



Laboratory Procedure Manual

Analyte: Inorganic mercury, Methyl mercury, Ethyl mercury

Matrix: Blood

Method: Blood mercury speciation TSID-GC-ICP-DRC-MS
(Triple Spike Isotope Dilution Gas Chromatography-
Inductively Coupled Plasma Dynamic Reaction Cell
Mass Spectrometry)

Method No: **DLS-3020.5**

Adopted:

Revised:

As performed by: Inorganic and Radiation Analytical Toxicology Branch
Division of Laboratory Sciences
National Center for Environmental Health

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

This document details the Lab Protocol for testing items in the following table:

Data File Name	Variable Name	SAS Label
IHgEM_G	LBXIHG	Mercury, inorganic ($\mu\text{g/L}$)
	LBDIHGSI	Mercury, inorganic ($\mu\text{mol/L}$)
	LBXBGE	Mercury, ethyl ($\mu\text{g/dL}$)
	LBXBGM	Mercury, methyl ($\mu\text{g/L}$)

1. CLINICAL RELEVANCE AND TEST PRINCIPLE

A. Clinical Relevance

Mercury (Hg) is widespread in the environment and found in its elemental form (Hg^0), inorganic forms such as mercurous (Hg^+) and mercuric (Hg^{2+}), and various organic forms such as methyl mercury (MeHg), ethyl mercury (EtHg), phenyl mercury (PhHg), and others. The health effects of mercury are diverse and depend on the form of mercury encountered and the severity and length of exposure. The relative order of increasing toxicity is: $\text{Hg}^0 < \text{Hg}^{2+} \ll \text{CH}_3\text{Hg}^+$ [1]. With large acute exposures to elemental mercury vapor, the lungs may be injured. At levels below those that can cause lung injury, low-dose or chronic inhalation may affect the nervous system. Symptoms include weakness, fatigue, loss of weight (with anorexia), gastrointestinal disturbances, salivation, tremors, and behavioral and personality changes, including depression and emotional instability [2]. Exposure to inorganic mercury usually occurs by ingestion. The most significant effect is on the kidneys, where mercury accumulates, leading to tubular necrosis. In addition, there may be an irritant or corrosive effect on the gastrointestinal tract involving stomatitis, ulceration, diarrhea, vomiting, and bleeding. Psychomotor and neuromuscular effects also may occur [3].

Methyl mercury is more toxic than inorganic mercury. The effects of methyl mercury include changes in vision, sensory disturbances in the arms and legs, cognitive disturbances, dermatitis, and muscle wasting. The critical organ for methyl mercury is the brain. Methyl mercury readily crosses the blood-brain barrier due to its lipid solubility and accumulates in the brain where it is slowly converted to inorganic mercury. Whether CNS damage is due to methyl mercury or inorganic mercury, or both, is still controversial [4]. Ethyl mercury is another organic form of mercury. Very little is actually known about ethyl mercury metabolism in humans, including whether it has the same potency as a neurotoxin, whether the blood concentration is ever significant, and even whether it crosses the blood-brain barrier. But the use of thimerosal, which metabolizes to ethyl mercury and thiosalicylate, as a vaccine preservative makes this subject very important.

In the general population, total blood mercury is due mostly to the dietary intake of organic forms, particularly methyl mercury and ranges from 0.2 to 5.8 $\mu\text{g}/\text{L}$ [5]. Urinary mercury is mainly comprised of inorganic mercury due generally to dental amalgam containing elemental mercury and occupational exposures ranging from 0.2 to 10 $\mu\text{g}/\text{L}$ [5].

The method described in this manual assesses mercury exposure, as defined by exposure to individual mercury species, by analyzing blood through the use of Solid Phase Micro Extraction (SPME) fiber for delivering sample to gas chromatography (GC) coupled to inductively coupled plasma-dynamic reaction cell-mass spectrometry (ICP-DRC-MS). Blood is chosen as a matrix because it might contain various organic mercury species as well as inorganic mercury while urine contains mostly inorganic mercury. This hyphenated method will provide accurate quantification of three mercury blood species: inorganic mercury (InHg), methyl mercury (MeHg), and ethyl mercury (EtHg).

Species Name	Abbreviation	Molecular Structure
Inorganic mercury	InHg	Hg^{2+}
Methyl mercury	MeHg	CH_3Hg^+
Ethyl mercury	EtHg	$\text{C}_2\text{H}_5\text{Hg}^+$

B. Test Principle

The quantification of InHg, MeHg, and EtHg is determined by using a triple spike isotope dilution (TSID) method employing gas chromatography (GC) to separate the species followed by introduction into an ICP-DRC-MS for detection. TSID is a specialized extension of the Isotope Dilution (ID) technique. TSID measures individual chemical species (inorganic, methyl and ethyl mercury species) in samples using ID principles. The blood sample is spiked with known amounts of each Hg species that have been enriched with isotopic variants of the target element of interest.

The first step of this method involves the addition ("spiking") of enriched isotopes ($^{199}\text{Hg}^{2+}$, $\text{CH}_3^{200}\text{Hg}^+$, and $\text{C}_2\text{H}_5^{201}\text{Hg}^+$) to the blood sample. Each Hg species spike is labeled with an enriched Hg isotope such that its isotopic pattern is unique to the species' chemical identity, i.e., the manner of isotope spiking is "species specific". Next, the spiked sample is digested in tetramethylammonium hydroxide (TMAH) which disassociates bound mercury species from proteins, polypeptides, and other biomolecules. The digested blood sample with freed mercury species is chemically reacted ("derivatized") with a reagent that adds 3-carbon chains (n-propyl groups) to the mercury atom of each species molecule without compromising species identity. This type of chemical derivatization results in loss of ionic charge and reduced polarity; the net effect is to make each mercury species molecule volatile so it can escape the liquid phase and accumulate in the gas phase ("headspace") directly above the sample. Derivatization is performed inside a partially filled vial sealed with a rubber septa cap that can be penetrated by a needle.

Solid Phase Microextraction (SPME) is a sampling technique that uses a thin polymer fiber with a hydrophobic coating; the method described here uses a SPME fiber with a 100 μm coating of polydimethylsiloxane (PDMS). The SPME assembly consists of the fiber inserted through the inside a stainless steel needle. A key design feature is the fiber can be mechanically withdrawn into the needle during vial septum penetration and then pushed out to expose the fiber to the headspace. During headspace exposure (the "extraction" step), the gaseous derivatized Hg species adsorb onto the PDMS coating of the SPME fiber. When other factors held are constant, the adsorbed mass increases as a function of sample concentration. After a predetermined time, the SPME fiber is retracted into the injection needle, and the needle is withdrawn from the sample vial. Subsequently, the needle moves to the injector port of the programmable temperature gradient gas chromatograph (GC) and, on programmatic command, performs a programmed temperature ramp injection sequence. This action transfers the propylated inorganic, methyl and ethyl Hg species to the head of a 30 m capillary GC column which, using He as the carrier gas, ramps the column temperature to 280°C. The order of chromatographic separation of the Hg species is based on increasing molecular weight: methylpropylmercury (derivatized methyl Hg), ethylpropylmercury (derivatized ethyl Hg), and dipropylmercury (derivatized inorganic Hg) is last. Hg species exiting the GC column are seen as chromatographic peaks detected using inductively-couple argon plasma (ICP) as the ion source and a quadrupole mass spectrometer (Q-MS) for mass specific quantification. Species identification is based on chromatographic retention time; species specific isotope ratios are calculated from integrated peak areas derived from m/z signals corresponding to ^{199}Hg , ^{200}Hg , ^{201}Hg and ^{202}Hg isotopes. The ICP-MS is equipped with a Dynamic Reaction Cell (DRC™) for minimizing polyatomic interferences. Operating the ICP-MS in DRC mode has an added benefit of enhancing Hg signal strength through an effect known as "collisional focusing" [6,7].

2. SAFETY PRECAUTIONS

Important

Precautionary information that is important to protecting personnel and safeguarding equipment will be presented inside a box, like this one, throughout the procedure where appropriate.

Follow universal precautions when handling blood samples. Wear gloves, a lab coat, and safety glasses while handling human blood, plasma, serum, urine, or other bodily fluid or tissue. Place disposable plastic, glass, and paper (e.g., pipette tips, autosampler tubes, and gloves) that come in contact with human biological fluids, such as blood, in a biohazard autoclave bag. Keep these bags in appropriate containers until they are sealed and autoclaved. When work is finished, wipe down all work surfaces where human biological fluid was handled with a 10% (v/v) sodium hypochlorite solution (or equivalent). Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.

PerkinElmer provides safety information that should be read before operating the instrument. This information is found in the PerkinElmer ELAN® 6100 ICP-DRC-MS System Safety Manual. Possible hazards include ultraviolet radiation, high voltages, radio-frequency radiation, and high temperatures.

Caution!

Exercise caution when handling and dispensing concentrated nitric and hydrochloric acid. Always remember to add acid to water. Nitric and hydrochloric acid are caustic chemicals that are capable of severe eye and skin damage. Wear powder-free gloves, a lab coat, and safety glasses. If nitric or hydrochloric acid comes in contact with any part of the body, quickly wash the exposed area with copious quantities of water for at least 15 minutes.

3. DATA SYSTEM MANAGEMENT

To maintain the integrity of specimen and analytical data generated by this method, eliminate hand entry of specimen identifiers or analytical results whenever possible, proofread all transcribed data, and regularly defragment and back up the ICP-MS computer's hard drive.

A. Data Entry and Transfer

Whenever possible, use bar code scanners to enter sample identifiers into the GC-ICP-DRC-MS computer software to avoid errors associated with the keyboard-entry process and to speed up sample processing. When bar code scanners cannot be used, proofread transcribed data after entry. Handle or transfer data electronically when reporting or moving data to other computerized data-handling software. In the Inorganic and Radiation Analytical Toxicology Branch, sample analysis results generated by this method are stored for long periods in Microsoft Access™ or MS SQL Server (Frontends) database software or an equivalent database. The results should include at least the analysis date, analytical run number, quality-control (QC) results for the run, results of specimen analysis by specimen identification (ID), and method identifier.

B. Routine Computer Hard Drive Maintenance

Defragment the computer hard drive regularly by using software such as Microsoft Windows® Disk Defragmenter (located in Start > Programs > Accessories > System Tools) or an equivalent defragmentation program to maximize computer performance and maintain data integrity for files on the hard drive. An entry will automatically be made in the Windows™ system event log when this process is done and will provide documentation of this step.

C. Electronic Data Backup

1. Schedule of Data Backups

- Weekly: Full data backups onto one or more recordable compact discs (CD-R) or digital video discs (DVD).
- Daily: Full data backups onto a secondary hard drive, if equipped.

2. Backup Procedures

a) Whenever making a backup (daily or weekly) include the directories and subdirectories:

- C:\elandata (include all subdirectories)
- C:\gc (must include subdirectories "data" and "methods", also include other relevant directories)

Before making weekly backups, saving a copy of the Windows™ event log in the active "elandata" directory will ensure archiving of all recent software system events (including communications between ICP-DRC-MS and ELAN software, as well as times of hard drive defragmentation, and other Windows™ system events).

b) Secondary Hard Disk Backups

- If available, use the computer's secondary hard disk to store backup files.
- Configure Microsoft Windows® Backup™ (Start > Programs > Accessories > System Tools) program to do a daily backup of the computer's data directories (see Backup Procedures)

c) Compact Disc (CD) Backups

- Use the CD writing program installed on the computer to create CD backups (e.g., "Roxio CD Creator"™, or equivalent software)..
- Use CD-R disks only (recordable CDs), not CD-RW disks (rewritable CDs).

d) Backup of Sensitive Data

- Make a backup for sensitive data on duplicate, recordable compact discs. Store the two CD-R disks in two different buildings.

D. Documentation of System Maintenance

1. **Computer Maintenance:** Record any maintenance of computer hardware, GC or ICP-DRC-MS software in the instrument logbook. Place other electronic records relating to integrity of the data and hard drive in the Windows™ event log. Back up the event log on a regular basis by saving a copy in the active "elandata" directory. The event log will then be backed up along with the ELAN data when backup CD-R disks and tapes are made.

2. **Instrument Maintenance:** Document system maintenance in hard copies of data records (i.e.,

daily maintenance checklists, PerkinElmer service records, and/or instrument log book) as well as in electronic records relating to instrument optimization (default.dac) and tuning (default.tun).

4. COLLECTING, STORING, AND HANDLING SPECIMENS; CRITERIA FOR REJECTING SPECIMENS

A. Specimen Type

Specimen type is whole blood. No special instructions for fasting or special diets are required of patient or study subjects.

B. Specimen Collection, Handling and Storage

- 1) The preferred volume of blood specimen is ≥ 0.5 mL; the minimum volume is 0.25 mL.
- 2) Acceptable containers for specimen acquisition include pre-screened polyethylene vials and pre-screened blood collection tubes, or other suitable pre-screened equivalent.
- 3) Samples should be stored in the dark at -20°C or lower temperature. Long term storage at -70°C or less is preferred.
- 4) Specimen handling conditions are outlined in the Division protocol for blood collection and handling (copies available in Branch, laboratory and Special Activities specimen handling offices). Collection, transport, and special requirements are discussed. If more than one blood collection tube is used to draw blood from a subject, the blood tube for trace metals tube should be drawn last. Draw the blood through a stainless steel needle into a pre-screened blood collection tube. Blood specimens should be transported and stored at $\leq 4^{\circ}\text{C}$. Once received, they can be frozen at $\leq -20^{\circ}\text{C}$ until time for analysis. Portions of the sample that remain after analytical aliquots are withdrawn can be refrozen at $\leq -20^{\circ}\text{C}$. Thawing and refreezing of samples before analysis is discouraged.

