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Site: Centredale
Break: 3-4
Other: 25648

APPENDIX B

Mass Descriptors for Modified Method 8290 and 1613

SDMS DocID 25648

Appendix B

Experiment: DIOXIN (5 Functions)

Operator	: User
Date	: 19-OCT-2000 12:12:15
Instrument	: AutoSpec

Function 1

Type	: SIR Voltage
Calibration file used	: TEST1005C_1
High mass	: 375.8
Low mass	: 293.0
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 19:00
End Time	: 28:25
Fast lock	: On
Number of channels	: 12
Cycle time (ms)	: 1060

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1 (Lock)	292.9825	80	20
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
2	303.9016	80	10
3	305.8987	80	10
4	315.9419	80	10
5	317.9389	80	10
6	319.8965	80	10
7	321.8936	80	10
8	327.8847	80	10
9	330.9792	80	10
10	331.9368	80	10
11	333.9339	80	10
12	375.8364	50	10

Septum flow : Off

Appendix B

Experiment: DIOXIN (5 Functions)

Operator	: User
Date	: 19-OCT-2000 12:12:15
Instrument	: AutoSpec

Function 2

Type	: SIR Voltage
Calibration file used	: TEST1005C_2
High mass	: 409.8
Low mass	: 339.9
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 28:25
End Time	: 37:30
Fast lock	: On
Number of channels	: 11
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	339.8597	80	20
2	341.8567	80	10
3	351.9000	80	10
4	353.8970	80	10
5 (Lock)	354.9792	80	10
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
6	354.9792	80	10
7	355.8546	80	10
8	357.8516	80	10
9	367.8949	80	10
10	369.8919	80	10
11	409.7974	80	10

Septum flow	: Off
Repeats	: 1

Appendix B

Experiment: DIOXIN (5 Functions)

Operator	: User
Date	: 19-OCT-2000 12:12:15
Instrument	: AutoSpec

Function 3

Type	: SIR Voltage
Calibration file used	: TEST1005C_3
High mass	: 445.8
Low mass	: 373.8
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 37:30
End Time	: 43:30
Fast lock	: On
Number of channels	: 11
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	373.8208	80	20
2	375.8178	80	10
3	383.8639	80	10
4	385.8610	80	10
5	389.8157	80	10
6	391.8127	80	10
7 (Lock)	392.9760	80	10
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
8	401.8559	80	10
9	403.8529	80	10
10	430.9729	80	10
11	445.7555	80	10

Septum flow	: Off
Repeats	: 1

Appendix B

Experiment: DIOXIN (5 Functions)

Operator	: User
Date	: 19-OCT-2000 12:12:15
Instrument	: AutoSpec

Function 4

Type	: SIR Voltage
Calibration file used	: TEST1005C_4
High mass	: 479.7
Low mass	: 407.8
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 43:30
End Time	: 48:00
Fast lock	: On
Number of channels	: 11
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	407.7818	80	20
2	409.7789	80	10
3	417.8253	80	10
4	419.8220	80	10
5	423.7766	80	10
6	425.7737	80	10
7 (Lock)	430.9729	80	10
Primary Span Lock (Peaks)	2.00		
Secondary Span Lock (Peaks)	2.00		
Lock Level (mV)	0		
Step Lock (Peaks)	0.020		
8	430.9729	80	10
9	435.8169	80	10
10	437.8140	80	10
11	479.7165	80	10

Septum flow	: Off
Repeats	: 1

Appendix B

Experiment: DIOXIN (5 Functions)

Operator	: User
Date	: 19-OCT-2000 12:12:15
Instrument	: AutoSpec

Function 5

Type	: SIR Voltage
Calibration file used	: TEST1005C_5
High mass	: 513.7
Low mass	: 441.7
Resolution	: 10000
Ionisation mode	: EI+
Accelerating Voltage	: 8000.0V
Magnet 1 control	: Current
Start Time	: 48:00
End Time	: 52:00
Fast lock	: On
Number of channels	: 9
Cycle time (ms)	: 1000

Channel	Mass	Ch Time (ms)	I/ch Time (ms)
1	441.7428	100	20
2 (Lock)	442.9728	100	10
Primary Span Lock (Peaks) 2.00			
Secondary Span Lock (Peaks) 2.00			
Lock Level (mV) 0			
Step Lock (Peaks) 0.020			
3	442.9728	100	10
4	443.7399	100	10
5	457.7377	100	10
6	459.7348	100	10
7	469.7779	100	10
8	471.7750	100	10
9	513.6775	100	10

Septum flow	: Off
Repeats	: 1

OCT 3 0 2000

STANDARD OPERATING PROCEDURE (SOP) FOR POLYCHLORINATED DIBENZO-P-DIOXIN/POLYCHLORINATED DIBENZOFURAN (PCDD/PCDF) SAMPLE PREPARATION USING MODIFIED METHODS 8290 AND 1613

Originated by: _____ Date: 10/30/00

Approved by: Karen L. Tracy
Technical Reviewer Date: 10/30/00

Approved by: Karen Riggs
Management Date: 10/30/00

Reviewed and Registered by the Quality Assurance Coordinator:

Charles D. Zaworie Date: 10/30/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This SOP describes routine procedures for preparing samples for PCDD/PCDF analysis. These procedures follow general guidelines described in EPA Methods 8290 and 1613, with some minor modifications/improvements. The purpose of this SOP is to provide a description of PCDD/PCDF sample preparation activities using modified Method 8290 and Method 1613 procedures and covers the following:

- Personnel qualifications
- Sample collection, handling and preservation
- Sample extraction and internal standard spiking
- Extract cleanup
- Final concentration activities.

III. References

A. SW-846, Method 8290. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRCG/HRMS), Revision 0, 1994.

B. EPA Method 1613: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B, 1994, EPA 821-B-94-005.

C. SOP ASAT.II-005-00. Standard Operating Procedure (SOP) for Polychlorinated Dibenzo-p-dioxin/Polychlorinated dibenzofuran (PCDD/PCDF) Desiccating Agent and Adsorbent Preparation and Storage.

D. SOP ASAT.II-006-00. Standard Operating Procedure (SOP) for Polychlorinated dibenzo-p-dioxin/Polychlorinated dibenzofuran (PCDD/PCDF) Standards and Reagents Preparation and Storage for Modified Method 8290 Analysis.

IV. Definitions

All references in this section are to SW846 Method 8290 unless otherwise indicated.

V. Procedures

A. Personnel Qualifications

Personnel assigned to laboratory activities meet the educational, work experience, and training requirements for their positions. Records on personnel qualifications and training are maintained in personnel files accessible for review during audit activities. Training is conducted in accordance with standard operating procedures and is available to all laboratory personnel. Employees must demonstrate proficiency at specific tasks and this capability is documented and kept in a central file.

B. Sample Collection, Handling, and Preservation

Sampl s will be treated as described in Method 1613 Section 8.0 (see Appendix A) or as required by the client.

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C. Sample Extraction and Spiking

The procedures for tissue, sediment, and water samples are described below. A quality control (QC) sample (Cambridge Isotope standard reference material EDF-2526 or EDF-2513) is extracted with field samples at a minimum of once every quarter. Procedures for other matrices will be distributed to staff in project specific workplans.

1. Sediment and Tissue Samples

   a. All glassware are detergent-washed, rinsed, solvent-rinsed (methanol, dichloromethane) and muffled overnight at 450°C. Soxhlets are assembled with 250-mL round bottom flasks (RB) and are also pre-extracted with the extraction solvent for three hours prior to use. This extract is archived until sample analysis is complete.

   b. The samples are mixed well and weighed into muffled 4-oz jars. For sediments, 10 g wet weight is typically used. For tissues, 20 g wet weight is required if lipid content is being determined; otherwise, 10 g wet weight is used. The samples are mixed with 5 to 10 g Varian Hydromatrix drying agent. The sample/Hydromatrix mixture is allowed to dry with occasional stirring until the mixture is free flowing and powdery. A method blank is prepared by adding Hydromatrix without sample to ensure that laboratory procedures and reagents are free from contamination.

   c. Approximately 1/4 inch of silica gel is added to the bottom of a pre-extracted glass thimble. The sample/Hydromatrix mixture is then added to the thimble. All samples are spiked with 1 ml of IS spiking solution. The matrix spike samples are spiked with 1 ml precision and recovery (PAR) spiking solution. The method detection limit verification samples (MDL) are spiked with 250 µL PAR spiking solution. Boiling chips are added, the Soxhlets are assembled, and the extractions started. Sediments are extracted using toluene. Tissues are extracted using a 50/50 solution of dichloromethane (DCM) and hexane.

   d. After 18 hours of extraction, the Soxhlets are allowed to cool and drain back into the 250-mL RB. New boiling chips are added and the extracts are concentrated to approximately 20 ml using Snyder columns.

2. Water Samples

   a. Water Samples with < 1% Solids

      1. All glassware are detergent-washed, rinsed, solvent-rinsed (methanol, dichloromethane) and muffled overnight at 450°C.

      2. The samples are allowed to come to room temperature.

3. A permanent marker is used to mark the sample volume on each sample container. The samples are then visibly inspected for solids.

4. If the samples are visually determined to be at or below 1% solids, the samples are transferred to a DCM rinsed 2-L separatory-funnel with 3 x 20-mL DCM rinses of the sample container.

5. A method blank, for water with < 1% solids, is prepared by adding 1 L reagent water and 60 ml of DCM to a DCM rinsed 2-L separatory-funnel.

6. All samples are spiked with 1 ml internal standard (IS) spiking solution while they are still in the sample container.

7. Matrix spike samples are also spiked with 1 ml PAR spiking solution.

8. Method detection limit (MDL) samples are also spiked with 250 µL PAR spiking solution.

9. The separatory-funnels are shaken for 2 minutes with periodic venting.

10. The phases are allowed to separate for 20 minutes and the bottom layer (DCM) is drained through a funnel containing approximately 15 g of DCM rinsed sodium sulfate into a 200-mL turboTube.

11. The sodium sulfate is then rinsed with an additional 20 ml of DCM and also collected in the 200-mL turboTube.

12. The 60 ml DCM extraction is repeated 2 more times, each time draining the DCM through the sodium sulfate into the 200-mL turboTube.

13. Using a TurboVap, the extracts are solvent exchanged into 4 ml of hexane.

14. The sample containers are filled to the marked volume with DI water. The DI water is then poured into a graduated cylinder and the volume is recorded

b. Waters with > 1% Solids

1. All glassware are detergent-washed, rinsed, solvent-rinsed (methanol, dichloromethane) and muffled overnight at 450°C.

2. The samples are allowed to come to room temperature.

3. A permanent marker is used to mark the sample volume on each sample container. The samples are then visibly inspected for solids.

4. If the samples are visually determined to be above 1% solids, each sample is filtered through a separate muffled 0.45 μm glass fiber filter.

5. All samples are spiked with 1 ml IS spiking solution while they are still in the sample container.

6. Matrix spike samples are also spiked with 1 ml PAR spiking solution while they are still in the sample container.

7. Method detection limit (MDL) samples are also spiked with 250 μL PAR spiking solution while they are still in the sample container.

8. Samples are shaken vigorously after they have been spiked with the appropriate spiking solutions and then allowed to sit at room temperature for 1 to 2 hours to allow the spike solutions to distribute through out the sample.

9. When samples have been received without any headspace in the sample container, approximately 20% of the sample is transferred to a glass, muffled secondary container before any of the spiking solutions are added to the sample container. The remaining sample is then spike according to the procedure above.

10. The glass fiber filter is wetted and sealed with reagent grade water onto the filtering apparatus. The samples are slowly poured through the glass fiber filters taking care not to disturb any settled solids at the bottom of the sample container. When the majority of the sample has passed through the filter, the remaining portion of the sample in the container is swirled to re-suspend the solids and then also poured through the glass fiber filter. A small amount of reagent grade water is used to rinse the sample container to ensure the complete transfer of solids to the filter.

11. However, if the solid content in the samples is visually determined to be too great for the 0.45 μm glass fiber filter, the samples are centrifuged in order to separate the water layer from the solids layer. The aqueous layer is then poured through a muffled glass fiber filter that has been wetted with reagent grade water. The sediment portion is transferred into a muffled 4-oz glass jar via a muffled stainless steal spatula and the centrifuge tube is rinsed with reagent grade water to remove any remaining solids. The centrifuge tube rinse water is poured through the same muffled glass fiber filter as the decanted aqueous layer. The solid

fraction is combined with Hydromatrix to form a free flowing mixture and then combined with the glass fiber filter in a Soxhlet apparatus. The combined solid fraction/glass fiber filter is extracted using the sediment extraction procedure above (step 1) without the addition of any spike solutions.

12. After the extraction has been completed, the concentrated solids extract is solvent exchanged using a rotoVap into 4 ml of hexane.

13. A method blank, for waters with > 1% solids, is prepared by pouring 1 L reagent water through a glass fiber filter, Soxhlet extracting the filter as described in the previous step. The 1 L of filtered reagent water and 60 ml of DCM are added to a DCM rinsed 2-L separatory-funnel.

14. The separatory-funnels are shaken for 2 minutes with periodic venting.

15. The phases are allowed to separate for 20 minutes and the bottom layer (DCM) is drained through a funnel containing approximately 15 g of DCM rinsed sodium sulfate into a 200-mL turboTube.

16. The sodium sulfate is then rinsed with an additional 20 ml of DCM and also collected in the 200-mL turboTube.

17. The 60 ml DCM extraction is repeated 2 more times, each time draining the DCM through the sodium sulfate into the 200-mL turboTube.

18. Using a TurboVap, the extracts are solvent exchanged into 4 ml of hexane.

19. The sample containers are filled to the marked volume with DI water. The DI water is then poured into a graduated cylinder and the volume is recorded

20. The water and solid fractions are combined before proceeding to any cleanup procedures.

D. Extract Cleanup

1. Partition

If the sample extract is suspected to be of high lipid content, the optional bulk acid silica cleanup (step 4 below) can be used prior to acid/base partitioning. If bulk acid cleanup is used, the cleanup standard (CS) referred to below must be added prior to the bulk acid silica cleanup rather than after transfer to the separatory funnels.

Acid/Base Partitioning:

a. The concentrated extracts are transferred to a hexane rinsed 125-mL separatory funnel using muffled pasteur pipettes with 3 x 4-mL hexane rinses of the round bottom (RB).

b. The sample extracts are spiked with 1 ml of cleanup standard (CS) spiking solution and brought up to approximately 50 ml with hexane.

c. The extracts are partitioned against one 30-mL aliquot and various 20-mL aliquots of concentrated sulfuric acid, until there is no visible color or a maximum of 4 acid washes has been reached.

d. The extracts are washed with 20 ml of 5% sodium chloride solution (NaCl), 15 ml of 20% potassium hydroxide (KOH) solution (for tissues, the first 20 ml 5% NaCl wash and the 20% KOH wash are omitted) and 2 x 20-mL of 5% NaCl solution.

e. The washed extracts are drained through a funnel containing hexane rinsed sodium sulfate with 3 x 6-mL hexane rinses of the separatory funnel and are collected in a 250-mL RB or a 200-mL turboTube.

f. The sodium sulfate is then rinsed with 20 ml hexane and collected in the same RB or turboTube.

If the extract is still discolored after the samples have been through the partitioning procedure, then follow with the optional acid silica column cleanup procedure (step 5 below) prior to performing the stack column cleanup procedure.

2. Silica/Alumina Column Cleanup (Stack Column Cleanup)

a. The sample extracts are rotovapped or turboVapped to near dry in a 46°C water bath and brought up in 4 ml of hexane.

b. Acid/base silica columns and alumina columns are prepared by cutting the tops from 25-mL muffled pipettes, plugging with muffled glass wool, adding activated silica to the 22-mark, base silica to the 18-mark, silica to the 16-mark, acid silica to the 1-mark and approximately ½ inch of muffled sodium sulfate on top.

c. Basic alumina columns are prepared by cutting the tops from 25-mL muffled pipettes, plugging with muffled glass wool; adding 6 g activated basic alumina and approximately ½ inch of muffled sodium sulfate on top.

d. Acid/base silica columns and alumina columns are rinsed separately with 20 ml of hexane. As the last of the hexane rinse approaches the top of the packing, the acid/base silica columns are stacked on top of the alumina columns. The sample extracts are applied to the columns using muffled Pasteur pipettes with 3 x 4-mL hexane rinses of the turboTube or RB. The stacked columns are eluted with 100 ml of hexane and collected in a muffled 125-mL glass jar. As the last of the 100 ml hexane eluant passes through the stacked columns the columns are separated and the alumina column is eluted with 40 ml DCM/hexane (50/50) and collected in a turboTube.

e. If the acid layer in the acid / base silica column is badly discolored through the entire acid silica layer, the stacked silica / alumina column cleanup is repeated once more.

3. Carbon Column Cleanup

a. The sample extracts are turboVapped to near dryness in a 46°C water bath and brought up in 1 ml of hexane.

b. Carbon/Celite columns are prepared by cutting the top and bottom from a 10 ml muffled pipette and fire polishing both ends, plugging one end with muffled glass wool, adding 0.55 g of carbon/celite mixture and plugging with muffled glass wool.

c. The columns are rinsed with 5 ml of toluene, 2 ml of 15:4:1 (DCM/methanol/toluene), 1 ml of 1:1 (cyclohexane/DCM), and 5 ml of hexane. Each rinse is added as each previous rinse approaches the top of the packed bed in order to avoid any mixing and dilution of solvents.

d. The sample extracts are applied using muffled Pasteur pipettes with 2 x 1-mL, 1 x 2-mL and 2 x 3-mL hexane rinses of the TurboVap tube.

e. The column is then rinsed with 2 ml of 1:1 and 2 ml of 15:4:1. As the last of the 15:4:1 passes through the column, the column is inverted and eluted with 30 ml of toluene. The eluate is collected in a 125-mL RB.

4. Bulk Acid Silica Cleanup (optional)

a. Extracts are transferred to a 500 ml Erlenmeyer flask that contains an $1^1/_2$ inch stir bar. The RB is rinsed 3 times with 4 ml of hexane and the rinses are added to the Erlenmeyer flask.

b. The extract volume is brought up to 200 ml by using hexane to wash down the walls of the Erlenmeyer flask.

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c. The flask is placed upon a stir plate, and the stir plate is started.

d. Five 10 g aliquots of acid silica are added to the extract and the extracts are stirred for 2 hours.

e. After the 2 hours of stirring, the hexane layer is decanted into a 500 ml RB (or 200 ml turboTube) through $NaSO_4$ that has been pre-rinsed with hexane.

f. 50 ml of hexane are added to the acid silica in the Erlenmeyer flask by washing the walls of the Erlenmeyer flask, stirred for an additional 10 minutes, and then this hexane layer is decanted in the RB (or turboTube) combining it with the original layer of hexane. This procedure is repeated two more times.

g. The NaSO4 is then rinsed with 20 ml of hexane. The hexane is also collected in the same RB (or turboTube) as the sample extract.

Procedures for the use of less acid silica are taken from Method 1613, Section 13.7.2.4 (see Appendix A).

5. Acid Silica Column Cleanup (optional)
   a. The sample extracts are rotovapped or turbovapped to near dry in a 46°C water bath and brought up in 4 ml of hexane.

   b. Acid silica columns are prepared by cutting the tops from 25-mL muffled pipettes, plugging with muffled glass wool, and activated silica to the 22-mark, acid silica to the 1-mark and approximately 1/2 inch sodium sulfate on top.

   c. The column is rinsed with 20 ml hexane. As the last of the hexane rinse approaches the top of the bed packing, a turboTube is placed under the column and the sample extracts are applied using muffled Pasteur pipettes with 3 x 4-mL hexane rinses of the RB or turboTube.

   d. The column is eluted with 90 ml of hexane and collected in the turboTube.

   If the acid silica layer is badly discolored, the acid silica column cleanup is repeated.

E. Final Concentration

1. The sample extracts are rotovapped to near dry in a 48°C water bath and brought up in 1 ml of hexane.

2. Muffled concentrator tubes are rinsed with DCM by vortexing for approximately 30 seconds and then the rinse is discarded.

3. After the concentrator tubes have dried, 20 µL of nonane is pipetted into the tubes using an Eppendorf pipette. The tubes are lightly tapped to remove any air bubbles and the meniscus is marked on the tube using permanent marker.

4. 200 µL of hexane is then pipetted into the tubes using an Eppendorf pipette and the tubes are lightly tapped to remove any air bubbles. The meniscus of the hexane is then marked on the tube using permanent marker.

5. The concentrator tubes are labeled with sample Ids using a permanent marker.

6. Leaving the solvents in the tubes, the sample extracts are transferred using muffled Pasteur pipettes with 3 x 1-mL hexane rinses of the RB.

7. The sample extracts are then blown down under nitrogen in a 45°C sand bath to the marked hexane meniscus.

8. The RB is then rinsed with 1 x 1-mL DCM and transferred to the concentrator tube, being sure to rinse the walls of the tube with the DCM rinses. This is also blown down under nitrogen to the marked hexane meniscus.

9. The RB is then rinsed with 1 x 0.5-mL DCM and transferred to the concentrator tube, being sure to rinse the walls of the tube with the DCM rinses. This rinse is also blown down to the marked hexane meniscus.

10. The sample extracts are then spiked with 10 µL RS spiking solution, vortexed for approximately 30 seconds, capped, and a sample information archive label affixed to each concentrator tube. If not going directly to analysis, the tubes containing the extracts are capped and stored in a freezer.

11. Prior to analysis, the labeled concentrator tubes are removed from the freezer and allowed to come to room temperature.

12. Label the pre-assembled GC vials that contain 300-µL inserts. There must be a gap in the labels on the vials for liquid volume inspection.

13. Put squeeze balls on pipettes. Visually inspect the concentrator tubes to ensure that at least a 200 µL volume is contained. If not, adjust to the marked line with DCM.

14. Vortex the concentrator tubes, making sure that the liquid rinses the side walls. 200 µL will reach about halfway up the tube.

15. Transfer the contents of the concentrator tubes to the correct GC vial inserts. It is not possible with a disposable to completely transfer all of the material (about 10 µL will remain).

16. Cap the GC vial and place it in a GC vial container. Continue with the remaining samples. When all original samples have been transferred, start the nitrogen blow down procedure.

17. On an N-Evap, push the Teflon tubing onto the ¼" metal tube/valve assemblies, and install new glass pipettes.

18. Check to see that the nitrogen is turned on at the tank, and that the pressure is set to 40 psi on the second stage of the tank regulator, and approximately 5 pi on the regulator just before the N-Evap.

19. Uncap the GC vials and place them in the sample holders, with the labeled gap towards you so that the solvent level may be inspected. Push the pipettes down until the tip just rests inside the vial opening. Check each blowdown valve to see that the flow is correct, so that evaporation occurs, but the level of the liquid is not greatly deformed. Blow the sample extracts down until they are almost dry.

20. Adjust the sample volume to 20 µL with nonane using a syringe. Vortex the GC vial and store it in the GC vial container. If the samples are not going directly to the instrument for analysis, store them in a freezer.

VI. Revision History

1. The extraction of a quarterly QC sample was added.
2. The procedure for spiking IS in water samples with < 1% solids was updated.

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Appendix A

Method 1613 References

Appendix A

determined. In this case, an isomer-specificity test standard containing the most closely eluted isomers listed in Table 5 (CIL EDF-4033, or equivalent) may be used.

7.16 QC Check Sample—A QC Check Sample should be obtained from a source independent of the calibration standards. Ideally, this check sample would be a certified reference material containing the CDDs/CDFs in known concentrations in a sample matrix similar to the matrix under test.

7.17 Stability of Solutions—Standard solutions used for quantitative purposes (Sections 7.9 through 7.15) should be analyzed periodically, and should be assayed against reference standards (Section 7.8.3) before further use.

8.0 Sample Collection, Preservation, Storage, and Holding Times

8.1 Collect samples in amber glass containers following conventional sampling practices (Reference 16). Aqueous samples that flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.

8.2 Maintain aqueous samples in the dark at 0-4°C from the time of collection until receipt at the laboratory. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 17). If sample pH is greater than 9, adjust to pH 7-9 with sulfuric acid.

Maintain solid, semi-solid, oily, and mixed-phase samples in the dark at <4°C from the time of collection until receipt at the laboratory.

Store aqueous samples in the dark at 0-4°C. Store solid, semi-solid, oily, mixed-phase, and tissue samples in the dark at <-10°C.

8.3 Fish and Tissue Samples

8.3.1 Fish may be cleaned, filleted, or processed in other ways in the field, such that the laboratory may expect to receive whole fish, fish fillets, or other tissues for analysis.

8.3.2 Fish collected in the field should be wrapped in aluminum foil, and must be maintained at a temperature less than 4°C from the time of collection until receipt at the laboratory.

8.3.3 Samples must be frozen upon receipt at the laboratory and maintained in the dark at <-10°C until prepared. Maintain unused sample in the dark at <-10°C.

8.4 Holding Times

8.4.1 There are no demonstrated maximum holding times associated with CDDs/CDFs in aqueous, solid, semi-solid, tissues, or other sample matrices. If stored in the dark at 0-4°C and preserved as given above (if required), aqueous samples may be stored for up to one year. Similarly, if stored in the dark at <-10°C, solid, semi-solid, multi-phase, and tissue samples may be stored for up to one year.

Appendix A

8.4.2 Store sample extracts in the dark at <-10°C until analyzed. If stored in the dark at <-10°C, sample extracts may be stored for up to one year.

9.0 Quality Assurance/Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 18). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

If the method is to be applied to sample matrix other than water (e.g., soils, filter cake, compost, tissue) the most appropriate alternate matrix (Sections 7.6.2 through 7.6.5) is substituted for the reagent water matrix (Section 7.6.1) in all performance tests.

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

9.1.2 In recognition of advances that are occurring in analytical technology, and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve separations or lower the costs of measurements. These options include alternate extraction, concentration, cleanup procedures, and changes in columns and detectors. Alternate determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL (40 CFR Part 136, Appendix B) is lower than one-third the regulatory compliance level or one-third the ML in this method, whichever is higher. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.

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

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

9.1.2.2.2 A listing of pollutant(s) measured, by name and CAS Registry number.

Appendix A

13.7.1.1 Prepare the column as given in Section 7.5.4.

13.7.1.2 Pre-elute the column with 100 mL of hexane. Drain the hexane layer to the top of the column, but do not expose the sodium sulfate.

13.7.1.3 Load the sample and rinses (Section 12.4.1.9.2) onto the column by draining each portion to the top of the bed. Elute the CDDs/CDFs from the column into the apparatus used for concentration (Section 12.4.1.7) using 200 mL of hexane.

13.7.1.4 Concentrate the cleaned up extract (Sections 12.6 through 12.7) to constant weight per Section 12.7.3.1. If more than 500 mg of material remains, repeat the cleanup using a fresh anthropogenic isolation column.

13.7.1.5 Redissolve the extract in a solvent suitable for the additional cleanups to be used (Sections 13.2 through 13.6 and 13.8).

13.7.1.6 Spike 1.0 mL of the cleanup standard (Section 7.11) into the residue/solvent.

13.7.1.7 Clean up the extract using the procedures in Sections 13.2 through 13.6 and 13.8. Alumina (Section 13.4) or Florisil (Section 13.8) and carbon (Section 13.5) are recommended as minimum additional cleanup steps.

13.7.1.8 Following cleanup, concentrate the extract to 10 µL as described in Section 12.7 and proceed with the analysis in Section 14.

13.7.2 Acidified silica gel (Reference 28)—Procedure alternate to the anthropogenic isolation column (Section 13.7.1) that is used for removal of lipids from the Soxhlet/SDS extraction (Section 12.4.1).

13.7.2.1 Adjust the volume of hexane in the bottle (Section 12.4.1.9.2) to approximately 200 mL.

13.7.2.2 Spike 1.0 mL of the cleanup standard (Section 7.11) into the residue/solvent.

13.7.2.3 Drop the stirring bar into the bottle, place the bottle on the stirring plate, and begin stirring.

13.7.2.4 Add 30-100 g of acid silica gel (Section 7.5.1.2) to the bottle while stirring, keeping the silica gel in motion. Stir for two to three hours.

NOTE: 30 grams of silica gel should be adequate for most samples and will minimize contamination from this source.

2000

STANDARD OPERATING PROCEDURE (SOP) FOR INTERNAL QA INSPECTION AND CORRECTIVE ACTION PROCEDURES FOR POLYCHLORINATED DIBENZO-P-DIOXIN/POLYCHLORINATED DIBENZOFURAN (PCDD/PCDF) AND RELATED COMPOUND ANALYTICAL PROGRAMS

Originated by: Charles V. Jaworie Date: 2/15/00

Approved by: Mary Schroeck Date: 2/15/00
Technical Reviewer

Approved by: Karen Riggs Date: 2/21/00
Management

Reviewed and Registered by the Quality Assurance Coordinator:

Charles V. Jaworie Date: 2/21/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This SOP describes routine procedures for internal Quality Assurance (QA) inspections and corrective action procedures for PCDD/PCDF and related compound analysis programs.

III. References

None

IV. Definitions

Critical Phases – Essential study events or procedures, which are observed by the Quality Assurance Officer or his/her designee to assess the integrity of a project.

Project Manager – The individual responsible for the overall conduct of a project.

Management –The individual defined as at least one level above the project manager for the purposes of routing Quality Assurance issues.

QA Officer –The individual assigned by management responsible for all aspect of quality assurance for the Atmospheric Science and Applied Technology department. This individual will be independent from the Project Manager and report directly to Management.

V. Procedures

A. Audits

Internal systems audits involve the evaluation of facilities, equipment, processes, and data packages for conformance to Battelle standards and project specific objectives. The Quality Assurance (QA) Officer or his/her designee conducts these audits. Any such audits or reviews are documented. To ensure that all issues have been addressed, the auditor also reviews all corrective actions taken.

1. Critical Phase Audits

The QA Officer will conduct critical phase inspections, when required by the project manager, to assess that facilities, equipment, personnel, methods, practices, records, and quality control, as they apply to regulated projects, are in conformance to approved protocols, SOPs, regulations, sponsor requirements, and Battelle policy.

Prior to conducting critical phase inspections in the laboratory or field, the QA Officer will review the method, current versions of applicable SOPs, personnel training records and any special sponsor requirements. All critical phase observations will be conducted in the immediate area where the work is taking place, unless requested by the project manager, or for safety purposes.

The QA officer will monitor the on-going activity to assure SOPs and/or approved methods are available to the personnel conducting the work and that the work is being done as described in those SOPs or methods.

The QA Officer will also check to see that appropriate calibration, standardization, and inspection of equipment (i.e., refrigerators, freezers, balances, etc.) has occurred prior to use and that all required equipment records are in place and current.

Data records will be examined to make sure that they are current, are being promptly recorded in ink for manual recordings, and that any error corrections are being made by following Good Laboratory Practice (GLP) techniques. The QA Officer will check to see that the procedure being observed is fully and accurately being documented.

All reagents, solutions, control and reference materials will be checked by the QA Officer to insure that they are appropriately labeled.

All findings and observations will be recorded on the form similar to Attachment A and will be kept on file in the Quality Assurance Unit following routing (see section A5).

2. Audits of Data Quality

All data reports, including the Laboratory Record Books (LRBs), will be reviewed by the project manager and/or his/her designee to ensure that the procedures have been fully documented. If required by the project, following the review of the project manager and/or his/her designee, all materials will be sent to the QA Officer for further review. The project manager and/or his/her designee and/or the QA Officer will review all raw data (or a percentage as required by the project/client) for completeness and accuracy. In addition to ensuring that the data adheres to all issues of GLP, if required, the following specific items will be emphasized. This list is not all-inclusive and additional items may be examined:

- verify the concentration and traceability of standard solutions
- ensure traceability of all sample extracts
- Initial calibration and continuing calibrations meet method and/or project requirements
- mass lock checks meet requirements
- transcription verification of extract number and sample weight from the prep LRB into OPUS forms and Excel spreadsheets for each sample
- all compounds of interest are accounted for
- double check for co-eluting ethers for the furans reported and that the compounds have been flagged appropriately
- percent recoveries for internal standards, Standard Reference Material (SRM), and any spike samples meet method and/or project requirements

- percent recoveries are flagged appropriately.

3. Facility Inspections
All laboratory facilities may be inspected annually by the QA Officer to ensure that the facilities adhere to all requirements set forth in the regulations. Equipment records for instruments used to generate data or that affect the integrity of data collected will be examined to assure that written records are being maintained according to SOP. All reagents and samples present in the laboratory will be reviewed to assure proper labeling and that the stated storage requirements are being met. The SOP manuals will be compared with the SOP manual Table of Contents to assess whether they are current.

In addition, a member of the Environmental Safety and Health (ES&H) Department conducts quarterly facility inspections. The laboratories will be inspected for general cleanliness and adherence to safety procedures and requirements. The results of these inspections are communicated to the laboratory personnel verbally and when deemed appropriate, the results may be provided in writing based on the severity of the inspection results.

B. Reports and Corrective Action(s)
Findings of Critical Phase audits, Technical Data audits, and Facility Inspections, by the QA Officer, will be documented on an Audit Comment Sheet similar to Attachment B. This form, together with a Quality Assurance Routing Sheet (similar to Attachment C) will then be sent to the project manager (or facility manager in the case of facility inspections). The project manager will review each finding (if any) and describe on the Audit Comment Sheet the corrective actions taken and sign the routing sheet to attest to the fact that these actions were implemented. The form will then be returned to QA officer who will verify that the corrective actions were adequate and have been implemented (confirmed by signature on the routing sheet). The findings will then be routed to management for review and signature and returned to the QA Officer for retention.

VI. Revision History
Not applicable

BATTELLE COPY

Attachment A

Critical Phase Inspection Form

Project Manager:
QA Officer:_____

Study Number:
Date:_____

Compliance Item Cited: See Attached Comments

No Adverse Findings

Phase	Protocol, SOP, Method, Equipment Used

Personnel:

Narrative:

AUDIT COMMENT SHEET

Study Number and Phase:
QA Auditor:

INSPECTION PARAMETER	COMMENT	CORRECTIVE ACTION/RESPONSE

Attachment C

Quality Assurance Routing Sheet

Project Number: **Project Manager**:

Activity: **Audit Type**:

Project Title:

Sponsor:

Auditor: **Date**:

Please complete the attached form indicating CORRECTIVE ACTION TAKEN (IF NEEDED), sign and date this Routing Sheet in the space provided beside your name, and return the entire set when completed to the Quality Assurance Unit no later than _____.

Route To	Signature	Date

2000

STANDARD OPERATING PROCEDURE (SOP) FOR POLYCHLORINATED DIBENZO-P-DIOXIN/POLYCHLORINATED DIBENZOFURAN (PCDD/PCDF) SAMPLE CONTAINER PREPARATION AND SHIPMENT

Originated by: _____ Date: 16 Feb 2000

Approved by: _____ Date: 2/15/00
Technical Reviewer

Approved by: _____ Date: 2/18/00
Management

Reviewed and Registered by the Quality Assurance Coordinator:

_____ Date: 2/18/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This SOP describes routine procedures for preparation and shipment of containers for PCDD/PCDF samples and contains the following:

- Sample container quality
- Shipping instructions for the sample container(s).

III. References

None.

IV. Definitions

Not applicable.

V. Procedures

A. Personnel Qualifications

Personnel assigned to laboratory activities meet the educational, work experience, and training requirements for their positions. Records on personnel qualifications and training are maintained in personnel files accessible for review during audit activities. Training is conducted in accordance with standard operating procedures and is available to all laboratory personnel. Employees must demonstrate proficiency at specific tasks and this capability is documented and kept in a central file.

B. Sample Container

All sample containers are amber glass with Teflon caps and are purchased certified, pre-cleaned (VWR, grade QA or equivalent) and are received along with a Certificate of Analysis. The supplier assigns a lot number to each container that allows the container to be traced to the appropriate Certificate of Analysis.

C. Sample Container Shipping Procedures

Each sample container is individually wrapped in bubblewrap. The wrapped containers are tightly packed into a cooler that is lined with bubble wrap and packing peanuts. Cool packs, at room temperature, are also added to the cooler, if required. A chain of custody form is then placed in a plastic sealed bag and taped to the inside top of the cooler. The cooler is tape/sealed shut and taken to the Battelle shipping department, along with a shipping memo that contains the address and client contact that the cooler is being sent to. The shipping department then sends the cooler and its contents to the client.

VI. Revision History

Not applicable.

STANDARD OPERATING PROCEDURE (SOP) FOR POLYCHLORINATED DIBENZO-P-DIOXIN/POLYCHLORINATED DIBENZOFURAN (PCDD/PCDF) DESICCATING AGENT AND ADSORBENT PREPARATION AND STORAGE

Originated by: _____ Date: 16 Feb 2000

Approved by: _____ Date: 2/15/00
Technical Reviewer

Approved by: _____ Date: 2/18/00
Management

Reviewed and Registered by the Quality Assurance Coordinator:

_____ Date: 2/18/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This SOP describes routine procedures for the preparation and storage of adsorbents and desiccating agents used in sample clean-up activities for PCDD/PCDF samples. These procedures follow general guidelines described in SW846 Method 8290 and EPA Method 1613, with some minor modifications/improvements. This SOP covers the following areas:

- Silica preparation and storage
- Acid silica preparation and storage
- Basic silica preparation and storage
- Alumina preparation and storage
- CarboPack C carbon preparation and storage
- Celite preparation and storage
- CarboPack C carbon/Celite mixture preparation and storage
- Hydromatrix (also known as Hidromatrix) preparation and storage
- Sodium Sulfate preparation and storage

III. References

A. SW-846, Method 8290. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRCG/HRMS), Revision 0, 1994.

B. EPA Method 1613: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B, 1994, EPA 821-B-94-005.

IV. Definitions

All sections referenced in this SOP are from SW846 Method 8290 unless otherwise indicated.

V. Procedures

A. Personnel Qualifications

Personnel assigned to laboratory activities meet the educational, work experience, and training requirements for their positions. Records on personnel qualifications and training are maintained in personnel files accessible for review during audit activities. Training is conducted in accordance with standard operating procedures and is available to all laboratory personnel. Employees must demonstrate proficiency at specific tasks and this capability is documented and kept in a central file.

B. Silica gel.

Silica gel (ICN Biomedicals, 100-200 mesh, grade 60 A, or equivalent) is activated and cleaned in a manner similar to that called for in Method 8290, Section 5. 2.3 using the following procedure. A muffled Pyrex tube is filled ¾ full with silica and rinsed with 350 mL methanol (EM Science, HPLC grade, or equivalent) and 350 mL dichloromethane (DCM)(B&J Brand, pesticide residue grade, or equivalent), consecutively, using nitrogen to push the solvent through the silica. While still saturated

with DCM, the silica is emptied into a muffled glass thimble, topped with DCM-rinsed muffled glasswool, and extracted with DCM in a Soxhlet apparatus for a minimum of 3 hours. After extraction, the silica is poured into a muffled Pyrex tube, placed under nitrogen, heated to 65 degrees Celsius for 30 minutes, and then activated at 160 degrees Celsius for 1 hour. After activation the silica is allowed to come to room temperature under nitrogen and then is stored in a dessicator in a muffled glass jar with a Teflon-lined screw cap (expiration is 1 year from date of preparation).

C. Acid silica gel
Acid silica gel is prepared in a manner similar to that called for in Method 8290, Section 5.2.5 using the following procedure: 35 grams of activated silica gel (prepared as in V.B above) is weighed into a muffled glass jar; 15 mL of concentrated sulfuric acid is then added to the same jar. The jar is then capped with a Teflon-lined screw cap and shaken until the mixture is free of lumps and free flowing. The mixture is stored in a muffled glass jar with a Teflon-lined screw cap, inside a dessicator (Expiration date is 1 year from preparation).

D. Basic silica gel
Basic silica gel is prepared in a manner similar to that called for in Method 8290, Section 5.2.4 using the following procedure: 35 grams of activated silica gel (prepared as in V.B above) is weighed into a muffled glass jar; 17 mL of 1 N NaOH solution is then added to the same jar. The jar is then capped with a Teflon-lined screw cap and shaken until the mixture is free of lumps and free flowing. The mixture is stored in a muffled glass jar with a Teflon-lined screw cap, inside a dessicator (Expiration date is 1 year from preparation).

E. Alumina
Alumina (Sigma, activity grade 1, type WB-2 basic, or equivalent) is activated in a manner using similar methods called for in Method 1613 B, Section 7.5.2.2. using the following procedure. A muffled Pyrex tube is filled ¾ full with alumina and kept under nitrogen; the tube is then placed in a tube furnace and slowly brought up to 500 °C. The alumina is kept at 500 °C for a minimum of 12 hours while being under nitrogen. After this activation period, the alumina is allowed to come to room temperature under nitrogen. Once cool the alumina is stored in a muffled Erlenmeyer flask, capped with a muffled glass stopper, and placed in an oven at 130 °C. Right before and during use the alumina is stored in a dessicator at room temperature. The alumina is re-activated if three days have passed since it's last activation.

F. CarboPack C Carbon
Carbopack C (Supelco, 60/80 mesh, or equivalent) following Method 1613 B, Section 7.5.3.1. requires no special cleanup prior to use. Stored in dessicator (expiration is 5 years from date of receipt).

G. Celite

Celite (Supelco, 545-AW, reagent grade, or equivalent) following Method 1613 B, Section 7.5.3.2. requires no special cleanup prior to use. Stored in dessicator (expiration is 5 years from date of receipt).

H. CarboPack C carbon/ Celite Mixture

The CarboPack C carbon (Supelco, 60/80 mesh) / Celite (Supelco, 545-AW, reagent grade) mixture is activated in a manner similar to that called for in Method 1613 B, Section 7.5.3.3. with a modification producing a 20 % CarboPack C to Celite mixture (w/w) using the following procedure: the required amounts of CarboPack C carbon and Celite are weighed into the same muffled glass jar to produce enough material to pack the desired number of columns with a small amount of material being left over. The jar is then capped with a Teflon-lined screw cap and rotated until a uniform mixture is obtained. The Teflon-lined screw cap is removed and the mixture is covered with muffled aluminum foil that is punctured with small holes. The mixture is then placed and stored in a muffle oven at 130 °C for a minimum of 6 hours. Prior to use, the carbon/Celite mixture is allowed to come to room temperature inside a dessicator and is capped with a Teflon-lined screw cap (expiration is 1 year from date of preparation).

I. Hydromatrix

Hydromatrix (Varian, Sample Preparation Products, part number 0019-8003, or equivalent) requires no special cleanup prior to use. Once opened the Hydromatrix is stored in a muffled glass jar with a Teflon-lined screw cap, inside a dessicator (expiration is 1 year from date of receipt).

J. Sodium Sulfate

Sodium sulfate (JT Baker, 12-60 mesh, Ultra Resi-Analyzed, or equivalent) is cleaned and dried in a manner similar to Section 5.4.1. by the following procedure: 1 kg is poured into a large muffled glass thimble and rinsed with 500 mL of DCM (B&J Brand, pesticide residue grade, or equivalent). The saturated sodium sulfate is allowed to dry inside the thimble for 1 hour. The partially dried sodium sulfate is poured into a shallow stainless steel tray and covered with muffled aluminum foil or a stainless steel lid and muffled for a minimum of 6 hours at 450 °C. The muffled sodium sulfate is stored in the muffling container at 130 °C. Prior to use, the muffled sodium sulfate is poured into a muffled glass jar with a Teflon-lined screw cap and allowed to come to room temperature inside a dessicator.

VI. Revision History

Not applicable.

STANDARD OPERATING PROCEDURE (SOP) FOR POLYCHLORINATED DIBENZO-P-DIOXIN/POLYCHLORINATED DIBENZOFURAN (PCDD/PCDF) STANDARDS AND REAGENTS PREPARATION AND STORAGE FOR MODIFIED METHOD 8290 ANALYSIS

Originated by: _____ Date: 16 Feb 2000

Approved by: _____ Date: 2/16/00
Technical Reviewer

Approved by: _____ Date: 2/18/00
Management

Reviewed and Registered by the Quality Assurance Coordinator:

_____ Date: 2/18/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This SOP describes routine procedures for preparation and storage of standards and reagents for PCDD/PCDF samples prepared using modified Method 8290 (ASAT.II-002). These procedures follow general guidelines described in EPA Method 1613, with some minor modifications/improvements. The descriptions in this SOP cover the following items:

- Internal Standard (IS) Spiking Solution
- Cleanup Standard (CS) Stock Solution
- Cleanup Standard (CS) Spiking Solution
- Precision and Recovery (PAR) Spiking Solution
- Recovery Standard (RS) Stock/Spiking Solution
- Other standards
- Potassium Hydroxide Solution
- Sodium Hydroxide Solution
- Sodium Chloride Solution
- Dichloromethane/Methanol/Toluene – 15:4:1 Solution
- Dichloromethane/Cyclohexane – 1:1 Solution
- Dichloromethane/Hexane – 1:1 Solution

III. References

A. EPA Method 1613: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B, 1994, EPA 821-B-94-005.

IV. Definitions

All references in this section are to EPA Method 1613 unless otherwise indicated.

V. Procedures

A. Personnel Qualifications

Personnel assigned to laboratory activities meet the educational, work experience, and training requirements for their positions. Records on personnel qualifications and training are maintained in personnel files accessible for review during audit activities. Training is conducted in accordance with standard operating procedures and is available to all laboratory personnel. Employees must demonstrate proficiency at specific tasks and this capability is documented and kept in a central file.

B. Standard Solutions

1. Internal Standard (IS) Spiking Solution

The IS spiking solution is made similar to Method 1613 B, Section 7.10.3 by the following procedure. IS stock solution (Cambridge Isotope Laboratories EDF-8999, or equivalent) is diluted 1:50 with acetone (B & J Brand pesticide residue grade, or eqivalent). Every sample, including each quality control (QC) sample, gets a 1 mL spike of IS spiking solution prior to extraction. This is equivalent of 20 µl of stock IS in each 1 mL of IS spiking solution. In order to minimize waste of these standards, the minimum volume possible of the IS spiking solution is prepared for each sample batch. The total

volume of the IS spiking solution prepared for a sample batch needs to be sufficient to deliver a 1 mL aliquot to each sample (including QC samples) with 2 mL being left over. For example, if the total number of samples (including QC samples) is 28, then 30 mL of IS spiking solution would be prepared. This often results in odd volumes of IS spiking solution being needed. Because of this, an accurate final volume for the IS spiking solution is determined using the weight and density of the components rather than by a direct volume measurement.

The IS stock solution volume is determined by taking the total mL of IS spiking solution to be prepared and multiplying by 20µL IS stock/mL. For this example, if 30 mL of IS spiking solution is being prepared, then 600 µL IS stock is needed. The IS stock volume is nominally measured using a calibrated Eppendorf pipettor and the stock is added to a tared, muffled, amber vial with a Teflon-lined screw cap. The weight of IS stock solution is noted in the lab record book. This weight should be within ±0.0003 of the theoretical weight for the volume of IS stock solution multiplied by the density of the solution, otherwise the total solution volume should be adjusted accordingly. For this example, the weight of 600µL of the IS stock solution, with a density of 0.718 g/mL, should read 0.4308 ± 0.0003 g. The theoretical IS spiking solution total weight is determined based on the volume of IS stock delivered and the amount of acetone needed to reach the correct total volume, and their respective densities. The solution is then brought up to the final volume with acetone by accurately reaching the theoretical weight of the IS spiking solution. This is accomplished by using a muffled pasture pipette to slowly add acetone to the IS stock aliquot until the theoretical weight of the solution is obtained within ±0.0003g. In the example for preparing 30 mL of the IS spiking solution, once 0.4308g of the stock IS was added, acetone would then be added until the total solution volume reached 23.6862 ± 0.0003g:

0.600 mL Stock IS (nonane ρ=0.718 g/mL)	Or	(0.4308g)
29.400 mL acetone (acetone ρ=0.791 g/mL)	Or	(23.2554g)
30.000 mL Total	Or	(23.6862g)

Once the final solution volume has been reached, the vial is capped. The capped vial is sealed with Teflon tape and the volume is marked using a permanent marker. The vial is then stored in a freezer. Prior to use, the IS spiking solution is kept capped and sealed and allowed to come to room temperature inside a fume hood. The IS spiking solution is good for a maximum period of five days from the date of preparation.

2. Cleanup Standard (CS) Stock Solution
The CS stock standard is prepared by a 1:50 dilution of CIL stock solution ED-907 (or equivalent) in nonane (Aldrich high purity, or equivalent). Usually only a few milliliters of this dilute solution are needed and, therefore, all volumes can be accurately measured using calibrated pipettors; no weighing is needed. The CS stock solution is prepared in a similar manner as Method 1613, Section 7.11. For example:

80 µL 2,3,7,8-TCDD$^{37}Cl_4$ stock (CIL ED-907)
1000µL + 1000µL + 1000µL + 920µL nonane
4.000 mL Total

The stock solution is measured using a calibrated Eppendorf pipettor and added to an amber vial with a Teflon-lined screw cap. This is brought up to volume with Aldrich high purity nonane (or equivalent) which is also measured using a calibrated Eppendorf pipettor. The vial is capped, then sealed with Teflon tape. The volume is marked using a permanent marker and stored in a freezer. Prior to use, the CS stock solution is kept capped and sealed and allowed to come to room temperature inside a fume hood. The solution is considered valid for a period of five years, at which time an assessment of the solution will be performed. If the assessment of the solution attests to the integrity of the quality and concentration of the stock, the expiration date of the stock may be extended for an additional five years.

3. Cleanup Standard (CS) Spiking Solution
Each sample extract, including QC sample extracts, gets 1 mL of CS spiking solution prior to any cleanup activities. The CS spiking solution is prepared in a similar fashion to the IS spiking solution (V.B.1) except that more of a dilution is necessary. The CS stock solution (from Section V.B.2) is diluted 1:5000 with acetone (B & J Brand pesticide residue grade, or equivalent). A minimum of 30 mL of the CS spiking solution is prepared at all times. In a fashion similar to the IS spiking solution, the CS spiking solution volumes are accurately determined using weights for the CS stock and acetone, and their respective densities rather than by a direct volume measurement. The stock CS volume is nominally determined using a calibrated Eppendorf pipetor and is then added to a tared amber vial with a Teflon-lined screw cap. The weight of CS stock solution is noted in the lab record book. This weight should be within ±0.0003 of the theoretical weight for the volume of CS stock solution multiplied by the density of the solution, otherwise the total solution volume should be adjusted accordingly. The theoretical CS spiking solution total weight is determined based on the volume of CS stock delivered and the amount of acetone needed to reach the correct total volume, and their respective densities. The solution is then accurately brought up to the final volume with acetone by reaching the theoretical weight of the CS spiking solution. This is accomplished by using a muffled pasture pipette to slowly add acetone to the CS stock aliquot until the theoretical weight of the spiking solution is obtained within ±0.0003g. For preparing 30 mL of the CS spiking solution, once 0.0043g of the stock CS is added, acetone would then be added until the total solution volume reached 23.7296 ± 0.0003g.

6 µL Stock CS (nonane ρ=0.718)	Or	(0.0043g)
29,994 µL acetone (acetone ρ=0.791)	Or	(23.7253g)
30.000 mL Total	Or	(23.7296g)

The CS spiking solution is then capped. The capped vial is sealed with Teflon tape and the volume is marked using a permanent marker. The vial is then stored in a freezer. Prior to use, the CS spiking solution is kept capped and sealed and allowed to come to room temperature inside a fume hood. The CS spiking solution is good for a maximum period of five days from the date of preparation. This standard may also be purchased directly from a vendor as long as it is a certified solution.

4. Precision and Recovery (PAR) Spiking Solution
For those samples that require a spike of PAR (matrix spike (MS), matrix spike duplicate (MSD), and method detection limit samples (MDLs), etc.) a 1:200 dilution of CIL stock solution EDF-7999 (or equivalent) is used. Usually only a few milliliters of this dilute solution are needed and, therefore, all volumes can be accurately measured using calibrated pipettors; no weighing is needed.. Each MS/MSD sample gets 1 mL of the PAR spiking solution (the equivalent of 5 µL of CIL EDF-7999), and each MDL sample gets 250 µL of the PAR spiking solution (the equivalent of 1.25 µL of CIL EDF-7999). The PAR spiking solution is prepared in a similar manner as Method 1613, Section 7.14. For example:

15 µL PAR stock (CIL EDF-7999)
1000µL + 1000µL + 985µL acetone
3.000 mL Total

The stock solution is measured using a calibrated Eppendorf pipettor and added to an amber vial with a Teflon-lined screw cap. This is brought up to volume with acetone (B & J Brand pesticide residue grade, or equivalent) which is also measured using a calibrated Eppendorf pipettor. The vial is capped, then sealed with Teflon tape. The volume is marked using a permanent marker and stored in a freezer. Prior to use, the PAR spiking solution is kept capped and sealed and allowed to come to room temperature inside a fume hood. The solution is good for a maximum period of five days from the date of preparation.

5. Recovery Standard (RS) Stock/Spiking Solution
Each sample extract, including QC sample extracts, gets 10 µL of RS standard added prior to analysis. The RS standard is prepared by a 1:250 dilution of CIL stock solutions ED-911(or equivalent) and ED-996 (or equivalent) in nonane (Aldrich high purity, or equivalent). Usually only a few milliliters of this dilute solution is needed and is, therefore, made using pipettors; no weighing is needed. Each sample gets 10 µL of the diluted RS (the equivalent of 0.04 µL of CIL ED-911 and ED-996). The RS spiking

solution is prepared in a similar manner as Method 1613, Section 7.14. For example:

20 µL 1,2,3,4-TCDD$^{13}C_{12}$ stock (CIL ED-911)
20 µL 1,2,3,7,8,9-HxCDD$^{13}C_{12}$ stock (CIL ED-996)
1000µL + 1000µL + 1000µL + 1000µL + 960µL nonane
5.000 mL Total

The stock solution is measured using a calibrated Eppendorf pipettor and added to an amber vial with a Teflon-lined screw cap. This is brought up to volume with Aldrich high purity nonane, which is also measured using a calibrated Eppendorf pipettor. The vial is capped, then sealed with Teflon tape. The volume is marked using a permanent marker and stored in a freezer. Prior to use, the RS spiking solution is kept capped and sealed and allowed to come to room temperature inside a fume hood. The solution is considered valid for a period of five years, at which time an assessment of the solution will be performed. If the assessment of the solutions attests to the integrity of the quality and concentration of the stock, the expiration date of the stock may be extended for an additional five years.

6. Other Standards
   Instrument calibration standards, window set mix, and the TCDD and TCDF column performance standards are purchased as certified mixes from CIL.

C. Reagent Solutions

1. Potassium Hydroxide Solution
   A 20 % weight per volume (w/v) potassium hydroxide (KOH) solution (Section 7.1.1) is prepared in organic – free reagent water (JT Baker, Ultra Resi-Analyzed, or equivalent). The solution is prepared by weighing 20 grams of KOH (EM Science,GR, pellets, or equivalent) into a muffled glass beaker. The KOH is then transferred to a Teflon bottle and 100 mL of water is slowly added to the bottle. The KOH is allowed to completely dissolve into the water before being used. The 20 % KOH solution is stored in the Teflon bottle with a Teflon cap at room temperature. The solution bottle is labeled with storage conditions and an expiration date of 1 year from date of preparation.

2. Sodium Hydroxide Solution
   A 1 N sodium hydroxide (NaOH) solution sited in Section 7.5.1.3. is prepared in organic-free reagent water (JT Baker, Ultra Resi-Analyzed, or equivalent). The 1 N NaOH solution is prepared by weighing 40 grams NaOH (JT Baker, Baker-analyzed, pellets, or equivalent) into a muffled glass beaker. The NaOH is transferred to a 1-L Teflon bottle and 1 L water is slowly added. The NaOH is allowed to completely dissolve into the water before being used. The 1 N NaOH solution is stored in the Teflon bottle with a Teflon cap at room temperature. The solution bottle is labeled with storage conditions and an expiration date of 1 year from date of preparation.

3. Sodium Chloride Solution
   A 5 % weight per volume (w/v) sodium chloride (NaCl) solution (Section 7.1.4) is prepared in organic-free reagent water (JT Baker, Ultra Resi-Analyzed, or equivalent). The solution is prepared by weighing 50 grams NaCl (EM Science, GR, crystals, or equivalent) into a muffled glass beaker. The NaCl is transferred to a 4-L reagent bottle and 1 L of water is slowly added. The NaCl is allowed to completely dissolve before being used. The 5 % NaCl solution is stored in the reagent bottle with a Teflon-lined cap at room temperature. The solution bottle is labeled with storage conditions and an expiration date of 1 year from date of preparation.

4. Dichloromethane/Methanol/Toluene – 15:4:1 Solution
   The 15:4:1 volume per volume (v/v) solution in Section 13.5.2. containing dichloromethane (B&J Brand, pesticide residue grade, or equivalent), methanol (EM Science, HPLC grade, or equivalent), and toluene (JT Baker, Ultra Resi-Analyzed, or equivalent) is made by measuring 150 mL of dichloromethane in a muffled glass graduated cylinder and then pouring the dichloromethane into a muffled glass 250 mL reagent bottle. Methanol (40 mL) is measured in a muffled graduated cylinder and poured into the same reagent bottle containing the dichloromethane. Toluene (10 mL) is measured in a muffled graduated cylinder and poured into the same glass reagent bottle containing the dichloromethane:methanol solution. The solution is capped with a Teflon-lined screw cap and stored at room temperature. The solution bottle is labeled with storage conditions and an expiration date of 1 year from date of preparation.

5. Dichloromethane/Cyclohexane – 1:1 Solution
   The 1:1 volume per volume (v/v) solution cited in Section 13.5.2. containing dichloromethane (B&J Brand, pesticide residue grade, or equivalent) and cyclohexane (B&J Brand, pesticide residue grade, or equivalent) is made by measuring 100 mL of dichloromethane in a muffled graduated cylinder and then pouring it into a muffled 250-mL glass bottle. Cyclohexane (100 mL)is measured in a muffled graduated cylinder and poured into the glass bottle containing the dichloromethane. The solution is capped with a Teflon-lined screw cap and stored at room temperature. The solution bottle is labeled with storage conditions and an expiration date of 1 year from date of preparation.

