNR samples, the ICB and CCB results need to be assessed to 0.15 ng/L or ten percent of the measured concentration in the sample. The results must be qualified or the affected samples re-analyzed if an ICB/CCB exceeds the highest of either of these values.

19.12 The analytical day must close with a CCV/OPR/CCB.

19.13 Since the method is done in real-time, it is EFGS' position that a single non-compliant QC sample result does not automatically invalidate a data set. All data points that can be explained and rerun with a passing result can be qualified. If the source of error cannot be corrected for a QC standard that day, none of the data can be validated. In the event that the system becomes out of control during the analysis day, all results bracketed between valid QC data points shall still be considered valid (CCV, OPR, CCB, etc).

19.14 The Control Limits are established from EPA 1631E.

20) Corrective Action

20.1 The data is reviewed as in the QC section (or matrix specific QC section) for all parameters that pass specific requirements. If the data does not meet QC requirements for waters, it is reanalyzed for confirmation; if the failing MD/MS/MSD results confirm, another sample is used for the batch. For all digestions, it is qualified or submitted for reruns. Data may be qualified (based on scientific peer review) by the Group Supervisor, Project Manager, Lab Manager, or QA Officer.

20.1.1 Continuing Calibration Verification (CCV): If a recovery falls outside acceptance criteria, recalibrate the instrument and reanalyze all affected samples since the last acceptable CCV or immediately analyze two additional CCVs. If both CCVs are within acceptance criteria, the samples may be reported without reanalysis and the analysis of the next bracket may continue beginning with a CCB.

20.1.1.1 If either of the two CCVs fails, the analysis must be terminated, the problem resolved, the instrument recalibrated and then all of the affected samples since the last acceptable CCV reanalyzed.

20.2 Control Chart data is generated through LIMS to monitor the performance of the CCV, LCS, MS, and MSD. This is done by the QA department.

20.3 Due to the real-time nature of the CVAFS method, failures must be investigated as they happen. If the source of the problem can be identified, and corrected, the samples may be rerun. If source of problem cannot be isolated, see the Senior Analyst, Group Supervisor, or Laboratory Manager for instructions.

20.4 The Senior Analyst, Group Supervisor, Laboratory Manager, or QA Officer must be informed if QC fails. It is also advisable to always alert the Project Managers.

21) List of Attachments

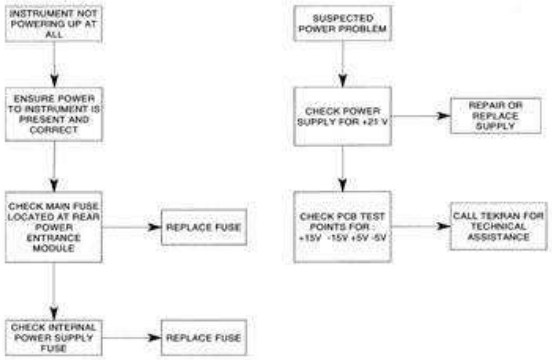
Table 1: QC Requirements for Total Mercury
Table 2: Troubleshooting Flow Chart

