Appendix III: MPSL-DFG SOPs

MPSL-DFG EPA Modifications and Laboratory Procedures			
Page	Procedure/Equipment	SOP Number	Revision Date
A	Modifications to EPA 3052		Feb 2006
B	Sample Container Preparation for Organics and Trace Metals, Including Mercury and Methylmercury	MPSL-101	Mar 2007
C	Sampling Marine and Freshwater Bivalves, Fish and Crabs for Trace Metal and Synthetic Organic Analysis	MPSL-102a Tis Collection	Mar 2007
E	Analysis of Mecury in Sediments and Tissue by Flow Injection Mercury System (FIMS)	MPSL-103 (formerly DFG SOP 103)	Feb 2000
D	Sample Receipt and Check-In	MPSL-104 Receipt and Check-in	Feb 2006
E	Protocol for Tissue Sample Preparation	MPSL-105 Tissue Preparation	Mar 2007

Appendix III A: Modifications to EPA 3052

Modification of EPA Method 3052

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Methods were modified from that described in EPA 3052 in order to reduce hazards to staff as well as more closely fit the requirements of the Microwave Assisted Reaction System (MARS) 5 unit.

It was determined through R&D that samples digested under the following conditions resulted in fully digested samples (modifications are listed according to section number):

7.2 All digestion vessels and vessel components are cleaned with hot 6% Double Distilled nitric acid for 8 hours, rinsed with reagent water and dried in a clean environment.

7.3.2 For tissue digestion, add 6 mL concentrated double distilled nitric acid to the vessel in a fume hood. For sediment digestion, add 5 ml concentrated double distilled nitric acid and 3 mL concentrated double distilled hydrofluoric acid to the vessel in a fume hood.

7.3.6 The following temperature and pressure settings are used for each matrix:
15 minute ramp to 195°C and 250 psi (controlled by temperature)
20 minute hold at temperature and pressure
Sediment samples (post boric addition):
5 minute ramp to 195°C and 250 psi (controlled by temperature)
15 minute hold at temperature and pressure

7.3.11 Transfer the sample into a pre-cleaned, pre-weighed 30 mL poly bottle. For tissues, bring the final solution weight to 20.00 ± 0.02 with reagent water. For sediments, record the solution volume.

Appendix III B: MPSL-101 Sample Container Preparation for Organics and Trace Metals, Including Mercury and Methylmercury

Method # MPSL-101

SAMPLE CONTAINER PREPARATION FOR ORGANICS AND TRACE METALS, INCLUDING MERCURY AND METHYLMERCURY

1.0 Scope and Application

1.1 This procedure describes the preparation of sample containers for the determination of synthetic organics and metals including but not limited to: aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), silver (Ag) and zinc (Zn) in tissue, sediment and water.

2.0 Summary of Method

2.1 Teflon, polyethylene, glass containers, and collection implements are detergent and acid cleaned prior to contact with tissue, sediment or water samples. Pre-cleaned containers may be purchased from the manufacturer in some instances.

3.0 Interferences

3.1 Special care must be used in selecting the acid(s) used for cleaning. Only reagent grade, or better, acids should be used. Prior to use, all acids should be checked for contamination.

3.2 If samples are to be analyzed for mercury, only Teflon or glass/quartz containers with Teflon-lined caps may be used. Use of other plastics, especially linear polyethylene, will result in Hg contamination through gas-phase diffusion through the container walls.

3.3 Colored plastics should be avoided, as they sometimes contain metal compounds as dyes (i.e., cadmium sulfide for yellow, ferric oxide for brown, etc.).

4.0 Apparatus and Materials

4.1 Crew Wipers: Fisher Scientific Part # 06-666-12

4.2 Disposable Filter Units, 250 mL: Nalge Nunc Inc. Part # 157-0045

4.3 Garbage Bag, clear 30 gallon

4.4 Glass Bottle Class 100 Amber, 4 L: I-Chem Part # 145-4000

4.5 Glass Bottle Class 200 Environmentally Cleaned, 250 mL: I-Chem Part # 229-0250

4.6 Glass Bottle Trace Clean, 250 mL: VWR Part # 15900-130

4.7 Glass Jar Class 100, 125 mL: I-Chem Part # 120-0125 (for use only when class 200 or 300 are not available)

4.8 Glass Jar Class 100, 500 mL: I-Chem Part # 121-0500 (for use only when class 200 or 300 are not available)

4.9 Glass Jar Class 200 Environmentally Cleaned, 125 mL: I-Chem Part # 220-0125

4.10 Glass Jar Class 200 Environmentally Cleaned, 500 mL: I-Chem Part # 221-0500

4.11 Glass Jar Class 300 Environmentally Cleaned, 125 mL: I-Chem Part # 320-0125

4.12 Glass Jar Class 300 Environmentally Cleaned, 500 mL: I-Chem Part # 321-0500

4.13 Heavy Duty Aluminum Foil

4.14 Homogenization Jar: Büchi Analytical Part # 26441

4.15 Immersion Heater: VWR Part # 33897-208

4.16 Lab Coats

4.17 Non-metal Scrub Brush

4.18 Non-metal Bottle Brush

4.19 Nylon Cable Ties, 7/16" wide x 7" long

4.20 Masterflex C-flex Tubing: ColeParmer Part # 06424-24

4.21 Plastic Knife

4.22 Polyethylene Bin, 63 L

4.23 Polyethylene Bin with Lid, 14.5"x10.5"x3.25": Cole Parmer Part # 06013-80

4.24 Polyethylene Bucket with Lid, medium: ColeParmer Part # 63530-12 and 63530-53

4.25 Polyethylene Bucket with Lid, small: ColeParmer Part # 63530-08 and 63530-52

4.26 Polyethylene Caps, 38mm-430: VWR Part # 16219-122

4.27 Polyethylene Gloves: VWR Part # 32915-166, 32915-188, and 32915-202

4.28 Polyethylene (HDPE) Bottle, 30 mL: Nalgene-Nunc, Inc. Part # 2089-0001

4.29 Polyethylene (HDPE) Bottle, 60 mL: Nalgene-Nunc, Inc. Part # 2089-0002

4.30 Polyethylene (HDPE) Jar, 30 mL: Nalgene-Nunc, Inc. Part # 2118-0001

4.31 Polyethylene (HDPE) Jar, 125 mL: Nalgene-Nunc, Inc. Part # 2118-0004

4.32 Polyethylene Scoop: VWR Part # 56920-400

4.33 Polypropylene Centrifuge Tubes, 15 mL: Fisher Scientific Part # 05-521

4.34 Polypropylene Cutter Tool: Büchi Analytical Part #24225

4.35 Polypropylene Diaphragm Seal: Büchi Analytical Part # 26900

4.36 Polypropylene "Snap Seal" Containers, 45 mL: Corning Part # 1730 2C

4.37 Polypropylene Spacer: Büchi Analytical Part # 26909

4.38 Precision Wipes: Fisher Scientific Part # 19-063-099

4.39 Sapphire Thermowell: CEM Part # 326280

4.40 Shoe covers: Cellucap Franklin Part # 28033

4.41 Steel Cutting Blade, Bottom: Büchi Analytical Part # 26907

4.42 Steel Cutting Blade, Top: Büchi Analytical Part # 26908

4.43 Syringe, 50 ml Luer Slip Norm-Ject: Air-Tite Part # A50

4.44 Teflon Centrifuge Tube, 30 mL: Nalge Nunc, Inc. Part # 3114-0030

4.45 Teflon HP500+ Control Cover: CEM Part # 431255

4.46 Teflon HP500+ Cover: CEM Part # 431250

4.47 Teflon HP500+ Liner: CEM Part # 431110

4.48 Teflon Sheet, 0.002"x12"x1000': Laird Plastics Part # 112486

4.49 Teflon Tape (plumbing tape)

4.50 Teflon Thermowell Nut: CEM Part #325028

4.51 Teflon Tubing, 0.0625" ID 0.125" OD: ColeParmer Part # 06406-62

4.52 Teflon Tubing, 0.1875" ID 0.25"OD: ColeParmer Part # 06406-66

4.53 Teflon Vial with cap, 60 mL: Savillex Part # 0202

4.54 Teflon Vial with cap, 180 mL: Savillex Part # 0103L-2-2- $^{1}/_{8}$"

4.55 Teflon Wash Bottle, 500 mL

4.56 Teflon Vent Nut: CEM Part # 431313

4.57 Titanium Cutter Screw: Büchi Analytical Part # 34376

4.58 Titanium Cutting Blade, Bottom: Büchi Analytical Part # 34307 DISCONTINUED

4.59 Titanium Cutting Blade, Top: Büchi Analytical Part # 34306 DISCONTINUED

4.60 Titanium Displacement Disc: Büchi Analytical Part # 26471

4.61 Ventilation Hood

4.62 Zipper-closure Polyethylene Bags, 4milx4"x6": Packaging Store Part # zl40406redline

4.63 Zipper-closure Polyethylene Bags, 4milx6"x8": Packaging Store Part # zl40608redline

4.64 Zipper-closure Polyethylene Bags, 4milx9"x12": Packaging Store Part # zl400912redline

4.65 Zipper-closure Polyethylene Bags, 4milx12"x15": Packaging Store Part # zl401215redline

4.66 Zipper-closure Polyethylene Bags, 4milx13"x18": Packaging Store Part # zl401318redline

5.0 Reagents

Reagent grade chemicals shall be used in all cleaning procedures. Unless otherwise indicated, it is intended that all reagents shall conform to the specification of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.1 Tap water (Tap)

5.2 Deionized water (DI)

5.3 Type II Water (MilliQ): Use for the preparation of all reagents and as dilution water. (reference ASTM D1193 for more on Type II water)

5.4 All-purpose Cleaner, 409™

5.5 Hydrochloric Acid (HCl), BAKER ANALYZED, 36.5-38.0% (12N): VWR Part # JT9535-3

5.6 Hydrochloric Acid (HCl), BAKER ANALYZED, 6N: VWR Part # JT5619-3

5.7 Hydrochloric Acid (HCl), 6N (50%): prepared by adding 1 part Baker 12N HCl to 1 part MilliQ

5.8 Hydrochloric Acid (HCl), 4N (33%): prepared by adding 1 part Baker 12N HCl to 2 parts MilliQ

5.9 Hydrochloric Acid (HCl), 1.2N (10%): prepared by adding 1 part Baker 12N HCl to 9 parts MilliQ

5.10 Hydrochloric Acid (HCl), 0.06N (0.5%): prepared by adding 1 part Baker 12N HCl to 99.5 parts MilliQ

5.11 Methanol: VWR Part # JT9263-3

5.12 Micro Detergent: ColeParmer Part # 18100-20

5.13 Nitric Acid (HNO_3), concentrated redistilled: Seastar Chemicals Part # BA-01

5.14 Nitric Acid (HNO_3), BAKER INSTRA-ANALYZED'*, 69.0–70.0% (15N): VWR Part # JT9598-34

5.15 Nitric Acid (HNO_3), 7.5N (50%): prepared by adding 1 part Baker HNO_3 to 1 part MilliQ

5.16 Nitric Acid (HNO_3), 6%: prepared by adding 1 part Seastar HNO_3 to 16.67 parts MilliQ

5.17 Nitric Acid (HNO_3), 1%: prepared by adding 1 part Seastar HNO_3 to 99 part MilliQ

5.18 Petroleum Ether: VWR Part # JT9265-3

6.0 Sample Collection, Preservation and Handling

6.1 All samples must be collected using a sampling plan that addresses the considerations discussed in each analytical procedure.