6. Dichloromethane/Hexane – 1:1 Solution
   The 1:1 volume per volume (v/v) solution cited in Section 12.4.1.2 & 13.4.7.2. containing dichloromethane (B&J Brand, pesticide residue grade, or equivalent) and hexane (B&J Brand, pesticide residue grade, or equivalent) is made by measuring 1000 mL of dichloromethane in a muffled graduated cylinder and then pouring it into a muffled 4-L glass bottle. Hexane (1000 mL)is measured in a muffled graduated cylinder and poured into the glass bottle containing the dicloromethane. The solution is capped with a Teflon-lined screw cap and stored at room temperature. The solution bottle is labeled with storage conditions and an expiration date of 1 year from date of preparation.

VI. Revision History

Not applicable.

OCT 30 2000

STANDARD OPERATING PROCEDURE (SOP) FOR THE SAMPLE CHAIN-OF-CUSTODY FOR DIOXIN/FURAN ANALYSIS

Originated by: _____ Date: 10/30/00

Approved by: _____ Date: 10/30/00
Technical Reviewer

Approved by: _____ Date: 10/30/00
Management

Reviewed and Registered by QAU:

_____ Date: 10/30/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I. Scope

This standard operating procedure defines chain-of-custody procedures for samples submitted to Battelle for polyhalogenated dibenzo-p-dioxin and dibenzofuran (dioxin/furan) analysis.

II. Purpose

According to Good Laboratory Practices (GLP) Regulations of the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and others, handling of samples must be conducted in a manner that ensures that:

- There is proper storage.
- Distribution is made in a manner designed to preclude the possibility of contamination, deterioration, or damage.
- Proper identification is maintained throughout the distribution process.
- The receipt and distribution of each batch is documented.

The above requirements are provided by the chain-of-custody procedures described in this standard operating procedure.

III. References

None.

IV. Definitions

Dioxin Laboratory is a limited access laboratory at Battelle designed exclusively for preparation of samples for dioxin/furan analysis. The Dioxin Laboratory is located in Room 5218.

Sample Preparation LRB is a bound laboratory record book used for documentation of sample preparation procedures.

Sample Name is the sample identification provided by the client for each sample (i.e., sludge AB). The sample name is generally listed on the label affixed to the sample container.

Sample Code is the unique sample identification number provided by Battelle for each sample received for dioxin/furan analysis.

V. Procedures

A. Sample Receipt

1. Samples for dioxin/furan analysis arrive at Battelle through Battelle's Shipping/Receiving Department. Upon notification of sample arrival, a staff member from the Dioxin

Laboratory will retrieve samples from the location of arrival. Alternatively, samples may be submitted directly to the Dioxin Laboratory by a Battelle Staff member.

2. Upon receipt of a sample(s) at the Dioxin Laboratory, the shipping container will be opened and the contents will be inspected to determine if receipt is acceptable. This inspection will include the following and will be recorded in the sample log-in laboratory record book (LRB):
   a.) The method of delivery will be documented and the package will be examined for the presence/absence of custody papers.
   b.) The shipping container will be inspected for the presence/absence and condition of custody seals.
   c.) The temperature of each shipping container will be measured and recorded to document if the samples were maintained at the appropriate temperature during shipment. The temperature of a cooler blank (if available), melt water, or the external temperature of the sample container should be measured and documented (thermometers or probes should <u>never</u> be inserted into a sample container).
   d.) Inspect each sample for the presence/absence and condition of custody seals. Check each sample for breaks or leaks.
   e.) Labels on sample containers will be compared with the accompanying sample documents or chain-of-custody records to ensure that all listed samples have been received. If any samples are missing, mislabeled, broken, or damaged, the Battelle project leader will be informed, who will then inform the client. The external chain-of-custody record will be signed and dated by the staff member receiving the samples. Any discrepancies or problems with the samples will be noted on the chain-of-custody record.
   f.) Aqueous samples that might contain total residual chlorine (TRC) should ideally be treated with sodium thiosulfate ($Na_2S_2O_3$) in the field. If that hasn't occurred, upon receipt in the laboratory the sample should be measured for TRC using a commercial test kit. If TRC is detected then $Na_2S_2O_3$ should be added at a ratio of 80 mg per liter of sample and documented on the COC and in the sample log-in LRB.
   g.) The pH of aqueous samples will be measured and recorded. If the pH is >9, the pH will be adjusted to a pH of 7-9 with sulfuric acid unless the project requirements state specifically otherwise.

3. In addition to sample names, all samples should be labeled with an expiration date and storage conditions required by the method, client, or study protocol. If expiration dates and storage conditions are not listed on the label affixed to the container, the Battelle staff member receiving the samples will affix a second label to each sample container with the expiration date and storage conditions. If expiration dates and storage conditions are not specified by the method, client, or study protocol, the following should be used:

	Expiration Date	Storage
Fish/adipose tissue:	5 years	<-20°C
All other matrices:	5 years	<4°C

4. Receipt of samples will be recorded in a LRB designated for sample receipt. One page will be used for each sample shipment. The following information will be recorded:

- Method of delivery (i.e., FedEx)
- Presence/absence and condition of custody seals on the shipping container and sample jars.
- Measurement for TRC and amount of $Na_2S_2O_3$ added if necessary.
- The temperature of each shipping container.
- The pH of each aqueous sample and adjustments made to sample pH.
- Any discrepancies between sample labels and COC sheets.
- Sample name
- Conditions of samples upon receipt (i.e., intact)
- Each sample will be given a unique log-in code based on the LRB in which the sample receipt is documented (i.e., 43201-10-2). The first group of numbers in this sample code is the LRB number, the second two digit number is the LRB page on which the sample code is documented, and the third number is the line on the LRB page which specifically refers to that sample (only one line is assigned per sample). This number will also be written on each jar.

5. A copy of the completed chain-of-custody records submitted with the samples will be made and attached to the sample receipt LRB. A copy will also be made for the project file. The original chain-of-custody record will be returned to the client if requested, or archived in the project files.

6. Samples will be stored under required conditions within the Dioxin Laboratory until preparation for analysis.

B. Sample Preparation

1. At the initiation of sample preparation for dioxin/furan analysis, each sample (or sample aliquot, if the entire sample is not used for sample preparation) will be assigned a unique sample code. An example of an acceptable code is one based on the LRB in which sample preparation is documented (i.e., 45674-24-5). The first group of numbers in this sample code is the LRB number, the second two digit number is the LRB page on which the sample code is documented, and the third number is the line on the LRB page which specifically refers to that sample (only one line is assigned per sample). Other sample codes are acceptable if they can be made unique for each sample and can be easily documented.

2. Date and name of staff member will be recorded in the sample preparation LRB for all sample preparation activities.

3. The sample extracts resulting from sample preparation will be labeled with the unique sample code and stored at < 4°C in a designated refrigerator within the Dioxin Laboratory, unless other storage conditions are specified by the method, client, or study protocol. A

LRB, referred to as the Extract Chain-of-Custody LRB, is used to document storage of sample extracts ready for analysis. Documentation will include a listing of the sample name, the unique sample code, and the date the extracts were stored and ready for analysis. An example of this documentation is shown in Attachment A.

C. Sample Analysis

1. The analyst will retrieve sample extracts for dioxin/furan analysis from the Dioxin Laboratory. The analyst will record his/her name and the date of extract retrieval on the page in the LRB assigned to that particular sample set (Attachment A).

2. Sample extracts will be brought by the analyst to the analysis laboratory. Extracts will remain in custody of the analyst at all times. At the end of each day, or if the analyst will be absent from the laboratory at any time during the day, sample extracts will be stored in a limited access storage access and documented in study records.

3. Mass spectrometry analysis of sample extracts will be recorded in daily log runs kept with each MS instrument. The log run will list the sample name, the unique sample code, and the MS file name as shown in Attachment B. The sample name, unique sample code, and MS file name will be recorded on each hard copy chromatogram generated by the analysis.

4. A copy of the run log will be entered in the project files at the completion of analysis.

5. At the completion of analysis, sample extracts will be returned to the Dioxin Laboratory for storage until final disposition. This return will be documented in the Extract Chain-of-Custody LRB by the analyst.

D. Sample Division

Samples which need to be divided and submitted for analyses other than dioxin/furan will be handled in the following manner:

1. At the initiation of sample preparation for dioxin/furan analysis, the aliquot(s) required for additional analyses will be removed from the bulk sample.

2. Aliquot removal will be documented in the sample preparation LRB.

3. The aliquot will be labeled with the original sample name and submitted to the laboratory responsible for the additional analyses. Documentation of transfer of sample aliquots to a second laboratory will be recorded in the sample preparation LRB.

4. It will be the responsibility of the laboratory conducting additional analyses to assign a unique sample code to the sample aliquot received for additional analyses, to label the sample with expiration date and storage conditions, and to initiate chain-of-custody procedures for the sample aliquot.

5. Remaining bulk sample will be stored in the Dioxin Laboratory until final disposition.

VI. Revision History

1. Additional sample login details were added in going to ASAT.II-007-01 including measuring cooler temperature upon sample receipt and measuring and adjusting pH of aqueous samples.

Attachment A

BATTELLE LABORATORY RECORD BOOK NO. 47905 99

Work Performed by: Savage Project No. C69200007-01 Date of Work: May 10, 2000

Title or Purpose: Perth Amboy Continued from: —

Samples ready for GC/MS analysis
Stored in Rm 5218, Freezer F-3

Extraction #
48248-17-02
03
04
05
06
07
08
09
10
11
12

Samples to 5282
Freezer 05-10-00
[illegible]

[illegible]
5/10/00

Entered by: [illegible] Date: 5/10/00 Continued to: —

Performances of this work observed by:
Date:
Date:

Disclosed to and understood by me:
[illegible] Date: 5/11/00
Date:

Attachment B

BATTELLE LABORATORY RECORD BOOK NO. 48279 33

Work Performed by: TRBar Project No. G003896-01BC Date of Work: 02-23-00

Title or Purpose: USEPA Region II Blue Creek Tissue Continued from: p32

4827933A	1	47725-45-12	CP3+WS+CP	(A) (Geo OK)	Lab DIOXIN
4827933B	1	48370-11-26	Method Blank		
	2	48370-11-02	111378		
	3	48370-11-03	111379		
	4	48370-11-04	111380		
	5	48370-11-05	111381		
	6	48370-11-06	111401		
	7	48370-11-09	111403		
	8	48370-11-10	111403 Duplicate		
	9	48370-11-11	111408		
	10	48370-11-12	111431		
	11	47725-45-12	CP3+WS+CP (Geo OK)		
	12	48370-11-26	Method Blank		
	13	48370-11-13	111432		
	14	48370-11-14	111437		
	15	48370-11-15	111441		
	16	48370-11-16	111442		
	17	48370-11-17	111444		
	18	48370-11-18	111446		
	19	48370-11-19	111464		
	20	48370-11-20	207280		
	21	48370-11-21	207282		
	22	47725-45-12	CP3+WS+CP (Geo OK)		

Cal date saved as DIOXCONCAL 07-FEB-00
opersquan date saved as G003896 01BC 23-FEB-00

(A) change Group I to end and II to start at 29:55

Entered by: Joe Tabor Date: 02-23-00 Continued to: p34

Performances of this work observed by: Date:

Date:

Disclosed to and understood by me: Karen Resnick Date: 2/23/00

Date:

OCT 30 2000

STANDARD OPERATING PROCEDURE FOR DIOXIN/FURAN TECHNICAL DATA REVIEW

Originated by: Karen L. Tracy Date: 10/24/00

Approved by: Mary Dehnock Date: 10/24/00
Technical Reviewer

Approved by: Karen Riggle Date: 10/27/00
Management

Reviewed and Registered by QAU: Charles P. Lawrie Date: 10/30/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This Standard Operating Procedure (SOP) describes the general procedures to be followed for the technical review of dioxin/furan analysis data.

III. References

A. SW-846, Method 8290. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS), Revision 0, 1994.
B. EPA Method 1613: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B, 1994, EPA 821-B-94-005.

IV. Definitions

None.

V. Procedures

A. Analyst Review of Analytical Data

1. Review the initial calibration curve and check the response factor summary sheet. Relative standard deviation (RSD) of response factors (RF) generated by isotope dilution should be ≤ 20%. The RSDs for RFs generated by internal standard should be ≤ 35%.

2. Check to verify that the PFK resolution checks meet the criteria found in EPA Method 1613B, Section 10.1.2.

3. Verify that the window set solution and column performance check meet the criteria found in EPA Method 1613B, Sections 10.3 and 10.4.

4. Check the continuing calibration results against EPA Method 1613B, Table 6 "VER" requirements. Flag any isomer analyte that fails this criteria and mark with a post-it note.

5. Review all chromatograms and analyte hits for the criteria in Method 1613, Revision B, Section 16.0. If the analyst deems a peak to be a valid hit even though it fails to meet one or more of the criteria found in Method 1613, Revision B, Section 16.0, an estimated maximum possible concentration can be calculated for this analyte following guidance in Method 8290, Revision 0, 1994, Section 7.9.5.2.1 and should be flagged as such in the raw data.

6. Once the analyst has completed review of the data as outlined above, he/she should place his/her initials and the date on the first chromatogram output for each run.

B. Secondary Technical Review of Analytical Data

1. Check the transcription of the extract number, sample identification, and sample weight/ volume from the preparation laboratory record book (LRB) onto the OPUS forms for each sample.

2. Check to see that all 2,3,7,8-isomers are accounted for, concentration reported, flagged for reasons not used, or obviously not present in each sample.

3. Spot-check continuing calibrations. Review any exceedances noted in V.A.4.

4. Double check that there are no co-eluting ethers contributing to the furans in each sample.

5. Check the lock mass check for each function to ensure that there are no variations > 20% that could impact reported results.

6. Flag any internal standard recoveries that are outside 40-135% (or 25-150% depending on project requirements) on the OPUS form. Tag these flagged forms with post-it notes for ease in highlighting final forms and report

7. Check calculations for all Estimated Maximum Possible Concentrations (EMPCs) and spot check the calculations for the concentrations detected.

8. Review HRMS LRB for the criteria listed in Section V.D.1.

9. Return the data to the analyst for corrections, updates, or reprints if there are any. The analyst should resave any data file that has been changed.

10. Review all corrections made by analyst.

11. Repeat steps 1-11 for second column confirmation data.

12. Format electronic spreadsheet data as required by project.

13. Flag any internal standard recoveries that are outside the 40 – 135% recovery limit (or 25 – 150% recovery limit, depending on project requirements) in the spreadsheet.

14. Add and flag any secondary column confirmation results into the spreadsheet.

15. Check that the QC check sample records have been updated within the quarter. If there are any new QC check sample results from the current project, add this information to the binder. The QC check samples will be Cambridge Isotopes standard reference materials (EDF-2526 or EDF-2513). Acceptability criteria are for concentrations in the QC check sample to be within 30% difference of consensus value

16. Document the secondary technical review on the data check list (Attachment A) and include in project records.

C. Technical Review of Dioxin/Furan Data Spreadsheets

1. Spot check to ensure that all calculations in the spreadsheets are correct (e.g. percent relative difference, percent recovery, standard deviation, method detection limit, etc.) and that sample data in the spreadsheet corresponds to the raw data.

2. Verify that correct units are used and that all header information is correct and complete and in the correct format required by the client.

3. Check that any data discussed in the report text is consistent with the data in the report tables.

4. Return to secondary technical reviewer for corrections, updates, or reprints.

5. Review all corrections made.

6. Document this review by initialing the concurrence box on the report internal review block.

D. Technical Review of Preparation Data for Dioxin/Furan Analysis

1. Review all laboratory record books (LRBs) used for the project. Check for completeness and accuracy of information, ensure that proper correction techniques have been used, sign the bottom of the page, and make a copy of the pages needed for the study file. LRBs include: Sample log-in, standard log-in, sample preparation, and extract chain-of-custody (COC).

2. Verify that the original COC and the miniaturized copy of the COC in the sample log-in LRB are identical.

E. Safety

N/A

VI. Revision History

None

Attachment A

Data Review Checklist
for
Samples Analyzed on :
OPUS Filename:

Initials/Date	Activity
	Check transcription of extract no., sample ID, and sample weight from prep LRB into OPUS forms for each sample.
	Check to see that all 2,3,7,8-isomers are accounted for: concentration reported, flagged for reason not used, or obviously not present in each sample.
	Double check that there are no ethers contributing to furans in each sample.
	Review lock mass checks for any variations, which could affect reported results.
	Flag any recoveries outside 40-135%/25-150% on OPUS form. Tag flagged forms with post-its for ease in highlighting final forms and report.
	Return data to analyst for corrections.
	Analyst makes corrections, updates, reprints and resaves OPUS data.
	Corrected OPUS data saved as ASCII text file. **ASCII text file Filename:**
	ASCII file pulled over to PC
	ASCII file imported to Quattro Pro or Excel **QPRO or Excel Filename:**
	Format spreadsheet data
	Flag in spreadsheet file any recoveries outside 40-135%/25-150% as on OPUS forms.
	Add and flag second column confirmation results in spreadsheet file.
	Check calculations for EMPCs.
	Review all LRBs used for project, check for completeness of information, ensure that the proper correction techniques have been used, sign the bottom of the page, and make a copy of the pages needed for the study file.
	Verify that the original COC and the miniturized copy of the COC in the sample log-in LRB are identical.
	Spot-check the calculations of concentrations.
	Verify that the daily calibrations, the window set mixture, and the percent valley are within the QC ranges allowed by the method.
	Print 2 copies of each finalized QPRO sample sheet. Place one in the OPUS printouts for each sample. Collect the others for the final report.
	Check to see if the QC check sample records have been updated within the last quarter. Add any new QC sample results from this project.

Manual Number: **BATTELLE COPY**

Battelle SOP Number: ASAT. II-011-00

OCT 30 2000

STANDARD OPERATING PROCEDURE
FOR USING ELECTRONIC AND MECHANICAL BALANCES

Originated by: _____ Date: 10/24/00

Approved by: Mary DeMoss _____ Date: 10/24/00
Technical Reviewer

Approved by: Karen Riggs _____ Date: 10/26/00
Management

Reviewed and Registered by the Quality Assurance Coordinator:

Charles D. Lawrie _____ Date: 10/30/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This Standard Operating Procedure (SOP) describes the general procedures to be followed for the operation, calibration, and maintenance of electronic and mechanical balances.

III. References

A. Balance manual, which if obtainable, should be readily available to users.

B. Battelle SOP ASAT.I-002-00, Use of Equipment Logbooks.

IV. Definitions

A. Critical weights are those that are critical to the scientific integrity of the experiment. Examples of critical weights would include the weights of samples, of chemicals used to prepare standards and of weights needed for calculations.

B. Non-critical weights are those that are estimates and/or used in applications in which the weight is not critical to the scientific integrity of the experiment. Recording of non-critical weights is not mandatory. Examples are weights determined for the rough splitting of samples, for balancing of centrifuge tubes, or for estimating weights of bulk reagents.

C. Responsible person is the individual assigned by management to be accountable for operations as described in equipment SOPs, including initiation and archival of Battelle Columbus Operations equipment logbooks.

V. Procedures

A. Operation of Balances Being Used for Determination of Critical Weights

1. No operator shall use the balance until properly trained. It is the responsibility of the user to ensure that the balance is functioning properly.

2. The operator should verify that the balance service interval sticker is current (i.e., the date of next service indication is a future or present date and not a past date). This sticker is provided by a trained service technician who is scheduled to do a yearly calibration of the balance. A balance which has not been properly calibrated should not be used.

3. Verify that the balance is level; if not, adjust the balance by using the built-in bubble indicators (if present).

4. Check the balance accuracy by using at least two different certified verification weights that are near the low and high end of the range to be weighed. The verification weights should have been certified within the last twelve months and should be ASTM class 2 weights or better. To test accuracy, the balance should read the verification weights to at least within 2 percent of their nominal weights unless other specifications are indicated in the equipment logbook. The balance must be checked for accuracy with the verification weights at the beginning of every month and recorded in the equipment log book. Additionally, at least once every day of use the balance should be checked for accuracy

with the verification weights and recorded in the study records. The accuracy of the balance must be rechecked with the verification weights if the balance is turned off or moved to a different platform.

5. Record the nominal and the determined values for the verification weights in the study record and/or equipment log. Check the determined values to make sure they are within the acceptable limits as established for the laboratory.

6. If the balance cannot pass the accuracy test, the particulars of the failure must be recorded in the equipment logbook, the balance must be labeled as out of calibration and not to be used, and the Responsible Person should be notified as soon as possible.

B. Operation of Balances Being Used for Non-Critical Weightings

1. No operator shall use any balance until properly trained on its use.

2. Mechanical balances may not have balance service interval stickers. They do not require accuracy verification with certified weights. It is recommended that these balances be checked for correct operation at zero grams before use and that the check weight identification, if used, and actual weights be documented in the study record or equipment logbook.

C. Maintenance

1. Whenever an operator suspects a balance is not functioning correctly, the operator shall label the balance "out of service" and the Responsible Person will be notified.

2. Balance repairs shall be performed by trained service technicians only.

3. An equipment log will be kept in accordance with Battelle SOP ASAT.I-002-00, Use of Equipment Logs. Equipment logs are required to be archived when the equipment is no longer in use or after the final quarter of 2002 and then every four years.

D. Safety

Care shall be taken not to damage the balance or calibration verification weights with fingerprints or residual chemicals on the pan. The use of gloves and weighing boats are recommended.

VI. Revision History

None

Attachment A

BATTELLE COLUMBUS OPERATIONS

EQUIPMENT LOGBOOK

BATTELLE X/I NUMBER _____

TYPE: _____

MAKE: _____

MODEL: _____

SERIAL NUMBER: _____

DEPARTMENT: _____

LOGBOOK DATES: FROM _____ TO _____

RESPONSIBLE PERSON: _____

SOP REFERENCE: _____

* For equipment comprised of components, use space below for additional X/I.

N/A = Not Applicable

Attachment B

ROUTINE MAINTENANCE - Battelle X/I or Serial No._____

Date	Initials	Describe the Nature of the Maintenance

Frequency/schedule _____
N/A = Not Applicable

Attachment C

NON-ROUTINE MAINTENANCE – Battelle X/I or Serial No._____

Date	Initials	Describe the Nature of the Problem, How & When Discovered, Remedial Action

N/A = Not Applicable

Attachment D

Balance Calibrations

Date	Initials	Ranges/Observed Mass (g)	Notes

*Frequency/schedule_____

N/A = Not application

BATTELLE X#:_____

Manual Number: **BATTELLE COPY**

Battelle SOP Number: ASAT. II-012-00

OCT 30 2000

STANDARD OPERATING PROCEDURE (SOP)
FOR THE USE OF REFRIGERATORS AND FREEZERS
USED FOR DIOXIN-RELATED PROJECTS

Originated by: _____ Date: 10/24/00

Approved by: _____ Date: 10/24/00
Technical Reviewer

Approved by: _____ Date: 10/26/00
Management

Reviewed and Registered by the Quality Assurance Coordinator:

_____ Date: 10/30/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I./II. Scope/Purpose

This Standard Operating Procedure (SOP) is applicable to the routine operation, monitoring, and maintenance of the refrigerators and freezers for dioxin-related projects.

III. References

Battelle SOP ASAT.I-002-00, Use of Equipment Logbooks

IV. Definitions

None.

V. Procedures

A. Files and Records

1. Completed monthly temperature/maintenance logs will be placed in a binder maintained by the person responsible or designee for the refrigerator(s)/freezer(s) until archived. Temperature/maintenance logs are required to be archived when the equipment is no longer in use or at the end of the last quarter of 2002 and then every four years.

2. The thermometer calibration records or equivalent documentation will be maintained by the responsible person, designee or instrument lab until archived. Thermometer calibration records are required to be archived when the equipment is no longer in use or at the end of the last quarter of 2002 and then every four years.

B. Temperature/Maintenance Log

1. The temperature/maintenance log (similar to Attachment A) shall be posted on the door of the refrigerator/freezer and shall contain the following information:
   a. The month and year of the temperature/maintenance log.

   b. Type of equipment (refrigerator or freezer).

   c. Manufacturer.

   d. BMI or serial number. If these numbers are not available, a permanent unique number will be assigned to the unit.

   e. The location.

   f. The operating range. The assigned operating ranges will be documented on the unit. Common ranges can be as follows: approximately 0-4°C for refrigerators, ≤-20°C for freezers, and ≤-70°C for ultra low freezers.

   g. The monitoring frequency for all refrigerator/freezer units used for dioxin related projects will be every Battelle business day.

h. The thermometer number assigned to the unit (if applicable).

i. The calibration due date of the thermometer assigned to the unit.

j. The person or designee to notify in case of problem or issue.

k. Any additional safety concerns (if applicable).

l. Routine and Non-routine maintenance. This information will be documented on either this log or in the refrigerator/freezer logbook.

2. At the end of each month the temperature log will be placed in A.1 above.

C. Monitoring

1. The temperature shall be checked every Battelle business day by the responsible person or designee. If the responsible person is to be absent, he/she needs to designate an alternate to record the temperature until the responsible person returns.

2. If the monitoring is documented according to Attachment A, temperature recording should be performed as follows:

a. If the temperature of the refrigerator/freezer is within the designated operating range, then the time, temperature, date and initials will be filled in the initial reading space on the temperature log. The second reading set of boxes will be left blank.

b. If the temperature of the refrigerator/freezer is outside the operating range, the time, temperature, date and initials will be recorded in the initial reading space on the temperature log. Within several hours, the temperature will be rechecked and appropriate information will be recorded in the second reading set of boxes.

c. If the temperature is still outside the operating range, the responsible person or designee will be notified. The person notified, along with the date and initials of the person monitoring will be documented.

d. The person responding to a deviation shall document the corrective action/comments, the date, and their initials appropriately.

e. If there is a known mechanical failure for an extended period, the contents of the unit will be moved at the responsible person's discretion. The new location(s), date, and initials of the person(s) transferring the material will be documented on the temperature/maintenance log or in the refrigerator/freezer logbook.

D. Use

1. It will be the responsibility of the person storing the material to place it in a refrigerator/freezer with the correct operating range.

2. If the refrigerator/freezer is equipped with a locking device it shall be kept locked as deemed necessary by the responsible person (e.g. radiolabeled material storage). Keys

or combinations to the refrigerator/freezer shall be made available to the personnel requiring access to the refrigerator/freezer.

E. Calibration and Maintenance

The thermometers shall be calibrated in accordance with SOP ASAT.II-013-00. Units shall be defrosted as needed. Compressor coils shall be maintenanced as needed by service personnel.

F. Safety

1. Certain refrigerator/freezers may represent a safety hazard due to poor ventilation, CO_2, presence of radioactive isotopes, etc. All units shall be properly labeled on the outside door with the appropriate means of notification of such hazards.

2. Refrigerated or frozen flammables must be stored in approved explosion-proof refrigerators/freezers.

3. All personnel entering refrigerators/freezers, including walk-in refrigerators/freezers must be sure that they have provided themselves with adequate protection and precautions such as protective clothing, eyewear, or supplemental ventilation to ensure undue risk is not taken.

G. Quality Control

The temperature/maintenance logs will be reviewed, dated and initialed for compliance with this SOP by the area supervisor or his/her designee on a weekly basis. If a deviation from this SOP is noted, the deviation will be documented and maintained with the refrigerator/freezer logbook.

VI. Revision History

This is the first version of this SOP.

ATTACHMENT A

BATTELLE TEMPERATURE LOG

Month/Year:_____ Operating Range:_____
Type Of Equipment:_____ Calibration Due Date:_____
Type Of Equipment:_____ BMI ID# Or SN:_____
Manufacturer:_____ Monitoring Frequency:_____
Manufacturer:_____ Therm/Chart Rec. ID#:_____
Room #:_____
Responsible Person to Notify:_____ Phone Number:_____

	Initial Reading			Second Reading				Initial Reading			Second Reading		
Date	Time	Temp	Date/Initial	Time	Temp	Date/Initial	Date	Time	Temp	Date/Initial	Time	Temp	Date/Initial
1							17						
2							18						
3							19						
4							20						
5							21						
6							22						
7							23						
8							24						
9							25						
10							26						
11							27						
12							28						
13							29						
14							30						
15							31						
16													

Problem/Responsible Person Notified	*Date/Initial*	**Corrective Action/Comments**	**Date/Initial**

Routine Maintenance:

Non-routine Maintenance:

Reviewed by (Date/Initials):_____ Week 1 Review (Date/Initials):_____

Week 2 Review (Date/Initials):_____ Week 3 Review (Date/Initials):_____

Week 4 Review (Date/Initials):_____ Week 5 Review (date/Initials):_____

OCT 30 2000

STANDARD OPERATING PROCEDURE (SOP) FOR THE USE AND CALIBRATION OF DIGITAL AND GLASS THERMOMETERS

Originated by: Karen L Gray Date: 10/26/00

Approved by: Mary Schrock Date: 10/26/00
Technical Reviewer

Approved by: Karen Riggs Date: 10/27/00
Management

Reviewed and Registered by QAU:

Charles P. Laurie Date: 10/30/00

Battelle
505 King Avenue
Columbus, Ohio 43201

I. Scope

This standard operating procedure (SOP) describes the use and calibration of digital and glass thermometers and describes the methods that will be used to cross-calibrate monitoring thermometers against NIST-traceable or standardized (calibrated) thermometers.

II. Purpose

The purpose of this SOP is to describe the use and calibration of digital and glass thermometers that are used to monitor the temperature of refrigerators, freezers, drying ovens, water baths, and shipping containers. This equipment is used primarily for the sample storage, sample and reagent drying, and sample processing.

Digital and glass thermometers are available from retail and scientific suppliers; the selection of a unit should be based on the required accuracy, range, and the intended use of the thermometer. Of specific importance are:

- Units: the thermometer must provide readings in °C (centigrade)
- Accuracy: units intended for monitoring refrigerators, freezers, water baths, or sample condition
  must be accurate to ± 1.0°C.
- Range: the temperature range must bracket the expected range of the intended use of the thermometer.

III. References

None.

IV. Definitions

None.

V. Procedures

Each laboratory thermometer will be identified with a unique identification number (ID). This ID may be the serial number that is stamped on the thermometer itself or some other unique ID. Laboratory thermometers used for routine monitoring of refrigerators, freezers, water baths, etc. should be assigned to a piece of equipment and should not "travel" among units, if possible. In addition, if a thermometer calibrated for one temperature is moved to a different type of unit, the thermometer must be recalibrated prior to use (e.g., if a thermometer assigned to a refrigerator is used to monitor a water bath then the calibration factor determined at 4°C cannot be applied to a water bath at 60°C without verification).

A. Digital Thermometers

1. Operation
The analyst must review the literature provided with the thermometer prior to use to determine proper use of the features. Of critical importance is selection of the correct units if the thermometer has an °F (Fahrenheit) option. Unless specified in the project-specific Quality Assurance Project Plan (QAPP) (or similar document), temperature is always recorded in °C. In most cases the thermometer operation follows these steps:
    a. Turn the unit on.
    b. Verify that the LED display is complete. This is typically a self-test of the unit as the reading "188.8".
    c. Allow the unit to equilibrate. Many units perform an initial self-test.
    d. Verify that °C units are selected.
    e. Begin temperature monitoring. The response of the thermometer may vary based on the temperature being measured. The analyst must observe the display to determine when the temperature has stabilized.
    f. When the digital display is stable (on fluctuation) for 15 seconds, then the temperature reading is considered stable and is recorded.
    g. Temperatures are recorded directly on the proper data sheet and in the proper laboratory record book (LRB) if needed.
    h. To preserve the battery, turn off the unit if the time between measurements is greater than 15 minutes if possible.

Some thermometers may include a low-battery warning. Back-up batteries should be carried with the unit to avoid the loss of data.

If the temperature-monitoring unit includes a positionable probe, the probe should be placed in the appropriate matrix to avoid sudden temperature fluctuations. These matrices are: water:ethylene glycol (1:1) for temperatures between 4°C and -20°C and sand for temperatures < -20°C.

2. Maintenance
Other than quarterly calibration against a NIST traceable thermometer and cleaning of the probe with water and lint-free paper, no maintenance is required for digital thermometers. Use of a digital thermometer must be discontinued if any part of the LED display fails. Care should be taken to protect the thermometer probe if it protrudes from the unit.

3. Training
Each trainee must read and fully understand this SOP, and then demonstrate to the satisfaction of the Laboratory Manager, or designee, that he/she understands the operation of the thermometer of interest.

4. Safety
   There are no safety considerations associated with the operation of a digital thermometer. The project manager should be consulted for safety issues related to samples or the sampling site.

B. Glass Thermometers

1. Operation
   In most cases thermometer operation follows these steps:
   a. Place the thermometer in the equipment that the measurement is being taken for. If the thermometer is to stay inside a piece of equipment submerge the bulb of the thermometer in a jar filled with a suitable material for maintaining temperature.
   b. Allow the thermometer to equilibrate for a minimum of 20 minutes.
   c. Take the measurement from the top of the meniscus formed by the liquid inside the thermometer and then adjusted by the correction factor for the thermometer.
   d. Temperatures are adjusted for their correction factor and recorded directly on the proper data sheet.

2. Maintenance
   The thermometer must be calibrated once every year against a NIST traceable thermometer. Thermometers which are cracked, broken, or have separated liquid columns cannot provide accurate measurements and should be discarded or returned to the manufacturer for maintenance, unless the thermometer is chilled and all of the liquid returns to the bulb.

3. Training
   Each trainee must read and fully understand this SOP, and then demonstrate to the satisfaction of the Laboratory Manager, or designee, that he/she understands the operation of the thermometer of interest.

4. Safety
   Thermometers are easily broken if extreme caution is not used in handling. Broken thermometers, particularly those with mercury as the column liquid, pose several potential safety hazards. Care must be taken to avoid the sharp edges and any contact with escaping mercury. If a thermometer breaks, (1) a mercury spill kit should be used immediately, (2) the Hazardous Waste Coordinator and/or a Hazardous Waste Handler should be contacted, and (3) personnel exposure should be assessed with the Health and Safety Officer.

C. Calibration of Digital and Glass Thermometers

1. National Institute of Standards and Technology (NIST) Thermometer Calibration
The NIST thermometer is calibrated annually by a professional thermometer service using approved ATSM methodology. These thermometers are very expensive and should not be used for routine monitoring. The calibration records received with these thermometers should be filed in the "Calibration" section of the Refrigerator Monitoring Log. These correction factors should be applied when performing the calibration described in this section.

2. Digital and Glass Thermometer Calibration
Each digital thermometer will be calibrated quarterly using a NIST traceable thermometer. Each glass thermometer will be calibrated annually using a NIST traceable thermometer.

To calibrate:

a. Place the digital or glass thermometer and the NIST-traceable thermometers in an area which can sustain the temperature of interest according to the following table. In most cases, both thermometers are immersed in a matrix that will not react to the temperature rise and fall associated with opening the equipment door. The matrix used during cross-calibration should be noted on the calibration form (Attachment A).

Area of Use	Desired Temperature	Suggested Matrix for cross-calibration
Freezers	Below -20°C	Place in container of sand
Refrigerators	4°C±2°C	Place in a beaker of water:ethylene glycol (1:1)
Water baths	60°C or 100°C	Water bath
Drying ovens	100°C	Drying oven Place in a beaker of sand

b. Shut the equipment door and allow the thermometers to stabilize for at least 30 minutes.

c. Read the NIST-traceable and laboratory thermometers and document the measured temperature to the nearest whole number on the Thermometer Calibration Form (Attachment A). Do not remove either thermometer from the stabilizing matrix during this

time.

d. Perform units conversion, if required, and apply the appropriate calibration factor (if any) to the NIST-traceable thermometer reading.

e. Calculate the difference in the readings between the corrected NIST-traceable thermometer and the laboratory thermometer to the nearest whole number. This is the correction factor that should be applied to all temperature measurements using the laboratory thermometer. This correction factor should not be more than 3°C, or the digital or glass thermometer should be replaced. It should be noted that this range is designated by the Laboratory Manager and is not based on a recognized method. The following example illustrates the procedure.

Thermometer ID	Unit ID	NIST ID	MATRIX	NIST Measurement (Units)	NIST Correction Factor (Units)	Corrected NIST Measurement (Units)	Laboratory Thermometer Measurement (Units)	Correction Factor (Units)	Date/ Initials
Ref. 3	Refrig. 3	ERTCO G-57811	H_2O	20	+0.08	20.08	19	20.08 - 19 = +1	2-23-95 rlb

f. A tag with the calculated correction factor and the expiration date (one year from the date of calibration for glass thermometers and three months from the date of calibration for digital thermometers) should be affixed to the thermometer and noted on the temperature log of the unit the thermometer is monitoring, if applicable.

3. Thermometer Care

NIST traceable thermometers should always be stored in their protective cases. Laboratory thermometers should be secured within the equipment being monitored. Thermometers, which are cracked, broken, or have separated liquid columns cannot provide accurate measurements. In the former two cases the thermometer should be discarded in the appropriate waste stream. In some cases it is possible to re-unite a split column by chilling the thermometer so that all liquid returns to the bulb. If this is unsuccessful the thermometer should be returned to the manufacturer for maintenance (NIST-traceable) or discarded in the appropriate waste stream.

4. Calibration Training

To complete training in the calibration of laboratory thermometers, laboratory personnel should

a. Read this SOP.
b. Observe a trained staff member perform the calibration and documentation procedures (Section V.C).

c. Perform the calibration and documentation procedures under the supervision of a trained person.

VI. Revision History

This is the first version of this SOP.

Attachment A

Thermometer Calibration Form

Thermometer ID	Unit ID	NIST ID	Matrix[1]	NIST Measurement	NIST Correction Factor	Corrected NIST Measurement	Laboratory Thermometer Measurement	Correction Factor	Date/ Initials

[1] Matrix in which thermometers were immersed during calibration.

[2] Correction Factor = [NIST calibrated thermometer Reading (°C)] - [Temperature Reading (°C)]

(°C + 9/5) + 32 = °F

BATTELLE MSL SOPs

Battelle
Putting Technology To Work

Marine Sciences Laboratory

EFFECTIVE DATE: 4-25-00

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-A-001-04

SAMPLE LOG-IN PROCEDURE

Approvals:		
AUTHOR: Laurie Niewolny	Laurie Niewolny	4/24/00
	Signature	Date
TECHNICAL REVIEWER: Carolynn Suslick	Carolynn Suslick	4/25/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	4-21-00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric Crecelius	4-24-00
	Signature	Date

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SAMPLE LOG-IN PROCEDURE

1.0 SCOPE AND APPLICATION

This method applies to sample receipt, log-in, preservation, storage, and disposal of all chemistry water, soil, sediment and tissue samples by the Battelle Marine Sciences Laboratory (MSL) Sample Custodian or designee.

2.0 DEFINITIONS

Log-In - The procedure by which samples are received and documented at the (MSL).

Sample Custodian - The person responsible for sample receipt and log-in.

CoC Form - Chain-of-custody form, accompanies samples from field to MSL and is kept with the sample files.

3.0 RESPONSIBLE STAFF

Sample Custodian or designee
Project Manager
Data Coordinator
Quality Assurance Officer or Representative

4.0 PROCEDURE

4.1 Sample Receipt

4.1.1 Samples arrive from the customer via a variety of delivery systems (e.g., United Parcel Service, Federal Express, Air Borne, Courier Service, General Delivery or from staff within MSL). The Sample Custodian is notified of sample arrival by either the shipping clerk or directly by the MSL staff member who has custody of the samples. The Log-In Checklist (see Attachment 1) is initiated.

4.2 Sample Log-In

4.2.1 The shipping container is inspected for a custody seal and opened. The container temperature is taken immediately and recorded on the Log-In Checklist. A calibrated thermometer or temperature probe (see MSL-W-003, Calibration and Use of Thermometers, for calibration instructions) is placed in the cooler in a representative location (not directly touching any ice or cold packs). If a temperature blank

sample is provided, this should be used to measure the container temperature at the time of receipt. Acceptable shipping temperatures are less than or equal to room temperature for metals in preserved water samples. For unpreserved water, tissue, sediment, or soil samples, container temperature(s) should be 4°C $\pm$ 2°C; however, solid samples (tissue, sediment, and soil) can be frozen.

4.2.2 Individual samples are then removed from the shipping container and inspected for the presence of sample custody seals, the intactness of the seals, damage, leakage, missing labels and/or other problems. These problems are noted on the Log-In Checklist. The sample identification numbers are compared to the CoC form that accompanies the samples to ascertain whether all samples are present and whether or not the labels on the containers match those on the CoC form. If no CoC form accompanies the samples, a MSL CoC form will be initiated and filled in with the sponsor codes listed on the sample labels.

4.2.3 At this point, the Project Manager should be notified of the number and condition of the samples (i.e., if any seals have been broken, sample containers are damaged, and/or the cooler temperature(s) are out of limits). The project manager is responsible for contacting the customer regarding problems that may affect the integrity of the samples or subsequent analyses. The discrepancy, its resolution, and the identity of the person contacted must be documented in the project file.

4.2.4 After complete inspection of the samples, the "Received By" section of the CoC form must be signed and dated, along with the receipt time, by the Sample Custodian.

4.2.5 From the Project Log-In/Central File Index (Log-In book), a project or initial set of samples is assigned a sequential central file number (CF#). The log-in book is a spiral notebook in MSL-5, Room 130. Subsequent sample sets received for ongoing projects are logged in with the same central file number. A group of samples can be one of several things: a set of samples from one sampling period or a continuous influx of samples from one project. The project manager usually determines this.

4.2.6 Each sample is then assigned an individual number after the central file number (e.g., 1205*1, 1205*2, etc...). Samples from an ongoing project are numbered beginning with the last number assigned. This number is called the MSL sample ID, and is recorded on the CoC form and on the corresponding sample container. All samples are referred to by the MSL sample ID from this point on until the data are reported, when the sponsor ID is matched back to the MSL sample ID.

4.2.7 The Sample Custodian records in the log-in book (see Attachment 2) the central file number, date samples are received, file name, project sample description, samples numbers, storage location and the custodian's initials.

4.3 Sample Filtration, Preservation and Storage

4.3.1 Samples are appropriately preserved to ensure sample holding times, depending on the matrix and analyses. In addition, container type and amount of sample are observed to make sure the integrity of the sample is maintained. Tables 1 and 2 provide recommended practices for sample preservation and container types. If there are any deviations, the deviation shall be documented and the Project Manager should be notified for direction.

Table 1. Preservation Recommendations

Analysis Requested	EPA 1600 Series and EPA 245 Suggested Practices	MSL Recommended Practices	Holding Time
Metals in water - except methyl Hg, Cr^{3+}, and Cr^{6+}	Acidify with ultrapure nitric acid to pH<2 (0.2%)	2mL concentrated ultrapure nitric acid/1L sample (0.2%) to pH<2	6 months
Total Hg in water	Acidify to 0.5% with ultrapure HCL or BrCl to pH<2 (0.5%)	5mL concentrated ultrapure HCl/1L sample (0.5%)	28 days
Methyl Hg in water	4 mL concentrated ultrapure HCl/1L freshwater sample or2mL concentrated sulfuric acid/1L seawater sample	5 mL concentrated ultrapure HCl/1L freshwater sample or 5mL 8M sulfuric acid/1L seawater sample	28 days
Cr^{3+} water samples	Add 1mL Cr^{3+} extraction solution to 100mL sample, vacuum filter through 0.4µm membrane, add 1mL concentrated ultrapure nitric acid to filter	Add 1mL Cr^{3+} extraction solution to 100mL sample, vacuum filter through 0.4µm membrane, add 1mL concentrated ultrapure nitric acid to filter	6 months
Cr^{6+} water samples	1mL 50% NaOH /125mL sample and refrigerate	1mL 50% NaOH /125mL sample and refrigerate	30 days
Arsenic speciation in water	Acidify pH<2 with HCl and refrigerate	2mL concentrated ultrapure HCL/1L sample (0.2%) and refrigerate	28 days

Table 1. Preservation Recommendations (continued)

Analysis Requested	EPA 1600 Series and EPA 245 Suggested Practices	MSL Recommended Practices	Holding Time
Selenium speciation in water	Not applicable	2mL concentrated ultrapure HCL/1L sample (0.2%) and refrigerate	28 days
Metals in tissue, sediment, and soil	Ship cold (4°C ± 2°C) then freeze dry	Freeze and/or refrigerate (4°C ± 2°C) then freeze dry	6 months
Total Hg in tissue, sediment, and soil	Ship cold (4°C ± 2°C) then freeze dry	Freeze and/or refrigerate (4°C ± 2°C) then freeze dry	28 days
Methyl Hg in tissue	Ship cold (4°C ± 2°C) then freeze dry	Freeze and/or refrigerate (4°C ± 2°C) then freeze dry	28 days
Methyl Hg in sediment	Ship cold (4°C ± 2°C) DO NOT FREEZE DRY	Freeze and/or refrigerate (4°C ± 2°C) DO NOT FREEZE DRY	28 days

Table 2. Container Types and Minimum Samples Amount

Analysis Requested	Container Type	Minimum Required Amount
Metals in water (except mercury)	fluoropolymer (FEP; Teflon™), polyethylene, polycarbonate, or polypropylene bottles with lids	150 mL
Metals in sediment and tissue	glass, polyethylene, polystyrene (SPEX) jars	10-20 g wet
Total and methyl Hg in water	fluoropolymer (FEP; Teflon™) or glass bottles with fluoropolymer or fluoropolymer-lined lids	500 mL
Total and methyl Hg in sediment and tissue	fluoropolymer (FEP; Teflon™), glass, or polystyrene (SPEX) jars	10-20 g wet

4.3.2 If water samples arrive unpreserved, the project manager is consulted to determine if filtration or preservation is required. Sample pH may be randomly measured to assure that samples are not preserved when other information is unavailable. Water samples have to be filtered before preservation with acid. Once filtration is complete, samples are acidified and all is noted on the Log-In Checklist form.

If the samples are to be analyzed for the Navy and are water samples for metals or mercury (Hg) analysis, the pH (see MSL-W-001, Calibration and Use of pH Meters) for all samples will be measured and the result and time of measurement documented using the form found in MSL-I-028, Navy Sample Analyses Plan. The pH of all other water samples for metals will be determined based on customer request.

4.3.3 Tissue, sediment and soil samples can be held in a refrigerator or freezer until sample preparation. If samples require freeze drying as per Project Manager instructions, samples are weighed and placed in the ultra-low temperature freezer (-68 ± 5 °C) located in MSL 5, Room 130.

4.3.4 All samples are placed in the location specified in the sample log-in book by the sample custodian. At this time, the Log-In Checklist is completed, signed and dated by the Sample Custodian.

4.3.5 Additional project information must be obtained from the project manager prior to the electronic log-in of the samples. This includes the customer name and sample analyses required. At this point, the sample information will be used to generate a computer spreadsheet of the sample log-in information called the Sample Log-In spreadsheet (see Attachment 3). A copy of the Sample Log-In form along with the completed Chain-of-Custody form(s), Log-In Checklist, and any custody information that accompanied the samples should be given to the Project Manager accompanied by a kit/addendum (see MSL-D-004, Data Reporting, Reduction, Back Up, and Archiving). The Project Manager will complete the kit/addendum and forward the packet to the Data Coordinator. The Data Coordinator will disperse copies of the kit/addendum to the appropriate analysts.

4.4 Sample Disposal

Sample disposition can either consist of returning samples to the customer or disposing of samples into an appropriate waste receptacle. Sample disposition requirements are documented on the Log-In Checklist. MSL sample disposition is mandated by the formal, Battelle Pacific Northwest National Laboratory-controlled document issued under the Standards Based Management System (SBMS) in the subject area of "Chemical Management System". The SBMS is a web-based policy and procedures resource for Battelle staff, which guides day-to-day operations. The Chemical Management System (CMS) tracks solution disposal (e.g., for samples, reagents, standards, etc.) Samples are labeled as to their disposition date when archived. A Sample Disposal Logbook is used to document Sample Disposal.

5.0 DATA ANALYSIS AND CALCULATION

Not applicable to this procedure

6.0 QUALITY CONTROL

The Project Manager reviews the CoC and Log-In Checklist for correctness and completeness before the kit/addendum is completed. When the QA reviews the data, the CoC and Log-In Checklist are reviewed again for correctness and completeness.

7.0 SAFETY

Precautions should be used when handling samples. Gloves, safety glasses and laboratory coats should be worn when handling samples of unknown content.

8.0 TRAINING REQUIREMENTS

Appropriate health & safety training is required to handle hazardous samples. All staff members who will be designated as a Sample Custodian shall first read this procedure. Documentation of training will be recorded on a training assignment form from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-W-003	Calibration and Use of Thermometers
MSL-W-001	Calibration and Use of pH Meters
MSL-I-028	Navy Sample Analyses Plan
MSL-D-004	Data Reporting, Reduction, Back Up, and Archiving
MSL-A-006	Marine Sciences Laboratory Training

Attachment 1
Example Log-In Checklist

LOG-IN CHECKLIST Reference SOP# MSL-A-001

Central File #: _____ Project Manager: _____

TO BE COMPLETED BY PROJECT MANAGER (prior to sample arrival when possible)

Matrix: _____ WP# _____

Yes	No	
☐	☐	Navy-type Project (requires high-level sample tracking procedures)
☐	☐	Filter Samples: Amount / Entire sample / Half of sample
☐	☐	Freeze dry sample(s) - samples will be weighed and placed in Ultra-low temp freezer (Lab# 130)
☐	☐	Special instructions: _____

Sample Preservation Instructions: _____

Date To Archive: _____ Date To Dispose: _____

TO BE COMPLETED UPON SAMPLE ARRIVAL/LOG-IN

Yes	No	N/A	Initial Appropriate Box When Completed	
☐	☐	☐	Custody seal present	
☐	☐	☐	Custody seal intact	
☐	☐	☐	Cooler(s) temperature (acceptable range 4°C±2°) (if multiple coolers, note temp. of each)	_____ °C _____ °C
☐	☐	☐	Project Manager notified of any discrepancies or if temperature outside acceptable range? Comments/Remedy: _____	
☐	☐	☐	All chain of custody forms signed and dated?	
☐	☐	☐	Samples Filtered?	

Sample condition(s): Acceptable / Other (explain)

Container type: Teflon / Poly / Glass / Sterile / Other _____

Notes: _____

Completed By: _____ Date/Time: _____

Attachment 1
Example Log-In Checklist (continued)

SAMPLE PRESERVATION

☐ Sample preserved upon arrival at MSL (noted on CoC / Sample / per PM Instruction)

☐ Random pH checked for ~10% of samples (use dip paper) Sample IDs: _____

☐ Complete pH check required for project (use pH meter and record on pH Record form)

If preservation necessary, record Acid Lot#

Type: ☐ 0.2% HNO3 Notes: _____

☐ 0.5% HCl (Hg samples) Notes: _____

☐ Refrigerate Notes: _____

☐ Freeze Notes: _____

☐ Other Notes: _____

Completed By: _____ **Date/Time:** _____

Attachment 2
Example Project Log-In/Central File Index (Log-In Book)

DATE	INITIALS	FILE NAME	PROJECT	SAMPLE DESCRIPTION	# OF SAMPLES	STORAGE LOCATION

Attachment 3
Example Sample Log-In Spreadsheet

cc: PM/CF Log-In File	**Project Title:**	**Example Sample Log-In Sheet**			**Project Manager:** **Date Received:**	
SPONSORR CODE	Battelle MSL Sample ID	Matrix	Storage Location	Parameters requested	**Collection Date**	Initials
Dump Site 1	***1205*1***	***Sediment***	***Hg Lab***	***MeHg***	***02/29/99***	***LN***

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Marine Sciences Laboratory EFFECTIVE DATE: 4-27-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-A-002-02

SAMPLE CHAIN-OF-CUSTODY

Approvals:		
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	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	4-26-00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E A Crecelius	4-27-00
	Signature	Date

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SAMPLE CHAIN-OF-CUSTODY

1.0 SCOPE AND APPLICATION

This procedure defines the methods for establishing the traceability of samples transferred to the Battelle Marine Science Laboratory (MSL) for chemical and/or biological testing. This process ensures the integrity of the samples from the time of collection through sample disposal. The sequential custody of samples will be documented using this procedure. Each custodian of the samples shall comply with the procedures described below.

2.0 DEFINITIONS

- **Custody** - Having control of the sample in one or more of the following manners: 1) physical possession; 2) in person's view after taking possession; 3) secured by a person in a manner that prevents tampering of sample; and/or 4) secured by a person in an area restricted to authorized personnel.
- **Sample Custodian** - The person assigned, at a given field site, laboratory, or testing facility, for having responsibility for custody of the sample.
- **LRB** - Laboratory Record Book
- **CoC** – Chain of Custody

3.0 RESPONSIBLE STAFF

Marine Sciences Laboratory (MSL) Staff as Sample Custodian or as Sample Recipient or as MSL Contact
Project Manager or Task Leader
MSL Manager
MSL Quality Assurance Officer or Representative

4.0 PROCEDURE

4.1 Custody Procedures in the Field or Laboratory

4.1.1 The sample custodian may be a member of the sampling crew or a person that works with those who are collecting the samples. The sample custodian ensures that sample labels are filled out and affixed to the appropriate sample containers before or at the time of sample collection.

Information on the sample labels may include, but not be limited to, a code number identifying the sample, date, time, and location of sample collection, and name of sample collector.

4.1.2 Once the samples are collected, the sample custodian records pertinent sample collection information on required raw data documentation (i.e., sample log, LRB, etc.). Information may include, but not be limited to, a code number identifying the sample, date, time, and location of sample collection, and name of sample collector.

Record in permanent ink all pertinent information about each sample on a Chain-of-Custody Form (Attachment 1 or 2). Press hard when making entries and assure transfer to carbon copies. Multiple samples collected on the same date may be recorded on one Chain-of-Custody Form, provided each sample is identified individually on the form.

Note: The Field Sample Chain of Custody (Attachment 1) is used primarily when transferring samples from the field to the MSL for processing. The Sample Custody Record (Attachment 2) is used when transferring samples from the field or the MSL to another laboratory or testing facility. For the purpose of this SOP, the term "Chain-of-Custody Form" can mean either of the two forms.

4.1.3 If required by a project-specific protocol, the sample custodian attaches custody seals to the samples or to the shipping container (e.g., ice chest) immediately on sample collection. The seal is attached in such a way that the sample cannot be opened without breaking the seal.

4.1.4 If there are special storage requirements (i.e., temperature requirements), the sample custodian ensures that samples are immediately stored using the required method and appropriate containers.

4.1.5 The sample custodian is responsible for the samples during delivery to the MSL, laboratory or testing facility until custody of the samples can be transferred to the sample recipient or until release of the samples during shipment (e.g., if samples have to be shipped via overnight carrier, etc.). If custody of the samples cannot be transferred to the sample recipient or shipped on the same day as sample collection, the samples must be stored in a locked or secured storage area until the transfer can be made.

Note: Chain-of-Custody Forms shall remain with samples during transfer.

4.2 Transferring Custody of Samples to a Laboratory or Testing Facility

4.2.1 Upon arrival at the laboratory or testing facility or just prior to releasing samples for shipment, the sample custodian examines the sample container(s) to ensure that the sample seals are intact and the sample containers have not been damaged.

4.2.2 The sample custodian relinquishes custody by signing, dating, and noting the time in the "Relinquished By" space on the Chain-of-Custody Form. The sample custodian tears off the bottom copy (pink) of the Chain-of-Custody Form and retains it for filing with project files.

4.2.3 The sample recipient takes custody of the samples by signing, dating, and noting the time in the "Received By" space on the Chain-of-Custody Form. The sample recipient now becomes the laboratory sample custodian, completing the transfer of sample custody. The contents of the shipping container must be checked against the information on the chain-of-custody form for anomalies. If any discrepancies are noted, or if laboratory acceptance criteria or project-specific criteria are not met, the laboratory must contact the client's designated point of contact for resolution of the problem. The discrepancy, its resolution, and the identity of the person contacted must be documented in the project file. If any seals have been broken and/or the sample containers are damaged, the sample recipient records the condition of the seals and containers in the remarks section of the Chain-of-Custody Form.

4.2.4 The Chain-of-Custody Forms travel with the samples during the transfer, and are filed in the laboratory or testing facility's project files.

4.3 Internal Chain of Custody

MSL does not routinely invoke a formal internal chain of custody process. Access to the building is limited by requiring all staff members to have electronic access cards to enter the building (refer to MSL-A-011, Marine Sciences Laboratory Access Control.) Visitors are issued daily badges. After hours site access is maintained by a gated fence to the grounds and the presence of a security guard. Non-analytical staff are not encouraged to be in areas when they have no reason to be there.

The laboratories are physically located in close proximity to one another and samples are within the physical control of the analysts during digestion and analysis activities. Samples are received in the shipping and receiving area, and logged in per procedure MSL-A-001, Sample Log-In Procedure, and stored until digestion (if required) and analysis. MSL does not store digestate for re-analysis. Instead, if a sample requires re-analysis it is digested from an archived sample.

Access to sample archive refrigerators and freezers is restricted.

A Log-in Checklist (see Appendix of MSL-A-001) is used to document sample sample receipt activities, verification of field sample preservation, sample filtration and preservation when required, and to document any deviations related to sample receipt and sample log-in.

MSL documentation provides the location of the sample post-receipt on the Sample Log-In Form. Digestion sheets provide a record of sample digestion dates. Analysis times are documented on raw data print outs. Sample disposition is determined by the client and is documented on the Log-In Checklist in the section completed by the Project Manager. Sample disposition processes are documented in MSL-I-026, Use of Laboratory Refrigerators and Freezers. When an internal chain of custody report is desired, it can be generated from data sheets in the sample analysis data file, and verified against the data file documentation by the Project Manager and MSL QA Officer.

4.4 Subdividing Samples

Once at the MSL laboratory or testing facility, if samples have to be subdivided and submitted to a subcontractor laboratory, this information will be noted on the original Chain-of-Custody Form (from sample collection), and a new Chain-of-Custody Form is initiated. With each transaction, the sample custodian relinquishes custody to the sample recipient, who then becomes the next sample custodian. (See Sections 4.2.2 through 4.2.4 above.) The requirements for chain of custody and sample disposition will be noted on the Chain-of-Custody form.

4.5 Disposal of Samples

4.5.1 When samples are disposed of by the subcontractor laboratory:

- If the subcontractor laboratory or testing facility is responsible for disposing of the samples, the subcontractor is asked to notify the MSL Project Manager before final disposition. The MSL Contact will notify the originator that the samples are scheduled to be destroyed, or will define customer requirements for an extended period of storage.

- After destruction of samples, the subcontractor laboratory or testing facility is asked to return a copy of the Chain-of-Custody Form to the MSL Contact for placement in project files. The originator may be forwarded a copy of the final Chain-of Custody documentation if requested.

