SOP #BR-0011

Determination of Methyl Mercury by Aqueous Phase Ethylation, Trap Pre-Collection, Isothermal GC Separation, and CVAFS Detection: BRL Procedure for EPA Method 1630 (Aqueous Samples) and EPA Method 1630, Modified (Solid Samples)

Brooks Rand Labs

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Scientist (if applicable)	06.06.11 Date

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Determination of Monomethylmercury by Aqueous Phase Ethylation, Pre-Concentration, Isothermal GC Separation, and CVAFS Detection: BRL Procedure for EPA Method 1630 (Waters) and EPA Method 1630, Modified (Solids)

1.0 Scope and Applications

1.1 Method BR-0011 is the performance based procedure followed at Brooks Rand Labs (BRL) as EPA Draft Method 1630. Unless specifically stated otherwise in this document, all apparatus, materials, reagents, standards and procedures as stated in EPA Method 1630 are used at BRL.

1.2 EPA Draft Method 1630 is for the determination of methyl mercury only in filtered and unfiltered aqueous samples. BRL Method BR-0011 is additionally used for the determination of methyl mercury in sediment, biota, and biomonitoring samples. BRL has developed specific sample preparation methods for these matrices. With the exception of the maximum volumes analyzed, the procedures followed for the analysis of sediment and biota preparations are identical to the procedures followed for aqueous preparations.

1.3 EPA Draft Method 1630 describes manual analysis of samples for monomethylmercury (MeHg) determination. Since its publication, Brooks Rand Labs has developed MERX, an automated system for the determination of MeHg. The analytical method in this SOP will refer to use of MERX unless otherwise stated. The manual methodology is retained as an addendum.

2.0 Summary of Method

2.1 Prior to instrumental analysis, all samples are prepared in a way to reduce matrix interference and preserve the MeHg that is present.

2.1.1 Aqueous samples are prepared by distillation at 138 °C under N_2 according to the procedure discussed in EPA Draft Method 1630, section 11.

2.1.1.1 An alternative to distillation, direct ethylation, has been developed and validated. For direct ethylation, aqueous samples are preserved as normal then analyzed as specified in EPA Draft Method 1630 after being buffered with citrate or acetate buffer to achieve the correct pH.

2.1.2 Sediment samples are prepared by dichloromethane (DCM) extraction.

2.1.3 Biota samples are prepared by alkaline digestion in 25% KOH in methanol and oven digestion at 65 °C for 3-4 hours, which can then be followed with distillation if a lower detection limit is required.

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2.2 MeHg is determined by an improved method (Liang, Bloom, and Horvat 1994). The prepared sample is buffered to pH 4.5-5 using acetate or citrate buffer, the MeHg is then ethylated with sodium tetraethylborate ($NaBEt_4$, the ethylation reagent). The ethylation reagent adds an ethyl group to the methyl mercury compound (CH_3Hg^+ $^-C_2H_5$) making volatile methylethyl mercury, it also adds two ethyl groups to the Hg^{2+} making volatile diethyl mercury. The volatile mercury species are liberated from the liquid and collected by purging with dry, Hg free nitrogen into a pyrex tube filled with Tenax™. The ethyl mercury derivatives are then thermally desorbed with Hg free argon and transferred to a GC column held in an oven at 36 °C, which separates the species chromatographically by mass. The ethylated Hg compounds are pyrolized at 900°C to Hg (0), then quantified by a cold vapor atomic fluorescence spectrophotometer (CVAFS). This method can by applied for the determination of MeHg in a variety of sample matrices and has been demonstrated as being very sensitive, precise, and accurate. Good agreement was obtained for the determination of MeHg in standards, CRMs and real samples in comparison to the manual system used at Brooks Rand Labs and less variability was seen in the automated system.

3.0 Definitions

3.1 A partial list of definitions is presented below. Most definitions can be found for specific terms in the sections where they are first mentioned. Many of the definitions mentioned in this method are based on those found in the glossary for EPA Method 1630. Please refer directly to this method for a more detailed list.

3.1.1 May: This action is allowed but not required.

3.1.2 May Not: This action is prohibited.

3.1.3 Must: This action is required.

3.1.4 Shall: This action is required.

3.1.5 Should: This action is suggested, but is not required.

4.0 Interferences

4.1 If properly applied, the distillation procedure will remove most if not all of the significant interferences. However, the concentration of hydrochloric acid (HCl) in the solution will affect the distillation of methyl mercury from the solution. Too little HCl will cause the distillation of methyl mercury to not be quantitative while too much HCl will cause the co-distillation of HCl fumes, which according to EPA Method 1630 can interfere with the ethylation process. This interference was investigated at BRL, see section 9.5.1.1 for more details.

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4.1.1 It has been found that there are few interferences in most aqueous samples and the direct ethylation technique can be used.

4.1.2 EPA Method 1630 dictates that fresh water samples must be preserved with between 0.3% to 0.5% (v/v) 11.6 M HCl (BRL preserves fresh water samples with 0.4% (v/v) 11.6 M HCl) and that salt water samples must be preserved with between 0.1% to 0.2% (v/v) 18 M sulfuric acid (H_2SO_4) (BRL preserves salt water samples with 0.2% (v/v) 18 M H_2SO_4).

4.2 Samples must not be preserved with nitric acid as it may cause partial decomposition of the analyte during distillation.

4.3 Positive artifact is possible with the distillation of samples that are high in inorganic mercury. Ambient organic matter may methylate 0.01% to 0.05% of the ambient inorganic mercury during distillation. In inorganic mercury contaminated waters this can significantly affect the results for methyl mercury. Solvent extraction may be preferable to distillation in samples that are high in divalent mercury (Hg(II)).

4.4 Residual DCM in sediment extractions has been shown to interfere with the recovery of methyl mercury. All DCM must be evaporated from the final back extraction to ensure that suppression does not occur. Strict adherence to the instructions found in Section 9.4.1 of this SOP should ensure that all DCM is removed from sample preparations.

4.5 Presence of the aqueous phase of the sediment prep after phase separation to isolate the organic phase can also cause interference. Strict adherence to the instructions found in Section 9.4.1 of this SOP should ensure that all DCM is removed from sample preparations.

4.6 Care must be taken when making the 1% sodium tetraethylborate solution as wax from the outside of the vial can cause reduced efficiency of the ethylation reagent. If wax gets in the solution, the solution must be discarded and remade.

4.7 Refer to EPA Method 1630, Section 4.0, for a detailed account of possible contamination routes, interferences to the analysis, and how these are avoided or minimized at BRL.

5.0 Safety

5.1 Refer to EPA Method 1630, section 5.0, for safety issues associated with the use of this method.

5.2 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability, and nervousness. Organo-mercurials may cause permanent brain damage. Because of this, only highly trained personnel familiar

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with the dangers and precautions to take when working with mercury compounds should ever handle standards and/or high level samples.

5.3 Material safety data sheets (MSDSs) are maintained for all chemicals used in this method. Scanned MSDS sheets are stored on the server and can be located by either chemical name or by CAS number.

5.4 Refer to the latest revision of the Chemical Hygiene Plan (CHP) for additional safety precautions and required protective equipment.

6.0 Apparatus and Materials Used at BRL

6.1 Refer to EPA Method 1630, Section 6.0 for a list of materials used in the method employed at BRL.

6.2 Routine preventative maintenance for the equipment used in this procedure is described in detail in the standard operating procedure BR-1205 (Preventative Maintenance).

6.3 Detailed instructions for the decontamination of bottles and other equipment are described in BRL SOPs BR-0400 and BR-0404.

6.4 Specific equipment used at BRL is listed below. Any modifications to EPA Method 1630 are described and explained.

6.4.1 Autosampler: An AI Scientific septum piercing autosampler with 168 slots is used at BRL. Other autosamplers may be used if they are compatible with the Guru software, have septum piercing capabilities, and can hold the 40 mL glass vials.

6.4.1.1 Reaction vessels: A 40 mL flat bottomed amber glass vial with a Teflon lined septum cap is used as the reaction vessel. Vials similar to I-Chem SB46-0040 or EP Scientific 341-40A are used.

6.4.2 MERX Methylmercury Autoanalyzer: The MERX methylmercury autoanalyzer is designed and manufactured by BRL. For a diagram, see Figure 1.

6.4.2.1 The Purge and Trap Module:

6.4.2.1.1 A purge vessel, a 50 mL glass tube with a port coming out of the top and another port coming off the base is used as the purge vessel. Mercury free nitrogen is purged into the reaction vessel forcing the sample into purge vessel from the port at the bottom. The nitrogen continues to pass through both the reaction vessel and the purge vessel during the entire purge time. As the sample is purged, the volatile mercury species are swept from the

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sample onto the preconcetration trap via the port at the top of the purge vessel.

6.4.2.1.2 The preconcentration trap is a Tenax trap measuring 10 cm long, 6.4 mm outside diameter x 4.0 mm inside diameter that is kinked at both ends and used for the collection of purged organomercury species.

6.4.2.2 The GC and Pyro Module: Isothermal gas chromatography system consisting of a GC column, GC oven, and pyrolitic column. For a diagram of the system see Figure 2.

6.4.2.2.1 Gas Chromatography (GC) Column: A GC column approximately 10 inches long with an ID of 1/8 inch packed with OV-3 and held at a constant 36 ºC temperature is used. Under a flow of high purity argon, organomercury species desorbed from the preconcetraion trap are carried by argon gas through the GC column where they are separated by mass.

6.4.2.2.2 Pyrolitic Column: The pyrolitic column is a quartz tube that is heated to 900 ºC. After GC separation, species pass through the pyrolitic column where they are thermally decomposed to elemental mercury before going through the detector.

6.4.3 Atomic fluorescence spectrophotometer (BRL part #AF-03): CVAFS systems are built by BRL (BRL Model III). Refer to the "Brooks Rand, LLC Model III Operations Manual" for instrument operating instructions.

6.4.4 Recorder: BRL uses direct data acquisition with the BRL Guru™ integration software instead of a chart recorder or integrator as described in EPA Method 1631E, section 6.6. Refer to the "Brooks Rand LLC Model III Operations Manual" for Guru™ software/integrator operating instructions and system requirements. Use of this integration software is faster, eliminates the expense of chart recorders and/or integrators, allows for storage of data in electronic form, and eliminates possible transcription errors.