C. Criteria for an Unacceptable Specimen

The criteria for an unacceptable specimen are either a low volume (< 0.25 mL) or suspected contamination due to improper collection procedures or collection devices. Specimen contact with dust or dirt may compromise test results. In all cases mentioned above, request a second blood specimen.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALBRATORS (STANDARDS), AND CONTROLS

A. Chemicals

- 1) Deionized (DI) water, ≥ 18 M Ω cm resistivity.
- 2) Sodium acetate anhydrous, (CAS# 127-09-3), (C₂H₃NaO₂--MW 82.04), (Sigma-Aldrich, Milwaukee, WI) or equivalent vendor.
- 3) Glacial acetic acid, (CAS# 64-19-7), (CH₃COOH--MW 60.05), reagent ACS grade (GFS Chemicals, Powell, OH) or equivalent vendor.
- 4) Double-distilled Hydrochloric Acid, (CAS# 7647-01-0), (HCl--MW 36.461--37.0%--12.1M) (GFS Chemicals Inc., Columbus, OH) or equivalent vendor.
- 5) Double-distilled Nitric Acid (CAS# 7697-37-2), (HNO₃--MW 63.013--70.0%--15.8M) (GFS Chemicals Inc., Columbus, OH) or equivalent vendor
- 6) Tetramethylammonium hydroxide, 25% w/w in methanol (CAS# 75-59-2), (C₄H₁₃NO--MW 91.15), (Alfa Aesar, Ward Hill, MA) or equivalent vendor.
- 7) Sodium tetra(n-propyl)borate, (CAS# 45067-99-0), (NaPr₄B -MW 206.16), (ABCR, Germany) or equivalent vendor.
- 8) Bleach (10% sodium hypochlorite solution) from any vendor.
- 9) Base whole blood, donated or purchased.
- 10) NIST SRM 955c - Toxic Metals in Caprine Blood (National Institute of Standards and Technology, Gaithersburg, MD), or equivalent reference material.
- 11) Inorganic Mercury (HgCl₂), (Applied Isotope Technologies, Inc. – Sunnyvale, CA) P/N 30528 or equivalent.
- 12) Methylmercury (CH₃HgCl), (Applied Isotope Technologies, Inc. – Sunnyvale, CA) P/N 30520 or equivalent.
- 13) Ethylmercury (CH₃CH₂HgCl), (Applied Isotope Technologies, Inc. – Sunnyvale, CA) P/N 35024 or equivalent.

B. Isotope Dilution Standards

- 1) ¹⁹⁹Hg-Isotopically-Enriched Inorganic Mercury (¹⁹⁹HgCl₂), (Applied Isotope Technologies, Inc. - Sunnyvale, CA) P/N 30530 or equivalent.
- 2) ²⁰⁰Hg-Isotopically-Enriched Methylmercury (CH₃²⁰⁰HgCl), (Applied Isotope Technologies, Inc. - Sunnyvale, CA) P/N 30521 or equivalent.
- 3) ²⁰¹Hg-Isotopically-Enriched Ethylmercury (CH₃CH₂HgCl), (Applied Isotope Technologies, Inc. - Sunnyvale, CA) P/N 35025 or equivalent.

C. Quality Control Material

Quality control (QC) materials are made from pools of whole blood obtained from a donor source or purchased from the vendor. See the Preparation of Quality Control Material section for details of preparation. The control "base" blood and two QC blood pools intended for the mercury speciation assay are designated as:

QC level	QC Designation ID
Base pool	BB-yy###
Low pool	LB-yy###
High pool	HB-yy###

Where substitutions are: yy = the last two digits of production year and ### = assigned pool identification number. QC material intended for bench quality control purposes needs to be "characterized" as described in the section Establish QC limits for each QC pool.

D. Instrumentation

1. Gas Chromatography System

- 1) Gas Chromatograph: PerkinElmer® Clarus 500, or equivalent system.
- 2) GC capillary column: PerkinElmer® Elite-5 30m, 0.25mmID, 0.25µm df (Catalog # N9316076, Shelton, CT), or equivalent.
- 3) GC Transfer Line, non-coated capillary column: PerkinElmer® Fused Silica Tubing 5m, 0.25mmID (Catalog # N9301356, S/N 920620, Shelton, CT), or equivalent.
- 4) GC-ICP-MS Heated Transfer Line accessory: Redshift® (P/N N0777440 for 115V or P/N N40777361 for 230V, Italy), or equivalent.
- 5) Solid Phase Micro Extraction (SPME) Fiber Assembly, specifically, Supelco Analytical 100µm Polydimethylsiloxane (PDMS) Coating (Red - Pack of 3), (Catalog # 57301, Supelco Analytical, Bellefonte, PA), or equivalent.

2. ICP-DRC-MS System

- 1) Inductively-coupled plasma mass spectrometer, specifically, the ELAN™ DRCII (PerkinElmer Instruments, Shelton CT), or equivalent.
- 2) Platinum (Pt) cones (Catalogue # SC2013-Pt and SC2014-Pt Perkin Elmer) or equivalent.
- 3) PerkinElmer ICP-MS injector (PerkinElmer) or equivalent.

3. Robotic Sample Processing Station

- 1) Robotic Liquid Sample Processing Workstation: LEAP® Technologies CombiPal® twin head system, or equivalent system.

Twin Head SPME Processing Workstation (LEAP Technologies, Inc.)

The Twin Head SPME CombiPal® 4(LEAP Technology, Inc.) sample processing workstation, mounted on top of the GC, has a unique design: features two (2) computer-controlled SPME fiber injection heads that run on a dual rail system. The two SPME Heads are independently-controlled and perform SPME fiber equilibration/injection

operations in tandem. This configuration allows for increased SPME fiber equilibration time up to 20 minutes per SPME head while maintaining a sample-to-sample injection cycle time of 10 minutes. The result is improved SPME efficiency and faster duty rates (6 injections per hour).

4. Equipment

- 1) Water purification system for providing ultrapure water with a resistivity $\geq 18 \text{ M}\Omega \text{ cm}$.
- 2) High-precision analytical balance capable of accurately weighing milligram amounts of material to the tenth of a milligram or better.
- 3) A pH meter with one hundredth's of a pH unit readout or better fitted with a pH probe that allows temperature compensation (pH probe).
- 4) Calibrated hand-held adjustable pipettors that cover the range of accurate liquid delivery from 5 μL to 5000 μL . Research Pro™ Eppendorf® electronic programmable pipettors (distributed by Brinkmann Instruments, Westbury NY) or equivalent.
- 5) Gas regulators for argon, helium and xenon (Airgas, Atlanta, GA) or equivalent.
- 6) Conventional oven (FREAS Model 605, Thermo scientific, Catalog No. 3166188), or equivalent.
- 7) Plastic tent (Captair Pyramid, Erlab, North Andover, MA) or equivalent for preparation of sodium tetra(n-propyl)borate solution.

5. Computer Software

- 1) The ELAN Instrument Control version 3.4 with Service Pack 2 (or later version) should be installed on the computer controlling the ELAN DRC II™.
- 2) Chromatography data handling software, specifically, TotalChrom™ Workstation, version 6.3.1 or later (PerkinElmer® Instruments, Shelton CT). Install PerkinElmer's TotalChrom Workstation package on the same computer containing the ELAN Instrument Control software*. Contact PerkinElmer for installation and configuration of TotalChrom. Or consult the TotalChrom™ Workstation User's Guide. This method assumes that version 6.3.1 of TotalChrom Workstation package is installed.
- 3) Operating software for CombiPal autosampler, specifically, Chronos (2007-2010 Axel Semrau GmbH & Co KG)
- 4) ChromLink™™ 2.1 software (PerkinElmer Instruments). The installation of ChromLink™™ is straightforward when using the supplied installation utility.
- 5) pdFactory Pro (FinePrint Software, LLC, www.fineprint.com) or equivalent software. This product is used for creating electronic Portable Document Files (pdf) directly from any Windows® compatible application print dialog box.
- 6) A custom Microsoft Excel® macro procedure named "Extract TC Data".

6. Supplies

- 1) 200 μL pipette tips, 960 tips per case (Eppendorf® catalogue # 2235137-1, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 2) 300 μL pipette tips, 960 tips per case (Eppendorf® catalogue # 2235144-3, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 3) 1000 μL pipette tips, 960 tips per case (Eppendorf® catalogue # 2249044-3, distributed by Brinkmann Instruments, Westbury NY), or equivalent.

- 4) 5 mL pipette tips, 500 tips per case (Eppendorf® catalogue # 2235081-1, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 5) 50 mL combitips plus, 100 tips per case (Eppendorf® catalogue # 2226660-8, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 6) 10 mL combitips plus, 25 tips per case (Eppendorf® catalogue # 2226650-1, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 7) 2.5 mL combitips plus, 100 tips per case (Eppendorf® catalogue # 2226630-6, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 8) 50-mL acid-cleaned volumetric flasks for triple spike solution preparation (polypropylene or Teflon flasks preferred). To acid-wash flasks, rinse with 1.2M hydrochloric acid followed by rigorous rinsing with DI water. Repeat this process several times depending on prior use of the containers.
- 9) 250-mL acid-cleaned volumetric flasks for derivatizing reagent (NaPr4B) preparation (polypropylene or Teflon flasks preferred). To acid-wash flasks, rinse with 1.2M hydrochloric acid followed by rigorous rinsing with DI water. Repeat this process several times depending on prior use of the containers.
- 10) Acid-cleaned 1L PE bottles for buffer solution preparation. To acid-wash flasks, rinse with 1.2M hydrochloric acid followed by rigorous rinsing with DI water. Repeat this process several times depending on prior use of the containers.
- 11) 50 mL polypropylene tubes pre-screened for Hg (Becton Dickinson) or equivalent.
- 12) 15 mL polypropylene tubes pre-screened for Hg (Becton Dickinson) or equivalent.
- 13) 2 mL polypropylene (PP) microcentrifuge tubes (Eppendorf® catalogue # 22363352, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 14) Tube racks for 2 mL microcentrifuge tubes (2236352-, distributed by Brinkmann Instruments, Westbury NY), or equivalent.
- 15) Six or more PAL Tray20mL (part# 65487454, CTC Analytics, PAL system accessories)
- 16) CombiPal autosampler vials 20 mL, glass, (Microliter Analytical Supplies, Inc. product #16-2000) or equivalent.
- 17) Head space caps for 20mL glass vials (Microliter Analytical Supplies, Inc. product #16-0050M) or equivalent
- 18) Kay-Dry™ paper towels and Kim-Wipe™ tissues (Kimberly-Clark Corp., Roswell GA, or equivalent vendor).
- 19) Teflon™-coated magnetic stir bars (4). (Catalog Number 58948-974 or equivalent), VWR Scientific Products, Buffalo Grove, IL.
- 20) Teflon™-coated magnetic stir bars. (Catalog Number 58947-140 or equivalent, VWR Scientific Products, Buffalo Grove, IL).
- 21) Cotton swabs (Hardwood Products Co. ME, or equivalent vendor).
- 22) Nitrile, powder-free examination gloves (N-Dex®, Best Manufacturing Co., Menlo, GA, or equivalent vendor).
- 23) Biohazard autoclave bags (Curtin-Matheson Scientific, Inc., Florence, KY, or equivalent)..

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Global Information

Volume units :	μL	Unidentified Peak Quant.	
Quantitation units :	ng	Calibration factor :	1.000e+06
Sample Volume :	1.000	Always use calib. Factor :	selected
Void time (min) :	0.000		
Calibration		RRT Calculation	
External Standard	selected	Use first peak in run as RRT reference:	selected
Reject outliers during calibration :	not checked		
Sample Amount Options			
Correct amounts for calibration standards :	not checked		
Convert unknown samples to concentration units:	checked		

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preparation of Stock Solutions

a) NaOAc Buffer Solution (0.1 M sodium acetate anhydrous)

- 1) Dissolve 16.41 g of sodium acetate anhydrous into approximately 1900 mL of DI water in a 2000 mL polypropylene vessel with a magnetic stir bar on a magnetic stir plate, and mix thoroughly.
- 2) Measure pH and adjust pH to approximately 4.75 with glacial acetic acid. Make final volume adjustment to 2000 mL with DI water.

b) Sodium tetra(n-propyl)borate (NaPr_4B , 2% w/v)

- 1) Place one unopened vial containing 5.00 g of sodium tetra(n-propyl)borate, a squirt bottle of DI water, and a clean 50 mL conical centrifuge tube filled with 10-20 mL of DI

water inside a glove box or tent. Fully purge glove box or tent with 100% nitrogen for approximately 5 minutes to remove oxygen.

- 2) Open the vial containing sodium tetra(n-propyl)borate and add 10-20 mL of DI water with constant swirling.
- 3) Pour the dissolved contents into the 50 mL conical centrifuge tube. Wash the reagent vial with additional DI water to dissolve remaining solids and quantitatively transfer remaining reagent to the 50 mL tube. Cap the 50mL tube and remove from tent. Once dissolved, the NaPr_4B solution may now be safely used in normal atmosphere.
- 4) Inside a chemical fume hood, quantitatively transfer reagent solution containing 5.00 g of NaPr_4B to a 250 mL UV-resistant volumetric flask. Dilute to the final volume with DI water. This makes a 2% (w/v) NaPr_4B solution. Store at 4°C.