- The MSL Contact records the date of receipt on the Chain-of-Custody

Form in the "Received by" section of the form space and indicates the samples were destroyed ending the chain of possession.

4.5.2 When samples are disposed of by the Marine Sciences Laboratory (MSL):

- If the laboratory or testing facility is not responsible for disposal of the samples, MSL personnel will obtain custody of the samples from the subcontractor laboratory or testing facility along with the Chain-of-Custody Form.

  For returned samples or samples that have never left MSL custody, the MSL Contact will notify the originator that the samples are scheduled to be destroyed, or will define customer requirements for an extended period of storage.

  If extended storage is not requested, then MSL will dispose of the samples following the guidelines specified in the Pacific Northwest National Laboratory's (PNNL's) Standards-Based Management System (SBMS). This system provides a framework for logging in reagents, chemicals and solutions into the associated Chemical Management System (CMS). This system provides the PNNL Laboratory with the policies and procedures regarding tracking and inventory, storage and disposal of completed samples and analytical wastes, as well as chemical use and disposal. The CMS is used to provide an up-to-date inventory to facilitate emergency response, monitor the location of various classes of materials and identify situations where acceptable limits for the building/facility determined by the assigned chemical hazard group and fire zone might be exceeded before a violation occurs.

- After destruction of samples, MSL personnel responsible for sample destruction returns a copy of the Chain-of-Custody Form to the MSL Contact and the Sample Disposal Log Book entry is updated.

- The MSL Contact records the date of receipt on the Chain-of-Custody Form in the "Received by" space next to the Sample Custodian's signature and indicates the samples were destroyed ending the chain of possession.

4.5.3 When samples are returned to the customer for disposal:

- Samples may be returned to the customer (or the sampling site) by customer request. Samples are shipped to meet Department of Transportation regulations Generally, the samples are shipped in the

same way that they were initially shipped to MSL. Sample disposition should be documented in the central file of each project.

4.5.4 The MSL Contact shall ensure that completed Chain-of-Custody Forms are filed in the appropriate project files. The originator may be forwarded a copy of the final Chain-of Custody documentation if requested.

5.0 DATA ANALYSIS AND CALCULATIONS

There are no calculations applicable to this procedure.

6.0 QUALITY CONTROL

It is the responsibility of each individual taking or relinquishing custody of the samples to ensure that Chain-of-Custody Forms are filled out accurately and completely for each transaction, and that the forms are filed in the appropriate project files.

If the Chain of Custody is broken at any time when the sample is in the control of MSL, this deviation must be documented in the data report narrative.

7.0 SAFETY

Not applicable.

8.0 TRAINING REQUIREMENTS

All staff responsible for sample custody (i.e., sample relinquisher or sample recipient) shall first read this procedure and document the training as a completed reading assignment on an Individual Training Assignment Form or a Group Training Documentation Form as described in MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-001	Sample Log-In Procedure
MSL-A-006	Marine Sciences Laboratory Training
MSL-A-011	Marine Sciences Laboratory Access Control
MSL-D-004	Data Reporting, Reduction, Back Up, and Archiving
MSL-I-026	Use of Laboratory Refrigerators and Freezers

Attachment 1

Battelle Marine Sciences Laboratory 1529 W. Sequim Bay Rd. Sequim, WA 98382	**EXAMPLE** **FIELD SAMPLING CHAIN OF CUSTODY**	Page ____ of ____

Shipped To:

Company:
Address:

Method of Shipment:
Shipped From: By:
Telephone:

SPECIAL INSTRUCTIONS:

Container No.: _____
Sampling Location: _____
Samples Collected By: Date: _____
Remarks:

_____
_____

SAMPLE IDENTIFCATION	

Relinquished by	Date/Time	Received by	Date/Time
Relinquished by	Date/Time	Received by	Date/Time
Relinquished by	Date/Time	Received by	Date/Time

ATTACHMENT 2

EXAMPLE
SAMPLE CUSTODY RECORD

Date _____
Page of

Battelle
Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

Project No.:											Laboratory:
Project Name:											Address:
Project Manager:											
Phone:					**Testing Parameters**						Attn:
										Number of Containers	
Lab No.	Sample No.	Collection Date	Matrix								Observations, Instructions
											total # of containers

Relinquished by: Signature Date Time Printed Name Company	Received by: Signature Date Time Printed Name Company	Shipment Method: **Special Requirements or Conditions:** **Sample Disposition:**
Relinquished by: Signature Date Time Printed Name Company	Received by: Signature Date Time Printed Name Company	Distribution: 1. 2 copies to the Laboratory 2. 1 copy project manager 3. Return completed original to Battelle Marine Sciences Laboratory

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EFFECTIVE DATE: 5-1-2000

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-A-005-04

DEVIATIONS FROM ESTABLISHED REQUIREMENTS

Approvals:		
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	Signature	Date
TECHNICAL GROUP MANAGER: Richard M. Ecker	Jan H. Nm for RMEcker	5-1-2000
	Signature	Date

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DEVIATIONS FROM ESTABLISHED REQUIREMENTS

1.0 SCOPE AND APPLICATION

This procedure provides the methods for addressing deviations from MSL procedures, protocols, work plans, and client requirements to ensure that they are properly documented, their effects evaluated and that immediate, as well as long-term corrective action is taken to preclude recurrence.

2.0 DEFINITIONS

Deviation – a deviation is defined as:

a) a failure to develop, document, or implement any applicable element of the QA program or activity established by mutual agreement with the client, or

b) the discovery after an activity is completed that there was a failure to follow established procedures, or

c) a situation in which the quality of an activity or document is indeterminate. For example, where the stated or implied purpose has not been met or where insufficient information exists to support the results that have been produced.

3.0 RESPONSIBLE STAFF

Staff Members
Project Manager
MSL QA Officer or Representative

4.0 PROCEDURE

4.1 General

Deviations are generally events resulting from unforeseen circumstances. Deviations occur when the quality of reportable data is indeterminate (i.e., no objective evidence is available to substantiate data quality or to indicate that established procedures and/or requirements were met). All departures from approved procedures, work plans, and protocols must be documented. If the probability of a procedure departure is known ahead of time and approved (in writing) by the Project Manager prior to procedure implementation, it is documented in the data sheet, daily log, project notebook summary, or other raw data record. If it is a deviation discovered after the fact – it is documented as a deviation. However, deviations from project control limits are not uncommon, especially when there are a number of control limits in a data set. For example,

the duplicate precision may have been exceeded, but all other control limits (matrix spikes, standard reference materials, blanks, and blank spikes) may indicate that the analysis is acceptable. Often these situations are determined when the data set is under initial review or during formal data audit review as per MSL procedure, MSL-Q-005, Quality Assurance Data Audits. The MSL QA Officer and the Project Manager will determine if there is a significant deviation when one or more control limits are exceeded in a data set. In some cases, the customer may be involved in these discussions. Deviations from project control limits will be identified in the narrative accompanying the data set or package or in a letter to the customer, and the impact of the deviation addressed. When a significant deviation occurs, the process described below will be followed.

4.2 Identification

Once a staff member has identified that a deviation has occurred he/she shall promptly notify the Project Manager of the deviation (usually within one working day of the discovery).

4.3 Documentation

4.3.1 The Project Manager shall:

- assure the deficiency is documented. The available means for documenting a minor deviation are discussed in Section 4.3.3. Serious deviations affecting the quality of the data or the outcome of a test or analysis are documented using a Quality Problem Report (QPR) form (Attachment 1) or the deviation section of the GLP study deviation documentation form (Attachment 2).
- determine the impact on the study or project,
- participate in determining the immediate and long term corrective action, and
- approve the actions identified in the corrective action and get the approval of other necessary management.

It is the Project Manager's responsibility to ensure the completion of the identified corrective action by the expected completion date, and request independent verification (if required)

4.3.2 When there has been an impact on data, the Project Manager shall assure that there is a cross reference in the raw data that indicates there is an associated Quality Problem Report, (i.e., refer to the QPR on each of the impacted data sheets, in the laboratory record book, and in any other documents used to transcribe the information or data).

4.3.3 Minor deviations not affecting the quality of the data or the outcome of a test or analysis may be documented on various forms used to process or analyze samples or conduct tests. Examples include:

- the sample log-in checklist

- a Chain of Custody form
- the Test Organism Receipt and Acclimation Record
- a toxicology test form:
  - -- Test Organism
  - -- Daily Log of Activities
  - -- Daily Water Quality Monitoring form
  - -- Daily Observation form
  - -- Test Termination form
- chemistry analysis forms
  - -- Sample Digestion form
  - -- Sample Extraction form
  - -- Sample Distillation form
  - -- Analyst Checklist form

Project Managers must be informed of minor deviations. In cases where an event initially appears to be minor, but for some reason becomes major and has a significant impact on the quality of the data or the outcome of a test or analysis, then a QPR must be initiated.

4.3.4 QPRs are sequentially numbered and tracked, and a new number must be issued by the MSL QA Officer. Numbers are based on the year and will begin each new calendar year in January at number 1. An example number is 2000-001, the first QPR issued in the year 2000.

4.4 Records

The Project Manager shall ensure that the completed QPR form is kept with the central project file and that a copy is provided to the MSL QA Officer or Representative.

4.5 Reporting to MSL Line Management

Once a quarter, the MSL QA Representative shall present to MSL Management a summary of all QPRs, any significant control limit data deficiencies, and an analysis of trends or recurring deficiencies.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

Tracking quality-affecting deviations allows MSL management to be informed regarding

deviations and to address recurring concerns.

7.0 SAFETY

There are no safety issues associated with implementing this procedure..

8.0 TRAINING REQUIREMENTS

All MSL staff must be trained to this procedure. Documentation of training shall be recorded on a training assignment form provided in procedure MSL-A-006, Marine Sciences Laboratory Training. All staff are empowered to identify quality-affecting deviations.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training
MSL-Q-005 Quality Assurance Data Audits

ATTACHMENT 1
Example Quality Problem Report Form

Originator	Date	QPR Number: Project Number:
Project Manager		Project Title:

Purchase Order Number/SOP/Work Plan

10CFR830.120 Related? Yes or No

Statement of the Deviation

Impact of the Deviation

Steps to Prevent Inadvertent Use of the Item or Process

Cause of Events Leading to the Problem

Planned Corrective Action for the Immediate Problem/Independent Verification Required? Yes or No

Planned Corrective Action to Prevent Recurrence/Independent Verification Required? Yes or No

Person Responsible for the Corrective Action	**Approval of Planned Corrective Action**
Last Name, First, MI	*Cognizant Manager or Designee*

Closing the Problem

Actions Completed as Planned *Name* *Date*	Intermediate Distribution:
Independent Verification Has Been Completed (if required) *MSL QA Representative* *Date*	Final Distribution: MSL QA Office

Attachment 2
Example GLP Study Deviation Documentation Form

GLP STUDY NUMBER
Project No. Page 7 of 1
Study Director:

Project Title

Entered by: _____ Date: _____

The following information is (check one)
[ ] miscellaneous documentation
[ ] a deviation from Protocol, Work Plan or QA Plan (give title)

_____

[ ] a deviation from SOP
No. _____

Description: _____

Impact on
Project: _____

APPROVED BY:

_____ _____
Study Director Date

EFFECTIVE DATE: 11-30-99

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Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-A-006-01

MARINE SCIENCES LABORATORY TRAINING

Approvals:		
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QA OFFICER: Deborah Coffey	Deborah Coffey	11-30-99
	Signature	Date
TECHNICAL GROUP MANAGER: Richard M. Ecker	for RM Ecker	11-30-99
	Signature	Date

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MARINE SCIENCES LABORATORY TRAINING

1.0 SCOPE AND APPLICATION

This SOP provides a uniform method for identifying, performing, and documenting the required indoctrination and training of staff working at the Marine Science Laboratory (MSL). This procedure applies to the training of all staff members who perform or manage activities that affect the quality of work for all Good Laboratory Practice (GLP) regulated projects and, when required by Line Management, on other projects or activities. Indoctrination and training needs are determined and initiated when:

- a new project or activity begins
- new personnel are assigned to the project or activity
- there is a significant change in the project or activity; e.g., scope of work, or quality assurance requirements
- the responsible manager determines that training should be performed, documented, and entered into the staff member's training file
- the training is specified by MSL clients

2.0 DEFINITIONS

Briefing - An informal overview of a topic rather than detailed instructions; e.g., a modification of a procedure with which the trainees are familiar.

Core Business Training - Applicable to an employee's customary work activity(s). Providing this training is normally a function of line management.

Generic Training - Training required of all MSL employees which is independent of the work assignment or job title of the employee.

Group Training Documentation Form - A form to identify and document training of one or more subjects to a group of individuals, using any of the three training methods (Attachment 3).

Individual Training Assignment Form - A form to document assignment and completion of required training of one or more subjects for an individual, using any of the three training methods (Attachment 2).

On-The-Job Training (OJT) - Training through performance of a specific job function under the supervision of an instructor or mentor who is familiar with the job, in accordance with an approved OJT plan (Attachment 4).

MSL Initial Personnel Training – New employee training addressing building emergency procedures, initial security briefing, electrical safety, hazard communication, laboratory hoods, Lock and Tag- General Employee Awareness, fire extinguisher training, computer security, and security badging.

MSL Qualification Form – A form summarizing an employee's education and experience to perform the assigned work (Attachment1). The form is updated whenever the scope of the employee's work changes.

New Employee QA Training – PNNL Quality Program Training available as an on-line, web-based computer training.

Project-Specific Training - Training apart from an employee's core business activity(s) that is related to a specific piece of work (project/activity)

3.0 RESPONSIBLE STAFF

- MSL Manager
- Line Manager, Project Manager/Study Director (i.e., Responsible Manager)
- Quality Assurance Representative
- Instructor
- Trainee

4.0 PROCEDURE

4.1 Evaluation and Documentation of Education and Previous Experience and Competency

Prior to allowing staff members to perform activities affecting quality, the Line Manager shall obtain documentation showing that staff members have the appropriate education and experience for the work they are to perform. Documentation shall include the following information:

- education completed (e.g., institution, dates attended, degree and major)
- work experience (years of experience, employer, major responsibilities)
- licenses and certifications (pertaining to the work assignment)
- related training and qualifications

A MSL Qualification form (Attachment 1) is required to document education and experience. A resume is not allowed in lieu of this form because training and qualifications are often assessed during audit activities. Resumes are protected documents under the Privacy Act and cannot be given to others.

4.2 Evaluation and Documentation of Proficiency

Prior to allowing staff members to perform activities affecting quality, the Line Manager shall determine if the evaluation and documentation of proficiency is required. If proficiency is a job requirement, then the approach to document proficiency shall be determined. This could include a review of certificates obtained from previous training activities, a review of proficiency documentation (run logs, data packages) showing that staff members have the appropriate proficiency to do the work they are to perform, or a demonstration or test of proficiency. Documentation might include the following information:

- length of previous work experience (employer and major responsibilities)
- licenses and certifications
- related training and qualifications
- proficiency test results (may include the periodic analysis of performance evaluation (PE) samples)
- documentation for a proficiency demonstration

4.3 Training Categories

Generic Training: Generic training is non-job specific. It applies to everyone at the MSL, regardless of the work they do. Examples of this kind of training are:

- new hire orientation (MSL Initial Personnel Training)
- lab safety and security indoctrination
- portable fire extinguisher use
- building emergency procedures

Core Business Training: Core business training is job specific. It applies to everyone at the MSL who customarily performs a specific job or activity. Examples of this kind of training are:

- radiation protection
- lead auditor certification
- 40-hour hazardous material worker training
- technical or administrative procedures

Project Specific Training: Project specific training applies to those kinds of activities required by a particular piece of work or project. Normally, the work is of a known duration and falls outside the staff member's customary (core) business activities. Examples of this kind of training are:

- quality assurance plans
- project management plans (e.g., work plans, protocols)
- technical or administrative procedures

Note: There are apparent overlaps between core business and project specific training in the area of technical and administrative procedures. Training responsibilities between line management and project management will vary, according to specific demands of the project and their relationship to the core business training requirements of staff assigned to the project.

4.4 Identification and Assignment of Training

4.4.1 The Line Manager shall:

- ensure personnel are qualified, indoctrinated, and trained prior to and commensurate with the work to be performed
- review each staff member's previous training records at time of hire and, at a minimum, annually thereafter
- identify all generic training required for new personnel and retraining needs for existing personnel
- identify all required training or retraining applicable to each staff member's assigned work activities (i.e., core business)
- assign identified training by preparing an Individual Training Assignment Form (Attachment 2) or a Group Training Documentation Form (Attachment 3), as appropriate
- verify that the trainee has an understanding of the training substance or material commensurate with the work to be performed
- ensure that personnel who need further training for a particular job perform work only with qualified supervision until training is completed
- assign new training or retraining as required training materials are revised or the nature/scope of work changes
- forward completed original training records to the MSL Quality Assurance Officer
- alert staff that trainees who receive required training external to the MSL are responsible for providing their manager with copies of documentation (e.g., certificates) verifying satisfactory completion of the course

4.4.2 The Project Manager/Study Director shall:

- verify each staff member's training is commensurate with work to be performed
- identify any required training that is project specific
- assign identified project-specific training by preparing an Individual Training Assignment Form (Attachment 2) or a Group Training Documentation Form (Attachment 3)

- verify that the trainee has an understanding of the training substance or material commensurate to the applicable work
- assign retraining or new training as applicable training materials/requirements are revised or the nature/scope of work changes
- forward completed original training records to the MSL Quality Assurance Officer
- retain a copy of completed training records in project files if required by a client
- alert staff that trainees who receive required training external to the MSL are responsible to provide their manager, LTC and the MSL Quality Assurance Officer with copies of documentation (e.g., certificates) verifying satisfactory completion of the course

4.4.3 The MSL Quality Assurance Officer shall forward a copy of completed training records to the Laboratory Training Coordinator (LTC) in Richland, Washington.

4.5 **On-The-Job Training**

4.5.1 Application: On-the-Job Training (OJT) is appropriate when:

- a procedure states that on-the-job training is required
- the trainee is required to demonstrate proficiency in a process or skill
- supervised experience or mentoring is necessary prior to allowing the individual to work independently; e.g., training which concerns the use of specialized test equipment

4.5.2 Responsibilities: The Responsible Manager or Instructor shall:

- use as-is or modify an existing OJT plan on file, or
- develop an original OJT plan in accordance with Attachment 4
- obtain all approval/concurrence signatures required on the OJT Plan
- conduct training in accordance with the OJT Plan
- document all briefing sessions on an Individual Training Assignment Form (Attachment 2) or a Group Training Documentation Form (Attachment 3)
- forward completed training records as required in 4.3.2.

4.6 **Briefing Sessions**

4.6.1 Application: Briefing sessions are appropriate when:

- a procedure states that briefing sessions are required
- training is meant to be an overview of a topic rather than detailed instruction

- training is to an Interim Change Notice or a revision of a procedure with which the trainees are familiar
- the amount of material to be presented is not large or complex in nature, but does require instructor input to ensure that the trainees adequately understand the subject

4.6.2 Responsibilities: The Responsible Manager or Instructor shall:

- document all briefing sessions on an Individual Training Assignment Form (Attachment 2) or a Group Training Documentation Form (Attachment 3)
- forward completed training records as required in 4.3.2.

4.7 **Reading Assignments**

4.7.1 Application: Reading assignments are appropriate when:

- a procedure states that reading assignment training is required
- the topic is adequately addressed in a document
- no instructor input is required to clarify the material
- the trainee is familiar with the basic concepts presented

4.7.2 Responsibilities: The Responsible Managers shall:

- document all reading assignments on an Individual Training Assignment Form (Attachment 2) or a Group Training Documentation Form (Attachment 3)
- forward completed training records as required in 4.3.2.

4.8 **Training Waivers**

4.8.1 Formal training may be waived in specific areas where the staff member has an acceptable level of proficiency based on previous experience or training, as determined by the Responsible Manager.

4.8.2 The Responsible Manager shall:

- complete the Training Waiver (Attachment 5), and
- attach or reference documented evidence of equivalent training or previous experience to the Training Waiver Form (e.g., certificate of completion, attendance sheet, resume, etc.)
- obtain approval signature of the next level manager (one-over-one)
- obtain concurrence of the MSL Quality Officer
- obtain concurrence of the MSL Safety Officer, if safety-related training

- forward completed original training records to the MSL Quality Assurance Officer
- forward a copy of completed training records to the Laboratory Training Coordinator in Richland, Washington

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable

6.0 QUALITY CONTROL

Not applicable

7.0 SAFETY

Not applicable

8.0 TRAINING REQUIREMENTS

All staff shall complete a Qualification and Training form when first hired and whenever the scope of the person's work changes (Attachment 1). All staff shall read this procedure and document required training as a reading assignment on an Individual Training Assignment Form (Attachment 2) or a Group Training Documentation Form (Attachment 3).

9.0 REFERENCES

None

ATTACHMENT 2 Page 1 of 2

Marine Sciences Laboratory
Qualification and Training Form

Section 1 - Personal Information:
Name: _____ Org. Code_____
E-Mail: _____ Phone_____ Fax:

If Contractor, please provide the following:(if not, leave blank)
Contract #(s)_____ Employer:_____
Address:

Section II – Qualifications: Summary of Education and Experience Applicable to Job Description

- ☐ Check if this form is being submitted to document a change in job scope (e.g., new organization new manager, new assignment), but no change in qualifications previously documented
- ☐ Check if person is returning to MSL (de-archive records)

Education: (list applicable degree(s), school, discipline)

Experience: (list applicable work activities and length of time)

Section III – Job Description (to be completed by Technical Group Leader):

POSITION: (check one) ☐ Administrative Staff ☐ Support Staff ☐ Management ☐ Technical Staff

AREA: (check all that apply) ☐ Statistical Analyst ☐ Contracts ☐ Quality Assurance ☐ Administrative Support ☐ Business Management ☐ Experimentalist ☐ Procurement ☐ Computer Support ☐ External Reviewer ☐ Training

Section IV - Additional Training: Manager to list other required training such as specific procedures, required reading, procedure testing, etc.

Section V – Signatures:

1. I certify that the information in Section II is factual, and that I have completed initial training and any assigned additional training.

Employee (Print Name) *Employee Signature* Date

2. I verify that the information in Section II is factual. (Leave this section black if employee is an MSL employee or if self- employed).

Contract Manager (Print Name) *Signature* Date

3. Based on the above, this individual is qualified to perform the assigned Job Description

MSL Technical Group Leader *Signature* Date

ATTACHMENT 2 **Page 1 of 2**

EXAMPLE INDIVIDUAL TRAINING ASSIGNMENT FORM

Trainee: Course Title and No.:

Payroll No.: Project/Organization No.:

Training Conducted By/
Reading Assigned By: Date:

Required training and method(s) used are indicated below. Please use permanent ink when indicating completion.

Title/Subject/Document	Rev. No.	Method Used*	Sign and Date When Completed

* Method: O= On-The-Job Training OJT Plan No.: N/A

B = Briefing Session
R = Reading Assignment

Responsible Manager: Sign and date below when this form has been reviewed for completeness and forward a copy (either original or reproducible) to the Laboratory Training Coordinator.

Signature: _____ Date: _____

ATTACHMENT 2

Trainee: Course Title and No.:

Payroll No.: Project/Organization No.:

Title/Subject/Document	Rev. No.	Method Used*	Sign and Date When Completed

ATTACHMENT 3 Page 1 of 2

EXAMPLE GROUP TRAINING DOCUMENTATION FORM

Training Date: Page ___ of ____

Training Method Used		Course No./Title/Subject/Rev No.	OJT Plan & Rev. No.
	On-The-Job		
	Briefing		
	Reading		

Retrain Date (if applicable):

Additional Information:

Training Conducted By/

Reading Assigned By: Date:

Printed Name	Payroll No.	Project/Org No.	Sign and Date When Completed

Responsible Manager: Sign and date below when this form has been reviewed for completeness and forward a copy (either original or reproducible) to the Laboratory Training Coordinator.

Signature: _____ Date:

____ Check to obtain credit for Instructor's Attendance (first time presented only).

ATTACHMENT 3 **Page 2 of 2**

EXAMPLE GROUP TRAINING DOCUMENTATION FORM

Training Date: Page 2 of 2

Printed Name	Payroll No.	Project/Org No.	Sign and Date When Completed

ATTACHMENT 4

EXAMPLE ON-THE-JOB TRAINING PLAN

Date Issued: Page 1 of
Project/Activity:
Training Plan No.: Rev. No.:
Lesson Title:

TRAINING PLAN OUTLINE

I. OBJECTIVES:

II. PRESENTATION:

III. EXAMINATION: (optional)

IV. TIME ALLOCATION:

V. RETRAINING PERIOD:

Prepared By: _____
Instructor Date

Concurred With: _____
Quality Assurance Date

Approved By: _____
Responsible Manager Date

OJT PLAN INSTRUCTIONS

Training Plans are used for on-the-job training sessions. The following are instructions for filling out the On-The-Job Training Plans.

1. Project or activity to which the training plan applies.

2. Training Plan Number (this number should be unique to each individual Training Plan, and prefixed by project number or by organization acronym).

3. Lesson title and revision number.

4. Objectives - describe what the trainee should be able to do or know upon completion of the training.

5. Presentation - identify within the training plan the information to be presented and any materials used which will achieve the course objective.

6. Examination - examinations are at the discretion of the Instructor. Indicate if an examination is required, and attach any testing or examination documents to the training plan.

7. Time Allocation - indicate when the training should be completed.

8. Retraining Period - indicate when retraining is required, if applicable.

9. Obtain all required signatures.

ATTACHMENT 5

EXAMPLE TRAINING WAIVER

Date Issued:

Print Name: Payroll Number:

Project/Organization Number:

Training to be Waived

Title:

Course Number: Revision Number:

Justification for Waiver
(Attach or reference documented evidence of equivalent training or previous experience)

Submitted By: _____ Date: _____
Responsible Manager

Concurrence: _____ Date: _____
Quality Assurance

Concurrence: _____ Date: _____
Safety Representative

Approved By: _____ Date: _____
One-Over-One Manager

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Marine Sciences Laboratory

EFFECTIVE DATE: 4-24-00

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-A-008-01

CONTROL OF STANDARDS, REAGENTS, SOLUTIONS, TEST/CONTROL ARTICLES AND SPECIMENS

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	4/12/00
	Signature	Date
TECHNICAL REVIEWER: Brenda Lasorsa	B Lasorsa	4/12/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	4/12/00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric Crecelius	4-24-00
	Signature	Date

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CONTROL OF STANDARDS, REAGENTS, SOLUTIONS, TEST/CONTROL ARTICLES AND SPECIMENS

1.0 SCOPE AND APPLICATION

This procedure describes the minimum requirements for receipt, labeling and when applicable, maintaining distribution control of standards, reagents, solutions, test articles, and specimens used on projects requiring implementation of U.S. Environmental Protection agency (EPA) or Food and Drug Administration (FDA) Good Laboratory Practices (GLP), or used for general Marine Sciences Laboratory (MSL) daily operations.

2.0 DEFINITIONS

Test Article Any food additive, color additive, drug, biological product, electronic product, or medical device for human use, or any other article subject to regulation.

Control Article Any food additive, color additive, drug, biological product, electronic product, or medical device for human use, or any article other than a test article, feed, or water that is administered to the test system in the course of a non-clinical laboratory study for the purpose of establishing a basis for comparison with the test article.

Reagent A substance, chemical, or solution used in the laboratory to detect, measure, or otherwise examine other substances, chemicals, or solutions.

Solution A single homogenous liquid, solid, or gas phase that is a mixture in which the components (liquid, solid, gas, or combinations thereof) are uniformly distributed throughout the mixture.

Specimen Any material derived from a test system for examination or analysis.

Standard Neat chemicals or purchased stock standards that are used as the basis for analyte quantitation or for the preparation of quality control (QC) samples.

3.0 RESPONSIBLE STAFF

Analyst/Technician
Study Director
QA Officer or Representative
Hazardous Material Custodian
Radiation Safety Officer
Shipping and Receiving Clerk
Sample Custodian

4.0 PROCEDURE

4.1 Procurement of Standards, Reagents, Solutions, Test/Control Articles or Specimens

Procurement of the items listed above is addressed in procedure, MSL-A-012, Procurement. All procurements are required to define applicable quality assurance (QA) requirements for quality-affecting items and be reviewed by the MSL QA Officer to ensure that quality specifications are conveyed in the procurement documentation. If items are hazardous or radioactive, then review of the procurement documentation by the Hazardous Material Custodian or Radiation Safety Officer is also required. The person ordering the item is responsible for ensuring that the item that is received meets all of the ordering specifications before accepting the item and authorizing payment.

Certificates of analysis or purity for standards, reagents, solutions, and test/control articles shall be requested.

Procurement of animals and feed for bioassays should include requirements for chain of custody of animals (as per MSL-A-002, Sample Chain of Custody) during shipping and documentation of any available feed analyses, feed storage recommendations, and expiration dates so that feed quality can be monitored. Animal shippers should be requested to document conditions of animals and environmental parameters (temperature) at the time of shipping for comparison with conditions encountered at the time of receipt.

4.2 Chemical Receipt

4.2.1 All chemicals that are received at the MSL shall be logged by the Shipping and Receiving Clerk according to the requirements of the Chemical Management System (CMS) described on the network Standards Based Management System (SBMS). Material safety data sheets and any certificates of analysis or purity shall be filed by the shipping/receiving clerk, and copied and provided to the analyst requesting the chemical.

4.2.2 Each chemical shall receive a unique sample inventory number (bar code) by the Shipping and Receiving Clerk. This number along with receipt date and initials of who ordered the chemical shall be placed on a removable label attached to the chemical container. The CMS tracks the following applicable information:

1) Inventory Number
2) Purchase Requisition Number
3) Receipt Date
4) Owner (staff member who ordered chemical)
5) Employee Number (payroll number)
6) Compound Name
7) Quantity
8) Manufacturer
9) Lot Number
10) Expiration Date

11) Storage Location
12) User Name

4.2.2 Once the chemicals have been depleted, the user shall place the removable label in the designated envelope in each laboratory. The Hazardous Material Custodian shall periodically check the envelopes and use the bar codes to delete the chemicals from the database inventory.

4.2.3 If the received chemical is radioactive, the MSL Radiation Safety Officer must survey the item.

4.3 **Reagents/Solutions**

4.3.1 All containers holding reagents and solutions shall be labeled with the material identity, concentration or titer, expiration date, special storage requirements, and date and initials of the person preparing it.

Material Identity - Try to avoid abbreviations; however, abbreviations are permitted provided the abbreviation is defined in the applicable notebook or wherever the documentation for reagent/solution preparation is maintained..

Concentration or Titer - Use appropriate units. Avoid ambiguity (i.e., use "wt/vol" or "ng/ l" versus "ppm".

Expiration Date - The last possible calendar date (month/day/year) that a material can be used in the laboratory.

Storage Requirements - Indicate special storage conditions. If special storage conditions are not indicated, ambient laboratory conditions are assumed.

Date and Initials - Indicate reagent/solution date and the initials of the person preparing the reagent.

4.3.2 At the time of receipt of the material, the manufacturer's label will be inspected by the staff member receiving the material for the information stated in 4.2.1. Any information found to be lacking will be determined and filled in.

4.3.3 All containers holding aliquots or dilutions of reagents, reagent solutions or solutions in the laboratory shall be labeled with the requirements in 4.1.1.

4.3.4 A period of five years will be listed (month/year) on the label for material where no expiration date was given by the manufacturer or could be determined. The label should also contain the reason for not being able to determine the expiration date (e.g., could not be determined).

4.3.5 Upon completion of the expiration period, the material will either be discarded or assessed to determine whether a new expiration could be justified. If a new expiration period is given, the justification must be documented on the label.

4.4 Test and Control Articles

4.4.1 An inventory of all test and control articles will be maintained in accordance with section 4.5 for GLP studies. For routine MSL studies, the CMS inventory process is adequate to meet requirements for tracking inventoried items.

4.4.2 All test and control articles will be identified by chemical name, Battelle invoice number, batch number, expiration date (if any is given) and storage conditions required to maintain the material's integrity. The label should clearly indicate "No Expiration Date Determined" on materials where no expiration date was given by the client. This information should be listed on all containers holding bulk and aliquots of the bulk.

4.4.3 Precautions shall be taken immediately following expiration of a test or control article to ensure that it is not left in a situation where it could be accidentally administered to a test system.

4.4.4 Out-dated test and control articles should either be disposed of or returned to the client as soon as possible. Documentation of the final disposition shall be documented on a Test/Control Article Inventory Form (Attachment 1).

4.4.5 Archived samples of test and control articles should be identified as such. Expiration dates on such materials will be identified only if specified by the client. These materials shall be kept until completion of the final report. At that time these materials should be returned to the client or discarded. Documentation of the final disposition shall be documented on a Test/Control Article Inventory Form (Attachment 1).

4.4.6 The earliest expiration date known of any of the components will apply in cases where test articles have been mixed with carriers.

4.5 Specimens

4.5.1 All specimens shall be labeled with the Battelle Study Number to identify the test system (if required), nature and any other information required to specifically identify the specimen. Specimens should also be labeled with the date of collection and if applicable, specimen identification and storage conditions required to maintain specimen integrity.

4.5.2 Following completion of the study, final disposition of the specimens shall be determined and documentation of the disposition placed with the study files in archives.

4.6 Inventory Control

4.6.1 A Test/Control Article Inventory Form (Attachment 1) shall be used to track all test and control articles for GLP studies. If required by the customer or the study director, this form can also be used to track other types of samples.

4.6.2 The Test/Control Article Inventory Forms will be kept next to the appropriate storage containers or kept in the project files until ready to be used.

4.6.3 The date, amount of article taken, the amount of article remaining and the signature of the person removing the article will be entered on the Test/Control Article Inventory Form at the time of removal.

4.7 Use of Standards, Reagents, Solutions, and Test Articles

4.7.1 Traceability

In order to maintain traceability and trackability, the lot or batch number of standards, reagents, solutions, and test articles will always be transferred to labels when these items are used to prepared secondary standards, diluted for use, transferred to a secondary container or any other use that requires transfer of the material to a new container and preparation of a new label. In addition, the identify of the material, the concentration or titer, expiration date, special storage requirements, and date and initials of the person preparing the material are required to be recorded. Lot or batch numbers are critical in the investigation of any contamination issues that might arise from the use of standards, reagents, solutions, and test articles.

4.7.2 Standard Expiration Dates

All standard and quality control (QC) materials must be labeled with expiration dates. Expiration dates must be assigned and documented for all standard and QC materials that do not have label listed expiration dates. The assigned date is based on best professional judgement of the analyst. Generally, most standards for routine analyses are used within a relatively short period. No standard should be used if the integrity of the standard is questionable. An acceptable check of a standard's integrity is to make a calibration curve using the standard and analyze a certified standard reference material (SRM). If the SRM result falls within the acceptance criteria, then the standard can be deemed acceptable. The data supporting this check must be available and retrievable, and somehow associated with the standard. Continued SRM analysis results are needed to certify the continued integrity of the standard. An expiration date must be assigned to the standard when the initial acceptability check is made. A period of 6 months to one year is the recommended maximum expiration date to be assigned and should be based on a knowledge of the standard material and its stability.

4.7.3 Extending a Standard's Expiration Date

When standards and QC materials approach an expiration date, a comparison test (with 3 replicates) against a new standard is needed to justify extending a standard's expiration date. If results are ± 10% of the value of an independent standard, then it is acceptable to re-label the tested, expired standard with a 1-year extension of its expiration date.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

QC for the steps outlined in this procedure is the responsibility of the technical staff. MSL QA staff will make independent assessments to evaluate and report to management whether this procedure is being correctly followed and implemented.

7.0 SAFETY

Applicable safety requirements are the following:

- Observe requirements for procurement, handling and tracking of hazardous and radioactive items. Ensure that trained staff are responsible for these items.
- Handle standards, reagents, solutions, test articles, and specimens in such a manner as to maintain the integrity of the item (e.g., observe storage recommendations), the tracking of the item, and the safe disposal of the item.
- Use appropriate personal protective equipment (i.e., gloves, goggles, safety glasses, face shields, gloves, laboratory coats, rubber aprons, gas cylinder carts, etc.) when handling, storing, moving and disposing of items.

8.0 TRAINING REQUIREMENTS

All personnel who will be handling or working with reagents, solutions, test/control articles, or specimens must first read this procedure. Documentation of training will be recorded on a reading assignment form from the procedure, MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-002	Sample Chain of Custody
MSL-A-006	Marine Sciences Laboratory Training
MSL-A-012	Procurement

ATTACHMENT 1

TEST/CONTROL ARTICLE INVENTORY FORM

TEST ARTICLE		STUDY NO.	
LOT NUMBER		CONCENTRATION	
MANUFACTURER		SPONSOR	
AMOUNT RECEIVED		DATE RECEIVED	
EXPIRATION DATE		SIGNATURE	

Inventory:

Date	Quantity Removed	Balance	Signature
Total Used			

Disposition:

[ ] Returned To: _____ Amount _____ Date _____ Initials
[ ] Transferred To: _____ Amount _____ Date _____ Initials
[ ] Destroyed Method:_____ Amount _____ Date _____ Initials

Study Director Approval: _____ Date: _____

Battelle

Marine Sciences Laboratory

EFFECTIVE DATE: August 18, 1999

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-A-010-00

DOCUMENT CONTROL

Approvals:

Sandra L. English	8-12-99
AUTHOR	DATE
[illegible]	8-17-99
REVIEWER	DATE
Sandra L. English	8-12-99
QUALITY ASSURANCE	DATE
[illegible] for RM Eckar	8-17-99
MSL MANAGER	DATE

1.0 SCOPE AND APPLICATION

1.1 The purpose of this procedure is to provide a uniform method for the hardcopy distribution of controlled procedures and other selected documents. Distribution is controlled to ensure that documents available for use are current.

1.2 This procedure applies to the control and distribution of hardcopies of procedures and other selected documents (as identified by line and project management) at the Marine Sciences Laboratory.

2.0 DEFINITIONS

3.0 RESPONSIBLE STAFF

Cognizant Manager – the line or project manager who deems it necessary to use document control to assure that current procedures or documents are available to staff or other recipients.

Coordinator – (designated by the Laboratory Manager) is responsible for the document control process and records to indicate it is implemented

Copyholder - is responsible for updating the affected document, and signing and dating and returning the transmittal/receipt acknowledgement within the specified time frame.

4.0 PROCEDURE

4.1 Planning

The Cognizant Manager shall:

- request that Document Control Coordinator perform the control and distribution function
- provide to the Coordinator a copy of the procedure or document and the requested distribution list

4.2 Distribution

4.2.1 The Coordinator shall:

- develop a distribution list for new documents based on input from the Cognizant Manager
- obtain and review existing distribution list when existing document is revised, update the list based on input from the Cognizant Manager
- develop a transmittal. The Controlled Document Transmittal/Receipt, EXHIBIT 1, is the preferred format. However, an alternate format may be used provided the ollowing information is included:

- document number and revision
- document title
- transmittal number or letter number and date
- Coordinator's or Cognizant Manager's signature
- specific instructions concerning the document(s) being transmitted
- disposition of obsolete documents
- receipt acknowledgement requirement.

4.2.2 When a new or revised document is issued to a set of controlled procedures or other document , the Coordinator shall prepare (or update) a Controlled Document List or a Table of Contents (see EXHIBIT 2 for examples of formats). As a minimum, the Controlled Document List or Table of Contents shall contain the following information:

- document number
- correct revision number
- changes issued
- document title
- effective date.

4.2.3 Prior to distribution the Coordinator shall indicate in red ink on the signature page or on the title page of:

- all documents for distribution (excluding the transmittal):

CONTROLLED DOCUMENT
COPY NO._____

NOTE: The copy number is not required on the signature or title page of individual procedures that are included in a controlled manual that has a copy number. These procedures are only required to have CONTROLLED DOCUMENT in red ink on the signature or title page.

4.3 Status Control

4.3.1 The Coordinator shall distribute, in accordance with the distribution list, the document package which includes a transmittal (EXHIBIT 1), a controlled document list or a table of contents (EXHIBIT 2), and the documents being issued. When receipt acknowledgement is not received within 30 days, delinquency notification shall be sent to all delinquent copyholders. Delinquency notification should be submitted as follows to delinquent copyholders:

- 30 days - to the copyholder (PNL and Non-PNL)
- 45 days - to the PNNL copyholder's Manager

4.3.2 If the Coordinator does not receive a response within 10 working days after the 45 day notice is sent, the PNNL copyholder's manual or procedure shall be recalled.

The Coordinator shall notify the PNNL copyholder's Manager of this action. Non-PNL copyholders, who have not responded to the 45 day request, will be sent notification stating that they have been removed from the distribution list.

5.0 DATA ANALYSIS AND CALCULATIONS

NA

6.0 QUALITY CONTROL

NA

7.0 SAFETY

NA

8.0 TRAINING REQUIREMENTS

The Coordinator shall read this procedure and document the training in accordance with MSL-A-006.

Copyholders for controlled documents shall be informed of their responsibilities via the Transmittal / Receipt acknowledgement. .

9.0 REFERENCES

None

ATTACHMENT 1

Controlled Document Transmittal/Receipt (CDT/R)		Page ___ of ___
Distribution Authority Signature	Transmittal Date	Transmittal No.
From QA Representative Battelle Marine Sciences Laboratory 1529 West Sequim Bay Road Sequim, WA 98382	To	

Document No.	Document Title

Receipt Acknowledgment:

I am verifying my receipt of the listed document(s), and that I have incorporated the transmitted document(s) in accordance with the instructions. **Please return the signed CDT/R within 15 working days to QA Representative, Battelle Marine Sciences Laboratory, 1529 West Sequim Bay Road, Sequim, WA 98382.**

Signature _____ Date _____

ATTACHMENT 2

QUALITY ASSURANCE MANUAL

CONTENTS

	Issue Date
MESSAGE FROM THE DIRECTOR	
INTRODUCTION	08/31/94
1. ORGANIZATION	08/31/94
1.1 Organization	08/31/94
2. QUALITY ASSURANCE PROGRAM	08/31/94
2.1 Quality Assurance Program	08/31/94
Appendix I, PNL-MA-70 Procedures	08/31/94
Appendix II, Project QA Planning Process	08/31/94
Appendix III, Quality Assurance Documents	08/31/94
2.2 Training	08/31/94
3. DESIGN CONTROL	08/31/94
3.1 Design Control	08/31/94
3.2 Computer Software	08/31/94
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4.1 Procurement Document Control	08/31/94
4.2 Work Package Control	08/31/94
NSTRUCTIONS, PROCEDURES, AND DRAWINGS	08/31/94
5.1 Instructions, Procedures, and Drawings	08/31/94
6 DOCUMENT CONTROL	08/31/94
6.1 Document Control	08/31/94
7 CONTROL OF PURCHASED ITEMS AND SERVICES	08/31/94
7.1 Control of Purchased Items and Services	08/31/94
7.2 Control of Work Package Items and Services	08/31/94
8 IDENTIFICATION AND CONTROL OF ITEMS	08/31/94
8.1 Identification and Control of Items	08/31/94
9 CONTROL OF PROCESSES 08/31/94	

CONTROLLED DOCUMENT LIST
PNL-MA-70 PROCEDURES

January 19, 1996

DOCUMENT NUMBER	REV NUM	NO. OF ICNS ISSUED	TITLE	EFFECTIVE DATE
**CAP				
CAP-70-401	1	0	PREPARATION OF REQUEST FOR PROPOSALS AND AWARD OF CONTRACTS/AGREEMENTS	08/31/94
CAP-70-701	1	0	PROPOSAL EVALUATION, SUPPLIER/ SUBCONTRACTOR SELECTION, AND CONTRACT/AGREEMENT ADMINISTRATION (POST AWARD)	08/31/94
**PAP				
PAP-70-101	1	0	COMMUNICATION AND COMMITMENT (INTERFACE) CONTROL	08/31/94
PAP-70-201	3	0	INDOCTRINATION AND TRAINING	08/31/94
PAP-70-203	3	0	QUALIFICATION AND CERTIFICATION OF INSPECTION AND TEST PERSONNEL	08/31/94
PAP-70-205	3	0	QUALITY ASSURANCE PLANS	08/31/94
PAP-70-206	1	1	CONTROLLING WORK RECEIVED FROM HANFORD CONTRACTORS	08/31/94
PAP-70-207	0	0	QUALITY PROGRAM REQUIREMENTS FOR SAFETY CLASS SYSTEMS, STRUCTURES, AND COMPONENTS	08/31/94
PAP-70-208	3	0	IMPACT LEVELS	08/31/94
PAP-70-301	1	0	HAND CALCULATIONS, GENERAL	08/31/94
PAP-70-302	3	0	ASSURANCE AND CONTROL OF ENGINEERING DESIGN	08/31/94
PAP-70-401	2	1	PURCHASE REQUISITIONS	08/31/94
PAP-70-402	1	0	CONTROL OF SUSPECT/COUNTERFEIT ITEMS	08/31/94
PAP-70-404	2	0	OBTAINING SERVICES	08/31/94
PAP-70-501	3	0	PREPARATION AND APPROVAL OF ADMINISTRATIVE PROCEDURES	08/31/94
PAP-70-601	3	0	DOCUMENT CONTROL	08/31/94

Battelle
Putting Technology To Work

EFFECTIVE DATE: 11/08/99

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-A-012-00

PROCUREMENT

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	11-02-99
	Signature	Date
TECHNICAL REVIEWER: Jan H. Slater	Jan H. Slater	11-2-99
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	11-02-99
	Signature	Date
TECHNICAL GROUP MANAGER: Richard M. Ecker	RM Ecker	11/8/99
	Signature	Date

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PROCUREMENT

1.0 SCOPE AND APPLICATION

This procedure describes guidelines for the specification of quality requirements or the level or acceptance criteria for procured items (materials and supplies), or services which have significant influence on the quality of work (chemical analyses and toxicological and other testing) conducted at the Battelle Marine Sciences Laboratory (MSL). Upon receipt, purchased materials must be inspected for conformance to quality requirements prior to use. Services must be evaluated to ensure that the services rendered meet the requirements and that defined products were received as specified.

2.0 DEFINITIONS

P-Card. A MasterCard credit card used to make Battelle MSL purchases from sources. P-cards are used for:

- Goods valued at $5,000 or less per transaction.
- Services valued at $2,500 or less per transaction. However, services performed on-site are prohibited, and
- Software valued at $2,500 or less per transaction.
-

3.0 RESPONSIBLE STAFF

- Individual initiating procurement action
- Finance and Business Administration staff
- Quality Assurance Officer or representative
- Sample Inventory Coordinator

4.0 PROCEDURE

4.1 Procurement Options

Items may be obtained by two approaches; use of a purchase requisition (PR) or use of a P-Card. A P-card is a credit card accepted by local suppliers in the same way a credit card is used. The MSL staff member holding the P-Card is responsible for monthly reconciliation of P-Card transactions and for maintaining the receipts. P-cards cannot generally be used for the procurement of services. Services that can be obtained using a P-card are restricted to Battelle suppliers appearing on a list of approved suppliers. Approved suppliers have qualified for the list by meeting certain requirements such as a pre-award audit and an evaluation of business operation practices and systems. P-card procurements are not subjected to a signature approval process. This fact requires that the P-Card

user be responsible for determining *prior to the purchase* if the item will have a significant influence on the quality of work and therefore may need defined quality requirements. The MSL Quality Assurance Officer or formally-designated representative for procurement will provide assistance in making this determination.

4.1.1 PR Procurements

A PR is initiated by completing a PR form which has sections of the form that assess:

- if QA requirements are necessary (Y or N box)
- if a QA Clause (refer to Section below) is provided
- identify the QA Representative involved in the determination of the need for QA requirements (requests a telephone number and date)
- and specifies who will be responsible for ensuring the item is inspected upon receipt for conformance with specifications and QA requirements (Author, QC or other)

4.1.2 P-Card Procurements

There is no internal control similar to the PR form controls mentioned above that is encountered by P-Card users. Therefore, P-card users assume responsibility for:

- determining if there are any applicable QA requirements, and what those requirements might be.
- P-Card users also assume a liability when using the P-card for quality-affecting purchases without ensuring that any applicable QA requirements have been specified, and users should be aware of this fact.

All P-Card users are advised to seek guidance from the QA Representative *prior to the purchase* to help determine the need for QA requirements and to assist in defining what requirements apply. It is difficult or impossible to obtain required information or meet specified requirements after the fact.

4.2 Procurement of Items

Examples of items that generally have a significant influence on the quality of MSL work, and therefore generally need defined quality requirements are the following:

- Standards and reference materials
- Reagents, chemicals and solutions
- Animals and feed
- Computer software and hardware, and
- Some miscellaneous items such as designed equipment

Items must be evaluated at the time of delivery to ensure that the item meets the purchase requirements and specifications. When items do not meet the specifications, delivery of the item should be refused or a quality problem report initiated and the process for resolving quality problems invoked (MSL-A-005, Deviations from Established Requirements).

4.2.1 Standards and Reference Materials

Standards and reference materials must be traceable to the National Institute of Standards and Technology (NIST; formerly the National Bureau of Standards or NBS) or other nationally-recognized standard (e.g., American Society for Testing Materials or ASTM). The traceability must be documented by a certificate or label that verifies this traceability. The certificate is often required to be copied and submitted as part of project records and the original should remain on file and available for review by auditors and to demonstrate traceability for the range of uses of the item.

4.2.2 Reagents, Chemicals and Solutions

The procurement of reagents, chemicals and solution should include requirements for shipping stocked inventory materials with the longest period to the expiration date (i.e., the freshest material) possible, with lot numbers specified. In some cases where extremely high purity material is requested, a request for purity documentation may be necessary.

Procurement procedures should require that a manufacturer's recommended expiration date be provided with every standard material. If manufacturer's expiration dates are not provided, the laboratory must assign an appropriate expiration date, based on professional judgement and in consideration of the shelf life for similar materials at similar concentrations. The technical basis for each such determination must be documented in the project file by the responsible analyst, and approved by the Project Manager.

MSL follows the Pacific Northwest National Laboratory's (PNNL's) Standards-Based Management System (SBMS) requirements for logging in reagents, chemicals and solutions into the associated Chemical Management System (CMS). This system provides the PNNL Laboratory with policies and procedures regarding tracking and inventory, storage, and disposal of completed samples and analytical wastes as well as chemical use and disposal. The CMS is used to provide an up-to-date inventory to facilitate emergency response, monitor the location of various classes of materials and identify situations where acceptable limits for the building/facility determined by the assigned chemical hazard group and fire zone might be exceeded before a violation occurs. An assigned Sample

Inventory Coordinator provides bar codes for each chemical item when it is received and assigns it to a location. The item then is tracked in the CMS until disposal. The system is also used to ensure that facility limits based on the chemical hazard group and the fire zone determination are not exceeded.

4.2.3 Animals and Feed

The procurement of animals and feed for bioassays should include requirements for chain of custody of animals during shipping and documentation of any available feed analyses, feed storage recommendations, and expiration dates so that feed quality can be monitored. Animal shippers should be requested to document conditions of animals and environmental parameters (temperature) at the time of shipping for comparison with conditions encountered at the time of receipt. In some cases, it might be important to include QA requirements for a minimum/maximum thermometer or temperature strip in the cooler at the time of shipping. Requirements regarding common carriers, Saturday delivery acceptability and locations, and other details might also be specified in QA requirements documents.

4.2.4 Computer Software and Hardware

QA requirements for the procurement of hardware must ensure that hardware is Y2K compliant (YR2000 and 2001) and compliant for other periods where clock or time information settings provided by the manufacturer might affect future hardware operation. QA requirements for the procurement of software should follow some general guidelines:

- Commercial software that has been developed under the manufacturer's QA Program and fully tested before release is preferable to other types of software developed under lesser or no QA Program
- Documents necessary to demonstrate that software was developed using a Life Cycle approach such as User's Manuals shall be requested when software is ordered.
- Licenses that come with the software and original documentation should be requested, obtained and protected.
- Software that requires a signed site license agreement can only be purchased by individuals with appropriate delegations.
- Hardware/Software that exceeds $5,000 can only be purchased with appropriate management approvals.
- Software procured as a product under a subcontract must specify detailed QA requirements for software development and use, and provide plans for testing, verification and validation tests and include acceptance criteria.

4.2.5 Miscellaneous Procurements

Miscellaneous procurements of items that have a significant influence on the quality of MSL work, generally need defined quality requirements. When the purchaser does not know if quality requirements should be specified, the rule is to request the MSL Quality Assurance Officer or representative to make this determination and document it as a note, letter or email.

4.3 **Procurement of Services**

The procurement of research-related or testing services requires a formal Statement of Work. The MSL Quality Assurance Officer or representative shall be requested to make a determination of whether or not QA requirements are needed and if needed, develop a QA Clause to insert into the Statement of Work. Requests for analytical support services always require a QA Clause to specify analysis methods, detection limits, reporting units, holding time specifications, QC sample requirements (QC sample type, frequency, and control limits), data package requirements and turn around times. Services obtained must be evaluated at the conclusion of the period of performance to ensure that the services obtained meet QA specifications. When the products of a service do not meet the specifications, the product should not be rated as "acceptable". A quality problem report is initiated and the process for resolving quality problems invoked (MSL-A-005, Deviations from Established Requirements).

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

Periodic surveillance should be conducted by the MSL's Quality Assurance Officer or Representative to ensure that procurement activities follow processes that result in the determination of necessary quality requirements, the specification of these requirements and ensuring that suppliers receive and comply with requirements. Results of periodic surveillances may become a part of the project files.

7.0 SAFETY

Not applicable to this procedure.

8.0 TRAINING REQUIREMENTS

Personnel who will be procuring items and services must be trained on this procedure

and the training documented as per MSL-A-006, Marine Sciences Laboratory Training. P-Card users must receive P-Card training before obtaining the card.

9.0 REFERENCES

MSL-A-005 Deviations from Established Requirements
MSL-A-006 Marine Sciences Laboratory Training

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Marine Sciences Laboratory

EFFECTIVE DATE: 5-11-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-A-014-00

SAMPLE CONTAINER REQUESTS

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	5/11/00
	Signature	Date
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	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	5/11/00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric Crecelius	5-11-00
	Signature	Date

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SAMPLE CONTAINER REQUESTS

1.0 SCOPE AND APPLICATION

This procedure defines the approach for fulfilling Battelle Marine Science Laboratory (MSL) customer sample container requests and for establishing container traceability in the case of container contamination. Laboratory procedures for preparing, supplying and tracking sampling containers, preservatives, and reagent water for field use are addressed.

2.0 DEFINITIONS

Field Blank	A field blank is a sample of analyte-free water transferred, at the project site, into an appropriate container for the purpose of distinguishing contamination from sample collection and handling procedures.
Temperature Blank	A temperature blank is a sample container filled with tap or deionized water which is shipped with the samples in the cooler and used upon arrival to determine cooler temperature.
Trip Blank	A trip blank is used to identify the presence of contamination attributable to transfer into a sample container during shipping and storage of samples. A trip blank is a sample of an analyte-free matrix that is transported from the laboratory to the sampling site with the empty sample containers. The trip blank is stored on-site with the sample containers and field samples and then transported back to the laboratory with the samples for analysis. The trip blank is received and processed as a sample by the laboratory.

3.0 RESPONSIBLE STAFF

Marine Sciences Laboratory (MSL) Staff as Sample Custodian or as Sample Recipient or MSL Contact
Project Manager or Task Leader
MSL Manager
MSL Quality Assurance Officer or Representative

4.0 PROCEDURE

4.1 Receiving the Request

4.1.1 Project Manager Point-of-Contact

MSL customers contact MSL Project Managers to request sample containers. Project Mangers work with the first-time and repeat customers to define sample matrices and analytes of interest, determine numbers of containers per analytes, and minimum sample volumes, discuss appropriate sample preservation requirements (refer to Table 1 in MSL-A-001, Sample Log-I n-Procedure) and determine when and where samples should be sent. Discussions regarding what information should be included when sample labels are filled out, and if sample labels are to be affixed to the appropriate sample containers before or at the time of sample collection are also needed to determine what needs to be shipped. Customers are advised that information on the sample labels may include, but not be limited to, a code number identifying the sample, date, time, and location of sample collection, and name of sample collector. Some sample collection efforts may require global positioning system accuracy for sample collection location information.

The Project Manager may also discuss sample chain of custody (CoC) concepts and the use of field sample chain of custody forms (Attachment 1) with the customer. Often when MSL supplies bottles, blank CoC forms are also provided.

In addition, the Project Manager may advise on sample shipping carriers, time of day to ship, and how to load and pack coolers for sample shipping. Bottles are sent in a cooler so that the sample cooler can be returned with the samples.

4.1.2 Bottle Request Documentation

Once the bottle request has been determined, the MSL Project Manager completes a bottle request form. An example request form is provided as Attachment 2. An alternative to using the form is to write an email to the person providing samples (Prep Lab or Mercury Lab Manager) documenting the request. The request defines the type, size, number, purpose, date needed, and any comments. The shipping address is also specified.

4.2 MSL Policies

MSL prefers that samples be collected and immediately shipped. Preservation in the field is discouraged for Hg and methylHg samples due to the high probability of contamination. Metals contamination is also a concern, but customer preference on this issue is used when defining bottle requests. In general, MSL does not ship

sample bottles containing acids. The reasons are that 1) the bottles may open during shipping, contaminating the cooler contents, and 2) shipping requirements for acids include labeling and hazardous specifications.

MSL recommends including both a temperature blank and a field bank and/or trip blank in the sample set. However, customer preference on including these blanks is observed. Some customers prefer to submit blind field and/or trip blanks.

MSL recommends that samples be shipped by Federal Express because of the electronic traceability of the shipment and positive past experiences in receiving next-day shipments.

MSL staff will accept Saturday deliveries if they are 1) scheduled ahead of time, and 2) delivered to a residence because site access is limited to common carriers on weekends.

4.3 Fulfilling the Request

Requests for sample bottles are forwarded to either or both the Prep Lab or Mercury Lab Managers. Bottles are packed in coolers and may include Blue Ice packets.

4.4 Documentation Requirements

Because it is not MSL's policy to ship acid-preserved sample bottles, lot numbers of acids are not routinely tracked. In a rare case of shipping bottles with acid preservatives, the lot number of the acid used and the acid type will be recorded on the sample request

Sample requests are initialed and dated when completed, and submitted to the Project Manager for inclusion in the project file. Since all bottle request coolers are sent by Fed Ex, Project Managers track sample shipments by the Fed Ex tracking numbers. The MSL computer network svstem allows access to Fed Ex tracking systems. Fed Ex shipping documentation is maintained by Project Managers.