6.2 All samples shall be collected and analyzed in a manner consistent with the sampling and analytical sections of this QA/QC document (MPSL QAP Appendix E).

7.0 Procedures

All chemicals must be handled appropriately according to the Moss Landing Marine Laboratories Health and Safety Plan. Rinsings must be neutralized to pH 5-10 prior to disposal through the sewer system.

Two forms of acid baths are used throughout these procedures: Cold Bath and Hot Bath. All acid baths must be lidded and secondarily contained. Allow hot acid to cool completely before removing cleaned equipment.

A cold bath may be created in any clean polyethylene container of appropriate size. A hot bath is created using a clean polyethylene bucket and lid, two 63 L polyethylene bins and an immersion heater. The two bins are put together, the outer serving as secondary containment. The acid filled bucket is placed inside the inner bin and water is added to surround the bucket, creating a water bath. The immersion heater is placed outside the acid bucket, but within the water bath. The immersion heater MUST be set in a Teflon cap or other heat resistant item of appropriate size to disperse the heat source and eliminate melting of the two outer bins.

7.1 Trace Metal (including, but not limited to: Al, As, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Se, Ag, Zn) Sample Containers

7.1.1 Carboy

7.1.1.1 Fill completely with dilute Micro/Tap solution and soak for three days.

7.1.1.2 Rinse three times in Tap and three times in DI.

7.1.1.3 Fill completely with 50% HCl and soak for three days.

7.1.1.4 Remove acid and rinse three to five times in MilliQ.

7.1.1.5 Fill with 10% HNO_3 and soak for three days.

7.1.1.6 Remove acid and rinse three to five times in MilliQ.

7.1.1.7 If carboy is to be used immediately, fill with MilliQ and soak for 3 days. Collect solution in cleaned Trace Metal and Mercury water sample containers and test for contaminants.

7.1.1.8 If carboy is to be stored, fill with 0.5% HCl. Double bag in new garbage bags. Label the outer bag with "Acid Cleaned" and the date of completion.

7.1.2 Carboy Spigots and Tubing

7.1.2.1 Soak in dilute Micro/Tap solution overnight.

7.1.2.2 Rinse three to five times in Tap and DI, making sure to work the spigot valve to rinse all surfaces.

7.1.2.3 Submerge in 4N HCl cold bath for three days.

7.1.2.4 Rinse three to five times in MilliQ, making sure to work the spigot valve to rinse all surfaces.

7.1.2.5 Dry completely on crew wipers, then bag in new appropriately sized zipper-closure polyethylene bags. Label outer bag "Acid Cleaned" along with the date of completion.

7.1.3 Syringes for Field Filtration (not for Hg use)

7.1.3.1 Pull plungers out of syringes and place the outer tube in a 10% HCl bath. Swirl to ensure ink removal.

7.1.3.2 Once ink is completely gone, rinse three times with each Tap and DI.

7.1.3.3 Submerge all syringe parts in 4N HCl cold bath for three days.

7.1.3.4 Rinse three to five times with MilliQ.

7.1.3.5 Allow to completely dry on clean Crew Wipers.

7.1.3.6 Reassemble dry syringes and double bag in new appropriately sized zipper-closure polyethylene bags. Label outer bag "Acid Cleaned" along with the date of completion and the number of syringes within.

7.1.4 Polyethylene Water Containers (not for Hg use)

7.1.4.1 Fill each new 60 mL bottle with a dilute Micro/Tap solution. Place in a clean dissection bin and soak for one day.

7.1.4.2 Rinse three times in Tap, followed by three rinses in DI.

7.1.4.3 Fill each bottle with 50% HCl, soak for three days. (Note: HCl may only be used up to 6 times before it must be appropriately discarded.)

7.1.4.4 Pour out HCl and rinse each bottle and lid three to five times in MilliQ.

7.1.4.5 Fill each bottle with 1% Seastar HNO_3, cap. Allow outside of bottle to dry.

7.1.4.6 Double bag each bottle in new appropriately sized zipper-closure polyethylene bags. Label each outer bag with the date.

7.1.5 Polyethylene Tissue Dissection Containers

7.1.5.1 Fill each new 60 mL or 125 mL jar with a dilute Micro/Tap solution. Place in a clean dissection bin and soak for one day.

7.1.5.2 Rinse three times in tap water, followed by three rinses in DI.

7.1.5.3 Fill each jar with 10% HCl, soak for three days. (Note: HCl may only be used up to 6 times before it must be appropriately discarded.)

7.1.5.4 Pour out HCl and rinse each jar and lid three times in MilliQ.

7.1.5.5 Fill with MilliQ and soak for three days.

7.1.5.6 Remove MilliQ and place cleaned jars in a dissection bin lined with clean crew wipers to dry.

7.1.5.7 Once completely dry, pair lids and jars and place in a new appropriately sized zipper-closure polyethylene bag. Label bag "Acid Cleaned" along with the date of completion.

7.1.6 Polyethylene Scoops

7.1.6.1 (Performed by field crew) Thoroughly scrub new and used scoops in dilute Micro/Tap to ensure no residue remains in nicks and scratches. If soil cannot be completely removed, discard scoop.

7.1.6.2 (Performed by field crew) Rinse three times in Tap. Dry.

7.1.6.3 (In the lab) Submerge in 4N HCl cold bath for 3 days.

7.1.6.4 Rinse three to five times with MilliQ.

7.1.6.5 Let dry completely and double bag in new appropriately sized zipper-closure polyethylene bags. Label each outer bag with the date and number of scoops within.

7.1.7 Polypropylene Knives for Aliquoting

7.1.7.1 Scrub knives in dilute Mirco/Tap solution.

7.1.7.2 Rinse three times with Tap, followed by three rinses in DI.

7.1.7.3 Allow to completely dry on Precision Wipes. Roll in Precision Wipes, then place in new appropriately sized zipper-closure polyethylene bags. Label outer bag with "Micro Clean" and the date of completion.

7.1.8 Teflon Digestion Vessel and Lids

7.1.8.1 Using a soft, sponge-like bottle brush, scrub each vessel and lid with a dilute Micro/Tap solution.

7.1.8.2 Rinse three times with Tap, followed by three rinses with DI.

7.1.8.3 Submerge in 6% Seastar HNO_3 bath, heated for a minimum of 8 hours in a hotbath.

7.1.8.4 Rinse three to five times in MilliQ.

7.1.8.5 Place on new Crew Wipers under fume hood to dry.

7.1.8.6 Once completely dry, place in clean appropriately sized zipper-closure polyethylene bag. Label bag with the date of completion. (Note: You may use bags that have formerly contained clean digestion vessels or lids.)

7.1.9 Teflon and Sapphire Digestion Nuts and Thermowells

7.1.9.1 Remove any rupture membranes that may still be in the Vent Nuts.

7.1.9.2 Rinse each item with a dilute Micro/Tap solution by rubbing them gently between your hands.

7.1.9.3 Rinse three times with Tap, followed by three rinses with DI.

7.1.9.4 Submerge in 6% Seastar HNO_3 bath, heated for a minimum of 8 hours in a hotbath. Use a new 4milx6"x8"Zipper-closure polyethylene bag filled with acid to contain and protect these small parts in the bath. (Note: You may reuse this bag as long as it does not come in contact with unclean surfaces.)

7.1.9.5 Rinse three to five times in MilliQ.

7.1.9.6 Place on new Crew Wipers under fume hood to dry.

7.1.9.7 Store completely dry nuts it an appropriately sized zipper-closure polyethylene bag. Label bag with the date of completion. (Note: You may use bags that have formerly contained clean nuts.)

7.1.9.8 Store thermowells in the tubes provided to reduce the chance of breakage.

7.1.10 Polyethylene Digestate Bottles

7.1.10.1 Fill each new 30 mL bottle with a dilute Micro/Tap solution. Place in a clean dissection bin and soak for one day.

7.1.10.2 Rinse three times in tap water, followed by three rinses in DI.

7.1.10.3 Fill each cup with 50% HCl, soak for three days. (Note: HCl may only be used up to 6 times before it must be appropriately discarded.)

7.1.10.4 Pour out HCl and rinse each bottle and lid three times in MilliQ.

7.1.10.5 Fill with MilliQ and soak for three days.

7.1.10.6 Remove MilliQ and place cleaned bottles and lids upside-down in a dissection bin lined with clean crew wipers to dry.

7.1.10.7 Once completely dry, pair lids and bottles and place in a new appropriately sized zipper-closure polyethylene bag. Label bag "Acid Cleaned" along with the date of completion.

7.1.11 Polypropylene Centrifuge Tubes, 15 mL ("ICP Tubes")

7.1.11.1 Soak tubes in dilute Micro/Tap bath for three days.

7.1.11.2 Rinse three times in Tap, followed by three rinses in DI.

7.1.11.3 Submerge tubes and caps in 50% HCl cold bath for three days.

7.1.11.4 Rinse each tube and cap three times with MilliQ.

7.1.11.5 Place tubes and caps on clean crew wipers to dry.

7.1.11.6 Once completely dry, place in a new appropriately sized zipper-closure polyethylene bag. Label bag "Acid Cleaned" along with the date of completion.

7.2 Mercury Only Sample Containers

7.2.1 Water Composite Bottles, 4L

7.2.1.1 Caps do not get micro cleaned.

7.2.1.2 Scrub the outside of each bottle with a dilute Micro/Tap solution, rinse with Tap.

7.2.1.3 Place a small volume of the Micro/Tap solution inside the bottle. Shake vigorously to coat all surfaces.

7.2.1.4 Rinse with Tap until no more suds appear.

7.2.1.5 Rinse three times with DI.

7.2.1.6 Fill each bottle with 3N HCl. Cap and let stand on counter for three days. (Note: Acid may be used for a total of six cleaning cycles.)