B. Preparation of Working Spike Solution

Caution!

Mercury compounds are toxic! Take extra care to avoid accidental dermal contact, ingestion or inhalation of these materials. Wear appropriate personal protective equipment. Above all, wear a laboratory coat and latex or nitrile gloves. Clean up any spill that might occur according to applicable hazardous material spill procedures.

Exercise the utmost care in executing all measurements precisely to obtain the best accuracy for the final concentrations. Use only pipettes, disposable tips and volumetric flasks that have been tested for accuracy and will deliver liquid volumes with a precision of $\pm 1\%$ or better.

1. Preparation of Working Triple Spike Standards Solution

On the day that the samples will be digested, prepare fresh working spike solution. Remove the vendor-supplied standard solutions of the following from the refrigerator and allow to reach room temperature: In^{199}Hg , Me^{200}Hg , and Et^{201}Hg . The following intermediate and working solutions should be prepared by weighing allowing the final concentrations of "spike" material in the working solution to be determined by weight. An Excel worksheet was developed to calculate the final concentrations of working triple spike standards solution, and the template for the worksheet can be found in Appendix B.

a) Intermediate standard solution

- 1) Pipette 50 μL each of In^{199}Hg , Me^{200}Hg , and Et^{201}Hg standards into separately labeled 2 mL centrifuge tubes. Add 400 μL of DI water to each and mix thoroughly. (Note that the volumes pipetted depend on the concentrations of the original stock solutions).

b) Working solution (containing mixture of 3 isotope standards):

- 1) Fill a clean 50 mL volumetric flask approximately half full with DI water.
- 2) Pipette 100 μL of each intermediate standard solution (In^{199}Hg , Me^{200}Hg , and Et^{201}Hg) into the flask. Complete the volume to the 50 mL mark with DI water. (Note that the volumes pipetted depend on the concentrations of the original stock solutions).
- 3) The concentration of each isotope standard in the working solution should be within a range of 0.7–1.5 $\mu\text{g Hg/L}$. Calculate the exact concentration of each isotope standard contained in the Working Triple Spike Standards Solution by dividing the starting concentrations (indicated by the isotope standard's certificate of analysis) by the dilution factors shown in Table 9-1 (dilution factors must be adjusted to produce isotope standard concentrations in the range of 0.7–1.5 $\mu\text{g Hg/L}$). Make appropriate adjustments to the calculations if volumes deviate from the prescribed amounts.

TABLE 9-1: Dilutions Factors for Preparing Working Triple Spike Standards Solution

Mercury Species	Calculation (units = mL)	Dilution Factor
In ¹⁹⁹ Hg	$0.4 \div 0.05 \times 50 \div 0.1 =$	4000
Me ²⁰⁰ Hg	$0.4 \div 0.05 \times 50 \div 0.1 =$	4000
Et ²⁰¹ Hg	$0.4 \div 0.05 \times 50 \div 0.1 =$	4000

C. Preparation of Quality Control Material

Order bovine blood from an appropriate vendor (or use donated human blood) and characterize for total Hg using this method or an equivalent method.

- Base pool preparation:** Human blood (or bovine blood) with the lowest mercury content should be used. Distribute base blood into pre-labeled 2 ml vials by 1.5 ml aliquots.
- Low and High pool preparation:** Analyze base blood for InHg, MeHg, and EtHg species. On the basis of this calculation, add appropriate volumes of InHg, MeHg, and EtHg (not enriched) species to obtain "low" and "high" pools. While maintaining constant stirring of each pool, aliquot approximately 1.5 mL of blood into a sufficient number of pre-labeled 2 mL vials to provide QC material for 1000 or more runs. Store aliquotted QC material at a temperature of $\leq -70^\circ$.

D. Sample preparation

Caution!

Work with open vials containing biological samples inside of a biological safety cabinet (BSC). Recap vials before removing them from BSC. Wear appropriate personal protective equipment (lab coat, safety glasses, and gloves).

- Preparation of blood samples for digestion:**
 - Pipette 100 μ L of blood samples into pre-labeled 2 mL tubes.
 - Add 100 μ L of Triple Spike Standards Solution. Recap and vortex each tube before continuing to the next tube.
 - Add 500 μ L of TMAH to all tubes. Cap and vortex.
- Digestion:**
 - Place rack containing capped tubes in an incubator or oven set to $80 \pm 3^\circ\text{C}$ for ≥ 20 hours.
- Derivatization of the digested samples:**
 - Aliquot 200 μ L of digested samples into pre-labeled 20 mL SPME headspace vials.
 - Add 7.7 mL of NaOAc Buffer Solution followed by 250 μ L of NaPr₄B Derivatization Reagent. Cap the vial immediately and mix.

E. Requirements for Batch Analysis of Samples and QC Material

Process a predetermined set of samples and QC material for batch analysis. One "batch" run is defined as the analysis of a contiguous set of samples bracketed by "Bench QC" material at the beginning and end of the set. Each Bench QC level (typically, a "high" and a "low") should be analyzed at the beginning and again at the end of the batch run. QC needs to be treated like the unknown samples, i.e., each QC sample is individually prepared and goes through all the steps as is done for unknown samples. It is not appropriate to report the QC results coming from the split analysis of a single QC sample if it has already been processed (i.e., diluted, centrifuged, filtered, digested, derivatized, etc.). Note that this limitation does not apply to the duplicate use QC material originating from the same original vial as long as they both are processed identically like unknown samples and *on the same day*.

- 1) Identify, gather, and thaw a predetermined number of sample tubes/vials containing the blood samples to be analyzed in a batch run.
- 2) For each batch of samples run, thaw one tube of low and high bench QC (often identified as "LB-yyxxx" and "HB-yyxxx"; for explanation of nomenclature, see Quality Control Material section).
- 3) Label the necessary number of 2 mL microcentrifuge vials with appropriate identification to ensure that they will be matched to their corresponding unknown samples and QC. Similarly, label an equal number of 20 mL SPME vials.
- 4) Use the TMAH digestion procedure for blood samples and QC material.
- 5) Use the preparation of digested samples for analysis procedure to prepare samples and QC material.
- 6) Cap all autosampler vials with the proper fitting septum caps.

The use of a barcode scanning device to electronically record sample identification from barcodes printed on vial labels should be utilized if available.

F. GC Instrument Program

- 1) Set the GC analytical run parameters according to TABLE 9-2. Refer to the Clarus 500 User Guide for programming specifics.
- 2) One or more parameters specified in TABLE 9-2 may be changed, if determined necessary, to meet analytical performance goals.

TABLE 9-2: GC Settings

Parameter	Setting
GC injector Temperature	Step #1: 1 min @ 220°C Step #2: linear ramp to 280°C, hold
Carrier gas flow rate (Helium)	2 mL/min
Oven Temperature	Step #1: 1 min @ 75°C Step #2: linear ramp to 250°C (at a rate of 45 °C/min)
Total flow ratio	28:1 @ 0.25 min
Transfer line Temperature (Aux - auxiliary zone)	250°C
(Aux - auxiliary pneumatics)	OFF (all)

G. CombiPal Autosampler Program

- 1) CombiPal is initially set up and optimized by LEAP technology service engineers. The settings can be optimized according to analyst needs (refer to PAL system user manual). The following procedures present the key steps that are taken to set up the CombiPal autosampler system for this method.
 - (a) Using the PAL control terminal, start at a window displaying "JOB QUEUE". Press ESC to return to the previous menu. Press function key F1 - "MENU". Rotate the outer knob to scroll through items in a menu list. To select a highlighted item press the central knob (ENTER button). Then use the outer knob to scroll through available options for that item or to change a numeric value. Then press the inner knob again to ENTER the displayed option.
 - (b) In the "MENU" window, select "Utilities" followed by "Tray" then select "Agitator" and enter parameters from TABLE 9-3. Then press "ESC" to get back to the "Tray" option, scroll to the right, select "Tray" (this "tray" is an option for first "Tray" selection), and enter parameters. X, Y, and Z parameters have to be optimized for each SPME arm.
 - (c) In the "Utilities" window select "Injector" and select "GCInj1". Enter the parameters. Also in the "Utilities" window select "Vial" and select "Standard". Enter the parameters from TABLE 9-3.

TABLE 9-3: CombiPal® Autosampler Settings

Parameter	Setting
Tray (Agitator)	
Needle Penetration	20.0 mm
Tray type	SMMTray
Offset X, Y, Z	0, 0, 55.5 mm
Stand by Temp	OFF
Actual Temp	25.7°C (changes)
Tray (Tray)	
Needle Penetration	12.0 mm
Tray type	VT32-20
Offset X, Y, Z	-55.3, -188.1, -2.0 mm
Injector (GCInj)	
Needle Penetration	35.0 mm
Vial (Standard)	
Needle Penetration	43.0 mm

- 2) CombiPal is operated by Chronos software. Click on the Chronos icon on the operating computer desktop. Chronos method files used for this method can be accessed through clicking on "Method Editor" on the Main Menu of Chronos. In the right corner, click on the "Load" tab which allows the analyst to upload the needed method file. There are five method files used in this method: SPME bakeout LEFT.cam, SPME bakeout RIGHT.cam, SPME conditioning LEFT.cam, SPME conditioning RIGHT.cam, TWIN SPME_cdc.cam. The settings can be changed by the analyst for better separation and analytical response.
- (a) To clean SPME fibers every day before starting a run,- SPME bakeout LEFT.cam and SPME bakeout RIGHT.cam are used. The settings should be as shown in TABLE 9-4.

TABLE 9-4: Chronos Settings (Baking)

Parameter	Setting
Bakeout Time (s)	420
GC Runtime + Cooling Down (s)	120
Needle Penetration Vial (mm)	34
Fiber Exposure (mm)	12
Needle Penetration (mm)	35

- (b) To condition new SPME fibers use - SPME conditioning LEFT.cam and SPME conditioning RIGHT.cam. The settings should be as shown in TABLE 9-5.

TABLE 9-5: Chronos Settings (Conditioning)

Parameter	Setting
Bakeout Time (s)	1800
GC Runtime + Cooling Down (s)	120
Needle Penetration Vial (mm)	34
Fiber Exposure (mm)	12
Needle Penetration(mm)	35

- (c) To perform a run, the file "TWIN SPME_cdc.cam" is used. The settings should be as shown in TABLE 9-6.

TABLE 9-6: Chronos Parameter Settings (for Run)

Parameter	Setting
Source Tray	Tray1,1
Time	1 s
Enrichment Time	1200 s
Desorption Time	420 s
GC Cooling Down	180 s
Needle Penetration Vial	34 mm
Fiber Exposure	12 mm
Needle Penetration Inlet	35 mm

H. ICP-DRC-MS Instrument Setup

To improve workflow efficiency, do the programming steps described in this section before the day of analysis.

1. Programming the DRC Gas Flow Delay Parameter

A special ELAN DRC™ setting, called "Flow Delay", needs to be changed from its default setting to avoid the problem of the ELAN software forcing a time delay of several seconds before collecting data at the start of a chromatographic run when in DRC mode. This setting can only be changed by entering the ELAN software's Service Mode. This change only needs to be done once per software installation or upgrade, or if the setting was deliberately changed by a field service engineer. It is a good idea to inform the service engineer who intends to perform work on the instrument of the importance of returning the "Flow Delay" to the non-default value of 1.

Important!

While in Service Mode, DO NOT make changes to any setting except for the one change described below.

- 1) From within the ELAN program and in the window entitled "Instrument Control Session", choose menu item "Options" > "Service Mode." You will be prompted to enter a Service Mode password. Enter the password "Elan6000" (omit the quotes and pay attention to capitalization) and click OK. If this password is not accepted, you will have to contact a supervisor or a PerkinElmer service technician.
- 2) You will be presented with a new tab called "Service" within the Instrument window. Maximize the window. At the bottom will be a row of tabs, click on "Gas". Look for the parameter called "Flow Delay" (Gas changes while in DRC Mode). If its setting is a value other than "1", click on the "Set Pauses..." button. Change the value in the field named "Flow Change" to 1. Click the "Apply" button then click the "Close" button. Choose menu item "Options" > "Exit Service Mode."

2. Programming the ELAN ".mth" file

- 1) If it is not already open, launch the ELAN program and in the window titled "Instrument Control Session", choose menu item "File" > "Review Files". Click the "Load" button for "Method" (the first item on the list). Navigate to the folder "C:\elandata\Method" and click on "GC_Hg.mth" file then click the "Open" button.
- 2) If the "GC_Hg" file cannot be found, or it has been changed or corrupted in a manner that makes its use questionable, then cancel the open file dialog box and close the Review Files window by clicking the "Done" button. Do the following steps; otherwise, proceed to step 3:
 - (a) Make the active method file the active window (do this by clicking on the tool bar icon that looks like a notepad with a "Cu" on it). Then click "File" > "New" on the menu bar and choose "Data Only" in the New Method window that appears. Click "OK" then maximize the window. Complete this window with the information in the TABLE 9-7.