Table 1: QC Requirements for Total Mercury

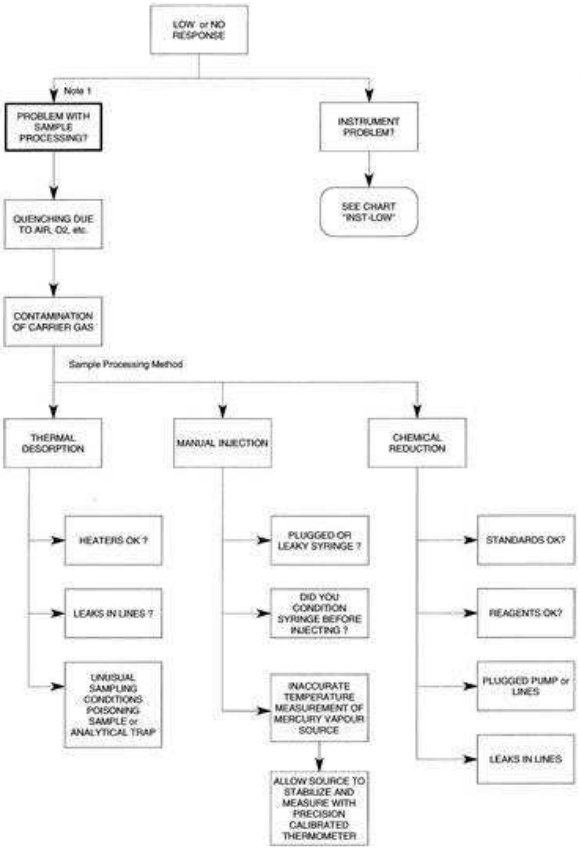
QC Parameter	Acceptance Criteria
Initial Calibration Verification (ICV)	79-121% Recovery
Continuing Calibration Verification (CCV)	77-123% Recovery
Ongoing Precision and Recovery (OPR)	77-123% Recovery
Initial Calibration Blank (ICB)/ Continuing Calibration Blank (CCB)	Individually, IBL and CCB ≤ 0.50ng/L, but the mean of all the IBLs shall be ≤ 0.25ng/L with a standard deviation of ≤ 0.10ng/L. For WI DNR, IBL and CCB individually must be ≤ 0.15 ng/L.
Laboratory Control Standard (LCS) or Quality Control Standard (QCS)	80-120% Recovery RSD ≤ 24%
Certified Reference Material	75-125% *Recovery* RPD ≤ 24%
Calibration Curve RSD (Referred to as "Corr. RSD CF" in Excel spreadsheet).	RSD of Calibration Response Factor ≤ 15%
Lowest Calibration Point	75-125%
1% BrCl Method Blank (BLK)	≤ 0.50ng/L (≤ 0.25ng/L for DOD projects, ≤ 0.15 ng/L for WI DNR) (individually)
Matrix Duplicate (MD) and Analytical Duplicate (AD)	≤ 24% RPD
Matrix Spike and Matrix Spike Duplicate (MS/MSD) ; Analytical Spike (AS) and Analytical Spike Duplicate (ASD)	71-125% Recovery ≤ 24% RPD

