

NOVEMBER 2000 REVISION

Quality Assurance Project Plan for Monitoring

Appendices

Prepared for:

Sacramento River Watershed Program

By

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Appendix A

Sampling and Analytical Responsibilities and Contacts

SAMPLING RESPONSIBILITIES AND CONTACTS

Agency or Company	Primary Contact (Phone #)	SRWP Monitoring Element
California Department of Fish and Game (Moss Landing Marine Lab)	Mark Stephenson (408) 633-0253	Event-based water samples at Greene's Landing: • Water Chemistry
Sacramento Regional County Sanitation District (Coordinated Monitoring Program)	Andrew Frankel (916) 875-9133	Sacramento River at Veterans Bridge, Freeport and River Mile 44: • Water Chemistry • Pathogens • Aquatic Toxicity
Pacific EcoRisk (Aquatic Toxicology Lab)	Stephen Clark (925) 313-8080	Water samples at all remaining locations: • Water Chemistry • Pathogens • Aquatic Toxicity
California Department of Fish and Game (Moss Landing Marine Lab)	Gary Ichikawa (831) 633-6032	• Fish Tissue at all sites
California Department of Fish and Game (Water Pollution Control Laboratory)	Jim Harrington (916) 358-2858	• Bioassessment at all sites

ANALYTICAL LABORATORIES AND CONTACTS

Laboratory	Address	Primary Contact (Phone #)	<u>Monitoring Element and Analytes</u>
APPL Labs	APPL, Inc. 4203 West Swift Street Fresno, CA 93772	Glen Brown (209) 275 2176	<ul style="list-style-type: none"> • <u>Water Chemistry</u> organophosphate, carbamate, and triazine pesticides
BioVir Laboratories Inc.	BioVir Laboratories Inc. 685 Stone Road Benicia, CA 94510	Rick Danielsen (800) 442-7342	<ul style="list-style-type: none"> • <u>Pathogens</u> <i>Cryptosporidium</i>, <i>Giardia</i>, Coliform bacteria
Frontier Geosciences	Frontier Geosciences 414 Pontius North Address Seattle, WA 98109	Misty Kennard (206) 622-6960	<ul style="list-style-type: none"> • <u>Water Chemistry</u> MeHg
Moss Landing Marine Lab	Moss Landing Marine Lab, 7711 Sandholdt Road, Moss Landing, CA 95039	Mark Stephenson (831) 633-0253	<ul style="list-style-type: none"> • <u>Water Chemistry</u> Hg, trace metals • <u>Fish Tissue</u> Hg
Pacific EcoRisk	Pacific EcoRisk 827 Arnold Dr., Suite 100 Martinez, CA 94553	Stephen Clark (916) 921-9600	<ul style="list-style-type: none"> • <u>Water Chemistry</u> conventional parameters • <u>Aquatic Toxicity/TIE</u> <i>C. dubia</i>
Sierra Foothill Laboratory	823 South Highway PO Box 1268 Jackson, CA 95642	Sandy Nurse (209) 223-2800	<ul style="list-style-type: none"> • <u>Water Chemistry</u> organic carbon
Water Pollution Control Laboratory	Dept. of Fish and Game, 2005 Nimbus Road, Rancho Cordova, CA 95670	Jim Harrington (916) 358-2858	<ul style="list-style-type: none"> • <u>Bioassessment</u> Benthic invertebrates, physical habitat
Water Pollution Control Laboratory	Dept. of Fish and Game, 2005 Nimbus Road, Rancho Cordova, CA 95670	David Crane (916) 358-2858	<ul style="list-style-type: none"> • <u>Fish Tissue</u> PCBs, chlorinated pesticides, dioxins, dibenzo-furans, co-planar PCBs

Appendix B

Calculations for Data Quality Assessments

Calculations for Data Quality Assessments

This appendix documents the calculations used to assess precision, accuracy, and completeness of the data.

Precision

Precision is a measure of the degree to which replicate measurements differ from one another. Precision assessed through calculation of field and laboratory duplicates, and matrix spike duplicates is expressed as the Relative Percent Difference (RPD).

RPD for laboratory and field duplicates is calculated as follows:

$$\text{RPD} = 100 \times \frac{|\text{replicate 1} - \text{replicate 2}|}{(\text{replicate 1} + \text{replicate 2}) \div 2}$$

RPD for matrix spike duplicates is calculated as follows:

$$\text{RPD} = 100 \times \frac{|\text{Recovery 1} - \text{Recovery 2}|}{(\text{Recovery 1} + \text{Recovery 2}) \div 2},$$

where *Recovery* is calculated as described for matrix spikes, below.

If assessed with three or more replicate measurements, precision should be expressed as Relative Standard Deviation (RSD). RSD is calculated as:

$$\text{RSD} = 100 \times \frac{\text{standard deviation of replicate measurements}}{\text{average of replicate measurements}}$$

Accuracy

Accuracy is the degree to which a measured value agrees with a true or expected value for a parameter. Accuracy is typically assessed using standard reference materials, laboratory control samples, and matrix spikes.

Recovery of laboratory control samples and standard reference materials is calculated as:

$$\% \text{ Recovery} = 100 \times \frac{\text{recovered concentration}}{\text{true spike concentration}}$$

Recovery of matrix spikes is calculated as:

$$\% \text{ Recovery} = 100 \times \frac{\text{total recovered concentration} - \text{sample concentration}}{\text{true spike concentration}}$$

When sample concentrations are less than the method detection limit, a value of "0" (zero) will be used as the sample result concentration for purposes of calculating spike recoveries.

Completeness

Completeness may be defined as the number of valid measurements compared to the total number of measurements collected. Completeness is calculated as:

$$\% \text{ Completeness} = 100 \times \frac{\text{number of valid measurements}}{\text{total number of measurements}}.$$

Appendix C

Supporting Documents for Water Quality Monitoring

**Field Sampling Procedures
(CDFG 1993)**

**Methylmercury Sampling Procedures
(CDFG 2000)**

DFG SOP—100
Field Sampling Methyl and Total Mercury in Water Based Upon
Frontier Geoscience's SOP 008 and Modified EPA Method 1669

Department of Fish and Game
Moss Landing Marine Labs
7711 Sandholt Rd.
Moss Landing, CA.
95039

Originated by: Mark Stephenson

February 26, 2000

1.0 SCOPE AND APPLICATION

- 1.1. This SOP describes the techniques used to collect and preserve water samples for mercury and methyl mercury in a way that neither contaminates, loses or changes the chemical form of the analytes of interest
- 1.2. The samples are collected in the field into previously cleaned and tested sample bottles of a material appropriate to the analysis to be conducted
- 1.3. Appropriate sampling technique may vary depending on the location, sample type, sampling objective, and weather.

2.0 SUMMARY OF METHOD

- 2.1. Sample bottles that have tested low for trace metals after the cleaning procedure are double bagged in a class 100 clean air bench or class 100 clean room. At the site the bottles are either filled with water using the “clean hands-dirty hands” technique (Frontier FGS 008.2) if an unfiltered sample is desired, or they are filled with water using a pumping system if a filtered sample is desired. Bottles are resealed tightly and re-bagged using the opposite series of steps as were used to open them. Bottles are shipped to the laboratory via over-night courier for further processing (filtration, etc.) and/or preservation.

3.0 INTERFERENCES

- 3.1. There are no known interferences.

4.0 SAFETY

- 4.1. The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the MSDS for each chemical they are working with.
- 4.2. All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease-causative agents.

5.0 EQUIPMENT

- 5.1. **Clean Filtration Bench.** A clean sampling bench for filtering mercury samples consists of a sheet of polyethylene that a fresh sheet of polyethylene film can be taped to. It has supports to hold one 4-l bottle and 6-250ml bottles. It has a bracket to hold an umbrella if it is raining or dusty.
- 5.2. **Sampling Pump.** A peristaltic pump is used to pump samples from the composite bottle to the smaller sample bottles. The pump tubing consists of a short piece of CFLEX tubing with teflon tubing tightly fitting into both ends of the C-flex tubing.
- 5.3 **Composite sample bottles.** Large 4 or 2.5 l bottles are used to collect composites but either have to be old acid bottles that have only been used to store Baker concentrated acid or new bottles that have been cleaned according to the cleaning procedure described below.
- 5.4 **Filters. Gelman No. 12178.** A random selection of the filters from each lot shall be initially tested to verify cleanliness. These filters normally will not require pre cleaning.
- 5.5 **IChem series 300, 250ml glass bottles** with lids with teflon liners are used

for sample bottles. These bottles normally will not require pre cleaning.

6.0 CLEANING PROCEDURES (6.1-6.3 from Gary Gill)

6.1 Composite Bottles

1. soak the whole bottle in hot (> 40 oC) lab detergent (i.e. micro) bath for 3+ days
2. Rinse with DI water
3. Fill with 7.5 N reagent grade HNO_3 and immerse in hot (> 40 oC) (weak or 7.5 N HNO_3) bath for 3+ days
4. Rinse with MQ water under class-100 conditions
5. Fill with high purity 0.5% HCl for storage; dry on clean bench if necessary, and double bag in new bags.

6.1.1 Alternate cleaning procedure developed by DFG for composite bottles

New 4 liter amber glass bottles with polyethylene caps will be cleaned by soaking them for 24 hours in 50% reagent grade HCL at room temperature. The HCL will be decanted and the bottle will be rinsed with 5 rinses of MilliQ water to remove traces of acid. Initially 50% of the bottles will be tested for Hg, thereafter, 10% will be tested if all the bottles blank out about .1 to .2 ng/L.

6.2 Teflon Tubing

1. Fill or immerse the tubing in a heated (> 40 oC) lab detergent (i.e. micro) bath for 3+ days
2. Rinse with large amounts of DI water
3. Fill with 7.5 N reagent grade HNO_3 and immerse in hot (> 40 oC) (7.5 N HNO_3) bath for 3+ days. We do this by coiling up the tubing into a small coil and holding it in place with Teflon tape. We use a large glass jar filled with 7.5 N HNO_3 for this treatment. Hold the tubing under the surface and rotate the tubing coil in the (cold) HNO_3 bath so that all the air pockets are removed. Then warm it up. Reverse the process when you remove the tubing from the bath.
4. Rinse with MQ water under class-100 conditions
5. Fill with high purity 0.5% HCl for storage; dry on clean bench if necessary and store new polyethylene bags.
6. Ten percent (may be reduced to 5% if cleanliness is proven) of the tubing

assemblies will be pretested for blanks by pouring or pumping 1 l of water through the tubing for a rinse and then pouring or pumping 250mls into a sample bottle.

6.3 C-Flex Tubing

C-flex can stand up to concentrated HCl, but not HNO₃. Cut it into approximately 1 - 1.5 ft. lengths and place several lengths vertically into a 1 L plastic graduated cylinder. Fill the cylinder with concentrated reagent grade HCl and let it stand cold for several days in a fume hood. The acid is then poured off and the tubing is rinsed in the cylinder in batch with numerous volumes of DI water. The cylinder is then filled with high-purity 0.5% HCl and left to stand cold for 3+ days on a clean bench. The weak acid solution is then poured off and the tubing is rinsed in batch in the cylinder in the clean room with lots of high purity MQ water. Dry it on a clean bench and store it in poly bags until use. 6. Ten percent (may be reduced to 5% if cleanliness is proven) of the tubing assemblies will be pretested for blanks by pouring or pumping 1 l of water through the tubing for a rinse and then pouring or pumping 250mls into a sample bottle.

6.4 Filters.

Filters do not normally require precleaning. However, a random selection of the filters from each lot (one in each batch of 50) shall be initially tested to verify cleanliness.

6.5 Sample Bottles.

IChem series 300, 250ml 1000 ml glass bottles with lids with teflon liners are used for sample bottles and normally do not require precleaning. Each lot is tested (10% of total) to verify low blanks by rinsing 3 times with MilliQ, filling the bottle with MilliQ and .5% HCl, letting stand for 24 hours, and analyzing for Hg.

7.0 REAGENTS

7.1. *Water:* MilliQ water will be used for all rinses or making dilutions of acid.

7.2. *Hydrochloric Acid.* Baker reagent grade acid will be used. It is preanalyzed for Hg if it is used for any purpose but cleaning glassware.

7.3. Bromine Monochloride, 0.2 N - 27 g of KBr are added to a 2.5-L bottle of pre-

tested concentrated HCl. A clean magnetic stir bar is placed in the bottle, and it is stirred for 1 hour in a fume hood. Next, 38 g of pre-analyzed, low Hg KBrO₃ is slowly added to the acid while stirring. When all of the KBrO₃ has been added, the solution should have gone from yellow to red to orange. Loosely cap the bottle, and allow to stir another hour before tightening the lid. CAUTION: THIS PROCESS GENERATES COPIOUS QUANTITIES OF FREE HALOGENS (Cl₂, Br, BrCl) WHICH ARE RELEASED FROM THE BOTTLE. ADD THE KBrO₃ *SLOWLY* AND IN A WELL OPERATING FUME HOOD!