TABLE 9-7 ELAN Timing Parameters

Parameter	Setting
Sweeps/Reading:	1
Readings/Replicate:	2725
Number of Replicates:	1
Tuning File:	C:\elandata\Tuning\default.tun
Optimization File:	C:\elandata\Optimization\gc_xe_drc.dac

- (b) On the first line of the worksheet-like table, click in the cell of row 1 of the "Analyte (*)" column. Type "Hg" then press "Enter" key. The row will suddenly be filled-in with mercury's "Begin Mass (amu)" of 201.971 (or something close) and several default parameters. Right click on "Hg" and a periodic table will appear. Select five Hg isotopes from mass ~197.9670 to ~201.9710 and click "OK". All five isotopes should be seen in the "Analyte (*)" column. Tab to next cells and fill in the information shown in TABLE 9-8.

TABLE 9-8: ELAN Analyte Parameters

Parameter	Setting
Analyte:	Hg
Begin Mass (amu):	197.9670* (first row, then proceed to the 4 other isotopes)
End Mass:	<leave empty>
Scan Mode:	Peak Hopping
MCA Channels:	1
Dwell Time:	25
Integration Time:	68125 (automatically determined by software)

*Actual mass may differ by a few hundredth of amu.

(c) Click on the "Processing" tab and enter the following information:

TABLE 9-9: ELAN Processing Parameters

Parameter	Setting
Detector:	Dual
Measurement Unit:	Cps
Process Spectral Peak:	Average
Process Signal Profile:	Average
Apply Smoothing:	Checked
Factor:	5
Auto Lens:	Off
Isotope Ratio Mode:	Off

(d) Skip the "Equation" tab. Click on "Sampling" tab and enter the following information:

TABLE 9-10: ELAN Sampling Parameters

Parameter	Setting
Peristaltic Pump Under Computer Control:	Unchecked
Sampling:	External

(e) Click on the "Report" tab and enter the information in TABLE 9-11.

TABLE 9-11: ELAN Report Parameters

Parameter	Setting
Report View Send to Printer:	Unchecked
Report Options Template:	<leave empty> *
Automatically Generate NetCDF File:	Checked C:\elandata\reportoutput\
Report to File Send to File:	Unchecked
Report Options Template:	<leave empty> *
Report File Name:	<leave empty> *
Report Format:	<leave empty> *
File Write Option	<leave empty> *

*Content of these fields is not important since Send To Printer/File is unchecked.

(f) Choose menu item "File" > "Save As" and navigate to "C:\elandata\Methods\" folder. Enter "GC_Hg" as the name of the method file and click the "Save" button.

3) The ELAN method "GC_Hg" is now loaded into memory.

3. Creating the ELAN Sample Table ".sam" file

- 1) If it is not already open, launch the ELAN program and in the window entitled "Instrument Control Session", choose menu item "File" > "Review Files". Click the "New" button for "Dataset" which is the second item on the list. Navigate to the folder "C:\gc\data\" and enter the file name "Hg<yymmdd>" where yy = last 2 digits of current year, mm = this month, and dd = date of run. Click the "Open" button. The new dataset folder has been created and is now active. Click on the "DONE" button in the Review Files Window.
- 2) Click on the tool bar icon that looks like three Erlenmeyer flasks. Choose "File" > "New" on the menu bar. A new window will appear entitled "Samples - [Untitled]". Click the "Batch" tab then click on the "Sample Template" button. A dialog box entitled "Sample Template Data" will appear. Enter the information as given in TABLE 9-12.

TABLE 9-12: Sample Template Data

Parameter	Setting
Sample ID:	001_ <sample name>
Measurement Action (*):	Run Sample
Method:	GC_Hg.mth
Sample type:	Sample
Wash Override (sec)	0

- 3) Parameters not mentioned in TABLE 9-12 can be left blank.
- 4) From the menu bar, choose "File" > "Save As" and save the file in the directory "C:\gc\data\" using the name "Hg<yyymmdd>.sam" where yy = last 2 digits of current year, mm = this month, and dd = date of run.
- 5) It is a good idea to save a copy of this file as a template, thereby avoiding the need to re-create it every time.

I. ICP-DRC-MS Performance Checks

The following performance checks should be recorded in the instrument log book.

1. Daily Performance Check

- 1) Each day, before samples are analyzed, aqueous blanks and a 10X dilution of Low Bench QC should be analyzed to ensure that the instrument is functioning properly. Prepare all the following using the same technique and supplies as samples, unless stated otherwise.
 - (a) **Aqueous Blank.** To prepare, add 200 μ L of TMAH into a 20 mL vial. To each vial add 7.7 mL of NaOAc Buffer solution and 250 μ L of derivatization reagent (NaPr4B). Cap the vial immediately and gently mix it. Aqueous blanks are ready to be analyzed.
 - (b) **10X Diluted Low Bench QC.** To prepare, mix 0.1mL of Low Bench QC (LB) in 0.9 mL of base blood. This sample is digested and then analyzed in the same manner as patient samples.
- 2) After SPME-GC-ICP-MS analysis of Aqueous Blank and 10X Diluted Low Bench QC are complete, open the Aqueous Blank and 10X Diluted Low Bench QC RAW files in TotalChrom. Examine the Aqueous Blank chromatograph for possible indicators of contamination. Then look at the 10X Diluted Low Bench QC chromatogram. This chromatogram should have visible peaks for the mass 202 isotopes of MeHg, EtHg, and InHg. The integrated area under each mercury species peak has to be greater than 300 (arbitrary units), otherwise there may not be sufficient sensitivity and the instrument may need to be optimized (see weekly performance check section).

2. Weekly Performance Check

- 1) Visual check of torch, injector, RF coil, and cones:
 - (a) Slide the vacuum chamber and interface away from the torch box, and visually check the cleanliness of these components. Notate any cleaning / replacing of consumables on the "Daily Maintenance / Performance Checklist". For details on cleaning procedures refer to IRAT Weekly Maintenance SOP.

- (b) **Injector:** There should not be deposits on the inside of the injector. If there are deposits, remove the injector and clean it with 1-5 % v/v nitric acid and a cotton swab. Alternatively, replace injector with a spare and clean the dirty one in an overnight soak in 5% v/v nitric acid (can be ultrasonicated, but is not typically necessary).
 - (c) **Torch:** Check for melting, cracking, and cleanliness. If necessary, replace with a spare and soak overnight in 5% v/v nitric acid bath. If torch is only dirty, replacement / cleaning can be deferred to the regular weekly maintenance day.
 - (d) **RF coil:** Check for excessive corrosion (flaking). Replace if necessary.
 - (e) **Sampler cone:** Check for excessive buildup of matrix, cracking, or pitting. If necessary, replace dirty cones with clean spare cones and clean.
- 2) Replace GC injector septum.
 - 3) Visually check level and condition of oil in roughing pumps. Appropriate level for oil is $\sim\frac{3}{4}$ full. If color indicates the need to change oil soon, do so at the next weekly maintenance. Oil is clear yellow when new. Light brown or "tea colored" is ok to use. Dark brown or "coffee colored" indicates need to replace.
 - 4) Weekly, the optimization of both plasma and mass spectrometer parameters of the ICP-MS is achieved by using a mixture of xenon (Xe) in argon (Ar). The ICP-MS system is optimized with Xe gas because of its similarities in ionization potential to Hg.
 - (a) Ignite the plasma and set the flow rate of the Xe gas mixture (0.01-0.1% Xe in Ar) to 0.01 - 0.25 mL/min (depending on Xe% in Ar) on the GC main display soon after plasma ignition to achieve Xe intensity of > 350,000 cps (counts per second). In the ELAN software, upload "gc_xe_daily_drc.mth". After the gas signal stabilizes, in the "Sample" window press "Analyze Sample" in "manual" mode. Perform X-Y optimization to obtain the highest possible Xe intensity (at least >100,000 cps). Proceed with the following steps to optimize other parameters in TABLE 9-13 for maximum Xe intensity.
 - (b) In the ELAN software upload "gc_xe_xy_drc.mth". Also in the ELAN in the window entitled "Instrument Control Session", choose menu item "File" > "Review Files". Click the "Load" button for "Optimization" (the sixth item on the list). Navigate to the folder "C:\elandata\Optimize" and click on "gc_xe_drc.dac" file then click the "Open" button.
 - (c) SmartTune™ is used for DRC Mode optimization. Optimization parameters can be selected from "edit list". The appropriate method file in the "method" section should be selected. The optimization for maximum Xe intensity should be performed (see IRAT Optimization SOP).
 - (d) After recommended parameters are optimized, save the optimized file as "gc_xe_drc.dac". The current optimized values will appear automatically and will be similar to the ones in TABLE 9-13.
 - (e) After optimization for maximum Xe intensity, navigate to the folder "C:\elandata\Method" and click on "gc_xe_daily_drc.mth" file then click the "Open" button. Record this data.

TABLE 9-13: ELAN Optimization Parameters

Parameter	Setting	Parameter	Setting
Nebulizer Gas Flow (NEB):	1.5	Cell Path Voltage Std	- 14†
Auxiliary Gas Flow:	1.2	Rpa	0
Plasma Gas Flow:	15	Rpq	.5†
Lens Voltage:	4.75*	Cell Gas A	0.3
ICP RF Power:	1450	Cell Gas B	0
Analog Stage Voltage:	-1950*	DRC Mode NEB	1.5
Pulse Stage Voltage:	950*	DRC Mode QRO	- 16.90†
Quadrupole Rod Offset Std	0	DRC Mode CRO	- 1.90†
Cell Rode Offset Std	23	DRC Mode CPV	- 51†
Discriminator Threshold	17	Axial Field Voltage	150†

†Suggested starting values only. Optimum parameters will depend on the outcome of the optimization procedure.

J. ICP-DRC-MS Warm Up

- 1) Launch the ELAN ICP-DRC-MS software and note whether all graphical indicators of instrument readiness are green. If not, take the appropriate actions described in the instrument's software and hardware manual.
- 2) Perform necessary maintenance checks as described in Chapter 5 of the ELAN DRC II Hardware Guide (e.g., argon supply, interface components, cleanliness, positioning, and interface pump oil condition). Note the base vacuum pressure in the INSTRUMENT window of the software. (Before igniting the plasma, the vacuum is typically about 8×10^{-6} torr). Keep a record any maintenance procedures along with the base vacuum pressure on the Daily Maintenance Checklist.
- 3) In the INSTRUMENT window of the ELAN software, click the "Front Panel" tab and click the plasma "Start" button to ignite the plasma. In the same window, the ignition sequence bar (blue progress bar) will start to expand from the right, indicating the approximate time before plasma ignition. The plasma may at first flicker but it should establish a more or less steady intensity after 5-10 seconds.

On a rare occasion, the plasma may ignite emitting an orange, violently flickering light, and electrical discharge noises will be heard. In this case, immediately shut off the plasma by pressing the yellow "Stop" button on the ICP-DRC-MS instrument's front control panel. Wait 30 seconds then investigate the cause of the plasma misfire. A more common occurrence is that the plasma may extinguish itself a few seconds after ignition. Promptly reignite by pressing the "Start" button on the ICP-DRC-MS instrument's front control panel. Usually, the plasma will stay lit after the second try. If not, investigate the cause of this instability (refer to the ELAN DRC II Hardware Guide).

- 4) Soon after the plasma ignites, perform daily performance.
- 5) Fill in the Daily Maintenance Checklist to record to the completed optimization procedures. If a tuning (mass-calibration) procedure was done, save it to the file "default.tun," and also in a separate file containing the analysis date "default_MMDDYY.tun" (where MM=month, DD=day, and YY=year).

K. GC-ICP-DRCII-MS System Startup

1. Entering Sample Names into the ELAN Sample Table

- 1) Click on the tool bar icon that looks like three Erlenmeyer flasks. If the current Samples window is not this run's sample file, then choose "File" > "Open" on the menu bar and navigate to and open this run's current data folder in "C:\gc\data\". Click on the file named "Hg<yyymmdd>.sam" (yy = year, mm = digit month, dd = date) and open it. The Samples window will be the one created in the section creating the ELAN Sample Table ".sam" file.
- 2) Fill in the name of each sample by double-clicking after the "_" (underscore) in the cell "sample ID". Type in the sample name and press "Enter" on the keyboard. In this manner, enter the name of blanks, quality control, and samples that will be analyzed in the run. If barcodes are used on the sample labels, use the barcode scanner attached to the ICP-DRC-MS computer to scan the sample ID from the barcode on each sample before placing it into position in CombiPal autosampler tray.
- 3) Fill in the samples table in similar fashion to what is shown in TABLE 9-14 below.

TABLE 9-14: ELAN Samples Table

A/S Loc.	Batch ID	Sample ID	Measurement Action	Method ...	Wash Speed (+/- rpm)
1		001_ Aq.Blk	Run Sample		0
1		002_ Aq.Blk	Run Sample		0
2		003_ LB 10X	Run Sample		0
2		004_ LB 10X	Run Sample		0
3		005_ LB-yyxxx	Run Sample		0
3		006_ HB-yyxxx	Run Sample		0
4		007_ Sample	Run Sample		0
4		008_ Sample	Run Sample		0
...rows for 18 samples and SRM material were omitted for brevity					
16		031_LB-yyxxx	Run Sample		0
16		032_HB-yyxxx	Run Sample		0

In the example table above, a run of 20 samples is shown so the last vial ends up being placed in A/S Location #16 (this location corresponds to autosampler location which is defined in Chronos, since there are two autosampler trays two #16 are used - "Left" and "Right". It is not necessary to put A/S loc in ELAN - only for analyst's benefit).

The numbers preceding the underscore character correspond to the order of injection. These numbers will later help the analyst find individual chromatograms based on injection number instead looking for specific sample names during post-run data processing in TotalChrom.

