

Group A Elements: Project Management

Element 1. Title and Approval Sheets

QUALITY ASSURANCE PROJECT PLAN

**INCORPORATING WILDLIFE
METHYLMERCURY EXPOSURE AND RISK
ESTIMATES USING BIOMAGNIFICATION
FACTORS INTO CALIFORNIA LAKE
MONITORING**

The Bioaccumulation Oversight Group (BOG)

Surface Water Ambient Monitoring Program

April 2012

Program Title	SWAMP Bioaccumulation Oversight Group Wildlife BMF Study	
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Effective Date	This Quality Assurance Project Plan (QAPP) is effective from April 2012 to May 2013 unless otherwise revised, approved and distributed accordingly at an earlier date.	
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QAPP Preface

This Quality Assurance Project Plan (QAPP) document defines procedures and criteria that will be used for this project conducted by SWAMP Bioaccumulation Oversight Group (BOG) in association with the US Geological Survey (USGS), California Department of Fish and Game Marine Pollution Studies Laboratory (MPSL-DFG), and the San Francisco Estuary Institute (SFEI). Included are criteria for data quality acceptability, procedures for sampling, testing (including deviations) and calibration, as well as preventative and corrective measures. The responsibilities of USGS, MPSL-DFG, and SFEI also are contained within. The BOG selects the sampling sites, the types and size of tissue samples, and the number of analyses to be conducted.

This work is funded through the Surface Water Ambient Monitoring Program (SWAMP) fiscal year 11/12 Bioaccumulation funding.

Approvals

The approvals below were submitted separately, preventing their inclusion in this signature block. Instead, they appear in Appendix VI of this document. Originals are kept on file by Autumn Bonnema of MPSL-DFG.

Rusty Fairey
Contract Manager

_____ Date _____

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Principal Investigator

_____ Date _____

Collin Eagles-Smith
Co-Principal Investigator

_____ Date _____

Jay Davis
Lead Scientist

_____ Date _____

Beverly van Buuren
SWAMP Quality Assurance Officer

_____ Date _____

Autumn Bonnema
Project Coordinator/ MPSL-DFG Quality Assurance Officer

_____ Date _____

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Element 3. Distribution List and Contact Information

A copy of this Quality Assurance Project Plan (QAPP), in hardcopy or electronic format, is to be received and retained by at least one person from each participating entity. At least one person from each participating entity (names shown with asterisk*) shall be responsible for receiving, retaining and distributing the QAPP to their respective staff within their own organization. Contact information for the primary contact person (listed first) for each participating organization also is provided below in Table 1.

Table 1. Contact Information

Name	Agency, Company or Organization
<u>SAN FRANCISCO ESTUARY INSTITUTE</u>	
Jay Davis*	SFEI 7770 Pardee Lane Oakland, CA 94621-1424 Phone: (415) 746-7368 Email: jay@sfei.org
<u>UNITED STATES GEOLOGICAL SURVEY, Forest and Rangeland Ecosystem Science Center</u>	
Collin Eagles-Smith	USGS
Branden Johnson*	3200 SW Jefferson Way Corvallis, OR Phone: (541) 750-0949 Email: ceagles-smith@usgs.gov
<u>UNITED STATES GEOLOGICAL SURVEY, Western Ecological Research Center</u>	
Josh Ackerman	USGS
Alex Hartman	1 Shields Avenue, UC Davis
Robin Keister*	Davis, CA 95616 Phone: (530) 752-0485 Email: jackermad@usgs.gov
<u>MARINE POLLUTION STUDIES LAB</u>	
<u>CALIFORNIA DEPARTMENT OF FISH AND GAME</u>	
Autumn Bonnema*	MPSL-DFG
Dylan Service	7544 Sandholdt Road Moss Landing, CA 95039 Phone: (831) 771-4177 Email: mstephenson@mlml.calstate.edu
<u>MOSS LANDING MARINE LABORATORIES</u>	
<u>QUALITY ASSURANCE RESEARCH GROUP</u>	
Beverly van Buuren*	QA Research Group, MLML
Eric von der Geest	c/o: 4320 Baker Ave. NW Seattle, WA 98107 Phone: (206) 297-1378 Email: bvanbuuren@mlml.calstate.edu

* Indicates person responsible for receiving, retaining, and distributing the final QAPP to staff within their organization

Element 4. Project Organization

The lines of communication between the participating entities, project organization and responsibilities are outlined in Table 2 and Figure 1.