8.0 Field Sampling Protocols Sampling Protocols for Mercury and Methyl Mercury Water Samples

8.0.1 Pre-sampling preparations

From information obtained at a site visit determine which equipment will be needed such as: type of sampler to be used. Have all Teflon or glass bottles used in clean sampling procedures double bagged in plastic and placed in appropriate containers for transport to the field site. Obtain the proper cleaning equipment and supplies such as: vinyl or polyethylene powderless gloves, Milli-Q water in liter bottles for rinsing, and waste containers.

8.0.1.1 Obtain processing equipment: splitter (composite bottle), pre cleaned Teflon tubing and C-flex tubing, pre-labeled clean sample bottles, or other equipment that should be kept clean.

8.0.1.2 Obtain filtration equipment: Filtration chamber, peristaltic pump and heads, peristaltic motor assembly.

8.0.1.3 Obtain and check equipment for ancillary measurements including: thermometers, pH meter, conductivity meter, dissolved oxygen meter, current meter (if discharge measurements are to be made), pH buffers, conductivity standards, alkalinity titration acids, de-ionized water if needed.

8.0.1.4 Check for need for special supplies including safety equipment, boots, first-aid kit, traffic control devices, coolers and ice.

8.0.1.5 Pre-label all environmental sample bottles with site identifier, unique sample number, date and type of sample.

8.1.2 General sampling procedures for collecting non contaminated mercury samples

8.1.2.1 Use a peristaltic pump for all samples requiring filtering. If the sample does not require filtering the sample can be collected by dipping the bottle into the stream or river by hand using methods in Frontier Geoscience's SOP FGS-008.

Two people are required for sample collection; determine which person is designated as clean hands and which person is designated as dirty hands for the duration of the sampling event. The personnel should all wear vinyl or polyethylene gloves. Gloves can be discarded during sampling if anything happens which may compromise the cleanliness of the gloves.

8.1.2.2 **Sample Compositing and Filtering**

8.1.2.2.1 Sample is collected in a amber glass 4l bottle. It is very critical that all the acid is rinsed out of the bottle before the sample is taken. It is dipped into the stream or water body to collect the sample (after being rinsed three times with ambient water) and is brought inside the water quality truck or sampling box for processing.

8.1.2.2.2 Personnel involved in sample processing don vinyl or polyethylene gloves.

8.1.2.2.3 During sampling the dirty hands person opens the bag holding the composite bottle and opens the outer plastic bag. The clean hands person opens the inner plastic bag and places the bottle on a plastic covered surface of the clean bench.

8.1.2.2.4 The Teflon or glass 250 ml sample bottles are taken from the double wrapped plastic bags in a manner exactly like that of the field sampling. The dirty hands person opens the first bag, and the clean hands person opens the inner bag around the bottle. The clean hands person then removes the bottle from the inner bag and places the bags and the bottle in a designated secure places in the box. This process is repeated until all bottles are lined up in the box with their tops still on. The top of the bottles are loosened so that they fit very loosely on top of the bottles so the clean hands person can remove the caps and pour or pump water into the bottles easier.

8.1.2.2.5 The clean hands person shakes the 4l sample in a steady and slow up and down motion for two full minutes. Samples that are not to be filtered (including TSS) are subsampled out of the bottle by pouring out of the large compositing bottle into the

250 ml bottles. The compositing bottle is shaken for 15 seconds between these subsamples. Each 250 ml bottle is rinsed 3 times with ambient before filling to the neck. The filled bottles are then capped.

- 8.1.2.2.6 The dirty hands collector uses new gloves and sets up the pump and opens the outer bag holding the tubing. The clean hands person opens the inner bag, takes out the tubing and holds the clean ends while the dirty hands person threads the tubing through a hole in the back of the clean box to the pump head.
- 8.1.2.2.7 Samples that need to be filtered are then processed. The clean hands person then puts the suction end of the clean tubing into a 1 l bottle of MilliQ water and holds on to the other end with a gloved hand making sure that the clean teflon tubing does not contact any surface of the box. One l of the MilliQ water is then pumped through the tubing and filter and discarded.
- 8.1.2.2.8 Equipment blanks are taken at this time (if required) by filling a sample bottle with the last 250mls of MilliQ. The Equipment or field blank is put through all the processes as the sample is put through. The clean end of the tubing used for suction is then placed into the 2.5 liter bottle. Approximately 50 mls of sample are then pumped through the system to get rid of any residual MilliQ. The 250 ml sample bottles are then filled.
- 8.1.2.2.9 The program requires the PI's to collect one sample in 20 in duplicate to send off to Frontier Geoscience for QA purposes. In this case a filtered sample will be pumped from the composite bottle or a unfiltered sample will be collected by shaking and aliquoting from the composite bottle.

8.1.2.4 **Preservation**

This step occurs in the laboratory within 48 hours after collection. The samples are kept cold during transport on blue ice and during storage in a refrigerator, at 1- 4 C, until preservation. The methyl mercury samples also have to be kept in the dark. In the lab the preservations are according to Frontier's method FGS 008.2 and are as follows:

Methyl Mercury - Freshwaters - Samples for determination of methyl mercury should be preserved to 0.4% (v/v) with HCl if their salinity is less than 1%.

Seawaters – Samples for the determination of methyl mercury should be preserved to 0.2% H₂SO₄ if their salinity is greater than 1%.

Total Mercury - Samples for determination of total mercury are preserved to 1-5% (v/v) with 0.2 N BrCl, but this should only occur in the laboratory. If samples for total mercury need to be preserved in the field, they should be preserved to 0.4% (v/v) with HCl. Acid preserved samples are stable indefinitely (> 6 months), although the current EPA-mandated holding time is still 28 days.

8.1.2.5 **Labeling Bottles**

Each bottle must have site identifier, date of collection, time of collection, unique sample number and sample type..

8.1.2.6 **Sample tracking**

Sample tracking: Field samples are then checked for proper labeling including site id, date, and time. Field are filled out with information on sample collectors and processing and other relevant information. Chain of custody forms are prepared for all samples transferred to another lab.

8.1.2.7 **Shipping**

Mercury and methyl mercury samples are double-bagged in plastic and placed in a cooler on blue ice. All bottles will be double bagged in 2 Ziploc bags and one bubble wrap bag.

**Evapo-Concentration Procedure
(CDFG 1993)**

Methylmercury Distillation of Aqueous Samples: FGS-013.2
(Frontier Geosciences 2000)

Methyl Mercury Distillation of Aqueous Samples

FGS-013.2

Frontier Geosciences Inc.
414 Pontius Avenue North
Seattle, WA 98109

Originated by: Nicolas S Bloom and Efrosini Tsalkitzis

Revised by: Paul Laskowski

January 3, 2000

1.0 SCOPE AND APPLICATION

- 1.1. This method is for the determination of methyl Hg in aqueous media (natural waters, precipitation, pore water, industrial and municipal effluents) at concentrations of less than 0.05 ng/L. Through the use of smaller aliquots (mL to mL range) contaminated waters and effluents of up to 1,000 ng/L can be directly measured. In general, using clean handling and reagents, method detection limits in the range of 0.01- 0.03 ng/L are routinely attainable.
- 1.2. Methyl mercury as defined by this method means all chloride-distillable methyl mercury forms and species found in aqueous solution and on aqueous suspended matter. This includes but is not limited to CH_3Hg^+ , CH_3HgCl , CH_3HgOH , and $\text{CH}_3\text{HgS-R}$.

2.0 SUMMARY OF METHOD

- 1.1. Samples are collected using ultra-clean sample handling protocols (Bloom, 1995) into rigorously cleaned Teflon™ bottles. Samples are preserved with 0.5% (v/v) pre-analyzed HCl, and stored in a cool, dark location until analysis. Before analysis, (Horvat, et. al, 1993; Bloom 1989; Bloom and Fitzgerald, 1988), the methyl Hg in an aliquot of the sample (typically 45 mL) is co-distilled into pure water. The distillates are then analyzed for methyl mercury by aqueous phase ethylation and GC – CVAFS as described in SOP FGS _____

3.0 INTERFERENCES

- 3.1. The use of the distillation pre-extraction procedure (Horvat, et. al, 1993) eliminates all method interferences from organic matter, particulates, and sulfides

which affected the methylene chloride extraction procedure (Bloom, 1989).

- 1.2. Under no circumstances should ordinary plastic (polyethylene, polypropylene or vinyl) containers be used, as they are very diffusive to Hg^0 gas from the air. The best containers are made of hot-acid cleaned Teflon™, although muffled (525°C for 6 hours) or rigorously acid cleaned Borosilicate or quartz glass bottles with Teflon™ caps may be used as well. It is critical that the bottles have very tightly sealing caps to avoid diffusion of atmospheric Hg through the threads.
- 1.3. The low detection limit of this method depends on the stringent cleaning of equipment used for sample collection and storage. The procedure for this is indicated below in section 6 ("sampling bottles").
- 1.4. It is important that the sample HCl concentration be between 0.2% and 0.6% (v/v) for effective distillation of the CH_3HgCl . If too high an acid concentration is present, HCl will co-distill, resulting in an interference with the ethylation procedure.
- 1.5. No HNO_3 or other oxidizing agents (Cl_2 , BrCl , $\text{CrO}_4^{=}$, etc.) may be present in the sample, or CH_3Hg may be destroyed. Particular care must be taken to eliminate the chlorine present in the municipal water that feeds the deionized water system, by passing it through an activated carbon bed.

4.0 SAFETY

- 4.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined. However, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. Exhibit particular caution in the preparation and use of sodium tetraethyl borate, as organo-boron compounds are known nerve toxins. Further, the solid material may spontaneously combust on exposure to air. Always handle this reagent in an approved fume hood.
- 4.2 Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan. This includes, but is not limited to, laboratory coat, safety goggles, and latex gloves under clean gloves.
- 4.3 The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and

exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the MSDS for each chemical they are working with.

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

5.0 EQUIPMENT

- 1.1. *Teflon™ Still:* The still (Figure 17.2) is constructed from 60 mL heavy wall Teflon™ vials (Savillex). The vial is fitted with a special cap, allowing insertion of 1/8" teflon tubing. Each vial should be engraved with a line at the 45.0 mL mark, determined by weighing 45.0 g of deionized water into the vial. One length of tubing goes from the purge nitrogen source into the distillation vessel, all the way to the bottom. The second length of tubing goes from the head space of the distillation vessel into the bottom of an identical vessel, which is the distillate receiver. The distillation vessel is heated in a temperature controlled aluminum block to approximately 145° C, while the receiving vessel is maintained in an ice/water bath. Nitrogen purge rate is 60 ± 20 mL/min. The exact block temperature is adjusted to obtain a distillation rate of 6-10 mL per hour.

- 1.2. *Sampling Bottles:* It is imperative for accurate sub-ng/L methyl mercury measurements that appropriately cleaned Teflon™ bottles with tight-fitting lids be used for all steps contacting the aqueous sample. New Teflon™ bottles are cleaned by heating to >95° C in 4 N HCl for at least 48 hours. The bottles are cooled, rinsed 3 times with ultra-clean water, and then filled with ultra-clean water containing 1% HCl in ultra-clean water. These bottles are capped and placed in a clean oven at 60° C overnight. After cooling, they are rinsed 3 more times, filled with ultra pure water plus 0.4% HCl, and placed in a low mercury class 100 clean-air station until dry. The bottles are then tightly capped and double bagged in new polyethylene zipper bags until needed. After the initial cleaning, bottles are cleaned by heating for about 6 hours in 4 N HCl, rinsing 4 times, and filling with high purity 0.4% HCl solution.

6.0 REAGENTS

- 6.1. *Water:* 18 megohm ultra-pure deionized water starting from a pre purified (distilled, R.O., etc.) source. As a final mercury and organic removal step, the

activated carbon cartridge on the 18 megohm system is placed between the final ion exchange bed and the 0.2 μ M filter. Water should be routinely monitored for Hg--especially after ion exchange beds are changed. Water should typically contain less than 0.2 ng/L total Hg, and remedial action should be taken if the total Hg exceeds 1 ng/L. Methyl Hg should be undetectable in reagent water.

6.2. *Hydrochloric Acid:* Trace-metal purified reagent HCl is purchased and pre-analyzed for Hg before use. It is possible to obtain acid containing less than 5 pg/mL Hg. When a lot number meeting this specification is found, several cases are purchased, and stored in a low Hg atmosphere (i.e., in clean lab or outside the building). Generally, lower values can be obtained in this manner, than by re-distilling acid in the laboratory. So called ULTRA-PURIFIED acids are often the most irreproducibly contaminated (for mercury) grade of acid commercially available and should be avoided.