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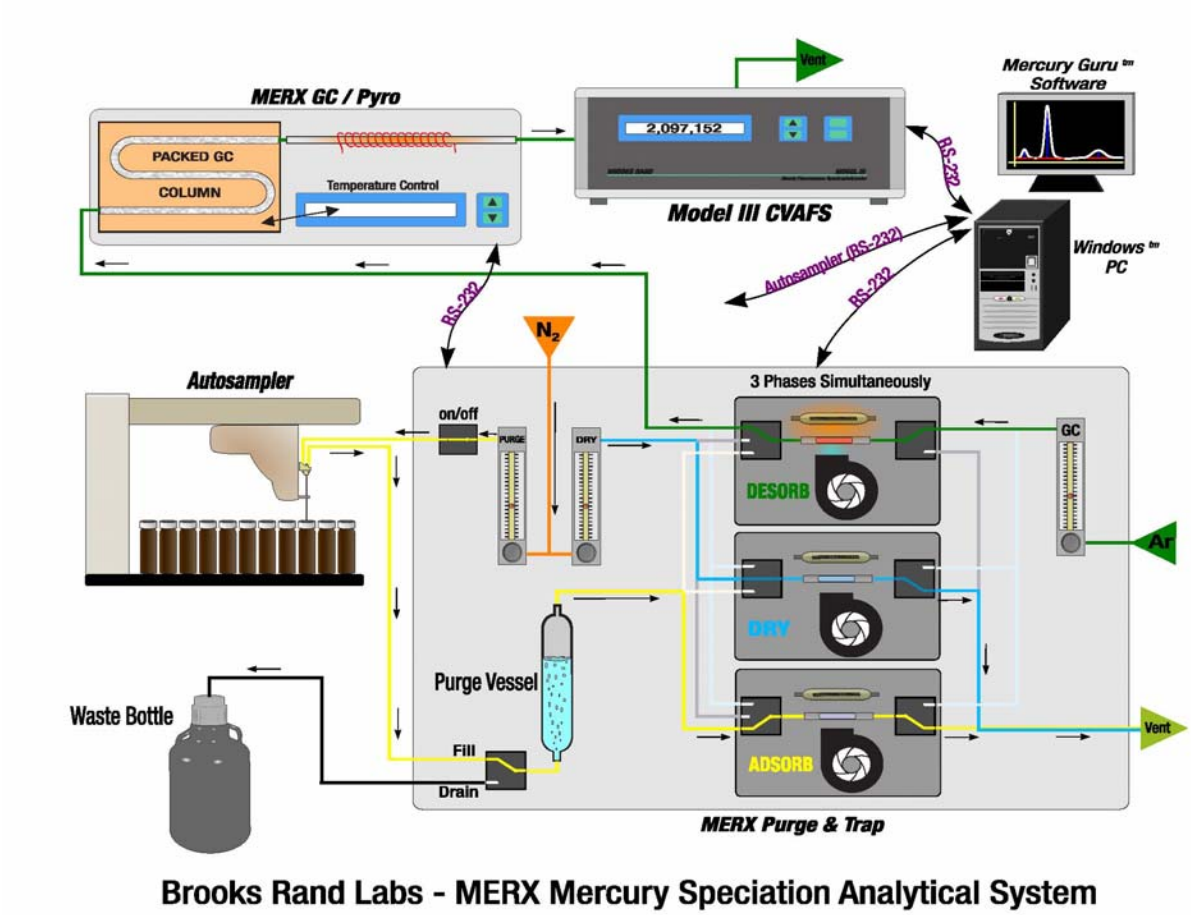

Figure 1. Diagram of sample flow path and connections for MERX MeHg analytical system.

7.0 Standards and Reagents

7.1 Refer to EPA Method 1630, Section 7.0 for a list of standards and reagents employed at BRL.

7.2 Water: Deionized water (DIW) is monitored on a daily basis during calibration of the instrument as part of the calibration blanks and the recoveries of the calibration standards.

7.3 MeHg Standard Solutions: Since MeHg is susceptible to photo oxidation, all standards should be stored in dark areas and minimally exposed to direct light during the working day.

7.3.1 Standard stock solution: 1 mg/mL MeHg is purchased from a known, accredited vendor. This standard is used to prepare the calibration standards. It is verified by in-house testing before being put into use and annually there-after.

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7.3.2 Intermediate stock solution: 1 µg/mL MeHg. Dilute 0.10 mL of 1 mg/mL stock solution, 0.2 mL of HCl, and 0.5 mL of HOAc with DIW to a final volume of 100 mL. This solution should be kept refrigerated and expires after one year.

7.3.3 Working standard: 10 ng/mL MeHg. Dilute 1.00 mL of 1 µg/mL intermediate stock solution, 0.2 mL of HCl, and 0.5 mL of HOAc with DIW to a final volume of 100 mL. This solution may be kept at room temperature and expires after one month.

7.3.4 Working standard: 1 ng/mL MeHg. Dilute 0.10 mL of 1 µg/mL intermediate stock solution, 0.2 mL of HCl, and 0.5 mL of HOAc with DIW to a final volume of 100 mL. This solution may be kept at room temperature and expires after one month.

7.3.5 Daily standard: 0.01 ng/mL MeHg. Dilute 0.05 mL of 1ng/mL working standard to 5 mL with DIW. This solution expires at the end of the day.

7.3.6 Independent calibration verification (ICV) solution: The ICV solution is made up monthly from an aliquot of the certified reference material NIST 1946 (fish tissue). Approximately 100 mg of NIST 1946 is accurately weighed into a 40 mL pre-tested glass vial. The reference material is digested with 10 mL of 25% KOH methanol solution at an oven temperature of 65 ºC for 3-4 hours (refer to section 9.3.1 for the alkaline digestion of biological materials). The digestate is then diluted to 40 mL with methanol. The final concentration of methyl mercury in this solution will be about 1 ng/mL. If NIST 1946 is no longer available, then a similar CRM certified for MeHg will be used in its place.

7.4 n-propylmercury (n-prHg) standard solutions

7.4.1 Standard stock solution: A 10,000 ng/mL n-propyl mercury standard is purchased from a known, accredited vendor.

7.4.2 Working standard: 100 ng/mL n-prHg. Dilute 1.00 mL of 10,000 ng/mL stock solution, 0.2 mL of HCl, and 0.5 mL of HOAc with DIW to a final volume of 100 mL. This solution may be kept at room temperature and expires after one month.

7.4.3 Daily standard: 1.0 ng/mL n-prHg. Dilute 0.05 mL of 100 ng/mL working standard to 5 mL with DIW. This solution expires at the end of the day.

7.5 Sodium tetraethylborate ($NaBEt_4$) solution: Dissolve 1.0 g of $NaBEt_4$ (stored in freezer) in 100 mL of 2.0% potassium hydroxide (KOH) solution that has been chilled to 0 ºC to produce a 1.0% working solution. This solution is aliquoted into individual 5.0 mL fluoropolymer vials. This reagent is stored at < -10 ºC and is thawed prior to use. The solution expires three hours after being thawed. A new batch of the ethylating reagent should be made as soon as there are any doubts about its quality (i.e. low recovery of

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matrix spikes). $NaBEt_4$ solids and solutions must not be used if they show any discoloration. The frozen $NaBEt_4$ solution has proven to be stable for at least one month if not thawed.

7.6 Sodium acetate buffer: A 2M acetate buffer is prepared by dissolving 136 g of reagent grade sodium acetate and 59 mL of glacial acetic acid in DIW to a final volume of 500 mL. 500 µL of 1% $NaBEt_4$ is added to this solution and it is then purged with N_2 gas overnight. The solution is stored in a fluoropolymer bottle and is tested for residual methyl mercury prior to use by analyzing the daily calibration blanks and standards.

7.7 Citrate buffer: Dissolve 100 g citric acid and 300 g sodium citrate in DIW. Bring to a final volume of 1 L with DIW.

7.8 Methanolic potassium hydroxide solution: Dissolve 250 g of reagent grade KOH pellets in high purity methanol to a final volume of 1 L. The solution is stored in a fluoropolymer bottle.

7.9 Gases: All gasses are passed through a gold-coated sand trap to remove traces of mercury prior to use.

7.9.1 Argon used as the carrier gas from trap desoption, through the GC and pyrolitic column to the detector and is from cryogenic bleed off.

7.9.2 Nitrogen used as a purge gas for sweeping derivatives from a bubbler is plumbed from cryogenic bleed-off.

7.10 20 % potassium chloride (KCl) / 0.2% L-Cysteine solution: Dissolve 10.0 g KCl and 0.1 g L-Cysteine in 50 mL of DIW. This solution must be discarded and replaced every 6 months or if crystals begin to form.

7.11 11.6 M hydrochloric acid (HCl): Used for the preservation of freshwater samples.

7.12 18 M sulfuric acid (H_2SO_4): Concentrated form used to preserve saltwater samples. This acid is also used to prepare 9 M H_2SO_4.

7.12.1 9M H_2SO_4: Mix equal parts DIW and pre-tested, concentrated H_2SO_4. Introduce the reagents slowly as this procedure generates a great deal of heat. Allow complete cooling before capping tightly. This solution can be stored indefinitely.

7.13 Potassium bromide/sulfuric acid solution (KBr/H_2SO_4): 18% (w/v) KBr + 5% (v/v) H_2SO_4. Dissolve 18.0 g of KBr into 100 mL of 5% H_2SO_4. This solution is stable for up to one year.

7.14 1 M copper sulfate solution ($CuSO_4$): Dissolve 24.97 g of copper (II) sulfate pentahydrate into 100 mL DIW. This solution is stable for up to one year.

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7.15 DCM (methylene chloride): DCM (HPLC Grade) may be purchased from an authorized vendor.

8.0 Sample Collection, Preservation, and Handling

8.1 Refer to EPA Method 1630, Section 8.0 and EPA Method 1669 (*Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels*) for a detailed description of sample collection, preservation, and storage methods.