- 4) When the sample table entries are verified to be correct choose "File" > "Save".
- 5) Print the ELAN Sample table by choosing the "File" > "Print Setup" > "Reports". In the ensuing dialog box, select the preferred printer and click "OK". Next, choose "File" > "Print" and then click the "Print" button. Refer to the printout of the ELAN Sample table for the correct vial positions when loading samples into the CombiPal autosampler tray.

L. Starting the Run

- 1) Create the daily data folder in C:\GC\Data under the name Hgyymmdd (i.e., Hg110422)
- 2) Launch ELAN Instrument Control program if it is not already up. Do not launch or start any other programs at this time.
- 3) Check that the correct ELAN method is loaded and active in the window "Instrument Control Session". If it is not correct, load the correct Method file. Check under the Sampling tab that "Peristaltic pump under computer control" is unchecked, and the pull-down menu "Sampling" indicates "External".
- 4) Check that the correct Sample file in the window "Instrument Control Session" is active. If it is not correct, load the correct Sample file.
- 5) Load created dataset file "Hgyymmdd"
- 6) Check that the GC methods are correctly programmed.
- 7) Check that CombiPal® autosampler methods are correctly programmed.
- 8) This step offers the advantage that the ELAN data files will be converted in real time to TotalChom™.raw" files that have names containing a date-time stamp corresponding to the actual time of injection.
 - (a) Launch TotalChrom Navigator. In the resulting TotalChrom Navigator window, choose menu item "Apps" > "ChromLink™" (alternatively, you may launch ChromLink™ from the operating system "Start" > "Programs" menu).
 - (b) In the ChromLink™ program window, choose the menu item "Configuration" > "Mass Details" and check the Nominal Name and Mass for mercury isotopes. If it is missing or the ELAN tune ("default.tun") file was re-optimized earlier then ChromLink™ needs to be configured (see Configuration of ELAN ChromLink™ on page 35 for details). To save time, the analyst may choose to close the TotalChom™ Navigator and ChromLink™ windows and skip step 6 in its entirety. Data file conversion via ChromLink™ can easily be done during post-run data reprocessing.
 - (c) In the ChromLink™ program window, click on the "Browse" button just right of the "ELAN ChromLink™ file location" field. Navigate to the current working folder, double-click on it then click the "OK" button so that ChromLink™ knows where to save its processed files.
 - (d) Otherwise, refer to step (b) of Data Processing and Analysis on page 36 for details on proper setting of the ELAN ChromLink™ window's parameter fields. In the ELAN ChromLink™ window, click the button "Start Processing ELAN Data Files" to put ChromLink™ in watch mode so it will process each data for each injection in real time. A new dialog box will open and indicate it is ready to convert data and waiting for the first file.
- 9) Launch Chronos program, which communicates with the CombiPal autosampler and ELAN software.
 - (a) In the main menu, click on "Sample List" then upload analysis method for SPME fiber cleaning "baking".
 - (b) Upload "SPME bakeout LEFT.cam" (to clean the left fiber) then select "Create Schedule". The Schedule window will appear with time intervals required for the measurement. Click "RUN" on the main menu, the run window appears and click "run" again to proceed with the fiber cleaning.
 - (c) Repeat step (b) to clean right fiber and use "SPME bakeout RIGHT.cam"

- (d) After fiber cleaning and daily performance, check to ensure that the QC and samples are ready for analysis using SPME fibers. On the "Main Menu" select "Sample List" then click "Load List". Upload Dual SPME .csl file. This file communicates with Left and Right fibers. The table similar to the one below (TABLE 9-15) will appear. The table should have Analysis Method "C:\Chronos\Methods\TWIN SPME_cdec.CAM" uploaded.

TABLE 9-15 Chronos Samples Table

Analysis Method	Source Vial	Incubation Time (s)	Enrichment Time (s)	Desorption Time (s)	GC cooling down (s)
C:\Chronos\Methods\ TWIN SPME_cdec.CAM	1	1	1200	420	180
	1	1	1200	420	180
	2	1	1200	420	180
	2	1	1200	420	180
Rows for source vials 3 to 31 are omitted					
	32	1	1200	420	180
	32	1	1200	420	180

- (e) "Source Vial" column shows which autosampler position will be first analyzed. There are two racks (left and right) with vial position 1 to 32. The left rack gets analyzed by the left SPME fiber and the right rack gets analyzed by the right SPME fiber. There are two numbers "1" in the source vial column. The first one always corresponds to left rack/left fiber.
- (f) Chronos communicates to the autosampler through "source vial" position. The analyst has to make sure that the samples in ELAN correspond to the locations seen by Chronos. For Example, if the analyst wants to analyze slots 1-5 on both racks (total of 10 samples), the rows below 10 should be deleted. To delete, select the rows and click "Remove Samples".
- 10) Check that the DRC gas is indeed flowing by making the ELAN's Instrument window active and clicking on the Diagnostics tab. Inspect the Cell Gas A or B whose value should be fluctuating at the current value ± 0.01 mL/min. If it is not, see section "Turning on the Reaction Cell Gas" for details on how to turn on the DRC gas flow.
 - 11) Check that all blanks, QC, and sample vials are loaded into their correct positions in the CombiPal autosampler tray as designated by the ELAN Sample window (or its printout) and in position seen by Chronos.
 - 12) Before samples are analyzed, the daily performance check should be analyzed to ensure that the instrument is functioning properly.
 - (a) Click on the ELAN "Instrument Control Session" window to make it active. Highlight the samples and click the "Analyze Batch" button. A Run Progress box will appear indicating that the ELAN software is now waiting for a signal from the Chronos that indicates the occurrence of an injection.

- (b) In Chronos: to analyze sample select "Create Schedule" then press "RUN" on the main menu and "RUN" again on the run window.
 - (c) View the chromatograms in TotalChrom and in the real time window of ELAN to ensure that there are no problems with the analysis. After the aqueous blanks and 10X LB been analyzed, the run can be started.
- 13) Open the ELAN "Instrument Control Session" Real-Time window by clicking the tool bar button that looks like a Gaussian distribution (the blue chromatographic peak). After the Real-Time window opens, click on the drop-down menu and select "Signal". Real-time data will now be displayed.
 - 14) The CombiPal® autosampler will seek the first vial and make an injection. A blue bar in the ELAN's progress box will now indicate that data is being collected. The system can now run unattended.
 - 15) Check the progress of the run after 2 or 3 injections. Note the chromatograms appearing in the ELAN's Real Time window. Adjust the signal scale in the Real Time window, as necessary. Compare the positions and peak heights of each mercury species. It helps to visually compare it to a printed reference chromatogram. If abnormalities in retention time, peak height, or peak shape are readily apparent, the analyst may need to stop the autosampler and abort the run in the Chronos program and then ELAN. Correct the problem(s) and restart the run.

Important

Remember to disable the ELAN's Auto Stop feature before re-enabling it otherwise the ELAN may perform an auto shutoff prematurely.

M. Instrument Shut Down

- 1) The autosampler will stop after all samples have been analyzed.
- 2) Shut off ICP-DRC-MS plasma.
- 3) At the controller computer, visit the ELAN Instrument Control Session application and open the "Dataset" window. Confirm that all samples ran successfully and that the corresponding data for each sample is listed in this window.
- 4) Remove the QC and sample vials from the GC tray. Discard them according to CDC biohazard waste disposal guidelines.

N. Post-Run Data Analysis

1. Configuration of TotalChrom Integration Method

The following information is presented as a starting point to help the analyst develop robust integration method parameters that will work best for most chromatography data. Many of these parameters will work just fine as presented below. However, the separation chemistry of GC columns can vary due to frequency of use, column replacement, or because of individual sample "oddities". Some parameters may need to be adjusted from time to time to maximize the ability of TotalChrom™ to properly integrate peaks and identify components with minimum operator intervention. Therefore, the analyst should pay particular attention to the chromatograms produced in every run and make necessary adjustments as warranted. The

analyst should be familiar with the TotalChrom's frequently used integration functions, which are described in Chapter 18 of *TotalChrom Workstation User's Guide: Volume II*.

- 1) The creation of a new method file in TotalChrom is done the first time TotalChrom is setup, or it will need to be recreated if the file "Hg.mth" cannot be found or has been corrupted. In the TotalChrom Navigator window, choose the menu item "Build" > "Method." In the next dialog box, click the "Create a new method" radio button and click "OK." The default method will load into the method editor.
- 2) Click on the "Components" item in the menu bar in Method Editor. If the menu item "Delete All Components" is not grayed out, select it and click "OK" when prompted to "Delete all components, calibration levels, and calibration replicates".
- 3) Choose the menu Item "Process" > "Integration". Click on the "Integration" tab in the "Process" window. Enter the information shown in TABLE 10-1. These values are to be used as a starting point, but the analyst may make appropriate changes to one or more of the integration parameters as necessary.

TABLE 10-1: Integration

Basic Parameters		Advanced Parameters	
	Value		Value
Bunching Factor :	1	Peak Separation Criteria	
Noise Threshold :	115	Width ratio :	0.2
Area Threshold :	577	Valley to peak ratio :	0.01
		Exponential Skim Criteria	
		Peak height ratio :	5.000
		Adjusted height ratio :	4.000
		Valley height ratio :	3.000

- 4) Click on the "Baseline Timed Events" tab. Enter the information shown below in TABLE 10-2. The analyst may make appropriate changes to one or more of the Baseline Timed Events as may prove necessary.

TABLE 10-2: Baseline Timed Events

Defined Events				
Time	Event	Value	Code	Level
0.000	Set Area Threshold	125	AT	
0.000	Set Noise Threshold	25	NT	
0.000	Smooth Peak Ends On	5	+SM	
2.222	Set Bunching Factor	4	BF	

- 5) Click on the "Optional Reports" tab. Uncheck the box for "Keep temporary files".
- 6) Click on the "Replot" tab. Enter the information shown in TABLE 10-3. The analyst may make appropriate changes to one or more of the Replot parameters as may prove necessary.

TABLE 10-3: Replot

Plots		Miscellaneous	
<i>Generate a separate replot :</i>	checked	<i>Start plot at end of delay :</i>	checked
<i>Number of pages:</i>	1	<i>Gradient overlay :</i>	not checked*
<i>Retention Labels :</i>	Top of Plot*	<i>Draw baselines :</i>	checked*
<i>Component Labels :</i>	Actual time	<i>Timed Events :</i>	checked*
<i>Scaling Type :</i>	Absolute Scaling	<i>Plot Title :</i>	Chromatogram
Scaling Parameters		<i>X axis label :</i>	Time [min]
<i>Scale Factor :</i>	1.000000*	<i>Y axis label :</i>	Response [mV]

*These parameters maybe altered to suit the analyst.

- 7) It is unnecessary to click on the "User Programs" tab because it is not used. Close the Process window by clicking on the "OK" button.
- 8) In the Method Editor window, choose the menu item "Components" > "Global Information." Click on the "Integration" tab in the "Process" window. Enter the information shown in TABLE 10-4:

TABLE 10-4: Global Information

Volume units :	µL	Unidentified Peak Quant.	
Quantitation units :	ng	Calibration factor :	1.000e+06
Sample Volume :	1.000	Always use calib. Factor :	selected
Void time (min) :	0.000	RRT Calculation	
Calibration External Standard	selected	Use first peak in run as RRT reference:	selected
Reject outliers during calibration :	not checked		
Sample Amount Options			
Correct amounts for calibration standards :	not checked		
Convert unknown samples to concentration units:	checked		

- 9) The "LIMS Results" tab is not used. Click the "OK" button to close the window. The parameters in TABLE 10-4 are starting points. The analyst may make appropriate changes to one or more of the Global Information parameters as may prove necessary.
- 10) In the Method Editor window, choose the menu item "Components" > "New Component." The white list box in the left portion of the window will be empty. Click in the empty field labeled "Name" and type "Hg⁰". Press the tab key and enter "1.614" in the field labeled "Retention time". Select the radio button labeled "Peak" if it is not already selected. Leave the other fields and check boxes unaltered. Click the "New Component" button. Enter each of the component names and parameters listed in TABLE 10-5.

TABLE 10-5: Method Editor - Components Settings

Name	Retention Time	Absolute window	Relative window	Find tallest peak in window
Hg ⁰	1.614	2	3	No
InHg	3.470	0	3	No
MeHg	2.640	0	3	Yes
Ethg	3.176	0	3	Yes

- 11) Click the "New Component" button before starting a new component. After entering the last component, click the "OK" button. The values for Retention Time, Absolute Window and Relative Window serve as starting points. The analyst may alter these values as actual chromatographic results may dictate.
- 12) In the Method Editor window, choose the menu item "Components" > "Defaults." Click on the "Identification" tab". Enter the information shown in TABLE 10-6.

TABLE 10-6: Components Defaults – Identification

Parameter	Setting	Parameter	Setting
Component Type :	Peak	Reference :	Blank
Absolute window :	0	Internal Standard :	Blank
Relative window :	3	Find tallest peak :	Not checked

- 13) Click on the "Calibration" tab in Components Defaults Window. Enter the information shown in TABLE 10-7.