5.0 DATA ANALYSIS AND CALCULATIONS

There are no calculations applicable to this procedure.

6.0 QUALITY CONTROL

It is the responsibility of each individual collecting field samples to ensure that Chain-of-Custody Forms are filled out accurately and completely for each transaction, and

that the forms are submitted with the returned sample bottles.

7.0 SAFETY

Not applicable.

8.0 TRAINING REQUIREMENTS

All staff responsible for initiating or filling requests for sample containers shall first read this procedure and document the training as a completed reading assignment on an Individual Training Assignment Form or a Group Training Documentation Form as described in MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-001	Sample Log-In Procedure
MSL-A-006	Marine Sciences Laboratory Training

Attachment 1

Battelle Marine Sciences Laboratory 1529 W. Sequim Bay Rd. Sequim, WA 98382	EXAMPLE FIELD SAMPLING CHAIN OF CUSTODY	Page ____ of ____

Shipped To:

Method of Shipment:
Shipped From: By:

Company:
Telephone:
Address:

SPECIAL INSTRUCTIONS:

Container No.: _____
Sampling Location: _____
Samples Collected By: Date: _____
Remarks:

_____
_____

SAMPLE IDENTIFCATION	

Relinquished by	Date/Time	Received by	Date/Time
Relinquished by	Date/Time	Received by	Date/Time
Relinquished by	Date/Time	Received by	Date/Time

ATTACHMENT 2

EXAMPLE

SAMPLE CONTAINER REQUEST FORM

Battelle
Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

MATERIALS REQUEST					
Project Manager					
For:			phone #:		
Project Name:					
Project #:					
CF #:					
WP#:					
Dollar amount to be charged to WP#:					
TYPE	SIZE	NUMBER	PURPOSE	DATE NEEDED	**COMMENTS**
CUSTOMER:					
Ship to:					
Lisa Lefkovitz					
Battelle-Duxbury Operations					
397 Washington St.					
Duxbury, MA 02332					
Phone #:					

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Marine Sciences Laboratory

EFFECTIVE DATE: 4-24-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-C-003-02

PERCENT DRY WEIGHT AND HOMOGENIZING DRY SEDIMENT, SOIL, AND TISSUE

Approvals:		
AUTHOR: Linda Bingler	Linda S. Bingler	April 24, 2000
	Signature	Date
TECHNICAL REVIEWER: Brenda Lasorsa	Brenda Lasorsa	4/24/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	4/24/00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric Crecelius	4-24-00
	Signature	Date

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PERCENT DRY WEIGHT AND HOMOGENIZING DRY SEDIMENT, SOIL, AND TISSUE

1.0 SCOPE AND APPLICATION

This method is applicable to the determination of percent dry weight and homogenization of dry sediment, soil and tissue samples.

2.0 DEFINITIONS

Desiccator - A sealed environment containing an adsorbing compound (such as silica gel) in which samples are placed while cooling to prevent adsorption of moisture.

3.0 RESPONSIBLE STAFF

- Trained technician-level or higher laboratory personnel.
- QA Officer or Representative- data verification

4.0 PROCEDURE

4.1 Equipment

- Spex jars (125 ml) and Spex balls acid cleaned in accordance with MSL-C-011, Glassware and Equipment Cleaning Procedures.
- Aluminum weigh boats.
- Freeze dryer equipped with a vacuum, capable of maintaining temperatures of –50°C.
- Ultra low freezer, capable of maintaining temperatures near –68°C.
- Analytical balance capable of weighing to 0.001 g.
- Spex Ball Miller/Shaker.
- Oven Maintained at 110°C.

4.2 Percent Dry Weight Determination

Two methods are available to determine percent dry weight.

4.2.1 Freeze-Dried samples

Weigh an appropriately-cleaned and labeled Spex jar and record the tare weight of the jar to the nearest 0.001g on the label under tare. Place 5-20 g of wet soil, sediment, or tissue into the tared jar. Weigh the jar containing the

sample and record the weight on the label as the tare + wet weight. Place the samples in a –68°C freezer for at least 2 hours or until completely frozen. Once frozen, put the samples in the freeze drier with the caps off, but make sure the jars can be associated with their respective caps. Record the project number, matrix, date, time and your initials in the Freezer Logbook (Attachment 1). Allow samples to dry; usually 2-4 days, depending on the sample. Samples are dry when they do not feel cold to the touch. Remove samples, cap, and weigh, recording weight on the label as tare + dry.

NOTE: Make sure the caps and jars are correctly matched to ensure correct weights.

Calculate percent dry weight using a spreadsheet (see Attachment 2 for an example).

4.2.2 Oven-Dried Samples

Weigh empty aluminum boats and record tare weight (see Attachment 2 for an example form). Place 5-20 g of wet soil, sediment, or tissue into the tared boat. Weigh the boat and record as tare + wet. Place samples into a 110°C oven and dry at least 8 hours or overnight. Remove the samples, place in a desiccator, and allow to reach room temperature. Weigh and record weight as tare + dry.

4.2.3 Interferences

Soil, sediment and tissue water content varies from sample to sample. Therefore, the length of time required for complete drying varies from sample to sample. Analysts should take appropriate steps to ensure samples are completely dry before final weight is determined.

4.3 Homogenizing Dry Samples (Ball Milling)

4.3.1 If samples are going to be analyzed for metals, after freeze drying, add 2-3 acid-cleaned Spex balls to each sample.

NOTE: Sediments with rocks or gravel need to be ball milled in a Spex ceramic jar with ceramic balls. Place a small amount of sediment in the ceramic jar and add the ceramic ball.

4.3.2 Place the Spex jar or ceramic ball mill on the shaker and shake for 2-15 minutes.

4.3.3 Clean the ceramic Spex mill between each sample by adding approximately 2 grams of clean silica sand, then placing the ball mill on the shaker for 2-5 minutes. Wipe the interior of the mill with a clean Kimwipe to remove any

remaining dust or particles.

4.3.4 Samples ball-milled in Spex jars do not require a cross-contamination blank because each sample is isolated from the other samples during the homogenization process. There is no route for cross-contamination.

4.3.5 Samples ball-milled in the ceramic ball mill must have one cross-contamination sample analyzed per batch (a batch consists of all samples of one matrix type ball-milled for one project). After cleaning the ball mill with silica sand, add approximately 2 grams of standard reference material (SRM) appropriate to the project matrix and ball mill for 2-15 minutes. Place the homogenized SRM into a clean Spex jar and digest and analyze with the samples.

Compare SRM and cross-contamination sample results with the certified mean and range values published for the SRM. Cross contamination in this process is not expected, and the results obtained should verify this assumption. However, cross contamination is indicated when the results vary from each other by greater than 25%. There is no effective corrective action to implement at this point because the samples are consumed by the process. The fact that cross contamination is indicated when the percent difference results are greater than or equal to 25% must be noted and conveyed to the customer in the data report.

5.0 DATA ANALYSIS AND CALCULATIONS

$$\frac{\text{Tare+Dry weight - tare weight}}{\text{Tare+Wet weight - tare weight}} \times 100 = \%\text{Dry Weight}$$

6.0 QUALITY CONTROL

If required by a project, replicate analyses may be performed. The number of replicates per batch or sample set are defined by the customer. Acceptance criteria for duplicate or replicate samples shall be ± 20% relative percent difference (RPD) or relative standard deviation (RSD) respectively, unless otherwise specified by client requirements.

7.0 SAFETY

Laboratory personnel should have access to data on potential risks of working with contaminated sediment and various hazardous substances, and procedures for minimizing accidents with these materials. Appropriate attire, including lab coats, goggles or safety glasses, and protective gloves should be worn when working in the vicinity of samples.

8.0 TRAINING REQUIREMENTS

All staff members who will be performing dry weight determinations shall first read this procedure prior to beginning work. Documentation of training shall be recorded on a training assignment form from procedure, MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006	Marine Sciences Laboratory Training
MSL-C-011	Glassware and Equipment Cleaning Procedures

Attachment 1
EXAMPLE FREEZE DRY LOG

CF No.	Number of Samples/ Description	Matrix	Dryer-1 (left) [check one]	Dryer-2 (right) [check one]	Date IN/ Time IN	Initials	Date OUT/ Time OUT	Initials

Attachment 2
Example Sample Preparation
for Percent Dry Weight Determination Form

Project: _____ Percent Dry Weight

Central File #: _____ Date: _____

Analyst: _____ Matrix: _____

Sample Number	Tare Mass (g)	Tare + Wet Mass (g)	Tare + Dry Mass (g)	Wet Mass (g)	Dry Mass (g)	% Dry Mass	% Wet Mass

Analyst: _____ Peer Reviewer: _____

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Marine Sciences Laboratory

EFFECTIVE DATE: 2-22-01

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-C-009-00

USE AND PERFORMANCE CHECKS OF BALANCES

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	2-19-01
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	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	2-19-01
	Signature	Date
TECHNICAL GROUP MANAGER: Dick Ecker	RM Ecker	2/22/01
	Signature	Date

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USE AND PERFORMANCE CHECKS OF BALANCES

1.0 APPLICATION AND SCOPE

This procedure describes the proper use of balances, the method for weighing, and the procedures for calibration verification performance checks of balances and maintenance.

Balances must be uniquely identified by a serial number (SN) and/or a balance number and the calibration and maintenance history of each balance tracked in a logbook.

Balances are calibrated in place, and therefore, should not be routinely moved. There are, of course, situations when a balance needs to be moved and the internal calibration routine should be used to ensure that the balance is operating properly. In addition, top-loading balances will require calibration verification using Class "S" weights before use when moved. Analytical balances require metrology calibration verification when moved, and this requirement *limits casual relocation* of these balances for routine tasks. Refer to the steps in Section 4 below.

2.0 DEFINITIONS

Calibration - periodic and documented comparison to known standards to determine the accuracy of M&TE (to determine as-found condition and to adjust the equipment or to provide a calibration curve). Balances are calibrated only by a Process Quality evaluated metrology facility.

Metrology -Metrology is the science that deals with measurement.

Performance Check - a check between calibration intervals to provide verification of balance accuracy. Performance checks do not take the place of calibration.

3.0 RESPONSIBLE STAFF

- Staff Members
- Wet Laboratory Manager
- Metrology facility staff
- QA Officer or Representative

4.0 PERFORMANCE CHECK PROCEDURE

MSL balances are calibrated on a semi-annual basis by an external metrology facility. The calibrated balance has a calibration sticker noting the current calibration interval and the date the calibration interval expires.

A performance check should be performed daily or each time the balance is used, depending on the program requirements,[1] using a certified set of calibrated weights. **Each balance should be checked at least daily, and preferably with each use (GLP programs require a with-each-use frequency check) by using at least two weights that bracket the expected weight range.** Balance calibration requirements are addressed in section 4.4.

4.1 Top Loading Balances

Top loading balances may be used to measure materials requiring two or three decimal points of accuracy, as specified in project plans.

The balance should be located on a stable surface away from drafts and vibration from any other source. If necessary, level the balance using the leveling bubble while adjusting the front legs.

4.1.1 If necessary, turn the balance on and allow to warm up to manufactures specifications. The required warm up time may vary for different balances, so consult the manufacturer's operations manual.

4.1.2 Ensure that the balance is within a valid calibration interval. Record the interval expiration date to document this step.

4.1.3 Allow the balance to go through the internal calibration check routine if the balance is just being powered up. If the balance is already on, initiate the internal calibration routine before use. Ensure that the internal calibration is acceptable before use.

4.1.4 Clean (e.g. with Kimwipe or brush) the balance pan and platform. Dry if necessary. Clean the balance after use so that the next user does not have to perform this step.

4.1.5 Zero the balance by pressing down on the tare bar. Do not lean on the counter while calibrating or weighing.

4.1.6 Obtain a calibration verification weight set and verify that it is within the 3-year calibration interval. With no load on the clean platform, and the display reading "0", select a Class "S" weight (below the expected weight of the material(s) to be measured) to begin the performance check.

Note: Never touch the Class "S" weights without using forceps or cotton gloves. Oils from the skin can corrode the weights, and change their certified weight value.

[1] GLP-level studies require documented performance checks with every balance use. Routine laboratory operations require minimum daily performance checks documented in a balance calibration logbook, so that all users are aware whether or not the daily calibration has been performed or needs to be performed.

4.1.7 Using clean forceps or cotton gloves, place the Class "S" weight on the platform. Record the weight results in the balance calibration and maintenance logbook. The data sheet should also require documentation of the balance used, and the calibration verification steps. Repeat this step using Class "S" weights above the expected weight as the material(s) to be weighed. Bracketing the quantity you plan to measure is adequate, but if various weight measurements will be used in a given day, then the balance may be checked using the certified weights in the range of the various measurements.

4.1.8 If the display varies more than the acceptable range criterion from the expected standard weight, check balance level and initiate the internal calibration. If the percent difference between the expected and observed reading is still greater than 1%, then the balance needs to be professionally serviced before use. Consult the laboratory manager or designee for assistance. Contact the MSL Quality Assurance Officer or Representative to initiate the services of the qualified metrology facility responsible for the service and calibration of the balances if necessary. The acceptable criterion shall be noted on the form or logbook, and will either be ±1% or otherwise determined by the program manager. The following equation is used to determine percent difference:

$$[(W_e - W_o)/W_e] * 100$$

where:

W_e = Certified Class "S" weight (expected weight)
W_o = weight determined by operator while checking performance (observed weight).

4.2 Analytical Balances

Analytical balances are used for determining more precise measurements, usually to four decimal places. Calibration of the balance (by a professional) is required whenever the balance is relocated, or when location conditions change.

4.2.1 The balance should be located on a stable surface away from drafts, vibrations and direct sunlight. If necessary, level the balance using the leveling bubble while adjusting the front legs.

4.2.2 While in the OFF position, if needed, remove the balance pan and clean with a Kimwipe, brush and deionized water. Dry, if necessary, using a Kimwipe or other soft drying paper. Ensure that the platform area is clean and dry.

4.2.3 If necessary turn the balance on and allow to warm up to manufactures specifications. This may vary so consult manufactures operations manual.

4.2.4 Press the tare button and the instrument will automatically re-zero itself. Make sure that the display shows "0".

4.2.5 Obtain calibration verification weight set and verify that it is within the 3-year calibration interval. With no load on the clean platform and the display reading

"0", select a Class "S" weight (below the expected weight of the material(s) to be measured) to begin the performance check.

4.2.6 Using clean forceps or cotton gloves, place the Class "S" weight on the platform. Record the weight results on the Balance Performance Check Form (Attachment 1) or on a balance maintenance logbook. If a balance maintenance logbook is used, the information contained in Attachment 1 needs to be recorded in the logbook. Repeat this step using Class "S" weights above the expected weight as the material(s) to be weighed. Bracketing the quantity you plan to measure is adequate, but if various weight measurements will be used in a given day, then the balance may be checked by the certified weights in the range of the various measurements.

4.2.7 If the display varies more than the acceptable range criterion from the expected standard weight, check balance level and initiate the internal calibration. If the percent difference between the expected and observed reading is still greater than 1%, then the balance needs to be professionally serviced before use. Consult the laboratory manager or designee for assistance. Contact the MSL Quality Assurance Officer or Representative to initiate the services of the qualified metrology facility responsible for the service and calibration of the balances if necessary. The acceptable criterion shall be noted on the form or logbook and will either be ±1% or otherwise determined by the program manager. The calculation in step 4.1.8 may be used to determine if the balance meets the calibration criterion.

4.3 Use of Balances

Qualified operators are responsible for checking the balance calibration prior to use. Routine maintenance of balances includes cleaning the balance-weighing chamber.

4.3.1 Materials to be weighed will be placed in an appropriate weighing vessel (e.g., plastic weigh boat,weigh papers, etc.).

4.3.2 The balance case doors will be closed during weighing to avoid drafts while the balance is equilibrating.

4.3.3 When using the analytical balance, materials should be added outside the balance case to avoid spillage onto the balance. The operator should tare the balance and weighing vessel, remove the weighing vessel, and place it on the bench. Place the weighing vessel and added material back on the balance pan for measurement.

4.3.4 If possible, try to avoid weighing very hot or very cold material because these can cause troublesome convection currents or the material itself can soak up water from the air, changing the measure. If it is necessary to weight hot or cold material, care should be taken to minimize drifting of weight readings.

4.3.5 Any balances that are dirty after use, will be cleaned. The pan of the analytical balance may be removed by lifting up. It can be cleaned with soap and warm

water. Dry thoroughly before replacing. Wipe top of balance and bench area with damp cloth or paper towel. For top loading balances, the surface will be cleaned by brushing off any debris left behind. Any liquid left on the balance after use will be removed with tissue or a soft cloth, and the instrument will be wiped dry.

4.4 Balance Calibration

Balances are calibrated by a Process Quality-approved metrology facility. The MSL QA Officer or Representative is responsible for scheduling and obtaining balance calibrations. Balances will be calibrated on an annual or semi-annual basis.

The QA Officer maintains a log that includes, but is not limited to, the following: balance manufacturer, model and serial number; calibration interval; and balance location. Inform the QA officer whenever balances are relocated or excessed, and when new balances are obtained so that the list is current.

Balance calibration reports supplied by the metrology facility will be maintained in the QA Office files.

5.0 DATA ANALYSIS AND CALCULATIONS

Percent difference between an expected Class "S" standard weight and the actual observed balance measurement is calculated as follows:

$$[(W_e - W_o)/W_e] * 100$$

where:

W_e = Certified Class "S" weight
W_o = weight determined by operator while checking performance.

6.0 QUALITY CONTROL

If the balance does not meet the 1% difference performance check criterion, then it needs to be professionally serviced before use.

7.0 SAFETY

Balances are very sensitive and should be handled carefully if moved. Operation of the balances creates no special safety hazards. However, care should be taken when weighing hazardous or potentially hazardous samples.

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all MSL staff performing the steps described in this procedure are properly trained and that documentation of the training exists prior to the performance of those procedures.

The trainee must read this procedure in its entirety. An experienced operator will then demonstrate performance checks, operation, data recording, and routine maintenance procedures to the trainee. Once proficiency has been verified, documentation of training will be recorded on a training assignment form obtained from procedure MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

ATTACHMENT 1

BATTELLE MARINE SCIENCES LABORATORY
Example Balance Performance Logbook Entry Form

Mass Balance:_____

Calib. Date:_____

Calibration acceptance criterion 1.0 % difference

Date	Calibration weight interval expiration date	Class "S" weight (g) (expected weight)	Measured or observed weight (g)	% Difference $\frac{W_e - W_o}{W_e} * 100$	Acceptable? Y or N	If N, corrective action or date out of service

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Marine Sciences Laboratory

EFFECTIVE DATE: 2-22-01

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-C-010-01

CALIBRATION AND USE OF PIPETTES

Approvals:		
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	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E A Crecelius	2-22-01
	Signature	Date

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CALIBRATION AND USE OF PIPETTES

1.0 APPLICATION AND SCOPE

This procedure describes how to calibrate and use several different types of laboratory pipettes used at the Battelle Marine Sciences Laboratory (MSL). Pipettes are designed for the transfer of known volumes of liquid from one container to another. There are three types of pipettes discussed in this procedure: volumetric, measuring, and the mechanical micropipette. It is important to define the necessary accuracy of the measure when choosing a pipette (refer to the definition section below). This procedure replaces MSL-M-050.

NOTE: Pipetting by mouth is not allowed. Always use a pipette bulb.

2.0 DEFINITIONS

2.1 Measuring Pipettes

Measuring pipettes are graduated in convenient units so that any volume can be delivered up to its maximum capacity. Measuring pipetttes are the least accurate of the three pipette types discussed here. Measuring pipettes are useful for measuring nominal concentrations of solutions used in making reagents.

2.2 Volumetric or Transfer Pipettes

Volumetric or transfer pipettes deliver a fixed volume. Volumetric pipettes are divided into two groups depending upon the method by which the volume in the pipette is determined:

"TC" (to contain): Volumetric pipettes are calibrated to contain a specified volume. These are sometimes referred to as "blow-out" pipettes.

"TD" (to deliver): Volumetric pipettes are calibrated to deliver a specified volume. These pipettes will have a small volume remaining in the pipette tip when the proper volume of liquid has been delivered.

Both types of volumetric pipettes are calibrated with water and must be re-calibrated for other solutions if extremely accurate work is to be performed.

2.2.1 Class "A" volumetric pipettes are calibrated to strict tolerances and are used for preparing standard solutions used for calibration and for precise measurements. The volume of Class "A" glass pipettes should be verified gravimetrically prior to use when preparing calibration standards or used for other accurate work.

2.2.2 Class "B" volumetric pipettes are calibrated to deliver at larger tolerances than Class "A" pipettes and should not be used when a precise measure is needed.

2.3 Mechanical Micropipette

The mechanical micropipette is similar to the volumetric pipette, in that it is designed to deliver a fixed volume of liquid in a single operation (although this volume is adjustable on some types of mechanical micropipettes). This style of pipette is a mechanical device with a plunger assembly that draws the liquid up into a disposable tip, and then delivers the appropriate volume when the plunger is depressed. Cleaning and use of this type of pipette should be done in accordance with the manufacturer's instructions. The calibration schedule for of this type of pipette is discussed in Section 4.3.1 of this procedure.

3.0 RESPONSIBLE STAFF

- Researcher/Technician for preparation of standards, reagents, solutions, test articles, specimens, or for sample preparation and analysis
- MSL QA Officer or Representative

4.0 PROCEDURE

4.1 Necessary Equipment

Pipettes
Deionized (DI) water

4.2 Calibration

It is seldom necessary to know the capacity of volumetric or measuring pipettes more accurately than the guaranteed tolerance. If there is need to determine the capacity of these two pipette types, references specifically describing these procedures should be reviewed. Most textbooks on quantitative analysis include a section on determination of volume. Also, most catalogs offering pipettes (VWR, Cole-Parmer, etc.) contain a section devoted to determining the capacity of pipettes calibrated "to deliver" and "to contain".

Mechanical micropipettes require frequent calibration to assure that their mechanical parts have not suffered damage that may cause changes in the volume of liquid delivered by the pipette. Mechanical micropipette calibration should be performed at least semi-annually (every six [6] months) and more frequently if needed as determined by use (refer to Table 1 below), substances pipetted, and anytime the performance of the pipette may be questionable.

NOTE: Mechanical micropipettes must be individually and uniquely identified so that their calibration status can be documented and tracked.

Table 1. Annual and Daily Calibration Schedule

Pipette Type	Annual Calibration	Corrective Action	Daily Use	Corrective Action
Measuring Pipettes	once per year (n= 5 or more)	clean, and retest; then remove from service	Calibration verification required for preparing standards or for use on Navy projects (n=1)	1. clean, and retest; 2. retest using 5 replicates, update annual calibration, or 3. remove from service
Volumetric or Transfer Pipettes	once per year (n= 5 or more)	clean, and retest; then remove from service	Calibration verification required for preparing standards or for use on Navy projects (n=1)	1. clean, and retest; 2. retest using 5 replicates, update annual calibration, or 3. remove from service
Mechanical Micropipettes	semi-annual- every six [6] months [n=10 to set initial acceptance criteria] (n= 5 or more)	1 clean, and retest; then 2. send to manufacturer for reconditioning 3. remove from service	Calibration verification required for preparing standards or for use on Navy projects (n=1)	1. clean, and retest; 2. retest using 5 replicates, update annual calibration, or 3. remove from service

Initial calibration and setting of acceptance criteria is based on 10 replicate measures. Routine calibration of mechanical micropipettes is accomplished by weighing at least five [5] aliquots of deionized water delivered by the pipette. The average weight is calculated as well as the standard deviation. From these two values, a coefficient of variation is calculated and used to estimate the accuracy and range of precision for each individual micropipette. The procedure for this determination is provided below. This procedure should bracket the volumes the pipette can deliver and the range for which the pipette is used.

4.2.1 Attach a disposable tip of the appropriate size to the mechanical micropipette to be calibrated. If the pipette is of the adjustable volume type, make sure the setting is appropriate for the tip being used and corresponds to the volume being measured.

Example: Some 5-10 mL mechanical pipettes have two different disposable tips, one tip for the dispensing of 1-5 mL of liquid, and a larger tip for the dispensing of a 5-10 mL volume.

4.2.2 Fill a beaker with deionized water.

4.2.3 Using a calibrated analytical balance (one which weighs to a milligram), weigh a small plastic weighing boat, and tare the balance to zero with the weigh boat on the balance tray.

4.2.4 Depress the plunger on the pipette until it just stops from resistance, place the tip of the pipette in the beaker of water, and slowly release the plunger to its fully extended position. This will draw an aliquot of water

into the pipette tip. Note: The exact pipetting procedure may vary from one pipette manufacturer to another.

4.2.5 Deliver the aliquot of water to the previously tared container by completely depressing the plunger on the pipette until it stops.

4.2.6 Record the weights of each pipette-delivered aliquot on a Pipette Calibration Form (Attachment 1).

4.2.7 Repeat the procedure of weighing aliquots of water at least five [5] times using the same pipette tip.

4.2.8 Calculate the average and standard deviation of all weighed aliquots of water (units of each should be in mL).

4.2.9 Calculate the relative accuracy (expressed as % error) and precision (expressed as the coefficient of variation, CV) of the pipette using the following formulas. In these calculations, the manufacturer's specified volume is the volume that the pipette is supposed to deliver.

$$\% \text{ Error} = \frac{(\text{Averaged Measured Volume} - \text{Manufacturer's Specified Volume})}{\text{Manufacturer's Specified Volume}} \times 100$$

$$CV = \frac{\text{Standard Deviation (SD) in Volume}}{\text{Average Volume}} \times 100$$

4.2.10 If the % error and the CV are near the accuracy and precision values specified by the manufacturer of the pipette, it is permissible to use the manufacturer's specified volume.

NOTE: The acceptable % error and CV may be 1) provided by the manufacturer, or 2) determined using a 1% or 2% difference from the mean based on at least ten (10) replicate measures to set the initial acceptance criteria, or 3) set by project-specific requirements.

4.2.11 If results more accurate than ± 2 % are desired or required, use the calibrated volume determined from averaging the water aliquot weights as described above.

4.2.12 If the % error and the CV are not within the manufacturer's specified volume or the experimentally-determined acceptance criteria, either remove the pipette from use or repeat the calibration process once to verify that the pipette does not meet the acceptance criteria and remove the pipette from use. Some pipettes can be returned to the manufacturer for cleaning and reconditioning.

4.2.13 If the pipette is sent for reconditioning, steps 4.2.1 – 4.1.12 above must be completed to verify pipette precision and accuracy before the pipette can be returned to service.

4.2.14 Ideally, pipettes should be labeled to indicate their calibration status, and range of use. In most cases this is impractical and a pipette logbook is used to document pipette calibration.

Logbook data should record the following:

- pipette serial number
- manufacturer
- volumes calibrated
- the standard deviation (SD) and the calculated CV for each calibrated volume
- date and initials of the person performing the calibration
- due date of next calibration, which is the expiration date of the current calibration

4.3 Pipette Volume Delivery Verification

4.3.1 All pipettes used for Navy work must be calibrated to a regular schedule and the calibration verified and documented daily when used. Pipettes used for other projects should be calibrated according to the schedule below and whenever there is reason to doubt the calibration or need to verify it. Calibration verification for volumetric pipettes and mechanical micropipette should be performed whenever these items are used to prepare standards.

4.3.2 Pipette volume delivery verification shall be made gravimetrically and recorded on either the applicable data sheet or in the calibration logbook. Acceptance criteria are the same as those used for semi-annual calibration

4.4 Cleaning the Pipettes

Pipettes containing dirt, grease, dried solutions and other visible contaminants will not give accurate results. The most important step in pipette cleaning begins just after the pipette is used. At that point, the person using the pipette knows what the contamination is and should take the necessary steps to remove the contamination. This will involve discharging the contaminating material with water, organic solvents, acid, alkali, or other agent capable of dissolving and removing a particular contaminant, followed by a clean water rinse and drying the pipette (air drying is acceptable).

4.4.1 After use, clean the pipette accordingly with the proper cleaning agent for the contaminant in the pipette. Once contaminants are removed, pipettes should be placed tip up in a tall container filled with a laboratory soap solution. Pipettes with the following contaminants may be cleaned as indicated:

a) **Grease and Oils**: Working in the hood, dissolve the material in acetone. Pour acetone into an organic waste bottle, rinse the pipette with deionized water, and place in soap solution.

b) **Aqueous Solutions**: Rinse each pipette with deionized water. Place them in soap solution.

c) **Solvent Solutions**: For compounds dissolved in a solvent (i.e. a pesticide dissolved in hexane), while working in the hood, rinse the pipette five times with the carrier solvent and adequately dispose of the solvent in a labeled compatible waste bottle. Rinse the pipette five times with acetone and properly dispose of the acetone waste. Place the pipette in soap solution.

d) **Blood, Body Fluids, and Albuminous Materials**: Rinse as soon as possible with deionized water. Soak in a 10% bleach solution. Rinse with deionized water and place in soap solution.

A number of procedures should **NEVER** be used to clean pipettes. **DO NOT** soak in strong alkaline solutions. **DO NOT** attempt to remove adherent organic residues by application of direct heat. **DO NOT** pipette cleaning solutions by mouth.

In a properly cleaned pipette, water will drain down the walls uniformly; it will not "break" or leave droplets of water on the glass. Water droplets or "breaks" are indications of unclean surfaces.

4.4.2 After several hours in the soap solution, remove pipettes and rinse them thoroughly (approximately 10 rinses) with deionized water.

4.5 Reading the Meniscus

Measurement of an accurate volume in a pipette depends upon an accurate reading of the meniscus. This curved shape of liquid at the top of a column is caused by the wetting of the container by the liquid. Readings are made at the lowest point of the meniscus. This lowest point of the meniscus should be on a plane with the middle of the graduation line of the pipette. On occasion, a liquid may be encountered which does not wet the glass. In these cases, the highest point of the convex liquid surface is read. When reading the meniscus, the pipette should be precisely vertical and the eye should be in the same horizontal plane as the graduation line and the meniscus.

4.6 Use of Pipettes

The following general instructions apply specifically to the manipulation of transfer (volumetric) pipettes, but with minor modification may also be used for other types of pipettes as well.

4.6.1 Liquids are drawn into the pipette by application of a slight vacuum. **The mouth should never be used for this suction.** The laboratory is supplied with a variety of suction devices that should be used in the pipetting procedures. Make sure that these devices are clean and dry. If liquid is accidentally drawn into the suction device, it should be immediately disassembled (if possible), cleaned and dried.

4.6.2 Keep the tip of the pipette below the surface of the liquid when drawing liquid into the pipette.

4.6.3 Fill the pipette above the graduation line. Make certain there are no air bubbles in the bulk of the liquid or foam at the surface.

4.6.4 Discontinue the suction with the liquid above the graduation mark.

4.6.5 Tilt the pipette slightly from vertical and remove any liquid on the outside of the pipette tip with clean filter paper or a Kimwipe.

4.6.6 While holding the pipette in a vertical position, release pressure on the index finger to allow the meniscus to slowly approach the graduation mark.

4.6.7 When the meniscus reaches the graduation mark, remove the drop of liquid on the tip of the pipette by touching it to the wall of the container.

4.6.8 Recheck the position of the meniscus. If it is not at the center of the calibration line, carry out the necessary steps to bring it to that point, by raising the level of the liquid in the pipette.

4.6.9 Transfer the pipette to the container to receive the liquid. Place the tip of the pipette into the receiving vessel. While holding the pipette in a vertical position, allow the liquid to flow from the pipette without restrictions.

4.6.10 While the liquid is flowing from the pipette, touch the tip of the pipette to the side of the container.

4.5.11 When the flow has stopped, and with the tip still in contact with the side of the container, wait two seconds and rotate the tip of the pipette (to remove any droplet adhering to the tip) while moving it horizontally away from the side of the container. Remove the pipette from the receiving container. Some pipettes are marked with a time in seconds. If so marked, this waiting time for the last drop of liquid should be used instead of the two-second interval.

4.5.12 The calibrated amount of liquid has been transferred. If it is a "to deliver" pipette, do not blow out remaining liquid in tip.

Note: In the case of color-coded pipettes, a frosted ring indicates complete blowout.

For these pipettes which are marked "to contain" or "TC", the procedures for pipetting are identical to those for the volumetric pipettes in the first ten steps above. When the flow to the receiving container has stopped, and with the tip still in contact with the sides of the container, wait ten seconds (or the amount indicated on the pipette) to allow the pipette to drain. Move the tip of the pipette away from the side of the container and blow a light puff of air through the pipette from a rubber bulb, displacing the last of the liquid from the pipette. The calibrated amount of liquid has been transferred.

When finished with the pipette, clean and rinse it as indicated previously.

5.0 DATA ANALYSIS AND CALCULATIONS

Calculations from Section 4.4.9:

$$\% \text{ Error} = \frac{(\text{Averaged Measured Volume} - \text{Manufacturer's Specified Volume})}{\text{Manufacturer Specified Volume}} \times 100$$

$$CV = \frac{\text{Standard Deviation in Volume}}{\text{Average Volume}} \times 100$$

Standard deviation (SD) – The SD is the positive square root of s^2, the variance.

NOTE: n-1 is used in the denominator of this calculation, not n.

For high quality mechanical micropipettes, the % error is generally ± 1 to ± 2 % and the CV is generally ± 1 %. Thus, expect the manufacturers specified tolerance to be a maximum error of ± 3% (or less) of the to-be-delivered volume. A rule of thumb is to set the allowable error $\leq$ 2%.

6.0 QUALITY CONTROL

The precision and accuracy of MSL pipettes shall be known and documented according to the schedule in Section 4.3.1. Calibration verification is necessary whenever standards are being prepared or used using mechanical micropipettes and volumetric pipettes. Calibration verification for other activities depends on the use of the pipette and the relative precision and accuracy required for the task.

For the calibration of mechanical micropipettes, the weighing of pipette-delivered aliquots of deionized water (DI water) is necessary. The procedure of weighing aliquots of water must be repeated a minimum of eight times for each volume being calibrated in order to assess the precision and accuracy of the pipette.

Pipettes not meeting the calibration requirement of a maximum error of 2-3% (depending on manufacturer's specifications) shall not be used in projects, and must be set aside for repair and cleaning, or discarded.

7.0 SAFETY

Caution must be exercised when pipetting harmful or caustic substances. Material Safety Data Sheets are available for all compounds and solvents used in the laboratory and should be consulted for safety concerns prior to the dispensing of any materials.

All analysts following this procedure should be aware of routine laboratory safety concerns, including the following:

- Protective clothing and safety glasses or goggles and face shields when necessary should be worn when appropriate.
- Proper care must be exercised when handling pipetting harmful or caustic substances

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this procedure are properly trained and that documentation of the training exists prior to the performance of those procedures.

To initiate training, all staff members must read this procedure and demonstrate proficiency in the use of these instruments prior to application in project use. A staff member unfamiliar with the use of pipettes will receive a verbal and visual demonstration by a certified co-worker. Once proficiency has been verified, documentation of training will be recorded on a training assignment form from procedure MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory Training

ATTACHMENT 1

Example Pipette Calibration Form

Pipette #:	Balance ID:
Pipette Make/Volume: Pipette Acceptance Criteria: Precision: Accuracy:	Within Calibration Period? Calibration Verification: Acceptable? Y or N Standard weight set used: Observed: Expected: % Diff:
Initials: Date:	
Measures:	Average of 5 reps = Standard Deviation = CV = Acceptable? Y or N If no, corrective actions taken:

Pipette #:	Balance ID:
Pipette Make/Volume: Pipette Acceptance Criteria: Precision: Accuracy:	Within Calibration Period? Calibration Verification: Acceptable? Y or N Standard weight set used: Observed: Expected: % Diff:
Initials: Date:	
Measures:	Average of 5 reps = Standard Deviation = CV = Acceptable? Y or N If no, corrective actions taken:

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EFFECTIVE DATE: 8/18/99

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-C-011-01

GLASSWARE AND EQUIPMENT CLEANING PROCEDURES

Approvals:

AUTHOR	8.4.99 DATE
REVIEWER	~~7/30/99~~ 8/5/99 DATE
QUALITY ASSURANCE	8-5-99 DATE
TECHNICAL GROUP MANAGER	8.4.99 DATE

GLASSWARE AND EQUIPMENT CLEANING PROCEDURES

1.0 SCOPE AND APPLICATION

This procedure describes the methods for cleaning the various types of laboratory glassware and equipment used in toxicological and chemical testing. Care needs to be taken to assure that all implements used in bioassay or toxicological tests are free of soap and contaminants which may interfere with study results.

2.0 DEFINITIONS

None.

3.0 RESPONSIBLE STAFF

- Staff Members.
- Project Manager/Study Director.

4.0 PROCEDURE

4.1 Toxicological glassware and equipment cleaning procedure

The following procedure will be used in cleaning various labware items:

4.1.1 Stainless Steel and Titanium

4.1.1.1 Wash well in hot water containing laboratory grade detergent, and rinse at least five times with tap water or deionized water.

4.1.1.2 Allow to air-dry.

4.1.1.3 Suit up with proper protective clothing for use with solvents: labcoat, full-length apron, faceshield or goggles, and solvent-resistant gloves. Rinse with methylene chloride under a fume hood and allow to dry under the hood.

4.1.1.4 Transfer cleaned items to dust-free storage or to testing area. Label stored items, either in boxed groups or individually, with a sticker or tape labeled with the date, your initials and "$MeCl_2$ rinsed" to denote that items have been cleaned and solvent rinsed.

4.1.2 Glassware and Aquaria

4.1.2.1 Wash well in hot water containing laboratory grade detergent, and rinse at least five times with tap water or deionized water.

4.1.2.2 Transfer items to drying racks or tables and allow to air-dry.

4.1.2.3 Soak all glass labware in 10% reagent grade nitric acid bath for a minimum of two hours. Protective clothing necessary for the insertion or removal of glassware from acid includes a labcoat, faceshield, full-length apron, and acid-resistant gloves.

4.1.2.4 Rinse at least five times with DI water, and allow glassware to dry completely before storing or packing in containers until ready for use.

4.1.2.5 Label clean, stored glassware with a sticker or tape clearly marked with the date, initials, and the words "acid washed and DI water rinsed."

4.1.3 Teflon, PVC, Nalgene, and Other Plastic Materials

4.1.3.1 Wash well in hot water containing laboratory grade detergent, and rinse at least five times with tap water.

4.1.3.2 Soak all labware in a 10% nitric acid bath for a minimum of two hours. Don required protective gear before placing items into or removing labware from the acid bath.

4.1.3.3 Rinse at least five times with DI water allow to dry completely. Store in clean containers until use, with a sticker or tape labeled with the date, initials, and the marking "acid washed DI water rinsed."

4.1.4 Rubber and Silicone

4.1.4.1 Wash well in hot water containing laboratory grade detergent, and rinse at least five times with tap water or deionized water.

4.1.4.2 Allow to air dry.

4.1.4.3 "Age" labware by soaking in seawater (static or flowing) for a minimum of four hours.

4.1.4.4 Rinse with DI water and allow to dry. Place items in a container and label with date, initials, and the marking "seawater soaked, DI rinsed."

4.2 Chemical testing sample container, glassware and equipment cleaning procedures

In general, glass bottles with Teflon lids are used for organic samples, while polypropylene is used for metals and other inorganics. If precleaned bottles are purchased, this must be noted and certificates indicating that the bottles are analyte free must be available and kept in the project file.

4.2.1 Sample Container Preparation

4.2.1.1 Inorganic Parameters

Clean plastic "Spex" jars used for sediment and tissue samples prior to sampling by filling the jars 3/4 full with 5% nitric acid and soaking for a minimum of 7 days with the lids on. Invert the sealed jars and allow to sit another 7 days. Empty the acid and rinse the jars 5 times with DI water. If the containers are for samples to be analyzed for metal speciation, 10% hydrochloric acid may be used.

Wash glass and teflon sampling containers with a laboratory laboratory grade detergent using hot tap water, rinse 3 times with DI water and soak in 1:1 nitric acid for a minimum of 72 hours. Then rinse containers 3 times with DI water and allow to air dry in a contaminant-free laminar flow hood.

In general, commercially cleaned sample containers are not recommended for Battelle's trace metals analyses since the levels monitored by these companies are well above the levels that are routinely determined at Battelle.

4.2.1.2 Organic Parameters

All sample containers used for samples to be analyzed for organic contaminants are glass. Wash containers with laboratory grade detergent and water. Rinse containers 3 times with DI water and then ash in a muffle furnace at 450°C (±50°C) for a minimum of 4 hours. Seal containers with teflon lined lids and store in a contaminant free area until use.

Alternatively, commercially cleaned sample containers that have been cleaned using EPA certified methods are acceptable for use. Certificates indicating that either individual sample containers or the container lot numbers are free from contaminants shall be kept in the central file with the project the containers are associated with.

4.2.2 Glassware Cleaning

4.2.2.1 Inorganic Parameters

Wash all glass, plastic and teflon equipment and glassware as described in section 4.2.1.1.

4.2.2.2 Organic Parameters

Clean all glassware used in the lab as described above in section 4.2.1.2. Just prior to extraction, rinse sample containers and equipment used for subsampling once with the solvent to be used in the extraction. Rinse all teflon and stainless steel equipment used during extractions with DI water 3 times and then rinse with the appropriate solvent (usually methylene chloride) once prior to use.

4.2.2.3 Additional analytical chemistry equipment

4.2.2.3.1 Polycarbonate filters

Soak polycarbonate filters (nucleopore, 0.45 micron) in 70% nitric acid in a sealed teflon container for a minimum of 5 days. Store filters in either house DI water (for metals) or Barnstead water (for organometallics). Samples should be soaked in the water for a minimum of 5 days prior to use. Filters can be stored indefinitely under water.

4.2.3.2 Polycarbonate Bottles

Polycarbonate bottles are often used for centrifugation of waters and sediments (generally for porewater preparation) or for storage of waters and sediments for metals and organometallic analyses. These bottles should be cleaned as follows:

Soak in 5% nitric acid overnight then rinse at least 3 times with either DI or Barnstead water. If bottles are to be used for samples requiring organometallic analyses, a methanol rinse is recommended followed by a minimum of 3 rinses with Barnstead water. Air dry bottles in a clean, laminar flow hood.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

All glassware used in testing must be free of contaminants which will interfere with the study, therefore, care must be taken in assuring that all labware is washed with a laboratory grade detergent and water, acid washed, and DI rinsed. Label all groups of clean labware with the date, initials of washer, and level of cleanliness (i.e. "laboratory grade detergent and water washed," "acid washed and DI rinsed") to avoid the use of improperly cleaned glassware.

7.0 SAFETY

7.1 Persons working in the wet laboratory must wear laboratory coats and close-toed shoes. Bare feet, sandals, or open-toed shoes are not allowed.

7.2 Persons using the acid bath or solvent rinse hood must wear a full-length apron, protective glasses and gloves.

7.3 The ventilation hood should be activated when using solvents.

8.0 TRAINING REQUIREMENTS

Persons using the wet laboratory must first read this SOP. A training instructor will be designated by the Project Manager and the trainee will be instructed both verbally and visually regarding proper procedures. Initial use of the acid bath and solvent rinsing should be done under supervision. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this SOP are followed. Upon successful completion of training, the training assignment form will be filled out and signed by the training instructor. Training will be documented in accordance with MSL-A-006.

9.0 REFERENCES

MSL-A-006. Marine Sciences Laboratory Training.

Battelle

Marine Sciences Laboratory

EFFECTIVE DATE: August 18, 1999

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-C-016-00

MAINTENANCE OF THE DEIONIZED WATER SYSTEM

Approvals:

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MAINTENANCE OF THE DEIONIZED WATER SYSTEM

1.0 SCOPE AND APPLICATION

This SOP describes the deionized water system for the Marine Sciences Upper Laboratory. The primary water purifying system is comprised of particulate filtration and mixed bed deionization. There is also ultraviolet sterilization.

The water received from the system is used for reagent and procedural blank preparation and final glassware rinses.

This procedure defines the periodic checks performed to assure the operation of the equipment and the adequacy of the water.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

- Designated Staff Member
- Facility Operations Staff Member

4.0 PROCEDURE

4.1 Background: The MSL has a large water storage tank (approximately 500 gallons) that is used to hold the deionized water. This is connected to a continuously circulating loop which supplies the individual laboratories. The water quality of the tank is monitored with a Myron L. Co in-line indicator. This indictor has lights which glow to indicate the acceptability of the water. The green light glows if the resistivity is >18 megaohms. The red light glows if the resistivity is < 18 megaohms and there is an automatic audible alarm. There is also a digital readout of the resistivity.

4.2 Each day the deionized water is used, a designated satff member checks the digital readout of the resistivity and records in the DI Water Record LogBook. This is a check of the water quality in the tank.

4.3 Each day, a Facilities and Operations staff member checks the monitor for the water quality in the circulating loop. This is a simple "light is on/ light is off" check that is recorded in the daily rounds log.

4.4 If there is a problem with the DI water (< 18megaohms) in the storage tank, the staff member notifies the Facility Operations staff and also informs all MSL research staff (usually via public announcement). If there has been any maintenance or repair on the system, the Facility and Operations staff inform the research staff.

4.5 The DI water resistivity meter is calibrated periodically by returning it to the manufacturer.

5.0 DATA ANALYSIS AND CALCULATIONS

Not Applicable

6.0 QUALITY CONTROL

Included in above information

7.0 SAFETY

Not applicable

8.0 TRAINING REQUIREMENTS

All MSL staff who perform monitoring or servicing of the DI water systems must first read this SOP prior to conducting their work (without supervision). Documentation of training will be recorded on a training assignment form from SOP MSL-A-006.

9.0 REFERENCES

MSL-A-006 Marine Sciences Laboratory

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EFFECTIVE DATE: 03-23-00

Marine Sciences Laboratory

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-D-001-03

RECORDING DATA ON DATA SHEETS AND LABORATORY NOTEBOOKS

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RECORDING DATA ON DATA SHEETS AND LABORATORY NOTEBOOKS

1.0 SCOPE AND APPLICATION

This procedure provides guidelines that are to be followed by Battelle Marine Sciences Laboratory (MSL) staff when recording raw data and maintaining laboratory notebooks and logbooks.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

All MSL staff recording data

4.0 PROCEDURE

The rules for recording data and commentary on data and methods in laboratory notebooks, data forms, logbooks and other media are intended to ensure legibility, accuracy, traceability, validity, and clarity of meaning.

4.1 General Data Recording Guidelines

4.1.1 **Legibility:** All entries must be legible. Writing or printing is acceptable.

4.1.2 **Recording Entries:** All entries should be made using reproducible (black or blue ink is recommended) pen. Water-soluble ink must not be used. **No pencil, felt tip, or red ink is permissible for routine entries.** Red ink may be used to indicate that quality assurance (QA) or quality control (QC) reviews have been performed and to show corrections that were made as a result of those reviews.

4.1.3 **Initialing and Dating Entries:** The originator(s) of all entries must be identified by initial(s) or signature(s) and the date of the entry. In most cases, there are specific places on the data sheet for initials and the date to identify the originator of entries or groups of entries. In laboratory notebooks and logbooks, initials should be cross-referenced to a printed name and signature.

4.1.4 **Dittos:** No ditto marks are allowed. Arrows or other symbols indicating "same" are not to be used to record sample i.d. numbers (Battelle Codes) and are to be kept to a minimum elsewhere.

4.1.5 **No-Data Entries:** All blanks with no data must contain a short horizontal or vertical line, or NA to indicate that the field is not applicable.

Exception: Where a large data field is included on the data sheet for an indeterminate amount of data, and data collection is terminated prior to the field's being filled, the end of the data collection shall be indicated by text such as "end of data collection". This comment shall be written just to the right and/or below the last data entry in the field. A single diagonal line shall be drawn through the blank area and the individual performing this step shall initial and date it. No horizontal or vertical lines need to be entered in the remaining blanks of the field.

Check Boxes: An " X" or a "✓" must be used in check boxes to indicate clearly that the item represented by the box has been selected or completed. The box must be left blank if the item is not complete. NA must be written in the box if the item is not applicable, to make it clear that the box was not overlooked.

4.1.6 **Abbreviations:** The use of abbreviations should be kept to a minimum. Only nationally accepted abbreviations (i.e., mph, ft, min, g/kg) and chemical formula abbreviations (i.e., NaOH, HCl) may be used without further clarification. Other abbreviations can be used providing they can be traced to corresponding explanations.

4.1.7 **Time of Day Entries:** In general, time of day should be expressed in terms of a 24-hour clock (i.e., 10:30 a.m. = 1030 or 10:30, 2:15 p.m. = 1415 or 14:15). If a 12-hour clock is used, the appropriate a.m. or p.m. designation must be recorded.

4.1.8 **Use of Photocopies:** There may be situations in which photocopies of raw data become part of the raw data file. Good Laboratory Practices (GLP) regulations permit the use of photocopies with the following restrictions:

a. *When photocopies of raw data sheets are placed in the study file as a substitute for originals:*

Each copy must be labeled with the words **"EXACT COPY"** and signed and dated by the person labeling the copy. NOTE: If an error is found on the copy, the original should be corrected, then new copies should be made and distributed. The incorrect ones should be destroyed. If the original is not available, then the copy should be corrected.

b. *When data are recorded on photocopies of raw data sheets as well as the originals:*

Both the original and the copy must be treated as raw data (i.e., completed in ink, data initialed and dated, error corrections initialed, dated and explained). Each sheet should reference the location of the other to ensure that the complete data set is traceable.

c. *When photocopies of data sheets are sent to another person or group to be used for reference only:*

The copy should be labeled with the words **"REFERENCE ONLY"**.

4.1.9 Work performed should be described or applicable procedures referenced.

4.1.10 When an instrument requiring calibration is used, it must be identified by serial number or instrument number. Instrument calibration status as well as the results of any continuing calibration checks should be recorded or cross-referenced.

4.1.11 When a standard or reagent is used, any information necessary to uniquely identify it (NIST number, lot number, batch number, etc.) must be recorded to provide traceability.

4.1.12 **Explanation/Assessment of Impact:**

a. When data are missing, and when project limits or specified ranges are exceeded, ensure that the space or value is marked, annotated, initialed and dated. The annotation needs to assess potential impacts and describe any corrective action(s) taken.

b. Methods are followed as written. Any deviation from method(s) must be documented on the data sheet or a separate sheet and submitted with the data package to the project manager, to enable the project manager to summarize the deviation for the client in the QA Narrative.

4.2 **Data Corrections**

4.2.1 Any errors will be crossed out with a single line so as to remain legible. Do not attempt to erase or write over entries, or use correction material. Each lined-out entry will be initialed, dated and, where necessary, annotated to provide an explanation or justification for the deletion or change. An abbreviation or symbol with a circle around it may be used to explain the correction as long as the abbreviation or symbol(s) can be traced to the corresponding explanation (i.e., referenced at the bottom of the page, listed at the beginning of a notebook.) The abbreviations listed on "Suggested Error Codes" (Attachment 1) are recommended; if used, the list should be attached in the notebook as described in 4.3.3 g, below. Other abbreviations or symbols can be used if none of the attached explanations suitably describe the error, but they must be defined.

4.2.2 If there is insufficient space for all or part of the correction information, enter a footnote callout near the incorrect data and enter the required information as a comment elsewhere on the data sheet or notebook page.

4.3 Laboratory Notebooks

4.3.1 **Laboratory Record Books (LRB):** When LRBs (official pre-paginated Battelle-issued laboratory notebooks with beige or maroon binding, tracked by a unique identification number) are used, the requirements of the Battelle Standards Based Management System (SBMS) subject area, Laboratory Record Books shall apply.

The Battelle policy is to use LRBs to ensure priority of invention and protect the intellectual property rights of Battelle, its staff, and clients. Staff must use LRBs to record novel ideas, processes, or approaches that are conceived or developed at any time during the course of a staff member's work. However, LRBs are not required for routine activities such as recording preventive maintenance, refrigerator or freezer temperatures, sample disposal, etc. Procedures for the use of both classified and unclassified LRBs are provided on the inside front cover of each LRB.

4.3.2 **Laboratory Notebooks:** For non-clinical GLP regulated studies where the original notebook will be archived, official Battelle-issued LRBs cannot be used. For these studies, pre-paginated, store-bought bound notebooks shall be used. In addition, store-bought bound notebooks can be used for documenting routine activities. In some cases, three-ring binders can be used as a project or laboratory notebook. Three-ring binder notebooks are paginated when the notebook is completed. All pages are counted and paginated in the format, "page x of y".

4.3.3 **Guidelines for Use of LRBs and Laboratory Notebooks:** The following requirements apply to the use of LRBs and laboratory notebooks:

a. The cover (or first page) of each book shall be identified with the study number and/or name and the responsible person.

b. The front pages of each book shall list a table of contents.

c. The first entries are:
   - Study title.
   - Brief description of the objectives of the study.
   - List of all person(s) making entries in the book: printed names with corresponding initials and signatures.

d. Study documentation shall be entered clearly, legibly, and in sufficient detail to permit duplication of the work by someone other than the person(s) originally performing it.

e. Each page must be dated by the month, day and year in which the data were recorded and signed by the person(s) performing the work or entering the data.

f. Record observations/data chronologically and completely. Describe (narrative or sketch) observations, experimental apparatus, equipment, materials, calculations and any procedures, data sheets, references, etc. that are used. Make the record self-explanatory.

g. Use the following steps if it is necessary to attach a loose sheet to the notebook:

- Attach the sheet to an unused page or portion of a page with tape or glue.
- Sign or initial and date the sheet across the page and the attached sheet so that the page can be matched if it comes loose.
- Identify on the sheet the study number, notebook number (if applicable) and page number to which it is attached (in case it comes loose).
- Make an entry in the notebook to introduce or describe the attached sheet.

h. Record as the last entry for a study a statement noting completion of the work or, if appropriate, reference to a subsequent LRB or laboratory notebook.

4.4 Guidelines for Review of LRBs, Laboratory Notebooks and Logbooks

4.4.1 The technical group or project manager identifies the technical reviewer(s).

4.4.2 The technical group or project manager defines the review cycle (not less than bi-annually) to be completed by reviewers for entries not associated with Intellectual Property (IP). IP entries must be reviewed and witnessed according to the SBMS Procedures for the Use of Laboratory Record Books.

4.4.3 The technical reviewer reviews the LRB, laboratory notebook or logbook in accordance with the following guidelines:

a. Ensure that data are traceable and understandable.
b. Ensure that all forms are complete.
c. Ensure that who recorded the data and when is evident.
d. Ensure that all instruments used are identified.
e. Ensure that all lined-out corrections are initialed and dated.
f. When project limits are exceeded, ensure that the value is marked and explained. The explanation needs to address whether the value exceeding the range resulted in a corrective action or an impact on the test.
g. When something is missing, ensure that an explanation and assessment of impact is provided.

4.4.4 Issues requiring resolution are identified and may become part of the notebook, logbook or project file.

4.4.5 The reviewer(s) sign and date the notebook or logbook and indicate that a review has been done.

4.4.6 Once corrections are completed the reviewer(s) sign the notebook and indicate the satisfactory resolution of concerns.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable.

6.0 QUALITY CONTROL

Not applicable.

7.0 SAFETY

Not applicable.

8.0 TRAINING REQUIREMENTS

Personnel who will be recording or reviewing data must first read this procedure. Documentation of training will be recorded on a reading assignment form from procedure MSL-A-006.

9.0 REFERENCES

None.

ATTACHMENT 1

SUGGESTED ERROR CODES

Error codes should be written near the correction and must be accompanied by the initials of the person making the change and the date of the change.

Symbol	Explanation of Correction
WL	Inadvertently recorded in the wrong location
CC	Changed for greater clarity
WO	Write over
SE	Spelling error
IR	Inadvertently not recorded at time of initial observation
CE	Calculation error
TE	Transcription error
RE	Recording error
EI	Entry not initialed, dated, and/or justified at the time of entry
TI	Incorrect time
DA	Incorrect date
UN	Incorrect units
ID	Incorrect sample ID
MD	See miscellaneous documentation form (refer to location of form)
WP	Peak misidentification (Gas chromatography only. Analyst judged peak to be incorrectly identified by instrument software.)

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Marine Sciences Laboratory

EFFECTIVE DATE: 4/26/00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-D-004-01

Data Reporting, Reduction, Back Up, and Archiving

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Data Reporting, Reduction, Back Up, and Archiving

1.0 SCOPE AND APPLICATION

This procedure describes routine data reduction, reporting, back up and archiving procedures used at the Battelle Marine Sciences Laboratory (MSL). Customer requirements guide the length of time records are required to be retained.

The National Environmental Laboratory Accreditation Program (NELAP) requires records to be maintained for five years from last use, and specifies that records stored only on electronic media must be supported by the hardware and software necessary for their retrieval. The Navy Quality Assurance (QA) Program specifies a 10-year data retention requirement. These long-time requirements necessitate that a hard copy data system be adopted to allow data to be easily accessed and used for historical data reconstruction requests. Electronic media requirements are difficult to meet because the hardware used to read the media must also be archived. This implies that analytical instrumentation needs to be archived to read raw data files from firmware, as well as PCs and floppy drives of various types and vintages. The advances in electronic data capture and storage, resulting in obsolete hardware over very short time periods, makes archiving both electronic data and hardware cost prohibitive and so, the MSL policy is to rely primarily on hard copy data storage.

2.0 DEFINITIONS

Data Audit – A qualitative and quantitative evaluation of the documentation and procedures associated with environmental measures to verify that the resulting data are of acceptable quality (i.e., meet specified criteria) and are traceable.

Data Reduction – The process of transforming raw data by statistical or mathematical functions, the application of calibration curves, concentration factors or sample characteristics (e.g., wet weight, dry weight, percent moisture) into a more useable form.

Data Validation – The process of substantiating specified performance criteria or control limits.

Data Verification – The process of confirming by examination and review of objective evidence that specified requirements have been met and data are traceable.