8.2 Aqueous samples may be collected in fluoropolymer, FLPE, or glass containers with fluoropolymer lined lids. Aqueous samples should be maintained at 0 – 4 ºC from the time of collection until preservation. Aqueous samples must be preserved within 2 days of collection. Freshwater samples are preserved with 4 mL/L of pre-tested concentrated HCl. Saline samples (salinity > 10 ppt) and urine samples are preserved with 2 mL/L of pre-tested 18 M H_2SO_4. Preserved samples are stable for up to 6 months if kept in the dark and cool. BRL defines "cool" as ≤ 12 °C. As long as a sample is field preserved and received by BRL ≤ 12 °C, then no qualification of the data is necessary. Once received by BRL, the policy is to store all aqueous MeHg samples from 0 – 4 °C.

8.3 Biota and sediment samples may be collected in glass or HDPE containers. Solid samples must be maintained at 0 – 4 °C from the time of collection until receipt by BRL. Upon receipt, solid samples are moved to freezers for storage. Solid samples may be held for at least 1 year if kept in the dark at < -15 °C.

NOTE: Due to uncertainty inherent in accurately measuring sample temperatures, it is understood that the measured temperature may be as much as ±2 °C from the actual temperature. Therefore, as long as water samples remain unfrozen, ±2 °C are allowed beyond the above ranges without requiring qualification of the data.

9.0 Sample Preparation

9.1 Refer to EPA Method 1630, Section 11.0 for a detailed description of the preparation of samples. Depending on the purposes and definitions of investigations of mercury biogeochemistry cycling, samples are prepared in the following methods prior to analysis.

9.2 Preparation of aqueous samples for MeHg analysis: Two isolation methods, distillation and solvent extraction, have been used at BRL for the determination of MeHg in aqueous samples. Good agreement was obtained in the comparison of the two methods for most water samples studied: For organic rich and/or high level sulfide containing samples, the distillation showed some advantages over the solvent extraction method with higher recoveries (85 ± 4%, Horvat, Bloom, and Liang, 1993). In addition, extraction consumes large quantities of organic solvent, which can result in environmental contamination. Therefore, if preparation of aqueous samples in needed, distillation is the

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preferred preparation method for aqueous samples at BRL. Direct ethylation has shown recoveries equivalent to distillation and may also be used.

9.2.1 Distillation of aqueous samples:

Reagents: 20% KCl in 0.2% L-Cysteine, 9 M H_2SO_4

Distillation devices: Vials and caps for distillation and distillate collection are made of fluoropolymer obtained by Savillex Corporation, USA. Caps have 1/8" ports for friction fit 1/8" fluoropolymer tubing.

Distillation procedures: An aliquot of water sample, typically 50 ml ± 1 mL, is transferred into a 60 mL fluoropolymer vial (for high MeHg concentration samples, smaller sample sizes should be used, but bring the final volumes to a known volume near 50 mL with 0.4% HCl solution). A maximum volume of 10 mL should be used for distilling urine samples, and they should then be diluted to 50 mL with unpreserved DIW. Urine samples can be batched with other water samples but at least one urine sample should have QC performed.

All blanks, including blank spikes (BS), should be prepared by weighing out 50 mL of a 0.4% HCl solution then spiked if appropriate. Add 0.2 mL of the 20% KCl/0.2% L-Cysteine solution and 0.5 mL of 9 M H_2SO_4 (9 M H_2SO_4 is not added to samples preserved with H_2SO_4). Start the distillation immediately after addition of reagents at a nitrogen flow rate of approximately 60-70 $mL \cdot min^{-1}$ (Note: all rotometers must be within ± 3 $mL \cdot min^{-1}$ of one another) and at a heating block temperature of 138 °C.

The distillate is collected in a 60 mL fluoropolymer vial containing 15 mL of chilled DIW, which must be cooled in an ice-water bath prior to the beginning of the distillation. The distillation is finished when the final distillate volume is 40 mL (total of 55 mL in each receiving vial), as measured against a reference vial. This typically takes from approximately 3 hours. Bring the final volume of the receiving vial to the 58 mL mark with DIW. Depending on its MeHg concentration, transfer an aliquot of the distillate into the methylation reaction vessel for analysis as described in section 10.3.

NOTE: Open the flow of nitrogen for up to one hour before beginning the distillation to purge the lines of room air and other possible contaminants.

Special considerations to take when distilling samples:

- *Turn on the nitrogen gas and begin heating up the distillation blocks.*
- *Check <u>all</u> of the rotometers to ensure that they are set at 50. If any are off, then continue to check <u>all</u> of them to ensure that they are correctly set.*
- *Rinse vials, caps, and tubing thoroughly with deionized water.*

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- *Be especially careful to not touch the rinsed tubing nor let any potentially contaminated object touch the tubing that is to be inserted into the receiving vial.*
- *Use Teflon® tape to secure any tubing that is not tight fitting.*
- *Once cap sets have been secured to the N_2 lines, they must be placed on and completely covered by clean bench liners.*
- *When measuring out the sample aliquot, it is acceptable to weigh out 50 mL (± 1 mL).*
- *Measure out the 15 mL of DIW for the receiving vial by pouring into a pre-cleaned 15-mL centrifuge tube.*
- *Chill the water in the receiving vial by placing them in the chill blocks for a short amount of time while adding reagents to the sample vials. The receiving vial water should not freeze. To this end, if the ice blocks have not been used for > 3 days, they should be allowed to sit out on the counter for ½ hour prior to use.*
- *Do not add reagents to more than 10 samples at a time.*
- *Leave all sample vials in the heating block until the entire block is done. Place the tubing from the receiving vials into the ice water while waiting for the completion of all distillations in the block.*
- *Only use deionized water when making ice to chill the receiving blocks. Always rinse the chill blocks and their containers with DI water before filling the container to freeze.*

Besides the typical distillation of aqueous samples, BRL also offers "ultra low-level" distillation. To achieve the lower detection limits associated with the ultra low-level method, the vials and tubing used in the distillation must be rigorously cleaned with BrCl followed by heating in the oven with 0.8% $NH_2OH{\cdot}HCl$ and triple rinsing. Refer to the bottle washing SOP (BR-0400) for a full description of the cleaning procedures for equipment used in this method.

9.2.2 For direct ethylation, samples are preserved within 48 hours of collection as normal. There is no further sample preparation necessary. N-prHg is used as a surrogate to monitor the efficiency of the method.

9.3 Preparation of biological materials for MeHg.

9.3.1 Alkaline digestion of biota samples: Weigh about 0.1 gram of biological material (wet, homogenous) into a 2.5 mL fluoropolymer vial. Add 1.0 mL of 25% KOH methanol solution and cap the vial tightly. Digest the sample in an oven at 65 °C for 3-4 hours. At some point during the digestion (30 minutes to 1.5 hours into the digestion), shake the samples to re-suspend the material. After digestion, bring the final volume to 2.5 mL with methanol prior to analysis. A maximum of 30 µL of the sample preparation may be analyzed due to the methanol content. The digestion may be scaled up if larger volumes are required. One gram may be weighed into a 20 mL vial, and 10 mL of 25% KOH methanol

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solution added prior to digestion. Sample preparations must be held for a minimum of 5 days prior to analysis to allow for full digestion of the samples. On the day of analysis, samples should be shaken thoroughly and allowed to resettle prior to analysis. Special care must be exhibited with biota certified reference materials as the fine particulate material can interfere with the analysis causing low recoveries. Sufficient time must be given for the fine particulate material to settle prior to analyzing the sample preparation.

9.3.2 Distillation (following alkaline digestion): If there is matrix interference or if a lower detection limit is desired, the alkaline digestate can be distilled (Horvat, Bloom, and Liang, 1993). Distillation allows for up to 2.0 mL of the digestate to be analyzed as opposed to only 30 µL if the digestate is directly analyzed, thereby significantly reducing the detection limits for the analysis of MeHg in biota samples.

Distill the alkaline digestion by transferring 0.5-2.0 mL of alkaline digestate into a 60 mL fluoropolymer vial, diluting up to 50 mL with 0.4% HCl solution and following the procedure outlined in section 9.2.1 for the distillation of aqueous samples.

Note: Brooks Rand Labs routinely uses 0.5 mL of the alkaline digestate unless otherwise specified on the Sample Processing Form.

9.3.3 Digestions: Biological digestates are more stable than distillates and may be stored up to 30 days prior to analysis. As previously stated in Section 9.3.1, biological digestates must be held for a minimum of 5 days prior to analysis.

9.4 Preparation of sediments and soils for MeHg.

9.4.1 Solvent extraction for sediments: Sediment samples should be extracted to avoid the potential for artifact formation of MeHg during distillation. Approximately 2.5 g of sediment sample is accurately weighed into a clean 30 mL fluoropolymer vial. Only for surrogate correction, each sample is spiked with 0.050 mL of a 100 ng/mL n-propyl mercury standard. 10 mL of the KBr/H_2SO_4 solution (Section 7.13) and 2 mL of the 1M $CuSO_4$ solution (Section 7.14) are added to the sample. 18-20 g of DCM are accurately weighed and added. The sample is intermittently shaken by hand over the next hour or placed on a shaker for an hour. After shaking for one hour, the samples are centrifuged for 15 minutes at 3200 rpm to assist in the separation of the aqueous layer from the organic layer. The sample is then passed through phase separating filter paper so that only the organic layer is collected. The organic layer is collected directly to a triple rinsed, pre-weighed 125 mL Teflon® bottle. The weight of the collected organic layer is measured and approximately 50 mL of DIW is added to the bottle along with a couple of Teflon® boiling chips to help prevent the DCM from "bumping" during the back extraction. The sample

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is then heated to 70 ºC until the dichloromethane layer has evaporated off. After the organic layer is no longer visible in the sample, the sample is heated for an additional 3 hours to ensure that all traces of DCM have been removed.

NOTE: Heating the sample for a minimum of 6 hours at 70 ºC has yielded good results for spike and CRM recoveries. Samples may be loosely capped for the final 3 hours and left overnight on the hotplate if a timer is used to turn off the heating element after 6 hours total time.