6.3. *1% APDC Solution:* To 100 mL of deionized water, add 1.0 gram of reagent grade APDC (ammonium pyrrolidine dithiocarbamate), and shake to dissolve. The solution is purified by extraction with three 10 mL aliquots of methylene chloride.

1.4. *Nitrogen:* Grade 4.5 (standard laboratory grade) nitrogen which has been further purified by the removal of Hg using a gold-coated sand trap.

7.0 SAMPLE COLLECTION, HANDLING AND PRESERVATION

1.1. Samples should only be collected into rigorously cleaned Teflon™ quartz, or borosilicate glass bottles with Teflon™ caps. Appropriate sample identification numbers are transcribed onto all subsequent laboratory tracking/reporting forms.

1.2. It is critical that the bottles have very tightly sealing caps to avoid diffusion of atmospheric Hg through the threads (Gill and Fitzgerald, 1985). As an added precaution, clean bottles, filled with high purity 0.5% HCl are dried, capped, double bagged in new zipper lock bags in the cleanroom, and stored in wooden or plastic boxes until use.

1.3. Samples are collected using rigorous ultra-clean protocols (Bloom, 1995; Gill and Fitzgerald, 1985), which are summarized as follows.

1.4. At least two persons, wearing fresh cleanroom gloves at all times are required on a sampling crew.

- 1.5. One person ("dirty hands") pulls a bagged bottle from the box, and opens the outer, dirty bag, avoiding touching inside that bag.
- 1.6. The other person ("clean hands") reaches in, opens the inner bag, and pulls out the sample bottle.
- 1.7. This bottle is opened with a dedicated, plastic shrouded wrench, and the acidified water discarded downstream of the sampling site.
- 1.8. The bottle is rinsed once with sample water, and then completely filled.
- 1.9. Preservative (i.e., 0.5% v/v low Hg HCl) may be added at this time, or within 48 hours in a clean laboratory.
- 1.10. The cap is replaced with the wrench, and the bottle re-bagged in the opposite order from which it was removed.
- 1.11. Cleanroom gloves are changed between samples and whenever something not known to be clean is touched.
- 1.12. Water is best obtained by a surface grab, using gloved hands, and facing into a flowing body of water (i.e., looking upstream or off the bow of a moving boat). If samples are to be taken from depth, the only non-contaminating method generally available is pumping. Two methods have been found to work in this regard. The first is to use rigorously cleaned Teflon™ tubing and a peristaltic pump with freshly cleaned (heating at 70° C in 5% HCl + 5% CH₃COOH) silicone tubing. Beware that once cleaned, silicone tubing quickly absorbs Hg from the air. The other method involves high volume pumping (i.e., 50 L/min) through neoprene hose. If this method is used, it is best to clean the system first by pumping several hundred litres of 5% HCl solution, and then pumping clean water for several hours. The second technique works largely because the rate of flow is so high that the contamination, if present, becomes imperceptibly diluted.
- 1.13. Discrete samplers, such as Niskin, Go-Flo, and Kemerer Bottles are to be avoided, as under even the best conditions, they are often found to grossly contaminate at the ng/L level.
- 1.14. Samples may be preserved by adding 0.5% (v/v) of HCl. Samples may also be sent back to the laboratory unpreserved if they are 1) collected in

Teflon™ bottles, 2) filled to the top with no head space, and 3) sent to the laboratory at 0-4° C by overnight delivery. The samples should be preserved with acid as above within 48 hours of collection.

- 1.15. Following preservation, samples must be stored at 0-4° C until analysis, and must be analyzed within 28 days of collection, according to US EPA storage criteria for aqueous mercury. Laboratory studies have indicated that Hg speciation in water samples is stable for at least 3 months under these conditions (Bloom, et. al, 1995).

8.0 PROCEDURES

- 8.1. 45.0 mL of sample, preserved with 0.4% (v/v) 12.2 N HCl, are placed into a clean distillation vessel. If the sample was unpreserved, then 0.2 mL of 12.2 N HCl are added at this time. A smaller aliquot volume may be used if higher levels of MHg are expected. These are diluted to 45mL with a 0.5% HCl solution.
- 8.2. The Sample ID and aliquot size of each sample is written into a sample preparation logbook. A copy of the relevant page(s) will follow the distillates through the lab.
- 8.3. To the sample add 200 uL of 1% APDC solution. The distillation cap is replaced, and the outlet tubing is connected to the receiving vessel.
- 8.4. To the empty receiving vessel 5.0 mL of deionized water are added, covering the bottom of the tubing from the still.
- 8.5. The receiving vial is labeled with the sample ID of the sample that was aliquotted into it's respective distillation vial.
- 8.6. The receiving vessel is placed into an ice/water bath. The purge nitrogen is turned on (set flow to 20 on flow meter), and the heating block brought up to 125° C.
- 8.7. Distillation should be maintained at a rate of 10-15 mL per hour, until a total volume of 40 ± 1 mL is achieved in the receiving vial. The distillation for a water sample of this volume should take approximately 3 hrs hours.
- 8.8. The pH of each sample is taken and noted on the vial if not between 5-7.

8.9. The sample may then be analyzed using aqueous phase ethylation, GC separation, and CVAFS detection. Normally, for water samples, the entire sample will be analyzed, and calculation based upon the actual volume of sample distilled, corrected by the empirically determined distillation recovery factor of 90.6% (see Horvat, et. al, 1993). For samples high in methylmercury, a subfraction of the total distillate may be analyzed.

9.0 QUALITY CONTROL

9.1. Maximum Sample Batch Size: 25 samples for standard level QC; 20 samples for high level QC.

9.2. Preparation Blanks: 3 per batch, standard deviation used to determine estimated MDL for each batch.

9.3. Blank Spike: 1 per batch, 75-125% recovery limit.

9.4. MD: 1 per batch, 25 RPD limit.

9.5. MS/MSD: 1 per batch, 70-130% recovery limit, 25 RPD limit.

10.0 CORRECTIVE ACTIONS

10.1. Corrective action to follow if quality assurance measure is out.

11.0 EQUATIONS

11.1. Equation 1.

11.2. Equation 2.

12.0 REFERENCES

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Methylmercury Analysis and Calibration: FGS-070.1
(Frontier Geosciences 2000)

Methyl Mercury Calibration and Analysis

FGS-070.1

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Seattle, WA 98109

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May 15, 2000

1.0 SCOPE AND APPLICATION

- 1.1. This standard operating procedure (SOP) is designed to ensure that reproducible, traceable procedures are followed in the standardization of the methyl mercury (MeHg) analyzers and in the analysis of samples for methyl mercury, as well as to establish the bounds wherein data will be considered acceptable.
- 1.2. This method provides for the determination of methyl mercury in a wide range of matrices including aqueous, biologic, and geologic media. In general, using clean handling and reagents, the typical detection limits for the method are in the range of 0.010-0.030 ng/L for aqueous samples, 0.005-0.020 ng/g for low-level solids by distillation, and around 1.0 ng/g for solids by extraction or digestion.
- 1.3. This SOP consists of three aspects: (1) preparation of methyl mercury standard solutions, (2) calibration of the methyl mercury analyzers, and (3) analysis of samples for methyl mercury in various matrices.

2.0 SUMMARY OF METHOD

2.1. Preparation of Methyl Mercury Standards

- 2.1.1. Methyl mercury standard solutions are prepared using ultra-clean class A volumetric glassware and gravimetrically calibrated pipettors. All reagents (including reagent water) are pre-tested and must have very low mercury concentrations.
- 2.1.2. All standard solutions, preparations, and calibrations must be recorded in the Mercury Standards Logbook upon receipt or creation and given a unique identification number.
- 2.1.3. Any standard and its original documents that are received should be labeled with the receipt date and the receiver's initials, all documentation should be given to the QA Office. The QA Office is responsible for

maintaining standard records as well as updating the folders in the laboratory where the copies are kept.

2.2. Methyl Mercury Analyzer Calibration Sequence

- 2.2.1. The calibration sequence for the determination of methyl mercury consists of a 5-point curve (0.005 ng, 0.010 ng, 0.050 ng, 0.100 ng, and 0.200 ng), an instrument calibration verification (ICV), an instrument calibration blank (ICB), and a quality control sample (QCS).
- 2.2.2. The calibration standard is made from a serial dilution of the initial stock solution obtained from Strem Chemical which has been verified against NIST 3133.
- 2.2.3. The calibration sequence determines the range of sample concentrations that are reportable.

2.2. Methyl Mercury Analysis

- 2.3.1 All methyl mercury runs receive a unique dataset identifier. This is composed of the instrument type and number, the date, and the calibration number for that day. The format is as follows: MHG7-000224-1, where:
 - 2.3.1.1 “MHG” refers to the type of analysis
 - 2.3.1.2 “7” refers to methyl mercury instrument number 7
 - 2.3.1.3 “000224” refers to the date, February 24, 2000, in YYMMDD format
 - 2.3.1.4 “1” refers to the first calibration of that day
- 2.3.2 Methyl mercury analyses are split into three categories: waters, sediments/soils, and tissues.
 - 2.3.2.1 For water and sediment/soil analysis, an aliquot of prepared sample is added to a bubbler and brought up to a volume of approximately 50 mL with reagent water. Acetate buffer and ethylating agent is added to each bubbler.
 - 2.3.2.2 For the analysis of tissues, approximately 50 mL of reagent water is added to each bubbler, followed by acetate buffer and ethylating agent.

2.3.2.3 For all analyses, the bubbler connections are sealed with Teflon end plugs. After a set reaction time, end plugs are removed, blanked carbotraps are securely placed at the end of the bubbler, and the bubbler is purged with nitrogen. Gaseous methyl mercury collects in the carbotrap. After the carbotrap is connected directly to the gas lines to dry any water vapor from it, it is then placed in the analytical train and “burned” to the analyzer.

2.3.2.4 For each sample, three peaks are produced that are recorded by strip chart recorder or integrator. The first is Hg^0 , the second is methyl mercury, the third is Hg (II) .

3.0 INTERFERENCES

- 3.1. BrCl will oxidize nearly all forms of mercury; therefore, pipettes used to aliquot BrCl or bubblers used/cleaned with BrCl should never be used for methyl mercury analysis.
- 3.2. Methyl mercury analysis is pH sensitive and distillation can cause acidification of samples; therefore, it is essential that the pH of the distillates be adjusted by the addition of acetate buffer. Each distillate should be tested for pH before analysis, making sure not to place pH paper directly into sample. If the pH is determined to be < 3 , 600 μL of acetate buffer should be added.
- 3.3. Because of the basic nature of tissue digests prepared with the KOH /methanol digestion, 600 μL of acetate buffer must be added. Also, it is recommended that aliquots of no more than 25 μL of the tissue digest be analyzed, unless otherwise instructed by a project manager or senior analyst.
- 3.4. When analyzing samples for methyl mercury, bubblers should be rinsed and new reagent water added between every sample.

4.0 SAFETY

- 4.1. **CAUTION:** Ethylating agent (NaBEt_4) is toxic, gives off toxic gases (triethylboron), and is spontaneously combustible. To discard unused portions of ethylating agent and empty bottles, place into a large beaker of ~10% 1N HCl in the hood -- triethylboron will bubble off to the air where it is eventually oxidized to harmless boric acid. Leave the acid beaker in the hood indefinitely, or boil down to _ volume to destroy residues before discarding as acid waste.
- 4.2. Personnel will don appropriate laboratory attire according to the Chemical Hygiene Plan. This includes, but is not limited to, laboratory coat, safety goggles, and latex gloves under clean gloves.

- 4.3. The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Chemists should refer to the MSDS for each chemical they are working with.
- 4.4. All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease-causative agents. Frontier will reimburse the expense of Hepatitis A and B immunizations for any laboratory staff member who desires this protection.