3.0 RESPONSIBLE STAFF

- Analysts
- Data Coordinator
- Project Manager or Task Leader (i.e., Responsible Manager)
- Quality Assurance Officer or Representative

4.0 PROCEDURE

The general data collection process and data management practices in use at the MSL are specified below. However, it must be recognized that most of the MSL data quality requirements are customer-driven and specific customer requests for data reporting formats, data back up, and data

and sample retention direct day-to-day activities. This procedure will provide overall policies and procedures that may, at times, be superseded by customer requirements. Customer requirements are defined by the MSL Project Manager in a document called a "kit" which is prepared based on any project requirements documents (i.e., test plans, sampling and analysis plans, QA project plans) once samples are received for analysis or testing. If there are any issues related to requirements for customer confidentiality (including national security concerns) or proprietary rights, then this requirement is identified when the "kit" is initiated and the kit information is restricted to a "need to know" basis. Data may be stamped "Confidential" in these situations.

4.1 Sample Log-In and Chain of Custody

Two procedures guide MSL operations for sample log-in and chain of custody:

- MSL-A-001 Sample Log-In Procedure
- MSL-A-002 Sample Chain of Custody

Samples or test organisms are logged in when received in the shipping area. If a Chain of Custody (CoC) form accompanies the samples or test organisms, this form is used to document the date and time of sample receipt and condition. If a CoC form is not shipped with samples, an MSL form will be initiated. For test organisms, a shipping form can be signed and dated and the condition of organisms noted. The sample labels are compared to the CoC and assigned an identification code plus sequential numbering of samples upon arrival. Cooler temperatures are taken and recorded on the CoC. If sample preservation is indicated by the type of analysis or customer specification, samples may be pH adjusted or the pH of a set of subsamples measured to ensure that samples needing to be at a pH of $\leq$ 2 pH units are acidified. This is recorded on the CoC. If samples require filtration, they will be filtered and this information recorded on a Log-In Checklist (discussed in MSL-A-001, Sample Log In Procedure. Samples are counted and assigned an MSL project number (i.e., a central file number and sequential numbering of the set of samples that were received). In some cases samples in a set may arrive on different days depending on the customer's needs and direction.

For analytical samples, an electronic spreadsheet is generated (Login Sheet) listing the customer or sampling identification (ID) number (sponsor code), the MSL sample ID Number (Battelle code), the sample matrix, the parameters requested, the date of sample collection, and the initials of the person logging in the samples (the Sample Custodian or Designee). The location of sample storage until preparation is also noted.

The "kit"(refer to the four pages in Appendix 1) is initiated at this time and generally includes:

- kit initiation date
- assigned central file number
- client name
- project title
- data due date
- work package number (charge code)
- any holding time specifications[1]

In the absence of customer-specified holding times, holding times defined in standard methods (e.g., EPA 1600 series methods; U.S. Army Corps of Engineers, 1994. Inland Testing Manual. EPA-823-B-94-002. U.S. EPA, Office of Water. Washington, D.C.) may be assigned by the MSL Project Manager.

- expected sample concentration level, if known (high, moderate, low)
- expected number of samples
- blank correction instructions
- matrix and summary of requested sample preparation activities, digestions, and analyses
- hazardous material designation
- project manager signature and date
- Project Workplan Sheet – page 2; specifies sample preparation instructions
- Metals – page 3; specifies matrix and analysis method(s)
- QA/Quality Control (QC) Requirement Sheet – page 4; specifies precision (number of replicates, number of spiked replicates), and accuracy (standard reference material [SRM] type and frequency, number of matrix spikes and concentrations levels] method detection limit [blank and blank spike frequency] and initial calibration verification (ICV) and continuing calibration verification [CCV]) sample frequencies. Project control limits may also be specified or attached. When the customer does not specify project control limits, MSL default limits will be used.
- The CoC forms are appended to the last sheet of the kit.

An addendum is prepared for anything that is added to the kit. Additions might include the receipt of another sample set to be included in the project sample set, changes to the analysis request, or deletion of some samples to be analyzed.

The kit and any addedums are copied and distributed by the Project Manager to all analysts involved. The original pages of the kit become part of the Chemistry Central File System.

Sample disposal instructions are specified by the Project Manager on the Log-In Checklist (discussed in MSL-A-001) in the section that is filled out by the Project Manager (prior to sample arrival when ever possible).

4.2 Sample Preparation; Including Sample Digestion, Distillation, and Extraction

One procedure guides MSL operations for data collection, MSL-D-001 Recording Data on Data Sheets and Laboratory Notebooks. Worksheets are used to track and document sample preparation activities (digestion, distillation, extraction). The method is documented as an MSL method. The worksheet information varies depending on the preparation procedure and the analysis. Example information contained on a worksheet may include dates of preparation activities, analyst, balance number used, spiking solution ID number, spike concentrations, spike volumes, ID number(s) of standard(s) used, sample ID numbers, sample weights and/or volumes, total sample weights and/or volumes.

4.3 Sample Analysis and Testing

Sample analysis documentation varies with the analysis and the method. Analysis documentation generally includes a sequence listing showing the run order for blanks, calibration standards, QC samples, samples, and continuing calibration standards. This information is then traceable to the instrument output. The documentation also includes the calibration file with a list of the standards, the calibration curve, and when appropriate a summary of the slope, intercept and correlation coefficient (r^2).

Analysts complete a data review checklist documenting results of the evaluation of batch results with method and project-specific data quality criteria. The checklists are also used to document any

deviations encountered during the analysis. Deviations may arise from analyst decisions to deviate from a method or choices made during method implementation. Analysts are empowered to make these types of decisions as long as they are documented on the checklist so that the Project Manager is informed of the deviations and has any applicable information and/or justification for these decisions. In many cases, the analyst will discuss the proposed deviation with the Project Manager prior to the procedural change. In other cases a deviation may arise from routine occurrences in an analytical laboratory such as autosampler problems, limited sample volume, power outages, etc.

Data review checklists are signed off by the analyst and reviewed with the next person in the data collection process who signs off that they have received and reviewed the checklist when they receive the data package. The third level of data review is provided during the data package review conducted by the MSL QA Officer as per MSL-Q-005, Quality Assurance Data Audits.

4.4 Results of Sample Analysis and Testing

The results of sample analysis and testing are often (and whenever possible to minimize error) collected directly from the instrument into spreadsheet (EXCEL) files. Instrument raw data are collected and included in the data package. In cases where data can not be directly entered into a spreadsheet from the instrument, data are hand entered into spreadsheet files. These data are more carefully assessed, often at a 100% verification level, during the MSL data audit process described below in Section 4.5.

Spreadsheet data are transferred from the analyst to the Data Coordinator or the Project Manager and summary data sheets are prepared.

Instrument data are printed out and hard copies of raw data are transferred from the analyst to the Data Coordinator or the Project Manager and accompany the summary data sheets in the data package submitted for the data audit process.

4.4.1 Project Notebooks

For bioassays, a project notebook is initiated when test organisms or sediments are received. The general format of the notebook varies with the test and with the level of assigned QA/QC (i.e., GLP or non-GLP). Project notebooks contain animal receipt, handling, and acclimation information as per MSL procedure MSL-T-002, Animal Receipt, Acclimation and Holding. Assessment of temperature, dissolved oxygen (D.O.), salinity, and pH is made routinely during organism receipt, acclimation and at test set-up, during testing, and at test termination. The following procedures are used for the calibration of instruments and making routine measures:

- MSL-W-001 Calibration and Use of pH Meters
- MSL-W-002 Calibration and Use of Dissolved Oxygen Meters
- MSL-W-003 Calibration and Use of Thermometers, and
- MSL-W-004 Calibration and Use of Refractometers
- MSL-W-007 Routine Water Quality Measures for Toxicity Tests
- MSL-W-008 Determination of Ammonia

Bioassay documentation includes Test Organism Receipt and Acclimation Records, Daily Logsheets, Water Quality Monitoring data sheets, Daily Observation Forms, Test Termination Forms, summary data sheets, procurement records, calibration documentation, and deviation

summaries, which are also often included in a customer narrative. The specific testing procedure(s) used is also included.

4.4.2 Instrument Data

Instrument electronic data are backed up monthly. However, some instrumentation does not have back up capabilities. Most electronic instrument data and data summaries are retained as hard copies and become part of the analytical data package or project notebook. Electronic analytical data files are provided to the Data Coordinator. These are backed up bimonthly and retained for five years. Central file hard copy data are retained indefinitely in the data archive.

4.4.3 Manipulated Data

4.4.3.1 Historical Practices

Diskette data storage was used prior to 1997. Since mid-1997, when a macro was developed, there are data files backed up to the MSL M: drive (limited access) with a duplicate copy stored on the hard drive of the Data Coordinator.

4.4.3.2 Current Practice

Spreadsheet data are manipulated by the Data Coordinator for the Project Manager to summarize results and prepare data reports for customers in a usable format. The Project Manager is responsible for a review of these data prior to submitting for QA Review. Summary data files (hard copies) are usually retained at least by the Data Coordinator, and often an additional copy is kept by the Project Manager. Copies under control of the Data Coordinator are the central file hard copies that are retained indefinitely in the data archive.

4.4.4 Data Files

Final chemistry central data files will include the following:

- the "kit" as described in Section 4.1
- original hard copies of raw data for calibrations, samples and QC measures, analyst work sheets, data output records
- reference to the procedure(s) or test method(s) used
- final report copies
- correspondence related to specific-project activities
- any audit or corrective action reports and responses
- evidence of data review

Central file copies are kept for 6 months in the office of the Data Coordinator and then transferred to the locked access archive storage location.

4.5 **MSL Data Audit Process**

All data files to be audited must be validated and reviewed by the technical staff before being submitted to the QA staff. The data validation process performed by technical personnel may include, but is not limited to, verification of transcription, double keyboarding, identification and removal of outliers or spurious data points with a reason for removal, assessment of quality control

(QC) results, and evaluation of scientific validity.

The MSL data audit process is primarily a data verification activity which is described in MSL-Q-005, Quality Assurance Data Audits. However, verification of validation activities also occurs. Complete data packages including all kit information, hard copies of instrument outputs, and summary data sheets are provided to the MSL QA Officer or designee for review. Analytical data packages are reviewed to a checklist. Project notebooks, because of their variability, are not reviewed to a checklist. However, the review process is essentially the same. Date are reviewed to ensure that the data are accurate, traceable, defensible, and complete, as compared to the planning documents and/or project requirements. The audit procedure is a randomized check that involves comparing selected reported values to the original data. This check can either be performed randomly or on a statistical basis. Results of the data audit are documented either on the checklist from MSL-Q-005 or in a summary statement. Concerns that can be corrected will be corrected before the data are released. Deviations will be required to be summarized and provided to the customer.

4.6 Release of Data

Data are released to the client by the MSL Project Manager after the MSL data audit process has been completed, any questions are resolved, and a final data summary (and when required, a narrative summary or letter to the customer) prepared. Preliminary data may be released to the customer as long as it is clearly identified as "preliminary and subject to change". The decision to release preliminary data is the decision of the Project Manager. In some cases, the MSL QA Officer or representative and the MSL Project manager sign the data release. These cases are identified by customer request. Data releases to the customer usually include an electronic data summary file and/or an e-mail data file transmittal. The amount of electronic data provided to the customer is based on the customers' needs.

MSL policy does not allow the release of customer data to anyone except the customer unless expressly directed by the customer.

4.7 Responding to Questions Regarding Data

Customers often have requests for clarification of the data (e.g., procedures used, approach for calculating method detection limits, assignment of data qualifiers and requests for changes to the data summary such as blank corrected data, expanded data packages, additional analyses, or wet weight/dry weight conversions.) These requests are directed to the specific Project Manager who has served as the point of contact for the work. Customer requests are processed as soon as possible by the Project Manager, and generally are not difficult to address in a timely manner.

4.8 Customer Complaints

The process for tracking and addressing customer complaints is the following:

- The Project Manager is the point of contact for any customer complaints.
- The customer contacts the Project Manager to discuss the concern. The contact is generally made by e-mail or telephone, although a formal written follow up letter may be sent as well.
- The MSL Project Manager will inform the Technical Group Leader of the issue(s), and discuss

the concern(s) with the Laboratory Managers as appropriate. Concerns will be responded to in writing. A determination will be made of an appropriate response (e.g., data review and re-calculation, sample re-analysis, re-sampling and analysis, revision of deliverables) which will be discussed with the customer prior to finalizing in a response letter.

- An MSL tracking system for customer complaints has not been created because customer complaints are rare. If the frequency of customer complaints increases (>2/year), a formal tracking system may be developed. The MSL QA Officer will monitor the number of annual customer complaints.

4.9 Data Archiving

The preferred approach to long-term data archiving is to provide the customer with requested floppy disk or electronic data files at the time the data summaries are provided. The responsibility for maintaining long-term data file archiving then lies with the customer, not MSL. However, MSL also recognizes that customers expect that data from archived files can be provided upon request. Requests from customers for summary data far out weigh requests for instrument data. However, there are cases when instrument data, especially from analytical data sets such as those obtained by ICP/MS, can be reanalyzed using different data libraries or scanning algorithms.

MSL policy is to retain electronic data files for five years. Hard copy data are stored indefinitely as per MSL procedure MSL-D-003, Archiving of Records, Data, and Retired SOPs which primarily addresses GLP requirements. Archives are controlled access (locked) storage rooms. Data are stored and retrieved by project number.

5.0 DATA ANALYSIS AND CALCULATIONS

Data analysis and calculations are addressed in individual procedures. Implementation of this procedure does not require any data analysis or calculation.

6.0 QUALITY CONTROL

Periodic surveillance should be conducted by the MSL's Quality Assurance Officer or Representative to ensure that data management and reporting activities follow processes that result in the determination of data quality to specifications. Results of periodic surveillances may become a part of the project files.

7.0 SAFETY

Not applicable to this procedure.

8.0 TRAINING REQUIREMENTS

Most MSL staff require training to this procedure. Project managers must be trained to this procedure. Training is assigned by the responsible manager. Documentation of training shall be recorded on a training assignment form provided in procedure MSL-A-006, Marine Sciences

Laboratory Training.

9.0 REFERENCES

MSL-A-001 Sample Log-In Procedure
MSL-A-002 Sample Chain of Custody
MSL-A-005 Deviations from Established Requirements
MSL-A-006 Marine Sciences Laboratory Training
MSL-D-001 Recording Data on Data Sheets and Laboratory Notebooks
MSL-D-003 Archiving of Records, Data, and Retired SOPs
MSL-Q-005 Quality Assurance Data Audits
MSL-T-002 Animal Receipt, Acclimation and Holding
MSL-W-001 Calibration and Use of pH Meters
MSL-W-002 Calibration and Use of Dissolved Oxygen Meters
MSL-W-003 Calibration and Use of Thermometers
MSL-W-004 Calibration and Use of Refractometers
MSL-W-007 Routine Water Quality Measures for Toxicity Tests
MSL-W-008 Determination of Ammonia

Appendix 1 – EXAMPLE KIT
INORGANICS PROCEDURE OUTLINE

Distribution	Date/Initials
[redacted]	

CENTRAL FILE NO: _____

PROJECT NAME: _____

CLIENT: _____

DUE DATE (Finished table to PMI): _____

Blank Correction: _____ Yes _____ No **Work Pkg. #** _____

Instructions:

Holding Time(s): 28 days (Hg) 6 months Other:

Data Quality Objectives - Frequency

ICV/CCVs: _____

RMs: _____

S: _____

MS/MSDs: _____

Reps: _____

Method Criteria
[redacted]

HAZARDOUS MATERIAL ☐ NO ☐ YES

COMMENTS: _____

Project Manager: _____ (Signature) _____ (Date)

Water	Sediment	Tissues
Filter for Diss. Metals	Sed Evap (HNO3/HF)	HNO3 / HF
Acidify	Hg Split	Hg Split
T R M	EPA 200.2 Dig	AquaRegia Dig.
PRECONCENTRATE	TAMU Dig (HNO3/HCl)	TAMU Dig (HNO3/HCl)
FePd	HNO3 Dig	HNO3 Dig
APDC	HNO3 + H2O2 Dig	HNO3 + H2O2 Dig
Hg - BrCl Digestion	HNO3 + HSO4 Dig	HNO3 + H2SO4 Dig
	% Moisture	HCl Dig As Spec.
	MeH g	NaOH Dig As Spec.
	Distillation	**% Moisture**
Diss Sulfide	Extraction	OTHER:
OTHER:	AVS/SEM	
	SEM Hg (in teflon!)	
	XRF Split	
	Grain Size	
	TOC	
	TV S	
	Pb 210	
	Cs137	
	OTHER:	

...ial yte	ICP-MS	ICP-AES	IC	CVAF/CVAA	GFAA	Hydride	OTHER:
Ag							
Al							
As							
B							
Ba							
Be							
Ca							
Cd							
Co							
Cr							
Cu							
Fe							
Li							
Mg							
Mn							
[illegible]							
[illegible]							
Ni							
P							
Pb							
Sb							
Se							
Sn							
Sr							
Tl							
U							
V							
Zn							

Comments:

	Frequency	DQO (%)	Metals	Spike Level	Sample ID
[illegible]					
Comments:					

[illegible]					
Comments:					

Blank Spikes					
Comments:					

Blank Spikes					
Comments:					

Replicates					
DUP					
TRIP					
Comments:					

[illegible]					
[illegible]					
[illegible]					
Comments:					

.HER:

Battelle
Marine Sciences Laboratory

EFFECTIVE DATE: 2/6/92

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-F-001-00

SEAWATER AND FRESHWATER SYSTEM MAINTENANCE

Approvals:

AUTHOR	DATE 2/5/92
TECHNICAL REVIEWER	DATE 2/6/92
QUALITY ASSURANCE	DATE 2/5/92
MARINE SCIENCE LABORATORY MANAGER	DATE 2/6/92

SEAWATER AND FRESHWATER SYSTEM MAINTENANCE

1.0 SCOPE AND APPLICATION

This SOP describes the types of routine maintenance that the Marine Sciences Laboratory need to perform for the seawater and freshwater systems. This SOP also describes the policies for documenting and maintaining preventative maintenance records.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

- Facilities Manager
- Facilities Staff

4.0 PROCEDURE

4.1 Preparation of Preventative Maintenance Standards

4.1.1 The Facilities Manager shall assure that Preventative Maintenance Standards (PMS) are prepared and issued for all routine maintenance performed for the freshwater and seawater systems and associated equipment. As a minimum, PMSs shall be prepared for the following:

Seawater

- seawater intake lines
- seawater head tank
- raw and filtered seawater lines
- steam generator and heated seawater system
- seawater intake system
- seawater pumps 1, 2 and 3
- seawater filtering system
- emergency generator
- waste water treatment system

Freshwater

- general tank discharge pump
- sanitary water system and well water pump
- sanitary water filters

4.1.2 Each PMS shall address the following items, as appropriate:

- equipment Item
- equipment location
- equipment description
- maintenance to be performed - should include required frequency of maintenance and if necessary a checklist/procedure that describes in detail what needs to be performed
- spare parts inventory
- signature and date line for staff member performing the work

4.2 Performance of Preventative Maintenance Standards

4.2.1 Prior to performance of a preventative maintenance standard, the Facilities Staff shall notify the technical staff when the preventative maintenance may interfere with any ongoing tests.

4.2.2 Facilities Staff shall perform the preventative maintenance in accordance with the PMSs. Once the preventative maintenance has been completed the Facilities Staff member shall sign and date the PMS and indicate whether or not the equipment was left in satisfactory operating condition.

4.2.3 The Facilities Staff shall notify technical staff when:

- the preventative maintenance is complete, and/or when
- a preventative maintenance standard could not be completed and that preventative maintenance could interfere with the conductance of any ongoing tests.

4.2.4 The Facilities Manager shall ensure that all completed PMSs are retained and filed in the designated facility office.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable

6.0 QUALITY CONTROL

Quality Control of the steps outlined in this procedure is the responsibility of the facilities staff. Quality Assurance staff will make independent assessments to evaluate and report to management whether this procedure is being correctly followed and implemented.

7.0 SAFETY

Routine safety precautions as described in PNL-MA-42 and PNL-MA-43.

8.0 TRAINING REQUIREMENTS

The Facilities Manager and all Facilities Staff must first read this SOP prior to performance of preventative maintenances. Documentation of training will be recorded on a training assignment form from SOP MSL-A-006.

9.0 REFERENCES

PNL-MA-42 Industrial Hygiene, Occupational Safety and Fire Protection Program
PNL-MA-43 Managers Guide to Safety
MSL-A-006 Marine Sciences Laboratory Training

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EFFECTIVE DATE: 03-08-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-I-004-03

SEDIMENT EVAPORATION DIGESTION

Approvals:		
AUTHOR: Linda Bingler	Signature	3-8-00 Date
TECHNICAL REVIEWER: Eric Crecelius	Signature	3-8-00 Date
QA OFFICER: Deborah Coffey	Signature	03-08-2000 Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Signature	3-8-00 Date

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SEDIMENT EVAPORATION DIGESTION

1.0 SCOPE AND APPLICATION

This method is applicable to the digestion of sediment samples for most metals. The digestion is designed to prepare sediment samples for analysis of crustal elements, but the method can also be used for most other metals.

The final acid matrix is 1% nitric acid (HNO_3) which can be analyzed by ICP/MS, ICP-AES or GFAA.

2.0 DEFINITIONS

UHP - Ultra High Purity grade compressed gas
ICP/MS- Inductively Coupled Plasma Mass Spectrometry
GFAA - Graphite Furnace Atomic Absorption
ICP-AES - Inductively Coupled Plasma Atomic Emission Spectrometry

3.0 RESPONSIBLE STAFF

- Researcher/Technician-sample preparation, digestion
- Analyst – sample analysis
- MSL QA Officer or Representative- data verification

4.0 PROCEDURE

4.1 Hardware and Reagents

4.1.1 Hardware

- Teflon bomb, 60 mL and capping station
- Oven, capable of being maintained at 130 °C $\pm$ 10 °C
- Pipettes with trace metal free tips
- Polybottles, 20 mL acid cleaned
- Balance capable of weighing to 1 mg
- Repipettor

4.1.2 Reagents

- HNO_3
- 30% Hydrogen Peroxide (H_2O_2)
- Hydrofluoric acid (HF)
- 10% HNO_3
- Deionized (DI) water

4.2 Step-By-Step Procedure

4.2.1 Wash bombs in accordance with section 4.2.1 of procedure, MSL-C-011, Glassware and Equipment Cleaning Procedures. Record bomb ID number engraved on the Teflon bombs and the tare weight of the bomb in the sediment

preparations log book (See Attachment 1 for an example of a sample preparation sheet). Use an anti-static device to reduce static inside the Teflon bomb, if needed. One could add 0.5 mL of DI water to bomb to capture the sample and reduce static effects. Carefully weigh approximately 250 mg of homogenized sediment (prepared in accordance with procedure, MSL-C-003, Percent Dry Weight and Homogenizing Dry Sediment, Soil, and Tissue) into the bomb. Record the sample weight (g) on the data sheet.

4.2.2 Using a pipette with an acid clean pipette tip, add 5 mL of concentrated HNO_3 (trace metal grade - i.e., Baker Instra-analyzed) and 3 mL of 30% H_2O_2 (reagent grade – i.e. Baker analyzed) and 5 mL of concentrated HF (reagent grade – i.e., Baker Instra-analyzed) trying to wash all the sediment off the sides of the bomb.

If the samples are organic-rich or contain high amounts of calcium carbonate ($CaCO_3$), add just the HNO_3 and H_2O_2. Then loosely cap and warm the samples on a hot plate for a minimum of 4 hours. Using a pipette with an acid clean pipette tip, add 5 mL of concentrated HF (trace metal grade - i.e., Baker Instra-Analyzed), trying to wash all the sediment off the side of the bomb.

4.2.3 Cap the bombs using the capping station and place in the oven at 130^0 C for at least 8 hours or overnight. Allow to cool completely.

4.2.4 Uncap the bombs and remove 5 ml to a clean 20 mL poly bottle for Hg analysis. Record weight of digestate prior to removal and also the remaining. Place bombs with remaining digestate on hotplate and evaporate to dryness.

4.2.5 Add 1 mL of concentrated HNO_3, swirling to wet the residue, and heat to dryness.

4.2.6 Add 2 mL of 10% HNO_3, swirl, and heat gently for approximately 5 minutes on a hotplate.

4.2.7 Add 18 mL of deionized water through repippettor to the bombs. Cap the distillation bomb, weigh and record the digestate + tare weight on the sample preparation data sheet. Swirl the bomb to get all the digestate off the sides and transfer digestate to an acid clean polyethylene bottle. Samples are ready to be analyzed.

4.2.8 To the Hg aliquot, add 5 mL deionized water to the 5 mL digestate aliquot removed. Samples are ready for Hg analysis.

5.0 DATA ANALYSIS AND CALCULATIONS

Calculate the solution volume using the following equation:

$$V_{mL} = \frac{\text{digestate weight (g)}}{\text{density (g/mL)}}$$

where,

density = 1.01 g/mL for the final digestate

S_g = ((A – B)/ A-tare weight (g)) x sample weight (g)

where,

A = weight (g) of digestate in bomb before 4.0 mL aliquot removal
B = weight (g) of digestate in bomb after 4.0 mL aliquot removal

This gives the sample weight in the aliquot used for all metals, except mercury.

The equation:

S_{gHg} = sample weight - S_g

gives the sample weight in the aliquot used for mercury.

An example calculation for ICP/MS factor at a 10x dilution is:

$$\text{Factor} = \frac{((\text{Final Vol. (mL)} * 10)/1000)}{\text{Sample Mass (g)}}$$

6.0 QUALITY CONTROL

Procedural/reagent blanks, replicates, matrix spikes, and certified reference materials, should be run at a frequency of 1 per 20 samples or 1 per batch (if less than 20 samples). When required by the client or MSL project manager, other QC checks for accuracy and precision shall be applied to each set or batch of samples. Criteria for the relative percent difference (RPD) of the duplicates or replicates shall be ± 20%, and percent recovery for matrix spikes and certified reference materials shall be ± 25% unless otherwise specified by the customer.

7.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of acids (particularly HF), including the following:

- Gloves, protective clothing, face shields and goggles or safety glasses should be worn when handling samples and acids.
- Use an acid hood to exhaust brown gas fumes.

8.0 TRAINING REQUIREMENTS

8.1 All staff performing sediment digestions should first read this procedure and conduct their first digestion under supervision of a staff member who has had previous

experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

8.2 All staff will receive training in the handling of corrosive chemicals and the use of fume hoods. Extra caution should be taken due to the unpredictable nature of some samples during the heating process.

9.0 REFERENCES

MSL-C-003 Percent Dry Weight and Homogenizing Dry Sediment, Soil, and Tissue
MSL-C-011 Glassware and Equipment Cleaning Procedures
MSL-A-006 Marine Sciences Laboratory Training

ATTACHMENT 1
Example Sediment Sample Digestion Form

SEDIMENT SAMPLE DIGESTION

Logbook Page # _____ Analyst: _____
Project: _____ Addendum # _____ Balance # _____
CF#: _____ Sample Matrix: _____
Digestion Method: _____

Date/Time Started: _____ Date/Time Completed: _____

Bomb ID	Sample ID	Digestate + Tare wt (g)	Tare Wt. (g)	Sample Wt. (g)	
					SRM:
					ID#
					SRM:
					ID#
					SRM:
					ID#
					SRM:
					ID#

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EFFECTIVE DATE: 4-24-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-I-006-01

MIXED ACID SEDIMENT DIGESTION

Approvals:		
AUTHOR: Linda Bingler		4-24-2000
	Signature	Date
TECHNICAL REVIEWER: Brenda Lasorsa		4/24/00
	Signature	Date
QA OFFICER: Deborah Coffey		4-24-00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius		4-24-00
	Signature	Date

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MIXED ACID SEDIMENT DIGESTION

1.0 SCOPE AND APPLICATION

These methods are applicable for the determination of all metals. The aqua regia method is applicable to the digestion of sediment when it is necessary to quantify high levels of silver. The nitric acid method is applicable to the digestion of sediments and tissues for total metals when crustal levels are not requested. The nitric acid :peroxide method is used when samples contain high amounts of organic material. In this procedure, sediment samples are prepared for analysis by ICP/MS, ICP, CVAAS, or GFAA through an acid digestion which releases bound metals into solution.

This procedure replaces the following procedures:

MSL-M-094	Aqua Regia Sediment and Tissue Digestion
MSL-I-005	Hot Nitric Acid Digestion of Sediments and Tissues
MSL-I-006	Aqua Regia Sediment and Tissue Digestion
MSL-I-007	Nitric Acid and Hydrogen Peroxide Tissue and Sediment Digestion

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

- Trained technician-level or higher laboratory personnel.
- QA Officer or Representative- data verification.

4.0 PROCEDURE

4.1 Hardware and Reagents

4.1.1 Hardware

- Teflon bomb, 60 ml (Savillex, Inc.) and capping station (CEM Corp.)
- Oven maintained at 130 °C ± 10 °C
- Acid cleaned, 25 mL, glass scintillation vials
- Acid cleaned teflon caps for 25 mL vials
- Acid-cleaned teflon-coated spatula
- An electric skillet
- Pipette with acid-cleaned plastic tips
- Polybottles, 20 mL, acid-cleaned
- Hot plate
- Balance capable of weighing to 0.1 mg
- Repipette dispenser

4.1.2 Reagents

- Nitric Acid (HNO_3), concentrated (Instra-Pure for Trace Metals).
- Hydrochloric Acid (HCl), concentrated (Baker Instra-Pure for Trace Metals).
- Trace metal quality hydrogen peroxide (H_2O_2 - 30%)
- Trace metal quality deionized (DI) water

4.2 **Procedure**

The appropriate Sample Digestion form (Attachment 1 or 2) shall be used to record sample digestion information. Before weighing samples, the heading information, including the specific digestion method, should be completely filled out.

AQUA REGIA DIGESTION

4.2.1 Sample Preparation

4.2.1.1 Record bomb ID number engraved on the side of the bomb and tare weight on the appropriate Sample Digestion form (Attachment 1 or 2). Carefully weigh approximately 200 mg of finely ground, freeze-dried sediment (prepared in accordance with MSL-C-003), record as sample weight to 0.1 mg, and add to a tared, acid-cleaned Teflon bomb. If static electricity is present and causes some sample to stick to the sides of the bomb, use an anti-static device to eliminate the electricity.

4.2.1.2 Using automatic pipettes and acid-cleaned pipette tips, add 5.0 mL of HCl and 3.5 mL of HNO_3. Tighten the bombs securely using a capping station. Place the bombs in an oven maintained at 130 °C ± 10 °C overnight or for a minimum of 8 hours. CAUTION: If bombs are not tightened securely, they will leak which will lead to result errors.

4.2.1.3 Remove bombs from oven and allow to cool. Add 15 mL of reagent grade water using a repipette dispenser.

4.2.1.4 Weigh and record as digestate + tare weight. Shake the bomb vigorously and transfer the solution to a 20 mL acid-cleaned polyethylene vial. Samples are now ready for analysis.

NITRIC ACID DIGESTION

4.2.2 Sample Preparation

The appropriate Sample Digestion form (Attachment 1 or 2) shall be used to record sample numbers, sample weight, and project specific information. This digestion can be performed in glass scintillation vials or Teflon bombs.

4.2.2.1 Label a vial with the sample number and record the tare weight or record bomb ID number engraved on the side of the bomb and tare weight on the Sample Digestion form.

4.2.2.2 Using a spatula, transfer approximately 0.2 grams of dry ball-milled sediment to the vial or bomb and record the weight.

4.2.2.3 Add 5.0 mL of HNO_3 and place a Teflon cap loosely on the vial or cap the bomb and tighten with a cap tightener.

4.2.2.4 Place the vials in an acid hood on an electric skillet or place the bombs in an oven maintained at 130 °C ± 10 °C overnight or a minimum of 8 hours.

4.2.2.5 Slowly heat the samples in vials by increasing power with the skillet control knob. DO NOT allow samples to foam over or go to dryness during this or any other step.

4.2.2.6 Continue heating the samples at a controlled rate for another 4 hours or until the brown gas cloud disappears.

4.2.2.7 Turn the skillet "off " and allow the samples to cool for 1 hour or overnight. If using Teflon bombs, remove the bombs from the oven and allow to cool.

4.2.2.8 Remove the Teflon cap, dilute the samples with deionized water from a repipette dispenser to the bottom of the neck of the vial or add 20 mL of reagent grade water to the bomb. Replace the Teflon cap and record the final weight. The samples are now ready to be analyzed.

NITRIC ACID:PEROXIDE DIGESTION

4.2.3 Sample Preparation

The appropriate Sample Digestion form (Attachments 1 or 2) shall be used to record sample numbers, sample weight, and project specific information.

4.2.3.1 Label a vial with the sample number and record the tare weight on the Sample Digestion form.

4.2.3.2 Using a spatula, transfer approximately 0.2 grams of dry ball-milled sediment to the vial and record the weight.

4.2.3.3 Add 5.0 mL of HNO_3 and place a Teflon cap loosely on the vial.

4.2.3.4 Place the vials in an acid hood on an electric skillet.

4.2.3.5 Slowly heat the samples by increasing power with the skillet control knob. DO NOT allow samples to foam over or go to dryness during this or any other step.

4.2.3.6 Continue heating the samples at a controlled rate for another 4 hours or until the brown gas cloud disappears.

4.2.3.7 Turn the skillet "off " and allow the samples to cool for 1 hour or overnight.

4.2.3.8 Remove the cap and add 3 mL of 30% hydrogen peroxide and replace the cap.

4.2.3.9 Repeat steps 4.2.3.4 through 4.2.3.7. Samples may foam up considerably. Take precautions to minimize foaming such as adding peroxide in 1 mL increments and heating just slightly between additions.

4.2.3.10 Remove the Teflon cap, dilute the samples with deionized water from a repipette dispenser to the bottom of the neck of the vial. Replace the Teflon cap and record the final weight. The samples are now ready to be analyzed.

5.0 DATA ANALYSIS AND CALCULATIONS

Calculate the solution volume using the following equation.

$$V_{mL} = \frac{\text{digestate weight (g)}}{\text{density (g/mL)}}$$

where,

density = 1.09 g/mL for the aqua regia final digestate
density = 1.12 g/mL for the HNO_3 final digestate
density = 1.08 g/mL for the HNO_3:H_2O_2 final digestate

6.0 QUALITY CONTROL

Procedural/reagent blanks, duplicates, matrix spikes, and certified reference materials, should be run at a frequency of 1 per 20 samples or 1 per batch (if less than 20 samples). When required by the customer or MSL project manager, other QC checks for accuracy and precision shall be applied to each batch of samples.

7.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including all safety protocols regarding use of acids, including the following:

- Gloves, protective clothing and safety glasses, goggles, or face shield should be worn when handling samples and acids.
- Use an acid hood to exhaust brown gas fumes.

8.0 TRAINING REQUIREMENTS

8.1 All staff performing sediment digestions should first read this SOP and conduct their first digestion under supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

8.2 All staff should have received training in the handling of corrosive chemicals and the use of fume hoods. Extra caution should be taken due to the unpredictable nature of some samples during the heating process.

9.0 REFERENCES

MSL-C-003 Percent Dry Weight and Homogenizing Dry Sediment, Soil, and Tissue
MSL-A-006 Marine Sciences Laboratory Training.

ATTACHMENT 1

EXAMPLE SEDIMENT SAMPLE DIGESTION

Date:

Logbook Page #

Analyst:

Project:

Addendum #

Balance #

CF #

Sample Matrix:

Digestion Method

SOP #

Bomb ID	Sample ID	Final digestate weight (g)	Post aliquot removal weight (g)	Digestate + Tare Wt (g)	Tare Wt. (g)	Sample Wt. (g)	
							SRM:
							ID#
							SRM
							ID#
							SRM
							ID#
							REAGENT
							LOT#
							REAGENT
							LOT#
							REAGENT
							LOT#
							Comments:
Analyst:			Peer Reviewer:				

Attachment 2

EXAMPLE TISSUE SAMPLE DIGESTION

Date:

Logbook Page #

Analyst:

Proje ct:

Addendum #

Balance #

CF #

Sample Matrix:

Digestion Method

SOP #

Bomb ID	Sample ID	Digestate + Tare wt (g)	Tare Wt. (g)	Sample Wt. (g)

SRM:

ID#

SRM

ID#

SRM

ID#

REAGENT

LOT#

REAGENT

LOT#

REAGENT

LOT#

Comments:

Analyst:_____

Peer Reviewer:_____

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EFFECTIVE DATE: 11-19-99

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-I-013-02

TOTAL MERCURY IN AQUEOUS SAMPLES BY COLD VAPOR ATOMIC FLUORESCENCE

Approvals:		
AUTHOR: Brenda Lasorsa	Signature	11/19/99 Date
TECHNICAL REVIEWER: Mary Ann Deuth	Signature	11/19/99 Date
QA OFFICER: Deborah Coffey	Signature	11/19/99 Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Signature	11-19-99 Date

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TOTAL MERCURY IN AQUEOUS SAMPLES BY COLD VAPOR ATOMIC FLUORESCENCE

1.0 SCOPE AND APPLICATION

This is a peer-reviewed, published procedure and is applicable to the determination of total mercury in aqueous samples. It is a modification of EPA Method 1631, which was largely developed at the Battelle Marine Sciences Laboratory (MSL). The modification of Method 1631 is limited to allowing for calibration using either the linear regression or response factor method. All samples must be subjected to bromium chloride (BrCl) oxidation prior to analysis.

2.0 SUMMARY OF METHOD

The method is a cold vapor atomic fluorescence technique, based upon the emission of 254 nm radiation by excited mercury (Hg^+) atoms in an inert gas stream. Mercuric ions in the oxidized sample are reduced to Hg^+ with stannous chloride ($SnCl_2$) and then purged onto gold-coated sand traps as a means of preconcentration and interference removal. Mercury vapor is thermally desorbed to a second "analytical" gold trap, and from that into the fluorescence cell. Fluorescence (peak area) is proportional to the quantity of mercury collected, which is quantified using a standard curve as a function of the quantity of sample purged.

Typical detection limit for the method is 0.100 ng/L as Hg.

3.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

3.1 Samples should be collected in Teflon bottles. All Teflon bottles must be washed and then boiled in concentrated HNO_3 for 48 hours. Vessels are thoroughly rinsed in tap water shown to contain negligible concentrations of mercury, then filled with 1% HCl in tap water and heated to 65°C±5°C for 12 hours. This water is poured off and the vessels are refilled with 1% HCl and stored full until use. Just prior to use, vessels are emptied and dried in a clean drying oven at 65°C±5°C. Vessels to be shipped are packed in clean polyethylene bags. Samples can be collected in acid-cleaned borosilicate or quartz glass bottles with Teflon lids. Under no circumstances should polyethylene, polypropylene, or vinyl containers be used.

3.2 Sample bottles should be rinsed once with sample water, and then filled. Samples should be preserved with 0.5% by volume of high purity HCl or refrigerated at 4°C±2°C until they can be acidified in the clean room, but not frozen.

4.0 DEFINITIONS

4.1 Atomic Fluorescence - Detection based on fluorescent emission from excited atoms of a particular element at a characteristic wavelength. The amount of fluorescence, quantified by integration of peak area, is proportional to the

concentration of the element of interest.

4.2 Acid-cleaned - Cleaned in nitric acid of the highest concentration and temperature which can be withstood by the item being cleaned. New glass and Teflon containers are boiled in concentrated nitric acid for 48 hours.

5.0 POTENTIAL INTERFERENCES

5.1 Due to the strong oxidation step, followed by dual gold amalgamation, there are no observed interferences with the method. The potential exists for destruction of the gold traps (and consequently low values) if free halogens are purged onto them or if they are overheated (>500°C). When these instructions are followed, neither of these problems is likely to occur.

5.2 Water vapor may collect on the gold traps, and be released on to the fluorescence cell where it condenses, giving a false peak due to scattering of the excitation radiation. This can be avoided by pre-drying the gold trap and by discarding traps which tend to absorb large quantities of water vapor.

5.3 As always with atomic fluorescence, the fluorescence intensity is strongly dependent upon the inertness of the carrier gas. The dual amalgamation technique eliminates quenching due to trace gases, but it still remains the analysts responsibility to ensure high purity inert carrier gas and a leak-free analytical system.

6.0 RESPONSIBLE STAFF

Analyst - analysis, calculations
QA Officer or Representative - data verification

7.0 APPARATUS AND REAGENTS

7.1 Apparatus

7.1.1 Cold Vapor Atomic Fluorescence Spectrophotometer (CVAF). The components of this detector include a four-watt low pressure mercury vapor lamp, a far UV quartz flow-through fluorescence cell (12 mm x 12 mm x 45 mm) with a 10 mm path length, and a UV-visible photomultiplier, sensitive to <230 nm isolated from outside light with a 254 nm interference filter. The carrier gas flow is controlled using a flowmeter with needle valve capable of keeping a constant flow of 25 ml/min.

7.1.2 Flowmeter/needle Valves. Flowmeter capable of controlling and measuring gas flow to the purge vessel at 200-500 ml/min.

7.1.3 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.

7.1.4 Acid Fume Pretrap. A 10 cm x 0.9 cm diameter Teflon tube containing 2-3 g of reagent grade, non-indicating 8-14 mesh soda lime, packed between silanized glass wool plugs. This trap is purged of Hg by placing it on the output of a clean cold vapor generator containing deionized water and 1 ml $SnCl_2$ and purging for 30 min. with N_2 at 100 ml/min.

7.1.5 Cold Vapor Generator ("Bubbler"). A 250 ml or 125 ml Florence flask with standard taper 24/40 neck, fitted with a purging stopper having a coarse glass fit which extends to within 0.2 cm of the flask bottom. Silicon stopcock grease can be used to insure a leak-proof seal. Normally a set of 3 or 4 generators are used concurrently.

7.1.6 Gold-coated Sand Column. Made from 10 cm lengths of 6.5 mm O.D. x 4 mm I.D. quartz tubing, with a coarse quartz frit or crimp 2.0 cm from one end. The tube is filled with 3.4 cm of gold-coated ashed (800°C for 6 hours) quartz sand (60/80 mesh). The end is then plugged with quartz wool. Gold is applied to the sand as a coating several atoms thick using an ion exchange gilding apparatus. The columns are heated to 450-500°C with a coil consisting of 24 gauge nichrome wire at a potential of 10 VAC.

7.1.7 Oxidation Bottles. Acid-cleaned, 125 ml Teflon bottles and caps.

7.1.8 Pipetters. All plastic pneumatic fixed and variable volume pipetters in the range of 5μl to 5 ml (calibrated).

7.1.9 Recorder. Multi-range chart-recorder/integrator with 0.1 - 5.0 mV input and variable speed.

7.2 Reagents

7.2.1 Water. Deep well tap water which has been determined to be very low (<0.02 ng/l) in mercury or deionized water (18 megaohm•cm or better). The water is routinely monitored for mercury through the analysis of blanks.

7.2.2 10% Stannous Chloride ($SNCl_2$). A solution containing 100 grams of $SnCl_2 \bullet 2H_2O$ and 100 ml of concentrated HCl, brought to a final volume of 1 liter with mercury-free water. This solution is purged a minimum of 6 hours with mercury-free nitrogen at 500 ml/min to remove all traces of mercury. Store tightly capped and in the dark.

7.2.3 Bromine Monochloride (BrCl). 27 g of KBr are added to a 2-liter bottle of concentrated HCl (<5 ng/l Hg). A clean magnetic stir bar (Teflon-coated) is placed in the bottle and the solution is stirred for one hour in a fume hood. Next, 38 g of pre-analyzed, low Hg KBr03 are slowly added to the acid as stirring continues. **CAUTION:** This process should always be carried out in a fume hood. The fumes from this reagent are very corrosive and a strong irritant. When all of the KBr03 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.

7.2.4 30% Hydroxylamine Hydrochloride. Dissolve 150 g of $NH_2OH \bullet HCl$ in low Hg water to make 500 ml.

7.2.5 Stock Mercury Standard. A commercially available 10,000 ng Hg/ml atomic absorption standard is used.

7.2.6 Working Standard A. 0.1 ml stock standard is diluted with Hg-free water containing 0.5% by volume BrCl, to a final volume of 100 ml in a Teflon bottle. This solution contains 10 ng/ml of Hg and should be restandardized or replaced annually.

7.2.7 Working Standard B. Dilute 1.0 ml of working standard to 100 ml with Hg-free water containing 0.5% (by volume) BrCl, using a 100 ml class A volumetric flask. This solution has a [Hg] 0.1 ng/ml and should be replaced semi-annually.

7.2.8 Nitrogen. Grade 4.5 nitrogen which has been further purified by the removal of Hg using an in-line gold coated sand trap.

7.2.9 Helium or Argon. Grade 5.0 inert gas which has been further purified by the removal of Hg using an in-line gold coated sand trap.

8.0 PROCEDURE

8.1 Sample Preparation

8.1.1 Place a 100±5 ml aliquot of the acidified sample in a 125 ml Teflon prep bottle, either by a pipette or by weight difference, recording the volume on a total mercury digestion worksheet (see Attachment 1). Shake the sample container well prior to poring or pipetting an aliquot into the prep bottle. Add 0.5 ml of BrCl reagent to each sample, and place the bottle in the clean air hood for a minimum of 12 hours.

8.2 Calibration

8.2.1 Add 75 ml low Hg tap water or deionized and 1 ml $SnCl_2$ solution to each bubbler and purge for 20 minutes with soda lime trap attached. Place

blanked gold traps on soda lime trap. Purge for 25 minutes and analyze as outlined in Section 8.3.2. The peak area unit value of these blank traps constitute the bubbler blank. Place clean gold traps on each soda lime trap and run sequential aliquots between 5 and 500 μl of working standard A (0.02 to 5 ng.) and 200 µl of working standard B. This is the normal linear range, however for most waters a smaller calibration range of 0.05 to 1 ng will suffice. Run a minimum of four sequential aliquots in addition to the blanks.

8.3 Analysis

8.3.1 Add 75 ml of low Hg water to each bubbler, followed by 1 ml of $SnCl_2$ solution. The bubbler is purged with N_2 at 350 ml/min for 10 minutes. Just prior to analysis, add 0.250 ml $NH_2OH \bullet HCl$, or half volume of $NH_2OH \bullet HCl$ as BrCl, to the samples and let react for 5 minutes. The yellow color should disappear as the $NH_2OH \bullet HCl$ reacts with the BrCl. To analyze samples, attach a fresh gold-coated sand trap to the soda lime trap, then add 50±5 ml of digestate to each bubbler (by weight difference). On a mercury datasheet (Attachment 2), record 1) the sample number and, 2) the weight of the sample bottle before and after adding an aliquot to the bubbler. The sample is then purged for 25 minutes. New samples may be sequentially added to the bubblers, up to a maximum of 3 consecutive samples. After 3 samples, rinse the bubblers with clean low Hg water and repeat the above sequence.

8.3.2 To analyze the mercury contained on the gold trap, the gold trap is placed inside a coil of nichrome wire and then inserted in-line with a mercury analyzer, incoming Hg-free He, and the second (analytical) gold-coated sand trap, which is permanently wound with nichrome wire. The He flow rate should be about 30 ml/min. 10 VAC is applied to the nichrome coil on the working gold-coated trap for 4 minutes, thermally desorbing the Hg as Hg° which is then resorbed by the analytical gold-coated sand column. The voltage to the working gold-coated sand trap is turned off, and a cooling stream of compressed air is directed towards the trap. 10 VAC is applied to the analytical gold-coated sand trap, and the integrator is activated. The analytical trap is heated for 2.0 minutes, or 1 minute beyond the point where the mercury peak returns to baseline.

8.3.3 Following the integration of the mercury peak, the voltage to the analytical trap is turned off. A stream of cooling air is directed towards the analytical trap. The sample gold-coated sand trap is removed from the gas stream, and the Teflon end plugs are replaced until it is needed to collect another sample. The next gold-coated sand trap containing a sample is placed inside the nichrome wire coil, placed in-line with the mercury analyzer incoming Hg-free, and the procedure is repeated. Under no circumstances should a sample gold-coated sand trap be

heated while the analytical column is still warm, or analyte may be lost by passing through the analytical column.

8.3.4 Peaks generated using this technique should be very sharp and almost symmetrical. The peak comes off at approximately 1 minute and has a half-height width of about 5 seconds. Broad or asymmetrical peaks are indicative of an analytical problem possibly including: low gas flow, water vapor on the column, or an analytical column damaged by chemical fumes or overheating. If the analytical column has been damaged, replace the column and the tubing downstream due to the possibility of gold migration on the downstream surfaces.

8.3.5 Cold vapor atomic fluorescence for mercury is linear over at least five orders of magnitude (Bloom and Fitzgerald, 1988). This method is virtually interference free, so the method of standard additions is not needed. To run a check standard, an aliquot of working standard solution in the range of 0.5 ng Hg is pipetted into a purged bubbler containing 1.0 ml of $SnCl_2$ solution, and analyzed as one would a sample.

8.3.6 Gold-coated sand traps should be kept track of by unique identifiers, so that any trap producing poor results can be quickly recognized and discarded. Occasionally, due to inadvertent contact with halogen fumes, bubbler solution, organic fumes, or overheating, a sample gold-coated sand trap may become damaged, giving low and irreproducible results. Suspect gold-coated sand traps should be checked with at least two consecutive standard runs before continued used.

8.3.7 The major cause of analytical problems with this method is from using the soda lime pretraps too long. These traps should be used for only one day's analytical work. Longer use risks irreproducibility, as the traps may begin retarding the flow of Hg°. Also, as the traps become very wet there is a risk of NaOH-saturated water drops coming off onto the gold-coated sand traps.

8.4 Instrument Maintenance.

8.4.1 This system requires very little preventative maintenance. Simple observations on the part of the analyst will tell when parts such as the lamp, cuvette, analytical trap and sample traps require cleaning or replacement.

- The bubblers should be stored with a 1% HCl solution when not in use.
- The soda lime traps must be changed every day and purged for a minimum of 20 minutes prior to loading the calibration curve. Low results from one particular bubbler indicate a leak in the soda lime trap, in which case the Teflon trap jacket should be replaced.

- Slight drops in sensitivity over the course of the day which seem to recover overnight indicate that the analytical trap needs to be replaced. This occurs about once every six months if the system is in constant use.

- Slight drops in sensitivity over the course of the day which do not recover overnight indicate that the lamp needs to be re-aligned or replaced. This occurs about once every six to twelve months. Follow the instructions in the manual for the Tekran detector to re-align the lamp. If this does not improve the sensitivity, replace the lamp.

- Erratic results that follow no obvious pattern indicate that the sample traps need to be replaced. This can happen any time, depending on the type of samples being analyzed. Under normal conditions, however, the traps can last a year or more.

- An upward drift in the baseline or increase baseline noise may indicate a dirty cuvette. Follow the instructions in the manual for the Tekran detector to replace it. The cuvettes can be cleaned in the hot nitric acid bath and re-used. This happens very infrequently, unless soda lime traps have been allowed to become saturated and moisture gets into the detector.

- The gold traps used to scrub the carrier gas should be burned off once a year or more frequently if poor quality carrier gas is encountered.

9.0 QUALITY CONTROL

All data quality objectives are summarized in Table 1.

9.1 Quality assurance data must be composed of a minimum of 3 bubbler blanks, 1 analytical blank, and 1 certified reference material (or 1 on-going precision and recovery (OPR)) sample. Such checks should be run at least once per day, or every 20 samples, whichever is first.

9.2 Samples containing high analyte concentrations may be run either following dilution, or on a separate run at a lower instrumental sensitivity.

9.3 Duplicate or triplicate analyses (depending upon client preference) should be run once every 20 samples or once per batch, whichever comes first.

9.4 No certified materials exist for Hg in water near ambient levels. National Institute of Standards and Technology (NIST)- certified standard materials for mercury in water should be diluted a minimum of 1:200 for analysis. An ongoing precision and recovery (OPR) sample may also be prepared by spiking tap or reagent water at 5 ng/liter.

9.5 Matrix spikes should be run once per 20 samples or once per batch, whichever comes first.

9.6 Continuing calibration verification standards should be analyzed every 10 samples at a concentration approximately the average sample concentration. CCV should be within 15% of the initial calibration.

9.7 Reagent blanks are determined by adding aliquots of BrCl and NH_2OH directly to the bubbler and purging them directly on to the gold trap. The same volumes of reagents that were added to the sample should be used and then the resulting nanogram peak response can be divided by the average sample volume in ml (usually 100 ml) and multiplied by 1000 to convert to a ng/liter reagent blank.

Table 1 Data Quality Objectives

QA Sample Type	Frequency	Acceptance Limit	Corrective Action
Laboratory Reagent Blank	1 per batch of 20 or fewer	<5 times the MDL	Reanalyze. If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the batch must be redigested.
Replicate Precision	1 per batch of 20 or fewer	≤ 21% for analytes >3 times the MDL No more than 35% of all RSDs can be > 21%[c]	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple DQO's requires redigestion and reanalysis of batch.
Certified Reference Material (CRM) or Standard Reference Material (SRM); Ongoing Precision and Recovery	1 per batch of 20 or fewer	77-123% of certified value	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple CRMs requires redigestion and reanalysis of batch.
Matrix Spike (MS) /Matrix Spike Duplicate (MSD)	1 per batch of 20 or fewer	71-125% recovery <24% RPD	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple MSs requires redigestion and reanalysis of batch.
Initial and Continuing Calibration Verification	every 10 samples	<15% of initial calibration on	Reanalyze. If CCV still fails, rerun the calibration curve and calculate all samples analyzed after the last passing calibration check using the new curve.

10.0 CALCULATIONS

10.1 Linear Regression Method: a regression line is plotted using the bubbler blank-

corrected peak area for each calibration point versus its concentration (in ng). Calculate the recovery for the lowest standard (0.05 ng) using slope. If the recovery of the lowest standard is in the range of 75-125% and the r^2 of the line is better than 0.997,the calibration is acceptable Sample concentrations are then calculated using the following equation:

$$[Hg]\ ng/L = \frac{(\text{sample peak area - blank area})}{(\text{slope} * v)}$$

(Where [Hg] is the Hg concentration in ng/L, blank area is the bubbler blank area, slope is the slope of the standard regression line in area/ng, and v is the sample volume analyzed.)

The final sample concentrations must be corrected for dilution by reagents and reagent blank as follows:

[Hg]ng/L = ([Hg]ng/L(analytical) * (total volume/initial volume)) - Blank

where: total volume = volume of sample + reagents, initial volume = volume of sample only, and Blank = reagent blank in ng/L as determined in Section 9.7

10.2 Calibration Response Factor Method: For each calibration point, calculate the calibration factor (CFx) by dividing the bubbler-blank-subtracted peak height or area by the concentration of the standard added (in ng). Calculate the mean calibration factor (CFm), the standard deviation of the calibration factor (SD), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CFm and calculate the recovery for the lowest standard (0.05 ng) using CFm. If the RSD is less than or equal to 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CFm may be used to calculate the concentration of Hg in samples. Sample concentrations are then calculated using the following equation:

$$[Hg]\ ng/L = \frac{(\text{sample peak area - blank area})}{(CFm * v)}$$

(Where [Hg] is the Hg concentration in ng/L, blank area is the average bubbler blank area, CFm is mean calibration factor in area/ng, and v is the sample volume analyzed.)

The final sample concentrations must be corrected for dilution by reagents and reagent blank as follows:

[Hg]ng/L = ([Hg]ng/L(analytical) * (total volume/initial volume)) - Blank

where: total volume = volume of sample + reagents, initial volume =

volume of sample only, and Blank = reagent blank in ng/L as determined in Section 9.7

10.3 Method Detection Limit (MDL):

10.3.1 Calculation from IDL:

The IDL is calculated by recording the results of a standard analyzed seven times, whose concentration is within 10 times the minimum peak detected.

IDL [Hg] ng = SD * t(0.01(1)(n-1))

(Where SD is the standard deviation of the [Hg] ng of the standards analyzed multiplied by the Student T value for 99% one tailed confidence interval with n-1 degrees of freedom.)

Method Detection Limit [Hg] ng/L = MDL/sample volume (L)

(Where MDL is the method detection limit [Hg] ng/L)

10.3.2 Direct determination:

The MDL is calculated based on seven to ten replicates of a low level digested sample where

$$MDL = SD([Hg]ng/L) * t_{(0.01(1)(n-1))}$$

11.0 SAFETY

The toxicity or carcinogenicity of each chemical used in this procedure 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. Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability and nervousness. Organo-mercurials may cause permanent brain damage.

Because of the toxicological and physical properties of Hg, pure standards should be handled only by trained personnel who are thoroughly familiar with handling and precautionary procedures and who understand the associated risks. It is recommended that the analyst purchase a dilute standard solution of the Hg (1,000 to 10,000 µg/ml) to avoid the necessity to handle highly concentrated solutions or pure standards. If primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn when high concentrations are handled.

A reference file of material safety data sheets (MSDSs) must be made available to all personnel involved in these analyses. Samples suspected to contain high concentrations of Hg are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. All analysts following this procedure should be aware of routine laboratory safety concerns. Protective clothing and eyeglasses should be worn when appropriate.

12.0 TRAINING REQUIREMENTS

All staff performing total mercury in water must first read this SOP and then demonstrate proficiency in the process prior to performing any project work. Proficiency may be documented through the successful analysis of certified reference materials, performance evaluation (PE) samples, or matrix spike/matrix spike duplicates pairs. Documentation of training will be recorded on a training assignment form obtained from MSL-A-006, Marine Sciences Laboratory Training.

13.0 REFERENCES

Bloom, N.S. 1983. "Determination of Silver in Marine Sediments by Zeeman-Corrected Graphite Furnace Atomic Absorption Spectroscopy." Atomic Spectroscopy. 4:204.

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Bloom, N.S., and E.A. Crecelius. 1983. "Determination of Mercury in Seawater at Subnanogram per Liter Levels." Mar. Chem. 14:49.