7.2.1.7 Empty bottles and rinse three to four times with MilliQ, and fill.

7.2.1.8 Pipette in 20 mL HCl, BAKER ANALYZED, top off with MQ, replace caps and let dry.

7.2.1.9 Once completely dry, double bag in new appropriately sized zipper-closure polyethylene bags. Label outer bag with the date of completion.

7.2.1.10 Place in original boxes, labeled with date of completion. Bag entire box in a new garbage bag.

7.2.2 Tubing Sets

7.2.2.1 Cable Ties

7.2.2.1.1 Soak new cable ties in dilute Micro/Tap solution for three days.

7.2.2.1.2 Remove and rinse three times with Tap, followed by three rinses in DI and three rinses in MilliQ.

7.2.2.1.3 Allow to completely dry on Crew Wipers, then place in new appropriately sized zipper-closure polyethylene bags. Label outer bag with "Micro Clean" and the date of completion.

7.2.2.2 Polyethylene Caps with Holes

7.2.2.2.1 Drill a hole slightly smaller than 0.25 inches in the top of each new cap.

7.2.2.2.2 Soak in dilute Micro/Tap solution for three days.

7.2.2.2.3 Rinse three times with Tap, followed by three rinses in DI.

7.2.2.2.4 Soak in 4N HCl for 3 days.

7.2.2.2.5 Rinse three to five times in MilliQ. Let dry on Crew Wipers.

7.2.2.2.6 Once completely dry, place in new appropriately sized zipper-closure polyethylene bags until assembly. Label outer bag with "Acid Clean" and the date of completion.

7.2.2.3 Teflon Tubing

7.2.2.3.1 Using clean utility shears, cut one 3 foot and one 2 foot piece of tubing for each tubing set to be made.

7.2.2.3.2 Soak in dilute Micro/Tap solution for 3 days, ensuring that the tube is completely filled.

Note: Use Teflon tape to bind the two ends of each piece of tubing together. This will increase safety throughout the procedure.

7.2.2.3.3 Rinse three times in Tap, followed by three rinses in DI.

7.2.2.3.4 Submerge in 50% HNO_3 hot bath for 8 hours, ensuring that tubing is completely filled.

7.2.2.3.5 Rinse cooled tubing three to four times in MilliQ and let dry on clean Crew Wipers.

Note: Drying time may be decreased significantly by blowing reagent grade argon through the tubing to remove the water.

7.2.2.3.6 Once completely dry, place in new appropriately sized zipper-closure polyethylene bags until assembly. Label outer bag with "Acid Clean" and the date of completion.

7.2.2.4 C-Flex Tubing

7.2.2.4.1 Using clean utility shears, cut one 2 foot and one 4 inch piece of tubing for each tubing set to be made.

7.2.2.4.2 Soak in dilute Micro/Tap solution for one day, ensuring that the tube is completely filled.

7.2.2.4.3 Rinse three times in Tap, followed by three rinses in DI.

7.2.2.4.4 Submerge for three days in 12N HCl under a fume hood.

7.2.2.4.5 Rinse three to four times in MilliQ.

7.2.2.4.6 Submerge for three days in 0.5% HCl under a fume hood.

7.2.2.4.7 Rinse three to four times in MilliQ. Let dry completely on clean Crew Wipers.

Note: Drying time may be decreased significantly by blowing reagent grade argon through the tubing to remove the water.

7.2.2.4.8 Once completely dry, place in new appropriately sized zipper-closure polyethylene bags until assembly. Label outer bag with "Acid Clean" and the date of completion.

7.2.2.5 Tubing Set Assembly (using cleaned parts described above)

7.2.2.5.1 Using two cable ties, attach 2 foot Teflon tubing to 2 foot C-flex.

7.2.2.5.2 Next attach 4 foot Teflon to the other end of the 2 foot C-flex, again with 2 cable ties.

7.2.2.5.3 Add the 4 inch C-flex to the open end of the 4 foot Teflon tubing with 2 cable ties.

7.2.2.5.4 Put a drilled Poly cap on the open end of the 2 foot Teflon.

7.2.2.5.5 Coil the assembled tubing set, and double bag in new appropriately sized zipper-closure polyethylene bags. Label outer bag with "Acid Clean" and the date of completion.

7.2.2.6 In-Lab Mercury Filters

7.2.2.6.1 Fill upper reservoir with 10% HCl. Cap and apply vacuum.

7.2.2.6.2 Detach filter apparatus from vacuum manifold. Place finger over the valve and shake the unit to clean all surfaces of the lower reservoir.

7.2.2.6.3 Repeat two more times. Acid can be used 6 times.

7.2.2.6.4 Repeat wash three times with MilliQ. Cap and apply vacuum.

7.2.2.6.5 Discard MilliQ after each rinse.

7.2.3 Water Sample Bottles, 250 mL

7.2.3.1 Rinse new bottles in DI. Place the caps only in a MilliQ bath for the duration of the bottle cleaning.

7.2.3.2 Submerge in 50% Baker HNO_3 hot bath for 8 hours, ensuring that each bottle is completely filled.

7.2.3.3 Rinse cooled bottles three to four times in MilliQ, then fill each with MilliQ.

7.2.3.4 Pipette in 1.25 mL 100% HCl, replace caps and let dry completely.

7.2.3.5 Double bag in new appropriately sized zipper-closure polyethylene bags. Label outer bag with the date of completion.

7.2.3.6 Place in original boxes, labeled with date of completion.

7.2.4 Polypropylene “Snap Seal” Containers, 45 mL (“Trikona Tubes”)

7.2.4.1 Rinse new tubes in dilute Micro/Tap.

7.2.4.2 Rinse three times in Tap, followed by three times in DI.

7.2.4.3 Submerge in 50% HNO_3 hot bath for 8 hours, ensuring that each tube is completely filled.

7.2.4.4 Rinse cooled tubes three to four times in MilliQ.

7.2.4.5 Let dry completely on clean Crew Wipers.

7.2.4.6 Place dry tubes in new appropriately sized zipper-closure polyethylene bags. Label outer bag with “Acid Clean” and the date of completion.

7.3 Methylmercury Only Sample Containers

7.3.1 Teflon Digestion or Distillation Vials

7.3.1.1 Scrub vials with 409™ to remove any organic residue. It may be necessary to also soak the vials in dilute Micro/Tap for 3 days.

7.3.1.2 Rinse three times in DI.

7.3.1.3 Submerge in 50% HCl bath. Heat overnight, or soak for 3 days in cold bath.

7.3.1.4 Rinse three to five times in MilliQ; dry completely on clean crew wipers.

7.3.1.5 Place dry tubes in new appropriately sized zipper-closure polyethylene bags. Label outer bag with “Acid Clean” and the date of completion.

7.3.2 Teflon Distillation Caps and Tubing

7.2.3.2 Submerge in 50% Baker HNO_3 hot bath for 8 hours, ensuring that each bottle is completely filled.

7.2.3.3 Rinse cooled bottles three to four times in MilliQ, then fill each with MilliQ.

7.2.3.4 Pipette in 1.25 mL 100% HCl, replace caps and let dry completely.

7.2.3.5 Double bag in new appropriately sized zipper-closure polyethylene bags. Label outer bag with the date of completion.

7.2.3.6 Place in original boxes, labeled with date of completion.

7.2.4 Polypropylene "Snap Seal" Containers, 45 mL ("Trikona Tubes")

7.2.4.1 Rinse new tubes in dilute Micro/Tap.

7.2.4.2 Rinse three times in Tap, followed by three times in DI.

7.2.4.3 Submerge in 50% HNO_3 hot bath for 8 hours, ensuring that each tube is completely filled.

7.2.4.4 Rinse cooled tubes three to four times in MilliQ.

7.2.4.5 Let dry completely on clean Crew Wipers.

7.2.4.6 Place dry tubes in new appropriately sized zipper-closure polyethylene bags. Label outer bag with "Acid Clean" and the date of completion.

7.3 Methylmercury Only Sample Containers

7.3.1 Teflon Digestion or Distillation Vials

7.3.1.1 Scrub vials with 409™ to remove any organic residue. It may be necessary to also soak the vials in dilute Micro/Tap for 3 days.

7.3.1.2 Rinse three times in DI.

7.3.1.3 Submerge in 50% HCl bath. Heat overnight, or soak for 3 days in cold bath.

7.3.1.4 Rinse three to five times in MilliQ; dry completely on clean crew wipers.

7.3.1.5 Place dry tubes in new appropriately sized zipper-closure polyethylene bags. Label outer bag with "Acid Clean" and the date of completion.

7.3.2 Teflon Distillation Caps and Tubing

7.3.2.1 Scrub caps and tubing with 409™ to remove any organic residue.

7.3.2.2 Rinse three times in DI.

7.3.2.3 Submerge in 10% HCl hotbath overnight. Use a Teflon squirt bottle to fill the tubing with acid.

7.3.2.4 Rinse three to five times in MilliQ; dry completely on clean crew wipers.

Note: Hang tubing over a clean hook against crew wipers to speed drying time.

7.3.2.5 Place in new appropriately sized zipper-closure polyethylene bags. Label outer bag with "Acid Clean" and the date of completion.

7.4 Organic Sample Containers

7.4.1 Aluminum Foil Sheets

7.4.1.1 Using a clean scalpel, cut a 4 foot long section of aluminum foil.

7.4.1.2 Fold in half, with dull side out. (The bright side may contain oils from the manufacturing process.)

7.4.1.3 Under a fume hood, rinse both exposed sides of the folded foil three times with Petroleum Ether. Make sure all exposed surfaces are well rinsed.

7.4.1.4 Set against a clean surface under the fume hood to dry.

7.4.1.5 Once completely dry, fold the sheet in quarters, ensuring the un-rinsed shiny side does not come in contact with the now cleaned dull side.

7.4.1.6 Place into a new appropriately sized zipper-closure polyethylene bag. Label bag "PE Cleaned" along with the date of completion and the number of sheets within.

7.4.2 Dissection Jars (125mL, 500mL Glass Jars)

NOTE: Clean 100 series jars as follows below. 200 and 300 series jars may be used as is from the manufacturer, with a clean Teflon square (section 7.5.2) over the threads.

7.4.2.1 Using a clean scalpel, cut three inch squares from a sheet of new Teflon.

7.4.2.2 Fit Teflon square to the jar and lid, ensuring that the threads are completely covered and no leaks will occur.

7.4.2.3 Under a fume hood, rinse each jar and lid three times with Petroleum Ether by putting a small of amount in the jar, sealing it and then shaking the jar to coat all sides.