5.0 EQUIPMENT

- 5.1. Micro-pipettors: All-plastic pneumatic fixed-volume and variable pipettors in the range of 10 μ L to 5.0 mL.
- 5.2. Cold Vapor Generators (Bubblers): 150-mL, tall, flat-bottom borosilicate glass flasks with standard taper 24/40 necks, fitted with spargers having coarse glass frits which extends to within 0.2 cm of the flask bottom.
- 5.3. Recorder: Any integrator or multi-range chart recorder with 0.1-5.000 mV input and variable speeds is acceptable.
- 5.4. Carbotraps: Borosilicate, silanized quartz tubes filled with low mercury Carbotrap and plugged with quartz wool
- 5.5. Atomic Fluorescence Spectrophotometer (AFS): To achieve the low detection levels and small, interference-free sample aliquots claimed by this method, a very sensitive (IDL < 1 pg Hg) AFS detector is required. Such systems can be built in-lab or purchased from Tekran Inc. (Toronto, Ontario).
- 5.6. Flow Meter/Needle Valve: Capable of controlling and measuring gas flow to the purge vessel at 200-500 mL/minute.
- 5.7. Teflon Fittings: Connections between components and columns are made using 6.4-mm O.D. Teflon FEP tubing and Teflon friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2-mm O.D. Teflon tubing due to its greater flexibility.
- 5.8. Isothermal GC Unit: A 1.3-m packed GC column is made to the following specification (Supelco Inc. custom product): The column is made of 0.25-in. O.D. borosilicate glass column tubing with 4-mm I.D. bore. The tube is formed into an 8-cm diameter coil of 1.0-m length with two 15-cm arms extending in parallel up from the coil. The column is silanized, and packed with 1 m (in the

coil section only) of preconditioned 60/80 mesh 15% OV-3 on Chromasorb WAW-DMSC, held in place with silanized glass wool plugs. The column is held in a small temperature-controlled isothermal oven made from a heating mantle (Glass-Col TM-580) interfaced with a Cole Parmer Digi-Sense temperature controller. The column is held at a constant temperature of $100 \pm 2^\circ \text{C}$ using the temperature controller.

- 5.9. Pyrolytic Organo-mercury Breakdown Column: This column consists of a 20-cm length of 7-mm O.D. by 4.5-mm I.D. quartz tubing with the central 10 cm packed with quartz wool. The column is wrapped with 1.5 m of 22-gage Nichrome wire which is electrically heated to about 700°C (bright orange) with 30-34 volts from an autotransformer.

6.0 REAGENTS

- 6.1. Reagent Water: Reagent water (18-M Ω minimum) must be ultra-pure deionized water starting from a pre-purified source. Reagent water used in the mercury lab is checked weekly for total mercury concentrations. The total mercury concentration must be $< 0.20 \text{ ng/L}$.
- 6.2. Hydrochloric Acid (HCl): Hydrochloric acid must be trace-metal purified and reagent grade. Total mercury concentration in HCl must be $< 5.00 \text{ ng/L}$.
- 6.3. Sodium Acetate: Neat, reagent grade. Pre-tested and verified to be low in mercury.
- 6.4. Glacial Acetic Acid: Reagent grade. Pre-tested and verified to be low in mercury.
- 6.5. Acetate Buffer: Place 500 mL of reagent water in a 1.0-L Teflon bottle. Add 272 g of sodium acetate and dissolve. Add 118 mL of glacial acetic acid and dilute to a final volume of 1.0 L. Add $\sim 0.5 \text{ mL}$ of ethylating reagent (tetraethyl borate solution) and purge overnight with nitrogen. This solution has an indefinite lifetime when stored in a Teflon bottle at room temperature
- 6.6. Potassium Hydroxide: Neat, pellets.
- 6.7. Sodium Tetraethyl Borate: 1-g vials.
- 6.8. Ethylating Agent (Sodium Tetraethyl Borate Solution): Ethylating agent can be made by following the procedure below.
- 6.8.1. Rinse approximately 20 7-mL Teflon vials with reagent water and arrange on a clean sheet of blotter paper in a class-100 clean air station to dry.

- 6.8.2. Pour 100 mL of reagent water into a 125-mL Teflon bottle and add 2.0 g of KOH pellets. Dissolve mixture and place in freezer until ice crystals just begin to form.
- 6.8.3. Turn on reagent water. Remove wax and tape seal from a 1.0-g ampoule of sodium tetraethyl borate. Open the ampoule and immediately transfer its contents to the 125-mL Teflon bottle, cap tightly, and give a few quick shakes.
- 6.8.4. Fill the now empty ampoule and its cap with reagent water and sink into a beaker containing water and HCl (the pure borate powder is pyrophoric and could spontaneously-combust when exposed to air if not stabilized by the addition of water).
- 6.8.5. Shake the borate solution again then transfer to the 7-mL Teflon vials, 4 mL at a time, leaving ample air space for freezing and capping tightly.
- 6.8.6. Place all the 7-mL vials in the freezer standing upright. If any borate solution is left over, dispose in the HCl solution that the ampoule was placed in. If any doubt arises about the quality of the ethylating reagent, make a new batch, as the old material often gives good results for reagent water spikes, but not for environmental samples. Do not use NaBEt₄ solid or solutions if they have a yellow color.
- 6.9. Acetate Buffer: Place 500 mL of reagent water in a 1.0-L Teflon bottle. Add 272 g of sodium acetate and dissolve. Add 118 mL of glacial acetic acid and dilute to a final volume of 1.0 L. Add ~0.5 mL of ethylating reagent (tetraethyl borate solution) and purge overnight with nitrogen. This solution has an indefinite lifetime when stored in a Teflon bottle at room temperature
- 6.10. Nitrogen (N₂): Grade 4.5 (standard laboratory grade) nitrogen which can be further purified of mercury using a gold or iodated carbon trap located in line between the gas output and bubbler.
- 6.11. Argon (Ar): No less than Grade 4.7 (high purity grade) argon that has been further purified by the removal of mercury using a gold or iodated carbon trap that is located in line between the gas output and the analyzer gas input.

7.0 PROCEDURES

7.1. Documentation of Standards and Reagents

- 7.1.1. All freshly prepared standards, as well as purchased standards, are logged into the Mercury Standards Logbook and are given a unique identification number. The ID is composed of the number of the logbook, followed by the page number where the standard was logged in, followed by the line number

given to that standard. For example, AQ2-1-13 would indicate a standard documented in the “Aquatics” Mercury Standard Logbook, Volume 2, on page 1, and line 13. The date received or the date created as well as the analyst’s initials should be noted on the standard bottle and on the certification information shipped with the standard.

7.1.2. The standard is entered into the first available line in the logbook and the following information is recorded:

7.1.2.1. Number – the standard is given a number one higher than the previous line number.

7.1.2.2. Stock concentration – for liquid standards; record the stock concentration and unique ID used to create working/spiking standards. For solid reagents, write the name of the compound used to create working standards. The final working/spiking standard is to be tested in triplicate on two separate days to determine that it is low in mercury.

7.1.2.3. Initial volume – record the amount of the original standard used to create working/spiking standards.

7.1.2.4. Final volume – record the final working/spiking standard volume.

7.1.2.5. Diluent – record the volume and type of diluent used to bring initial volume up to final volume.

7.1.2.6. Dataset ID – after a standard has been tested, record all dataset IDs that contain the standard test results.

7.1.3. All reagents used for total mercury determination are recorded in the Mercury Reagent Testing Logbook. The name of the reagent, the date made, and the initials of the person who made the reagent are recorded. Next, the solution is given to an analyst to test (with the exception of ethylating agent) to assure that the reagent is low in mercury content. When the solution tests low, the analyst enters into the logbook that the solution tested low and records the corresponding dataset ID. The actual reagent bottle gets labeled with the reagent name, the initials of the person who prepared the solution, the date made, and if the solution tested low. If a reagent does not test sufficiently low, it is re-tested once. If it is still testing high, it is marked “not for laboratory use,” and a note is made in the Reagent Testing Logbook. The reagent is then appropriately disposed of and a new batch is made.

7.2. Preparation of Methyl Mercury Standard Solutions

7.2.1. Methyl mercury standard solutions are prepared using ultra-clean class-A volumetric glassware and gravimetrically calibrated pipettes. All waters and

reagents are pre-tested and must have very low mercury concentrations. Solution preparations and calibrations must be recorded in the Laboratory Standard Solution Preparation Logbook.

7.2.2. Methyl Mercury Initial Stock Solution: Methyl mercury initial stock solution is prepared by serial dilution of an initial concentrated methyl mercury chloride (approximately 4000 mg/L), obtained from Strem Chemical. The methyl mercury initial stock solution does not have a specific titer. Due to the contamination danger, the methyl mercury chloride is not weighed, but rather, the entire 5 gram bottle is added to 1,000 mL of reagent water preserved to 0.2% with HCl in a Teflon bottle.

7.2.3. Methyl Mercury Stock Standard Solution: Methyl mercury stock standard solution is prepared by a 1:4000 dilution of the initial stock solution. The diluent for the dilution is reagent water containing 5% acetic acid and 0.2% HCl. This solution's concentration is approximately 1000 ng/mL and must be calibrated against NIST-3133 to determine the concentration of methyl mercury. The solution should be discarded and a new one made if the fraction of methyl mercury in the solution drops below 98.0%. Laboratory stock standard solution should be recalibrated yearly.

7.2.4. Methyl Mercury Working Standard Solution: Two different concentrations of methyl mercury working standard solutions are made; one contains 1.00 ng/mL of methyl mercury, and the other contains 0.05 ng/mL of methyl mercury. Because of changes in concentration over time, the concentration of methyl mercury in the stock solution should be calibrated. The appropriate volume of methyl mercury stock standard solution is then added to reagent water containing 5% acetic acid and 0.2% HCl to bring the working standard to the desired concentration. After preparation, the new working standard is tested in replication (usually three replicates) against the old valid working standard calibration. The mean of at least three determinations of concentration should fall within 5% of the predicted value. Methyl mercury working standard solution should be remade quarterly.

7.2.5. Quality Control Sample (DORM-2): A Quality Control Sample (QCS) containing 4,470 ng/g is prepared by digesting 1.000 gram of the certified reference material DORM-2 in 20 mL of 25% KOH/methanol, and then diluting to 1000.00 mL with methanol.

7.3. Calibration of Methyl Mercury Stock Standard and Working Standard Solutions

7.3.1. Calibrate the methyl mercury stock standard solution by diluting 0.100 mL of it into 0.900 mL of BrCl solution in a small Teflon vial and allowing it to oxidize for a minimum of 4 hours. Next, dilute 0.100 mL of NIST-3133 certified total Hg stock solution the same way. The total Hg in the dilution of methyl mercury stock standard solution can be compared to that of NIST-

3133 analyzed using dual amalgamation/CV-AFS (Frontier SOP #FGS-069). A mean of at least 7 replicate analyses of the stock solution is necessary to accurately quantify the total Hg concentration of the methyl mercury stock standard solution. Next, at least two labile Hg(II) determinations must be made of the methyl mercury stock standard solution. These are analyzed by direct reduction of an aliquot of methyl mercury stock standard solution by stannous chloride (SnCl_2), with no prior BrCl oxidation. The methyl mercury concentration in the stock solution is then equal to the concentration of the total mercury minus the concentration of the labile Hg(II). The concentration of methyl mercury working standard solution is calibrated in the same manner.

7.4. Instrument Start-up

7.4.1. Begin by blanking your sample carbotraps. To do this you close the argon gas valve, attach one trap at a time to the analytical train, open the argon gas valve and burn to instrument for 30 seconds. Continue to burn traps while doing steps 7.4.2 and 7.4.3, making sure traps remain in analytical train until cool enough to remove safely.

7.4.2. Remove Ethylating Agent from freezer and allow to thaw for 15 minutes, then place in freezer.

7.4.3. Rinse each bubbler and bubbler top, and fill with approximately 50 mL of reagent water.

7.4.4. The analyst should record the following information on the strip chart or integrator print-out:

7.4.4.1. Corresponding dataset ID

7.4.4.2. Analyst name (also signature)

7.4.4.3. The baseline ratios (usually $X=1$ and $X=20$)

7.4.4.4. The date, analysis start time, and the analysis end time (at the end of the day)

7.4.4.5. The strip chart drum speed (usually 1 mm/min)

7.5. Analyzer Calibration Sequence

7.5.1. The sequence starts with a 5-point (0.005 ng, 0.010 ng, 0.050 ng, 0.100 ng, and 0.200 ng) standard calibration curve using the methyl mercury working standard solution. This 5-point curve defines the acceptable concentration of the samples being analyzed.

7.5.2. Immediately following the standard calibration curve, a quality control sample (QCS) is analyzed, followed by an initial calibration verification (ICV) standard and an initial calibration blank (ICB). For most projects, at least three preparation blanks are run following the ICB.

7.6. To calibrate the instrument, follow the protocols below:

7.6.1. Close connections to bubblers with Teflon end plugs.

7.6.2. Add 300 μ L of acetate buffer to each bubbler.

7.6.3. Using the 0.050 ng/mL MeHg working standard solution, add 100 μ L to the first bubbler. Then, using the 1.00 ng/mL MeHg working standard solution, add 50 μ L, 100 μ L, and 200 μ L to the remaining bubblers sequentially from left to right.

7.6.4. Remove ethylating agent from the freezer and add 38.5 μ L to each bubbler, immediately returning the ethylating agent to the freezer. Seal bubbler tops using Keck Clips and/or friction seal to insure nominal sample leakage and swirl gently.

7.6.5. Allow to react for 17 minutes.

7.6.6. Remove end plugs, connect blanked carbotraps to bubbler outlet using through plugs, connect gas lines, and purge with nitrogen for 17 minutes, ensuring that no water droplets enter the carbotraps.