Table 2. Positions and duties

Position	Name	Responsibilities
Contract Manager	Rusty Fairey MPSL-MLML	Approve reports and invoices for payment.
Principal Investigator	Josh Ackerman USGS WERC	
Co-Principal Investigator	Collin Eagles-Smith USGS FRESC Tom Maurer USFWS	
Project Manager	Mark Stephenson MPSL-DFG	Project management and oversight.
Lead Scientist	Jay Davis SFEI	Advisory Roll; Data reporting
Project Coordinator	Autumn Bonnema, MPSL-DFG	Generation of a QAPP, Project coordination; ensures all laboratory activities are completed within proper timeframes.
Program QA Officer	Beverly van Buuren QA Research Group, MLML	Approve QAPP and oversee SWAMP projects' QA/QC
Laboratory QA Officer	Mark Herzog USGS WERC Branden Johnson USGS FRESC Autumn Bonnema, MPSL-DFG	Ensures that the laboratory quality assurance plan and quality assurance project plan criteria are met through routine monitoring and auditing of the systems. Ensure that data meets project's objective through verification of results.
Sample Collection Coordinator	Josh Ackerman USGS WERC Collin Eagles-Smith USGS FRESC Billy Jakl MPSL-DFG	Sampling coordination, operations, and implementing field-sampling procedures.
Laboratory Director	Wes Heim MPSL-DFG	Organizing, coordinating, planning and designing research projects and supervising laboratory staff; Data validation, management and reporting
Sample Custodian	Tully Rohrer USGS Stephen Martenuk MPSL-DFG additional staff	Sample storage. Not responsible for any deliverables.
Technicians	Technical staff USGS MPSL-DFG	Conduct tissue dissection, digestion, and chemical analyses. Responsible for chemistry data submission

4.1. Involved parties and roles

Rusty Fairey of Marine Pollution Studies Lab - Moss Landing Marine Laboratories (MPSL-MLML) will be the Contract Manager (CM) for this project. The CM will approve reports and invoices for payment.

Josh Ackerman of USGS WERC will serve as the Principal Investigator (PI) for this project. The PI

Collin Eagles-Smith (USGS FRESC) and Tom Maurer (USFWS) will serve as Co-Principal Investigators (CPI) for the project.

Mark Stephenson of MPSL-DFG will serve as the Project Manager (PM) for the project. The PM will 1) review and approve the QAPP, 2) review, evaluate and document project reports, and 3) verify the completeness of all tasks.

Jay Davis of San Francisco Estuary Institute (SFEI) is the Lead Scientist (LS) and primary contact of this project. The LS will 1) generate the Sampling and Analysis Plan (SAP), 2) approve the QAPP, and 3) provide the BOG with a final report on completion of this project.

Autumn Bonnema of MPSL-DFG is the Project Coordinator (PC). The PC will 1) prepare the QAPP, 2) ensure that laboratory technicians have processing instructions and 3) ensure all laboratory activities are completed within the proper timelines. In addition, the PC may assist field crew in preparation and logistics.

Dylan Service of MPSL-DFG is in charge of directing fish collection for this project. He will 1) oversee preparation for sampling, including vehicle maintenance and 2) oversee sample and field data collection.

Collin Eagles-Smith and Josh Ackerman are in charge of directing grebe tissue collections for this project. They will 1) oversee preparation for sampling, including vehicle maintenance and 2) oversee sample and field data collection.

Stephen Martenuk is responsible for sample storage and custody at MPSL. His duties will be to oversee compositing of tissue samples. Tully Rohrer will do the same for samples processed at USGS.