TABLE 10-7: Components Defaults – Calibration

Parameter	Setting	Amount
Calibration Type :	Use Calibration Factor	1.0000E-6
Scaling :	None	
Weighing :	None	
Response :	Area	

- 14) The "User Values/LIMS" tab is not used. Close the "Components Defaults" window by clicking the "OK" button.
- 15) In the Method Editor window, Choose "File" > "Save As." A window appears inviting you to enter any information pertinent to this method which will be saved with the method. Enter your name and the date this method was created. Click "OK" and a "TotalChrom File-Save-As" dialog box opens. Navigate the directory tree to get to the folder C:\GC\Methods). Double-click on this folder. In the "File name:" field, enter "Hg-Template.mth". If there is already a file in that folder with the same name, highlight that file and right-click the mouse. Choose "Rename" and give the file a new name (e.g. add "backup" to the name). Now, you can click the "Save" button. Close the "Method Editor" window.

2. Configuration of ELAN ChromLink™

ELAN ChromLink™ should be configured after initial installation of the program or when the ELAN tune ("default.tun") file is re-optimized, and when there is available at least one recent ELAN NetCDF file (with the ".nc" extension) containing data for the mass of interest that was collected since last update of the "default.tun" file.

- 1) Launch TotalChrom Navigator. In the resulting TotalChrom Navigator window, choose menu item "Apps" > "ChromLink™" (alternatively, you may launch ChromLink™™ from the operating system Start > Programs menu).
 - (a) Inside the ELAN ChromLink™ window, click on menu item "Configuration" > "Default TotalChrom Method". Click on the "Browse..." button and navigate to the directory C:\gc\methods\. Select "Hg-TC.mth" and click the "Open" button. "C:\gc\methods\Hg-TC.mth" will now be the ChromLink™ default method. Click "OK" to close the "Default TotalChrom Method" window.
 - (b) Inside the ELAN ChromLink™ window, click on the "Set" button. A window entitled "Operating Mode" will open. Inside the ELAN ChromLink™ window, click on the "Set" button. A window entitled "Operating Mode" will open. Click on the "Automatic - process all ELAN NetCDF files in specified location" radio button. The lower radio buttons will gray out. Click the "OK" button to close the window.
 - (c) Click on the "Browse" button by the "ELAN NetCDF file - location/file to be converted" space. Choose the path "C:\Elandata\reportOutput". The location/file to be converted will now read "C:\Elandata\reportOutput*.nc".
 - (d) Click on the "Browse..." button for "ELAN NetCDF ChromLink™ file location (sequence and raw files generated by ChromLink™)" field. An open file dialog box will open; choose the file to which all the data should be place (usually the data file that was created that day).

- (e) Click on the "Start Processing ELAN Data Files" button. A window entitled "Processing ELAN Data" will appear.
- 2) At this time, ChromLink™™ may be closed by selecting "File" > "Exit." Click "OK" at the dialog box asking if you want to quit ChromLink™™.
- 3) In addition to configuring ChromLink™™ itself, it is necessary to alter one value in the "seed" method file that ChromLink™™ uses to set a select number of parameters to certain default values. This step only needs to be done once following the installation of ChromLink™™.
 - (a) In the TotalChrom Navigator window, choose the menu item "Build" > "Sequence" and a dialog box called "Startup" will appear. Click on the radio button labeled "Load sequence stored on disk" then click the "OK" button. Navigate to the folder on the C drive that contains the ChromLink™™ program file (usually in C:\PenExe\ChromLink™™ but if it is not there, check under the C:\Program Files directory). Click on the sequence file "seed.seq" to highlight it (if this file is missing, reinstall ChromLink™™). Click the "Open" button. A spreadsheet style sequence table will present itself in a window called "Sequence Information - Channel A". There will be a minimized window for channel B data; ignore this window.
 - (b) Choose menu item "File" > "Save." Close the Sequence Editor window by choosing "File" > "Exit" from the menu bar.

3. Data Processing and Analysis

Refer to Figure 2 "Post-Run Data Processing Work Flow Diagram" (page 39) for a summary representation of the important aspects of post-run data processing.

- 1) Open Microsoft Windows® File Explorer and open the current working GC data directory (e.g., C:\GC\Data\- 2) If it is not already open, launch TotalChrom.
- 3) If ChromLink™™ was not run in real-time data collection mode during the run as described in step 6 under Starting the Run (see page 30), then do the following:
 - (a) In the TotalChrom Navigator window, choose menu item "App" > "ChromLink™™." Choose the menu item "Configuration" > "Mass Details" and check the Nominal Name and Mass for mercury. If it is missing or altered then ChromLink™™ needs to be configured (see Configuration of ELAN ChromLink™™ on page 36 for details).
 - (b) Check that the Mode field indicates "Automatic - Process all NetCDF files in specified location". If it does not, click the Set button to the right of this field and in the resulting "Operating Mode" dialog box, click the "Automatic - process all ELAN NetCDF files in specified location" radio button, then click the OK button. Next, check that the Field labeled "ELAN NetCDF file - location/file to be converted" indicates the correct data folder. This should be "C:\elandata\Reportoutput*.nc". If it is not, click the Browse button to the right of it and navigate to that folder. Double-click on that folder then click the "OK" button to close the front most dialog box. Last, click the Browse button to the right of the field labeled "ELAN ChromLink™™ file location..." In the dialog box "Select TotalChrom Data Location", navigate to the folder containing the run data and double-click on it. Click the OK button to close that dialog box. In the ELAN ChromLink™™ window, click the button "Start Processing ELAN Data

Files" to start processing of the run data. A new dialog box will open and provide current information on the status of the data conversion.

- (c) When data conversion by ChromLink™ is completed within a minute or two, a message in the step field will indicate "Successfully Finished". Click the Close button. At this point, you may close the ELAN ChromLink™ application by choosing "File" > "Exit" or clicking on the window "x" box. In the resulting "OK to quit?" confirmation dialog box, click the "OK" button.
- 4) In the TotalChrom Navigator window, choose the menu item "Build" > "Method." Click the "Load method stored on disk" radio button and click "OK." In the TotalChrom File-Open" dialog box, find C:\GC\Methods folder and open the "GC_Hg.mth" file. The template method file should now be loaded.

If instead of loading the method file, an error message appears stating that the file is unavailable because it is in use and asks if you would like to open it in Read-Only mode, click the "No" button. Cancel the Open-File dialog box. Exit the Graphic Method Editor. In the Navigator window, choose menu item "Admin" > "CAM Administrator." A window will appear with two panes. In the left pane, click on the "+" sign in front of "TotalChrom Servers" to expand it. Click on the computer icon on the next line that just appeared to highlight it. In the right pane, under the heading "Resource/Instrument", select the first item. If there is more than one item, select every item by shift-clicking on each item. Every item should now be highlighted. Choose "Edit" > "Remove Locks" (or press the Delete key on the keyboard). Next, click on the "+" sign in front of "Users" to expand it. Click to highlight your TotalChrom user name that appeared. In the right pane, under the heading "Resource/Instrument", select every item and Choose "Edit" > "Remove Locks." This action serves to unlock files and make them available for editing. If in the future, TotalChrom™ gives an error message that files cannot be edited because they are locked, use CAM Administrator to unlock them. Choose "File" > "Exit" to quit CAM Administrator. Start again at the beginning of this step to open the Method Editor.

- 5) Choose "File" > "Save As." At the next window you will be invited to enter information about the method. You may enter pertinent information but this is optional. Click the "OK" button and a TotalChrom File-Save-As" dialog box opens. Navigate the directory tree to get to the folder that contains the ELAN data files for this run (typically in the folder C:\GC\Data\). Double-click on this folder. In the "File name:" field, enter the same name as it exactly appears for the folder that will contain it (i.e. Hg<yyymmdd> convention where yy=2-digit year, mm=2-digit month, dd=2-digit date). Click the "Save" button then close the "Method Editor" window.
- 6) In the TotalChrom Navigator window, choose the menu item "Build" > "Graphic Edit." A TotalChrom File-Open" dialog box appears, but click on cancel to close it. On the Graphic Method Editor's menu bar, choose "File" > "Open" and navigate the file-open dialog box to the folder containing the method file created in the preceding step. Click on that file and click the "Open" button. Return to the Graphic Method Editor's menu bar and choose "File" > "New Data File." Navigate to C:\GC\Data\ and double-click on the folder containing the run data. Find and click on a data file (indicated by the ".raw" extension) that corresponds to the "-LB0972". When this file appears in the File Name field, click the "Open" button. If a message box appears with the warning "Unable to open this file: default.mth", click "OK" to clear the message (you do not have to go to CAM Administration to unlock it). Do the same if another message warning box appears (i.e. click "OK" again to clear it). You should be in the "Graphic Method Editor - <path to method file>" window and see a chromatogram.
- 7) Under the menu item "Calibration" > "Show Windows" there should be a check mark beside "Show Windows." Retention window bars (looks like "H" style error bars) will be

present when it is checked. Each retention time window bar should be located above the chromatographic baseline and contain an identified peak within its bounds. If there are any bars at the bottom of the chromatogram located below the baseline, choose menu item "Calibration" > "Edit Components." Click on the first mercury species peak that falls outside its retention time window to select it. In the group of data fields located on the right side of the window, click on the "Name" dropdown arrow (located on the right side of the data entry field) and choose the appropriate species by name. It is usually not necessary to alter the retention time window's "Absolute" and "Relative" window parameters, but you may do so if experience dictates that a change will be beneficial. Click the "Next" or "Prev" button. Repeat these steps for each mercury species peak that was not properly identified because it was outside its retention time window. When the editing of peak retention time windows is complete, click on the menu bar item "Return". Next, choose "File" > "Save" followed by "File > Exit".

- 8) "In the TotalChrom Navigator window, choose the menu item "Build" > "Sequence" and a dialog box called "Startup" will appear. Click on the radio button labeled "Load sequence stored on disk" then click the "OK" button. The following steps should be used to create the sequence used for reprocessing.
 - (a) Navigate to the folder containing the run data and click on the sequence file (ends with ".seq") corresponding to the run (named "Hgyymmdd.seq" where yy=2-digit year, mm=2-digit month, dd=2-digit date). Click the "Open" button. A spreadsheet style sequence table will present itself in a window called "Sequence Information - Channel A". There will be a minimized window for channel B data, ignore this window. Look for the "Method" column and click on the first cell in row 1 in this column. Right click the mouse and a contextual menu will appear; choose "Browse". In the resulting File-Select dialog box, navigate and choose the method file (ending in ".mth") created earlier. Click on the Select button. The path and name of the new method file will replace the default information in this cell. Right click this cell again and choose Fill Down. The new file name information will fill down to every cell in the "Method" column. Look for the "Rpt Fmt File" column and follow the same process that was followed with the "Method" column. Instead of choosing the method file (ending in ".mth"), choose the report file (ending in ".rpt"). If the report file is not listed, click on the "default.rpt" file. In the bar that contains the file's path, change the "default" to "Hgyymmdd." Right click this cell again and choose Fill Down. The new file name information will fill down to every cell in the "Rpt Fmt File" column.
- 9) In the TotalChrom Navigator window, choose the menu item "Reprocess" > "Batch." A new window appears entitled "Batch Reprocessing". Choose menu item "File" > "Sequence" and another window appears entitled "From Sequence". Locate the top field labeled "Sequence file" and look for a button with an open folder icon immediately to the right of the field. Click this button and navigate, if necessary, to the folder containing the run's sequence files. Click on the sequence file and click the "Open button." Upon return to the previous window, set Start Analysis to "Peak Detection" and End Analysis to "Quantitation". Set Batch Printer to "pdfFactory Pro". Change Batch Execution to "ndlb-168462" or anything other than "Interactive". Check "overwrite existing result files" and select "Update existing raw file header with new sequence". All other parameters should remain unchanged. The parameters are shown in TABLE 10-8.
- 10) Click the "OK" button. Reprocessing of the chromatographic raw data will commence. The bottom panel in the window will update with each file's name as it is processed. When processing is done, this panel will be clear of files. Close this window.

TABLE 10-8: TotalChrom™ Navigator - Reprocess Batch

Parameter Name	Parameter Setting
Starting Row	: 1
Ending Row	: <number of rows in sequence file>
Channel A	: Checked
Channel B	: Not Checked
Start Analysis	: Peak Detection
End Analysis	: Quantitation
Batch Execution	: "ndlab-168462" or equivalent
Batch Printer	: pdfFactoryPro
Batch Plotter	: None
Enable Optional Reports in Method	: Not Checked Grayed Out
Use Method is Result File	
Overwrite Existing Result Files	: Checked
Raw File Treatment	: Update existing raw file header with new sequence

- 11) In the TotalChrom Navigator window, choose the menu item "Reprocess" > "Results." A new window should open called "Reprocess Results". If you get an error message telling you that you can only open this in read-only mode, then unlock the files (follow the procedure described in step 4 of this section). Select from the menu "File" > "Open." In the open file dialog box, click on the "Files of type:" dropdown menu and select "IDX files (*.idx)". Navigate to the folder containing this run's data and click on the newest file (in the format of "Hg<yymmdd>-<today's date>-<time of reprocessing>"). Click "Open." In the Reprocess Results window will be presented a chromatogram for the first sample in the sequence. Carefully inspect the chromatogram one peak at a time for correct peak identification and accurate baseline. If you are satisfied that there are no integration problems, proceed to the next sample's chromatogram by selecting "File" > "Next File" from the menu bar or pressing "F3." Examine all chromatograms in this manner and make corrections in peak identity and integration as necessary. Make notes concerning issues encountered with individual chromatograms and changes that were made. If a chromatogram is changed or edited in any way, be sure to select "File" > "Save" to save your changes. See the chapter entitled "Developing Processing Parameters in the Method" in the PerkinElmer TotalChrom Workstation Users Guide for a detailed explanation on how to use integration events to optimize the integration of a chromatogram. After review of each and every chromatogram, select "File" > "Exit" from the Reprocess Results menu bar.
- 12) Repeat step 9 except set both Start and End Analysis to "Report Generation". Set Batch Printer to "pdfFactory Pro". A new window will open. When reprocessing has completed, click the "Save" button on the pdfFactory Pro window. In the Save As dialog box that appears, navigate to the run's data folder and create a new pdf file named "Hg<yymmdd> report". Be sure to include a space between "report" and the first word of the new file. Click the "Save" button. This pdf file is to be kept and backed up, for archival purposes, in the same folder with all the other chromatographic data files for this run. Click the "Close" button to close pdfFactory Pro window.