The sample is then diluted to 100 mL with additional DIW. No more than 5 mL of the back extraction is analyzed. The extracted sample is analyzed following the same procedure used for the analysis of water distillations.

MeHg Sediment Prep step by step instructions

- *Take samples out the night before to give it time to thaw. (On the weekends, put it in the sediment fridge, not in the prep lab)*
- *Rinse funnels and vials from the acid vat A (4BLK, 1SRM (0.500g) per every 20 samples, 1BS @ MRL, Samples+ 1 sets of DUP, MS&MSD per 10 samples)*
- *Turn on the hot plate to 65 ℃ with the thermometer in water (not in sand)*
- *Decant the samples-if needed and not previously decanted.*
- *Weigh out approximately 2.50g of samples.*
- *Spike and add reagents*
  **2mL 1M $CuSO_4$, 10mL 18% KBr/ 5% H_2SO_4*
- *Weigh your vials (with cap on) and record it in the Original column.*
- *Add approximately 20g of DCM (methylene chloride). (Next to DCM bottle, there's 20mL vial with green tape. Top of the tape is approximately 20g for DCM.) Crank the tops on so they won't leak during the next step. Shake the vials.*
- *Weigh the vials and record on the 2nd column of your Prep vials. As you weigh, shake the vials!*
- *Shake vials vigorously once and put it on the shaker table for 1 hour. (Best time to grab some lunch!)*
- *While shaker is going for an hour, fold the phase separator paper, wash and label the 125mL bottles being careful to line up top of the tape to the faint line on the bottle. This will be used later on as a guide to bring samples up to final volume. With the caps on and label on, weigh the bottles and record on the prep sheet.*
- *centrifuge for 15 min at 3200rpm.*
- *Grab all the equipment and set it up in the hood. (Styrofoam bottle holder, 125mL bottles, funnels, filters, garbage can, dirty bin, waste bucket with no soda ash!)*

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- *Put the bottles in the holder, funnel on, and filter paper on. Filter the samples. DCM can eat up the filters so as soon as it's done dripping, take the funnel out. Dispose the waste into the waste bucket, throw the filter paper into the garbage can and put the funnel into dirty bin with the vials. Close caps to 125mL bottle, reweigh and record.*
- *Add a couple of boiling stones and 50mL of preheated DIW. (There's EE tubes used to measure out 50mL) Put the caps on lightly and at an angle (~45 degrees) and put it on the hot plate at 65℃ with the water thermometer for 6 hours.*
- *Bring it up to 100mL final volume. Bottom of labeling tape is where 100mL is. (Measured and proved that bottom of the tape is 100mL!)*

The green waste goes into Satellite waste container in the Prep Lab on the left side of the fume hood

9.5 Holding times for sample preparations.

9.5.1 Distillations: Water and sediment distillates and distillates of biota digestates are stable for up to 48 hours if stored at room temperature and in the dark. Distillates must not be refrigerated or frozen.

9.5.1.1 EPA Method 1630 specifies that distillates with a pH <3.5 must be discarded. BRL has found that good recoveries can still be obtained for these samples if an adequate pH is obtained prior to ethylation. This is done by adding extra buffer to the samples prior to analysis. The following procedure was instated to ensure proper pH is attained before analysis.

9.5.1.1.1 The pH of all samples is tested using pH strips after prep. The pH of each distillate is recorded on the prep log.

9.5.1.1.2 If the pH is 2.5 or less, 0.600 mL of acetate buffer should be added to the sample after it is weighed out into the analytical reaction vial.

9.5.1.1.3 The buffered sample should be capped and shaken then 0.050 mL removed for pH testing (via 4-6 pH strip).

9.5.1.1.3.1 If the pH is 4 or higher, no further buffering is needed

9.5.1.1.3.2 If the pH is less than 4, add 0.300 mL of acetate buffer and retest the pH. Repeat this step until pH 4 is acquired.

9.5.1.1.4 The analyst must record on the bench sheet the amount of acetate buffer added to the sample if it is more than 0.300 mL.

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9.5.2 Extractions: Water and sediment extractions (once back extracted into water) are stable for up to 48 hours if stored at room temperature in the dark.

9.5.2.1 The holding time limit for intermediate organic extraction (prior to back extraction) has been examined and was shown to be stable for up to one week. Currently, Brooks Rand Labs performs the entire extraction procedure within one day unless explicit approval is given in advance by the Lab Manager.

10.0 Instrument Calibration and Sample Analysis

10.1 Refer to EPA Method 1630, Sections 10.0, 11.0, and 12.0 for a detailed description of the analysis of samples and the calculation of results.

10.2 Instrument Calibration: BRL follows EPA method 1630, Section 10.0 for the instrument calibration with the same exceptions as for sample analysis. Standards, typically 0.5, 1, 2, 10, 50, 250, and 1000 pg MeHg are added into reaction vessels containing 40 mL of DIW and buffer. Two different buffer types are used, 300 µL of 2 M acetate buffer or 2 mL of citrate buffer. Only the ethylation blanks analyzed prior to the calibration standards are used for blank correcting the calibration and sample results. Any other ethylation blanks analyzed following the calibration standards are used only to check for carryover or other potential system contamination.

10.3 Instrumental Analysis: BRL has adopted the following modifications.

For samples, add appropriate sample volumes plus DIW as necessary for a final volume of 40 mL (for sample preparation see Section 9 of this SOP). Some acid can be carried over during distillation causing some distillates may have low pH (< 4). In that event, follow instructions in section 9.5.1.1 to bring the final pH to 4 – 5.5, which is the optimum pH for ethylation. An aliquot of 50 µl of $NaBEt_4$ is added, a squirt bottle of DIW is used to fill the vial until there is a convex meniscus, the sample is tightly capped, shaken, then placed in the appropriate position on the autoanalyzer.

Note: Based on a study conducted at BRL, it has been determined that samples can be analyzed up to 4 days after ethylation and yield acceptable recoveries. Details of this study are saved on the server at Curie\Lab\R&D\MeHg Autoanalyzer R&D\Ethylation Holding Time Study

Place the samples in the autosampler. Under the "Automation" tab in Guru, make sure all settings are correct then press "Start Batch". It will commence a 10 minute trap blanking cycle then begin to take up sample.

Peak integration is begun in conjunction with the heating of the Tenax trap. The typical heating time for the MERX system is between 9 and 9.9 seconds. The organomercury species are desorbed and carried through the GC column that is held in an oven at 36 °C. The species elute in order of increasing molecular weight, pass through the pyrolitic

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column, held at approximately 900 ºC at which point all organomercury species are converted into Hg(0), and detected by CVAFS. The reaction vessels are used only once then discarded.

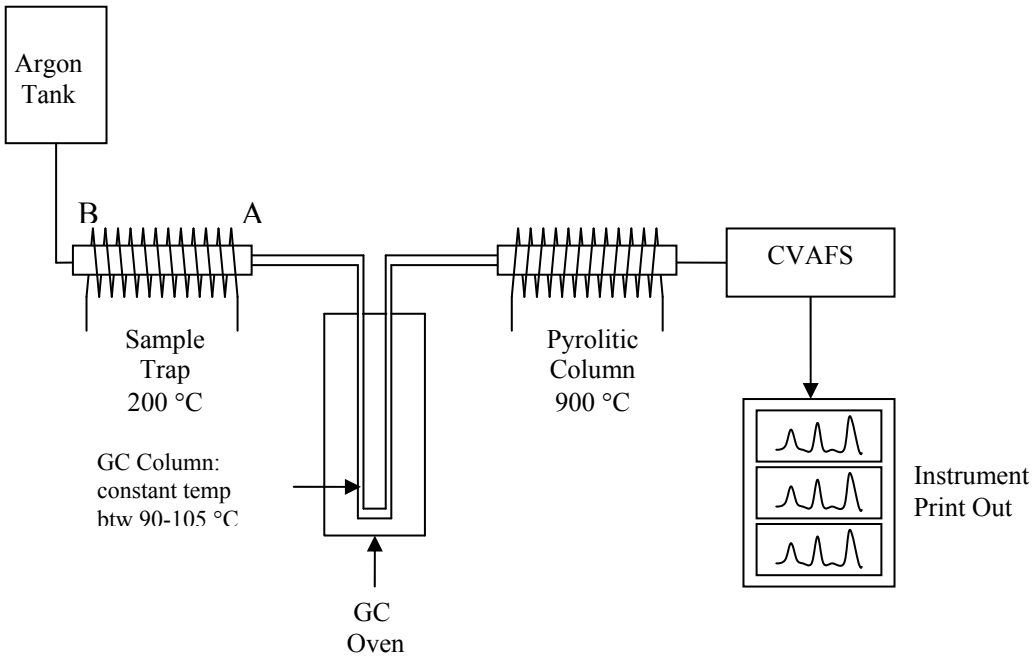

Figure 2: A schematic diagram of the isothermal Gas chromatograph system

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Quick MeHg MERX Analysis Guide

Setting Up MERX before Analysis

- Open Guru Template – It is suggested a template is created with the typical setting as well as the typical calibration curve entered in.
- Save as Data (File → Save As Data)
- Ensure purge and trap, GC and pyro, AFS, and autosampler are powered on.
    - **NOTE**: the detector should stay on all of the time or be turned on 24-48 hours before using the instrument.
- Connect instruments to computer (Instrument → Connect; then press Accept)
- Turn gases on at regulator to 17 psi
    - In the Automation tab, there is a set of 3 check boxes to turn the gases on, click those boxes and check the gas flows at the rotometers. It should be at typical settings of:
        - Purge: 50
        - Dry: 30
        - GC: 35

- Auto Zero the detector
- Measure Noise (Instrument → Measure Noise)
- Check run duration (5 min), purge duration(5 min), drying duration (3 min) and heating time (9.9 seconds) to make sure they are at the desired settings. (In the Batch Information tab, times listed)
    - For samples with surrogate n-prHg spikes, a run duration of 7 minutes and a purge duration of 6 minutes should be used.
- Set the number of vials to be analyzed (this can be adjusted while the system is analyzing). Also ensure that the first vial position number is correct (default is position 1).
- Put your prepared vials on the autosampler. **see instructions below.
- Press Start Batch.
- At this point you can continue to prepare samples to put on the autosampler or update the Run Information in Guru.