Collin Eagles-Smith and Josh Ackerman will serve as the Laboratory Directors (LD) for the USGS component of this project. Their specific duties will be to 1) review and approve the QAPP, 2) provide oversight for mercury analyses on grebe tissues to be done for this project, and 3) ensure that all USGS activities are completed within the proper timelines.

Wes Heim will also serve as the Laboratory Director (LD) for the MPSL-DFG component of this project. His specific duties will be to 1) review and approve the QAPP, 2) provide oversight

for mercury analyses on fish tissues to be done for this project, and 3) ensure that all MPSL-DFG activities are completed within the proper timelines.

The following serve in an advisory role and are not responsible for any deliverables: Terry Fleming (EPA), Bob Brodberg (Office of Environmental Health Hazard Assessment (OEHHA)), Karen Taberski (RWQCB2), Mary Hamilton (RWQCB3), Michael Lyons (RWQCB4), Chris Foe (RWQCB5), Cassandra Lamerdin (MPSL-MLML), Jennifer Salisbury (State Water Resources Control Board (SWRCB)), Gary Ichikawa (Department of Fish and Game), Dylan Service (MPSL-DFG), Alex Hartman (USGS) and Jennifer Hunt (SFEI).

4.2. Quality Assurance Officer (QAO) Role

The Laboratory Quality Assurance Officers fulfill the functions and authority of a project quality assurance officer (QAO). Autumn Bonnema is the MPSL-DFG QAO, Mark Herzog is the USGS WERC QAO, and Branden Johnson is the USGS FRESC QAO. The role of the Laboratory QAO is to ensure that quality control for sample processing and data analysis procedures described in this QAPP are maintained throughout the project. The Program QAO (Beverly van Buuren, MLML) acts in a consulting role to the Laboratory QAOs and ensures the project meets all SWAMP QA/QC criteria (QAPrP, 2008).

The Laboratory QAOs will review and assess all procedures during the life of this project against QAPP requirements, and assess whether the procedures are performed according to protocol. The Laboratory QAOs will report all findings (including qualified data) to the Program QAO and the PM, including all requests for corrective action. The Laboratory and Program QAOs have the authority to stop all actions if there are significant deviations from required procedures or evidence of a systematic failure.

A conflict of interest does not exist between the Laboratory QAOs and the work outlined in this QAPP as neither Laboratory QAO participates in any of the chemical analyses of the project. There is not a conflict of interest with one person fulfilling the roles of Laboratory QAO and Project Coordinator (PC), as laboratory decisions are not made by the PC and no other duties overlap. The role of the PC is detailed above.