Table 2: Troubleshooting Flow Chart

INSTPOW.ABC\TopChart

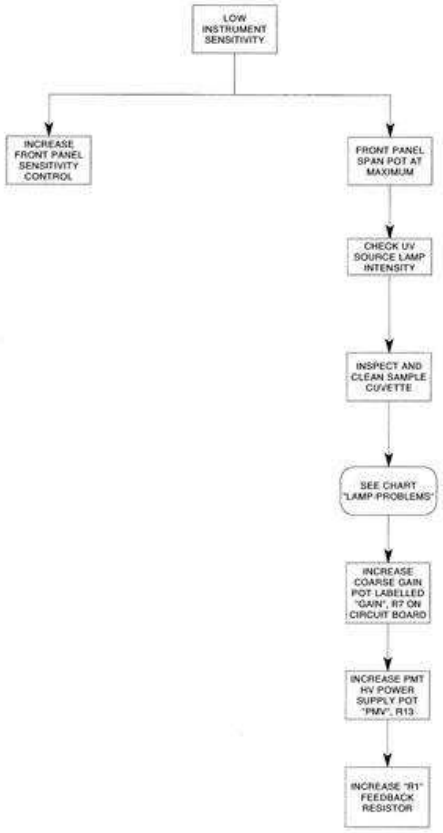


LOWRESP.ABC\TopChart

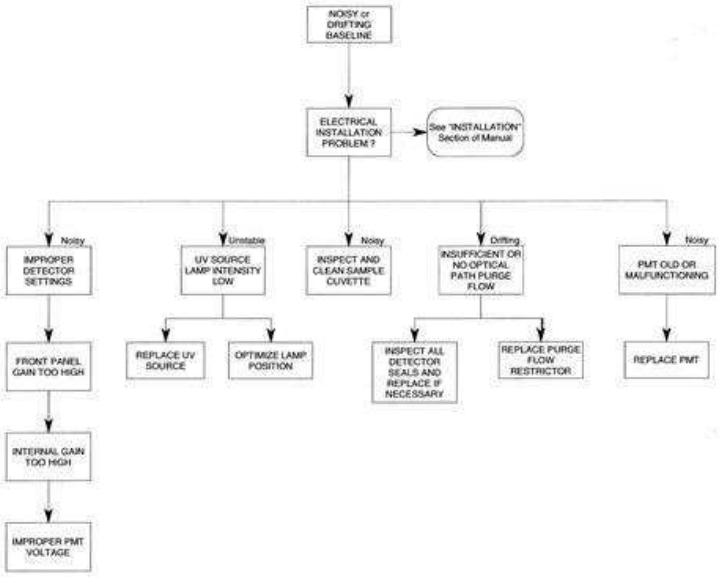


NOTES: 1) Most problems with low responses can be traced to chemical/physical problems with auxiliary equipment.

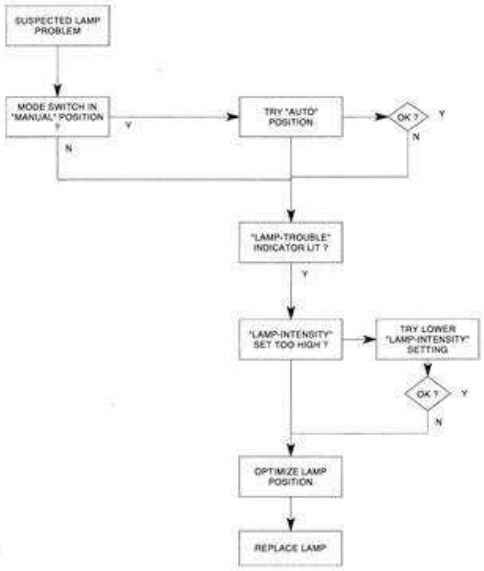
INSTLOW.ABC\TopChart



NOISEBAS.ABC\TopChart



LAMPPROB.ABC\TopChart



EFTM-T-TM-SOP2837 Total Recoverable Metals Digestion for Solid, Animal or Plant Materials
EFAFS-T-AFS-SOP2807 Preparation of Solids Samples for Total Mercury Analysis by Modified Cold Aqua-Regia Digestion
EFHS-S-HS-SOP2991 Waste Disposal Procedures for Client Sample Waste
EFSR-P-SP-SOP2796 Oxidation of Aqueous Samples for Total Mercury Analysis
EFSR-S-CS-SOP2794 Ultra-Clean Aqueous Sample Collection
EFAFS-T-AFS-SOP2795 Digestion of Tissues for Total Mercury Analysis Using Nitric Acid and Sulfuric Acids (70:30)
EFQA-P-DR-SOP2801 Data Review and Validation and Monthly Logbook Reviews
EFTM-T-TM-SOP2821 HF/HNO3/ HCl Bomb Digestion of Solids for Total Mercury Followed by Repeated HNO3 Evaporation for Other Metals
EFAFS-T-AFS-SOP2985 Digestion for Gas/Air Samples Collected on Fluegas Sorbent for Total Mercury™ Traps
EFAFS-T-AFS-SOP2800 Digestion of KCl Traps for Total Mercury
EFQA-R-MT-SOP2710 Balance Verification, Calibration & Maintenance
EFQA-R-EQ-SOP2711 Pipette and Dispenser: Operation, Calibration & Maintenance
EFAFS-S-SB-SOP5132 Cleaning of Sampling Equipment and Bottles for Mercury Analysis
EFQA-S-IT-WI7061 Instrument and LIMS Software Inventory

End of document

Version history

Version	Approval	Revision information
4	03.NOV.2015	
5	19.MAY.2016	

6	09.MAY.2017	