Note: It is easiest to clean four jars simultaneously. Use each volume of PE once in each of the jars; repeat. After cleaning the fourth jar, discard PE into evaporation bin under the hood, or into designated solvent waste container.

7.4.2.4 Set jars aside in the hood to dry.

7.4.2.5 When completely dry, match the lids to the jar and place back in the original box. Label box "PE Cleaned" along with the date of completion.

7.5 "Split" Sample Containers (for metals and organics)

7.5.1 Teflon sheets

7.5.1.1 Cut new Teflon to desired length (1 or 2 feet long depending on application)

7.5.1.2 Submerge crumpled sheets in a 10% Micro/Tap bath overnight.

7.5.1.3 Remove sheets from micro bath and flatten. Rinse all surfaces of each sheet three times in tap water, followed by three rinses in deionized water.

7.5.1.4 Crumple rinsed sheets and submerge in 10% HCl in a hot bath; heat at least 8 hours.

7.5.1.5 Remove sheets from acid bath and flatten. Rinse all surfaces of each sheet five times in MilliQ.

7.5.1.6 Layer rinsed Teflon sheets on new Crew Wipers, with new Precision Wipes between each sheet. Cover stack with new Precision Wipes. Let dry.

7.5.1.7 Once the sheets are completely dry, rinse each surface three times with Petroleum Ether.

7.5.1.8 Place on clean Crew Wipers and Precision Wipes, as before, under hood and let dry.

7.5.1.9 Once the sheets are completely dry, fold sheets and place into a new appropriately sized zipper-closure polyethylene bag. Label bag "PE Cleaned" along with the date of completion and the number of sheets within.

7.5.2 Teflon Squares for Dissection Jars

7.5.2.1 Using a cutting board and scalpel, cut Teflon sheet into 3-inch squares.

7.5.2.2 Soak in 6% Seastar HNO_3 coldbath overnight.

7.5.2.3 Rinse three times with MilliQ.

7.5.2.4 Rinse three times with Methanol, followed by three rinses with Petroleum Ether.

7.5.2.5 Lay on clean crew wipers to dry.

7.5.2.6 Once the squares are completely dry, place into a new appropriately sized zipper-closure polyethylene bag. Label bag "PE Cleaned" along with the date of completion.

7.5.3 Dissection Jars (125mL, 500mL Glass Jars)

NOTE: Clean 100 series jars as follows below. 200 and 300 series jars may be used as is from the manufacturer, with a clean Teflon square (section 7.5.2) over the threads.

7.5.3.1 Using a clean scalpel, cut three inch squares from a sheet of new Teflon.

7.5.3.2 Fit Teflon square to the jar and lid, ensuring that the threads are completely covered and no leaks will occur.

7.5.3.3 Under a fume hood, rinse each jar and lid three times with 6% HNO_3 by putting a small of amount in the jar, sealing it and then shaking the jar to coat all sides.

Note: It is easiest to clean four jars simultaneously. Use each volume of each chemical once in each of the jars; repeat. After cleaning the fourth jar, discard into the appropriate evaporation bin under the hood or into designated waste container.

7.5.3.4 Rinse each jar three times in MilliQ.

7.5.3.5 Rinse each jar three times in Methanol, let dry completely.

7.5.3.6 Rinse each jar three times in Petroleum Ether; set aside in the hood to dry.

7.5.3.7 When completely dry, match the lids to the jar and place back in the original box. Label box "Split Cleaned" along with the date of completion.

7.5.4 Homogenization Parts (Büchi) including glass, polypropylene, titanium and stainless steel

7.5.4.1 Scrub with dilute Micro/Tap, followed by 3 rinses with DI.

7.5.4.2 Rinse 3 times with 6% Seastar HNO_3 using a Teflon squirt bottle.

7.5.4.3 Rinse 3 times with MilliQ.

7.5.4.4 Rinse 3 times with Methanol, followed by 3 times with Petroleum Ether.

7.5.4.5 Allow parts to dry completely before assembly and homogenization.

8.0 Analytical Procedure

8.1 Tissue Preparation procedures can be found in Method # MPSL-105.

8.2 Trace Metal and Mercury Only digestion procedures can be found in EPA 3052, modified, and Method # MPSL-106, respectively.

8.3 Trace Metals are analyzed with ICP-MS according to EPA 200.8.

8.4 Mercury samples are analyzed by FIMS according to Method # MPSL-103 or by DMA and EPA 7473.

8.5 Methylmercury tissue samples are extracted and analyzed according to Method # MPSL-109.

8.6 Methylmercury sediment samples are extracted and analyzed according to Method # MPSL-110 and modified EPA 1630, respectively.

9.0 Quality Control

9.1 See individual methods.

10.0 Method Performance

10.1 System blanks are performed on Mercury Sample 250 mL and 4 L bottles and tubing sets to guarantee thorough cleaning.

10.2 Carboys are tested for all metals after cleaning.

11.0 References

Appendix III C: MPSL-102a Sampling Marine and Freshwater Bivalves, Fish and Crabs for Trace Metal and Synthetic Organic Analysis

Method # MPSL-102a

SAMPLING MARINE AND FRESHWATER BIVALVES, FISH AND CRABS FOR TRACE METAL AND SYNTHETIC ORGANIC ANALYSIS

1.0 Scope and Application

1.1 The following procedures describe techniques of sampling marine mussels and crabs, freshwater clams, marine and freshwater fish for trace metal (TM) and synthetic organic (SO) analyses.

2.0 Summary of Method

2.1 Collect mussels, clams, crabs, or fish. Mussels or clams to be transplanted are placed in polypropylene mesh bags and deployed. Mussels and clams to be analyzed for metals are double-bagged in plastic zipper-closure bags. Bivalves to be analyzed for organics are wrapped in PE cleaned aluminum foil prior to placement in the zipper-closure bags. Fish are wrapped whole or proportioned where necessary in cleaned Teflon sheets or aluminum foil and subsequently placed into zipper-closure bags. Crabs for TM and/or SO are double-bagged in plastic zipper-closure bags.

2.2 Each sample should be labeled with Date, Station Name, and any other information available to help identify the sample once in the lab.

2.3 After collection, samples are transported back to the laboratory in coolers with ice or dry ice. If ice is used, care must be taken to ensure that ice melt does not come into direct contact with samples.

3.0 Interferences

3.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and truck engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination.

3.2 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines, causing inaccurate analytical results. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot.

3.3 Polypropylene and polyethylene surfaces are a potential source of contamination for SO specimens and should not be used whenever possible.

4.0 Apparatus and Materials

Procedures for equipment preparation can be found in Method # MPSL-101.

4.1 Anchor Chains

4.2 Backpack Shocker (electro-fishing)

4.3 Boats (electro-fishing and/or for setting nets)

4.4 Bone Saw

4.5 Camera, digital

4.6 Cast Nets (10' and 12')

4.7 Data Sheets (see MPSL QAP Appendix E for example)

4.8 Daypacks

4.9 Depth Finder

4.10 Dip Nets

4.11 Dry Ice or Ice

4.12 Gill Nets (various sizes)

4.13 GPS

4.14 Heavy Duty Aluminum Foil, prepared

4.15 Heavy Duty plastic bags, Clear 30 gallon

4.16 Inflatable Buoy

4.17 Labels, gummed waterproof: Diversified Biotech Part #: LCRY-1258

4.18 Nylon Cable Ties, 7/16" wide x 7" long

4.19 Other (minnow traps, set lines, throw nets, etc)

4.20 Otter Trawl (various widths as appropriate)

4.21 Permanent Marking Pen

4.22 Plastic bucket, 30 gallon

4.23 Plastic Ice Chests

4.24 Polyethylene Gloves: VWR Part # 32915-166, 32915-188, and 32915-202

4.25 Polypropylene Mesh, 76mm wide with 13mm mesh

4.26 Polypropylene Mesh, 50mm wide with 7mm mesh

4.27 Polypropylene Line, 16mm

4.28 Rods and Reels

4.29 Screw in Earth Anchor, 4-6" diameter

4.30 Scuba Gear

4.31 Seines (various size mesh and lengths as appropriate)

4.32 Stainless Steel Dive Knives

4.33 Trap Nets (hoop or fyke nets)

4.34 Teflon Forceps

4.35 Teflon Sheet, prepared

4.36 Teflon Wash Bottle, 500 mL

4.37 Wading Gear

4.38 Zipper-closure Polyethylene Bags, 4milx13"x18": Packaging Store Part # zl401318redline

5.0 Reagents

5.1 Tap water (Tap)

5.2 Deionized water (DI)

5.3 Type II water (ASTM D1193): Use Type II water, also known as MilliQ, for the preparation of all reagents and as dilution water.

5.4 Micro Detergent: ColeParmer Part # 18100-20

5.5 Methanol: VWR Part # JT9263-3

5.6 Petroleum Ether: VWR Part # JT9265-3

6.0 Sample Collection, Preservation and Handling

6.1 All sampling equipment will be made of non-contaminating materials and will be inspected prior to entering the field. Nets will be inspected for holes and repaired prior to being used. Boats (including the electroshocking boat) will be visually checked for safety equipment and damage prior to being taken into the field for sample collection.

6.2 To avoid cross-contamination, all equipment used in sample collection should be thoroughly cleaned before each sample is processed. Ideally, instruments are made of a material that can be easily cleaned (e.g. Stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with ambient water, rinsed with a high-purity solvent (methanol or petroleum ether), and finally rinsed with MilliQ. Waste detergent and solvent solutions must be collected and taken back to the laboratory.

6.3 Samples are handled with polyethylene-gloved hands only. The samples should be sealed in appropriate containers immediately.

6.4 Mussels and clams to be analyzed for metals are double-bagged in zipper-closure bags. Bivalves to be analyzed for organics are wrapped in prepared aluminum foil prior to placement in zipper-closure bags.

6.5 Fish are wrapped in part or whole in prepared Teflon sheets and subsequently placed into zipper-closure bags.

6.6 Crabs analyzed for metals and/or organics are double-bagged in plastic zipper-closure bags.

6.7 Data is recorded for each site samples are transplanted to or collected from. Data includes, but is not limited to station name, sample identification number, site location (GPS), date collected or transplanted, collectors names, water depth, photo number, ocean/atmospheric conditions (if appropriate), description of site, and drawing if necessary.

6.8 A chain of custody form (MPSL QAP Appendix E) will accompany all samples that are brought to the lab. All samples that are processed in the lab MUST be checked in according to Method # MPSL-104.