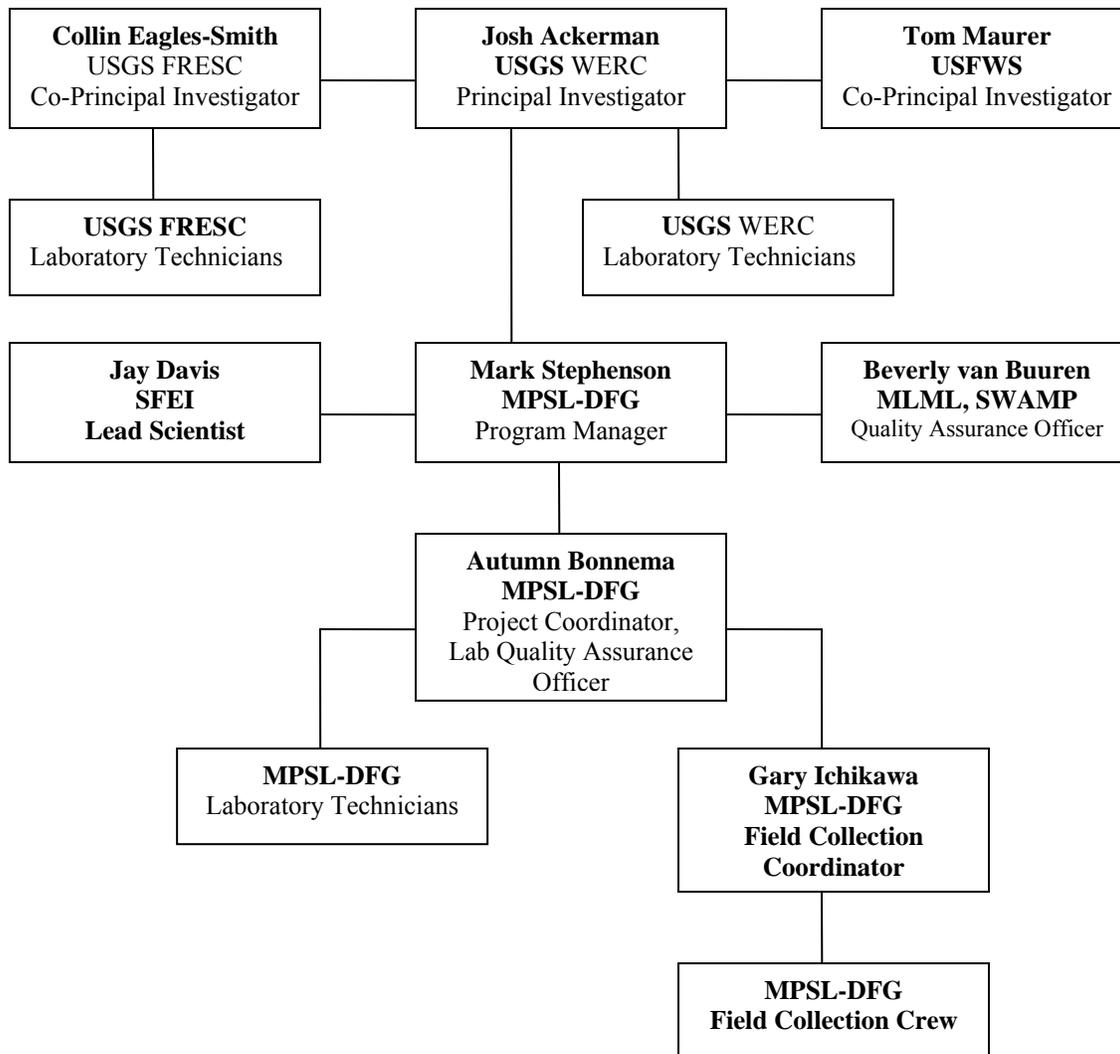
4.3. Persons responsible for QAPP update and maintenance

Revisions and updates to this QAPP will be carried out by Autumn Bonnema (PC), with technical input of the PM and the Laboratory and Program QAOs. All changes will be considered draft until reviewed and approved by the PM and the SWAMP QAO. Finalized revisions will be submitted for approval to the SWAMP QAO, if necessary.

Copies of this QAPP will be distributed to all parties involved in the project. Any future amended QAPPs will be held and distributed in the same fashion. All originals of these first and subsequent amended QAPPs will be held on site at SFEI, USGS and MPSL-DFG.

4.4. Organizational chart and responsibilities

Figure 1. Organizational Chart



Element 5. Problem Definition/Background

5.1. Problem statement

5.1.1. Addressing Multiple Beneficial Uses

Bioaccumulation in California water bodies has an adverse impact on both the fishing and aquatic life beneficial uses (Davis et al. 2007). The fishing beneficial use is affected by human exposure to bioaccumulative contaminants through consumption of sport fish. The aquatic life beneficial use is affected by exposure of wildlife to bioaccumulative contaminants, primarily piscivorous species exposed through consumption of small fish. Different indicators are used to monitor these different types of exposure. Monitoring of status and trends in human exposure is accomplished through sampling and analyzing sport fish. On the other hand, monitoring of status and trends in wildlife exposure can be accomplished through sampling and analysis of wildlife prey (small fish, other prey species) or tissues of the species of concern (e.g., bird eggs or other tissues of juvenile or adults of the species at risk).