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

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

MSL-A-006 Marine Sciences Laboratory Training

Attachment 1

Example Total Mercury Digestion Worksheet

PROJECT ID: ____________________ **SPIKING STANDARD NUMBER:** ____________

DIGESTION DATE: ______________ **SPIKE CONCENTRATION:** ______________

ANALYST: ______________________ **SPIKE VOLUME:** ______________________

BALANCE ID#: __________________ **STANDARD:** __________________________

BrCl Volume: ____________________ **NH2OH-HCl Volume:** ____________________

:

SAMPLE ID	BOTTLE WT grams	BOTTLE + SAMPLE grams	SAMPLE WT grams	SAMPLE VOL ml	TOTAL VOL ml	(SPIKE) ng/l

ATTACHMENT 2

MERCURY DATA SHEET

TYPE: ER TOTAL METHYL

DATE: _____

ANALYST: _____

PROJECT NAME(S): _____

_____

CF ID#s: _____

CALIBRATION FACTOR: _____

Y-INTERCEPT or BUBBLER BLANK: _____

CALIBRATION TYPE: _____

R-SQUARED: _____

MATRIX TYPE: WATER SEDIMENT TISSUE

REPORT#	SAMPLE ID	BUBBLER	SAMPLE VOLUME	UNITS	[Hg]	COMMENTS

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Marine Sciences Laboratory

EFFECTIVE DATE: 7-13-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-I-015-05

METHYLMERCURY IN TISSUES AND SEDIMENTS BY COLD VAPOR ATOMIC FLUORESCENCE (CVAF)

Approvals:		
AUTHOR: Brenda Lasorsa	Brenda Lasorsa	7/12/00
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	Signature	Date
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	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E A Crecelius	7-13-00
	Signature	Date

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METHYLMERCURY IN TISSUES AND SEDIMENTS BY COLD VAPOR ATOMIC FLUORESCENCE (CVAF)

1.0 SCOPE AND APPLICATION

This is a peer-reviewed, published procedure (Bloom, 1989) for the determination of methylmercury in a wide range of biological and geological matrices. Tissue samples are digested with potassium hydroxide (KOH)/methanol (MeOH) reagent prior to analysis and sediment/soil samples are distilled or extracted into a clean water matrix.

The quantification method is a cold vapor atomic fluorescence technique, based upon the emission of 254 nm radiation by excited mercury (Hg) atoms in an inert gas stream. The samples are homogenized and then digested or distilled. An ethylating agent is added to the digestate/distillate/extract to form a volatile methyl-ethylmercury derivative, and then purged onto graphitized carbon traps as a means of preconcentration and interference removal. The sample is then isothermally chromatographed, pyrolitically broken down to elemental mercury, and detected using a cold vapor atomic fluorescence detector.

Typical detection limits for the method are 0.001 µg/g (1 ppb) for tissues and 0.02 ng/g (0.02 ppb) for sediment.

2.0 DEFINITIONS

Atomic Fluorescence - Detection based on fluorescent emission from excited atoms of a particular element at a characteristic wavelength. The amount of fluorescence, quantified by integration of peak height, is proportional to the concentration of the atom of interest.

Acid-cleaned - Cleaned in nitric acid of the highest concentration and temperature which can be withstood by the item being cleaned. Glass and Teflon containers are boiled in concentrated nitric acid for 48 hours, rinsed and then filled with 0.5% hydrochloric acid (HCl) and warmed at 60°C overnight.

3.0 RESPONSIBLE STAFF

Researcher/Technician - sample preparation, digestion.
Analyst - analysis, calculations
QA Officer or Representative - data verification

4.0 PROCEDURE

4.1 Sample Collection, Preservation and Handling

4.1.1 Sediment and tissue samples should be collected into acid-cleaned Teflon, polystyrene, or glass bottles with Teflon-lined lids. Tissue samples should be collected into acid-cleaned

Teflon, glass, or methacrylate jars with Teflon-lined lids. Under no circumstances should polyethylene, polypropylene, or vinyl containers be used. All Teflon vials and glass jars are washed and then boiled in concentrated nitric acid (HNO_3) for 48 hours. Vials and jars are thoroughly rinsed in tap water shown to contain negligible concentrations of methylmercury and then stored in the clean room until use. Polystyrene jars are soaked in 10% HNO_3 for 72 hours and triple-rinsed in deionized water. Alternatively, glass containers can be purchased pre-cleaned to Environmental Protection Agency (EPA) specifications by the vendor.

4.1.2 Tissue samples shall be pre-frozen and shipped in such a manner that they remain frozen upon arrival at Battelle. Samples are transferred to a <-68° C freezer for 24 hours and then freeze-dried. Unless samples have been freeze-dried, they are to be frozen at <-5°C until use. A maximum holding time of 1 year at <-5°C is recommended. If percent moisture data are required or data are to be reported on a dry weight basis, then tissue samples should be collected in a pre-weighed container and then reweighed prior freezing so that percent moisture can be determined. Sediment sample vials should be filled so as to leave no headspace if the samples are to be analyzed within 48 hours of collection. If samples are to be stored for longer periods, the sample vials should be filled two-thirds full and then frozen at a temperature of at least <-5°C. Overfilling the sample container will cause the sample container to break upon freezing, rendering the sample useless.

4.1.3 All dissection, homogenization, and other handling of the samples should be done by gloved personnel in a clean station. All dissection utensils should be made of stainless steel and soap and water washed prior to use. Dissect and/or homogenize at least 10 grams of sample.

4.2 Apparatus and Reagents

4.2.1 Apparatus

Cold Vapor Atomic Fluorescence Spectrophotometer (CVAF): The components of this detector include a four-watt low pressure mercury vapor lamp, a far UV quartz flow-through fluorescence cell, (12 mm X 12 mm X 45 mm), with a 10 mm path length, and a UV-visible photomultiplier, sensitive to <230 nm, isolated from outside light with a 254 nm interference filter. The carrier gas flow is controlled using a flowmeter with needle valve capable of keeping a constant flow of 25 ml/min.

Flowmeter/needle valve: Flowmeter capable of controlling and measuring gas flow to the purge vessel at 200-500 ml/min.

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.

Carbo trap®: Made from 12 cm lengths of 7.0 mm O.D. X 5.0 mm I.D. quartz tubing, with a coarse quartz frit or crimp 3.0 cm from one end. Traps should be ashed, acid-cleaned, and silanized before use. The trap is filled with 6.0 cm of 45 mesh Carbo trap® (Supelco) graphitized carbon substrate and then plugged with silanized glass wool.

Cold vapor generator: A 250 mL or 125 mL Florence flask with standard taper 24/40 neck, fitted with a purging stopper having a coarse glass frit which extends to within 0.2 cm of the flask bottom.

Gold-coated sand column: Made from 10 cm lengths of 6.5 mm O.D. X 4 mm I.D. quartz tubing, with a coarse quartz frit or crimp 2.0 cm from one end. The tube is filled with 3.4 cm of gold-coated ashed (800°C for 6 hours) quartz sand (60/80 mesh). The end is then plugged with quartz wool. Gold is applied to the sand as a coating several atoms thick using an ion exchange gilding apparatus. The columns are used as purifying pretraps on the carrier gas.

GC system: A one meter long coiled glass column (1/4" OD, 4 mm ID) packed with 15% OV-3 on 60/80 Chromosorb WAW/DMCS (Supelco) held at 120°C in a small bench-top GC oven. In-flow and out-flow ports are 1/4" Teflon fittings. The in-flow end is plumbed with a 1/4" Teflon tube to accept connection of carbotraps. Connected to the outflow is the pyrolytic breakdown column, which consists of a 20 cm quartz tube (1/4" OD, 4 mm ID) with the center 8 cm packed with quartz wool and wound with heavy gauge (18 g) nichrome wire. Current is applied to the wire so that it glows deep orange and maintains a temperature of about 700°C. This column is kept at temperature throughout the analysis.

Distillation apparatus: 1) A solid aluminum heating block with 10-20 wells milled to fit 60 mL Teflon vials. This block must be temperature controlled to maintain a constant, even 125°C. 2) Ice baths to hold receiving vessels at 2°C.

Distillation vials: 25.6 mL Teflon vials with caps having 1/8" in- and out-flow ports. These ports are threaded with 1/8" tubing such that one port is open and the other contains a section of tubing that extends from the floor of the vial to about 5" outside the vial.

Digestion vials: Acid-cleaned, 22 mL Teflon or glass vials.

Centrifuge tubes: 40 mL teflon; acid cleaned.

Pipettes: All plastic pneumatic fixed and variable volume pipettes in the range of 5 µL to 10 mL. (calibrated).

Recorder: Multi-range chart-recorder/integrator with 0.1- 5.0 mV input and variable speed.

4.2.2 **Reagents**

Water: Well water which has been determined to be very low (<0.02 ng/L) in mercury. The water is continuously monitored for mercury by analyzing blanks. Alternatively, Barnstead, Milli-Q, or any other deionized (DI) water that has been tested for methylmercury content may be used.

25% KOH/MeOH: With constant stirring, carefully add 125 grams of pelleted KOH to 500 mL low mercury (<10 ng/L Hg) MeOH in a 500 mL Teflon bottle. Use caution...this mixture is exothermic.

8 M H_2SO_4: Dilute 448 mL concentrated sulfuric acid (H_2SO_4) to 1 liter with deionized water.

20% Potassium chloride (KCl): Dissolve 10 grams KCl in 100 mL deionized water.

Potassium Bromide (KBr) Solution: Dissolve 180 grams reagent grade KBr (Baker Analyzed best) in 50 mL H_2SO_4 and dilute to 1000 mL with reagent water. Add 0.2 g reagent grade NH_2OH HCl as a preservative. Keep this solution refrigerated and in the dark. Discard if the solution develops a yellow color.

1M Copper sulfate ($CuSO_4$) Solution: Dissolve 25 $CuSO_4$ in 100 mL reagent water. This reagent has an indefinite shelf life at room temperature.

Methylene Chloride (CH_2Cl_2): B&J Brand or Fisher HPLC Grade. It is essential that this reagent NOT contain any sort of stabilizer, as these can interfere with the analysis.

1% Sodium tetraethylborate solution (ethylating reagent): Dissolve 1 g sodium tetraethylborate in 100 mL of low mercury tap water. Pour this solution into 20 mL Teflon bottles and freeze until needed.

Methylmercury Primary Standard Solution: Dissolve 1.252 grams of reagent grade methylmercury chloride in isopropanol, dilute to exactly 1.00 L with isopropanol. This solution contains 1000 mg/L (as Hg) methylmercury. This solution is stable indefinitely when stored in an amber glass bottle with a Teflon lid at room temperature. Do not keep this concentrated stock solution in the trace mercury laboratory. Alternatively, this standard may be purchased from a chemical supply house and tested for titre.

Methylmercury Secondary Standard Solution. Dilute 500 µL of primary standard solution to exactly 250 mL with low Hg water preserved with 0.5% acetic acid and 0.2% hydrochloric acid. This solution contains 2.0 µg/ml methylmercury. The secondary standard solution is stable indefinitely when stored in a Teflon bottle and refrigerated.

Working Standard Solutions. Dilute 0.020 to 0.400 mL of the 2 µg/mL mercury standard to 500 mL with Hg-free water preserved with 0.5% acetic acid and 0.2% hydrochloric acid. These solutions contain 0.08 to 1.6 ng/mL methylmercury. These concentrations are only suggestions and are only meant to suggest an appropriate calibration range. A minimum of two different standards should be used to calibrate the instruments, as there is no source of additional calibration materials for use in calibration verification. This solution should be made on an annual basis. Alternatively, the titre of the standards can be checked annually by analyzing them for total Hg and labile mercury and calculating the MeHg concentration as total Hg – labile Hg.

Helium or argon: Grade 5.0 inert gas which has been further purified by the removal of Hg using an in-line gold coated sand trap.

Acetate buffer: Dissolve 272 grams of reagent grade sodium acetate and 118 mL of reagent grade glacial acetic acid in low Hg water. The final volume should be 1 liter. This solution has an indefinite shelf life when stored in a Teflon bottle at room temperature.

3 Potential Interferences

4.3.1 The tissue digestate interferes with the ethylation reaction. This is overcome by digesting similar sample weights, spiking samples with a known concentration of methylmercury, and analyzing the smallest aliquot necessary for accurate results.

4.3.2 Water vapor may collect on the Carbo trap® columns, and be released onto the fluorescence cell where it condenses, giving a false peak due to scattering of the excitation radiation. This can be avoided by pre-drying the Carbo trap®.

4.3.3 A clean fluorescence signal is strongly dependent upon the inertness of the carrier gas. Carrier gas inertness is insured by using grade 5 gas, and by using pretraps for oxygen, water, organics, and mercury.

4.3.4 The sediment distillation may result in the production of methylmercury in samples with high inorganic mercury and high organic carbon concentrations. This methylation artifact can significantly increase the methylmercury concentration in sediment samples. Any sample suspected of having high inorganic Hg and high TOC should be prepared for analysis using the extraction technique.

4.4 Sample preparation

4.4.1 **Tissue:** Weigh 0.1 to 1 grams of homogenized wet tissue or 0.05 to 0.20 grams of freeze-dried tissue directly in a 22 mL glass or Teflon vial, weighing the vial to the nearest milligram. Record the weight of each sample aliquot on a methylmercury digestion datasheet (Attachment 1). Return the unused sample to the freezer. Add 10.0 mL of the KOH/methanol reagent to each sample, cap, shake, and place in an oven at 60 °C ± 5°C for 2-4 hours, if tissue samples, until all soft tissue has solubilized. Allow the samples to cool and add 10 mL of methanol, shake the samples to mix thoroughly. For samples with undissolved solids, such as sediments, allow the solids to settle completely prior to analysis. Masses and volumes may be scaled down to accommodate smaller sample sizes when limited material is available.

4.4.2 **Sediment/Soil (distillation):** Prepare a set of "receiving" vials by adding 3 mL deionized water to a distillation vial and capping it with a distillation cap. Prepare one receiver for each sample. Place the receivers in a rack and immerse the rack in ice water to pre-chill the receiving water. Accurately weigh 0.2 to 2 g of each sample into labelled "sending" distillation vials and record on Attachment 2. Add 3 mL DI water, 50 µL 8 M H_2SO_4 and 200 µL 20% KCl. Swirl the sample until the sample is liquified and add another 20 mL DI water. The sending vial is then capped and connected to a receiving vial via the 1/8" Teflon tube. The sending vial is then placed in the heating block and immediately connected to nitrogen carrier gas flowing at 40 mL/min. The block is then heated to 125 °C until 20 mL of distillate has collected in the receiving vial. It is essential to 1) keep the receiving vials chilled throughout this process, and 2) remove the receiving vial as soon as the 20 mL level is reached. Failure to do so will result in acid distilling across into the sample, rendering the ethylation portion of the analysis ineffective. If the distillate pH is <2, the sample should be re-distilled. Once distillation is complete, bring the final volume of the sample to 25.6 mL by filling the receiver to the top with DI water.

4.4.3 **Sediment/Soil (extraction):**. Accurately weigh 0.2 to 5 g (wet) of each sample into labelled 50 ml centrifuge tube and record on Attachment 3. Add 5 ml potassium bromide solution, ml 1M $CuSO_4$ and 10 ml CH_2Cl_2. Swirl the sample until the sample is wetted and cap. Once all samples have been prepared, pack the centrifuge tubes snugly in teflon cans and place them sideways on the shaker table. Shake the samples vigorously for 1 hour. The samples are then centrifuged for 30 minutes at 2000 rpm. Higher speeds may be used for samples that fail to separate well using normal speeds. Immediately following centrifugation, 2 mls of the CH_2Cl_2 layer are pipeted into a clean 50 ml teflon distillation vial and 45 ml of reagent water are added. The distillation vial is capped and placed in the heating block and immediately connected to nitrogen carrier gas flowing at 60 ml/min. The block is then heated to 45°C until all of the CH_2Cl_2 has evaporated. It is essential to purge the CH_2Cl_2 completely from the sample, or low recoveries will result.

4.5 **Analysis**

4.5.1 **Tissue**: Add 75±10 ml of low Hg water to the cold vapor generator, then add 200 µl of acetate buffer and agitate to mix thoroughly. Add 20 - 100 µl of the digestate to the cold vapor generator using the following method: Insert the pipette tip just slightly into the digestate liquid and gradually draw the liquid into the pipette tip once, to the desired volume. Do not rinse the tip with sample, or use the same tip for multiple aliquots, as the KOH/MeOH sticks to the sides of the pipette tip, which will cause irreproducible volumes. Next insert the pipette tip with the digestate aliquot into the water of the cold vapor generator, and pipette the sample aliquot into the water. Rinse the pipette tip several times with the cold vapor generator water, to make sure the total sample aliquot is transferred to the cold vapor generator. Swirl the cold vapor generator to mix the water and sample thoroughly. Pipette 125 µl of the ethylating reagent directly into the water in the cold vapor generator. Do not pipette the ethylating reagent on the glass sides of the cold vapor generator, but directly into the water. If the ethylating agent is pipetted on the side of the cold vapor generator, add another 125 µl aliquot to the water. Seal the cold vapor generator and swirl to thoroughly mix the water, sample, and ethylating reagent. Allow the ethylating reagent to react for 17 minutes. Record the aliquot volume on a methylmercury datasheet (Attachment 4).

4.5.2 **Sediment/Soil:** Add 75 ± 10 ml of low Hg water to the cold vapor generator, then add 200 µl of acetate buffer and agitate to mix thoroughly. Pour or pippette 1-45 ml of the distillate/extract into the cold vapor generator and swirl the cold vapor generator to mix the water and sample thoroughly. Pipette 125 µl of the ethylating reagent directly into the water in the cold vapor generator. Do not pipette the ethylating reagent on the glass sides of the cold vapor generator, but directly into the water. If the ethylating agent is pipetted on the side of the cold vapor generator, add another 125 µl aliquot to the water. Seal the cold vapor generator and swirl to thoroughly mix the water, sample, and ethylating reagent. Allow the ethylating reagent to react for 17 minutes. Record the aliquot volume on a methylmercury datasheet (Attachment 4).

4.5.3 Place the Carbo trap® on the cold vapor generator with the silanized glass wool end towards the cold vapor generator. Purge the sample for 17 minutes with a flow rate of 200 - 400 ml/min. with argon. At the end of this period of time, allow argon to flow through the Carbo trap® for an additional 5 minutes, to dry the trap.

4.5.4 Place the dry Carbo trap® through the nichrome wire coil and in-line with the CVAFS system. The silanized glass wool plug should be facing the gas chromatograph (GC) column. Allow the CVAFS system to purge for a few seconds with helium, then apply 10 VAC (via an autotransformer) to the nichrome wire coil around the Carbo trap® for 35 seconds and activate the integrator for a 6 minute run. Record the height of the methyl-ethylmercury peak on a methylmercury datasheet (Attachment 4). Mercury species are eluted by boiling points, with the following peaks being typical in tissue or sediment analysis.

1) A peak at about 1minute corresponding to elemental Hg (Hg^0). Hg^0 is usually a decomposition product of diethylmercury, as Hg is not trapped by the Carbo trap®. A small Hg^0 peak is always present.

2) A peak at about 3 minutes corresponding to methyl-ethylmercury. This is the ethylation product of methyl mercury.

3) A peak at about 4.5 minutes corresponding to diethylmercury, resulting from the ethylation of inorganic Hg(II) in the reagents and sample. If dimethylmercury was present, it would be seen as a peak at about 1.5 minutes. If this is observed, an aliquot of that sample should be run without ethylation, as the triethylboron byproduct is coeluted with the dimethylmercury, resulting in peak attenuation. Dimethylmercury is volatile without ethylation.

4.6 Calibration

Cold vapor atomic fluorescence for mercury is linear over at least five orders of magnitude (Bloom and Fitzgerald, 1988). A 4-point (minimum) calibration curve should be done on a daily basis, bracketing the expected analyte concentrations. Spiked samples should be routinely run to check for matrix interferences of the digestate as well as distillation/extraction efficiency. Using this method the matrix effect should not be greater than 10% compared to spike samples in low Hg tap water.

5.0 DATA ANALYSIS AND CALCULATIONS

5.1 Calculations may be made using a best fit linear regression analysis of the standards and blanks. Using least squares best fit method, calculate the slope and y intercept of the line for the standards and blank.

$$[\text{MeHg}]\ \text{ng/g} = \frac{(\text{sample peak ht-y intercept}) * V}{\text{slope} * v * \text{sample wt (g)} * 1000}$$

Where:

slope is the slope of the standard regression line plotted on the axes of picograms MeHg vs peak height

sample peak ht is the sample peak height

V is the total distillate or digestate volume in mL

v is the sample distillate or digestate aliquot analyzed in mL

sample wt is the sample weight digested or distilled in grams

The concentration as calculated above is generally then corrected for the reagent blank which is prepared, analyzed, and calculated in the same manner as the samples, using an average sample mass and volume.

Distilled samples (soils and sediments) are also corrected for the mean distillation efficiency, which is a correction factor based on the mean matrix spike recovery for all samples of a particular matrix type.

Depending on how the data are reported, a correction for % dry weight should also be made if needed.

5.2 **Instrument and Method Detection Limits (IDL and MDL):**

To determine general matrix-specific MDLs: Analyze 7 replicates of a low level sample (clean sand for sediment or phytoplankton for tissue). Take the standard deviation of the 7 replicates and multiply it by the Student's T-value for 7 replicates as outlined in MSL-Q-007.

In cases when an empirically-derived MDL is not practical (when a clean enough sample can not be found to produce a meaningful MDL for a specific matrix), a theoretical MDL may be calculated. To determine a theoretical sample-specific MDL: Analyze 7 replicates of a low-level liquid standard (0.5 µg/l). Take the standard deviation of the 7 replicates and multiply it the Student's T-value for 7 replicates to derive an instrument detection limit (IDL). Put the IDL through the sample concentration calculation (divide by volume analyzed and then multiply by total sample volume and divide by total sample mass).

Battelle MSL does not routinely employ reporting limits (RL). It is MSL policy to report all values detected above the empirically determined MDL (or calculated MDL in the case of tissues or sediments when no empirical MDL can realistically be determined). If a client specifically requests that data be reported below the MDL, the affected data should be flagged as detected below the detection limit according to the client's requested flagging convention.

6.0 QUALITY CONTROL

6.1 All quality control data shall be maintained and available for easy reference or inspection.

6.2 Calibration data must be composed of a minimum of 1 blank and 4 standards. Such a calibration should be run at least once per day and continuing calibration verification standards, at a level near the mid-range of the samples, should be run every 10 samples. For Navy samples, high and low continuing calibration verification standards should be run and these are chosen based on the sample concentration.

6.3 Samples containing high analyte concentrations may be run either following dilution, or on a separate run at a lower instrumental sensitivity. Alternatively, a high standard may be run to brac

the peak height of the sample and show system linearity through that peak height. The calibration extension standard must match the original calibration within 15%.

6.4 Duplicate or triplicate analyses (depending upon client preference) should be run once every 20 samples or once per batch, whichever comes first.

6.5 Natural Resources Council Canada (NRCC) or International Atomic Energy Agency (IAEA) certified standard materials for methylmercury in tissues and sediments should be analyzed at a frequency of once per 20 samples or once per batch, whichever comes first. Note that IAEA-356 was certified using the distillation technique, and that SRM exhibits the methylation. Therefore, IAEA-356 analyzed by distillation should yield the certified value but, when analyzed using extraction, it will yield only 65-85% of the certified value.

6.6 Procedural spike recoveries should be run once per 10 samples or once per batch, whichever comes first.

Table 1 Data Quality Objectives (DQOs)

QA Sample Type	Frequency	Acceptance Limit	Corrective Action
Laboratory Reagent Blank	1 per batch of 20 or fewer	<5 times the method detection limit (MDL)	Reanalyze. If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the batch must be redistilled and reanalyzed.
Replicate Precision	1 per batch of 20 or fewer	≤ 35% for analytes >5 times the MDL No more than 35% of all relative standard deviations (RSDs) can be > 35%	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple DQOs requires redistillation and reanalysis of batch.
Certified Reference Material (CRM) or Standard Reference Material (SRM); or Ongoing Precision and Recovery sample	1 per batch of 20 or fewer	67-133% of certified value	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple CRMs requires redistillation and reanalysis of batch.
Matrix Spike (MS) /Matrix Spike Duplicate (MSD)	1 per batch of 20 or fewer	65-135% recovery <35% relative percent difference (RPD)	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple MSs requires redistillation and reanalysis of batch.
Initial and Continuing Calibration Verification (ICV and CCV)	every 10 samples	<20% of initial calibration on	Reanalyze. If CCV still fails, rerun the calibration curve and calculate all samples analyzed after the last passing calibration check using the new curve.

7.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including the following:

Protective clothing and saftey glasses or goggles should be worn when appropriate.

Proper care must be exercised when handling samples.

8.0 TRAINING REQUIREMENTS

All staff performing total methylmercury in tissues and sediments by CVAF must first read this procedure and then demonstrate proficiency in the process prior to performing the work. Documentation of training will be recorded on a training assignment form obtained from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

Bloom, N.S. 1983. "Determination of Silver in Marine Sediments by Zeeman-Corrected Graphite Furnace Atomic Absorption Spectroscopy." Atomic Spectroscopy. 4:204.

Bloom, N.S. 1989. "Determination of Picogram Levels of Methylmercury by Aqueous Phase Ethylation, Followed by Cryogenic Gas Chromatography with Cold Vapor Atomic Fluorescence Detection." Can. J. Fish Aq. Sci. 7:1131.

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

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

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

MSL-A-006-01, Marine Sciences Laboratory Training

ATTACHMENT 1
Example Methylmercury Digestion Worksheet

PROJECT ID: ____________________ **SPIKE:** ______________________________

DIGESTION DATE: _______________ **SPIKE VOLUME:** ______________________

BALANCE ID#: __________________ **STANDARD:** __________________________

SAMPLE ID	BOTTLE WT grams	BOTTLE + SAMPLE grams	SAMPLE WT grams	TOTAL VOL ml	(SPIKE) ng/l

KOH/METHANOL VOLUME: ______________ METHANOL VOLUME: ____________

ATTACHMENT 2
Example Methylmercury Distillation Worksheet – Sediment

Methylmercury Distillation Worksheet – Sediment

PROJECT ID: _____ **SPIKE:** _____

DIGESTION DATE: _____ **SPIKE VOLUME:** _____

BALANCE ID#: _____ **TEMP/FLOWRATE:** _____

SAMPLE ID	PAN TARE	PAN + WET	PAN + DRY	% DRY	SAMPLE WT grams	(SPIKE) ng/l

FINAL VOLUME: _____ ANALYST: _____

VOL.10% KCl: _____

ATTACHMENT 3
Example Methylmercury Extraction Worksheet

Methylmercury Extraction Worksheet - Sediment

PROJECT ID:_____ **SPIKE:** _____

EXTRACTION DATE: _____ **SPIKE VOLUME:** _____

BALANCE ID#:_____

SAMPLE ID	PAN TARE	PAN + WET	PAN + DRY	% DRY	Sample Wt grams (Wet)	Initial Methylene Chloride Mass	Initial Methylene Chloride Volume	Extracted Methylene Chloride Mass	Extracted Methylene Chloride Volume	(Spike) ng/l

ATTACHMENT 4
Example Methylmercury Data Sheet

MERCURY DATA SHEET

TYPE: ER TOTAL METHYL

DATE: _____

ANALYST: _____

PROJECT NAME(S): _____

_____

CF ID#s: _____

CALIBRATION FACTOR: _____

Y-INTERCEPT or BUBBLER BLANK: _____

CALIBRATION TYPE: _____

R-SQUARED: _____

MATRIX TYPE: WATER SEDIMENT TISSUE

REPORT#	SAMPLE ID	BUBBLER	SAMPLE VOLUME	UNITS	[Hg]	COMMENTS

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Marine Sciences Laboratory

EFFECTIVE DATE: 7-13-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-I-016-03
TOTAL MERCURY IN TISSUES AND SEDIMENTS BY COLD VAPOR ATOMIC ABSORPTION (CVAA)

Approvals:		
AUTHOR: Brenda Lasorsa	Brenda Lasorsa	7/12/00
	Signature	Date
TECHNICAL REVIEWER: Mary Ann Deuth	Mary Ann Deuth	7/12/00
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	7/12/00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E A Crecelius	7-13-00
	Signature	Date

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TOTAL MERCURY IN TISSUES AND SEDIMENTS BY COLD VAPOR ATOMIC ABSORPTION (CVAA)

1.0 SCOPE AND APPLICATION

This method is applicable to the determination, in parts per million, of total mercury (Hg) in acid-digested sediment and tissue samples by cold vapor atomic absorption (CVAA). This procedure replaces Battelle procedure, MSL-M-031, and it is a modification of EPA Method 245.5. The modification is in the digestion, because the EPA 245.5 digestion uses potassium permanganate, which is a source of Hg contamination. This method uses the digestion method outlined in the NOAA Technical Memorandum NOS ORCA 130 "Sampling and Analytical Methods of the National Status and Trends Program Mussel Watch Project: 1993-1996 Update". G.G Lauenstein and A.Y. Cantillo, eds.

2.0 SUMMARY OF METHOD

Mercury ions in a digestate are reduced by acidic $SnCl_2$ to Hg^+, then carried through a flow cell on a stream of inert gas, i.e. argon. A photometric detector measures the luminous intensity of monochromatic light that has passed through the sample and compares it with the luminous intensity of the same light that has passed through a reference beam. Mercury atoms absorb light at 253.7 nm. The attenuation of the light is directly proportional to the concentration of the mercury vapor, which is quantified using a standard curve. The typical detection limit for the method is 0.001 ug/g.

3.0 RESPONSIBLE STAFF

- Technician - sample digestion
- Analyst- sample analysis
- QA Officer or Representative- data verification

4.0 PROCEDURE

4.1 Sample preparation

Samples shall be digested using an appropriate strong acid digestion which results in complete sample dissolution. It has been found that the traditional potassium permanganate digestion used in many standard methods for mercury analysis results in unacceptably high and inconsistent blank levels and should be avoided. Digestions done in a closed Teflon vessel (bomb) are recommended, as many open vessel and microwave techniques result in loss of Hg during venting.

4.2 Apparatus and Reagents

4.2.1 Thermo Separation Products "mercuryModule" (mercury vapor generator).

4.2.2 Thermo Separation Products "mercuryMonitor 3200" (elemental mercury detector).

4.2.3 Thermo Separation Products "autoMetric 3000" (autosampler).

4.2.4 Computer with HgTalk program, screen and printer.

4.2.5 Rinse system for sample siphon - tubing, rinse water bottle, waste water bottle and flow regulating valve.

4.2.6 Acid cleaned (cold 50% HNO_3 for at least 2 days) test tubes, 13 X 100 mm.

4.2.7 Hydrocarbon trap for purifying gas flow into instrument.

4.2.8 Drying trap (4-8 mesh, reagent soda lime is preferred over the magnesium perchlorate which was recommended by the company. The soda lime lasts longer, is less hazardous, and causes less interference).

4.2.9 Pre-pure Argon gas.

4.2.10 Stannous chloride, 10% - add 50 g of $SnCl_2$ to 100 ml low Hg water and 50 ml of HCl in a specially marked Teflon bottle then fill to 500 ml with low Hg water. Bubble nitrogen gas through mixture for at least 6 hours at a very low flow. Transfer solution to 2 specially marked brown bottles. 2% $SnCl_2$ - dilute 50 ml of this 10% $SnCl_2$ with 200 ml of 10% HCl.

4.2.11 Nitric Acid, 3% - add 60 ml of HNO_3 to low Hg water and bring to 2L volume.

4.2.12 Mercury stock standard, 1000 mg/L - purchased from High Purity Standards, Inc.; expiration is usually 1 year.

4.2.13 Mercury intermediate standard, 10 mg/L - dilute 1 ml of 1000 mg/L stock standard into 100 ml of 1% HCl and store at room temperature in a Teflon bottle dedicated for use with the intermediate standard. As long as the standard is stored in Teflon, the standard will be stable for a period of at least 3 months and expiration can be expected to be the same as the stock standard.

4.2.14 Mercury working standards, dilute the intermediate standard (10 mg/L Hg) into 5 concentrations (i.e. 0.5 ug/L, 1.25 ug/L, 5.0 ug/L, 7.5 ug/L and 12.5 ug/L) and store at room temperature in Teflon bottles dedicated for use with standards. As long as the standards are stored in Teflon, the standards will be stable for a period of at least 6 months and expiration can generally be expected to be the same as the stock standard. Check the standards against the certified reference materials (CRMs) run in each analytical batch to verify that the working standards are retaining their titre.

4.3 Interferences

4.3.1 Take appropriate precautions to prevent stannous chloride contamination during sample handling or of any equipment associated with this analysis. If contamination should occur, mercury will be volatilized and lost.

4.3.2 Particle size in samples must be less than 10 µm.

4.3.3 Samples must be totally digested or organic material will interfere with detection.

4.4 Analysis

This analysis is entirely computer based. Software use and troubleshooting will not be reiterated in this procedure, because this information can be found in the software manual.

4.4.1 Allow the mercury monitor at least one hour to warm up before analysis. Turn on the mercury generator, the printer and especially the autosampler before the HgTalk program is loaded or the connection will not be made.

4.4.2 Change the soda lime trap after approximately 100 samples or if the analyzer has not been run for several days. Check the reagent bottles for proper amounts, empty the hazardous waste bottle and the rinse waste bottle, then turn on the argon gas to a pressure of 80 psi. Make sure the reagent pressure gauge reads 5.0 ±1 psi (regulator is on the rear panel) and the flow meter reads about 0.2.

4.4.3 Open HgTalk. There are 3 files necessary for analysis; "method", "sequence", and "data". The method file contains the calibration, integration parameters, and the reports possible. The method file currently being used is called "Hgmeth.hmh". The sequence file is the operation and calculation of the analysis and must be created and named for each analysis set. Each sample has its own data file, which is created during the analysis.

4.4.4 After entering HgTalk and checking that the information in the method file is correct, a sequence file must be created. It is best to have the information organized and calculated before entering the information into the file since this program does not multiply dilution factors or allow the creation of new columns. With the 5 ml loop in the mercury generator, there should be at least 6 ml of sample in the test tube, which does not hold more than 9 ml. The sample can be straight digestate, however this usually does not leave enough digestate for another analysis. 3 ml of digestate and 3 ml of 3% HNO_3 or 1 ml of digestate and 5 ml of 3% HNO_3 are the common dilutions used currently. With the information of sample, weight, and volume times dilution factor ready (see attachment #1 for an example), the new sequence can now be created.

A. Open "edit sequence" and choose "new".
B. Fill in "sequence header" with method file name, create new data file name, operator name and date.
C. Choose "edit", "create entries", answer "yes" to function overwrite, and fill in number of standards and samples.
D. Fill in the sequence spreadsheet that appears with requested information. See Attachment #2 for an example.
E. Choose "SaveAS" and name file. Exit.

F. In the first 6 sample tubes, place a calibration blank and each of the five working standards. These samples will be used to generate the calibration.

G. Pipette the correct volume of 3% HNO_3 into clean test tubes and add appropriate amount of digestate to achieve the desired dilution. Rinse pipette tip several times in the resulting solution. Be sure to pour the correct standard into the vials numbered in the sequence file. Check that the sample vials match the vial numbers in the sequence. Place the tray on the autosampler and check the flow of the rinse water.

H. Load file and choose "run", then "start".

4.4.5 After each calibration standard has been run and the instrument has calculated the calibration curve, verify that the calibration is linear to an r^2 of >0.995. All points on the calibration line must fall within 15% of the line, with the exception of the lowest point, which must not deviate from the line by more than 25%. If these criteria are not met, abort the run and re-run the calibration. If the calibration continues to fail, remake the working standards, repeat the calibration, and continue.

4.4.6 If the absorbance of the sample is higher than the highest calibration standard, reduce the volume appropriately to not less than 5 µl. If the absorbance is still higher than the highest calibration standard, dilute the digestate in a larger acid cleaned vial. If the absorbance of most of the samples in the batch exceed the highest calibration point, the calibration may be extended by running a higher calibration standard. The analyzer is linear to the range of a 50 µg/liter standard. If the result of the 50 µg/liter standard is within 10% of the original calibration, the system may be considered linear in that range and the data may be calculated using the original calibration.

4.4.7 Clean the analyzer by running 2-4 samples of 3% HNO_3, then DI H_2O through the machine at the end of each day.

4.5 Instrument Maintenance

4.5.1 The instrument is maintained by the analyst, with the assistance of service personnel at Thermo-Separation Products.

The following items are checked daily and changed weekly (under constant use):

- soda lime
- reagents (stannous chloride, 3% HNO_3, and rinse water)

The following items are checked weekly and changed bimonthly (under constant use):

- carbon trap
- filters

The following items are checked weekly and changed as needed:

- sample injection syringe
- tubing
- connectors
- lamp

4.5.2 The autosampler arm should be cleaned and lubricated bimonthly.

5.0 DATA ANALYSIS AND CALCULATIONS

5.1 The computer program calculates the concentration of Hg in the sample from either peak height or area, which is determined in the method file, by the following equations:

$$[Hg] = ((PH_s * RF_1) + Rf_0) * V_d / W_d / 0.001*DF$$

where

[Hg] = Mercury concentration (µg/g dry wt)
Ph_s = Sample peak height or peak area
RF_1 = Response Factor 1 (slope of the regression line in µg/area or height)
Rf_o = Response Factor (y-intercept)
V_d = Digested volume (ml)
W_d = Digested weight (g)
DF = Dilution Factor

5.2 Data Generation, Review, and Archiving and Software Maintenance

5.2.1 Instrument-generated data are overchecked using Excel software. All data points are recalculated from the raw peak area and compared to the instrument-generated value. The final data report is rechecked by the QA Officer during the data verification process.

5.2.2 Software is maintained by the analyst with the help of service personnel at Thermo-Separation Products. Backup instrument software is maintained on diskette. Data reduction is done using Excel software purchased and maintained under Battelle's software licensing agreement. Backup copies of all data are maintained on the mercury lab's shared drive, the m-drive.

5.2.3 Electronic data are backed up monthly and also maintained on the analyzer for 1 year. Electronic copies of the data summaries are maintained on the m-drive for two years and then either deleted, forwarded to the client, or archived on diskette as required by the client. Hard copies of all data are maintained in the central file system for at least 10 years.

6.0 QUALITY CONTROL

6.1 One method blank, a sample duplicate, and matrix spike (on a representative sample, to check for matrix interference) should be analyzed per batch of samples or as

specified by the customer. A method blank consists of all reagents used in the digestion procedure and it is digested and analyzed as a sample.

6.2 One standard reference material (SRM) should be digested and analyzed with each batch of samples or as specified by the customer, following step 4.4.4.

6.3 An initial calibration verification and continuing calibration verifications should be run every ten samples and must be within 15% of the original calibration. If this check fails, run a duplicate and if that fails rerun the calibration curve and all samples analyzed after the last passing calibration check.

6.4 Data quality objectives, acceptance limits, and corrective actions are outlined in the Table 1 below.

6.5 Method Detection Limits (MDLs) for total mercury are determined by two methods:

6.5.1 To determine general matrix specific MDLs: Analyze 7 replicates of a low level sample (clean sand for sediment or phytoplankton for tissue). Take the standard deviation of the 7 replicates and multiply it by the Student's T-value for 7 replicates as outlined in MSL-Q-007.

6.5.2 In cases when an empirically derived MDL is not practical (when a clean enough sample can not be found to produce a meaningful MDL for a specific matrix), a theoretical MDL may be calculated. To determine a theoretical sample-specific MDL: Analyze 7 replicates of a low-level liquid standard (0.5 µg/l). Take the standard deviation of the 7 replicates and multiply it by the Student's T-value for 7 replicates to derive an instrument detection limit (IDL). Put the IDL through the sample concentration calculation (divide by volume analyzed and then multiply by total sample volume and divide by total sample mass).

Battelle MSL does not routinely employ reporting limits (RL). It is MSL policy to report all values detected above the empirically determined MDL (or calculated MDL in the case of tissues or sediments when no empirical MDL can realistically be determined). If a client specifically requests that data be reported below the MDL, the affected data should be flagged as detected below the detection limit according to the client's requested flagging convention.

Table 1. Data Quality Objectives

QC Sample Type	Frequency	Acceptance Limit	Corrective Action
Laboratory Method Blank	1 per batch of 20 or fewer	<5 times the MDL	Reanalyze. If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the batch must be redigested.
Replicate Precision	1 per batch of 20 or fewer	25% for analytes >3 times the MDL No more than 35% of all RSDs can be > 25%[c]	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple DQO's requires redigestion and reanalysis of batch.
Certified Reference Material (CRM) or Standard Reference Material (SRM)	1 per batch of 20 or fewer	75-125% of certified value	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple DQOs requires redigestion and reanalysis of batch.
Matrix Spike (MS)	1 per batch of 20 or fewer	75-125% recovery	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple DQOs requires redigestion and reanalysis of batch.
Initial and Continuing Calibration Verification	every 10 samples	<15% of initial calibration on	Reanalyze. Rerun the calibration curve and all samples analyzed after the last passing calibration check.

7.0 SAFETY

All analysts following this procedure should be aware of routine laboratory safety concerns, including the following:

- Protective clothing and eyeglasses should be worn when appropriate.
- Proper care must be exercised when handling samples.

8.0 TRAINING REQUIREMENTS

All staff performing total mercury in tissues and sediments must first read this procedure and then demonstrate proficiency in the process prior to performing any project work. Proficiency may be demonstrated and documented through successful analysis of certified reference materials, performance evaluation (PE) samples, or matrix spike/matrix spike duplicates. Documentation of training will be recorded on a training form obtained from MSL-A-006, Marine Sciences Laboratory Training.

9.0 REFERENCES

Thermo Separation Products HgTalk Mercury Analysis Software

Thermo Separation Products Operator's Manuals for Mercury Vapor Generator and Detector

MSL-A-006, Marine Sciences Laboratory Training.

ATTACHMENT 1
EXAMPLE SPREADSHEET

PROJECT ID: Spreadsheet Examples

ANALYSIS: TSP HG ANALYSER

ANALYST: Deuth

ANALYSIS DATE: 1-Jan-97

FILE #: 100TEST

MATRIX: _____

VIAL #	FILE #	SAMPLE ID	DIGEST WT g	DIGEST VOL ml	ANALYZED VOL ml	TUBE VOL ml	DILUT FACTOR	CALC VOL ml
1		0 - Cal Blank						
2		0.5 ug/l						
3		1.25 ug/l						
4		5.0 ug/l						
5		7.25 ug/l						
6		12.5 ug/l						
7		Blk HNO3						
8		ICV Std #113						
9	100TEST	_____	0.500	23.091	3.000	6	2	46.18
10	100TEST	_____	0.500	22.768	0.200	6.2	31	705.82
11	100TEST	2616	0.502	22.905	0.100	6.1	61	1397.19
12	100TEST	2617	0.505	22.929	0.500	6.5	13	298.08
13	100TEST	2618	0.325	22.922	1.000	7	7	160.45
14	100TEST	2619	0.499	22.866	1.000	7	7	160.06
15	100TEST	_____	0.500	22.946	1.000	7	7	160.62
16	100TEST	_____	0.500	23.017	1.000	7	7	161.12
17	100TEST	2621	0.504	23.024	1.000	7	7	161.17
18	100TEST	_____	0.500	22.942	0.100	6.1	61	1399.48
19	100TEST	CCV Std #1160						
20	100TEST	2622	0.113	23.249	0.100	6.1	61	1418.19
21	100TEST	2623	0.078	23.259	0.100	6.1	61	1418.78
22	100TEST	2624	0.081	23.189	0.500	6.5	13	301.46
23	100TEST	2625	0.085	23.231	0.500	6.5	13	302.00
24	100TEST	2626	0.157	23.409	1.000	7	7	163.86
25	100TEST	2627	0.087	23.359	1.000	7	7	163.51
26	100TEST	2628	0.077	23.296	1.000	7	7	163.07
27	100TEST	2629	0.108	23.365	1.000	7	7	163.56
28	100TEST	CCV Std #1160						
29	100TEST	Blank	0.200	19.727	3.000	6	2	39.45
30	100TEST	PACS-1	0.200	23.904	0.100	6.1	61	1458.13
31	100TEST	2603 R1	0.200	23.899	0.500	6.5	13	310.69
32	100TEST	2603 R2	0.200	23.943	0.500	6.5	13	311.26
33	100TEST	2604	0.201	23.866	0.500	6.5	13	310.25
34	100TEST	2605	0.202	23.926	0.500	6.5	13	311.03
35	100TEST	2605 MS	0.200	23.938	0.025	6.025	241	5769.08
36	100TEST	2606	0.199	23.884	0.500	6.5	13	310.49
37	100TEST	2612	0.201	23.908	0.500	6.5	13	310.80
38	100TEST	2613	0.200	23.876	0.500	6.5	13	310.39
39	100TEST	CCV Std #1159						

ATTACHMENT 2
EXAMPLE SEQUENCE FILE

File Header Edit Global Change Validate

OVR	SDG	Vial#	Sample ID	Phase	Type	Level	Amount	Dilu. Fact
1		2/1	Cal Blank	LIQUID	blank	0	1	1
2		2/2	0.5 ug/l	LIQUID	standard	1	1	1
3		2/3	1.25 ug/l	LIQUID	standard	2	1	1
4		2/4	5.0 ug/l	LIQUID	standard	3	1	1
5		2/5	7.25 ug/l	LIQUID	standard	4	1	1
6		2/6	12.5 ug/l	LIQUID	standard	5	1	1
7		2/7	Blk HNO3	SOLID	sample		1	1
8		2/8	Std #113	LIQUID	ICV		1	1
9	100TEST	2/9	_____	SOLID	sample		0.500	45.540
10	100TEST	2/10	_____	SOLID	sample		0.500	712.250
11	100TEST	2/11	2616	SOLID	sample		0.502	1398.660
12	100TEST	2/12	2617	SOLID	sample		0.505	297.000
13	100TEST	2/13	2618	SOLID	sample		0.325	0.990
14	100TEST	2/14	2619	SOLID	sample		0.499	160.060
15	100TEST	2/15	_____	SOLID	sample		0.500	160.620
16	100TEST	2/16	_____	SOLID	sample		0.500	160.620
17	100TEST	2/17	2621	SOLID	sample		0.504	161.120
18	100TEST	2/18	_____	SOLID	sample		0.500	161.170
19	100TEST	2/19	Std #1160	LIQUID	CCV		1.000	1399.480
20	100TEST	2/20	2622	SOLID	sample		0.113	1.000
21	100TEST	2/21	2623	SOLID	sample		0.078	1418.190
22	100TEST	2/22	2624	SOLID	sample		0.081	1418.780
23	100TEST	2/23	2625	SOLID	sample		0.085	301.460
24	100TEST	2/24	2626	SOLID	sample		0.157	302.000
25	100TEST	2/25	2627	SOLID	sample		0.087	163.860
26	100TEST	2/26	2628	SOLID	sample		0.077	163.070
27	100TEST	2/27	2629	SOLID	sample		0.108	163.560
28	100TEST	2/28	Std #1160	LIQUID	CCV		1.000	1.000
29	100TEST	2/29	Blank	SOLID	sample		0.200	39.450
30	100TEST	2/30	PACS-1	SOLID	sample		0.200	1458.130
31	100TEST	2/31	2603 R1	SOLID	sample		0.200	310.690
32	100TEST	2/32	2603 R2	SOLID	sample		0.200	311.260
33	100TEST	2/33	2604	SOLID	sample		0.201	310.250
34	100TEST	2/34	2605	SOLID	sample		0.202	311.030
35	100TEST	2/35	2605 MS	SOLID	sample		0.200	5769.080
36	100TEST	2/36	2606	SOLID	sample		0.199	310.490
37	100TEST	2/37	2612	SOLID	sample		0.201	310.800
38	100TEST	2/38	2613	SOLID	sample		0.200	310.390
39	100TEST	2/39	STd #1159	LIQUID	CCV		1.000	1.000

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Marine Sciences Laboratory

EFFECTIVE DATE: 7-13-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-I-022-04

DETERMINATION OF ELEMENTS IN AQUEOUS AND DIGESTATE SAMPLES BY ICP/MS

Approvals:		
AUTHOR: Eric Crecelius	E A Crecelius	7-13-00
	Signature	Date
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	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	7-13-00
	Signature	Date
TECHNICAL GROUP MANAGER: Richard M. Ecker	RM Ecker	7/13/00
	Signature	Date

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DETERMINATION OF ELEMENTS IN AQUEOUS AND DIGESTATE SAMPLES BY ICP/MS

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the determination of dissolved elements in environmental waters, waste water and digestates of tissue and sediment by inductively coupled plasma mass spectrometry (ICP/MS). The expected instrument detection limits (± DLs) are element dependent; however, for many elements the manufacturer suggested IDLs are in the range of 0.1 to 0.001 µg/L. This standard operating procedure is a modification of EPA Method 1638 and the chemical analyses section of EPA Method 1640.

1.2 Summary of Method

1.2.1 This method describes the multi-element determination of elements by ICP/MS. Sample material in solution is introduced by pneumatic nebulization into a radio-frequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are detected by a continuous dynode electron multiplier assembly and the ion information processed by a data handling system. Interferences relating to the technique (section 4.2) must be recognized and corrected for. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix must be corrected for by the use of internal standardization.

1.2.2 Dissolved elements are determined after suitable filtration and acid preservation. Acid digestion is not necessary prior to analysis. In order to reduce potential interferences, the dissolved solids level should not exceed 0.5% (w/v). Marine waters need to be diluted or processed to remove dissolved solid before analysis.

1.2.3 Acid digestion procedures are required prior to the determination of total recoverable elements in water or analysis of solids such as tissue and sediment. In order to reduce potential interferences, the dissolved solids level should not exceed 0.5% (w/v).

2.0 DEFINITIONS

Dissolved - Material that will pass through a 0.45 µm membrane filter assembly, prior to sample acidification.

Total Recoverable - The concentration of analyte determined on an unfiltered sample following treatment with hot dilute mineral acid.

Instrument Detection Limit (IDL) - The IDL is the minimum concentration of an analyte that a particular instrument can measure and report with 99% confidence that the analyte concentration is greater than zero.

Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL is determined from analysis of replicate samples in a given matrix containing the target analyte.

Linear Detection Range (LDR) - The concentration range over which the analytical working curve remains linear.

Internal Standard - Pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same solution. The internal standard must be an analyte that is not a sample component. The concentration of the internal standard is 20 µg/L in the samples and standard. The internal standard is added at a rate of 200 µL of 1000 µg/L standard to 9.8 mL of sample.

Tuning Solution - A solution which is used to determine acceptable instrument performance prior to calibration and sample analyses. This solution is purchased from the instrument manufacturer and analyzed without dilution.

Blank Spike - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The blank spike is analyzed exactly like a sample, and its purpose is to determine whether the method is within accepted control limits. Blank spike concentration is usually equal to one of the calibration solutions.

Matrix Spike - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The matrix spike is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the matrix spike corrected for the concentrations found.

Standard Reference Material (SRM) – A sample obtained from an independent source that is certified to contain known concentrations of metals.

3.0 RESPONSIBLE STAFF

Researcher/Technician-sample preparation, digestion
Analyst – sample analysis
MSL QA Officer or Representative- data verification

4.0 PROCEDURE

4.1 Sample Collection, Preservation and Storage

4.1.1 For the determination of dissolved elements, the sample should be filtered through a 0.45 µm membrane filter. Use a portion of the sample to rinse the filter assembly, discard and then collect the required volume of filtrate. Acidify the filtrate with 1.0 µl/ml concentrated nitric acid immediately following filtration to a pH less than 2.0.

4.1.2 For the determination of total recoverable elements in aqueous samples, acidify with 1.0 µl/ml concentrated nitric acid at the time of collection to a pH less than 2.0. The sample should not be filtered prior to analysis.

NOTE: Samples that cannot be acid preserved at the time of collection, should be acidified with nitric acid to a pH of less than 2 upon receipt in the laboratory (normally, 1.0 µl/ml concentrated nitric acid is sufficient for most ambient and drinking water samples). Following acidification, the sample should be held for sixteen hours before withdrawing an aliquot for sample processing. Samples are stored at 20 °C in the sample preparation Laboratory, MSL5, Room 231, which has filtered air.

4.1.3 Sediment and tissue samples shall be digested according to the project specific requirements.

4.1.4 For the total recoverable digestion procedure, 9.6 mL of sample water is heated at 85°C for 2.5 hours with 0.2 mL of nitric acid and 0.2 mL of internal standard in a sealed 15 mL plastic centrifuge tube.

4.2 Potential Interferences

4.2.1 Isobaric Elemental Interferences - are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. Of the analytical isotopes recommended for use with this method, only molybdenum^{98} (ruthenium) and selenium^{82} (krypton) have isobaric elemental interferences. If alternative analytical isotopes having higher natural abundance are selected in order to achieve greater sensitivity, an isobaric interference may occur. All data obtained under such conditions must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. A record of this correction process should be included with the report of the data. It should be noted that such corrections will only be as accurate as the accuracy of the isotope ratio used in the elemental equation for data calculations. Relevant isotope ratios and instrument bias factors should be established prior to the application of any corrections.

4.2.2 Abundance Sensitivity - is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadruple operating pressure. Wing overlap

interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences,should be recognized and the spectrometer resolution adjusted to minimize them.

4.2.3 Polyatomic Ion Interferences - are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components (i.e. hydrochloric acid). Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions.

4.2.4 Physical Interferences - are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g. viscosity effects) at the point of aerosol formation and transport to the plasma (e.g. surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore, ion transmission. Dissolved solids levels not exceeding 0.5% (w/v) have been recommended to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects. Internal standards should have similar analytical behavior to the elements being determined.

4.2.5 Memory Interferences - result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to ten times the upper end of the linear range for a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of ten of the method detection limit, should be noted. Memory interferences may also be assessed within an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal values drop consecutively, the analyst should be alerted to the possibility of a memory effect, and should examine the analyte concentration in the previous sample to determine if this was high. If a memory interference is suspected, the sample should be re-analyzed after a long rinse period.

4.2.6 Where necessary, precautions should be taken to protect the electron multiplier from exposure to high ion counts or changes in instrument response or damage to the multiplier may result. Samples having high concentrations beyond the linear range and with isotopes falling within scanning windows should be diluted prior to analysis.

4.2.7 For the determination of trace levels of elements, contamination and loss are of prime concern. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area, designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by contributing contaminants through surface desorption or leaching and depleting element concentrations through adsorption processes. All re-useable labware (glass, quartz, polyethylene, Teflon etc.) including the sample container should be cleaned prior to use.

4.3 Apparatus and Reagents

- ICP/MS capable of scanning the mass range 5-250 amu with a minimum resolution capability of 1 amu peak width at 5% peak height. Instrument may be fitted with a conventional or extended dynamic range detection system.

- Argon gas supply (high-purity grade, 99.99%).

- A variable-speed peristaltic pump, mass-flow controller for the nebulizer gas supply.

- Various volumetric flasks, graduated cylinders and calibrated pipettes.

- Air displacement pipetter capable of delivering between 0.005 to 10.0 mLs.

- Nitric acid, concentrated (sp.gr. 1.41) Fisher optima.

- Standard Stock Solutions - May be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99 - 99.999% pure).

- Certified standards are purchased annually. The primary standards contain either 1000 ppm or 10-ppm concentrations of metals. Secondary standards (1.0 and 0.1 ppm) are prepared from these primary standards. The secondary standards are assigned unique numbers and information is recorded in the standard logbook in the ICP/MS laboratory. The secondary standards have the same shelf life as the primary standards. Instrument calibration standards (generally 1.0, 10, 25, and 100 ppb) are prepared the day of analysis.

- Multi-Element Stock Standard Solutions - Care must be taken in the preparation of multi-element stock standards that the elements are compatible and stable. Some certified multi-element standards may be purchased. Stock standards should be checked for the presence of impurities which might influence the accuracy of the standard. Freshly

prepared standards should be transferred to acid-cleaned, Teflon bottles for storage and monitored periodically for stability.

- Calibration standards - Dilute each of the stock multi-element standard solutions to levels appropriate to the operating range of the instrument using deionized (DI) water containing 2% (v/v) nitric acid. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve.

- Internal Standard Stock Solution - Use this solution to fortify blanks, calibration standards and samples. The number of internal standards used depends upon the masses of the analytes and their ionization potentials. Elements used as internal standards include Li, Ga, In, Lu, Tb, and Bi. A concentration of 20 µg/L, for example, or an appropriate concentration of each element in the sample is used. Care must be taken to avoid using internal standards that are present at significant concentrations in the samples. Count rates for internal standards should be on the order of 10^5 counts per second.

- Tuning Solution -This solution is used for instrument tuning and mass calibration prior to analysis. Multi-element tuning solution is purchased from the instrument manufacturer and analyzed directly without dilution. Tuning solution routinely used includes Mg, Rh and Pb. The tuning solution contains 10 µg/L of each element.

4.4 Step-by-Step Procedure

4.4.1 Sample Preparation

4.4.1.1 For the determination of ground and surface waters, an aliquot of the acid-preserved sample is spiked with the same concentration of internal standards as in the calibration standards and mixed.

NOTE: If a precipitate is formed during acidification, transport or storage, the sample aliquot must be treated using the procedure for total recoverable elements prior to analysis.

4.4.1.2 For the determination of elements in digestates, usually a 1 ml aliquot of digestate is spiked with the internal standards and diluted to 10 ml with 2% nitric acid.

4.4.2 Calibration

4.4.2.1 Initiate proper operating configuration of instrument and data system. Allow a period of not less than thirty minutes for the instrument to warm up. During this process conduct sensitivity and stability checks using the tuning solution. As recommended by the instrument manufacturer, mass calibration and resolution checks are only conducted if major maintenance work has been performed.

4.4.2.2 Instrument stability and sensitivity must be demonstrated by running the10 µg/L tuning solution. Results must exceed the manufacturers minimum specification (30,000 intensity units for rhodium and 5000 intensity units for magnesium and lead for the ELAN 5000) and stability <10% RSD.

4.4.2.3 Prior to initial calibration, set up proper instrument software routines for quantitative analysis. The instrument must be calibrated for the analytes to be determined using the calibration blank and calibration standards normally prepared at three or more concentration levels (typically 1.0, 10, 50, and 100 µg/L). The instrument is normally calibrated in the "weighted linear" or "linear through zero" mode. Three or more calibration levels are used. Three replicate integrations are required for data acquisition. Use the average of the integrations for instrument calibration and data reporting.

4.4.2.4 The rinse water should be used to flush the system between solution changes for blanks, standards and samples. Allow sufficient rinse time to remove traces of the previous sample (at least 30 seconds). Solutions should be aspirated for thirty seconds prior to the acquisition of data to allow equilibrium establishment.

4.4.2.5 Internal standardization is normally used in all analyses to correct for instrument drift and physical interferences. Internal standards must be present in all samples, standards and blanks at identical levels. The concentration of the internal standard should be sufficiently high that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample.

4.4.2.6 Check the performance of the instrument and verify the calibration using data gathered from analyses of calibration blanks, calibration standards and the quality control sample.