6.9 Samples are maintained at -20°c and extracted or digested as soon as possible.

7.0 Procedure

7.1 Sample collection - mussels and clams

7.1.1 The mussels to be transplanted (*Mytilus californianus*) are collected from Trinidad Head (Humboldt Bay Intensive Survey), Montana de Oro (Diablo Canyon Intensive Survey), and Bodega Head (all other statewide transplants). The freshwater clam (*Corbicula fluminea*) source is Lake Isabella or the Sacramento River. Analyze mussel and clam samples for background contaminates prior to transplanting.

7.1.2 Polyethylene gloves are worn while prying mussels off rocks with dive knives. Note: polyethylene gloves should always be worn when handling samples. Mussels of 55mm to 65mm in length are recommended. Fifty mussels are collected for each TM and each SO sample.

7.1.3 Collected mussels are carried out of collection site in zipper-closure bags placed in cleaned nylon daypacks. For the collection of resident samples where only one or two samples are being collected the mussels are double bagged directly into a labeled zipper-closure bag. Samples for SO are wrapped first in prepared aluminum foil.

7.1.4 Clams (*Corbicula fluminea*) measuring 20 to 30mm are collected by dragging the clam dredge along the bottom of the lake or river. The clams are poured out of the dredge into a 30 gallon plastic bag. Clams can also be collected by gloved hands in shallow waters and placed in labeled zipper-closure bags. 25-200 clams are collected depending on availability and necessity for analyses.

7.1.5 Data is recorded for each site samples are collected from. Data includes, but is not limited to station name, date collected, collectors names, water depth, GPS readings, photo, ocean/atmospheric conditions (if appropriate), description of site, and drawing if necessary.

7.2 Transplanted sample deployment

7.2.1 With polyethylene gloves, fifty transplant mussels are placed in each 76mm X 13mm polypropylene mesh bag. Each bag represents one TM or one SO sample. A knot is tied at each end of mesh bag and reinforced with a cable tie. On one end another cable tie is placed under the cable tie which will be used to secure the bag to the line for transplant deployment. The mussels in the mesh bag are divided into three groups of approximately equal size and sectioned with two more cable ties.

7.2.2 Once bagged, the mussels are placed in a 30 gallon plastic bag and stored in a cooler (cooled with ice) for no more than 48 hours. The ice is placed in zipper-closure bags to avoid contamination.

7.2.3 If marine samples are held for longer than 48 hours they are placed in holding tanks with running seawater at the lab. Control samples for both SO and TM are also held in the tank.

7.2.4 For freshwater clams: clams (25-200) are placed in 50mm X 7mm polypropylene mesh bags using identical procedures to those used with mussels (section 7.2.1). If clams need to be stored for more than 48 hours, the mesh bags are deployed either in a clean source or in holding tanks with running freshwater at the lab until actual sample deployment.

7.2.5 The mussels are attached to an open water transplant system that consists of a buoy system constructed with a heavy weight anchor (about 100lbs) or screw-in earth anchor, 13mm polypropylene line, and a 30cm diameter subsurface buoy. The sample bags are attached with cable ties to the buoy line about 15 feet below the water surface. In some cases the sample is hung on suspended polypropylene lines about 15 feet below the water surface between pier pilings or other surface structures. Creosote-coated wooden piers are avoided because they are a potential source of contamination. In some cases the mussels are hung below a floating dock. In shallow waters a wooden or PVC stake is hammered into the substrate and the mussel bags are attached by cable ties to the stake.

7.2.6 The clams are deployed by attaching the mesh bag with cable ties to wooden or PVC stakes hammered into substrate or screw in earth anchors. The bags containing clams are typically deployed 15cm or more off the bottom. In areas of swift water, polypropylene line is also attached to the staked bags and a permanent object (piling, tree or rock).

7.2.7 Transplants are usually deployed for 1-4 months. Ideally mussels are transplanted in early September and retrieved in late December and early January. Clams are usually transplanted in March or April and retrieved in May or June.

7.2.8 Data is recorded for each site samples are transplanted to or collected from. Data includes, but is not limited to station name, date collected or transplanted, collectors names, water depth, GPS readings, photo, ocean/atmospheric conditions (if appropriate), description of site, and drawing if necessary.

7.3 Sample Retrieval

7.3.1 The transplanted or resident and control mussels analyzed for TM are double bagged in appropriately sized and labeled zipper-closure bags.

7.3.2 All mussels to be analyzed for SO are wrapped in prepared aluminum foil (Method # DFG 101). The foil packet is double bagged in appropriately sized and labeled zipper-closure bags. Note: samples should only contact the dull side of the foil.

7.3.3 The bags containing samples are clearly and uniquely identified using a water-proof marking pen or pre-made label. Information items include ID number, station name, depth (if from a multiple sample buoy), program identification, date of collection, species and type of analysis to be performed.

7.3.4 The samples are placed in non-metallic ice chests and frozen using dry ice or regular ice. (Dry ice is used when the collecting trip takes more than two days.) At the lab, samples should be stored at or below -20°c until processed.

7.4 Sample Collection – Fish

7.4.1 Fish are collected using the appropriate gear for the desired species and existing water conditions.

7.4.1.1 Electro-fisher boat- The electro-fisher boat is run by a trained operator, making sure that all on board follow appropriate safety rules. Once on site, adjustment of the voltage, amps, and pulse for the ambient water is made and recorded. The stainless steel fish well is rinsed with ambient water, drained and refilled. The shocked target fish are placed with a nylon net in the well with circulating ambient water. The nylon net is washed with a detergent and rinsed with ambient water prior to use. Electro-fishing will continue until the appropriate number and size of fish are collected.

7.4.1.2 Backpack electro-fisher- The backpack shocker is operated by a trained person, making sure that all others helping follow appropriate safety rules. The backpack shocker is used in freshwater areas where an electro-fisher boat can not access. Once on site, adjustment of the voltage, amps, and pulse for the ambient water is made and recorded. The shocked target fish are captured with a nylon net and placed in a 30 gallon plastic bag. The nylon net is washed with a detergent and rinsed with ambient water prior to use. Electro-fishing will continue until the appropriate number and size of fish are collected.

7.4.1.3 Fyke or hoop net- Six-36 inch diameter hoops connected with 1 inch square mesh net is used to collect fish, primarily catfish. The net is placed parallel to shore with the open hoop end facing downstream. The net is placed in areas of slow moving water. A partially opened can of cat food is placed in the upstream end of the net. Between 2-6 nets are placed at a site overnight. Upon retrieval a grappling hook is used to pull up the downstream anchor. The hoops and net are pulled together and placed on a 30

gallon plastic bag in the boat. With polyethylene gloves the desired fish are placed in a 30 gallon plastic bag and kept in an ice chest with ice until the appropriate number and size of fish are collected.

7.4.1.4 Otter-trawl- A 14 foot otter trawl with 24 inch wooden doors or a 20 foot otter trawl with 30 inch doors and 80 feet of line is towed behind a boat for water depths less than 25 feet. For water depths greater than 25 feet another 80 feet of line is added to capture fish on or near the substrate. Fifteen minute tows at 2-3 knots speed are made. The beginning and ending times are noted on data sheets. The trawl is pulled over the side of the boat to avoid engine exhaust. The captured fish are emptied into a 30 gallon plastic bag for sorting. Desired fish are placed with polyethylene gloves into another 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.5 Gill nets- A 100 yard monofilament gill net of the appropriate mesh size for the desired fish is set out over the bow of the boat parallel to shore. The net is retrieved after being set for 1-4 hours. The boat engine is turned off and the net is pulled over the side or bow of the boat. The net is retrieved starting from the down-current end. If the current is too strong to pull in by hand, then the boat is slowly motored forward and the net is pulled over the bow. Before the net is brought into the boat, the fish are picked out of the net and placed in a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.6 Beach seines- In areas of shallow water, beach seines of the appropriate length, height, and mesh size are used. One sampler in a wetsuit or waders pulls the beach seine out from shore. The weighted side of the seine must drag on the bottom while the float side is on the surface. The offshore sampler pulls the seine out as far as necessary and then pulls the seine parallel to shore and then back to shore, forming a half circle. Another sampler is holding the other end on shore while this is occurring. When the offshore sampler reaches shore the two samplers come together with the seine. The seine is pulled onto shore making sure the weighted side drags the bottom. When the seine is completely pulled onshore, the target fish are collected with polyethylene gloves and placed in a 30 gallon plastic bag and kept in an ice chest with ice. The beach seine is rinsed off in the ambient water and placed in the rinsed 30 gallon plastic bucket.

7.4.1.7 Cast net- A 10 or 12 foot cast net is used to collect fish off a pier, boat, or shallow water. The cast net is rinsed in ambient water prior to use and stored in a covered plastic bucket. The target fish are sampled with polyethylene gloves and placed in a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.8 Hook and line- Fish are caught off a pier, boat, or shore by hook and line. Hooked fish are taken off with polyethylene gloves and placed in a Ziploc™ bag or a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.9 Spear fishing- Certain species of fish are captured more easily by SCUBA divers spearing the fish. Only appropriately trained divers following the dive safety program guidelines are used for this method of collection. Generally, fish in the kelp beds are more easily captured by spearing. The fish are shot in the head area to prevent the fillets from being damaged or contaminated. Spear tips are washed with a detergent and rinsed with ambient water prior to use.

7.4.2 As a general rule, five fish of medium size or three fish of larger size are collected as composites for analysis. The smallest fish length cannot be any smaller than 75% of the largest fish length. Five fish usually provides sufficient quantities of tissue for the dissection of 150 grams of fish flesh for organic and inorganic analysis. The medium size is more desirable to enable similar samples to be collected in succeeding collections.

7.4.3 When only small fish are available, sufficient numbers are collected to provide 150 grams of fish flesh for analysis. If the fish are too small to excise flesh, the whole fish, minus the head, tail, and guts are analyzed as composites.

7.4.4 Species of fish collected are chosen for their importance as indicator species, availability or the type of analysis desired. For example, livers are generally analyzed for heavy metals. Fish without well-defined livers, such as carp or goldfish, are not collected when heavy metal analyses are desired.

7.4.5 Fish collected, too large to fit in clean bags (>500 mm) are initially dissected in the field. At the dock, the fish are laid out on a clean plastic bag and a large cross section from behind the pectoral fins to the gut is cut with a cleaned bone saw or meat cleaver. The bone saw is cleaned (micro, DI, methanol) between fish and a new plastic bag is used. The internal organs are not cut into, to prevent contamination. For bat rays, a section of the wing is cut and saved. These sections are wrapped in prepared Teflon sheets, double bagged and packed in dry ice before transfer to the freezer. During lab dissection, a subsection of the cross section is removed, discarding any tissue exposed by field dissection.