The Surface Water Ambient Monitoring Program (SWAMP) via the Bioaccumulation Oversight Group (BOG) has recently completed state-wide surveys of contaminants in sport fish tissue from over 250 lakes in California and throughout coastal waters. However, this impressive effort only focused on human health issues. Because many fish-eating wildlife such as grebes, terns, cormorants, and mergansers eat fish smaller than those that were sampled by BOG, and since fish mercury concentrations are not always indicative of wildlife exposure to mercury, the current BOG surveys do not address whether wildlife beneficial uses may be impaired by mercury in these water bodies.

5.1.2. Addressing Multiple Monitoring Objectives and Assessment Questions for Aquatic Life Beneficial Uses

The BOG has developed a set of monitoring objectives and assessment questions for a statewide program evaluating the impacts of bioaccumulation on the fishing beneficial use. This assessment framework is consistent with frameworks developed for other components of SWAMP, and is intended to guide the bioaccumulation monitoring program over the long-term. The four objectives can be summarized as 1) status; 2) trends; 3) sources and pathways; and 4) effectiveness of management actions.

Over the long-term, the primary emphasis of the statewide bioaccumulation monitoring program will be on evaluating status and trends. Bioaccumulation monitoring is a very effective and essential tool for evaluating status, and is most cost-effective tool for evaluating trends for many contaminants. Monitoring status and trends in bioaccumulation will provide some information on sources and pathways and effectiveness of management actions at a broader geographic scale. However, other types of monitoring (i.e., water and sediment monitoring) and other programs (regional TMDL programs) are also needed for addressing sources and pathways and effectiveness of management actions.

In the near-term, the primary emphasis of the statewide bioaccumulation monitoring program will be on evaluating Objective 1 (status). The reasons for this are:

1. a systematic statewide assessment of status has never been performed and is urgently needed;
2. we are starting a new program and establishing a foundation for future assessments of trends;
3. past monitoring of sport fish established very few time series that are useful in trend analysis that this program could have built upon.

5.1.3. Addressing Multiple Habitat Types

SWAMP has defined the following categories of water bodies:

- lakes and reservoirs;
- bays and estuaries;
- coastal waters;
- large rivers;
- wadeable streams; and
- wetlands.

Due to their vast number, high fishing pressure, and a relative lack of information on bioaccumulation (Davis et al. 2007), lakes and reservoirs were identified as the first priority for sport fish monitoring. Coastal waters, including bays and estuaries, were selected as the next priority, due to their importance for sport fishing and a relative lack of past monitoring. Rivers and streams were the last in the series of water body types to be covered with a statewide screening study. Wetlands were not covered due to the low fishing pressure in those habitats.

Following the sequence established for the fishing beneficial use, assessment of the impact of bioaccumulation on aquatic life beneficial uses is also beginning with a focus on lakes and reservoirs. Methylmercury exposure and risk was identified as the greatest concern in this habitat type, and reproduction in piscivorous birds as the taxa and lifestage at greatest risk. The logistics of performing surveys of exposure and risk in wildlife, require much greater effort and time at each water body, and thus do not readily allow for statewide surveys of the same breadth as were performed for sport fish. However, a two-year study covering 24 lakes was considered to be feasible within the scope of available funding and staffing, and is expected to be sufficient to answer some critical general questions with regard to aquatic life beneficial uses. Including other contaminants or habitats is not feasible with existing funding at this time.

Bioaccumulation is likely having negative impacts on aquatic life beneficial uses in all of the habitat types identified by SWAMP, including wetlands, which are among the most important habitats for wildlife. Whether SWAMP will perform surveys in the other habitat types has not yet been determined. The results of this preliminary assessment of methylmercury impacts in lakes and reservoirs will be valuable in informing the decision on the priority of further assessments.

In summary, focusing on one habitat type (lakes and reservoirs), one objective (status), and one category of beneficial use (aquatic life) will allow us to provide reasonable coverage and provide an informative assessment of bioaccumulation in these habitats in a two-year study.