4.4.2.7 After the calibration has been established, it must be initially verified for all analytes by analyzing a second source standard (from a different lot than the standard used to calibrate the instrument). This is called the independent calibration verification and is performed at the mid-point of the calibration curve. In addition, a low level sample (LLS) and a high level sample (HLS) are used for calibration verfication for Navy project samples. If measurements exceed the QC criteria for the project, the source of the problem should be identified and appropriate action taken which could include instrument recalibration.

4.4.2.8 To verify that the instrument is properly calibrated on a continuing basis, run a check sample such as a blank spike (continuing calibration standard) or certified reference material at a frequency of one every 20 samples or as required by project QC

requirements. If the indicated concentration of any analyte deviates outside the project quality control (QC) range, contact the project manager for appropriate corrective action.

4.4.2.9 All masses which might affect data quality must be monitored during the analytical run. This information should be used to correct the data for identified interferences.

4.4.2.10 The rinse water should be used to flush the system between samples. Allow sufficient time to remove traces of the previous sample or a minimum of thirty seconds. Samples should be aspirated for thirty seconds prior to the collection of data.

4.4.2.11 Samples having concentrations higher than the established linear dynamic range should be diluted into range and re-analyzed. The sample should first be analyzed for the trace elements in the sample, protecting the detector from the high concentration elements, if necessary, by the selection of appropriate scanning windows. The sample should then be diluted for the determination of the remaining elements. Alternatively, the dynamic range may be adjusted by selecting an alternative isotope of lower natural abundance, provided quality control data for that isotope have been established.

4.5 Instrument Maintenance, Operation and Preventive Maintenance

The ICP/MS is maintained by the Perkin Elmer service engineer under a maintenance agreement. All maintenance is recorded in the ICP/MS maintenance book and stored in Room 228. The service engineer conducts preventive maintenance on a schedule recommended by Perkin Elmer. The MSL ICP/MS operator daily checks and records the argon supply pressure, the base and operating vacuum, the temperature of the cooling chiller, and the nebulizer flow. A sensitivity and stability check is made each day that the instrument is operated. If the sensitivity for 10 µg/L ppb Rh is below 30,000 cps or if Rh precision (relative standard deviation; RSD) is greater than 10%, corrective action is taken before quantitative analysis is conducted. Corrective action includes changing the auto sampler pump tubing, cleaning or changing cones, adjusting the nebulizer flow, and calling the service engineer for additional corrective action or to schedule a site visit.

4.6 Software Maintenance and Verification

The ICP/MS software is upgraded and maintained by the service engineer under the maintenance agreement. When software problems occur, the service engineer reinstalls the software. Changes in software are recorded in the ICP/MS maintenance logbook. No tapes are used. Hard copies of the raw data are stored in the Central Files for more than 10 years.

Verification of the software can be done by two methods. First, the QC results for certified reference materials (CRMs) are a verification check because significant errors in the software would result in consistently unsatisfactory results for CRMs, which are run daily. Secondly, the software can be verified by hand calculating the

concentration using raw counts for the calibration standards and the unknown samples and comparing this result to the software result.

5.0 DATA ANALYSIS AND CALCULATIONS

5.1 Reported values should be calibration blank subtracted. For aqueous samples prepared by the digestion, multiply solution concentrations by the dilution factor.

5.2 Data values should be corrected for instrument drift or sample matrix induced interferences by the application of internal standardization. Corrections for characterized spectral interferences should be applied to the data.

5.3 If an element has more than one monitored isotope, examination of the concentrations calculated for each isotope, or the isotope ratios, will provide useful information for the analyst in detecting a possible spectral interference. Consideration should be given to both primary and secondary isotopes in the evaluation of the element concentration.

5.4 The dilution factor is calculated for digestates and must be entered into the sample file in order for the results to be in the desired units.

The ICP/MS gives results in µg/L. For sediments and tissues the results are usually desired in µg/g dry weight. To provide results in µg/g for digestates a dilution factor can be entered that will convert µg/L to µg/g. That factor is:

(Final Sample Volume x dilution)/(1000 x sample weight)

For example:

Final Sample Volume	=	22.64 mL
Diluted 1:11	=	11.1
Sample Weight	=	0.1994 g

(22.64 x 11.1)/(1000 x 0.1994)

The factor for converting µg/L to µg/g for this particular sample would be 1.26.

5.5 Data Generation, Data Review, Data Reporting, and Archiving

The ICP/MS data output is in the form of concentrations of metals in units of ppb for water and ppm for tissue and sediment. These raw data, on a paper printout, are stored in the chemistry central files for at least 10 years. An electronic copy of the metal concentrations is also made on a 3.5" disk and transferred to an Excel spreadsheet using a PC computer. The Excel spreadsheet is used to prepare a table for the client report. The project manager and the MSL QA officer review the table to determine if the raw data were accurately reduced in the final report table. All raw data and versions of the report tables are archived.

6.0 QUALITY CONTROL

All data quality objectives are summarized in Table 1.

Table 1. Data Quality Objectives

QA Sample Type	Frequency	Acceptance Limit	Corrective Action
Laboratory Reagent Blank	1 per batch of 20 or fewer	<10 times the MDL	Reanalyze. If confirmed and all samples are >10 times the blank, no corrective action is required. If samples are <10 times the blank, the batch must be redigested.
Replicate Precision	1 per batch of 20 or fewer	≤ 25% for analytes >10 times the MDL No more than 25% of all RSDs can be > 25%	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple DQO's requires redigestion and reanalysis of batch.
Certified Reference Material (CRM) or Standard Reference Material (SRM); Ongoing Precision and Recovery	1 per batch of 20 or fewer	75-125% of certified value	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple CRMs requires redigestion and reanalysis of batch.
Matrix Spike (MS) /Matrix Spike Duplicate (MSD)	1 per batch of 20 or fewer	75-125% recovery <24% RPD	Reanalyze. Failure to meet criteria shall be reported in Data Summary. Failure of multiple MSs requires redigestion and reanalysis of batch.
Initial and Continuing Calibration Verification	every 10 samples	<15% of initial calibration on	Reanalyze. If CCV still fails, rerun the calibration curve and calculate all samples analyzed after the last passing calibration check using the new curve.

6.1 Calibration blank - consists of 2% (v/v) nitric acid in DI water and is used to establish the analytical calibration curve.

6.2 Laboratory reagent blank - must contain all the reagents in the same volumes as used in processing the samples. The reagent blank must be carried through the entire sample digestion and preparation scheme. The laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and to assess spectral background. Blanks should be analyzed at a frequency of 1 per 20 samples or in a batch of samples if less than 20.

6.3 Rinse water - 2% (v/v) nitric acid in DI water is used to flush the instrument between samples in order to reduce memory interferences.

6.4 Instrument and Method Detection Limits (IDL and MDL):

6.4.1 Instrument Detection Limits (IDL) for ICPMS are determined annually in water by spiking seven or more replicates of reagent water at a minimum of two concentrations (0.1 and 1.0 µg/L or one to five times the expected IDL) for the metals in EPA Method 6020 (April 16, 1996). The IDL is calculated as follows:

IDL = (t) x (S)

where, t = students' T value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates)
S = standard deviation of the replicate analyses

6.4.2 Method Detection Limits (MDL) for water should be established for all analytes, using blank spikes at a concentration of one to five times the estimated detection limit. To determine MDL values, take seven blank spikes and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = (t) x (S)

where, t = students' T value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates)
S = standard deviation of the replicate analyses

Method detection limits should be determined annually or whenever a significant change in background or instrument response is expected (e.g. detector change).

6.4.2 Meaningful MDLs often cannot be determined in an actual tissue or sediment because the concentrations of most trace elements are naturally present at relatively high concentrations in the material and therefore high MDLs will result. In cases when an empirically derived MDL is not practical, a theoretical MDL may be calculated. MDLs for tissue and sediment can be calculated from the IDLs determined in water using the appropriate dilution factor. For ICPMS the dilution factor is normally 1000 X, based on 0.2 g tissue or sediment diluted to 20 mL in the digestion procedure, then diluted 10 X at the time of analysis by ICPMS.

6.4.3 Battelle MSL does not routinely employ reporting limits (RL). It is MSL policy to report all values detected above the empirically determined MDL (or calculated MDL in the case of tissues or sediments when no empirical MDL can realistically be determined). If a client specifically requests that data be reported below the MDL, the affected data should be flagged as detected below the detection limit according to the client's requested flagging convention.

6.6 Linear calibration ranges - linear calibration ranges are primarily detector limited. The upper limit of the linear calibration range should be established for each analyte by determining the signal responses from a minimum of three different concentration standards, one of which is close to the upper limit of the linear range. The linear calibration range which may be used for the analysis of samples should be judged by the analyst from the resulting data. Linear range is checked annually and after major maintenance such as replacement of the detector. The acceptance criterion for each calibration curve is an r^2 of 0.995.

6.7 Matrix spikes should be analyzed at a frequency of 1 per 20 samples or per batch if less than 20. The analyte spike concentration should be about that in the unspiked

sample. Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unspiked sample. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = (C_s - C)/S \times 100$$

where,
- R = percent recovery
- C_s = spiked sample concentration
- C = sample background concentration
- S = concentration equivalent of spike added to sample

If the recovery of any analyte falls outside the designated range, and the laboratory performance for that analyte is within established criteria, the recovery problem encountered with the spiked sample is judged to be matrix related, not system related. The result for that analyte in the spiked sample must be labeled suspect due to matrix effects.

6.8 Internal Standards Responses - The analyst is expected to monitor the responses from the internal standards throughout the sample set being analyzed. Ratios of the internal standards response against each other should be monitored routinely in order to detect potential problems caused by mass dependent drift, errors incurred in fortifying solutions or increases in the concentrations of individual internal standards caused by background contributions from the sample. The absolute response of any one internal standard should not deviate more than 60-125% of the original response in the calibration blank. If deviations greater than this are observed, the instrument should be checked for proper function of the autosamples, dirty cones and proper tuning.

7.0 SAFETY

7.1 Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material data handling sheets should be available to all personnel involved in the chemical analysis.

7.2 Analytical plasma sources emit radiofrequency radiation in addition to intense ultraviolet (UV) radiation. Suitable precautions should be taken to protect personnel from such hazards.

8.0 TRAINING REQUIREMENTS

All staff performing analysis of elements by ICP/MS must first read this procedure and then demonstrate proficiency in the process prior to performing the work. Proficiency will include demonstrating that 1) an SRM is within the certified range; and 2) spike recoveries are within acceptable recovery criteria. Documentation of training will be recorded on training assignment and on-the-job training forms from MSL-A-006, Marine Sciences Laboratory Training. Records of training are kept by the laboratory Quality Assurance Officer.

9.0 REFERENCES

U.S. Environmental Protection Agency. 1996. Method 1638: Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma – Mass Spectrometry. Office of Water and Office of Science and Technology, Washington, D.C.

U.S. Environmental Protection Agency. 1997. Method 1640: Determination of Trace Elements in Water by Preconcentration and Inductively Coupled Plasma-Mass Spectrometry. Office of Water and Office of Science and Technology, Washington, D.C.

MSL-A-006-01 Marine Sciences Laboratory Training.

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Marine Sciences Laboratory

EFFECTIVE DATE: 4-26-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE

MSL-I-024-02

MIXED ACID TISSUE DIGESTION

Approvals:		
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	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric Crecelius	4-25-00
	Signature	Date

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MIXED ACID TISSUE DIGESTION

1.0 SCOPE AND APPLICATION

These methods are applicable to the digestion of tissues for all metals of interest. The aqua regia digestion is used when it is necessary to quantify high levels of silver (Ag), and when metals affected by chloride (Cl) interference are requested for analysis. The Texas A&M University (TAMU) digestion is used when As and Se are requested and the client won't allow the use of hydroflouric acid (HF). The nitric acid (HNO_3):HF method is used for the determination of all metals. The HNO_3 digestion is used for the determination of all metals, except Ag. The HNO_3:peroxide (H_2O_2) digestion is used to determine all metals, except Ag. In this procedure, tissue samples are prepared for analysis by ICP/MS, ICP, CVAAS, or GFAA through an acid digestion which releases bound metals into solution.

This procedure replaces procedure, MSL-M-094, and it reflects a title change from MSL-024-00, which was titled, Aqua Regia Evaporation Tissue Digestion.

2.0 DEFINITIONS

CVAAS	Cold Vapor Atomic Absorption Spectroscopy
ICP	Inductively Coupled Plamsa
ICP/MS	Inductively Coupled Plamsa/Mass Spectroscopy
GFAA	Graphite Furnace Atomic Absorption

3.0 RESPONSIBLE STAFF

Technical Staff – sample preparation
Analyst - analysis, calculations
QA Officer or Representative - data verification

4.0 PROCEDURE

4.1 Hardware and Reagents

4.1.1 Hardware

- Teflon bomb, 60 mL (Savillex, Inc.) and capping station (CEM Corp.)
- Oven maintained at 130 °C ± 10 °C
- Pipette with acid-cleaned plastic tips
- Polybottles, 20 mL - acid-cleaned
- Hot plate
- Balance capable of weighing to 0.1 mg
- Repipette dispenser

4.1.2 Reagents

Suppliers listed in parentheses are recommended suppliers and may be substituted if necessary. All reagent concentrations should be verified by a clean blank.

- Nitric Acid (HNO_3), concentrated (Baker Instra-Analyzed for trace metals or equivalent).
- Hydrochloric Acid (HCl), concentrated (Baker Instra-Analyzed for trace metals or equivalent).
- 4:1 mixture of nitric:perchloric (HNO_3:$HCLO_4$) acids (Baker Instra-Analyzed for trace metals or equivalent).
- 30% Hydrogen Peroxide (H_2O_2; reagent grade for trace metals),
- Hydroflouric Acid (HF; Baker Instra-Analyzed for trace metals)

4.2 **Procedure**

The Tissue Sample Digestion form (Attachment 1) shall be used to record sample digestion information. Before weighing samples, the header information, including the specific digestion method, shall be completely filled out.

Note: Avoid dust and air borne particle contamination.

4.2.1 Aqua Regia Tissue Digestion

4.2.1.1 Record bomb ID number on the side of the bomb and tare weight on the Sample Digestion form. Carefully weigh approximately 500 mg of finely ground freeze-dried tissue (prepared in accordance with MSL-C-003, Percent Dry Weight and Homogenizing Dry Sediment, Soil, and Tissue), record as sample weight to 0.1 mg and add to a tared, acid-cleaned Teflon bomb. If static electricity is present and causes some sample to stick to the sides of the bomb, use an anti-static device to eliminate the electricity.

4.2.1.2 Using automatic pipettes and acid-cleaned pipette tips, add 5.0 mL of HCl and 3.5 mL of HNO_3.

4.2.1.3 Tighten the bombs securely using a capping station. Place the bombs in an oven maintained at 130 °C ±10 °C overnight or a minimum of 8 hours. CAUTION: If bombs are not tightened securely, they will leak which will lead to errors in the results.

4.2.1.4 Remove bombs from the oven and allow them to cool. Add 15 mL reagent grade water using a repipette dispenser.

4.2.1.5 Weigh and record as digestate + tare weight. Shake the bomb vigorously and transfer the solution to a 20 mL acid-cleaned polyethylene vial. Samples are now ready for analysis.

4.2.2 TAMU Tissue Digestion

4.2.2.1 Record bomb ID number from the side of the bomb and tare weight on the Tissue Sample Digestion form. Carefully weigh approximately 500 mg of finely ground freeze-dried tissue (prepared in accordance with MSL-C-003), record as sample weight to 0.1 mg and add to a tared, acid-cleaned Teflon bomb. If static electricity is present and causes some sample to stick to the sides of the bomb, use an anti-static device to eliminate the electricity.

4.2.2.2 Using automatic pipettes and acid-cleaned pipette tips, add 3 mL 4:1 HNO_3 : $HClO_4$. Screw on the caps, but do not tighten. Place the bombs on a warm hot plate (with water bath) in a fume hood for 4 hours. This step allows the release of fumes that could create high pressure conditions within the bomb. Allow to cool before handling.

4.2.2.3 Tighten the bombs securely using a capping station. Place the bombs in an oven maintained at 130 °C ±10 °C for 4 hours. **CAUTION**: If bombs are not tightened securely, they will leak which will lead to errors in the results.

4.2.2.4 Remove bombs from oven and allow to cool. Add 20 mL of reagent grade water using a repipette dispenser.

4.2.2.5 Weigh and record as digestate + tare weight. Shake the bomb vigorously and transfer the solution to a 20 mL acid-cleaned polyethylene vial. Samples are now ready for analysis.

4.2.3 Nitric Acid: Hydrofluoric Acid (HNO3:HF) Tissue Digestion

4.2.3.1 Record bomb ID number from the side of the bomb and tare weight on the Tissue Sample Digestion form. Carefully weigh approximately 300 mg of finely ground freeze-dried tissue (prepared in accordance with MSL-C-003), record as sample weight to 0.1 mg and add to a tared, acid-cleaned Teflon bomb. If static electricity is present and causes some sample to stick to the sides of the bomb, use an anti-static device to eliminate the electricity.

4.2.3.2. Using automatic pipettes and acid-cleaned pipette tips, add 5.0 mL of HNO3 and 0.5 mL of HF to each bomb. Screw on the caps and tighten with a bomb tightener.

4.2.3.3. Place the bombs in an oven maintained at 130 °C ± 10 °C for a minimum of 8 hours or overnight. CAUTION: If bombs are not tightened securely, they will leak which will lead to errors in the results.

4.2.3.4. Remove bombs from oven and allow to cool. Add 15 mL of reagent grade water using a repipette dispenser.

4.2.3.5. Weigh and record as digestate + tare weight. Shake the bomb vigorously and transfer the solution to a 20 mL acid-cleaned polyethylene vial. Samples are now ready for analysis.

4.2.4 Nitric Acid (HNO_3) Tissue Digestion

This method can be performed using Teflon bombs or using 30 mL glass scintillation vials. Teflon bombs are sealed and placed in an oven overnight or a minimum of 8 hours. Glass vials with loose lids are placed on a hot plate in a ventilated hood for 4 hours or until the brown cloud inside each vial has dissipated.

4.2.4.1 Record bomb ID number from the side of the bomb and tare weight (or just glass vial tare weight) on the Tissue Sample Digestion form. Carefully weigh approximately 300 mg of finely ground freeze-dried tissue (prepared in accordance with MSL-C-003), record as sample weight to 0.1 mg and add to a tared, acid-cleaned Teflon bomb or tared, acid-cleaned glass vial. If static electricity is present and causes some sample to stick to the sides of the bomb, use an anti-static device to eliminate the electricity.

4.2.4.2 Using automatic pipettes and acid-cleaned pipette tips, add 5.0 mL of HNO_3 to each bomb or glass vial. For Teflon bombs, screw on the caps and tighten with a bomb tightener. For glass vials, replace lids loosely.

4.2.4.3 Place the bombs in an oven maintained at 130 °C ± 10 °C for a minimum of 8 hours or overnight. CAUTION: If bombs are not tightened securely, they will leak which will lead to result errors. Place glass vials on a cold hot plate and slowly increase the temperature until approximately 225°C (350°F). Cook at this temperature until the brown cloud in each vial dissipates or 4 hours. Do not let samples go to dryness.

4.2.4.4 Remove bombs from oven or glass vials from hot plate and allow to cool. Add reagent grade water to the neck of each glass vial or 15 mL to each Teflon bomb using a repipette dispenser. Replace sample container lids.

4.2.4.5 Weigh and record as digestate + tare weight. Samples in glass vials are now ready for analysis. Shake the bomb vigorously and transfer the solution to a 20 mL acid-cleaned polyethylene vial. Samples are now ready for analysis.

4.2.5 Nitric Acid: Hydrogen Peroxide (HNO_3:H_2O_2) Tissue Digestion

4.2.5.1 Record glass vial tare weight on the Tissue Sample Digestion form. Carefully weigh approximately 300 mg of finely ground freeze-dried tissue (prepared in accordance with MSL-C-003), record as sample weight to 0.1 mg and add to a tared, acid-cleaned glass vial. If static electricity is present and causes some

sample to stick to the sides of the vial, use an anti-static device to eliminate the electricity.

4.2.5.2 Using automatic pipettes and acid-cleaned pipette tips, add 5.0 mL of HNO_3 to each glass vial. Replace lids loosely.

4.2.5.3 Place glass vials on a hot plate and warm them for approximately two hours or until foaming stops. Cool samples completely.

4.2.5.4 Slowly add 3 mL of H_2O_2 in 1 mL increments. Allow the chemical reaction to go to completion between additions. Warming the samples slightly helps the reaction go to completion quicker, but be careful that samples do not foam up and out of the sample container. Return the samples to a cold hot plate and increase the temperature to approximately 225°C (350°F) slowly. Cook at this temperature until the brown cloud in each vial dissipates or 4 hours. Do not let samples go to dryness.

4.2.5.5 Remove glass vials from hot plate and allow to cool. Add reagent grade water to the neck of each vial. Replace sample container lids.

4.2.5.6 Weigh and record as digestate + tare weight. Samples are now ready for analysis.

5.0 DATA ANALYSIS AND CALCULATIONS

Calculate the solution volume using the following equations:

$$V_{mL} = \frac{\text{digestate weight (g)}}{\text{density (g/mL)}}$$

where,

density = 1.05 g/mL for the aqua regia final digestate.
density = 1.04 g/mL for the TAMU final digestate.
density = 1.13 g/mL for the HNO3:HF final digestate
density = 1.12 g/mL for the HNO3 final digestate
density = 1.08 g/mL for the HNO3:H2O2 final digestate

6.0 QUALITY CONTROL

Procedural and/or reagent blanks, replicates, matrix spikes, and certified reference materials, should be prepared and analyzed at a frequency of 1 per 20 samples or 1 per batch (if less than 20 samples). When required by the customer or MSL Project Manager, other QC checks for accuracy and precision shall also be applied to each batch of samples.

7.0 SAFETY

All technicians and analysts following this procedure should be aware of routine laboratory safety concerns, especially all safety protocols regarding use of acids, including the following:

- Gloves, protective clothing, face shields, and safety glasses or goggles should be worn when handling digestates and acids.
- Use an acid hood to exhaust brown gas fumes.

8.0 TRAINING REQUIREMENTS

8.1 All staff performing tissue and sediment digestions should first read this procedure and conduct their first digestion under supervision of a staff member who has had previous experience conducting the procedure. Staff should demonstrate proficiency in the process prior to performing the work. Documentation of training will be performed in accordance with MSL-A-006, Marine Sciences Laboratory Training.

8.2 All staff should have received training in the handling of corrosive chemicals and the use of fume hoods. Extra caution should be taken due to the unpredictable nature of some samples during the heating process.

9.0 REFERENCES

MSL-C-003 Percent Dry Weight and Homogenizing Dry Sediment, Soil, and Tissue
MSL-A-006 Marine Sciences Laboratory Training

ATTACHMENT 1
Example Tissue Sample Digestion Form

EXAMPLE TISSUE SAMPLE DIGESTION **Date:**

Logbook Page # **Analyst:**

Project **Addendum #** **Balance #**

CF # **Sample Matrix:**

Digestion Method **SOP #**

		Digestate +	Tare	Sample			
Bomb ID	Sample ID	Tare wt (g)	Wt. (g)	Wt. (g)			
							SRM:
							ID#
							SRM
							ID#
							SRM
							ID#
							REAGENT
							LOT#
							REAGENT
							LOT#
							REAGENT
							LOT#
							Comments:

Analyst:_____ Peer Reviewer:_____

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Marine Sciences Laboratory

EFFECTIVE DATE: 11-22-99

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-I-026-01

USE OF LABORATORY REFRIGERATORS AND FREEZERS

Approvals:		
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	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	11-18-99
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	Eric A. Crecelius	11-18-99
	Signature	Date

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USE OF LABORATORY REFRIGERATORS AND FREEZERS

1.0 SCOPE AND APPLICATION

Laboratory refrigerators and freezers at Battelle are used for the storage of project samples, standards, chemical solutions, and reagents. The purpose of this procedure is to outline the proper use and maintenance of these units. The activities described herein are considered minimum requirements. Additional project-specific requirements are detailed in project work plans and will be performed in addition to, not instead of, these requirements.

This procedure describes the use and maintenance of refrigerator and freezer units operated by various technical sections within the Battelle Marine Sciences Laboratory (MSL). Documentation strategies of these sections differ according to need, size, organization, and space limitations. In this procedure, section file is defined as the filing system used by an organizational department for storing records, whether this is a filing cabinet or a logbook.

2.0 DEFINITIONS

Refrigerator - Stand alone or walk-in unit for storage of samples at 4°C ± 2 °C.

Freezer - Stand alone or walk-in unit for storage of samples at -20 °C ± 4 °C.

Low-temperature freezer - Stand alone or walk-in unit for storage of samples at -80 °C ± 4 °C.

3.0 RESPONSIBLE STAFF

The following procedures are performed and documented by any staff member designated by an appropriate Laboratory Manager or group supervisor.

4.0 PROCEDURE

Each laboratory refrigerator and freezer is equipped with a thermometer capable of measuring the intended temperature range of the unit. Each thermometer is assigned an identification number (ID #), which is documented on the temperature-monitoring log and on the thermometer itself. The accuracy of this thermometer is calibrated annually with a National Institute of Standards & Technology (NIST)-standardized (traceable) thermometer. Calibration will be performed more often if the integrity of the thermometer is suspect (as determined during weekly monitoring). The correction factor determined during the calibration exercise is applied during routine temperature monitoring. The correction factor will be documented on the monitoring log. Calibration and temperature monitoring need not be performed on refrigerator and freezer units that are not in use. Inactive units will be documented in the monitoring records.

Each laboratory refrigerator or freezer is labeled with an identification number, which is directly traceable to its location and the organizational unit responsible for its use and maintenance. Thermometers are placed in a secure location within each refrigerator and freezer unit. Refrigerator thermometers are immersed in an aqueous solution (e.g., Milli-Q water). The temperature of most units is recorded five times a week. Units which are strictly used for long- term storage of samples and extracts are monitored a minimum of three times each week.

NOTE: Monitoring will be performed on Monday, Wednesday, and Friday (if one of these days falls on a holiday, the day can be shifted +/- I day).

The frequency of temperature monitoring may be increased to meet project specific requirements. Additional requirements are documented in the project specific work plan. Attachments 1 is an example of the form used for monitoring refrigerator and freezer units. The acceptable temperature range for each unit has been determined by the Lab Manager or group supervisor and is recorded on these forms. Temperature monitoring records are maintained on the door of each unit and are filed in the section files when complete. The walk-in refrigerator/freezer unit has a continuously recording monitor, which is checked daily by security personnel. Deviations from acceptable temperature ranges are reported immediately to the facilities maintenance staff and the laboratory manager. The Laboratory Manager directs the appropriate corrective action.

The Laboratory Manager or his/her designee is responsible for maintaining a log or file containing the following information:

A master list defining ID # and location of all section refrigerator and freezer units (a copy of this list should be supplied to the Quality Assurance Unit and updated, as needed),

- Thermometer calibration records
- Miscellaneous documentation (e.g., documentation of outliers and corrective action)
- Refrigerator/freezer maintenance log forms
- Temperature monitoring records for each appropriate refrigerator and freezer unit.

Samples logged into the walk-in refrigerator or freezer unit for storage will be assigned placement based on date of arrival to MSL. Sample groups will be labeled with appropriate month and year of arrival and will be reviewed for disposal on a six-month rotation unless otherwise requested by Project Manager. When the Project Manager makes a request for samples be archived, samples will be moved to the designated area marked "Archive" and a notation will be made in the central file.

MSL sample disposition is mandated by the formal, Battelle Pacific Northwest National Laboratory-controlled document issued under the Standards Based Management System (SBMS) in the subject area of "Chemical Management System". The SBMS is a web-based policy and procedures resource for Battelle staff which guides day-to-day

operations. The Chemical Management System (CMS) tracks solution disposal (e.g., for samples, reagents, standards, etc.) Samples are labeled as to their disposition date when archived. A Sample Disposal Logbook is used to document Sample Disposal.

Maintenance is performed when:

- Spills occur in the refrigerator or freezer unit (spills should be cleaned up promptly and the Laboratory Manager informed)
- Freezer units require defrosting
- Corrective actions are required.

5.0 DATA ANALYSIS AND CALCULATIONS

Not Applicable

6.0 QUALITY CONTROL

In the event a refrigerator or freezer deviates consistently from the acceptable temperature range, corrective action must be taken. Corrective action may include:

- Verifying the integrity of the thermometer
- Rechecking the temperature once the unit has stabilized
- Cleaning the unit seals, vents, and grill
- Defrosting
- Arranging for an authorized professional service call

All corrective action and results of corrective action will be documented in the maintenance log.

7.0 SAFETY

No food meant for human consumption is stored in laboratory refrigerators or freezers. While laboratory refrigerators and freezers themselves pose no danger, all laboratory personnel should assume that the contents are hazardous, unless the exact identification is known. Gloves, lab coats, and safety goggles are worn when handling hazardous materials.

At the end of the storage period, all unneeded samples are disposed of in the appropriate waste stream.

8.0 TRAINING REQUIREMENTS

All laboratory personnel using refrigerators or freezers for the storage of samples, reagents, and standards will read this procedure. Documentation of training will be recorded on a training assignment form from MSL-A-006, Marine Sciences Laboratory Training.

9.0 References

MSL-A-006 Marine Sciences Laboratory Training.

ATTACHMENT 1

Example of Temperature Monitoring Record for Refrigerators

REFRIGERATOR/FREEZER TEMPERATURE LOG

Equipment _____ Acceptable Range _____ Location _____

ID# _____ Date _____

Temperature C___ F___ Thermometer ID_____

Day	JAN		FEB		MAR		April		May		JUNE	
	Temp	Initial	Temp	Initial	Temp	Initial	Temp	Initial	Temp	Initial	Temp	Initial
1									Weekend			
2	Weekend								Weekend			
3	Weekend						Weekend					
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EFFECTIVE DATE: 7-13-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-I-027-03

DETERMINATION OF METALS IN AQUEOUS AND DIGESTATE SAMPLES BY ICP/AES

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DETERMINATION OF METALS IN AQUEOUS AND DIGESTATE SAMPLES BY ICP/AES

1.0 SCOPE AND APPLICATION

This procedure is to be used by personnel working in the Battelle Marine Sciences Laboratory (MSL) Inorganic Laboratory for analysis of environmental samples by Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) using the Perkin-Elmer Optima 3000. These procedures conform to specifications in U.S. Environmental Protection Agency (EPA) Method 200.7, EPA SW-846, Method 6010B, and the Superfund Contract Laboratory Program (CLP) Statement of Work.

1.1 Dissolved elements are determined in filtered and acidified samples. Appropriate steps must be taken in all analyses to ensure that potential interferences are taken into account. This is especially true when dissolved solids exceed 1,500 mg/L.

1.2 Total elements are determined after appropriate digestion procedures are performed. Since digestion techniques increase the dissolved solids content of the samples, appropriate steps must be taken to correct for potential interference effects.

1.3 Table 1 lists elements along with recommended wavelengths and typical inter-element interferences.

1.4 This procedure provides general operating instructions for trace metals analysis using the Perkin-Elmer Optima 3000. It is not intended to replace the instruction manual provided by the manufacturer. It is assumed that persons using this procedure have a good working knowledge of atomic spectroscopy and are experienced in the use of computer-controlled atomic spectrophotometric instrumentation.

TABLE 1. Elements Determined by ICP-AES; Recommended Wavelengths and Typical Inter-element Interferences

Element	Wave Length (nm)	Possible IECs* Required
Ag	328.068	Fe, Mn,
Al	308.2	Mn, Mo, V
As	193.696	V, Al, Fe, Ca, Mg, Mo
B	249.772	Fe
Ba	455.403	
Be	313.042	Ti, V
Ca	317.933	Cu, Fe
Cd	226.502	Co, Fe,
Cd	228.802	As
Co	228.616	Fe
Co	238.892	Ca, Fe, Mo, V
Cr	267.715	Fe, Mn, Mg, V
Cu	324.754	Fe, Mg, Mn, V
Fe	259.939	
K	766.491	Ca
Li	670.784	-
Mg	279.077	Ca, Fe, Mn
Mn	257.610	Fe, Mg
Mo	202.030	Fe
Na	588.995	-
Ni	231.604	Al, Fe
Ni	221.648	Fe
Pb	220.353	Al, Ca, Fe,
Se	196.026	Fe,
Sr	407.771	Ca
Ti	334.941	Ca
V	292,404	Cr, Fe, Mn, Mo, Ti
Zn	206.2	Co, Cr, Fe, Ti
Zn	213.856	Ca, Cu, Fe, Ni, Ti

* Inter-element correction factors

2.0 SUMMARY OF METHOD

This method describes a technique for the simultaneous, multi-element determination of trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where dissociation and excitation occurs. Characteristic atomic-line emission spectra are produced by a radio-frequency ICP. The spectra are dispersed by an echelle-grating spectrometer and the intensities of the emission lines are monitored by a segmented-array charge coupled-device detector (SCD). The SCD provides simultaneous detection with simultaneous background measurement. The polychromator has an echelle grating designed to use multiple diffraction orders. To separate the orders and create a two-dimensional diffraction pattern, the echelle grating is combined with a cross-dispersing element for the ultraviolet spectrum and a prism is used in the visible range. A background correction technique is required to compensate for variable background contribution to the determination of trace elements. Background must be measured adjacent to analyte lines on samples during analysis. The positions used for background measurements must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences should also be recognized and appropriate corrections made. A more detailed discussion of interferences and correction techniques appears in Section 6.

The position selected for the background intensity measurement, may be on either, or both, sides of the analyte line.

3.0 DEFINITIONS

Dissolved - Material that will pass through a 0.45 µm membrane filter assembly, prior to sample acidification.

Suspended - Those elements (metals) which are retained by a 0.45 µm membrane filter.

Total Recoverable - The concentration of analyte determined on an unfiltered sample following treatment with hot dilute mineral acid.

Instrument Detection Limit (IDL) - The IDL is the minimum concentration of an analyte that a particular instrument can measure and report with 99% confidence that the analyte concentration is greater than zero.

Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero and determined from analysis of a sample in a given matrix containing analyte.

Linear Detection Range (LDR) - The concentration range over which the analytical working curve remains linear.

Reagent Blank - A volume of deionized, distilled water of the same acid matrix as the calibration standards carried through the entire analytical scheme.

Calibration Blank - A volume of deionized, distilled water acidified with nitric acid (HNO_3) and hydrochloric acid (HCl).

Blank Spike - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The blank spike is analyzed exactly like a sample, and its purpose is to determine whether the method is within accepted control limits. The blank spike concentration is usually equal to one of the calibration solutions.

Matrix Spike - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The matrix spike is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the matrix spike corrected for the concentrations found.

4.0 RESPONSIBLE STAFF

- Researcher/Technician-sample preparation, digestion
- Analyst – sample analysis
- MSL QA Officer or Representative- data verification

5.0 PROCEDURE

5.1 Sample Collection, Preservation, Storage, and Digestion

5.1.1 For recommendations on sample collection and preservation requirements, refer to the procedure MSL-A-001, Sample Log In Procedure.

5.1.2 For the determination of dissolved elements, the sample should be filtered through a 0.45 µm membrane filter. Use a portion of the sample to rinse the filter assembly, discard and then collect the required volume of filtrate. Acidify the filtrate with 1.0 µl/ml concentrated HNO_3 immediately following filtration to a pH less than 2.0.

5.1.3 For the determination of total recoverable elements in aqueous samples, acidify with 1.0 µl/ml concentrated HNO_3 at the time of collection to a pH less than 2.0. The sample should not be filtered prior to analysis.

NOTE: Samples that cannot be acid preserved at the time of collection, should be acidified with HNO_3 to a pH of less than 2.0 upon receipt in the laboratory (normally, 1.0 µl/ml concentrated HNO_3 is sufficient for most ambient and drinking water samples).

Following acidification, the sample should be held for sixteen hours before withdrawing an aliquot for sample processing. Samples are stored at 20 °C in the Sample Preparation Laboratory, MSL 5, Room 231, which has filtered air.

5.1.4 Sediment and tissue samples shall be digested according to the project-specific requirements.

5.1.5 For the total recoverable digestion procedure, 10.0 mL of sample water is heated at 85 °C for 2.5 hours with 0.2 mL of HNO_3 and 0.5 mL of HCL in a sealed 15 mL plastic centrifuge tube.

5.2 Potential Interferences

Several types of interference effects may contribute to inaccuracies in the determination of trace elements. These are summarized in the following sections:

5.2.1 Spectral interferences can be categorized as 1) overlap of a spectral line from another element; 2) unresolved overlap of molecular band spectra; 3) background contribution from continuous or recombination phenomena; and 4) background contribution from stray light from the line emission of high concentration elements. The first of these effects can be compensated for by utilizing a computer correction of the raw data, requiring the monitoring and measurement of the interfering element. The second effect may require selection of an alternate wavelength. The third and fourth effects can usually be compensated for by a background correction adjacent to the analyte line. In addition, users of simultaneous multi-element instrumentation must assume the responsibility of verifying the absence of spectral interference from an element that could occur in a sample, but for which there is no channel in the instrument array.

Winge *et. al.*,1985 provides some of the interference effects for the recommended wave lengths listed in Table 1. The data in this reference are intended for use only as a rudimentary guideline for the indication of potential spectral interferences. A list of possible inter-element interferences observed in using the Optima 3000 appears in Table 1. Typically, linear relations between concentration and intensity for the analytes and the interferant can be assumed.

The suggested use of this information is as follows: Assume that arsenic (at 193.696 nm) is to be determined in a sample containing approximately 10 mg/L of aluminum. According to the reference Winge *et. al.*,1985, 100 mg/L of aluminum would yield a false signal for arsenic equivalent to approximately 1.3 mg/L. Therefore, 10 mg/L of aluminum would result in a false signal for arsenic equivalent to approximately 0.13 mg/L. The reader is cautioned that the Optima 3000 may exhibit somewhat different levels of interference than those discussed by Winge *et. al.*,1985. Actual interference effects for the Optima 3000 were investigated by performing wavelength scans while aspirating single-element solutions of analytes and observing the effects produced on every individual analytical channel. Spectra thus acquired were then plotted such that blank, analyte and interferant scans were overlaid. These overlays were used to determine the optimum placement of background correction points and to check for spectral interferences requiring inter-element corrections. Generally,

interferences were discernable if they produced peaks or background shifts corresponding to 2-5 percent of the peaks generated by the analyte.

5.2.2 Physical interferences are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies especially in samples containing high dissolved solids and/or acid concentrations. The Optima 3000 uses a peristaltic pump to lessen these interferences. If these types of interferences are operative, they must be reduced by dilution of the sample and/or utilization of standard addition (MSA)/or internal standard techniques. Another problem which can occur from high dissolved solids in samples is salt buildup at the tip of the nebulizer. This affects aerosol flow rate causing instrumental drift. The Optima 3000 uses a cross-flow type nebulizer which exhibits excellent resistance to clogging when aspirating samples containing high levels of dissolved solids. In extreme situations sample dilution is used to further control this problem.

5.2.3 Chemical interferences are characterized by molecular compound formation, ionization effects, and solute vaporization effects. Normally these effects are not pronounced with the ICP technique; however, if observed they can be minimized by careful selection of operating conditions (that is, incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. These types of interferences can be highly dependent on matrix type and the specific analyte element.

5.3 Apparatus and Reagents

- Optima 3000 Computer-controlled ICP-AES Spectrophotometer with automatic sample changer. Data acquisition/instrument control system. The ICAP uses a Pentium III 450 MHz personal computer with enhanced color graphics running WINLAB 32 software. This system is used for controlling the spectrometer and automatic sample changer and calculating and applying dilution, background correction, inter-element correction and internal standard correction factors.

- Argon gas supply (high-purity grade, 99.99%)

- Nitrogen gas supply (for optics purge), with pressure regulating valve(s) to deliver gas at the pressure and volume required by the instrument.

- Various volumetric flasks, graduated cylinders and calibrated pipettes.

- HNO_3, concentrated, trace metal grade

- HCl, concentrated, trace metal grade

- Standard stock solutions are purchased or prepared from ultra-high purity grade chemicals or metals at least once each year, or as needed during the year. All stock solutions, metals or salts must have documented purity and a traceable certification to the National Institute of Standards and Technology (NIST).

- Certified standards are purchased annually. The primary standards contain either 1000 ppm or 10 ppm concentrations of metals. Secondary standards are prepared from these primary standards. The secondary standards are assigned unique numbers and information is recorded in the standard logbook. The secondary standards have the same shelf life as the primary standards.

- Certified multi-element stock standard solutions are purchased annually. (refer to Appendix 1 for typical concentrations).

- Working standards are prepared by diluting stock multi-element standard solutions. Working calibration standards are required to be prepared daily, using deionized (DI) water containing 5% (v/v) HCL and 2% (v/v) HNO_3.

- Class A, volumetric glassware shall be used in performing all dilutions.

- Three types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve while the reagent blank (preparation blank), is used to detect possible contamination resulting from varying amounts of the acids used in the sample processing, and the rinse blank is used to flush the instrument between samples to reduce memory interference.

- The calibration blank is prepared by diluting 200 mL of concentrated HNO_3 and 500 mL of concentrated HCl to 10 L with deionized water. This solution will be used as a diluent in the preparation of all working calibration and quality control (QC) standards and will be used to rinse the sample introduction system of the ICP between sample analyses.

- The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

- In addition to the calibration standards, several QC standards are analyzed to verify and monitor instrument calibration and stability and to validate inter-element and background corrections. Detailed information regarding QC standards is presented in Section 7, Quality Control.

5.4 Step-by-Step Procedure

5.4.1 Sample Preparation

5.4.1.1 For the determination of ground and surface waters, HCL and HNO_3 acid are added as to matrix match with the standards.

5.4.1.2 For the determination of elements in digestates, depending on the amount of digestate, the samples are either run direct or diluted to matrix match with the diluent used in making the standards.

5.4.2 Instrument Start-Up and Calibration

5.4.2.1 Check that argon and nitrogen gas supplies are on and adjusted correctly. Turn on power to the instrument in accordance with manufacturer's instructions and initiate the appropriate operating configuration of the computer and controlling software. Check the peristaltic pump tubing and replace if it is worn. Ignite the plasma and aspirate blank/rinse solution for 1 hour to allow the instrument to come to thermal equilibrium.

5.4.2.2 Following the manufacturer's recommendations, the instrument is optimized using the manganese channel while aspirating a 10 ppm manganese solution. After profiling record the peak intensity, time, date and optical position in the maintenance log.

5.4.2.3 Prior to initial calibration, set up proper instrument software routines for analysis. Standardize the instrument according to instrument manufacturers' recommended procedures, using two solutions consisting of a blank and a high standard for each of the standards used in analysis. Calibration standards need to be prepared such that the major interfering analytes are not in the same solution as the affected analyte. Three replicates are required and the calculated average intensity of the three replicates is used for both standardization and sample analysis. Flush the system with the calibration blank between each analysis

5.4.2.4 A typical analysis sequence for a calibration by ICP-AES is shown below, sample numbers analyzed after sequence #33 may be greater than10:

1. Calibration Blank
2. Cal Std A
3. Cal Std B
4. Cal Std C
5. ICV1
6. ICV2
7. ICB
8. CRI (if required)
9. Histd A
10. Histd B
11. Histd C
12. ICSA
13. ICSAB
14. CCV
15. CCB
16. Prep blank
17. SRM/ LCS
18. Sample 1
19. Sample 1 serial dilution
20. Sample 1 post-digestion spike (if required)
21. Sample 1dup
22. Sample 1 spike
23. Sample 1 spike dup

24. Sample 2
25. Sample 3
26. CCV
27. CCB
28. Sample 4
29. Sample 5
30. Sample 6
31. Sample 7
32. Sample 8
33. Sample 9
34. Sample 10
35. CRI (if required)
36. ICSA
37. ICSAB
38. CCV
39. CCB

5.5 Instrument Maintenance, Operation and Preventive Maintenance

The ICP-AES is maintained by the Perkin Elmer service engineer under a maintenance agreement. All maintenance is recorded in the ICP-AES maintenance book and stored in MSL 5, Room 114. The service engineer conducts preventive maintenance on a schedule recommended by Perkin Elmer. The MSL ICP-AES operator daily checks the argon supply pressure and the nitrogen supply pressure. Corrective action includes changing the auto sampler pump tubing, adjusting the nebulizer flow, and contacting the service engineer for additional corrective action or to schedule a site visit when needed.

5.6 Software Maintenance

The ICP-AES software is upgraded and maintained by the service engineer under the maintenance agreement.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 For aqueous samples prepared by the digestion, multiply the solution concentrations by the dilution factor.

6.2 The sample preparation weight, volume and any dilutions are entered in the sample information file and the instrument uses these values to convert the concentrations from µg/L to µg/g for the analysis of tissues or sediments.

6.3 Data Generation, Data Review, Data Reporting, and Archiving

6.3.1 The ICP-AES data output is in the form of concentrations of metals in units of µg/L or mg/L for water and µg/g for tissue and sediment. These raw data, on a paper printout, are stored in the chemistry central files for at least 10 years. The software uses an exporting program to convert the results file to an electronic file to be i nported into an Excel spreadsheet using a PC computer. An Excel

spreadsheet is used to prepare a summary data table for the client report. The project manager and the MSL QA Officer review the table to determine if the raw data were accurately reduced in the final report table. All raw data and versions of the report tables are archived.

6.3.2 The result library is checked and archived into Zip files monthly using the manufacturer's software. The result files are then deleted from the hard disk and the libraries are packed. The Zip files are also copied on to Zip disks and stored in case of computer malfunction.

7.0 QUALITY CONTROL

The data acquired from QC sample analysis are used to estimate and evaluate the quality and information content of analytical data and to determine the necessity or the effect of corrective action procedures. The summary values used to estimate quality and information content include precision, accuracy, detection limit, and other quantifiable and qualitative indicators.

7.1 General Requirements

7.1.1 All QC data are maintained and available for easy reference or inspection.

7.1.2 Chemicals are dated upon receipt of shipment, and expiration dates noted Chemicals are replaced as needed, or before the shelf life, as indicated by the expiration date, has been exceeded.

7.2 Specific Requirements

The following steps should be performed at least once with each analytical batch, or at a frequency of a minimum of once per twenty sample batch.

7.2.1 ICP Serial Dilution Analysis.

A serial dilution is performed to verify that there are no chemical or physical interferences present.

Prior to reporting concentration data for the analyte elements, the ICP Serial dilution analysis must be performed and reported along with the sample results.

The ICP Serial Dilution Analysis must be performed on every group of samples of a similar matrix type (i.e., water, soil) and concentration (i.e., low, medium, high) for each batch of samples, or for every 20 samples received, whichever is more frequent. Samples identified as field blanks cannot be used for serial dilution analysis. If the analyte concentration is sufficiently high (minimally a factor of 10 above the instrumental detection limit in the undiluted sample), an analysis of a 1:5 fold dilution must agree within 10 percent of the original determination.

7.2.2 Method of Standard Additions (MSA)/ Internal Standard

The MSA should be used if an interference is suspected or a new matrix is encountered. When the method of standard additions is used, standards are added at one or more levels to a portion of prepared sample. This technique compensates for enhancement or depression of an analyte signal by a matrix. It will not correct for additive interferences, such as contamination, interelement interferences, or baseline shift. This technique is valid in the linear range when the interference is constant over the range, the added analyte responds the same as the endogenous analyte, and the signal is corrected for additive interferences. The simplest version of this technique is the single addition method. This procedure calls for two identical aliquots of the sample to be taken. To the first aliquot, a small volume of standard is added; while to the second, a volume of acid blank is added equal to the standard addition. The sample concentration is calculated by: multiplying the intensity value for the unfortified aliquot by the volume (liters) and concentration (µg/L, mg/L or µg/g) of the standard addition to make the numerator; the difference in intensities for the fortified sample and unfortified sample is multiplied by the volume (liters) of the sample aliquot for the denominator. The quotient is the sample concentration.

For more than one fortified portion of the prepared sample, linear regression analysis can be applied using a computer or calculator program to obtain the concentration of the sample solution.

An alternative to using method of standard additions is the internal standard technique. Add on or more elements not in the samples and verified not to cause an interelement spectral interferences to the samples, standards and blanks; yittrium or scandium are often used. The concentration should be sufficient for optimum precision but not so high as to alter the salt concentration of the matrix. The element intensity is used by the instrument as an internal standard to ratio the analyte intensity signals for both calibration and quantitation. This technique is very useful in overcoming matrix interferences in high solids matrices.

7.2.3 ICP Interference Check Sample Analysis

To verify inter-element and background correction factors, an ICP Interference Check Sample must be analyzed at the beginning and end of each sample analysis run (or a minimum of twice per 8-hour working day, whichever is more frequent). Results for the check sample analysis during the analytical runs must fall within the control limit of ± 20 percent of the true value for the analytes included in the Interference Check Sample. If the control limit is exceeded, terminate the analysis, correct the problem, recalibrate, verify the calibration, and reanalyze the samples

7.2.4 Instrument and Method Detection Limits (IDL and MDL):

7.2.4.1 Instrument Detection Limits (IDL) for ICP-AES are determined annually in water by spiking seven or more replicates of reagent water at one to five times the expected IDL for all analytes of interest. The IDL is calculated as follows:

IDL = (t) x (S)

where, t = students' T value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates)
S = standard deviation of the replicate analyses

7.2.4.2 Method Detection Limits (MDL) for water should be established for all analytes, using blank spikes at a concentration of one to five times the estimated detection limit. To determine MDL values, take seven blank spikes and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = (t) x (S)

where, t = students' T value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates)
S = standard deviation of the replicate analyses

Method detection limits should be determined annually or whenever a significant change in background or instrument response is expected (e.g. detector change).

7.2.4.3 Meaningful MDLs often cannot be determined in an actual tissue or sediment because the concentrations of most trace elements are naturally present at relatively high concentrations in the material and therefore high MDLs will result. In cases when an empirically derived MDL is not practical, a theoretical MDL may be calculated. MDLs for tissue and sediment can be calculated from the IDLs determined in water and mathematically processing them through the same dilution factor that applies to actual tissue or sediment samples (volume analyzed, total mass digested, and total volume digested).

7.2.4.4 Battelle MSL does not routinely employ reporting limits (RL). It is MSL policy to report all values detected above the empirically determined MDL (or calculated MDL in the case of tissues or sediments when no empirical MDL can realistically be determined). If a client specifically requests that data be reported below the MDL, the affected data should be flagged as detected below the detection limit according to the client's requested flagging convention.

7.2.5 Linear Dynamic Range (LDR) Determination

At least once annually, the linear operating range for each analyte will be determined. This process includes normal instrument and QC validation of the calibration and instrument performance. Standards containing expected linear range levels of analytes are analyzed as ordinary samples. A ± 5% recovery of the

analyte indicates linear behavior. If the recovery does not meet the required criteria, successively lower concentrations of the analyte are analyzed until the ± 5% recovery requirement is met.

Example: A 100 mg/L standard needs to recover between 95-105 mg/L, in order to establish linearity. If the analysis of this standard falls outside of these criteria, then lower the concentration till it meets the ± 5% criteria. When the recovery meets the criteria, the LDR is established.

7.2.6 Accuracy Evaluation

A QC sample, used to evaluate accuracy (Standard Reference Material (SRM), matrix spike, or blank spike) should be analyzed at a frequency of 1 per 20 samples or per batch if the batch is less than 20 samples. For matrix spike samples, the analyte spike concentration should be about that in the unspiked sample. Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unspiked sample. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = (C_s - C)/S \times 100$$

where,
- R = percent recovery
- C_s = spiked sample concentration
- C = sample background concentration
- S = concentration equivalent of spike added to sample

If the recovery of any analyte falls outside the designated range, and the laboratory performance for that analyte is within established criteria, the recovery problem encountered with the spiked sample is judged to be matrix related, not system related. The result for that analyte in the spiked sample must be qualified as suspect due to matrix effects.

7.2.7 Initial Calibration Verification (ICV)

Frequency:	once (immediately after standardization)
Limits:	± 5% for Method 200.7 ± 10% for CLP and Method 6010B
Concentration:	in calibration range, approximately mid-range.
Source:	NIST or independent of calibration standards with value certified by NIST or EPA.
Action:	for Method 200.7 values between ± 5% and ± 10% are noted. For all methods for CLP and 6010B, when analytes exceed the ± 10% criteria, recalibrate and reanalyze all affected samples

7.2.8 Initial Calibration Blank (ICB)

Frequency:	once (immediately after ICV)
Action:	If the blank is greater than 3 X MDL, rerun twice and average the results. If the average is not within three

standard deviations of the background mean, terminate the analysis, correct problem, recalibrate and rerun affected samples.

7.2.9 Continuing Calibration Verification (CCV)

Frequency:	prior to first sample, every 10 samples and after last sample in batch
Limits:	± 5% for Method 200.7 ± 10% for CLP and Method 6010B
Concentration:	mid-range
Source:	same as calibration standards
Action:	for Method 200.7 values between ± 5% and ± 10% are noted. For all methods for CLP and 6010B analytes, when the ± 10% criteria is exceeded, recalibrate and reanalyze all affected samples

7.2.10 Continuing Calibration Blank (CCB)

Frequency:	prior to first sample, every 10 samples and after last sample in batch
Action:	If the blank is greater than 3 X MDL, rerun twice and average the results. If the average is not within three standard deviations of the background mean, terminate the analysis, correct problem, recalibrate and rerun affected samples.

7.2.11 Detection Limit Standard (CRI) (if required)

Frequency:	twice (immediately after ICB and after last sample)
Limits:	none established
Concentration:	2x the client's required target detection limit (or 2x the MDL for elements for which no client-required detection limit is specified).

7.2.12 Highest Mixed Calibration Standard (HISTD), when required

Frequency:	once
Limits:	95 to 105% or as specified for the method in use.
Concentration:	equal to highest concentration calibration standard
Action:	when ± 5% criteria limit is exceeded, recalibrate

If the concentration of the samples exceeds the concentration of the highest standard, then a standard needs to be added that is greater than the concentration of the samples or the samples need to be diluted and rerun

7.2.13 Reagent Blank

Frequency:	one for every 20 samples of each matrix
Limits:	less than 5x the MDL
Action:	Reanalyze. If the analyte concentration is >5x the MDL and the analyte concentration in any of the samples is less than 10 times that of the reagent blank value, the entire sample batch must be redigested

7.2.14 Matrix Spike (pre-digest)

Frequency:	once for every 20 samples of each matrix
Limits:	75 - 125%
Action:	flag all related samples

7.2.15 Post Digestion Spike (if required)

Frequency:	once for every 20 samples of each matrix
Limits:	75 - 125%
Action:	none specified

7.2.16 Duplicate Sample

Frequency:	once for every 20 samples of each matrix
Limits:	RPD (relative percent difference) = ± 20% (if the analyte concentration is > 5 times the MDL)
Action:	flag all affected samples

7.2.17 Laboratory Control Sample (LCS)

Frequency:	one per sample batch
Limits:	aqueous, 80-120%; soil within established acceptance windows
Action:	may redigest all affected samples depending on the decision of the Project Manager.

8.0 SAFETY

8.1 Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material safety data sheets are available to all personnel involved in the chemical analysis.

8.2 The minimum level of hand protection when handling samples or hazardous chemicals is poly gloves.

8.3 Care should be taken when performing repair or routine maintenance on the instrument. Some parts and surfaces of the instrument are unavoidably

contaminated during normal use. Proper protective equipment must be used when contact with contaminated surfaces is possible.

8.4 The Optima 3000 uses refrigerated, liquefied argon and nitrogen gasses. Both of these gasses are inert and pose a suffocation hazard only. Liquid gasses are extremely cold and will cause immediate frostbite on contact with skin. Proper protective equipment must be worn when contact with liquid gasses is a possibility. All regulators, valves and connections should be checked for leaks whenever a connection is changed. Leaks must be repaired before system is put into use.

9.0 TRAINING REQUIREMENTS

All staff performing analysis of elements by ICP-AES must first read this procedure and then demonstrate proficiency in the process prior to performing the work. Proficiency will include demonstrating that 1) a SRM is within the certified range; and 2) spike recoveries are within acceptable recovery criteria. Documentation of training will be recorded on training assignment and on-the-job training forms from procedure MSL-A-006, Marine Sciences Laboratory Training. Records of this training will be kept by the MSL Quality Assurance Officer.

10.0 REFERENCES

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MSL-A-001 Sample Log In Procedure

MSL-A-006 Marine Sciences Laboratory Training

APPENDIX 1

ICP INTERMEDIATE CALIBRATION STANDARDS		
ANALYTE	CONCENTRATION (PPM)[1]	CALIBRATION STD
Ag	20	CALSTD A
Al	500	CALSTD C
As	100	CALSTD A
B	100	CALSTD B
Ba	100	CALSTD A
Be	50	CALSTD A
Ca	500	CALSTD C
Cd	50	CALSTD A
Co	100	CALSTD A
Cr	100	CALSTD A
Cu	100	CALSTD A
Fe	500	CALSTD C
K	500	CALSTD C
Li	100	CALSTD B
Mg	500	CALSTD C
Mn	100	CALSTD A
Mo	100	CALSTD B
Na	500	CALSTD C
Ni	100	CALSTD A
Pb	100	CALSTD A
Se	100	CALSTD A
Sn	100	CALSTD A
Sr	100	CALSTD B
Ti	100	CALSTD B
Tl	100	CALSTD A
V	100	CALSTD B
Zn	100	CALSTD A

1. Typical analyte concentration

2. Dilute 10X for HISTD and 20X for CCV

3. Solution is purchased pre-made from a reputable commercial source and expires according to the manufacturer.

ICP INTERMEDIATE ICV1 AND ICV2* STANDARDS	
ANALYTE	CONCENTRATION (PPM)[1]
Ag	1.5
Al	6,20*
As	6
B	6
Ba	6
Be	3
Ca	6,20*
Cd	3
Co	6
Cr	6
Cu	6
Fe	6,20*
K	20*
Li	6
Mg	6,20*
Mn	6
Mo	6
Na	20*
Ni	6
Pb	6
Sb	6
Se	6
Sn	6
Sr	6
Ti	6
Tl	6
V	6
Zn	6

1. Typical analyte concentration

2. Solutions expire at earliest analyte expiration date.

ICP INTERMEDIATE ICSA SOLUTION	
ANALYTE	CONCENTRATION (PPM)[1]
Al	5000
Ca	5000
Fe	2000
Mg	5000

1. Typical analyte concentration
2. Dilute 10X, 20X, OR 50X depending on sample concentrations for ICSA and ICSAB
3. Solution is purchased pre-made from a reputable commercial source and expires according to the manufacturer.