Preparing vials for MeHg analysis on MERX

Water samples prepared via Distillation

- Add sample to vial (record exact amount of sample added, max volume of 30mL)
- Add 0.300 mL of Acetate Buffer or appropriate amount for samples
- Add 0.050 mL of NaBEt4
- Bring up to volume with Reagent Water (use a squirt bottle)
- Cap tightly
- Invert rapidly to mix contents

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Water samples for direct ethylation

- Pipette 10 mL of sample into vial
- Add 2 mL of citrate buffer
- Add 50 pg of n-propylmercury (0.050 mL of 1 ng/mL n-prHg standard)
- Dilute to ~40 mL with DIW
- Add 0.050 mL $NaBEt_4$
- Bring up to volume with Reagent Water (use a squirt bottle)
- Cap tightly
- Invert rapidly to mix contents

Biota or sediment samples

- Add approximately 40 mL of Reagent Water to vial (exact amount doesn't matter)
- Add sample to vial (record exact amount of sample added)
- Add 0.300 mL of Acetate Buffer or appropriate amount for samples
- Add 0.050 mL of NaBEt4
- Bring up to volume with Reagent Water (use a squirt bottle)
- Cap tightly
- Invert rapidly to mix contents

11.0 Calculations

BRL uses the following formulas for the calculation of monomethyl mercury in a given sample.

11.1 Mean Calibration Coefficient:

A calibration coefficient (CF) is calculated for each standard used in the calibration as follows:

$$CF = CS_{pgMeHg} / (CS_{PH} - EB_{PH})$$

Where CS_{pgMeHg} is the calibration standard measured in picograms of methyl mercury, CS_{PH} is the peak height obtained during the analysis of the standard, and EB_{PH} is the mean peak height obtained during the analyses of all of the ethylation blanks that were analyzed prior to the calibration standards. The mean calibration coefficient (CF_{avg}) is then calculated for all of the standards used in the calibration.

11.2 Measured methyl mercury in the sample preparation:

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The amount of methyl mercury present in the analyzed volume of the sample preparation is calculated using the equation:

$$MeHg_{measured\ pg} = (A_{PH} - EB_{PH}) \cdot CF_{avg}$$

Where A_{PH} is the peak height obtained during the analysis of the sample preparation.

11.3 Total methyl mercury in the sample preparation:

The total amount of methyl mercury present in the sample preparation is calculated using the equation:

$$MeHg_{total\ pg} = [(MeHg_{measured\ pg}) / V_A] \cdot V_D$$

Where V_D is the final dilution volume of the sample preparation in mL and V_A is the volume analyzed of the sample preparation in mL.

11.4 Concentration of methyl mercury in the sample:

The final concentration of methyl mercury in the sample is calculated using the equation:

$$MeHg_{conc} = (MeHg_{total\ pg} - MB_{total\ pg}) / V_o$$

Where $MB_{total\ pg}$ is the average total picograms of methyl mercury present in the method blanks and V_o is either the volume of the prepared sample measured in mL (aqueous samples) or the weight of the prepared sample measured in mg (solid samples). Therefore, the final concentration of methyl mercury in the sample is reported in units of ng/L for aqueous samples and in units of ng/g for solid samples.

NOTE: The total picograms of methylmercury present in each method blank is calculated using the same formula used to calculate the total picograms of methyl mercury in the sample preparation.

Sediment and soil results are typically dry weight corrected by dividing the wet weight result by the percent total solids result. Biota results are typically reported on a wet weight basis, but can be reported dry weight corrected upon request.

11.5 Empirically derived correction factor

BRL routinely recovery corrects results for distilled samples, as per EPA Draft Method 1630, to account for the fact that the distillation procedure is not 100% efficient in recovering methyl mercury. Results are multiplied by an empirically derived correction factor that is based on the average recovery of the appropriate quality control sample (Laboratory Fortified Blanks for aqueous distillates and the certified reference material (CRM) BCR 580 (Marine Sediment) for sediment distillates). If an appropriate QCS

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sample is not available, the correction factor is based on the average recovery of the spikes made to samples with a similar matrix to the sample of concern.

The correction factor is calculated using the following equation:

$$F = 100 / R$$

Where F is the empirically derived correction factor and R is the running mean of the recoveries of the last 30 quality control samples or matrix spikes. The empirically derived correction factor is updated quarterly or any time that there is a significant change in performance.

BRL does not use the IPR and CCV samples to calculate the correction factor since, unlike the client samples and quality control samples, these samples are not distilled.

No correction factor is necessary for samples that are analyzed by direct ethylation.

11.6 Surrogate spike correction

11.6.1 Sample preps can be spiked with n-propylmercury that is used as surrogate spike.

11.6.2 N-prHg concentrations are calculated in the same way as the MeHg in the samples.

11.6.3 Soil and sediment samples can be corrected by the surrogate concentration by the following equation:

$$\text{Final } MeHg_{conc\ f} = MeHg_{conc\ i} / R_{n\text{-}prHg}$$

Where $MeHg_{conc\ f}$ is the surrogate recovery corrected concentration. $MeHg_{conc\ I}$ is the initial concentration of MeHg before recovery correction. $R_{n\text{-}prHg}$ is the percent recovery of the n-propylmercury expressed as a decimal.

Percent recovery of n-prHg is determined by the following equation:

Percent Recovery = (spiked sample result (conc.)) / (amount spiked)

12.0 Quality Control

12.1 Refer to EPA Method 1630, Section 9.0 for a detailed description of the quality control procedures employed at BRL for this method.

12.2 All quality control data should be maintained and available for easy reference and/or inspection.

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12.3 Each analyst must perform an initial demonstration of capability (IDOC) for the analysis of methyl mercury prior to the analysis of any client samples. The IDOC consists of an initial precision and recovery (IPR) study following the procedure in EPA Draft Method 1630, Section 9.2.2. The acceptance criteria and run sequence for the IDOC can be found in Table 3 in Section 17 of this SOP.

12.4 Calibration data must be composed of a minimum of 1 ethylation blank (BRL analyzes 3 ethylation blanks prior to analyzing the calibration standards) and a minimum of 5, preferably 7, standards (See Section 7.3 and 10.2 of this SOP for the standards used to calibrate instruments at BRL). Such a calibration should be run daily, prior to analysis, or whenever stock standards have been remade, conditions have changed, or initial calibration check (ICV) or ongoing precision and recovery (CCV) as defined in Section 12.5 do not yield acceptable recoveries.

12.5 The CCV solution prepared by spiking the reaction vessel with 25 pg methyl mercury using the calibration standard and followed by an ethylation blank must be analyzed after every 10 client samples and at the end of the analysis of each analytical batch. Additionally, BRL analyzes an independent calibration check (ICV) solution prepared by performing a KOH/Methanol digest of the certified reference material NIST 1946 (Refer to Section 7.3.6) and spiking this solution directly into the reaction vessel prior to the analysis of each analytical batch. The criterion for the recovery of the CCV solution is 67-133% and the recovery criterion for the recovery of the ICV solution is 80-120%. All ethylation blanks must contain no more than the level of methyl mercury found in the low calibration standard.

12.6 Matrix spike/matrix spike duplicate (MS/MSD) analysis should be performed once per every 10 client samples or once per batch, whichever is greater. A matrix spike sample is defined as an aliquot of homogenized sample that has a known amount of analyte added to it. The target spiking concentration is 2 – 5 times the level of the native sample. If there is no historic data on which to base the spike concentration, then the following default spiking levels should be used:

Aqueous Samples:	1 ng/L
Sediment Samples:	2 ng/g
Fish Samples:	750 ng/g
Other Biota:	200 ng/g

The matrix spike sample is processed through the entire preparation and analytical procedure. Bias is then determined by calculating the percent recovery of the known amount using the following formula:

Percent Recovery = 100 ∗ (spiked sample result (conc.) - sample result (conc.)) / (amount spiked)

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The criterion for spike recovery is determined by control charts and is different for each matrix type. The specific matrix spike recovery criteria for each matrix type and preparation procedure can be found in Tables 5 and 6 in Section 17 of this SOP.

The relative percent difference between the MS and the MSD is calculated using the following formula:

$$RPD = 200 \cdot (\left| MS\text{-}MSD \right|) / (MS + MSD)$$

The RPD for the MS/MSD pair must meet the criterion for each of the matrix types found in Tables 5 and 6 in Section 17 of this SOP.

12.7 Method duplicates are prepared and analyzed upon client request. For solid matrices method duplicates should be performed in conjunction with the MS/MSD samples and whenever the heterogeneity of a sample is deemed great enough that it may cause problems with the analysis of the sample. The relative percent difference (RPD) between duplicate samples is calculated using the same formula as used to calculate the RPD between the MS and MSD samples. The specific RPD criteria for each matrix type and preparation procedure can be found in Tables 5 and 6 in Section 17 of this SOP. If the acceptance criterion for duplicate analysis is not met for either samples or matrix spike samples, then the system performance is unacceptable. Associated samples must be qualified or the problem must be corrected and the samples reanalyzed.

12.8 Field duplicates are analyzed at the client's discretion. The acceptance criterion for field duplicate analysis is the same as that used for method duplicate analysis. The client must be notified immediately anytime that the acceptance criterion for field duplicates is not met.

12.9 Four method blanks (MB) should be prepared and analyzed with each batch. For water distillation batches, the method blanks are prepared using reagent water acidified to 0.4% HCl in order to simulate the preservation of the samples. For sediment and biota batches, the method blanks are prepared just using the reagents. All method blank results must meet the acceptance criteria set forth in Tables 5 and 6 of Section 17.

12.10 Blank spikes (BS) are prepared and analyzed with each distillation batch at a minimum frequency of one per batch. BSs are prepared by spiking a method blank sample with the calibration standard at a concentration of approximately 1.0 ng/L. The BS is then distilled as per an aqueous sample. The acceptance criterion for the recovery of the BS (recovery corrected) is identical to the acceptance criterion for the recovery of CCV samples.