7.4.6 Field data (MPSL QAP Appendix E) recorded include, but are not limited to site name, sample identification number, site location (GPS), date of collection, time of collection, names of collectors, method of collection, type of sample, water depth, water and atmospheric conditions, fish total lengths (fork lengths where appropriate), photo number and a note of other fish caught.

7.4.7 The fish are then wrapped in aluminum foil or Teflon sheets if thylates are analyzed. The wrapped fish are then double-bagged in zipper-closure bags with the inner bag labeled.

The fish are put on dry ice and transported to the laboratory where they are kept frozen until they are processed for chemical analysis.

7.5 Sample Collection- Crabs

7.5.1 Crab/lobster traps- Polyethylene traps are baited to collect crabs or lobsters. Traps are left for 1-2 hours. The crabs are placed in a zipper-closure bag or a 30 gallon plastic bag and kept in an ice chest with ice.

8.0 Analytical Procedure

8.1 Tissue Preparation procedures can be found in Method # MPSL-105.

8.2 Trace Metal and Mercury Only digestion procedures can be found in EPA 3052, modified, and Method # MPSL-106, respectively.

8.3 Trace Metals are analyzed with ICP-MS according to EPA 200.8.

8.4 Mercury samples are analyzed by FIMS according to Method # MPSL-103 or by DMA and EPA 7473.

8.5 Methylmercury tissue samples are extracted and analyzed according to Method # MPSL-109.

9.0 Quality Control

9.1 Field Replicates: project specific requirements are referenced for field replication.

9.2 A record of sample transport, receipt and storage is maintained and available for easy reference.

10.0 References

10.1 Flegal, R.A. 1982. In: Wastes in the Ocean, Vol VI: Near Shore Waste Disposal. B.H. Ketchum (ed.). John Wiley and Sons Inc. Publishers, New York, 1982.

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10.3 Gordon, R.M., G.A. Knauer and J.H. Martin. 1980a. *Mytilus californianus* as a bioindicator of trace metal pollution: variability and statistical considerations. Mar. Poll. Bull. 9:195-198.

10.4 Hayes, S. P. and P. T. Phillips. 1986. California State Mussel Watch: Marine water quality monitoring program 1984-85. State Water Resources Control Board Water Quality Monitoring Report No. 86-3WQ.

10.5 EPA. 1995. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1: Fish Sampling and Analysis. EPA 823-R-95-007.

Appendix III D: MPSL-103 Analysis of Mercury in Sediments and Tissue by Flow Injection Mercury System (FIMS)

Analysis of Mercury in Sediments and Tissue by Flow Injection Mercury System (FIMS)
MPSL-103
(Formerly known as DFG SOP-103)

Department of Fish and Game
Marine Pollution Studies Group
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Moss Landing, CA 95039

Adapted from FGS 069.1 by: Mark Stephenson
February 21, 2000

1.0 SCOPE AND APPLICATION

1.1. This SOP is designed to ensure that reproducible, traceable procedures are followed in the standardization of Perkin Elmer FIMS mercury analyzer, and to establish the bounds wherein data will be considered acceptable. This SOP consists of two aspects: (1) preparation of mercury standard solutions; (2) calibration sequence of the mercury analyzer.

2.0 SUMMARY OF METHOD

Sediment and tissue are analyzed for mercury by: (1) digesting using Frontier Geoscience's methods for sediment (FGS-066) and tissue (FGS-011.2); and (2) analyzing the solutions by flow injection using the Perkin Elmer FIMS system.

3.0 INTERFERENCES There have been some reports of interferences by chlorine gas in the digestate.

4.0 SAFETY

4.1. **CAUTION**: The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined. However, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. Exhibit particular caution in the preparation and use of bromine monochloride, as it releases extremely irritating, corrosive fumes similar in effect to free chlorine. Always handle this reagent in an approved fume hood.

5.0 EQUIPMENT

Perkin Elmer FIMS system with autosampler

6.0 REAGENTS

6.1. Reagent Water- Reagent water (18MΩ minimum) is ultra pure deionized water starting from a pre-purified source. MilliQ water is the reagent grade water that is used for the preparation of all total mercury standards and reagents. MilliQ water must be checked weekly for total mercury concentrations. The total mercury concentration in MilliQ water must be < 0.20 ng/L.

6.2. Rinsing Water- MilliQ water is used for rinsing of analytical equipment (i.e. bubblers, frits). MilliQ water must be checked weekly for total mercury concentration. The total mercury concentration in MilliQ water must be < 1.00 ng/L.

6.3. Hydrochloric Acid- Hydrochloric acid (Baker reagent grade HCl) and must be pre-analyzed for total mercury concentration prior to use. Total mercury concentration in HCl must be < 5.00 ng/L.

6.4. Stannous Chloride (1.1%) Baker Reagent Grade

7.0 PROCEDURES

7.1. Preparation of Mercury Standard Solutions

7.1.1. Working standards are prepared by diluting 1000 ppm mercury (II) oxide (VWR, 0.1% Hg^{o} in dilute nitric acid (w:v)) with blank water (0.5% HCl in MQ, (v:v)) to final concentrations from 150 ppt to 5 ppb by weight. Method blanks are drawn from the same batch of blank water used in the preparation of working standards. PACS (2) and NBS 1944 are used as the Standard Reference Material (SRM) for sediments, and DORM (2) is the SRM used for tissues. SRM's are digested and prepared for analysis identically to samples. Aliquots are drawn from digested mercury samples and diluted with blank water until sample concentrations fall within working standard end member concentrations.

7.2. Mercury Analyzer Calibration Sequence

7.2.1. The following sequence will be used for all projects, except in cases where project specific additional requirements are stated. The sequence starts with a 5 point standard calibration curve which must cover greater than the entire range expected from the samples that are to be analyzed that day. If a sample is higher than the largest standard run, a higher standard or a smaller sample aliquot must be run.

7.2.2. Following the standard calibration curve, an initial calibration verification (ICV), and an initial calibration blank (ICB) are run followed by a minimum of 3 prep

blanks. A standard reference material (SRM) of the same matrix and digested in the same manner as the samples to be analyzed should follow the prep blanks. In the event that an SRM does not exist, a blank spike is prepared instead.

7.2.3. A maximum of 10 samples, including the above preparation blanks and SRM/blank spike, are run between ICV/ICB and the continuing calibration verification (CCV)/continuing calibration blank (CCB) pair. There may not be more than 10 samples between any CCV/CCB sets. In each batch of 20-25 samples, various matrix QC may be performed: Matrix Duplicate (MD); Matrix Spike (MS) and a matrix spike duplicate (MSD). The analysis day should end with a CCV/CCB.

7.3. ANALYZING SAMPLES

7.3.1. When running either water or solid samples, it is imperative to check the project sheets. Within these sheets the analyst will find a summary of all the information to run the samples properly. Determine if the samples are to be analyzed in a "High QA" format, the QC required by the project, as well as gathering any information about spiking levels and suggested aliquot size. Be aware that all samples considered to be High QA need to be run prior to any Standard QA samples that are to be analyzed on the same analytical day. Also, it is important for the analyst to note on the project sheets the date that the samples are analyzed and if any reruns are required. If at all possible, analyze the samples in the order that they appear on the COC's for water samples, or in the order that they were digested. The first samples analyzed should always be the blanks then the SRM, followed by actual samples. After every 10 samples including the Blanks and SRM, perform a CCV/CCB pair, and close out the run with a CCV/CCB.

Mercury Samples are analyzed by Atomic Spectroscopy using a Perkin Elmer Flow Injection Mercury System (FIMS-100) with the software application AA WinLab. A peristaltic pump set to 85 mL/min is used to transport various liquids through the system. The peristaltic pump, in conjunction with an autosampler (Perkin Elmer AS-90) draws a 4 mL aliquot of the sample solution into the mixing block. The reducing reagent (1.1 % Tin (II) chloride in 3 % HCl (v:v)) is pumped simultaneously mixing with the sample and a spontaneous reaction takes place, reducing the ionic mercury to metallic mercury. The carrier gas (liquid argon) then carries the mercury vapor to the gas/liquid separator at a flow rate of ~50 mL/min. The liquid is pumped to waste, the gas phase continues on to the FIMS-cell, which is the radiation beam of the spectrometer. The radiation source is a low pressure mercury lamp. The detector is a photocell with maximum sensitivity at 254 nm. The FIMS-cell has an inner diameter of 4 mm and an optical pathlength of 260mm. The cell is heated to 50 °C.

8.0 QUALITY ASSURANCE

8.1. The acceptable recoveries must be met in order to consider a data set valid. All data points noted on the analysis day's spreadsheet as invalid for know reasons may be discarded, if rerun during the same analysis day. In the event that the system becomes out of control during the analysis day, all results between valid QC data points shall still be considered valid.

Condition	Corrective Action
r for the calibration curve is less than 0.995	3-5, 10-13
Instrument blank is 10% greater than the IDL	3-5, 10-13
Continuing Calibration Check value differs by more than 20% from the most recent calibration.	1,3-6,10-13
QC Check Sample differs by > 30% form its expected value	1-6,10-13
The current method blank is greater than the MDL	2-6,10-13
The Percent Recovery of the current Spiked Method Blank falls outside the PQL control limits	2-6,10-13
The Relative Percent Difference of the current sample duplicate pair exceeds 30%	2-6,10-13
Percent Recovery for either of the current Matrix Spike (MS) and MS Duplicate (MSD) falls outside ±30% of the mean.	2-6,10-13
The sample matrix interfered with the ability to make an accurate determination	report with Data Qualifier "J"
The value determined is less than the MDL	= MDL with Data Qualifier "U"
The value determined is less than the PQL, but is greater than the MDL.	= PQL with Data Qualifier "I"

9.0 CORRECTIVE ACTION

Key to Corrective Actions in Table 8.1:

1. Re-analyze the samples of the analytical set
2. Re-process (re-extract or digest) the samples of the analytical set
3. Perform corrective maintenance
4. Re-calibrate instrument
5. Prepare fresh standards and re calibrate
6. Repeat the analysis of those samples analyzed since last acceptable check of this kind
7. Check calculations
8. Re-evaluate system
9. Qualify reported results
10. Determine cause of contamination/failure
11. Check water blank source

12. Clean Instrument
13. Check reagent sources

9.1. If insufficient amounts remain to repeat analysis for samples analyzed after the last acceptable CCV, use best professional judgment to estimate values. Bracket those samples from previous acceptance QC check, report the results with the Data Qualifier "J", and provide a narrative explanation.