7.6.7. Connect traps directly to the gas lines for 7 minutes to remove water vapor from the traps.

7.6.8. Attach carbotraps to analytical train and burn for 30 seconds each in sequential order. Do not remove trap until all three peaks have been recorded.

7.6.9. Rinse bubblers and frits with reagent water and add approximately 50 mL of reagent water to each bubbler.

7.6.10. Add 300 μ L of acetate buffer to bubblers 1, 3, and 4. Add 600 μ L of acetate buffer to bubbler 2.

7.6.11. Add 200 μ L of 0.050 ng/mL MeHg working standard solution to the first bubbler. Open the QCS (DORM-2) bottle and aspirate some of the fumes into a pipette. Without conditioning the pipette, draw up 25 μ L of the digest

allowing the pipette tip to touch only just below (1 mm) the surface of the liquid. Immediately inject the sample into the second bubbler, below the surface of the water. Rinse the pipette tip several times into the bubbler water. Add 100 μL of 1.00 ng/mL MeHg working standard solution to the third bubbler for the initial calibration verification. No standard is added to the fourth bubbler, the initial calibration blank.

7.6.12. Remove ethylating agent from the freezer and add 38.5 μL to each bubbler, immediately returning the ethylating agent to the freezer. Seal bubbler tops using Keck Clips and/or friction seal to insure nominal sample leakage and swirl gently.

7.6.13. Follow steps 7.6.5 through 7.6.8.

7.7. Pre-analysis and Organization

7.7.1. When analyzing samples, it is imperative to check the project sheets. Within these sheets the analyst will find a summary of all the information to verify the samples were prepared correctly as well as the information needed to run the samples properly. These sheets can list the QC specifications required for the samples, suggested aliquot size, project manager information, as well as listing information about spiking levels.

7.7.2. The analyst should locate the samples to be analyzed and their chain of custody (COC) forms (plus internal COCs or digestion bench sheets, if applicable).

7.7.3. The analyst should compare the project sheet to the COCs and digestion bench sheets, and confirm that all samples are accounted for. Notify the project manager of any discrepancies.

7.7.4. The analyst should organize the samples to be analyzed in the order listed on the COC or the digestion bench sheet, or in numerical order, depending on the preference of the PM. The first samples analyzed should be the preparation blanks then the digested SRM if running solid samples, (or the blank spike if analyzing waters) followed by actual samples. If there are total and dissolved samples with the same sample ID, run these side by side to ease in checking that total concentration is greater than dissolved concentration. Field blanks, equipment blanks, and trip blanks should all be analyzed before their associated samples.

7.7.5. Be aware that all samples specified as being High QA should be analyzed prior to any Standard QA projects that are being analyzed on the same instrument on the same day.

7.8. Analyzing Aqueous Samples

7.8.1. All aqueous samples should be prepared according to Frontier SOP #FGS-013 (Distillation of Aqueous Samples for Methyl Mercury Analysis).

7.8.2. While drying the standard curve carbotraps, the analyst should prepare the first batch of samples.

7.8.2.1. Rinse bubblers and frits with reagent water.

7.8.2.2. Add 300 μ L of acetate buffer directly to the distillation vial, unless sample pH is known to be low (3 or less), in which case add 600 μ L of acetate buffer.

7.8.2.3. Dilute vial up to volume with reagent water, and pour the entire sample into bubbler.

7.8.2.4. Remove ethylating agent from the freezer and add 38.5 μ L to each bubbler, immediately returning the ethylating agent to the freezer. Seal bubbler tops using Keck Clips and/or friction seal to insure nominal sample leakage and swirl gently.

7.8.3. Follow steps 7.6.5 through 7.6.8 of calibration process.

7.8.4. Sample IDs, aliquot volume, peak height/peak area, distillation factor (0.906), dilution factor (if applicable), and blank correction associated with each sample and analysis sequences should be entered into the methyl mercury spreadsheet template.

7.8.5. While drying and burning one set of sample traps, the analyst should begin preparing the next round of water samples in the same fashion to maximize efficiency.

7.8.6. Quality Control Procedures for Aqueous Samples

7.8.6.1. An analytical batch is defined as 20 or fewer field samples. QC samples are not included, such as PBWs, the ICV, the ICB, CCVs, CCBs, or matrix QC (MD, MS, or MSD). An analytical day is defined as 12 hours or less.

7.8.6.2. One CCV/CCB must be performed every 10 analytical runs (a run being one bubbler).

7.8.6.3. One matrix spike/matrix spike duplicate (MS/MSD) pair must be analyzed for every 20 samples. Therefore, a minimum of one MS/MSD pair is required per analytical batch. MS/MSDs are spiked according to

the project manager instructions. Aqueous samples are spiked by lab assistants during preparations.

7.8.6.4. Upon request, a matrix duplicate (MD) or matrix triplicate (MT) sample may be requested by the project manager, but otherwise it is not required. MD and MT samples should be analyzed using the same sample aliquots as the ambient sample.

7.8.6.5. At least one blank spike must be prepared and analyzed per analytical batch. The spiking levels may vary depending upon the client, and will be performed during preparation for distillation. Refer to project sheets/project managers for specific spiking levels.

7.8.6.6. At the end of the analytical day a CCV and CCB must be analyzed.

7.9. Analyzing Sediment/Soil Samples

7.9.1. For analysis of sediment/soil samples prepared by Frontier SOP #FGS-017 (Methyl Mercury Distillation of Low Level Solids) or Frontier SOP #FGS-045 (Preparation of Sediments by Acidic KBr Extraction Into Methylene Chloride for Determination of Methyl Mercury), follow steps listed in section 7.8.2 – 7.8.5.

7.9.2. Quality control requirements for the analysis of sediment/soil samples are the same as is listed in sections 7.8.6.1 – 7.8.6.4 and section 7.8.6.6. When samples are spiking following preparation, spiking levels should be 1-5 times the native analyte concentration.

7.10. Analyzing Tissue Samples

7.10.1. For analysis of tissue samples prepared by Frontier SOP #FGS-017 (Methyl Mercury Distillation of Low Level Solids), follow steps listed in section 7.8.2 – 7.8.5.

7.10.2. For analysis of tissue samples prepared by Frontier SOP #FGS-010 (KOH/Methanol Digestion of Solids for Methyl Mercury), follow steps listed in section 7.8.2 – 7.8.5., with the following exceptions:

7.10.2.1. Add 600 µL of acetate buffer to each bubbler.

7.10.2.2. Consult project sheet and/or PM as to proper aliquot volume.

7.10.2.3. As the tissue digest liquid has low surface tension and tends to want to drip out of a pipette, an altered pipette technique is necessary:

7.10.2.3.1. Open the digest bottle and aspirate some of the fumes into a pipette.

7.10.2.3.2. Without conditioning the pipette, draw up proper aliquot (typically 25 µL) of the digest, allowing the pipette tip to touch only just below the surface (1 mm) of the liquid.

7.10.2.3.3. Immediately inject the sample into the bubbler below the surface of the water, then rinse the pipette tip several times into the bubbler water.

7.10.3. Quality Control Procedures for Tissue Samples

7.10.3.1. Quality control requirements for the analysis of tissue samples are the same as is listed in sections 7.8.6.1 – 7.8.6.4 and section 7.8.6.6.

7.10.3.2. Spiking levels for MS and MSD samples should be 1-5 times the native analyte concentration.

8.0 QUALITY ASSURANCE

8.1. Analysts are to verify QC sample results in “real-time” as they come off the instrument. This allows for correction of any analytical problems immediately.

8.2. The acceptable recoveries must be met in order to consider a data set valid. Of particular importance to the client is Frontier’s position that a **single non-compliant result on a QC sample does not automatically invalidate a data set**. All data points noted on the analysis day’s spreadsheet as invalid for known reasons may be discarded if rerun during the same analysis day. In the event that the system becomes out of control during the analysis day, all results sandwiched between valid QC data points shall still be considered valid.

Quality Control Limits for Determination of Methyl Hg*

QC Item	Acceptance Criteria
Calibration curve correlation coefficient value (minimum 5-points calibration curve)	0.995
QCS (SRM or LCS)	70-130% recovery
ICB and CCBs	Mean < 0.025 ng/L
CCVs	80-120% recovery
MS/MSD	70-130% recovery with RPD 25, spiking level ambient level
MD	25 RPD for values > 10x the est. MDL

PBW	Mean \leq 0.025 ng/L
PBS	Mean < 0.02 ng/g
PBT	Mean < 2.0 ng/g

***Client QC requirements may be more stringent. Analysts should refer to project sheets prior to analysis.**

10.0 CORRECTIVE ACTION

- 10.1. The quality control data gathered throughout the analytical day provides an indication of overall data quality. Therefore, corrective action is required if quality assurance measures are outside of acceptable limits. First, a careful re-examination of the calculations is performed to assure that there are no numerical errors. The project manager is informed of the data issue, and they decide what, if any corrective action, including reruns, is warranted. The Quality Assurance Officer oversees this process and has the final say in what corrective action is to be performed.
- 10.2. If insufficient sample volume remains to repeat analysis for samples analyzed after the last acceptable CCV, use best professional judgment to estimate values. Bracket those samples from previous acceptable QC checks, and provide a narrative explanation on the dataset coversheet. Estimated values must be flagged on the dataset and the report to the client.
- 10.3. The above corrective actions apply only to events which have unknown causes. If the analyst is aware of the cause, no corrective action is necessary other than reanalyzing the sample.

11.0 EQUATIONS

11.1. Methyl Mercury in Water

- 11.1.1. Average all bubbler blanks (B) using the peak height/peak area values from the strip chart.
- 11.1.2. The slope of the calibration curve (A) is calculated using the chart units per ng of mercury. A standard statistical package is used to determine the slope, using the five initial calibration points. The calibration points are first corrected by subtraction of the mean of the bubbler blanks. The statistical program forces the regression line through zero (0,0). Average the results for the preparation blanks (PB), from the chart values of at least three preparation blanks.
- 11.1.3. To calculate methyl mercury in waters (ng/L), use the following equations.

$$11.1.3.1. \text{ MeHg/Aliquot (ng)} = [(\text{Peak Height or Peak Area}) - B] / A$$

$$11.1.3.2. \text{ MeHg Gross (ng/L)} = [(\text{MeHg/Aliquot}) / V_a / D] * 1000$$

$$11.1.3.3. \text{ MeHg Net (ng/L)} = [(\text{MeHg Gross}) / 0.906] - (PB)$$

Where:

- **B** is the average bubbler blank peak height or peak area
- **A** is the slope of the calibration curve (in ng/units)
- **V_a** is the volume of sample analyzed (the aliquot size) in mL
- **D** takes into account any dilution of the sample and is expressed as a fraction (0.2 = 1/5 dilution)
- **PB** is the average of the preparation blanks in ng/L

11.2. Total Mercury in Solids

11.2.1. To calculate total mercury in a solid digestion or extraction (ng/g), use the following equations:

$$11.2.1.1. \text{ THg/Aliquot (ng)} = [(\text{Peak Height Or Peak Area}) - B] / A$$

$$11.2.1.2. \text{ THg Gross (ng/L)} = [(\text{THg/Aliquot}) / V_a / D] * 1000$$

$$11.2.1.3. \text{ THg/Digest (ng)} = [(\text{THg Gross} - PB) * V_d / 1000]$$

$$11.2.1.4. \text{ THg Solid (ng/g)} = (\text{THg/Digest}) / m$$

Where:

- **B** is the average bubbler blank peak height or peak area
- **A** is the slope of the calibration curve (in ng/units)
- **V_a** is the volume of sample analyzed (the aliquot size) in mL
- **V_d** is the final digested volume of the digest

- **D** takes into account any dilution of the sample and is expressed as a fraction ($0.2 = 1/5$ dilution)
- **m** is the mass of the sample which can be expressed as either a dry or wet weight.
- **PB** is the average of the preparation blanks in ng/L

11.2.2. To calculate total mercury in a solid distillation (ng/g), use the following equations:

$$11.2.2.1. \text{ THg/Aliquot (ng)} = [(\text{Peak Height Or Peak Area}) - B] / A$$

$$11.2.2.2. \text{ THg Gross (ng/L)} = [(\text{THg/Aliquot}) / V_a / D] * 1000$$

$$11.2.2.3. \text{ MeHg Net (ng/L)} = [(\text{MeHg Gross}) / 0.906] - (PB)$$

$$11.2.2.4. \text{ THg/Digest (ng)} = [(\text{THg Gross} - PB) * V_d / 1000]$$

$$11.2.2.5. \text{ THg Solid (ng/g)} = (\text{THg/Digest}) / m$$

Where:

- **B** is the average bubbler blank peak height or peak area
- **A** is the slope of the calibration curve (in ng/units)
- **V_a** is the volume of sample analyzed (the aliquot size) in mL
- **V_d** is the final digested volume of the digest
- **D** takes into account any dilution of the sample and is expressed as a fraction ($0.2 = 1/5$ dilution)
- **m** is the mass of the sample which can be expressed as either a dry or wet weight.
- **PB** is the average of the preparation blanks in ng/L

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Total Hardness
(Pacific EcoRisk 1995)

TOTAL HARDNESS

Standard Operating Procedure

1.0 THEORY OF OPERATION

After the sample is buffered to pH 10.1, ManVer 2 Hardness Indicator is added, and forms a red complex with a portion of the calcium and magnesium in the sample. EDTA titrant reacts first with the free calcium and magnesium ions, then with those bound to the indicator, causing it to change to a blue color at the end point.