12.11 Appropriate certified reference materials (CRM) for MeHg are prepared for all batches containing tissue or sediment samples. It is BRL policy to prepare one CRM with every solid batch. Two entirely different CRMs may be prepared if different matrix types are analyzed together. Criteria for CRM recoveries are determined by control charts. If control charts are not available then CRM results should be within 35% of the certified

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value (following recovery correction) for the analysis to be considered valid. CRM accuracy results not meeting this criterion shall be reprepared and reanalyzed or qualified at the discretion of the Laboratory Director. Currently, there are not any water based CRMs available.

12.12 Surrogate spikes are used to evaluate the efficiency of the procedure. Surrogates must be performed in every sample if direct ethylation is performed. They also may be used with sediment batches to efficiency correct the data. Refer to Tables 5 and 6 for acceptance criteria regarding surrogate spike recoveries.

13.0 Method Performance

13.1 Refer to EPA Method 1630, section 13.0, for information regarding the verification of this method.

13.2 The detection limits reported in Table 1 were achieved by performing a full MDL study as described in 40 CFR 136, Appendix B. The warning and control limits listed in Table 2 show the limits achievable at BRL.

14.0 Pollution Prevention

14.1 Refer to EPA Method 1630, section 14.0, for EPA recommendations regarding pollution prevention techniques.

14.2 Whenever feasible, lab personnel should use pollution prevention techniques to limit waste generation. The cost involved in purifying acids makes such recycling unpractical at BRL. Instead, every effort is made to reduce volumes necessary to still produce the best possible results. When making standards, they should be prepared in volumes consistent with their use in the laboratory to minimize the volume of expired standards to be disposed.

15.0 Waste Management

15.1 Refer to EPA Method 1630, section 15.0, for information and references related to managing waste produced by application of this method.

15.2 All waste is disposed of in accordance with state and federal regulations either by sewer disposal (only if concentrations are below the King County sewer limits) or through a licensed and bonded hazardous waste disposal facility such as Philips Services.

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16.0 References

16.1 Liang, L.; Bloom, N.S.; and Horvat, M. (1994) "Simultaneous Determination of Mercury Speciation in Biological Materials by GC/CVAFS After Ethylation and Room-Temperature Precollection." *Clin. Chem.* 40/4: 602-607.

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16.7 Horvat, M.; May, K.; Stoeppler, M.; and Byrne, A.R. (1988) *Appl. Organomet. Chem.* 2: 515.

16.8 EPA Draft Method 1630 (January 2001) "Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS."

16.9 EPA Method 1669 (April 1995) "Sampling Ambient Water for Trace Metals At EPA Water Quality Criteria Levels."

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17.0 Tables and Benchsheets

Table 1 Current method detection limits and minimum levels determined at BRL for the analysis of methyl mercury using EPA Method 1630

Matrix	Preparation Method	Method Detection Limit (MDL)[1]	Minimum Level (ML)
Water	Distillation	0.020 ng/L	0.050 ng/L
Water (ultra-low)	Distillation	0.010 ng/L	0.025 ng/L
Sediment/Sludge	Extraction	0.008 ng/g	0.025 ng/g
Biota	Digestion	1.0 ng/g	3.0 ng/g
Biota	Digestion/Distillation	0.07 ng/g	0.20 ng/g

NOTES:
1. MDL as determined by the procedure 40 CFR Part 136, Appendix B.
   MDL and ML reported here for distillations are for recovery corrected results.
2. The ultra-low method requires rigorously cleaned distillation vials and a lower calibration achievable only on the auto-analyzer system.

Table 2 Summary of recent control chart data ending December 2010 (minimum of 30 data points) for the analysis of methyl mercury using EPA Method 1630

QA Sample	Matrix	Mean[1] Recovery (%)	Warning Limit (%) Mean ± 2 StDev	Control Limit (%) Mean ± 3 StDev
ICV	ALL	98	80 - 116	70 - 125
CCV	ALL	102	87 - 118	79 - 125
Matrix Spikes	Water	106	84 - 128	73 - 139
Matrix Spikes[2]	Sed/Sludge	93	67 - 119	54 - 132
Matrix Spike	Biota	115	96 - 133	87 - 143
BS	Water	97	70 - 125	56 - 138
CRM[2,3]	Sed/Sludge	92	73 - 111	64 - 121
CRM[5]	Biota	89	73 - 105	65 - 113
QA Sample	**Matrix**	**Mean RPD**	**Warning Limit (%) Mean ± 2 StDev**	**Control Limit (%) Mean ± 3 StDev**
Duplicates	Water	6	20	26
Duplicates[2]	Sed/Sludge	9	25	32
Duplicates	Biota	10	23	30

NOTES:
1. Recoveries for distillations (water samples) have been recovery corrected using an empirically derived correction factor.
2. Control limits for sediments prepared by DCM extraction.
3. SQC-1238 (Sediment) is the CRM used for sediments. Use of this SRM began in August 2010. Control charts were generated from that time up to April 2011.
4. DORM-3 (Dogfish Muscle) is the CRM used for most biota samples.

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Table 3 Quality Control Acceptance Criteria and General Analytical Run Sequence for the Initial Demonstration of Capability for the Analysis of Methyl Mercury

Run	Run Name	Section Name	Analyze	Requirements
1 2 3	Rinse Rinse Rinse	Blanking Equipment	DIW + analytical reagents	n/a
4 5 6	IBL IBL IBL	Calibration	Ethylation Blank Ethylation Blank Ethylation Blank	≤ the MRL
7 8 9 10 11 12 13	0.5 pg std 1 pg std 2 pg std 10 pg std 50 pg std 250 pg std 1000 pg std	Calibration[1]	0.5 pg std 1 pg std 2 pg std 10 pg std 50 pg std 250 pg std 1000 pg std	RSD of Avg. CF ≤ 15% Recovery of Low Standard 65-135%
14	ICV	Independent Calibration Verification	~30 pg	Recovery 80-120%
15 16 17 18 19 20	Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank	Contamination Check	Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank	≤ the MRL
21 22 23 24 25 26 27	MDL sample MDL sample MDL sample MDL sample MDL sample MDL sample MDL sample	Method Detection Limit[2,3]	Appropriate matrix spiked at a level of 1 – 5 times the expected MDL	Calculated MDL no greater than 5 times the spike level
28	CCV std (25pg)	Continuing Calibration Verification	25 pg	Recovery 67-133%
29	Ethylation Blank	Contamination Check	Ethylation Blank	≤ the MRL
30 31 32 33	IPR std IPR std IPR std IPR std	Initial Precision and Recovery	ICV solution	Ave. recovery 69-131%, RSD ≤ 31%
34	CCV std (25pg)	Continuing Calibration Verification	25 pg	Recovery 67-133%
35	Ethylation Blank	Contamination Check	Ethylation Blank	≤ the MRL

NOTES:
1. All standards and samples are corrected for mean ethylation blank. Lower calibrations can be achieved as long as ethylation blank criteria are still met.
2. All samples are corrected for mean method blank.
3. Distilled samples are recovery corrected prior to calculating the MDL.

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Table 4 Quality Control Acceptance Criteria and General Analytical Run Sequence for the Analysis of Methyl Mercury

RUN	Analyze	Description	Requirements
1 2 3	Rinse Rinse Rinse	DIW + analytical reagents	n/a
4 5 6	IBL IBL IBL	Contamination Check	≤ the MRL
7 8 9 10 11 12 13	0.5 pg std 1 pg std 2 pg std 10 pg std 50 pg std 250 pg std 1000 pg std	Calibration Curve[1]	RSD of Avg. CF ≤ 15% Recovery of Low Standard 65-135%
14	ICV (independent calib. verific.) (prepared with NIST 1946; around 30 pg MeHg)	Precision and Recovery	80 – 120% recovery
15 16 17 18 19 20	Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank Ethylation Blank	Contamination Check	≤ the MRL
21 22 23 24	Method Blank 1 (MB-1) Method Blank 2 (MB-2) Method Blank 3 (MB-3) Method Blank 4 (MB-4)	Contamination Check	Refer to specific water and solid criteria found in Tables 5 and 6.
Next	Known Blanks	Trip, Field, or Equipment Blanks	Result < ML or < 1/5th associated sample results
Next	BS or CRM	Precision and Recovery	Rec = 70 – 130%[3] for aqueous, Rec = 65 – 135% for sediment samples, and Rec = 65-135% for biota samples
Next	Sample 01[2] Sample 01MD	Native Sample Duplicate Sample	RPD ≤ 35% or ±MRL for aqueous samples and ±2xMRL for solids if results are ≤5xMRL
Next	Sample 01MS Sample 01MSD	Matrix Spike Matrix Spike Duplicate	Rec = 65 – 135%[3] for aqueous; Rec = 65 – 135% for sediment and biota samples; RPD ≤ 35%
Next	Sample 02 through Sample 10	Client Sample	
Next	CCV std (25 pg)	Continuing Calibration Check	67 – 133% recovery
Next	Ethylation Blank	Contamination Check	≤ the MRL
Next	Sample 11MS Sample 11MSD	Matrix Spike Matrix Spike Duplicate	Rec = 65 – 135%[3] for aqueous; Rec = 65 – 135% for sediment and biota samples; RPD ≤ 35%
Next	Sample 12 through Sample 20	Client Sample	
Last	CCV std (25 pg)	Continuing Calibration Check	67 – 133% recovery
Next	Ethylation Blank	Contamination Check	≤ the MRL

NOTES:

1. The calibration curve may be adjusted depending on the expected range of samples (i.e. seds and biota 10pg-5000pg). Lower calibrations can be achieved as long as ethylation blank criteria are still met.
2. Any known field or equipment blanks should not be spiked and should be analyzed prior to other samples. The acceptance criterion for these samples is a result < the ML.
3. Recovery corrected.