- 13) Open Microsoft Excel and choose "GCICPMS" > "Extract TC Data" from the menu. In the open file dialog box, navigate to the folder containing the run's data files. A box titled "Choose a Sequence File" should appear. Navigate to the folder that contains the sequence file that you are working with. Double click on the sequence file. Immediately a macro will run that will extract the data and put it into a format that is easily exported into the database. Just before the macro finishes, a "Save As" dialog box will open giving you the opportunity to save the file as an Excel workbook. Give the file a name as follows: "Hg<yymmdd> results". The multi-tabbed Excel workbook contains a worksheet suitable for data exportation to the Frontends (MS SQL Server 7™) database.
- 14) Calculated "spike" concentrations for enriched InHg, MeHg and EtHg in triple spike standards solution should be entered in the column "ppb Spike" in Microsoft Excel. Then, in Microsoft Excel toolbar choose "GCICPMS" > "Deconvolute" from the menu. Sample concentration values will be calculated and appear in the column "ppb Result".
- 15) The data processing portion on the instrument controller computer is now complete. At this point you may close Microsoft Excel® and TotalChrom Navigator.
- 16) Throughout the sample analysis it is important to ensure that three mercury species peaks (InHg, MeHg, and EtHg) are well resolved. Thus, the following criterion is followed to accept/reject the sample analysis. The Microsoft Excel document containing concentration results displays retention times for each mercury species in the column labeled "RT". Mercury species elute from a chromatographic column in the following order MeHg, EtHg, InHg with relative retention times t_{r1} , t_{r2} , t_{r3} , respectfully. For the purpose of establishing relevant criterion, relative retention time for InHg (t_{r3}) is set to 1. Then the ratio $(t_{r2}/t_{r1}) = 0.91 \pm 0.13$ and $(t_{r3}/t_{r1}) = 0.77 \pm 0.12$ (\pm represents three times standard deviation). If the relative retention time ratios fall outside the allowable error- the samples should be repeated.

O. Recording of Sample and QC Data

1. Transferring the Data to the Branch Database

- 1) Transfer the "Hg<yymmdd> results".xls file via recordable media (e.g., encrypted USB flash drive) to the appropriate subdirectory on the network drive where exported data are stored. (Note that directories are named according to instrument/year/month/ and study name or ID, for example, \\cdc\project\CCEHIP_NCEH_DLS_IRATB_COMMON\Nutritional\Instruments\ELAN_DRCM\2011\04\Hg110423 Results - Study 2006-09").
- 2) From a computer that has access to the Frontends database used for tracking data, log in using your user ID and password.
- 3) Click the "Add Sample Results to Database" button. New buttons will appear. Click the "Import Instrument Data File" button. For "Instrument", choose "ELAN-DRC2M" (or the appropriate instrument). For "Assay", choose "HgSpecGC". Enter the appropriate Analyst and Study for these two fields. It is not necessary to fill-in the "IS Lot Number" Field. Select the location of the data file on the network drive and press the "Open" button.
- 4) In the "Imported Results" table, pressing the "Find X's" button will show only those samples whose sample ID is not recognized as a valid QC pool ID or sample ID for this study. (Sample IDs are set up when the study is logged into the database.) If necessary, corrections to sample IDs and dilution factors can be made in this table (e.g., correction of transcription errors and adjustment for level of dilution). If samples were diluted for analysis, both the sample ID and the dilution factor need to be edited in

this table before the values are transferred to the database. First, change the dilution factor to reflect the way that the sample was analyzed then edit the sample ID to remove any comments about the level of dilution at which the sample was analyzed. (The replace command is useful here).

- 5) When corrections to sample IDs are made, press the "Recheck" button to evaluate the sample IDs. Any sample or analyte row marked "Not Recognized" will not be transferred to the database when the "Transfer" button is pressed.

P. QC Data

Store the results of the QC samples analyzed in each run in the Frontends database when all other data for the run is imported from the ELAN software. Refer to "Recording of Data" described above for how to import data into the Frontends database. The database allows for the printing of several types of QC reports.

R. Final Data Review

Analysis Printouts and Analyst Run Report

If the samples analyzed are part of a study that has an associated study folder, place the analysis printouts in the study folder(s). Store the results of the patient samples analyzed in each run in the Frontends database when all other data for the run is imported from the ELAN software. Refer to "Recording of Data" described above for how to import data into the Frontends database. The database allows for the printing of a run summary report that indicates whether any particular patient-sample results are outside of the normal concentration reference range or whether any measurement failed precision limits.

Plotting QC Results

When the Frontends database is used, QC plots are updated automatically when the data are imported into the database. Monitor these plots regularly for any trends in the bench QC results. If trends are observed, contact the laboratory supervisor.

Supervisor Review

The Frontends database allows the supervisor to review the QC and sample results directly in the database. The data from each analytical run is stored in pdf format on the CDC shared drive under the instrument that the analysis was performed on.

S. Replacement and Periodic Maintenance of Key Components

1. ICP-MS Maintenance

Part numbers listed below are PerkinElmer part numbers from their 2010-2011 Consumables Catalog.

- 1) Cross-Flow II Replacement Liquid and Gas Tip Ferrules (part #09920518 and #09920515, respectively). Keep at least two spares on hand, if feasible.
- 2) Injector Support/Torch Base (part #N812-0116). Keep at least one spare on hand, if feasible.
- 3) Torch O-Ring Kit (packages of four, part #N812-0100). Keep approximately four spare packages on hand, if feasible.

- 4) Quartz torch. At least one spare torch should be on hand (part #N812-2006).
- 5) 0.8-mm i.d. sample injector (part #N8126039). At least one spare injector should be on hand, if feasible.
- 6) RF coil (part #WE02-1816). One spare should be on hand, if feasible.
- 7) Platinum (Pt) Skimmer (part WE027803) and sampler cones (part WE027802). Keep at least two spares of each on hand, if feasible.
- 8) Skimmer and sampler cone O-rings (part #N812-0512 and #N812-0511, respectively). Keep at least 10 spares of each on hand, if feasible.
- 9) Series II replacement Ion lens (part #WE018034). Keep two spares on hand, if feasible.
- 10) Pump oil for the roughing pump (part #N812-2004). Keep two bottles on hand in general lab storage, if feasible.

T. GC Maintenance

- 1) Septa (part # 20654, Supelco, Bellefonte, PA) in the GC injector should be changed weekly.
- 2) Glass inlet (part # 2631405, Supelco) in the GC injector should be checked weekly, if condensation or any particulates are found (often parts of septa) should be replaced.
- 3) GC column (Catalog # N9316076) should be replaced when the quality of chromatographs decreases.
- 4) GC Transfer Line non-coated column (Catalog # N9301356) should be changed when the quality of chromatographs worsens.
- 5) Graphite ferrules (part # 09903700, Perkin Elmer Instruments, Shelton, CT).
- 6) Vu-Union Vespel (part # 20428, Restek).

U. Limits of Detection

The limit of detection (LOD) for mercury species in blood specimens are calculated using data from ≥ 60 separate analytical runs. To determine this method's LOD's, prepared blood samples were used containing known amounts of inorganic, methyl and ethyl mercury at four different concentrations (levels #1–4). Level #1 had concentrations of each mercury species that were close to the anticipated LOD; Level #4 had concentrations $\sim 10X$ greater than those of Level #1. Upon final completion of this study's analytical phase, the LOD for each species was obtained from summary statistics of the pooled results. The standard deviation of pooled results at each level was plotted against its concentration and the points fitted by least squares linear regression. The Y-axis intercept (standard deviation), which is in concentration units, is multiplied by 3. It is this value that is defined as the LOD. For reporting purposes, results below the LOD are reported as "< LOD".

TABLE 14-1: Limits of Detection (LOD)

Species Chemical Name	Abbreviated Name	Limit of Detection, $\mu\text{g/L}$

Inorganic Mercury	BHGI	0.27
Methyl Mercury	BHGM	0.12
Ethyl Mercury	BHGE	0.16

9. REPORTABLE RANGE OF RESULTS

Only analyte results having values within the analytical concentration range may be reported without requiring dilution of the sample. The analytical concentration range for this method is LOD to 30 µg/L. Samples having results greater than the upper limit of the analytical range must be diluted to bring the analyte within the analytical concentration range and reanalyzed using this method. The final result is the diluted sample result arithmetically corrected for the dilution. Any sample that has an analytical result above 10µg/L (i.e. 2UB) for any analyte should be repeated and confirmed. Total mercury concentration values produced by DLS 3001 method will be available for the analyst to predict needed dilution factors prior to the analysis of blood samples by this method (DLS 3020).

10. QUALITY CONTROL PROCEDURES

The Inorganic and Radiation Analytical Toxicology Branch uses the method described in this protocol for environmental and occupational health screening studies. The analyst inserts bench QC specimens two times in each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. Taking these samples through the complete analytical process assesses all levels of the analyte concentrations. The data from these materials are then used to estimate methodological imprecision and to assess the magnitude of any time-associated trends. The bench QC pools used in this method comprise two levels of concentration spanning the "low-normal" and "high-normal" ranges for each mercury species. Both of these pools are analyzed before any patient samples are analyzed to ensure that all systems are functioning properly. These bench QCs should be analyzed again at the end of the run.

A. Establish QC limits for each QC pool.

A run to assess the homogeneity of the pools is performed after the pools are aliquotted into individual vials. Vials are randomly chosen and randomly analyzed, and the first and last vials dispensed are always included in the homogeneity study. Unlike the characterization of the QC, the homogeneity study can be completed in a single run. Once analysis is complete, the data is evaluated in terms of QC recovery to determine whether or not trends exist in QC during the dispensing of the pool. If the pool does not vary from beginning to end or problem vials can be identified and eliminated, the characterization of the QC is the next step. If problems do exist, the source(s) of the problem has to be identified and the pool has to be re-made and dispensed again.

To complete the characterization that will allow you to assess limits for each pool, analyze a minimum of twenty samples of each pool (low and high) on 20 different days, preferably among all of the instruments that will be used to analyze this method. During the 20 characterization runs, analyze samples from previously characterized QCs or pools with target values assigned by outside laboratories to evaluate each run's QC. Once analysis is complete, calculate the mean and standard deviation for each pool from the concentration results. These values will be used to establish the limits for each pool.

B. Precision and Accuracy

QC Results Evaluation. After completing a run, consult the QC limits to determine whether the run is "in control." The QC rules apply to the average of the beginning and ending analyses of each of the bench QC pools. The QC rules are as follows:

- 1) If both the low-and the high-QC results are within the 2s limits, accept the run.
- 2) If one of two QC results is outside the 2s limits, apply the rules below and reject the run if any condition is met.

1_{3s} - Average of both low QCs OR average of both high QCs is outside of a 3s limit.

2_{2s} - Average of both low QCs AND average of both high QCs is outside of 2s limit on the same side of the mean.

R_{4s} sequential - Average of both low QCs AND average of both high QCs is outside of 2s limit on opposite sides of the mean.

10_x sequential - The previous nine average QCs results (for the previous nine runs) were on the same side of the mean for either the low OR high QC.

If the run is declared "out of control," the analysis results for all patient samples analyzed during that run are invalid for reporting.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If an analyte result for a QC material falls outside of the limits for mean or range, the following steps should be taken, if possible:

- Check the chromatograms for each blank, QC, and sample for proper peak integration and identification. Change integration parameters or manually reintegrate peaks, if necessary, and reprocess the run in TotalChom™.
- Setup a new run for the reanalysis of the patient samples affected by the previous failed run. Be sure to use freshly prepared QC material.

If these steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions. No analytical results should be reported for runs that are not in statistical control.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

There are no interfering substances or conditions for this assay.

13. REFERENCE RANGES (NORMAL VALUES)

The reference range for each mercury species (see TABLE 18-1) is based on literature reports and from periodic review of accumulated data collected during the analysis of blood samples representing a normal, healthy population believed to be free of unusual exposure to mercury. Where data is absent or scant, reference ranges are based on the scientific literature, if available.

TABLE 18-1: Reference ranges for Mercury Species

Species Chemical Name	Reference Range¹, µg/L
Inorganic Hg	
Methyl Hg	< 10 µg/L (within the established linearity for the method)
Ethyl Hg ²	

¹ Above ranges are estimates based on data from the Fourth National Report on Exposure to Environmental Chemicals.

² There are no established reference ranges for EtHg.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

The analyst should report any patient results confirmed to be greater than the second upper boundary (defined in the laboratory database as the "2UB" and currently 10 µg/L) to the QC reviewer as an "elevated result". The protocol for supervisors reporting elevated results to medical personnel is defined according to the study protocol. Levels of concern for mercury in blood are >100 µg/L for children (6 years and younger) and >200 µg/L for adults. These values are based on total Hg results.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may reach and maintain ambient temperature during analysis. Take stringent precautions to avoid external contamination. After the samples are analyzed, return them to ≤ -20°C freezer storage as soon as possible.