2.0 PROCEDURE

- 1.) Select a sample volume and EDTA titration cartridge corresponding to the expected hardness concentration from the following Table:

Range (mg/L as CaCO ₃)	Sample Volume (mL)	Titration Cartridge (M EDTA)	Digit Multiplier
10-40	100	0.0800	0.1
40-160	25	0.0800	0.4
100-400	100	0.800	1.0
200-800	50	0.800	2.0
500-2000	20	0.800	5.0
1000-4000	10	0.800	10.0

- 2.) Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.
- 3.) Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.
- 4.) Use a graduated cylinder or pipet to measure the appropriate sample volume (see above table). Dilute to 100mL with deionized water and transfer the sample into a 250 mL beaker with a stir bar.
- 5.) Place sample on stir plate, add two mL of Buffer Solution, Hardness 1, and slowly stir.
- 6.) Add the hardness indicator to the sample and mix.
- 6.) Place delivery tube into the solution and stir sample while titrating with EDTA from red to pure blue. Record the number of digits required.
Note: Titrate slowly near the endpoint because the reaction is slow, especially in cold samples.
- 7.) Use the following formula to calculate the final concentration:

$$\text{Digits Required} \times \text{Digit Multiplier} = \text{mg/L Total Hardness as CaCO}_3$$

- 8.) **Record sample hardness in log book.**

Total Alkalinity
(Pacific EcoRisk 1995)

TOTAL ALKALINITY

Standard Operating Procedure

1.0 THEORY OF OPERATION

The sample is titrated with sulfuric acid to a colorimetric endpoint corresponding to a specific pH. T (total) alkalinity is determined by titration to a pH of 4.5, and includes all carbonate, bicarbonate and hydroxide.

2.0 PROCEDURE

- 1.) Select a sample volume and Sulfuric Acid titration cartridge corresponding to the expected alkalinity concentration from the following Table:

Range (mg/L as CaCO ₃)	Sample Volume (mL)	Titration Cartridge (N NaOH)	Digit Multiplier
10-40	100	0.1600	0.1
40-160	25	0.1600	0.4
100-400	100	1.600	1.0
200-800	50	1.600	2.0
500-2000	20	1.600	5.0
1000-4000	10	1.600	10.0

- 2.) Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.
- 3.) Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.
- 4.) Use a graduated cylinder or pipet to measure the appropriate sample volume (see above table). Dilute to 100mL (if necessary) with deionized water and transfer the sample into a 250 mL beaker with a stir bar
- 5.) Place sample on stir plate, measure pH of sample and record in log book and then add the contents of one Bromcresol Green-Methyl Red Powder Pillow, and slowly stir.
- 6.) If the solution turns green, titrate to pH 4.5 (solution should reach a light pink end point). Place the delivery tube tip into the solution and stir sample while titrating with sulfuric acid. Record the number of digits required
- 7.) Use the following formula to calculate the final concentration:

$$\text{Digits Required} \times \text{Digit Multiplier} = \text{mg/L as CaCO}_3 \text{ Total Alkalinity}$$

Record sample alkalinity in log book.

**Total Dissolved Solids: EPA Method 160.1
(EPA 1971)**

METHOD #:	160.1	Approved for NPDES (Issued 1971)
TITLE:		Residue, Filterable (Gravimetric, Dried at 180°C)
ANALYTE:		Residue, Filterable
INSTRUMENTATION:		Drying Oven
STORET No.		70300

1.0 Scope and Application

- 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
- 1.2 The practical range of the determination is 10 mg/L to 20,000 mg/L

2.0 Summary of Method

- 2.1 A well-mixed sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to constant weight at 180°C.
- 2.2 If Residue, Non-Filterable is being determined, the filtrate from that method may be used for Residue, Filterable.

3.0 Definitions

- 3.1 Filterable residue is defined as those solids capable of passing through a glass fiber filter and dried to constant weight at 180°C.

4.0 Sample Handling and Preservation

- 4.1 Preservation of the sample is not practical; analysis should begin as possible. Refrigeration or icing to 4°C, to minimize micro-biological decomposition of solids, is recommended.

5.0 Interferences

- 5.1 Highly mineralized waters containing significant concentrations of calcium, magnesium, chloride and/or sulfate may be hygroscopic and will require prolonged drying, desiccation and rapid weighing.
- 5.2 Samples containing high concentrations of bicarbonate will require careful and possibly prolonged drying at 180°C to insure that all the bicarbonate is converted to carbonate.
- 5.3 Too much residue in the evaporating dish will crust over and entrap water that will not be driven off during drying. Total residue should be limited to about 200 mg.

6.0 Apparatus

- 6.1 Glass fiber filter discs, 4.7 cm or 2.1 cm, without organic binder, Reeve Angel type 934-AH, Gelman type A/E, or equivalent
- 6.2 Filter holder, membrane filter funnel or Gooch crucible adapter
- 6.3 Suction flask, 500 mL
- 6.4 Gooch crucibles, 25 mL (if 2.1 cm filter is used)
- 6.5 Evaporating dishes, porcelain, 100 mL volume. (Vycor or platinum dishes may be substituted)
- 6.6 Steam bath
- 6.7 Drying oven, $180^{\circ}\text{C} \pm 2^{\circ}\text{C}$
- 6.8 Desiccator
- 6.9 Analytical balance, capable of weighing to 0.1 mg

7.0 Procedure

- 7.1 Preparation of glass fiber filter disc: Place the disc on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible. While vacuum is applied, wash the disc with three successive 20 mL volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Discard washings.
- 7.2 Preparation of evaporating dishes: If Volatile Residue is also to be measured heat the clean dish to $550 \pm 50^{\circ}\text{C}$ for one hour in a muffle furnace. If only Filterable Residue is to be measured heat the clean dish to $180 \pm 2^{\circ}\text{C}$ for one hour. Cool in desiccator and store until needed. Weigh immediately before use.
- 7.3 Assemble the filtering apparatus and begin suction. Shake the sample vigorously and rapidly transfer 100 mL to the funnel by means of a 100 mL graduated cylinder. If total filterable residue is low, a larger volume may be filtered.
- 7.4 Filter the sample through the glass fiber filter, rinse with three 10 mL portions of distilled water and continue to apply vacuum for about 3 minutes after filtration is complete to remove as much water as possible.
- 7.5 Transfer 100 mL (or a larger volume) of the filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath.
- 7.6 Dry the evaporated sample for at least one hour at $180 \pm 2^{\circ}\text{C}$. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained or until weight loss is less than 0.5 mg.

8.0 Calculation

- 8.1 Calculate filterable residue as follows:

$$\text{Filterable residue, mg/L} = \frac{(A - B) \times 1,000}{C}$$

where:

A = weight of dried residue + dish in mg

B = weight of dish in mg

C = volume of sample used in mL

9.0 Precision and Accuracy

9.1 Precision and accuracy are not available at this time.

Bibliography

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**Total Suspended Solids: EPA Method 160.2
(EPA 1971)**

Approved for NPDES (Issued 1971)

Residue, Non-Filterable (Gravimetric, Dried at 103-105°C)

Residue ,Non-Filterable

Drying Oven

00530

- 1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
- 1.2 The practical range of the determination is 4 mg/L to 20,000 mg/L.

- 2.1 A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C.
- 2.2 The filtrate from this method may be used for Residue, Filterable.

3.1 Residue, non-filterable, is defined as those solids which are retained by a glass fiber filter and dried to constant weight at 103-105°C.

- 4.1 Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
- 4.2 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.

- 5.1 Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results.
- 5.2 Samples high in Filterable Residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter (7.5) minimizes this potential interference.

6.0 Apparatus

- 6.1 Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent.
NOTE: Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size", collection efficiencies and effective retention are used to define this property in glass fiber filters. Values for these parameters vary for the filters listed above.
- 6.2 Filter support: filtering apparatus with reservoir and a coarse (40-60 microns) fritted disc as a filter support.
NOTE: Many funnel designs are available in glass or porcelain. Some of the most common are Hirsch or Buchner funnels, membrane filter holders and Gooch crucibles. All are available with coarse fritted disc.
- 6.3 Suction flask.
- 6.4 Drying oven, 103-105°C.
- 6.5 Desiccator.
- 6.6 Analytical balance, capable of weighing to 0.1 mg.

7.0 Procedure

- 7.1 Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus or insert into bottom of a suitable Gooch crucible with wrinkled surface up. While vacuum is applied, wash the disc with three successive 20 mL volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103-105°C for one hour. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighing, handle the filter or crucible/filter with forceps or tongs only.
- 7.2 Selection of Sample Volume
For a 4.7 cm diameter filter, filter 100 mL of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 mL/cm² of filter area and collect at least a weight of residue proportional to the 1.0 mg stated above.
NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, the following scheme is recommended: Use an unweighed glass fiber filter of choice affixed in the filter assembly. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five mL increments for timing are suggested. Continue to record the time and volume increments until filtration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.
- 7.3 Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.
- 7.4 Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected in 7.2 to the filter using a graduated cylinder. Remove

all traces of water by continuing to apply vacuum after sample has passed through.

- 7.5 With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.

NOTE: Total volume of wash water used should equal approximately 2 mL per cm². For a 4.7 cm filter the total volume is 30 mL.

- 7.6 Carefully remove the filter from the filter support. Alternatively, remove crucible and filter from crucible adapter. Dry at least one hour at 103-105°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

8.0 Calculations

- 8.1 Calculate non-filterable residue as follows:

$$\text{Non-filterable residue, mg/L} = \frac{(A - B) \times 1,000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg

B = weight of filter (or filter and crucible) in mg

C = mL of sample filtered

9.0 Precision and Accuracy

- 9.1 Precision data are not available at this time.

- 9.2 Accuracy data on actual samples cannot be obtained.

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Organic Carbon by Persulfate-UV Oxidation: SM 5310C
(Sierra Foothill Laboratory, 2000)

Forms:
Labels, Log Sheets, Data Reports

Appendix D

Supporting Documents for Aquatic Toxicity Monitoring

**Quality Assurance/Quality Control Manual, May 2000:
(Pacific EcoRisk 2000)**

Quality Assurance/Quality Control Manual

(May 2000 Revision)

QA Policy Statement

Pacific EcoRisk maintains a QA Plan that provides a detailed description of quality assurance and quality control (QA/QC) procedures for all testing and chemical analyses performed by Pacific EcoRisk. These procedures address all aspects of toxicity testing that can potentially affect data quality and interpretation, including sampling and handling of test materials, collection, holding and conditioning of test organisms, test conditions and procedures, calibration of instruments, experimental design, reference toxicant testing, record keeping, and statistical evaluation of data. The content of the QA plan is reviewed and revised on a regular basis.

The staff is composed entirely of degreed professional scientists who have considerable experience performing both routine regulatory testing as well as extensive expertise in research and methods development for more specific non-routine studies.

Pacific EcoRisk follows Laboratory Standard Operating Procedures in accordance to methods established by the U.S. Environmental Protection Agency (EPA), ASTM, and Standard Methods. Quality Control Procedures are documented and follow EPA protocols. As a result of the quality of the data, Pacific EcoRisk is able to provide technical support related to NPDES, Water Quality Criteria Development, (Dredging) 404 Certification, and Ecological Risk Assessments.

The primary objective of the Pacific EcoRisk Quality Assurance Plan is to ensure that all of the data generated and reported are scientifically valid, legally defensible and of known accuracy, precision, representativeness and comparability.

All of the testing performed at Pacific EcoRisk meet the following Criteria.

- Methods and procedures conform to the specifications and requirements of the appropriate regulatory agencies (EPA, Regional Water Quality Control Boards, U.S. ACOE, DTSC, etc.)
- When applicable, all measures of precision, accuracy, representativeness, and comparability are reported in the data package.
- All data is reviewed relative to the quality control plan. Corrective actions are implemented when data fail to meet established quality control criteria.
- Standard operating procedures have been developed and are used in order to ensure that good quality data is collected.
- All final reports are reviewed in order to meet the clients objectives with respect to quality and completeness.