ICP INTERMEDIATE ICSB SOLUTION	
ANALYTE	CONCENTRATION (PPM)[1]
Ag	100
Ba	50
Be	50
Cd	100
Co	50
Cr	50
Cu	50
Mn	50
Ni	100
Pb	100
V	50
Zn	100

1. Typical analyte concentration
2. Dilute100x for ICSAB

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Marine Sciences Laboratory

EFFECTIVE DATE: 7-13-00

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-I-029-03

DETERMINATION OF METALS IN AQUEOUS AND DIGESTATE SAMPLES BY GFAA

Approvals:		
AUTHOR: Jordana R. Wood		7-12-00
	Signature	Date
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	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	7-12-00
	Signature	Date
TECHNICAL GROUP MANAGER: Eric Crecelius	E A Crecelius	7-13-00
	Signature	Date

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DETERMINATION OF METALS IN AQUEOUS AND DIGESTATE SAMPLES BY GFAA

1.0 SCOPE AND APPLICATION

This procedure is to be used by personnel working in the Battelle Marine Sciences Laboratory (MSL) Inorganic Laboratory for analysis of environmental samples by Graphite Furnace Atomic Absorption Spectroscopy (GFAA) using Perkin-Elmer 5100ZL, 4100ZL, 5100Z or a 5000Z. These procedures conform to specifications in U.S. Environmental Protection Agency (EPA) Method 200.9, EPA SW-846, 7000 series, and the Superfund Contract Laboratory Program (CLP) Statement of Work.

ANALYTE	200 SERIES	7000 SERIES
Arsenic	206.2	7060A
Antimony	204.2	7041
Beryllium	210.2	7091
Cadmium	213.2	7131A
Chromium	218.2	7191
Lead	239.2	7421
Nickel	249.2	7521
Selenium	270.2	7740
Thallium	279.2	7841
Silver	272.2	7761

2.0 SUMMARY OF METHOD

The GFAA spectroscopy method is routinely used to quantify arsenic (As), selenium (Se), thallium (Tl), and lead (Pb) in environmental samples including aqueous samples for total or dissolved metals, and solid extracts that are prepared using a nitric acid (HNO3) digestion procedure. A hydrochloric (HCL)- HNO3 digestion can also be used for As and Pb. Other elements such as antimony (Sb), beryllium (Be), nickel (Ni), silver (Ag), and chromium (Cr) can also be analyzed by this method with programs specifically developed for each element. Although these are the elements most commonly analyzed by this method, GFAAS technique is not limited to these elements or matricies.

After preparing a sample in a HNO_3 matrix, an aliquot is injected into a graphite furnace and subjected to a temperature program including the following steps: 1) Drying, 2) Char, 3) Atomization, 4) Clean out, and 5) Cooling. Using a lamp (light source) emitting light at a wavelength characteristic to the element to be determined, the absorption of light is measured during sample atomization. The analysis is performed using a Zeeman background correction. The absorbance is converted to a concentration

value using a standard curve with a blank and three (3) or more standards prepared prior to sample analysis. The result is converted to µg/L for aqueous samples or to µg/g for soil samples

3.0 DEFINITIONS

Dissolved - Material that will pass through a 0.45 µm membrane filter assembly, prior to sample acidification.

Suspended - Those elements (metals) which are retained by a 0.45 µm membrane filter.

Total Recoverable - The concentration of analyte determined on an unfiltered sample following treatment with hot dilute mineral acid.

Instrument Detection Limit (IDL) - The IDL is the minimum concentration of an analyte that a particular instrument can measure and report with 99% confidence that the analyte concentration is greater than zero.

Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero and determined from analysis of a sample in a given matrix containing analyte.

Matrix Modifier – Salts used in AA to mitigate effects of chemical interferents, viscosity and surface tension.

Reagent Blank - A volume of deionized, distilled water of the same acid matrix as the calibration standards carried through the entire analytical scheme.

Calibration Blank - A volume of deionized, distilled water acidified with the same acids as used in sample preparation.

Laboratory Control Sample - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LCS is carried through the entire preparation scheme as the samples, analyzed exactly like a sample, and its purpose is to determine whether the method is within accepted control limits.

Matrix Spike - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The matrix spike is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the matrix spike corrected for the concentrations found.

4.0 RESPONSIBLE STAFF

- Researcher/Technician-sample preparation, digestion
- Analyst – sample analysis
- MSL QA Officer or Representative- data verification

5.0 PROCEDURE

5.1 Sample Collection, Preservation, Storage, and Digestion

5.1.1 For recommendations on sample collection and preservation requirements, refer to the procedure MSL-A-001, Sample Log In Procedure.

5.1.2 For the determination of dissolved elements, the sample should be filtered through a 0.45 µm membrane filter. Use a portion of the sample to rinse the filter assembly, discard and then collect the required volume of filtrate. Acidify the filtrate with 1.0 µl/ml concentrated HNO_3 immediately following filtration to a pH less than 2.0.

5.1.3 For the determination of total recoverable elements in aqueous samples, acidify with 1.0 µl/ml concentrated HNO_3 at the time of collection to a pH less than 2.0. The sample should not be filtered prior to analysis.

NOTE: Samples that cannot be acid preserved at the time of collection, should be acidified with HNO_3 to a pH of less than 2.0 upon receipt in the laboratory (normally, 1.0 µl/ml concentrated HNO_3 is sufficient for most ambient and drinking water samples.) Following acidification, the sample should be held for at least sixteen hours before withdrawing an aliquot for sample processing. Samples are stored at 20 °C in the Sample Preparation Laboratory, MSL 5, Room 231, which has filtered air.

5.1.4 Sediment and tissue samples shall be digested according to the project-specific requirements.

5.1.5 For the total recoverable digestion procedure, 10.0 mL of sample water is heated at 85 °C for 2.5 hours with 0.2 mL of HNO_3 in a sealed 15 mL plastic centrifuge tube.

5.2 Apparatus and Reagents

- Perkin Elmer GFAAS Models 4100ZL, 5100Z, 5100ZL, and 5000Z equipped with electrothermal atomizer, Zeeman background correction, and autosamplers.

- Electrode discharge lamp (EDL) power supply and individual hollow cathode lamps (HCL) or EDL lamps for each analyte to be determined

- Water chiller recirculator for the furnace cooling, or connections to house water used for cooling of the furnace

- Computer with either GEM software (4100ZL) or AAWINLAB software, for controlling the spectrophotometer and auto-sampler, for calculating analyte

concentration, for applying dilution and background correction factors. This does not apply to the 5000Z.

- Argon gas supply (high-purity grade, 99.99%)
- Various volumetric flasks, graduated cylinders and calibrated pipettes.
- HNO_3, concentrated, trace metal grade
- HCl, concentrated, trace metal grade
- Matrix modifier solutions
- Standard stock solutions are purchased or prepared from ultra-high purity grade chemicals or metals at least once each year, or as needed during the year. All stock solutions, metals or salts must have documented purity and a traceable certification to the National Institute of Standards and Technology (NIST).
- Certified standards are purchased annually. The primary standards contain either 1000 ppm or 10 ppm concentrations of metals. Secondary standards are prepared from these primary standards. The secondary standards are assigned unique numbers and information is recorded in the standard logbook. The secondary standards are assigned a expiration date based on the earliest expiration date of the stock standards used in preparation.
- Certified multi-element stock standard solutions are purchased annually.
- Working standards are prepared by diluting stock multi-element standard solutions. Working calibration standards are required to be prepared daily, using deionized (DI) water containing the acid matrix of the samples to be analyzed.
- Class A, volumetric glassware shall be used in performing all dilutions.
- Two types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve while the reagent blank (preparation blank), is used to detect possible contamination resulting from varying amounts of the acids used in the sample processing.
- The calibration blank is prepared by diluting 40 mL of concentrated HNO_3 and 2 L with deionized water. This solution will be used as a diluent in the preparation of all working calibration and quality control (QC) standards. Unless another matrix is being analyzed.
- The reagent blank must contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.
- In addition to the calibration standards, several QC standards are analyzed to verify and monitor instrument calibration and stability and to validate inter-

element and background corrections. Detailed information regarding QC standards is presented in Section 7, Quality Control.

5.4 Step-by-Step Procedure

5.4.1 Sample Preparation

5.4.1.1 For the determination of ground and surface waters, HNO_3 acid is added as to matrix match with the standards.

5.4.1.2 For the determination of elements in digestates, depending on the amount of digestate, the samples are either run direct or diluted to matrix match with the diluent used in making the standards.

5.4.2 INSTRUMENT START-UP and Calibration

5.4.2.1 Check that argon supply is on and adjusted correctly. Turn on power to the instrument in accordance with manufacturer's instructions and initiate the appropriate operating configuration of the computer and controlling software. Check that the cooling system or water in on.

5.4.2.2 Install the appropriate lamp in the instrument before recalling analytical method file. Allow lamps to warm up before standardization. HCLs must warm up for a minimum of 15 minutes, EDLs require a minimum of 30-45 minutes. The lamps must be aligned for maximum energy and the gain must be set.

5.4.2.3 After installing a new tube into the furnace, it must be preconditioned before analyses can commence.

5.4.2.4 Check that the capillary tube enters the graphite tube correctly and that it delivers the sample on the L'vov platform from a height of about 1 mm to avoid splashing. The instrument must be calibrated starting with the standard blank, on which it auto-zeroes, followed by three or more calibration standards analyzed, within the linear dynamic range of the analyte, in sequence. Method of additions calibration can also be used to establish a calibration curve. Immediately after calibration, print a hard copy of the graph of the standard curve. The instrument software will fit a linear or nonlinear equation to the calibration curve. The correlation coefficient must be 0.995 or greater for the calibration curve to be acceptable, otherwise corrective action must be taken, and the GFAA need to be recalibrated. All subsequent sample results are obtained using this curve.

5.4.2.5 For method specific projects, duplicate injections are required. The relative standard deviation (RSD) between the two burns must be less than 20% for all samples with analysis results greater than the MDL.

5.4.3 AUTOSAMPLER TRAY LOADING

5.4.3.1 Sample identification numbers and dilution factors must first be entered in the instrument sample information file or ID/Weight file for Perkin-Elmer instruments. This file is used as a guide to loading the auto sampler tray.

5.4.3.2 Sample spiking and dilutions of 1:20 or less may be carried out in the autosampler cups or vials using calibrated pipettes. All other dilutions must be performed using Class A volumetric flasks and pipettes.

5.5 Instrument Maintenance, Operation and Preventive Maintenance

Instrument maintenance is provided by Perkin Elmer on an as-needed basis. The MSL GFAA operator checks the argon supply pressure. Daily maintenance includes inspection of the furnace tube, contact rings, and optical windows

Due to the differences between various makes and models of satisfactory instruments, detailed operating instructions are provided in the specific instrument manuals. Sensitivity, instrument detection limit, precision, linear dynamic range and interference effects must be investigated and established for each analyte to be quantified. All measurements must be within the calibration range. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements and to maintain quality control data confirming instrument performance and analytical results. Flexibility in the selection of operating conditions must be maintained to allow optimization for various matrices and analytical requirements.

5.6 Software Maintenance

The GFAA software is upgraded and maintained by the service engineer.

6.0 DATA ANALYSIS AND CALCULATIONS

6.1 For aqueous samples prepared by the digestion, multiply the solution concentrations by the dilution factor.

6.2 The sample preparation weight, volume and any dilutions are entered in the sample information file and the instrument uses these values to convert the concentrations from µg/L to µg/g for the analysis of tissues or sediments.

6.3 Data Generation, Data Review, Data Reporting, and Archiving

6.3.1 The GFAA data output is in the form of concentrations of metals in units of µg/L or mg/L for water and µg/g for tissue and sediment. These raw data, on a paper printout, are stored in the chemistry central files for at least 10 years. The software uses an exporting program to convert the results file to an electronic file to be imported into an Excel spreadsheet using a PC computer. An Excel spreadsheet is used to prepare a summary data table for the client report. The project manager and the MSL QA Officer review the table to determine if the raw

data were accurately reduced in the final report table. All raw data and versions of the report tables are archived.

6.3.2 The result library is checked and archived into Zip files monthly using the manufacturer's software. The result files are then deleted from the hard disk and the libraries are packed. The Zip files are also copied on to Zip disks and stored in case of computer malfunction.

7.0 QUALITY CONTROL

The data acquired from QC procedures are used to estimate and evaluate the information content of analytical data and to determine the necessity or the effect of corrective action procedures. The summary values used to estimate information content include precision, accuracy, detection limit, and other quantifiable and qualitative indicators.

The following QC criteria are derived from the cited methods. However, clients often request other project-specific criteria.

7.1 GENERAL REQUIREMENTS

7.1.1 All QC data are maintained and available for easy reference or inspection.

7.1.2 Chemicals are dated upon receipt of shipment, and expiration dates noted Chemicals are replaced as needed, or before the shelf life, as indicated by the expiration date, has been exceeded.

7.2 SPECIFIC REQUIREMENTS

The following steps should be performed at least once with each analytical batch, or at a frequency of a minimum of once per twenty-sample batch.

7.2.1 GFAA Dilution Test.

For each analytical batch select one typical sample for serial dilution to determine whether interferences are present. The concentration of the analyte should be at least 25 times the estimated detection limit. Determine the concentration in the undiluted sample. Dilute the sample by a minimum of 5 fold (1 + 4) and reanalyze. If all of the samples are below 10 times the detection limit, perform the spike recovery analysis. Agreement within 10% between the concentration for the undiluted and diluted sample shows that there is an absence of interferences and such samples may be analyzed without using method of standard additions (MSA).

7.2.2 Recovery Test

If results from the dilution test do not agree, a matrix interference may be suspected and a spiked sample should be analyzed to help confirm the finding from the dilution test.

Spike a representative sample with the analyte being analyzed at approximately mid-range of the calibration. Calculate the spike recovery. If the recovery is less than 85% or greater than 115%, corrective action must be taken. See the following table for actions.

For CLP or 7000 method requirements an analytical post spike will be done on each unknown sample.

ANALYTICAL SPIKE CRITERIA FOR GFAA ANALYSIS

SAMPLE CONCENTRATION	ANALYTICAL SPIKE % RECOVERY	REPORT		ACTION
ND	85 - 115%	Yes	ND	None
ND	> 115%	Yes	ND	None
ND	40 - 85%	Yes	ND, flagged	None
ND or <MDL	< 40%	No	-->	Dilute once & reanalyze
	if still < 40 %	Yes	ND **If > MDL, then flag data	None
≥ MDL	85 - 115%	Yes	Report concen-tration	---
≥ MDL	< 85% or > 115%	No	-->	MSA

ND -Not Detected
RDL- Reported Detection Limit
MSA- Method of Standard Addition

** The dilution needs only be performed once. If dilution fails the result is flagged as appropriate and reported.

- If the sample concentration is high enough to be quantitated using a dilution, a dilution can be done in lieu of an MSA.
- For project specified CLP work, if the sample concentration is less than 50% of the spike and the spike recovery is less than 85% or greater than 115% report the results and flag the data.

7.2.3 Method of Standard Addition (MSA)

A sample is run using a series of standard additions. Only single injections of each solution are required when using MSA. To equal volumes of sample are added a series of standard solutions containing different quantities of the analyte, and all solutions are diluted to the same final volume. For example addition one should be approximately 50% of the expected absorbance from the endogenous analyte in the sample. Additions 2 and 3 should be prepared so that the concentrations are approximately 100% and 150% of the expected sample concentration. The absorbance of each solution is determined and then plotted on the vertical axis of a graph, with the concentrations of the known standards plotted on the horizontal axis. When the resulting line is extrapolated to zero absorbance, the interception of the abiscissa is endogenous concentration of the analyte in the sample. A linear regression program may be used to obtain the intercept concentration.

The correlation coefficient must be ≥ 0.995 for MSA to quantitate a sample. Otherwise rerun MSA once, and report the value from the MSA with the highest correlation coefficient.

7.2.4 Instrument and Method Detection Limits (IDL and MDL):

7.2.4.1 Instrument Detection Limits (IDL) for GFAA are determined annually in water by spiking seven or more replicates of reagent water at one to five times the expected IDL) for all analytes of interest. The IDL is calculated as follows:

$$IDL = (t) \times (S)$$

where, t = students' T value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates)
S = standard deviation of the replicate analyses

7.2.4.2 Method Detection Limits (MDL) for water should be established for all analytes, using blank spikes at a concentration of one to five times the estimated detection limit. To determine MDL values, take seven blank spikes and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where, t = students' T value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates)
S = standard deviation of the replicate analyses

Method detection limits should be determined annually or whenever a significant change in background or instrument response is expected (e.g. detector change).

7.2.4.3 Meaningful MDLs often cannot be determined in an actual tissue or sediment because the concentrations of most trace elements are naturally present at relatively high concentrations in the material and therefore high MDLs will result. In cases when an empirically derived MDL is not practical, a theoretical MDL may be calculated. MDLs for tissue and sediment can be calculated from the IDLs determined in water and mathematically processing them through the same dilution factor that applies to actual tissue or sediment samples (volume analyzed, total mass digested, and total volume digested).

7.2.4.4 Battelle MSL does not routinely employ reporting limits (RL). It is MSL policy to report all values detected above the empirically determined MDL (or calculated MDL in the case of tissues or sediments when no empirical MDL can realistically be determined). If a client specifically requests that data be reported below the MDL, the affected data should be flagged as detected below the detection limit according to the client's requested flagging convention.

7.2.5 Accuracy Evaluation

A QC sample, used to evaluate accuracy (Standard Reference Material (SRM), matrix spike, or blank spike) should be analyzed at a frequency of 1 per 20 samples or per batch if the batch is less than 20 samples. For matrix spike samples, the analyte spike concentration should be about that in the unspiked sample. Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unspiked sample. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = (C_s - C)/S \times 100$$

where,
- R = percent recovery
- C_s = spiked sample concentration
- C = sample background concentration
- S = concentration equivalent of spike added to sample

If the recovery of any analyte falls outside the designated range, and the laboratory performance for that analyte is within established criteria, the recovery problem encountered with the spiked sample is judged to be matrix related, not system related. The result for that analyte in the spiked sample must be qualified as suspect due to matrix effects.

7.2.6 Initial Calibration Verification (ICV)

Frequency:	once (immediately after calibration)
Limits:	± 10%
Concentration:	in calibration range, approximately mid-range.

Source:	Standard must be of a different source than the calibration standards.
Action:	For all methods for when analytes exceed the ± 10% criteria, recalibrate and reanalyze all affected samples

7.2.7 Initial Calibration Blank (ICB)

Frequency:	once (immediately after ICV)
Action:	If concentration is > 5 times MDL, stop recalibrate and rerun

7.2.8 Continuing Calibration Verification (CCV)

Frequency:	prior to first sample, every 10 samples and after last sample in batch
Limits:	± 10% for Method 200.9 ± 20% for 7000A
Concentration:	mid-range
Source:	same as calibration standards
Action:	For all methods when the criteria is exceeded, recalibrate and reanalyze all affected samples

7.2.9 Continuing Calibration Blank (CCB)

Frequency:	prior to first sample, every 10 samples and after last sample in batch
Action:	If concentration is > 5 times MDL, stop recalibrate and rerun

7.2.10 Detection Limit Standard (CRA) (if required)

Frequency:	Once (beginning of analysis)
Limits:	none established
Concentration:	2x the client's required target detection limit (or 2x the MDL for elements for which no client-required detection limit is specified).

7.2.11 Reagent Blank

Frequency:	one for every 20 samples of each matrix
Limits:	less than 5x the MDL
Action:	if the analyte concentration is >5x the MDL and the analyte concentration in any of the samples is less than 10 times that of the reagent blank value, the entire sample batch must be redigested.

7.2.13 Matrix Spike (pre-digest)

Frequency:	once for every 20 samples of each matrix
Limits:	75 - 125%

Action: flag all related samples

7.2.14 Duplicate Sample

Frequency: once for every 20 samples of each matrix
Limits: RPD (relative percent difference) = ± 20% (if the analyte concentration is > 5 times the MDL)
Action: flag all affected samples

7.2.15 Laboratory Control Sample (LCS)

Frequency: one per sample batch
Limits: aqueous, 80-120%; soil within established acceptance windows
Action: may redigest all affected samples depending on the decision of the Project Manager.

8.0 SAFETY

8.1 Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material data handling sheets are available to all personnel involved in the chemical analysis.

8.2 The minimum level of hand protection when handling samples or hazardous chemicals is poly gloves.

8.3 Care should be taken when performing repair or routine maintenance on the instrument. Some parts and surfaces of the instrument are unavoidably contaminated during normal use. Proper protective equipment must be used when contact with contaminated surfaces is possible.

8.4 The GFAAs uses refrigerated, liquefied argon. This gas is inert and poses a suffocation hazard only. Liquid gasses are extremely cold and will cause immediate frostbite on contact with skin. Proper protective equipment must be worn when contact with liquid gasses is a possibility. All regulators, valves and connections should be checked for leaks whenever a connection is changed. Leaks must be repaired before system is put into use.

8.5 The Zeeman background correction of the GFAAs uses a strong magnet to minimize background interferences. People with pacemakers or similar devices that are affected by strong magnetic fields should not be near the Zeeman furnaces during atomization.

9.0 TRAINING REQUIREMENTS

All staff performing analysis of elements by GFAA must first read this procedure and then demonstrate proficiency in the process prior to performing the work.

Proficiency will include demonstrating that 1) a SRM is within the certified range; and 2) spike recoveries are within acceptable recovery criteria. Documentation of training will be recorded on training assignment and on-the-job training forms from procedure MSL-A-006, Marine Sciences Laboratory Training. Records of training are kept by the MSL Quality Assurance Officer.

10.0 REFERENCES

U.S. Environmental Protection Agency, 1994. Statement of Work for Inorganic Analysis - Multi-media Multiconcentration. U.S. EPA, Washington, D.C.

U.S. Environmental Protection Agency, 1994 (revised). Methods for Chemical Analysis of Water and Wastes. EPA/600/5-94/020. U. S. EPA, Cincinnati, Ohio.

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MSL-A-001 Sample Log In Procedure

MSL-A-006 Marine Sciences Laboratory Training

Battelle
Marine Sciences Laboratory

EFFECTIVE DATE: 1/24/92

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-M-045-00

CALIBRATION AND USE OF pH METERS

Approvals:

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TECHNICAL REVIEWER	DATE
R Calla	1/16/92
QUALITY ASSURANCE	DATE
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MARINE SCIENCE LABORATORY MANAGER	DATE

CALIBRATION AND USE OF pH METERS

1.0 SCOPE AND APPLICATION

This procedure describes the methods needed to calibrate and use the Orion pH meter, Model SA-250. pH meters are used for the routine measurement and monitoring of pH during laboratory testing.

2.0 DEFINITIONS

None.

3.0 RESPONSIBLE STAFF

- Staff Members
- Reviewer (Project Manager or designee)

4.0 PROCEDURE

4.1 Necessary Equipment

- pH meter and probe
- Deionized water in a wash bottle
- pH buffer (4, 7, 10) solutions
- Electrode Storage Solution

4.2 Calibration

When performing calibration of pH meters (daily, before each use), staff members shall comply with the following:

4.2.1 Slide power switch to **ON** position.

4.2.2 If **LO BAT** indicator on LCD remains on, the battery must be replaced.

4.2.3 Slide **mode** switch to **mV**. Display should read **0 ± 0.3.**

4.2.4 Slide **mode** switch to **temp**. Display should read **25.0**. If **25.0** is not displayed, use the scroll keys, until **25.0** is displayed and press **enter**.

4.2.5 Slide **mode** switch to **pH .01**. Press **iso**. Display should read the letters **ISO** then a value of **7.00**. If **7.00** is not displayed, scroll until **7.00** is displayed press **enter**.

4.2.6 Press **slope**. Display should read the letters **SLP** then a value of **100.0**. If **100.0** is not displayed, scroll until **100.0** is displayed and press **enter**.

4.2.7 Press **sample**. Observe the letters **pH** then a steady reading of **7.00 ± 0.02** should be obtained. If not, press **cal** and scroll until **7.00** is displayed and press **enter**. Press **sample** and observe a reading of **7.00**.

4.2.8 Slide **mode** switch to either **pH 1** or **pH .01**. Choose two buffers that will bracket your expected sample range (i.e., pH buffer 7 and either pH buffer 4 or 10).

4.2.9 Place electrode into fresh pH 7 buffer (buffers will be changed daily).

4.2.10 Press **cal**. The display will alternate between **1** and the pH value of the buffer, indicating this is the first buffer and a value has not been entered.

4.2.11 Wait for a stable pH display. Scroll in the correct value and press **enter**. The correct value of the pH 7 buffer, for calibration purposes, is 7.02. When the display reads 7.02 steadily for several seconds, press **enter** and record this reading on the pH Meter Daily Calibration Record (Attachment 1).

The display will freeze for 3 seconds then advance to **2** indicating the meter is ready for the second buffer.

4.2.12 Rinse electrode with deionized water, shake, and place into the second buffer (pH 10). Buffers will be changed daily. Wait for a stable pH display. Scroll in the correct value (pH of 10.05 for calibration) and press **enter**. Record this reading on the pH Meter Daily Calibration Record.

After the second buffer value has been entered the letters **PH** will be displayed. The meter is now calibrated and automatically advances to the **sample** mode.

4.2.13 Recheck the pH 7 buffer; the meter should read within 0.01 units of original setting. If the readings are more than 0.01 units above or below the buffer value, recalibrate, otherwise proceed with pH measurements.

4.2.14 Record pH buffer lot numbers and expiration date for all pH buffers used on the pH Meter Daily Calibration Record. Errors shall be crossed out with a single line, initialed, dated and the reason for the error shall be noted (error codes are listed in SOP MSL-D-001).

4.3 As each pH Meter Daily Calibration Record is completed, the Reviewer shall examine the document for technical adequacy and then indicate review by signing and dating the form.

4.4 Upon completion of the pH Meter Daily Calibration Record, staff members shall transfer them to a labeled three-ring binder or place them in the project files.

4.5 <u>Use of pH Meters</u>

When using pH meters, staff members shall make sure instrument is calibrated and then shall comply with the following:

4.5.1 After calibration is performed in accordance with section 4.2, rinse the electrode with deionized water and shake.

4.5.2 Place the electrode into the solution to be measured deep enough so that the bulb is covered. Stir gently until the pH reading stabilizes.

4.5.3 Record pH measurement to nearest 0.01 unit on water quality data sheets.

4.5.4 Rinse electrode with filtered seawater or deionized water and gently shake off between samples. Use caution not to touch or scratch the tip of the electrode as a slight scratch on the glass bulb can render the electrode useless.

4.5.5 The electrode should be stored in filtered seawater or deionized water when there is a time delay between readings.

4.5.6 When probe is not in use, the electrode should be stored in electrode storage solution.

4.5.7 The electrode storage solution should be changed weekly. If storage solution is not available, use about 200 mL of pH 7 buffer and add approximately 1 gram KCl crystals as a temporary substitute.

5.0 DATA ANALYSIS AND CALCULATIONS

Not Applicable

6.0 QUALITY CONTROL

Calibration of pH meters must occur daily prior to the instruments' use in pH measurements. Buffers will be changed daily, and the Lot numbers of these buffers documented.

7.0 SAFETY

Care must be taken when shaking water off the pH electrode between samples. Touching or scratching the tip of the electrode can render the probe useless.

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this SOP are properly trained and that documentation of the training exists prior to the performance of those procedures.

To initiate training, all persons must read this SOP and demonstrate proficiency in the use of this instrument prior to application in project use. A staff member unfamiliar with the use of pH meters will receive a verbal and visual demonstration by a certified co-worker. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this SOP are followed. Once proficiency has been verified, documentation of training will be recorded on a training assignment form obtained from SOP MSL-A-006.

9.0 REFERENCES

- MSL-A-006 Marine Science Laboratory Training
- MSL-D-001 Recording Data on Data Sheets and Laboratory Notebooks

ATTACHMENT 1

BATTELLE MARINE SCIENCES LABORATORY

pH METER DAILY CALIBRATION RECORD
SOP USED: pH METER MSL-M-045

pH buffers used:
pH 4.0 - Lot No._____ Expiration date_____
pH 7.0 - Lot No._____ Expiration date_____
pH 10.0 - Lot No._____ Expiration date_____
Electrode Storage Solution -
Lot No._____ Expiration date_____

Note: pH buffers to be changed daily. Electrode storage solution to be changed weekly.

Date	Time	Model/ Serial No.	pH 4.0 Reading	pH 7.0 Reading	pH 10.0 Reading	Buffers Changed	Initials

Reviewed by _____ Date _____

Battelle
Marine Sciences Laboratory

EFFECTIVE DATE: 1/24/92

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-M-046-00

CALIBRATION AND USE OF DISSOLVED OXYGEN METERS

Approvals:

Lisa M Karle AUTHOR	1-16-92 DATE
Rhonda K. Karls TECHNICAL REVIEWER	01.16.92 DATE
[illegible] QUALITY ASSURANCE	1/16/92 DATE
RM Eaker MARINE SCIENCE LABORATORY MANAGER	1/24/92 DATE

CALIBRATION AND USE OF DISSOLVED OXYGEN METERS

1.0 APPLICATION AND SCOPE

This procedure describes the methods needed to calibrate and use Dissolved Oxygen (D.O.) meters. D.O. meters are used for the routine measurement and monitoring of dissolved oxygen.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

- Staff Members
- Reviewer (Project Manager or designee)

4.0 PROCEDURE FOR MODEL 57 METER

4.1 Necessary Equipment

- Dissolved Oxygen (D.O.) meter and probe
- Deionized water (DI water)

4.2 Preparing the Instrument

It is important that the meter be placed in the intended operating position: vertical, tilted, or on its back - before it is prepared for calibration and use. Readjustment may be necessary when the instrument operating position is changed. After establishing the instrument operating position, proceed as follows:

4.2.1 Turn the meter on by moving the upper switch to the **RED LINE** position, and allow approximately ten minutes for the instrument to warm up.

4.2.2 While in the **RED LINE** position, check to see if the black meter needle rests directly on the red line across the scale. If necessary, use the **RED LINE** control knob to adjust the needle over the red line.

4.2.3 Move switch to the **ZERO** setting and, if necessary, adjust the needle to 0.0 on the meter using the **ZERO** control knob.

4.2.4 Set switch to 0 - 10 mg/L scale.

4.2.5 Switch **SALINITY** control knob to **FRESH** (0 parts per thousand).

4.3 Calibration

The accuracy of the D.O. meters shall be checked daily before the first water quality measurement and after the last water quality measurement is read. Each D.O. meter has a specific record of daily calibration information. **PRIOR TO DAILY D.O. METER CALIBRATION, BE CERTAIN THAT THE MODEL AND SERIAL NUMBER OF THE METER TO BE CALIBRATED AGREES WITH THE NUMBERS ON THE METER RECORD SHEET.** Staff members shall comply with the following when performing accuracy checks of the D.O. meters:

4.3.1 Air saturate a volume of deionized water (300-500 mL) by aerating for at least 30 minutes at a relatively constant temperature with an air stone.

4.3.2 Place the probe in the aerated deionized (DI) water and stir. Check the temperature of this aerated DI water using a calibrated thermometer (the **TEMP** setting on the D.O. meter can be used to determine the water temperature, but may or may not be very accurate).

4.3.3 Using the **SALINITY TABLE** for the Solubility of Oxygen (mg/L) in water, note the proper D.O. reading under the 0 parts per thousand column for the DI water temperature. Example: 8.73 mg/L D.O. at 22°C.

4.3.4 While swirling the probe in the aerated water, adjust the meter to read the appropriate dissolved oxygen concentration from 4.3.3 using the **CALIBRATION** control knob. Continue stirring the probe in the DI water for several seconds after adjustment to verify calibration stability.

4.3.5 Read display and record this value as "Initial Reading" on the Dissolved Oxygen Meter Daily Calibration Record form (Attachment 1). Errors shall be crossed out with a single line, initialed, dated and the reason for the error shall be noted (error codes are listed in SOP MSL-D-001).

4.3.6 As each Dissolved Oxygen Meter Daily Calibration Record is completed, Reviewer shall check the document for technical adequacy and then indicate review by signing and dating the form.

4.3.7 Upon completion of the Dissolved Oxygen Meter Daily Calibration Record, staff members shall transfer the sheets to the appropriately labeled three-ring binder for each particular meter.

4.4 Use of Dissolved Oxygen Meters

When using Dissolved Oxygen meters, staff members shall make sure instrument is calibrated and then shall comply with the following:

4.4.1 Determine the salinity by using a refractometer.

4.4.2 Adjust the **SALINITY** control knob to the salinity of the sample. Place probe in the sample and stir.

4.4.3 Allow sufficient time for probe to stabilize to sample temperature and dissolved oxygen. Read dissolved oxygen concentration in mg/L and record (to nearest 0.1 unit for Model 57) on water quality data sheets.

4.4.4 If the probe is transferred between solutions of uniform temperature, rinse the probe in clean water and insert the probe into the next sample, stir, and read the dissolved oxygen concentration in mg/L when the needle has stabilized. Leave the instrument on between measurements to avoid the necessity of recalibrating the meter.

NOTE: D.O. probe should be kept moist.

4.5 Preparing the Probe

As a function of Dissolved Oxygen Meter maintenance, under normal conditions the probe membrane is changed monthly (average replacement interval is 2-4 weeks) and recorded by the Staff member responsible. However, if erratic readings or evidence of membrane damage occur, replacement of the probe membrane and KCl solution may be necessary.

4.5.1 If there is no prepared KCl probe solution (use of this manufactured solution is preferable), the O_2 Probe Solution from the manufacturer (saturated KCl) can be prepared by dissolving the KCl crystals in a dropper bottle with deionized water. Squeeze bottles from the manufacturer are supplied with a pre-measured amount of KCl. If the manufacturer solution is not available, prepare saturated KCl by dissolving 5.25g of KCl in 30ml of deionized water and 1/16ml of photo-flo. Fill the bottle to the fill line with the KCl solution.

4.5.2 Unscrew the sensor guard from the probe and then remove the O ring and membrane. Thoroughly rinse the sensor with KCl solution.

4.5.3 Fill the probe with KCl as follows:

a) Grasp the probe in your left hand. The pressure compensating vent should be to the right. Successively fill the sensor body with electrolyte while pumping the diaphragm with the eraser end of a pencil or similar soft, blunt tool. Continue filling and pumping until no more air bubbles appear.

b) Secure a membrane under your left thumb. Add more KCl to the probe until a large convex meniscus completely covers the gold cathode. NOTE: Handle membrane material with care; keeping it clean and dust-free, touching it only at the ends.

c) With the thumb and forefinger of your other hand, grasp the free end of the membrane.

d) Place the membrane over the meniscus without trapping any air.

e) Roll the O ring over the end of the probe. There should be no wrinkles in the membrane or trapped air bubbles. Some wrinkles may be removed by lightly tugging on the edges of the membrane beyond the O ring.

f) Trim off excess membrane with scissors or a sharp knife. Check that the stainless steel temperature sensor is not covered by excess membrane.

4.5.4 Shake off excess KCl and reinstall the sensor guard.

4.5.5 For temporary storage of the probe, place in aerated distilled water. In the case of less frequent use, a plastic bottle is provided with the probe for convenient storage. Place a small piece of moist towel or sponge in the bottle and insert the probe into the open end. This keeps the electrolyte from drying out.

5.0 DATA ANALYSIS AND CALCULATIONS

Not Applicable

6.0 QUALITY ASSURANCE

Dissolved oxygen meters must be calibrated prior to the instrument's use. The DO probe membrane shall be changed on a monthly basis or sooner if membrane damage occurs. All wrinkles and air bubbles must be removed from under the membrane for accurate dissolved oxygen readings.

7.0 SAFETY

Not applicable

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this SOP are properly trained and that documentation of the training exists prior to the performance of those procedures.

To initiate training, all persons must read this SOP and demonstrate proficiency in the use of this instrument prior to application in project use. A staff member unfamiliar with the use of dissolved oxygen meters will receive a verbal and visual demonstration by a certified co-worker. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this SOP are followed. Once proficiency has been verified, documentation of training will be recorded on a training assignment form obtained from SOP MSL-A-006.

9.0 REFERENCES

- MSL-A-006 Marine Science Laboratory Training
- MSL-D-001 Recording Data on Data Sheets and Laboratory Notebooks

ATTACHMENT 1

BATTELLE MARINE SCIENCES LABORATORY
Dissolved Oxygen Meter Daily Calibration Record

Dissolved Oxygen Meter Model _____

Serial Number _____

Dates Membrane Changed _____ _____ SOP Used: MSL-M-046

NOTE: MODEL AND SERIAL NUMBER OF DO METER MUST MATCH AS ABOVE!!
SALINITY TABLE INFORMATION SHOULD AGREE WITH INITIAL READING FOR CALIBRATION

Date _____ Initials _____

Initial Reading _____ at _____ °C Final Reading _____ at _____ °C

Date _____ Initials _____

Initial Reading _____ at _____ °C Final Reading _____ at _____ °C

Date _____ Initials _____

Initial Reading _____ at _____ °C Final Reading _____ at _____ °C

Date _____ Initials _____

Initial Reading _____ at _____ °C Final Reading _____ at _____ °C

Date _____ Initials _____

Initial Reading _____ at _____ °C Final Reading _____ at _____ °C

Date _____ Initials _____

Initial Reading _____ at _____ °C Final Reading _____ at _____ °C

Reviewed by _____ Date _____

Battelle
Marine Sciences Laboratory

EFFECTIVE DATE: 1/24/92

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-M-048-00

CALIBRATION AND USE OF REFRACTOMETERS

Approvals:

Lisa M Karle	1-16-92
AUTHOR	DATE
Rhonda K. Karls	01.16.92
TECHNICAL REVIEWER	DATE
[illegible]	1/16/92
QUALITY ASSURANCE	DATE
[illegible]	1/24/92
MARINE SCIENCE LABORATORY MANAGER	DATE

CALIBRATION AND USE OF REFRACTOMETERS

1.0 APPLICATION AND SCOPE

This procedure describes the methods needed to calibrate and use all models of refractometer. The refractometer is used to measure the salinity of seawater during water quality monitoring.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

- Staff Members
- Reviewer (Project Manager or designee)

4.0 PROCEDURE

4.1 Necessary Equipment

- Refractometer
- International Association of Physical Oceanographers (IAPO) Standard Seawater, 35.0 ‰ salinity
- Distilled water

4.2 Calibration

When performing monthly calibration of refractometer, staff members shall comply with the following:

4.2.1 Place IAPO Standard seawater in refractometer and read the salinity on the scale. Record reading on Calibration Record (Attachment 1) if reading is 35.0 ‰.

4.2.2 If the meter does not read 35.0 for the IAPO Seawater:

a) Refer to the owner's manual to readjust the refractometer to read 35.0 ‰ for this seawater. Record reading on Calibration Record (Exhibit 1).

b) Attach a label to the refractometer informing the operator that no correction factor is needed and of the calibration expiration date.

If refractometer cannot be readjusted:

1) Record reading on Calibration Record and calculate correction factor by variance from 35.0 ‰.

2) Attach a label to the refractometer informing the operator of the correction factor to be applied to the device (i.e. subtract 1.0 ‰ from the reading for true salinity).

3) Identify on the label the calibration expiration date.

NOTE: The calibration expiration date shall be one month from calibration date.

4.2.3 As each Calibration Record is completed (monthly), the Reviewer shall view the document for technical adequacy and then indicate review by signing and dating the form.

Errors shall be crossed out with a single line, initialed, dated and the reason for the error shall be noted (error codes are listed in SOP MSL-D-001).

4.2.4 Upon completion of the Calibration Record, staff members shall transfer them to a labeled three-ring binder or place them in the project files.

4.3 Use of Refractometer

When using refractometer, staff members shall make sure refractometer is calibrated and then shall comply with the following:

4.3.1 With the cover in place, fill the refractometer from the top until the sample being measured covers the lens. Care should be taken to minimize warming of refractometer body with whole hand. Instead, hold between thumb and fingers.

4.3.2 Adjust the focus by turning the focus dial to the right or left until the scale is clearly visible.

4.3.3 The salinity is read at the white:black interface as parts per thousand (°/₀₀). Measurements should be recorded to the nearest 0.5 °/₀₀, with any necessary correction factors applied, on water quality data sheets.

4.3.4 Lens and cover will be dried between measurements.

5.0 DATA ANALYSIS AND CALCULATION

Not Applicable

6.0 QUALITY CONTROL

Calibration of refractometers shall be performed at a minimum of once a month, using IAPO Standard seawater. The instruments must be calibrated to agree with the 35.0 °/₀₀ IAPO Standard or labeled with the appropriate correction factor if a non-adjustable refractometer is used.

7.0 SAFETY

Not Applicable

8.0 TRAINING REQUIREMENTS

It is the responsibility of the technical managers to ensure that all staff performing the procedures described in this SOP are properly trained and that documentation of the training exists prior to the performance of those procedures.

To initiate training, all persons must read this SOP and demonstrate proficiency in the use of this instrument prior to application in project use. A staff member unfamiliar with the use of refractometers will receive a verbal and visual demonstration by a certified co-worker. A proficiency test will consist of the training instructor observing the trainee to ensure that procedures described in this SOP are followed. Once proficiency has been verified, documentation of training will be recorded on a training assignment form obtained from SOP MSL-A-006.

9.0 REFERENCES

- MSL-A-006 Marine Sciences Laboratory Training
- MSL-D-001 Recording Data on Data Sheets and Laboratory Notebooks

ATTACHMENT 1

BATTELLE MARINE SCIENCES LABORATORY

REFRACTOMETER (MONTHLY CALIBRATION RECORD)

SOP : MSL-M-048

SEAWATER STANDARD: _____

SALINITY (°/₀₀): _____

MODEL	SERIAL NUMBER	SALINITY (°/₀₀)	CORRECTION FACTOR	DATE CALIBRATED	INITIALS

REVIEWED BY: _____ DATE: _____

Battelle
Marine Sciences Laboratory

EFFECTIVE DATE: 11/22/94

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-M-056-01

STOCK AND STANDARD SOLUTION PREPARATION

Approvals:

AUTHOR	11/22/94 DATE
TECHNICAL REVIEWER	22 NOV 94 DATE
QUALITY ASSURANCE	11/22/94 DATE
MARINE SCIENCE LABORATORY MANAGER	11-22-94 DATE

STOCK AND STANDARD SOLUTION PREPARATION

1.0 SCOPE AND APPLICATION

This SOP covers preparation of stock and standard solutions for both organic and inorganic calibration standards, internal standards, and spiking solutions. Since any error in preparing these solutions will reflect directly on the accuracy of analytical results, solutions must be prepared as exactly as possible. Only compounds of the highest purity commercially available will be used in the preparation of stock solutions.

2.0 DEFINITIONS

None

3.0 RESPONSIBLE STAFF

Laboratory Supervisor. A Technical Specialist or Scientist in the Marine Chemistry Group having expertise in the principles involved with this procedure and in the use of laboratory operations in general. Responsible for 1) ensuring that analysts are trained in the handling of solvents and using balances; 2) checking the analysts' work to ensure that samples are handled appropriately and that all measurements are recorded properly; 3) making decisions regarding problems with the analysis or deviations from the SOP; and 4) defending the data in a Quality Assurance Audit.

Analyst. A Technician, Technical Specialist, or Scientist assigned to prepare stock and/or standard solutions using this procedure. Responsible for 1) understanding the proper handling of compounds of interest and solvents; and 2) recording information regarding solution preparation in the appropriate logbooks.

4.0 PROCEDURE

4.1 Upon receipt of the stock solution, the date received must be noted on the container and/or certificate accompanying the standard. The person receiving the standard should also initial both the bottle and the certificate.

4.2 Stock solutions can be prepared from compounds which are liquids or solids at room temperature or from commercially available stock solution.

4.2.1 Check calibration logbook for the balance to be used to see if a performance check was performed that day. If not, check the balance prior to use in accordance with SOP MSL-M-049.

4.2.2 Transfer appropriate amount of compound desired to a tared weighing pan or glass container. Record weight to the nearest 0.001g.

4.2.3 If stock is to be made up in a container different than the one into which the compound was originally weighed, transfer the material to the appropriate container. Rinse pan or original container with appropriate solvent or reagent. Add rinse to the new container.

4.2.4 Measure volume and dilute with appropriate solvent or reagent to achieve desired concentration. Desired volume accuracy is ±1 percent.

4.1.4 Transfer to appropriate storage container (if necessary) and store in controlled access area until needed.

4.3 Standards are prepared by calculating the amount of stock or stocks needed to achieve the desired concentration, and diluting to the appropriate volume. Always use the most accurate method of measurement possible.

4.4 Stock and standard solutions will be stored in vials or sealed in ampules whenever possible. Label information should include the stock or standard ID number, chemical name, concentration, solvent, date prepared, expiration date (see Section 6.0), and the preparer's initials. Most solutions will be stored in the freezer.

4.5 Documentation

4.5.1 Each stock or standard solution prepared will be given a unique laboratory ID number determined by sequential numbering in the appropriate Chemistry Standards Logbook for that set of analytes.

4.5.2 All documentation relating to stock and standards preparation will be recorded in the Chemistry Standards Logbook, on the Stock and Standard Solution Preparation Forms. See attachments 1 and 2.

4.5.3 Completing the Stock Solution Preparation Form or the Standard Solution Preparation Form is self-explanatory. Use one form for each solution or mixture of solutions prepared. Serial dilutions may all be included on one form.

5.0 CALCULATIONS

Calculations required by this SOP will vary, depending on amount and concentration of solution desired.

6.0 QUALITY CONTROL

6.1 For inert organic analytes, the expiration date for stocks or standards sealed in ampules are either, 1) as assigned by the manufacturer; or 2) when an expiration date is not supplied by the manufacturer, one year from receipt at the MSL. Expiration dates assigned to working standards cannot be later than the expiration date of the associated parent stock or standard.

For inorganic analytes, the expiration date for stocks or standards are as assigned by the manufacturer. Expiration dates assigned to working standards cannot be later than the expiration date of the associated parent stock or standard.

Expiration dates must be noted both on the original preparation form and on the container. Expiration dates for organic analytes in sealed ampules and for inorganic analytes may be extended at the discretion of the laboratory supervisor. If an extension is made, then the justification should be documented by the laboratory supervisor using a Documentation form (see SOP MSL-A-005) with an explanation for the extension. In addition, the expired standard should be run against another standard of know quality and quantity to verify that the expired standard concentration is still acceptable (within ±10% of each other). This documentation should be included with the Documentation form and kept with the standard certification. The laboratory supervisor shall assign a new expiration date. For organics, the maximum expiration date for working standards is one year from preparation. For inorganics, the maximum extended expiration date is one year from the original expiration date.

6.2 All calculations and dilutions will be checked and approved by the laboratory supervisor.

6.3 Routine surveillance will be conducted by the MSL Quality Assurance Representative to ensure that stock and standard solution preparation is performed in accordance with this SOP.

7.0 SAFETY

Before handling any potentially toxic chemical compounds, the individual preparing the solutions should make himself aware of any hazards and employ the appropriate safety precautions. Lab coats, gloves, and eye protection should be worn, and respirators are available to laboratory personnel if necessary.

8.0 TRAINING REQUIREMENTS

The individual preparing the solutions must read this SOP and also the SOP for balance calibration (SOP MSL-M-049). Individuals must show a proficiency for preparing stocks and standards. This will be demonstrated by comparison of results for the new stocks and standards to the results from the old standards. Documentation of training will be recorded on training assignment and on-the-job training forms from SOP MSL-A-006.

9.0 REFERENCES

MSL-M-049. Use and Performance Checks of Balances

MSL-A-006. Marine Sciences Laboratory Training

Attachment 1

STANDARDS & REAGENTS PREP LOGBOOK #1 **LOGBOOK PAGE#:**_____

Analyst:_____**Date of Preparation:**_____

Chemical I.D. #:_____Expires on Date:_____
Name of Chemical Prepared:_____
_____
_____

Name of Stock Chemical #1:_____
Manufacturor:_____Lot #:_____Exp. Date:_____
Weight used (g):_____Volume used (mL):_____
Balance I.D.:_____Pipette I.D.:_____

Name of Stock Chemical #2:_____
Manufacturor:_____Lot #:_____Exp. Date:_____
Weight used (g):_____Volume used (mL):_____
Balance I.D.:_____Pipette I.D.:_____

Name of Stock Chemical #3:_____
Manufacturor:_____Lot #:_____Exp. Date:_____
Weight used (g):_____Volume used (mL):_____
Balance I.D.:_____Pipette I.D.:_____

Name of Stock Chemical #4:_____
Manufacturor:_____Lot #:_____Exp. Date:_____
Weight used (g):_____Volume used (mL):_____
Balance I.D.:_____Pipette I.D.:_____

Name of Stock Chemical #5:_____
Manufacturor:_____Lot #:_____Exp. Date:_____
Weight used (g):_____Volume used (mL):_____
Balance I.D.:_____Pipette I.D.:_____

Type of Diluent Used for Preparation:_____
Manufacturor:_____Lot #:_____Exp. Date:_____
Weight used (g):_____Volume used (mL):_____
Balance I.D.:_____Pipette I.D.:_____

Final Volume of Chemical Solution Prepared (mL):_____

Comments:_____
_____
_____

ORGANIC STANDARD PREPARATION

Page_____of_____

Standard Lab ID # _____ Prep. date: _____ Exp date: _____

Stock used or supplier and lot	stock exp. date or date rec.	Stock / chemical name	Stock concentration	Stock volume	Diluted to	Solvent -Mfg and lot #	Standard concentration

Solution prepared by _____ Storage location _____

purpose of standard _____

comments

Attachment 2

BIENNIAL REVIEW OF PROCEDURES

Battelle Pacific Northwest National Laboratories, Marine Sciences Laboratory

Standard Operating Procedure No.: MSL-O-006-00

Title: HPLC CLEANUP OF SEDIMENT AND TISSUE EXTRACTS FOR SEMIVOLATILE POLLUTANTS

This procedure has been reviewed with the following results:

Designated Reviewer	Date	Acceptable As Is	Revision Required	Delete Procedure
TGM or MSL Manager	Date	Acceptable As Is	Revision Required	Delete Procedure

This procedure has been reviewed with the following results:

Designated Reviewer	Date	Acceptable As Is	Revision Required	Delete Procedure
TGM or MSL Manager	Date	Acceptable As Is	Revision Required	Delete Procedure

This procedure has been reviewed with the following results:

Designated Reviewer	Date	Acceptable As Is	Revision Required	Delete Procedure
TGM or MSL Manager	Date	Acceptable As Is	Revision Required	Delete Procedure

Battelle
Putting Technology To Work

Marine Sciences Laboratory

EFFECTIVE DATE: 11-24-99

Battelle Pacific Northwest National Laboratories
Marine Sciences Laboratory

STANDARD OPERATING PROCEDURE
MSL-Q-002-03

QUALITY ASSURANCE INSPECTIONS OF MSL SYSTEM AND STUDY ACTIVITIES

Approvals:		
AUTHOR: Deborah Coffey	Deborah Coffey	11-24-99
	Signature	Date
TECHNICAL REVIEWER: Richard M. Ecker	RM Ecker	11/24/99
	Signature	Date
QA OFFICER: Deborah Coffey	Deborah Coffey	11-24-99
	Signature	Date
TECHNICAL GROUP MANAGER: Richard M. Ecker	RM Ecker	11/24/99
	Signature	Date

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QUALITY ASSURANCE INSPECTIONS OF MSL SYSTEM AND STUDY ACTIVITIES

1.0 SCOPE AND APPLICATION

This procedure describes the steps to be followed by the Battelle Marine Sciences Laboratory (MSL) Quality Assurance Unit (QAU) for scheduling inspections, conducting inspections and reporting findings to management on regulated Good Laboratory Practice (GLP) studies, MSL projects, or MSL systems. These inspections are necessary to assure the integrity of a particular study, project, or system activity.

2.0 DEFINITIONS

Critical Phase Inspection - An inspection performed on events or procedures conducted during the course of a study that are considered essential by the QAU and Project Manager/Study Director to observe at a specified frequency to assess the compliance and integrity of a study.

Surveillances - Surveillances are formal verification activities that are performed to determine verification with a requirement or procedure, and include formal corrective action and follow-up.

Assessments - Assessments are informal verification activities. The purpose of assessments is to determine the status and to report the factual evidence and is not intended to be a verification activity with formal corrective action response, follow-up, etc. Assessments are generally requested by MSL management to assess the status of a particular activity.

3.0 RESPONSIBLE STAFF

MSL Quality Assurance Unit (Officer) or Representative
Project Manager/Study Director
MSL Managers

4.0 PROCEDURE

The QAU or QA Officer shall conduct verification activities (surveillances and assessments) to assess that facilities, equipment, personnel, methods, practices, records and quality control are in conformance to approved planning documents, procedures, regulations, client requirements and Battelle policy.

4.1 Non-GLP Verification Activities

4.1.1 Quarterly, with input from Project Managers and MSL Managers, the QA Unit or Officer shall prepare a QA Verification Plan (see Attachment 1). This schedule will include verifications based on client needs, management requests and routine internal verifications (i.e., checking standards logs, sample preparation forms, quality control (QC) checklists, equipment calibration and maintenance, and so on.)

4.1.2 Surveillances will be performed in accordance with QP-24, Internal Audits. The purpose of surveillances is to determine verification with a requirement and include formal corrective action and follow-up.

4.1.3 Assessments will be performed to determine the status and to report the factual evidence. Assessments are not intended to be a verification activity with formal corrective action response, follow-up, or verification. Assessments are generally requested by MSL management to assess the status of a particular activity but can also be determined to be necessary by the QA Unit or Officer.

4.1.4 In addition, informal walk-throughs will be performed by the QA Unit or Officer, at a minimum, on a monthly basis. The subject of these walk-throughs can be any activity that could affect data or system quality, but will generally be conducted to review raw data sheets. Indication of raw data sheet review will be evidenced by approval of the QA Representative directly on the data sheet. Results of walk-throughs will provide input into the need for surveillances or assessments.

4.1.5 All verification activities will be reported and submitted to Line Management and any appropriate Project Managers.

4.1.6 Data audits shall be performed to all data submitted to clients in accordance with MSL-Q-005, Quality Assurance Data Audits.

4.2 **GLP Studies - Scheduling Critical Phase Inspections**

4.2.1 Unless defined by the protocol, the QAU assigned to the study, along with the Study Director, will predetermine the list of critical events to be inspected from the study protocol and schedule. The following list represents common critical phases. This list is not considered to be complete and must be individually assessed for each study.

- test/control article receipt records and storage
- test system receipt, identification, randomization
- test/control article calculations and dose preparation
- laboratory/field set-up operations
- test system dosing, observations, weights, handling
- sample/specimen collection and handling

4.2.2 Inspections will be scheduled by the QAU based on a study schedule provided by the Study Director. In the event that study events cannot be pinpointed to a calendar day, verbal communication between the Study Director or study personnel and the QAU will provide a means for determining when events will take place.

4.2.3 For studies lasting less than 3 months, all critical phases will be inspected at least once. For studies lasting longer than 3 months, all reoccurring critical phase events will be inspected once every 3 months.

4.3 GLP Studies - Conducting Critical Phase Inspections

4.3.1 Prior to conducting critical phase inspections, the QAU shall:

- review the protocol and SOP(s) covering the activities to be inspected
- prepare a checklist which addresses the key requirements to be inspected

4.3.2 During the critical phase inspection the QAU shall, at a minimum, inspect for the following:

- Are current SOPs available to the person conducting the work?
- Do the activities observed reflect those described in the SOP(s)?

When appropriate,

- Is the equipment calibrated?
- Are equipment maintenance records available and current?
- Are reagents and solutions labeled appropriately?
- Is the training of individuals performing the procedure documented?
- Are test, control, and reference articles maintained appropriately?

4.4 GLP Studies - Reporting Critical Phase Inspections

4.4.1 The QAU shall provide a verbal report to key staff members and the Study Director (or his/her designee) at, or immediately after, the critical phase inspection.

4.4.2 The QAU shall document critical phase inspections on a surveillance report in accordance with QP-24, Internal Audits. The surveillance report shall contain all results of the inspections and the corrective action(s) required.

4.4.3 The surveillance report shall be distributed, as a minimum, to the Study Director and the MSL Manager.

5.0 DATA ANALYSIS AND CALCULATIONS

Not applicable

6.0 QUALITY CONTROL

Not applicable

7.0 SAFETY

Not applicable

8.0 TRAINING REQUIREMENTS

All MSL Quality Assurance Personnel and Project Manager/Study Directors must first read this procedure prior to GLP study initiation and prior to performing any verification activity. Documentation of training will be recorded on a training assignment form from procedure MSL-A-006.

9.0 REFERENCES

QP-24	Internal Audits (from Process Quality homepage, Process Quality Instructions)
MSL-A-006	Marine Sciences Laboratory Training
MSL-Q-005	Quality Assurance Data Audits

Attachment 1
Example Format for Reporting Assessment Results

The format below is recommended for reporting assessment results:

PLAN

Number	A tracking number assigned by the organization.
Topic	Describe what activities are to be assessed and why the assessment is being done
Scope	Identify any requirements documents (procedure, subject area, client requirement, management practice, policy) that is the basis for the assessment.
Assessment Method	Identify the planned assessment method (staff interviews, document reviews, data reviews, direct observation)
Assessor(s)	Identify who is to perform the assessment
Responsible Line Manager	Identify the managers(s) who has(ve) responsibility for this activity, operation, project.

PERFORM

Location	Identify the building, area, laboratory or site where the assessment was performed
Time	Identify the date and time the assessment was performed
Results	Identify the results of the assessment; summarize the activities performed by discussing the background of the activities examined, the strengths and weaknesses observed, list any noncompliances and provide recommendations
Lead Assessor:	_____ **Date:**_____ *Signature*

REVIEW

Manager	Results are reviewed by the appropriate managers, including the MSL Manager.
Review Assessment Report is Closed:	_____ **Date:**_____ *MSL Manager Signature*

CORRECTIVE ACTION

Assigned Responsibility	Managers assign responsibility for identified corrective actions. Corrective action is to be completed within 10 30 60 days Corrective action is to be verified in a follow-on assessment Yes No
Corrective Action Completed:	_____ **Date:**_____ *Responsible Person Signature*