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Table 5 Quality Control Acceptance Criteria and Corrective Action Guidelines for the Analysis of Methyl Mercury in Aqueous Samples by Distillation or Direct Ethylation

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Ethylation Blank	Contamination from bubblers	4 prior to calib.; following calib. and end of batch	≤ the MRL	Clean and test bubblers until criteria met prior to any analysis
Calibration Standards	Acceptability of the Calibration Curve	Daily, prior to analysis of samples or whenever the CCV fails	RSD of response factors ≤ 15%; Recovery of Low Standard = 65 – 135%	Reanalyze suspect calibration standard. If criteria still not met, then remake standards and recalibrate the instrument
Independent Calib. Ver. (ICV) Continuing Calib. Ver. (CCV)	Accuracy	Following Cal.; Beginning and end and 1 per 10 sample preparations	ICV Rec. = 80-120% CCV Rec. = 67-133%	Correct problem (recalibrate, remake standard, etc.) and reanalyze ICV/CCV. If criteria met, reanalyze samples backwards (if possible) until 2 consecutive results with RPD ≤ 20%
Carryover Check Ethylation Blank	Contamination due to carryover in the bubbler/trap	Following any unusually high result and CCV. Currently ≥ 2x the high standard	≤ the MRL	Clean and continue to test bubbler/trap combo until criteria met prior to further use. Reanalyze samples that were analyzed in same bubbler/trap following high result
Method Blank	Contamination from reagents, lab ware, etc.	4 per batch	Avg ≤ 0.045 ng/L StDev ≤ 0.015 ng/L or < 1/10th of associated samples	Correct problem. All samples associated with a contaminated method blank must be reanalyzed.
Blank Spike (BS)	Accuracy	1 per 20 samples	Recovery = 70 – 130%*; RPD ≤ 30%	Reanalyze remaining volume. Correct problem prior to continuing analysis
Matrix Spike/Spike Duplicate	Accuracy and Precision within a given matrix	1 per 10 client samples	Recovery = 65 – 135%*; RPD ≤ 35%	If **recoveries** similar but fail recovery criteria, an interference is present in the sample and the result must be qualified. If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.
Method Duplicates	Precision	Per client request	RPD ≤ 35% or ± MRL if sample < 5x MRL	Correct problem and reanalyze all associated samples.
Surrogate Spike	Efficiency	Every sample analyzed via direct ethylation	Recovery 70-130%	Reanalyze at the same or less volume; do perform an MS on the sample.

* Recovery Criteria for Matrix Spikes and BS samples are based on recovery corrected results.

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Table 6 Quality Control Acceptance Criteria and Corrective Action Guidelines for the Analysis of Methyl Mercury in Solid Samples by Distillation, Extraction, and/or Digestion

QC Sample	Measure	Minimum Frequency	Criteria	Corrective Action
Ethylation Blank	Contamination from bubblers	4 prior to calib.; following calib. and end of batch	≤ the MRL	Clean and test bubblers until criteria met prior to any analysis
Calibration Standards	Acceptability of the Calibration Curve	Daily, prior to analysis of samples or whenever the CCV fails	RSD of response factors ≤ 15%; Recovery of Low Standard = 65 – 135%	Reanalyze suspect calibration standard. If criteria still not met, then remake standards and recalibrate the instrument
Independent Calib. Ver. (ICV) Continuing Calib. Ver. (CCV)	Accuracy	Following Cal.; Beginning and end and 1 per 10 sample preparations	ICV Rec. = 80-120% CCV Rec. = 67-133%	Correct problem (recalibrate, remake standard, etc.) and reanalyze ICV/CCV. If criteria met, reanalyze samples backwards (if possible) until 2 consecutive results with RPD ≤ 20%
Carryover Check Ethylation Blank	Contamination due to carryover in the bubbler/trap	Following any unusually high result. Currently ≥ 2x the high standard	≤ the MRL before further analysis of samples on same bubbler/trap	Clean and continue to test bubbler/trap combo until criteria met prior to further use. Reanalyze samples that were analyzed in same bubbler/trap following high result
Method Blank	Contamination from reagents, lab ware, etc.	4 per batch	Avg ≤ 2 x MDL StD ≤ 2/3rd MDL or < 1/10th of associated samples	Correct problem. All samples associated with a contaminated method blank must be reanalyzed.
Certified Reference Material (CRM)	Accuracy	1 per 20 samples	Soil Rec=65-135% Biota Rec=65-135%*	Correct problem prior to continuing analysis
Matrix Spike/Spike Duplicate	Accuracy and Precision within a given matrix	1 per 10 client samples	Soil Rec=65-135%; RPD ≤ 35% Biota Rec=65-135%*; RPD ≤ 35%	If **recoveries** similar but fail recovery criteria, an interference is present in the sample and the result must be qualified. If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.
Method Duplicate	Precision within a given matrix	In association with MS/MSD	RPD ≤ 35% or ± 2x MRL if sample < 5x MRL	If **RPD** criteria not met, then the system is not in control. Correct problem and reanalyze all associated samples.
Surrogate Spike**	Efficiency	Every sample analyzed via direct ethylation	If recovery 50-150%, then sample results are corrected for the surrogate recovery.	Reanalyze at the same or less volume; perform an MS on the sample.

* Recovery Criteria for Distilled Matrix Spikes and CRM samples are based on recovery corrected results.
** If surrogate spikes are performed, then the following criteria is applied.

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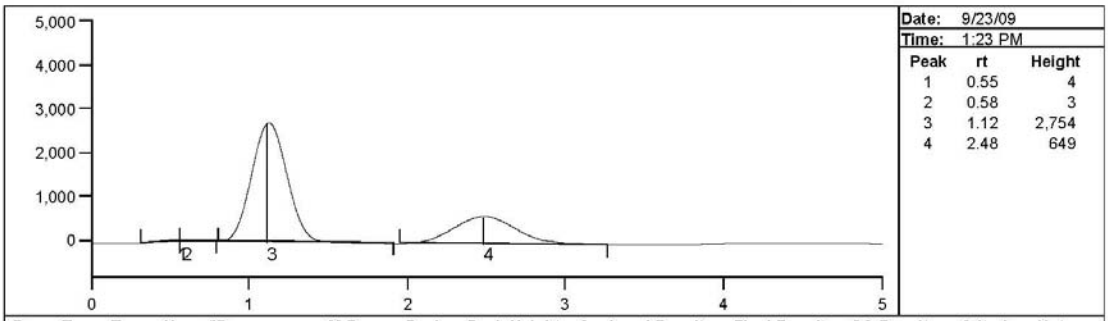
Peak Report

Batch Number: B091247
Method Number: BR-0011

Project Number(s): 0900780
Instrument ID: BR-11

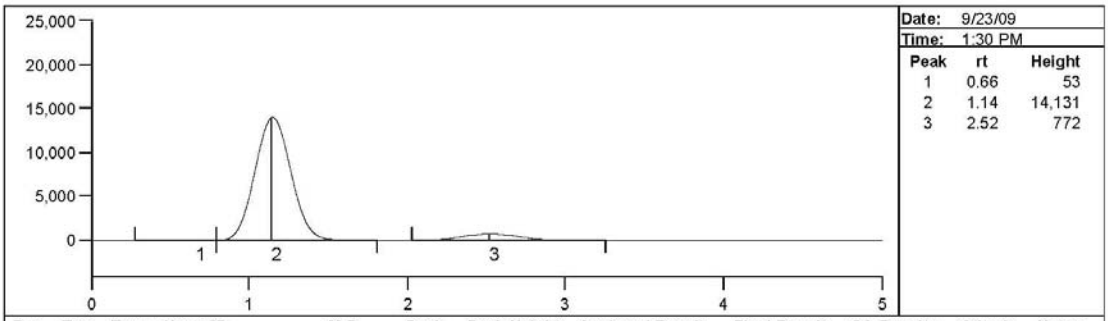
Date Analyzed: 9/23/09
Analyst Name: MSU



Date: 9/23/09
Time: 1:23 PM

Peak	rt	Height
1	0.55	4
2	0.58	3
3	1.12	2,754
4	2.48	649

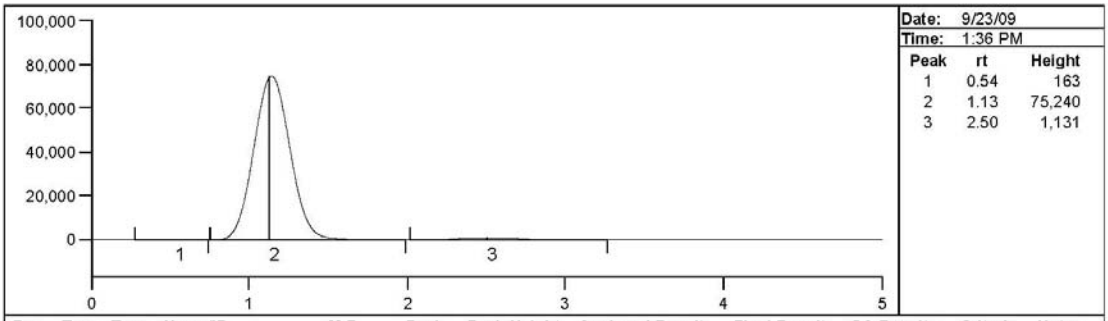
Run	Trap	Type	Name/ID	M B	Peak	Peak Height	Analyzed Result	Final Result	QA Results	Criteria	Notes
10	A	STD	SEQ-CAL4		3	2,754	9.94		99.4	75-125	accept
Notes											



Date: 9/23/09
Time: 1:30 PM

Peak	rt	Height
1	0.66	53
2	1.14	14,131
3	2.52	772

Run	Trap	Type	Name/ID	M B	Peak	Peak Height	Analyzed Result	Final Result	QA Results	Criteria	Notes
11	B	STD	SEQ-CAL5		2	14,131	51.6		103	75-125	accept
Notes											



Date: 9/23/09
Time: 1:36 PM

Peak	rt	Height
1	0.54	163
2	1.13	75,240
3	2.50	1,131

Run	Trap	Type	Name/ID	M B	Peak	Peak Height	Analyzed Result	Final Result	QA Results	Criteria	Notes
12	C	STD	SEQ-CAL6		2	75,240	276		110.	75-125	accept
Notes											

Page 4 of 25 (Peak Report)

Mercury Guru ver 4.0 © 1995-2008 Brooks Rand LLC

Figure 3 Examples of Typical Chromatograms Generated by the Mercury Guru Software During the Analysis of Methyl Mercury Using EPA Method 1630