The experienced staff, the modern facility, and strict adherence to the QA program contribute to an overall commitment to provide high quality data in a timely fashion. As a result of the quality of the data, Pacific EcoRisk is capable of providing technical support related to NPDES, Water Quality Criteria Development, 404 Certification (Dredging), and Ecological Risk Assessments.

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Quality Assurance

Introduction

This Quality Assurance (QA) Manual outlines the procedures employed for assuring the integrity of the data produced and used at Pacific EcoRisk. The establishment of a continuing program to ensure the reliability and validity of results is one of the fundamental responsibilities of the laboratory.

Standard Operating Procedures

A Standard Operating Procedure (SOP) is available for all laboratory procedures that require specific knowledge and/or adherence to a specific sequence of procedural steps. This includes but is not restricted to the following:

- Sample collection, preservation, and holding time
- Sample custody, receipt, and document control
- Analytical methods
- Instrument calibration and maintenance
- Test Methods
- Reference Toxicant Preparation
- Safety
- Hazardous Waste Holding and Disposal

Any laboratory personnel participating in or performing any testing-related activity in the lab must be intimately familiar with the relevant SOPs. A copy of each SOP shall be maintained in each of the following: Office File in clearly labeled file folder(s); Laboratory File in clearly-labeled file folder(s) or posted above work station; and Laboratory SOP Manual.

Quality Assurance Objectives for Measurement of Data

Any data are only as good as the scientists measuring and recording that data. To ensure that the data generated by this laboratory meet the highest standards, all laboratory staff participating in any lab-related activity that affects the quality of the tests being performed shall be familiar with the Standard Operating Procedures established for that activity. Furthermore, each staff person shall demonstrate, in the presence of the QA/QC Officer, their hands-on competency in following the SOPs and performance of specific tasks. Upon successful demonstration of such competency, the staff person will initial the Staff Training Check Off List for that task, again under the supervision of the QA/QC Officer.

Documentation*Chain-of-Custody*

The purpose of using a chain-of-custody record is to maintain an accurate written record that can be used to trace the possession of the sample from the moment of its collection through its final analysis and disposal. In addition, the chain-of-custody record documents that the samples are handled only by authorized and properly-trained personnel. Chain-of-custody begins in the field. The sample collector is responsible for the care and custody of the samples until they are transferred to the appropriate laboratory or given to an assigned custodian.

Samples must be accompanied by a chain-of-custody record that includes the name of the study, location of collection (or station number and location), date and time of collection, type of sample, number of containers, analysis required, and the collectors' signatures. When turning over possession of samples, the person relinquishing the sample(s) and the recipient must both sign, date, and record the time of the transfer. For certain projects, an additional sample transfer sheet is initiated to track the sample through the laboratory during storage, sample preparation and generation of raw data. Samples are discarded only when it is certain that all tests and analyses have been properly performed and recorded.

Sample Log-In

All samples will be assigned a unique sample log number upon arrival at the laboratory. This number is used throughout the project. For effluent and receiving/ambient water samples, measurement of initial temperature, pH, D.O. salinity, conductivity, and total ammonia is recorded on the sample log-in sheets.

Raw Data Collection

All raw data collected during the course of a test is dated and signed/initialed by each individual working on that particular test. This serves as documentation of who collected specific data on the test data sheets. When a project is completed, the data are reviewed by the lab director and/or the QA/QC officer before a final report is released. The final report includes method summaries, results, raw data, quality control information and chain-of-custody records as applicable.

Collection and Handling of Samples

Each sample is collected according to established guidelines for the specific type of sample and

sampling location. Each sample must have a identification tag or label securely attached to the sample container at the time the sample is collected. The sample tag must contain the following information: 1) sampling station name and location, 2) date, time and duration of sample collection, 3) type of sample (i.e. grab vs. composite) and 4) name of sample collector(s). Record the date, time, duration and volume of sample collection. The sampling point and the type of sample collected should be the same as specified in the project description or permit. Labels are written legibly with waterproof ink.

All field measurements, records and notes (including temperature, salinity, etc.) are logged in bound field notebooks. Sufficient information is recorded in detail in the field notebook to completely reconstruct the sampling event(s).

Precautions should be taken to ensure that methods for collection and storage of samples (including materials used) do not contribute to sample toxicity (i.e. use appropriately cleaned sample containers, etc.). Samples may be shipped in glass or plastic (polyethylene of polypropylene) bottles, or in disposable Cubitainers. All samples should be shipped on ice and stored in the laboratory at 4°C.

Collection and Preparation of Dilution Water

The type of dilution water is determined on an individual basis for each testing event and for each sample. Dilution and control water (reference water) for tests shall be obtained from an unaffected (clean and non-toxic) natural water (i.e. Commercial spring water) or by formulation in laboratory (following EPA protocols). Collection of natural dilution and control waters shall be from reference sites that are remote from pollution sources and acceptable to the Regional Water Quality Control Board. Reference waters should be filtered through a 1um filter prior to storage and through a 0.2 um filter prior to testing. Field collected water is stored at 4°C and may be held for periods of up to two weeks, as long as acceptable control responses are obtained. The minimum requirement for field collected dilution water is that the test organisms survive, develop, and reproduce normally in it.

Collection and Preparation of Receiving Water

When “receiving” waters are being evaluated in bioassay testing as the dilution water, they must be collected within 96 hours of test initiation. Receiving water is stored at 4°C. Receiving water is gently agitated to evenly suspend particulates before subsampling or preparing test solutions. Receiving water is not filtered, except in the case of tests that require filtration where particulate concentrations or naturally occurring biota interfere with the accurate analysis of the test endpoint (i.e. algal tests).

In instances where the receiving water dissolved oxygen (D.O.) is below acceptable levels (less than 60% of saturation at test temperature), sample is gently aerated so as to raise the D.O. to between 60% and 100% of saturation. Precautions should be taken to ensure that methods for collection, preparation and storage of dilution water (including materials used) do not increase dilution water toxicity.

Sample Holding and Disposal

All effluent and sediment samples will be held at 4°C in the dark for the duration of the testing period. Effluent samples must be used to initiate a test within 36 hours of completion of sample collection unless otherwise stated.

All samples will be disposed of properly in accordance with accepted procedures. All samples that fall under the description of hazardous waste will be stored in the proper containers for later pick-up and disposal by a commercial hazardous waste disposal company.

Lab Water

Reverse-Osmosis, deionized water is used in the laboratory for the preparation of dilution water, preparation of reference toxicant reagent solutions and the final rinsing of glassware. Conductivity of the deionized water is monitored daily to ensure the water is properly treated.

Glassware and Labware Cleaning

Volumetric glassware, Class A, is used for all precise measurements of volume. All glassware and test chambers are cleaned in accordance with EPA guidelines, this consists of the following procedures:

- 1) rinse thoroughly with tap water
- 2) scrub with Alconox and rinse thoroughly with tap water,
- 3) rinse with a reagent grade organic solvent (i.e. methanol or acetone),
- 4) triple rinse with deionized water (18 M ohm),
- 5) soak for 24 hours in 2N hydrochloric acid, and
- 6) rinse six times with deionized water. Disposable containers must be punctured after use to prevent reuse.

Instrumentation

All instruments are adjusted and calibrated to the manufacturer's specifications. Instrument maintenance is performed as specified by the manufacturer or more frequently when necessary. All instruments that require expert technicians are maintained under service contracts with the manufacturers, or with reliable service engineers.

Quality Control

Introduction

Quality Control (QC) consists of specific activities and procedures designed to measure and control the quality of the data being produced. Pacific EcoRisk is committed to the generation of high quality data and employs strict QC in the laboratory.

Instrument Calibration and Standardization

Requirements for instrument calibration and standardization for use in toxicity tests and routine water quality analyses are briefly described below. Detailed descriptions of these chemical analyses are described in Laboratory Standard Operating Procedures.

Temperature - Temperature is measured to the nearest degree Centigrade using digital or mercury thermometers. Laboratory thermometers are calibrated semi-annually against a Standard Thermometer that has been certified as factory-calibrated against a National Institute of Standards and Technology (NIST) thermometer.

Conductivity - Conductivity is measured to the nearest $\mu\text{S}/\text{cm}^2$ using a calibrated conductivity meter. Maintain the meter and probe according to factory specifications. Standards are kept refrigerated in clean, sealed glass bottles. Handling of standards is minimized by using subsamples for multiple calibrations.

Salinity - Salinity is measured to the nearest g/L using a calibrated salinity meter. Meter and probe are maintained according to factory specifications. Keep standards refrigerated in clean, sealed glass bottles. Handling of standards is minimized by using subsamples for multiple calibrations.

pH -pH is measured to the nearest 0.01 pH unit using an appropriate meter and probe. Meter and probe are maintained according to factory specifications. Probe is calibrated before each use using buffer solutions that bracket the pH range of the samples.

Dissolved Oxygen -Dissolved oxygen is measured to the nearest 0.1 ppm with an appropriate meter and probe. Meter and probe are maintained according to factory specifications. Calibrate before each set of measurements using water saturated air or oxygen saturated water as specified in the manufacturers instructions for the probe. Calibrate the “Zero” on the probe using a 0 ppm oxygen solution (e.g. 3.81 g analytical grade sodium borate in a liter of distilled water saturated with crystalline sodium sulphite).

Irradiance (Light) - Irradiance is measured using an appropriate meter and an irradiance sensor that measures photosynthetically active radiation (PAR, photons) in units of $\mu\text{Einsteins}^{-2}\text{sec}^{-1}$. Meter is factory calibrated at intervals recommended by the manufacturer.

Total Ammonia - Ammonia is measured to the nearest 0.1 mg/L using an appropriate meter and probe. Meter and probe are maintained according to factory specifications. The probe is calibrated before each use using standard ammonia solutions that bracket the concentration range of the samples.

Total Residual Chlorine -Chlorine is measured to the nearest 0.1 mg/L using an appropriate meter and probe. Meter and probe are maintained according to factory specifications. The probe is calibrated before each use in accordance to manufactures instructions.

Weights and Volumes - Calibration of the balance is checked before each use with weights traceable to NIST standards. Balance is certified annually by a service representative and maintained according to factory specifications. Calibration weights are inspected at each calibration and discard if corroded or otherwise suspect. Flasks and pipettes/pipettors are calibrated by weighing volumes of distilled water on an analytical balance.

Precision and Accuracy

Reference toxicant tests are performed to assess the precision and accuracy of the bioassays performed for each set of organisms tested in the laboratory. Reference toxicant tests indicate the sensitivity of the organisms being used and the suitability of the test methodology. Reference toxicant tests are performed on in-house cultures once a month as well as simultaneously with each test as necessary. A single reference toxicant test is acceptable for comparison with multiple test samples if all organisms tested are from a single batch of organisms. A database on all reference toxicant tests performed at this laboratory is kept and used as a tool to evaluate the acceptability of any new reference toxicant test performed in conjunction with testing as well as to monitor the health of in-house cultures. Reference toxicant control charts are kept to measure the precision of the laboratory in performing testing with a specific organism; the test accuracy is estimated through the comparison of a specific reference toxicant test with the established in-house database.

Internal Quality Control Checks

The following procedures are performed as internal quality control checks to ensure that all infrastructural functions and generation of data in the laboratory are within acceptable

performance ranges: water bath temperature monitoring, DI water quality monitoring, review of test data, instrumentation calibration log entries, test organisms acclimation and husbandry log, sample acquisition log, calibration of equipment according to SOPs, and continual training of laboratory personnel.

External Quality Control Checks (Performance Evaluations)

Pacific EcoRisk participates in performance evaluations administered by the California State Department of Health Services Environmental Laboratory Accreditation Program and the U.S. EPA DMR-QA Program. These performance evaluations serve as external quality control programs to further substantiate the defensibility and validity of the data generated.

Acquisition, Reduction, Validation and Reporting of Data

All data is entered in permanent, insoluble, and reproducible ink. All data entries are initialed and dated with any incorrect data struck out with one line, initialed and dated. During the data reduction process, all calculations are double-checked by qualified personnel and initialed. Sample chain-of-custody records are checked to ensure the following information is available: information on sample type, date, place, and time of collection, and name of the sample collector; date and place of laboratory natural water collection (if applicable). Test reports are reviewed to ensure they include: dates of sample receipt and testing, test method descriptions (including references) and results of tests; reference toxicant results (if applicable); listing of acceptability criteria and whether results met these criteria; copies of raw data, chain-of-custody record(s), and statistical printouts. Reports shall also include descriptions of any deviations from protocols and discussion of deviation impact on study results. Any reports that are amended or revised are labeled and dated so as to be distinguishable from any earlier versions.

Quality Assurance Reports

In-house lab audits will be performed by the QA/QC officer to evaluate the performance of the laboratory with respect to adherence to established SOPs and maintenance of internal quality control requirements. Audit reports will include suggestions for corrective action and if corrective action is needed, will include a brief description on the implementation of the corrective action. In addition to lab audits, all personnel are tested and signed off for procedures that they are approved to perform.