10.0 EQUATIONS

10.1. Mercury in sediment and tissue

Concentration=(1/slope)*(peak height sample-peak height blank)*DF

% Recovery SRMs = (Observed concentration*100)/ certified concentration

Spike % recovery=
(ng Hg in spiked sample-ng Hg in unspiked sample)*100/ng Hg added

Equation assumes that sample weights of unspiked and spiked are equal.
Care should be taken to equalize these weights when aliquoting.
If the weights are unequal then the dry weight in the unspiked sample is used to calculate the ng in the sample portion of the spiked sample by the equation:
(conc. Hg unspiked*sample weight of spiked sample)=ng in unspiked sample

RPD duplicates = absolute value of$((X_1-X_2)/((X_2+X_1)/2))*100$

Appendix III E: MPSL-104 Sample Receipt and Check-In

Method # MPSL-104

SAMPLE RECEIPT AND CHECK-IN

1.0 Scope and Application

1.1 This method describes the cataloging and handling of samples as they arrive at the laboratory for processing and analysis

2.0 Summary of Method

2.1 A record of sample transport, receipt and storage is maintained and available for easy reference.

2.2 Each sample is assigned a unique lab identification number. The number is recorded in a logbook as well as on the sample itself.

2.3 Each sample is preserved according to the applicable analytical method and is stored accordingly. The preservation and storage is recorded in the logbook.

3.0 Interferences

3.1 Not Applicable

4.0 Apparatus and Materials

4.1 Bound logbook with numbered pages

4.2 Permanent Pen

4.3 Permanent Marker (i.e. Sharpie)

4.4 Digital Probe thermometer: Fisher Part # 15-077-32

4.5 3-Ring Binder

4.6 Copy Machine

5.0 Reagents

5.1 Not Applicable

6.0 Sample Collection

6.1 Water Samples are collected according to EPA 1669, modified, according to analytical or project specific methods.

6.2 Tissue samples are collected according to Method MPSL-102a, or according to analytical or project specific methods.

6.3 Sediment samples are collected according to Method MPSL-102b, or according to analytical or project specific methods.

7.0 Procedure

7.1 Samples accompanied by a Chain of Custody Record (COC) are delivered to the laboratory from the field crew. Samples may be hand delivered or shipped via FedEx or another overnight shipping service provided the samples maintain the appropriate temperatures during shipment.

7.2 Cooler temperature is measured prior to the removal of any sample. The probe of the digital thermometer is placed amongst the samples. Temperature is allowed to equilibrate prior to recording on the COC and logbook. It is noted when samples were delivered by the field crew and placed directly into the refrigerator or freezer, rendering a cooler temperature unobtainable.

7.3 The COC is reviewed for preservation and requested handling of the samples.

7.4 A new page in the log book is used for each COC. Entries MUST include the following:

7.4.1 Date of entry.

7.4.2 Project Name and Number

7.4.3 Unique 9-digit Lab Number

7.4.3.1 The first four digits are the year in which the sample was received.

7.4.3.2 The second four digits are sequential numbers beginning with 0001. Each successive sample receives the next number.

7.4.3.3 A single letter is appended to each Lab Number to indicate the matrix type (-w = water, -s = sediment, -t = tissue, -c = chlorophyll a).

7.4.4 Date and time (if provided) of sample collection. Time shall be recorded using a 24-hour clock.

7.4.5 Sample Identification; station information taken directly from the COC

7.4.6 Analyte of suite of analytes requested for each sample.

7.4.7 At the end of the entry, the following are recorded:

7.4.7.1 Type- Specify the matrix of the samples. List all that apply.

7.4.7.2 Preservation/Storage- List for each matrix/analyte combination.

7.4.7.3 From- the name of the person last in possession of the samples (signed the COC)

7.4.7.4 Received by- the name of the person at the lab who first received the samples

7.4.7.5 Date and Time of sample receipt as well as cooler temperature upon arrival.

7.4.7.6 Checked by- the name of the person that verified the contents of the cooler with the COC and assigned the lab numbers.

7.4.7.7 Any comments pertaining to the samples (special instructions, anomalies, etc.).

7.5 Water samples are preserved according to the specific analytical methods (EPA 1630, 1631E and 1638). Preserved samples are given to the analysts along with copies of the COC and log-book entry.

7.6 Tissue, sediment and chlorophyll a samples are stored in a walk-in freezer at -20°C until dissection and/or digestion can occur.

7.7 At least one copy is made of each COC and log book entry. One copy MUST be kept in the COC binder. Other copies may be stored with the samples themselves, or given to the analyst.

7.8 All entries are entered and maintained in a MS Access database.

8.0 Analytical Procedure

8.1 Trace Metal tissue and sediment digestions are performed according to EPA 3052M, modified.

8.2 Mercury Only tissue and sediment digestion procedures can be found in Method # MPSL-106 and Method # MPSL-107, respectively.

8.3 Trace Metals are analyzed with ICP-MS according to EPA 200.8 (tissues and sediments) and EPA 1638, modified (waters).

8.4 Mercury tissue and sediment samples are analyzed by FIMS according to Method # MPSL-103 or by DMA and EPA 7473.

8.5 Mercury water samples are analyzed according to EPA 1631E, modified.

8.6 Methylmercury tissue samples are extracted and analyzed according to SOP-CALFED.D03.

8.7 Methylmercury water samples are analyzed according to EPA 1630, modified.

9.0 Quality Control

9.1 MS Access database does not allow duplicate Lab Numbers

9.2 Each COC, along with a copy of the pertinent portion of the logbook, is retained for reference.

Appendix III E: MPSL-105 Laboratory Preparation of Trace Metal and Synthetic Organic Samples of Tissues in Marine and Freshwater Bivalves and Fish

Method # MPSL-105

LABORATORY PREPARATION OF TRACE METAL AND SYNTHETIC ORGANIC SAMPLES OF TISSUES IN MARINE AND FRESHWATER BIVALVES AND FISH

1.0 Scope and Application

1.1 The following procedures describe techniques for the laboratory preparation of marine and freshwater tissues for trace metal (TM) and synthetic organic (SO) analysis.

2.0 Summary of Method

2.1 Laboratory processing is carried out under "clean room" conditions, with a positive pressure filtered air supply, non-contaminating laboratory surfaces, and a supply of deionized (DI) and Type II water (MilliQ).

2.2 All tools that come in contact with the sample are washed with Micro and water, rinsed with tap water and then DI. It is important to use tap water because DI alone will not remove Micro detergent.

2.3 Dissection information (initial jar weight, total weight, and tissue weight) is recorded in individual log books as well as project specific dissection sheets. Other information specific to each type of dissection is also recorded.

2.4 Personnel MUST wear polyethylene gloves at all times when handling samples and prepared dissection equipment.

2.5 All samples are dissected and placed in prepared containers appropriate for the analyses requested.

2.6 Any anomalies (parasites, injuries, etc) are recorded in all cases.

2.7 Dissected samples are homogenized to obtain a uniform sample. Aliquots of homogenate are distributed according to analyte and are acid-digested or solvent-extracted.

3.0 Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines, causing inaccurate analytical results. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot.

3.2 Polypropylene and polyethylene surfaces are a potential source of contamination for SO specimens and should not be used whenever possible.

3.3 TO MINIMIZE CONTAMINATION, ALL SAMPLES ARE PROCESSED UNDER "CLEAN ROOM" CONDITIONS. Criteria enumerated in Flegal (1982) are recommended. Shoe covers and lab coats are worn in the laboratory to minimize transport of contaminants into the laboratory. The trace metal laboratory has no metallic surfaces, with bench tops, sinks and fume hoods constructed of acid resistant plastic to avoid metal contamination. A filtered air supply (class 100) which provides a positive pressure clean air environment is an important feature for reducing contamination from particulates.

4.0 Apparatus and Materials

Procedures for equipment preparation can be found in Method # MPSL-101.

4.1 Brinkmann Polytron model PT 10-35

4.2 Büchi Mixer B-400

4.3 Disposable Scalpel, #10: Fisher Scientific Part # 08-927-5A

4.4 Ear Protection

4.5 Fillet knives

4.6 Glass Jar Class 100, 500 mL, prepared

4.7 Glass Jar Class 200, 500 mL, prepared

4.8 Glass Jar Class 300, 500 mL, prepared

4.9 Glass Jar Class 100, 125 mL, prepared

4.10 Glass Jar Class 200, 125 mL, prepared

4.11 Glass Jar Class 300, 125 mL, prepared

4.12 Glass Jar Class 200, 60 mL: I-Chem Part # 220-0060

4.13 Glass Jar Class 300, 60 mL: I-Chem Part # 320-0060

4.14 Heavy Duty Beakers, 1000 mL

4.15 Heavy Duty Beakers, 400 mL

4.16 Garbage Bags, Clear 30 gallon

4.17 Lab Coats

4.18 Plastic Knives, prepared

4.19 Polyethylene Gloves: VWR Part # 32915-166, 32915-188, and 32915-202

4.20 Polyethylene (HDPE) jar, 30 mL, prepared

4.21 Polyethylene (HDPE) jar, 125 mL, prepared

4.22 Shoe Covers: Cellucap Franklin Part # 28033

4.23 Teflon Forceps, prepared

4.24 Titanium Bars

4.25 Titanium Generator: Brinkmann Part # PTA 20

5.0 Reagents

5.1 Tap water (Tap)

5.2 Deionized water (DI)

5.3 Type II water (ASTM D1193): Use Type II water, also known as MilliQ, for the preparation of all reagents and as dilution water.

5.4 Micro Detergent: ColeParmer Part # 18100-20

5.5 Methanol: VWR Part # JT9263-3

5.6 Petroleum Ether: VWR Part # JT9265-3

5.7 Hydrochloric Acid (HCl), BAKER ANALYZED, 36.5-38.0%: VWR Part # JT9535-3

5.8 Hydrochloric Acid (HCl), 50%: prepared by adding 1 part Baker HCl to 1 part MilliQ

5.9 Nitric Acid (HNO_3), BAKER INSTRA-ANALYZED'*, 69.0–70.0%: VWR Part # JT9598-34

5.10 Nitric Acid (HNO_3), 50%: prepared by adding 1 part Baker HNO_3 to 1 part MilliQ

6.0 Sample Collection, Preservation and Handling

6.1 Samples should be collected according to Method # MSPL-102a, # MPSL-102b, and EPA 1669, modified.

6.2 All dissection equipment and containers must be prepared according to Method # MPSL-101.

6.3 Tissue dissections should be carried out by or under the supervision of a competent biologist. Each organism should be rinsed free of dirt with deionized water and handled with prepared stainless steel, quartz, or Teflon instruments. Fish or other samples processed as "whole body" must only come in contact with MilliQ water to reduce contamination. The SO specimens should come in contact with prepared glass, aluminum foil or Teflon surfaces only (Method # MPSL-101).