16. ALTERNATE METHODS FOR PERFORMING TEST AND STORING SPECIMENS IF TEST SYSTEM FAILS

If the analytical system fails, freezer storage (≤ -20°C) is recommended until functionality is restored.

17. TEST-RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

For critical calls, the supervisor should notify the supervising physician or principal investigator as soon as possible. The most expeditious means should be used (e.g., telephone or E-mail).

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

The analyst who receives specimens or samples delivered to Inorganic and Radiation Analytical Toxicology Branch sets up a "Specimen Folder." Fill out a tracking form and place it in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens.

Use standard electronic record keeping means (e.g., Microsoft Access™, optical disk, or tape backup) to track specimens. Maintain records, including related quality assurance (QA) and QC data, for 3 years or longer. Keep duplicate records (off site, if sensitive or critical) in electronic or hardcopy format. Use only numerical identifiers (e.g., case ID numbers); all personal identifiers are available only to the medical supervisor or project coordinator to safeguard confidentiality.

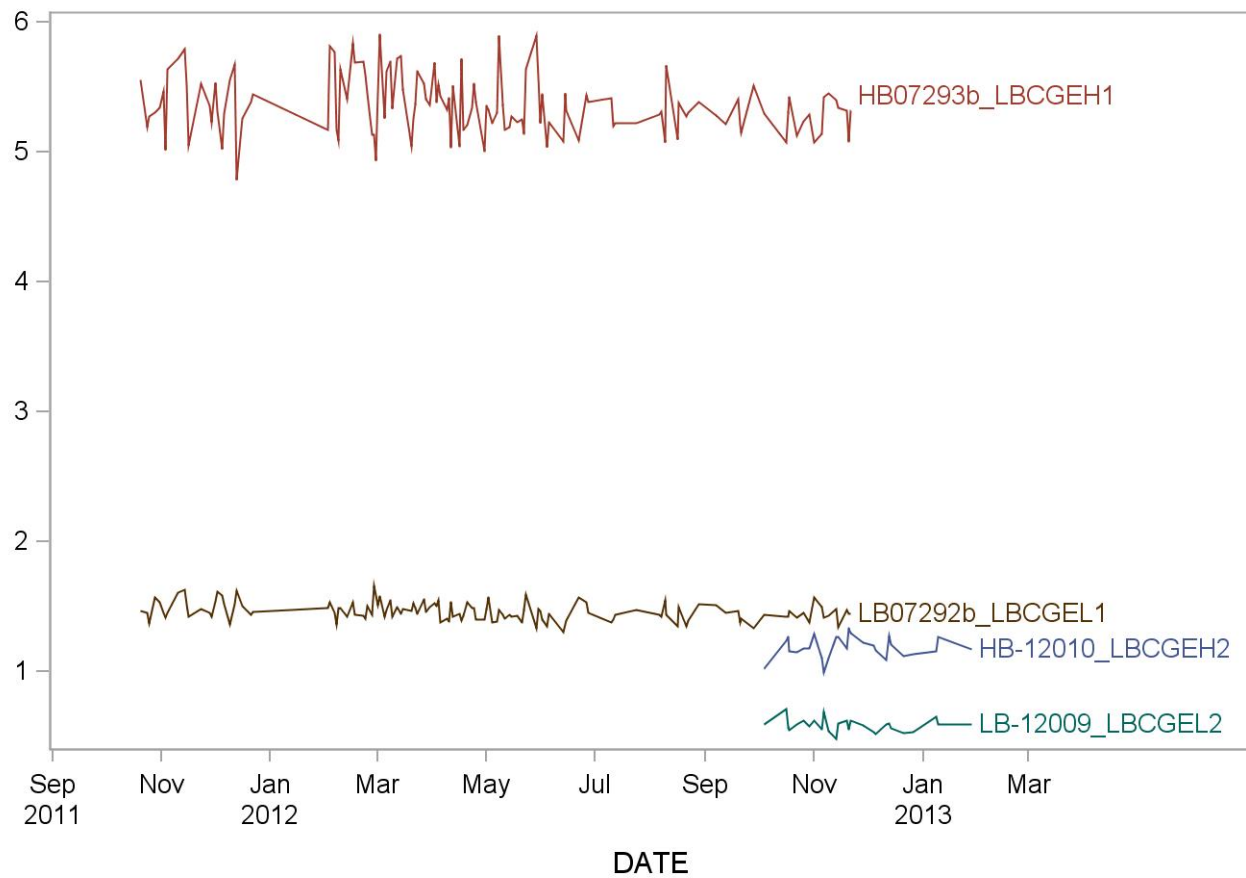
19. SUMMARY STATISTICS AND QC GRAPHS

See following pages.

Summary Statistics for Ethyl mercury, blood (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB07293b_LBCGEH1	168	20OCT11	21NOV12	5.3584	0.2389	4.5
LB07292b_LBCGEL1	168	20OCT11	21NOV12	1.4610	0.0730	5.0
HB-12010_LBCGEH2	34	04OCT12	28JAN13	1.1882	0.1145	9.6
LB-12009_LBCGEL2	34	04OCT12	28JAN13	0.5832	0.0545	9.3

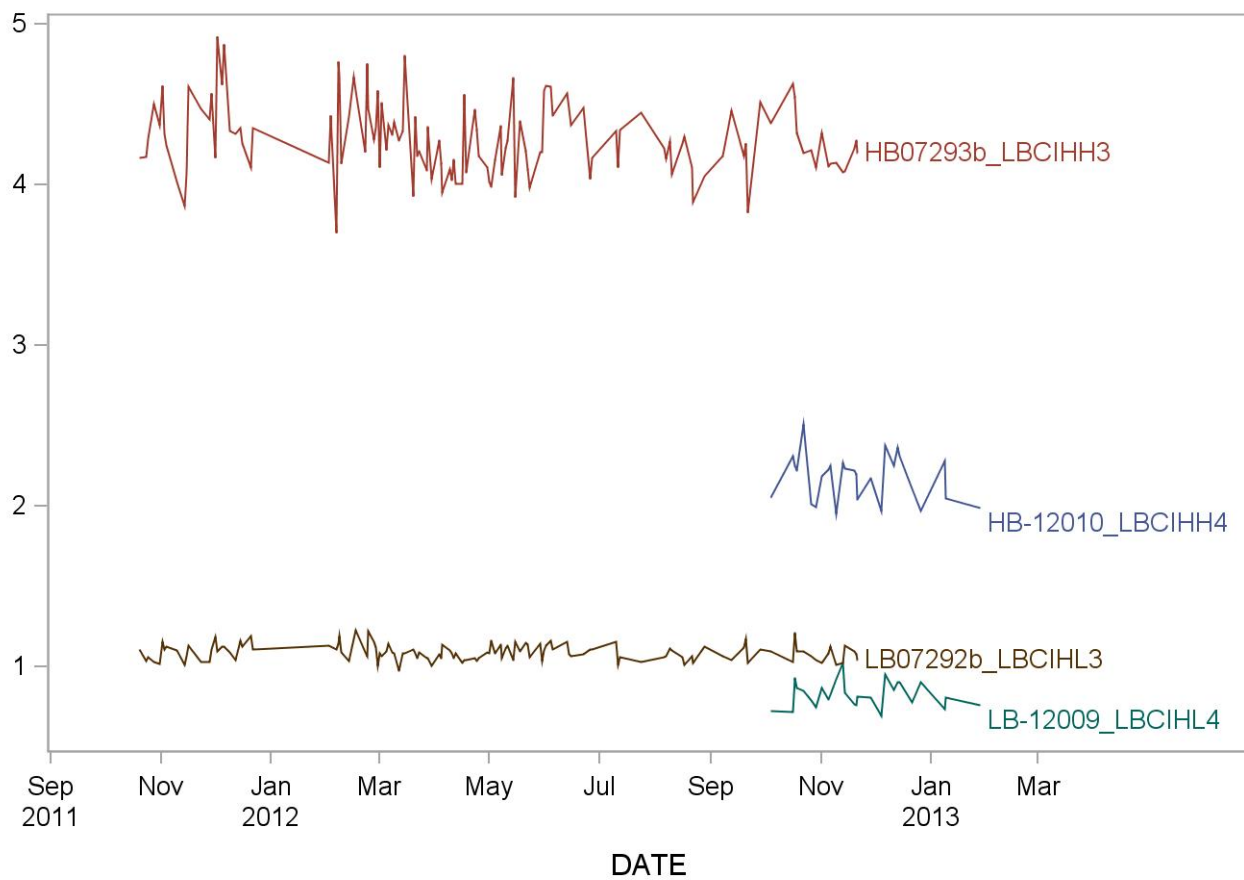
2011-2012 Ethyl mercury, blood (ug/L) Quality Control



Summary Statistics for Inorganic mercury, blood (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB07293b_LBCIHH3	168	20OCT11	21NOV12	4.283	0.255	6.0
LB07292b_LBCIHL3	168	20OCT11	21NOV12	1.090	0.053	4.9
HB-12010_LBCIHH4	34	04OCT12	28JAN13	2.176	0.173	7.9
LB-12009_LBCIHL4	34	04OCT12	28JAN13	0.826	0.086	10.4

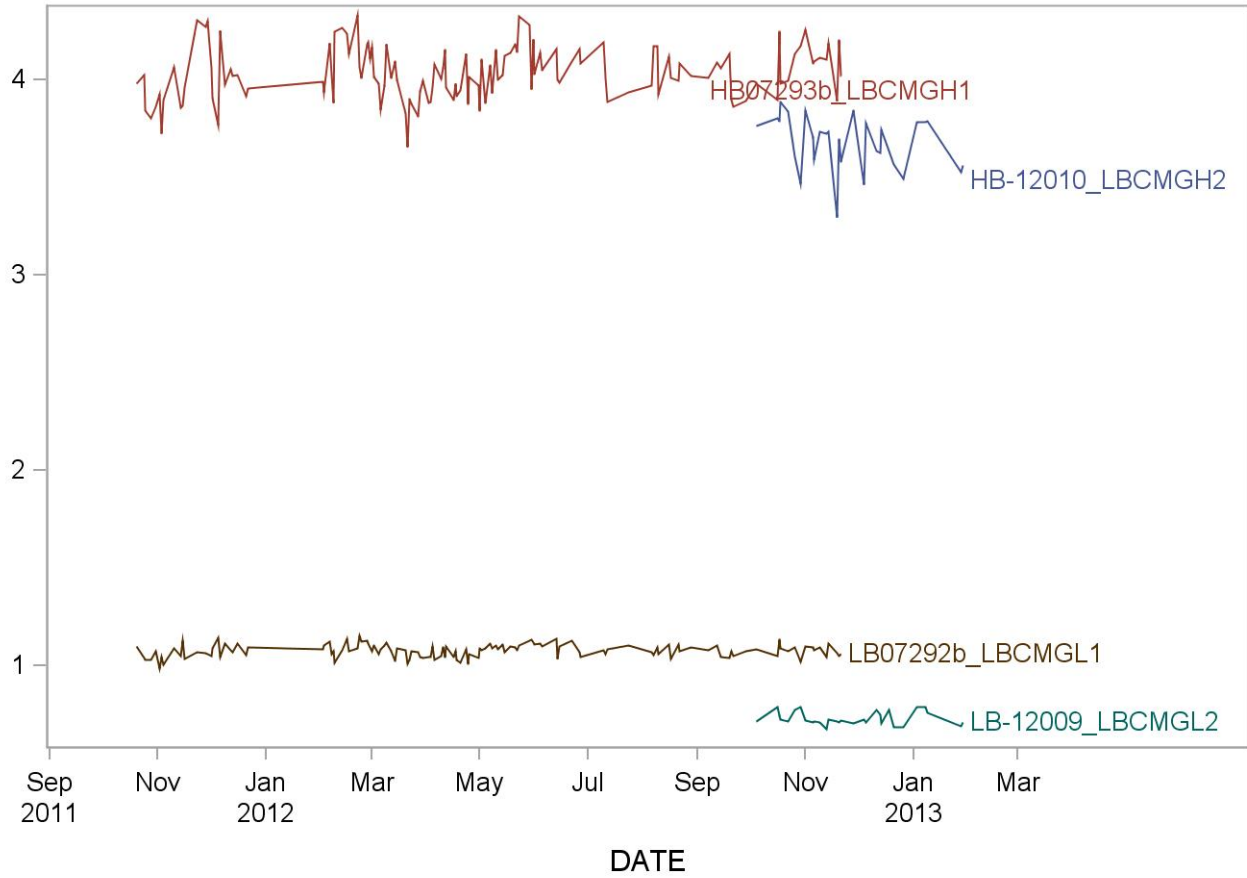
2011-2012 Inorganic mercury, blood (ug/L) Quality Control



Summary Statistics for Methyl mercury, blood (ug/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
HB07293b_LBCMGH1	170	20OCT11	21NOV12	4.0276	0.1483	3.7
LB07292b_LBCMGL1	170	20OCT11	21NOV12	1.0772	0.0374	3.5
HB-12010_LBCMGH2	37	04OCT12	29JAN13	3.6905	0.1533	4.2
LB-12009_LBCMGL2	37	04OCT12	29JAN13	0.7285	0.0360	4.9

2011-2012 Methyl mercury, blood (ug/L) Quality Control



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