Laboratory Organization and Personnel Responsibilities

Laboratory staff consists of a Lab Director, Lab Manager, QA/QC Officer, Project Managers, Laboratory Scientists and Lab Technicians. Their responsibilities are as follows:

Director - Oversees general operation of the laboratory. Coordinates activities of Project Managers. Consults daily with Lab Manager and QA/QC officer to evaluate lab operations.

Lab Manager - Oversees daily operation of the laboratory. Coordinates activities of Project Managers. Consults daily with Lab Director and QA/QC officer to evaluate lab operations.

QA/QC Officer - Responsible for the development and implementation of the QA/QC plan. Consults daily with lab manager to evaluate lab operations. Performs quarterly audits.

Project Manager - Responsible for the design and performance of individual projects. This includes overseeing all laboratory scientists participating in the project, adherence to Standard Operating Procedures, QA/QC plan, interpretation of data, and preparation of Final Reports.

Laboratory Scientists - Responsible for the daily performance of all tests, maintenance of organism cultures, performance of chemical analyses, and data acquisition and recording.

Lab Technicians - Responsible for the cleaning of all glassware and performance of routine chemical analyses.

Quality Assurance Officer, Duties, Responsibility, and Authority

Specific Duties

- Reviews the overall QA/QC effort and provides a quarterly QA report to Laboratory management.
- Reviews all internal QC charts and outside QC programs to ensure that the quality of the data is maintained over time. Makes recommendations based upon these trends in order to consistently provide data that is of the highest quality.
- Maintains a file of all Corrective Actions reports.
- Maintains a file of all laboratory accreditation information.
- Reviews all laboratory notebooks and logs to insure that the information is uniform, clear and precise.

Responsibility and Authority

- Develops and reviews quality control programs including statistical procedures and techniques for the maintenance of quality control standards.
- Monitors quality assurance activities to determine conformance with the guidelines established in the laboratory SOP manuals.
- Evaluates new ideas and current developments relative to the field of quality control and quality assurance, and recommends means for their implementation.
- Has the authority to stop a project.
- Evaluates data quality and maintains records on related quality control charts and other pertinent information.
- Coordinates and/or conducts quality assurance investigations (intra- and inter- laboratory programs).

Bibliography

The following bibliography is a listing of the most commonly accepted guidelines for test methods used in this laboratory. All personnel participating in any test described in one or more of these guidelines are expected to be familiar with these guidelines.

- American Public Health Association (APHA). 1989. Standard Methods for Examination of Water and Wastewater, 17th ed. APHA, Washington.
- American Society for Testing and Materials (ASTM). 1993. ASTM Standards on Aquatic Toxicology and Hazard Evaluation. PCN: 03-54703-16. ASTM, Philadelphia.

Acute Toxicity Testing

- Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, Third Edition. EPA/600/4-85/013. U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH.
- Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, Fourth Edition. EPA/600/4-90/027F. U.S. EPA, Office of Research and Development, Washington, D.C.
- Guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. ASTM E729-88a. American Society for Testing and Materials, Philadelphia, PA.

Chronic Toxicity Testing

- Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, Second Edition. EPA/600/4-89/001. U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, OH.
- Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, Third Edition. EPA/600/4-91/002. U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati, OH.
- Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA/600/4-87/028. U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH.
- Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA/600/4-91/003. U.S. EPA, Environmental Monitoring

and Support Laboratory, Cincinnati, OH. (Second Edition)

- Guide for conducting toxicity tests starting with embryos of four species of saltwater bivalve molluscs. ASTM E724-89. American Society for Testing and Materials, Philadelphia, PA.
- Short-term methods for estimating the chronic toxicity of effluents and receiving waters to West Coast marine and estuarine organisms. EPA/600/R-95/136. U.S. EPA, Office of Research and Development, Washington, D.C.

Toxicity Identification Evaluations/ Toxicity Reduction Evaluations

- Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures (Second Edition). EPA-600/6-91/003. U.S. EPA, Environmental Research Laboratory, Duluth, MN.
- Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/080. U.S. EPA, Office of Research and Development, Washington, D.C.
- Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/081. U.S. EPA, Office of Research and Development, Washington, D.C.
- Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants. EPA/600/2-88/062. U.S. EPA, Water Engineering Research Laboratory, Cincinnati, OH.
- Sediment toxicity identification evaluation: Phase I (characterization), Phase II (identification), and Phase III (confirmation). Modifications of effluent procedures. EPA-600/6-91/007. U.S. EPA, Environmental research Laboratory, Duluth, MN.

Sediment Toxicity & Bioaccumulation Testing

- Long-term management strategy (LTMS) for the placement of dredged material in the San Francisco Bay Region (Draft). U.S. EPA Region 9, U.S. Army Corps of Engineers, San Francisco Bay Conservation and Development Commission, San Francisco Bay Regional Water Quality Control Board, California State Water Resources Control Board.
- Evaluation of dredge material proposed for ocean disposal - Testing Manual. EPA-503/8-91/001. U.S. EPA-U.S. Army Corps of Engineers, Washington, D.C.
- Evaluation of dredged material proposed for discharge in waters of the U.S. - Inland Testing

- Manual. EPA-823/B-94/002. U.S. EPA-U.S. Army Corps of Engineers, Washington, D.C.
- QA/QC guidance for sampling and analysis of sediments, water, and tissues for dredged material evaluations. Phase 1 - Chemical evaluations. EPA 823-B-95-001. U.S. EPA, Office of Water, Washington, D.C.
 - Methods for measuring the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA-600/R-94/025. U.S. EPA, Environmental Research Laboratory, Narragansett, RI.
 - Guidance manual: bedded sediment bioaccumulation tests. EPA-600/X-89/302. U.S. EPA Environmental Research Laboratory, Newport, OR.
 - Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-94/024. U.S. EPA, Office of Research and Development, Washington, D.C.
 - Standard guide for conducting sediment toxicity tests with freshwater invertebrates. ASTM E1383-94. American Society for Testing and Materials, Philadelphia, PA.
 - Standard guide for collection, storage, characterization, and manipulation of sediments for toxicity testing. ASTM E1391-90. American Society Testing & Materials, Philadelphia, PA.
 - Public Notice 93-2: Testing Guidelines for Dredged Material Disposal at San Francisco Bay Sites. US Army Corps of Engineers. Regulatory Branch, San Francisco, CA.

***Ceriodaphnia dubia* 7-Day Survival and Reproduction Bioassay
Standard Operating Procedures
(Pacific EcoRisk 1997)**

Ceriodaphnia dubia
7 Day Survival and Reproduction Bioassay
Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA/600/4-89/001 and EPA-600-4-91-002)

1.0 INTRODUCTION

This test is based on a seven day static-renewal exposure of < 24 hr old *Ceriodaphnia dubia* to different concentrations of effluents and/or receiving waters. The test endpoints are survival and reproduction.

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Selenastrum, YCT and the vitamins Thiamin, Biotin and B₁₂
2. pH, D.O., and conductivity/salinity meter, needed to document test water quality.
3. ASTM certified Thermometer, for documenting test water temperature.
4. Test Containers, 30 mL plastic cups. cups must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
5. De-Ionized water, for rinsing of probes, etc.
6. Wash Bottles, for rinsing of probes, etc.

7. Volumetric and Graduated Flasks and Pipettes, for making up dilution series and reference toxicant test solutions.
8. Transfer Pipettes, for transfer of cerios to and from test containers.
9. Cubitainers may be necessary for the client's collection of effluent.
10. ACS Reagent CuSO_4 (Copper Sulfate), for use as reference toxicant.

2.2 Ordering and Holding of Test Organisms

1. Test organisms should be ordered far enough in advance so as to ensure arrival of < 24 hrs old animals on test set-up day. Approximately 25-33% more animals should be ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.
2. Order cerios from: - Aquatic Research Organisms (603)926-1650
3. For additional instruction on the receipt and handling of the test organisms, see the "Test Organism Receipt and Handling S.O.P."

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or "blue ice type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in measurements should be taken. For instruction on the log-in of incoming samples, see the "Test Sample(s) Log-In Procedures". The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 1 Liter of sample will be needed each day.

3.0 TEST INITIATION

Before test initiation begins, read the attached "Summary of Test Conditions for *Ceriodaphnia dubia*".

1. Prepare the test cups for each concentration. You will need 10 containers per treatment. Label the cups with their treatment and replicate I.D. using a Sharpie pen.
2. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels.

3. Label (6) 250 mL beakers (I.e., Control, 5, 10, 25, 50,100) with the appropriate test treatments.
4. Add the following nutrient concentrations into each sample treatment prior to distribution to test containers.

Selenastrum	0.1 mL/15 mL (3×10^7 cells/mL)
YCT	7 mL/L
Thiamin	0.1 mL/L
Biotin	0.1 mL/L
B ₁₂	0.1 mL/L

5. For each treatment, record the initial water quality (pH, D.O., conductivity, and temperature) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels.
6. Beginning with the Control treatment and working up through the concentration series, pour 15 mL of sample water into each of the 10 cups.
7. Identify 10 adult cerio females from the brood board cultures that have had 8 or more offspring. Each of the neonates from one adult will be used to load one replicate (ie all A replicates) from each treatment concentration (this is done so that any anomalies, such as a high proportion of males in a particular replicate, can be addressed by omitting that particular replicate from the statistical analysis.
8. Try to arrange the work schedule so that loading of organisms into the test containers will take place around 2-3 p.m. Using a transfer pipette, capture and randomly allocate 1 cerio into each of the test containers.
9. Let the replicate containers (with the animals within) sit for one hour and then re-examine each replicate, removing any organism(s) that appear to have been unduly stressed or injured from the loading procedure, and replace with a freshly caught organism.

4.0 MAINTAINING THE TEST

1. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels.

2. Prepare the appropriate test solutions and add the appropriate nutrients. Record pH, D.O. and conductivity before pouring into new test cups.
3. Transfer each organism into its designated chamber and save and aliquot of each test concentration to measure the “old” pH and D.O.. Check these measurements to make sure that the water quality is within acceptable limits.
4. Count the neonates in each chamber and record the number on the data sheet.
5. Place transferred organisms and test media back into the trough.

5.0 TEST TERMINATION

Test are terminated when 60% or more of the surviving females in the controls have produced their third brood. All observations on organisms’ survival and number of offspring should be completed within two hours of test termination.

1. Count the neonates in each chamber and record the number on the data sheet.
2. Save and aliquot of each test concentration to measure the “old” pH, D.O.
3. Record all appropriate data and begin data reduction process for report preparation.

5.1 Test Acceptability

Test acceptability criteria for the *Ceriodaphnia dubia* chronic test include 80% or greater control survival and an average of 15 or more young per surviving female.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the *Ceriodaphnia* reference toxicity test is presented in the “Chronic *Ceriodaphnia dubia* Reference Toxicity Test” SOP.

7.0 DATA ANALYSIS

The two endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a TOXCALC data file labeled for identification of the specific test. Statistical analysis are performed in accordance with EPA guidelines for statistical analysis.

8.0 QUALITY CONTROL

1. Control water, consisting of commercial spring waters for tests and cultures.
2. All equipment is calibrated and operated as described in each applicable equipment SOP.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

9.0 SAFETY

The *Ceriodaphnia dubia* 7 Day chronic toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. After the ref-tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

Flow Charts of TIE Procedures

**The Use of Ion Exchange Resins to Determine the Biototoxicity and
Concentration of Dissolved Trace Metals in Natural Waters
(Connor 1991)**

Forms:
Labels, Log Sheets, Data Reports

Appendix E

Supporting Documents for Pathogen Monitoring

Method 1623:
***Cryptosporidium* and *Giardia* in Water**
by Filtration/IMS/FA
(USEPA 1999)

Appendix F

Supporting Documents for Benthic Invertebrate Monitoring

California Stream Bioassessment Procedures:

Habitat Assessment and Biological Sampling (CDFG 1996)

Macroinvertebrate Laboratory and Data Analyses (CDFG 1996)

**Methods For Collecting Benthic Invertebrate Samples
As Part Of The National Water-Quality Assessment Program
(USGS 1993)**

Appendix G

Supporting Documents for Fish Tissue Monitoring

**CDFG Fish Sampling and Sample Handling Protocols
(SFBRWQCB 1995)**

**Analytical Protocols:
PCBs and Chlorinated Pesticides in Fish Tissue
(California Department of Fish and Game
Water Pollution Control Laboratory, Rancho Cordova)**

**Analytical Protocols:
Mercury in Fish Tissue
(CDFG Marine Pollution Laboratory, Moss Landing)**

Forms:
Labels, Log Sheets, Data Reports

Appendix H

Example Chain of Custody Form