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Brooks Rand Labs

MMHg Water Prep Benchsheet

Prepped By: _____ SOP/Rev #: BR-0011 / _____

Prep Date: _____ Batch: _____

Sample ID	Sample Mass (g)	Slot #	Time On	Time Off	pH	Sample ID	Sample Mass (g)	Slot #	Time On	Time Off	pH

Batch QC ID	Source	Spike vol (uL)	Spike conc (ng/mL)	Spike ID	Spike Witness	Reagent	ID
						0.5 mL H2SO4	
						0.2 mL KCL/ L-cysteine	
						HCl	
						Hot Block Temp	
						Final Dilution Vol	
Comments:						Balance ID	

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Example Log
Example Log

Brooks Rand Labs

MMHg Biota Prep Benchsheet

SOP/Rev BR-0011 Rev _____

Prepped By: Batch: _____

Preparation Start Date/Time*: _____ Preparation End Date/Time**: _____

* Time is when the first reagents are added. ** Time is when the last sample is brought upto volume

Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)	Sample ID	Sample Mass (g)

Batch QC ID	Sample Source	Spike vol (uL)	Spike conc (ng/mL)	Spike/CRM ID	Spike Wit-ness	Target Temp/Time:	65 C/4 hours
						Oven ID:	
						Oven Temp (measured / corrected):	/
						time on:	
						time off:	
						Balance ID:	
						Thermometer ID:	
						Volume:	

Reagent	ID	Comments:
1 mL KOH/Me		
Methanol		

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Example Log

Brooks Rand Labs

MMHg Sed/Soil Prep Benchsheet SOP / Revision #: BR-0011 Rev _____

Prepped By: _____ **Batch:** _____

Preparation Start Date/Time*: _____ Preparation End Date/Time**: _____

* Time is when the first reagents are added. ** Time is when the last sample is brought upto volume

Sample ID	Sample mass (g)	Original vial (g)	vial plus DCM (g)	**EXINFO 1** EXTRACT. DCM (g)	**EXINFO 2** Empty Bottle (g)	**EXINFO 3** Bottle + DCM (g)	Recovered DCM (for LIMS calcs)	Rec. DCM/ext. DCM
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!
				0.000			0.000	#DIV/0!

Batch QC ID	Sample Source	Spike vol (uL)	Spike conc (ng/mL)	Spike/CRM ID	Spike Witness
BS1		63	1.0		
MS/MSD1					
MS/MSD2					
MS/MSD3					
SRM1/2	SQC-1238				

	Reagent ID
CuSO4	
KBr/H2SO4	
DCM	
Balance ID:	
Thermometer ID:	
(measured / corrected):	/
Date/Time On:	
Date/Time Off:	
Final Dilution Vol:	

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(Example Log) **MeHg-Autoanalyzer Analysis Sheet** Page 1 of ____

Sequence:_____ Batch:_____ Analyst:_____

Instrument ID#:_____ Standards:1 ng/mL:_____ 0.01 ng/mL:_____

Date:_____ Buffer:_____ $NaBEt_4$:_____

ICV:_____

	Run/ Vial Position	Sample ID	Analyzed volume (mL)	Dilution Factor	Comments **$NaBEt_4$ ID and time out**
	1	EB-1	---		
	2	EB-2	---		
	3	EB-3	---		
	4	CB-1	---		
	5	CB-2	---		
	6	CB-3	---		
	7	0.5 pg	0.050		0.01 ng/mL
	8	1 pg	0.100		0.01 ng/mL
	9	2 pg	0.200		0.01 ng/mL
	10	10 pg	1.00		0.01 ng/mL
	11	50 pg	0.050		1.0 ng/mL
	12	250 pg	0.250		1.0 ng/mL
	13	1000 pg	1.00		1.0 ng/mL
	14	ICV	0.030		NIST 1946
	15	CCB-1	---		
	16	CCB-2	---		
	17	CCB-3	---		

Comments:_____

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(Example Log) **MeHg Analysis Sheet** Page ____ of ____

Sequence:_____ Analyst:_____ Date:_____

	Run/ Vial Position	Sample ID	Analyzed volume (mL)	Dilution Factor	Comments **$NaBEt_4$ ID and time out**

Comments:_____

_____

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Appendix A: Brooks Rand Labs' Procedure for EPA Draft Method 1630 (01/01)

Differences Between EPA Method 1630 and Brooks Rand Labs SOP #BR-0011

The following differences exist between EPA Method 1630 (with relevant sections marked in boldface) and the practices employed by Brooks Rand Labs (SOP #BR-0011):

- **Section 1.1** of EPA Draft Method 1630 (hitherto referred to as simply 1630) states that the scope of the method is for the determination of methyl mercury in aqueous samples. Brooks Rand Labs has made use of several peer reviewed papers to expand the scope of the method to include sediment and biota. Therefore, any description of the preparation or analysis of sediment or biota samples found in this SOP will NOT be found in 1630 and should be considered a difference.

- According to 1630, Carbotraps® are heated for 45 seconds at a temperature of 450-500 ºC (**Section 6.5.4**). Brooks Rand Labs uses Tenax traps instead of Carbotraps®. The Tenax traps are heated for 30 seconds at a temperature of 200 ºC.

- 1630 describes the GC column as being 1 meter long (**Section 6.5.6.1**); whereas, the GC column used at Brooks Rand Labs is 50 cm long.

- **Section 6.5.6.4** of 1630 states that the GC column should be heated to a temperature of 110 ºC (± 2 ºC). At Brooks Rand Labs the GC column is set at a temperature of 90-105 ºC.

- **Section 7.8** of 1630 states that frozen ethylation reagent ($NaBEt_4$) is good for one week. Brooks Rand Labs has found frozen $NaBEt_4$ to be stable for up to one month as long as it has not been thawed.

- **Sections 9.4.1, 10.1.1.2, 11.2.1, and 11.2.2** of 1630 state that the bubblers with ethylation blanks, standards, and samples should have 300 µL of acetate buffer and 40 µL of $NaBEt_4$ added to them prior to purging. Brooks Rand Labs adds the 300 µL of acetate buffer, but adds 50 µL of $NaBEt_4$.

- The calibration standards used in 1630 are listed, in order of analysis, as 5 pg, 50 pg, 100 pg, 200 pg, and 1000 pg (**Section 10.1.1.2**). Brooks Rand Labs calibrates the instrument using the following standards: 2 pg, 10 pg, 50 pg, 250 pg, and 1000 pg.

- **Section 11.1.1** of 1630 makes no mention of adding additional H_2SO_4 to the sample prior to distillation. Brooks Rand Labs routinely adds 0.5 mL of 9M H_2SO_4 to each distillation vial prior to beginning the distillation.

- **Section 11.1.1** of 1630 states that 200 µL of 1% APDC solution is used as the chelator and is added to the distillation vial just prior to beginning the distillation. Brooks Rand Labs uses a

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20% KCl / 0.2% L-Cysteine solution as the chelator and adds 200 µL of this solution to the distillation vial.

- 1630 states that once the sample preparation and all of the reagents have been added to the reaction vessel (bubbler), they should be allowed to react for 17 minutes so that all of the methyl mercury is converted to volatile methylethyl mercury prior to purging (**Sections 10.1.1.2 and 11.2.4**). Brooks Rand Labs has found that 15 minutes are sufficient for this process.

- 1630 states that the bubbler contents should be purged onto the trap for 17 minutes (**Section 11.2.5**). Brooks Rand Labs has found that 15 minutes are sufficient for this process.

- Following purging, 1630 states that any adsorbed water should be removed from the Carbotraps® by drying the trap for 7 minutes with N_2 gas (**Section 11.2.6**). Brooks Rand Labs has found that 5 minutes is sufficient for drying the Tenax traps.

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Appendix B: Manual MeHg Analysis

INSTRUMENT CALIBRATION AND SAMPLE ANALYSIS

Instrument Calibration: BRL follows EPA method 1630, Section 10.0 for the instrument calibration with the same exceptions as for sample analysis. Standards, typically 2, 10, 50, 250, and 1000 pg MeHg are added into reaction vessels containing 50-75 mL of DIW and 300 µL of 2M acetate buffer.

Instrumental Analysis: BRL has adopted the following modifications.

For samples, add appropriate sample volumes plus DIW as necessary for a final bubbler volume of 50 mL to 75 mL (for sample preparation see Section 7 of this SOP). Some acid can be carried over during distillation causing some distillates may have low pH (< 4). In that event, up to 1 mL of acetate buffer should be added to bring the final pH to 4 – 5.5, which is the optimum pH for ethylation. An aliquot of 50 µl of $NaBEt_4$ is added, the 4-way valve-cap inserted and clamped, and the vessel swirled to wash any droplets back into solution. Allow the mixture to react without purging for 15 minutes.

Place a trapping column on the bubbler with the kinked side toward the bubbler. It is best to load the kinked side then reverse the flow into the GC column so the trapped species do not have to flow all the way through the Tenax trap. The bubbler is then purged with N_2 at a flow rate of 250 $mL \cdot min^{-1}$ (rotometer set at 35) for 15 minutes. The organomercury compounds are swept from solution and collected onto the trapping column. Then the trap is taken off and hooked directly to the gas-line for 5 minutes, to remove water condensation from the trap. Afterwards, connect the trapping column in-line with the GC column with the kinked side toward the GC.

When using a Tenax trap, special attention must be paid to the orientation of the trap. The trap is placed so that the end facing the bubbler output is now facing the GC column input to avoid the organomercury species passing through the entire length of the heating trap column and decomposing to Hg(0) (Liang, Bloom, and Bloom 1994). Under a argon flow rate of 45 $mL \cdot min^{-1}$ (rotometer set at 45), apply appropriate voltage to the coil around the column so that the column reaches 200 °C from room temperature within 30 seconds. Peak integration is begun in conjunction with the heating of the trap. The organomercury species are desorbed and carried through the GC column that is held in an oven at 90-105 °C. The species elute in order of increasing molecular weight, pass through the pyrolitic column, held at approximately 900 °C at which point all organomercury species are converted into Hg(0), and detected by CVAFS. The sparging/reaction vessels must be triple rinsed with DIW after each use to flush out reaction by-products that can interfere with subsequent purgings.

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