6.4 Samples should be maintained at -20°C and extracted or digested as soon as possible.

7.0 Procedure

7.1 Dissection

7.1.1 Bivalve Dissection

7.1.1.1 For both TM and SO: Frozen mussels are thawed, removed from the bags, and cleaned of epiphytic organisms, byssal threads and debris under running DI. Dissections are conducted on cleaned Teflon cutting boards.

7.1.1.2 The gametogenic condition of each sample is recorded in the logbook and dissection sheet a "ripe", "partial" or "not ripe".

7.1.1.3 For both TM and SO: The first 15 shell lengths are recorded. Lengths are measured across the longest part of each shell.

7.1.1.4 TM Bivalve Dissection

7.1.1.4.1 Forty-five mussels are dissected per sample. These are divided into 3 groups of 15. Each group of 15 creates A, B, and C replicates. If there are fewer than 45 mussels the mussels are divided into three equal samples. The total number of mussels in each jar is recorded.

7.1.1.4.2 The adductor muscle is severed with a scalpel and the shell is pried open with the plastic end of the scalpel. The gonads are then excised. The weight of the gonads from the first 15 mussels is recorded. These and all subsequent gonads can then be thrown away.

Note: Gonads are not removed from clams.

7.1.1.4.3 The remainder of the soft part is removed from shell and placed in a pre-weighed, prepared polypropylene 125mL jar. The final sample weight for each jar is recorded. All jars must be properly labeled on both the lid and the jar itself.

7.1.1.5 SO Bivalve Dissection

7.1.1.5.1 The adductor muscle is severed and the shell is pried open with clean titanium blade. The entire body, including gonads, is placed in a pre-weighed, prepared glass jar. All forty-five individuals are placed in the same jar. All jars must be properly labeled on both the lid and the jar itself.

7.1.1.6 "Split" Bivalve Dissection

7.1.1.6.1 Samples are dissected as TM samples with the following exceptions:

7.1.1.6.1.1 All gonads from each sample of 45 mussels are excised and retained in prepared 125mL glass jar. The combined weight of all 45 gonads is recorded.

7.1.1.6.1.2 The remainder of the tissue from each of the 3 replicates is dissected into prepared 125mL glass jars.

7.1.2 Fish Dissection

7.1.2.1 Large fish requiring dissection are partially thawed, then washed with DI water. It may be necessary to rub more vigorously in order to remove mucous. Place the rinsed fish in a clean, Teflon lined bin.

7.1.2.2 Total fish length and fork length are measured to the nearest millimeter. The body is then placed on a clean Teflon sheet on the balance and weighed. All lengths and weights are recorded.

7.1.2.3 Scaly fish (Large Mouth Bass, Perch, etc.) are de-scaled from the tail to the operculum above the lateral line with the titanium rod, and are dissected "skin-on". The skin is removed from scale-less fish in the same section as above, and the fish are dissected "skin-off". (EPA Guidelines) If the contract requires aging, 10 scales are taken from the appropriate region of the fish and placed in labeled coin envelopes for later age determination.

7.1.2.4 Fish are filleted to expose the flesh. It is important to maintain the cleanliness of the tissue for analysis, therefore any "skin-off" flesh that has been in direct contact with the skin or with instruments in contact with skin must be eliminated from the sample.

Trim the edges of the fillet with a clean scalpel or fillet knife to remove this contaminated tissue.

7.1.2.5 Fillets are cut into small pieces, less than 1 square inch for homogenization purposes.

7.1.2.6 Record the individual fillet weight. For composite samples, equal fillet weights are taken from each individual.

7.1.2.7 As much flesh as possible should be removed for each sample to meet the requirements for each analysis as well as have tissue retained for archive. Generally, 150-200g total sample weight is ideal.

7.1.2.8 If possible, the sex of each individual is determined and recorded.

7.1.2.9 If the contract requires liver analysis, the livers are removed from the predator species by opening the body cavity with the incision scalpel. The liver is freed by cutting with a fresh dissection scalpel and removed with a clean forceps. The livers are rinsed with MilliQ and placed in a prepared, pre-weighed sample jar. Individual liver weights recorded.

7.1.2.10 At this time vertebrae may be taken from ictalurids for aging. The first unfused vertebra is removed and placed in a 25mL beaker, covered with water and placed in the refrigerator until the flesh has broken down enough to be cleaned away. The vertebrae are placed in a coin envelope and may later be used for age determination.

7.1.2.11 Sections of fish, rather than whole body, may be delivered from the sampling crew. The lengths and weight will have already been recorded by the collection team. Tissue is dissected as before, however any exposed flesh must be eliminated from the sample.

7.1.2.12 Whole-bodied fish are thawed under MilliQ. They may be stripped of mucous by using prepared forceps. At no time may the whole body fish touch any unclean surface or instrument.

7.1.2.13 Total length, fork length and weight are recorded.

7.1.2.14 The body is cut into pieces smaller than 1 square inch for homogenization. It may be necessary to use a prepared bone saw to cut through larger vertebrae.

7.1.2.15 All samples are refrozen after dissection and maintained at -20°C until homogenization and/or analysis. It may be possible to homogenize fish samples immediately after dissection, but is not necessary.

7.2 Homogenization

7.2.1 TM Bivalve Homogenization

7.2.1.1 Samples are homogenized in the original sample jar using the Polytron and Titanium Generator.

Note: Ear Protection should be worn when operating any homogenizer.

7.2.1.2 Clean the generator by running it in a dilute Micro/Tap Solution. Rinse by running the generator in a 2 separate Tap baths, followed by 3 DI baths and 1 MQ bath. Allow to dry. Extra rinses may be necessary if tissue can be seen in any of the baths. If tissue is found in the DI or MQ baths, begin again with Tap water.

7.2.1.3 The tissue is homogenized to a paste-like consistency. No chunks of clearly defined tissue should be left in homogenate.

Note: operate the Polytron at the lowest speed possible to avoid heating the sample or splattering tissue.

7.2.1.4 The generator is cleaned with new solution baths between reps as well as between stations.

7.2.1.5 Samples must be refrozen at -20°C until acid-digestion can take place.

7.2.2 SO Bivalve Homogenization

7.2.2.1 Samples are homogenized in the original sample jar using the Polytron and either Stainless Steel or Titanium Generator.

Note: Ear Protection should be worn when operating any homogenizer.

7.2.2.2 Clean the generator by running it in 3 separate DI baths and 1 MQ bath, followed by 3 wash bottle rinses each with Methanol and Petroleum Ether. Extra rinses may be necessary if tissue can be seen in any of the baths. If tissue is found in the MQ bath, begin again with DI water.

7.2.2.3 The tissue is homogenized to a paste-like consistency. No chunks of clearly defined tissue should be left in homogenate.

Note: operate the Polytron at the lowest speed possible to avoid heating the sample or splattering tissue.

7.2.2.4 The generator is cleaned with new solution baths between stations.

7.2.2.5 Samples must be refrozen at -20°C until transfer to analytical lab and solvent extraction can occur.

7.2.3 "Split" Bivalve (TM and SO) Homogenization

7.2.3.1 Samples are homogenized as TM with the following exceptions:

7.2.3.1.1 The TM cleaned titanium generator is washed 3 times with 6% HNO_3 prior to the 3 MQ rinses, and is further rinsed 3 times each with Methanol and Petroleum Ether.

7.2.3.1.2 The retained gonads are homogenized in addition to the 3 replicates.

7.2.3.2 Homogenized samples are aliquoted for SO, ensuring enough tissue remains for TM analysis. Equal portions of body tissue are taken from each of the 3 replicates. The ratio of gonad:body weight is calculated for the entire sample, and the ratio is applied to the SO aliquot body weight to determine the amount of gonad material to add back in. Once all tissue is present in the SO sample, it is homogenized by hand with a prepared titanium rod.

7.2.4 Fish

7.2.4.1 Fish samples are removed from the freezer and are allowed to thaw long enough to be transferred to split-clean Büchi sample jar.

7.2.4.2 Prior to and after homogenization the blades and drive shaft of the Buchi are scrubbed with Micro, and rinsed 3 times each in tap and DI.

7.2.4.3 To TM clean the titanium blades, rinse 3 times in MilliQ.

7.2.4.4 To SO clean the steel blades, rinse 3 times in MilliQ, followed by 3 rinses each in methanol and PE. Air dry.

7.2.4.5 To split clean titanium blades, rinse 3 times in 6% HNO_3, followed by 3 rinses in MilliQ. Follow up with 3 rinses each in methanol and PE. Air dry.

7.2.4.6 Assemble the homogenizer according to manufacturer specifications.

7.2.4.7 Place sample jar on tray; close and lock the homogenizer door.

7.2.4.8 Raise the sample jar into position with the on/off toggle. When the jar reaches the appropriate height, the blades will begin rotation and come in contact with the sample.

7.2.4.9 It is important to PULSE the cutting unit in the sample by briefly releasing the toggle. This allows the entire sample to be homogenized, and not get pushed against the sides of the container, as well as keeping the friction to a minimum. It is imperative the sample not get hot.

7.2.4.10 Once the sample has fully homogenized, it may be aliquoted with a prepared titanium rod into the appropriate prepared sample containers for each analysis.

7.2.4.11 Samples are frozen at -20°C until acid-digestion or transfer to analytical lab and solvent extraction can occur.

8.0 Analytical Procedure

8.1 Trace Metal and Mercury Only digestion procedures can be found in EPA 3052, modified, and Method # MPSL-106, respectively.

8.2 Trace Metals are analyzed with ICP-MS according to EPA 200.8.

8.3 Mercury samples are analyzed by FIMS according to Method # MPSL-103 or by DMA and EPA 7473.

8.4 Methylmercury tissue samples are extracted and analyzed according to Method # MPSL-109.

9.0 Quality Control

9.1 Sample Archive: All remaining sample homogenates and extracts can be archived at -20°C for future analysis.

9.2 A record of sample transport, receipt and storage is maintained and available for easy reference.

9.3 All samples are prepared in a clean room to avoid airborne contamination.

10.0 Method Performance

10.1 See individual analytical methods.

11.0 References

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