5.2. Decisions or outcomes

In response to information needs articulated by the state and regional Water Boards, three management questions have been articulated to guide the 2012 screening survey of the status of bioaccumulation in wildlife in California Lakes. Questions relating to 303(d) listing (included in the lakes survey) and spatial patterns (included in the coast survey) were not a priority for managers and were not included in this survey.

5.2.1. Management Question 1 (MQ1)

Does methylmercury pose significant risks to aquatic life in a representative sample of California lakes and reservoirs?

Answering this question is critical to determining the degree of impairment of the wildlife beneficial use across the state due to bioaccumulation. This question places emphasis on characterizing the status of the wildlife beneficial use through monitoring of wildlife and prey fish exposure. A systematic statewide survey of wildlife risk to Hg in freshwater lakes has never been performed.

The data needed to answer this question are average Hg concentrations in Western grebe blood and eggs, and prey fish from various lakes throughout California. Western grebes are useful wildlife indicators for this task because they breed widely throughout California, are upper-trophic level fish-eating birds, and are flightless during breeding, making their Hg concentrations reflective of conditions within individual lakes. Monitoring species that accumulate high concentrations of contaminants (“indicator species”) is valuable in answering this question: if concentrations in these species are below thresholds, this is a strong indication that an area has low concentrations.

5.2.2. Management Question 2 (MQ2)

Can a biomagnification factor approach be applied on a statewide basis to estimate risks to birds based on concentrations measured in small fish?

The Surface Water Ambient Monitoring Program (SWAMP) via the Bioaccumulation Oversight Group (BOG) has recently completed state-wide surveys of contaminants in sport fish tissue from over 250 lakes in California and throughout coastal waters. However, this impressive effort only focused on human health issues. Because many fish-eating wildlife such as grebes, terns, cormorants, and mergansers eat fish smaller than those that were sampled by BOG, and since fish mercury concentrations are not always indicative of wildlife exposure to mercury, the current BOG surveys do not address whether wildlife beneficial uses may be impaired by mercury in these water bodies.

When properly derived, biomagnification factors are valuable because they provide managers and regulators with a quantitative tool to estimate mercury concentrations across environmental

matrices, thus enabling them to adequately estimate wildlife exposure without the need for comprehensive sampling at all sites of interest. Biomagnification factors (BMF) are derived for biota from the organism's diet, and are calculated by dividing the chemical concentration in the predator by the chemical concentration in the predator's diet. This biomagnification factor can then be used for translating small fish mercury concentrations to bird mercury concentrations.

5.2.3. Management Question 3 (MQ3)

What are appropriate TMDL monitoring requirements to address methylmercury exposure in wildlife?

Understanding the transferability of biomagnification factors across lakes and species will be important for understanding whether small or large fish provide a useful index of wildlife exposure. The approach used here will test whether a single, broad translator adequately captures wildlife risk, or if region or lake specific coefficients are needed.

5.2.4. Overall Approach

To answer these questions, over two consecutive field seasons in 2012 and 2013, we will sample birds and small fish simultaneously at 24 lakes throughout California during the breeding season when birds are particularly vulnerable to potential mercury-induced reproductive impairment. Specifically, the study will have four main components:

- 1) Sample grebes at 24 California lakes over 2 years to determine mercury levels in a species near the top of the food chain, and compare these data to known effects-thresholds for birds.
- 2) Simultaneously with grebe sampling, collect small fish (<100 mm) at these same 24 lakes over 2 years to determine if mercury concentrations are above current wildlife diet objectives.
- 3) Use these data in Objectives 1 and 2 to calculate a bird biomagnification factor, evaluate the biomagnification factor's usefulness for estimating wildlife exposure, and assess whether the biomagnification factor differs by lake type or geographic region.

Simultaneously with grebe and small fish sampling, collect sport fish at these same 24 lakes over 2 years to assess correlations of mercury concentrations in sport fish, small fish, and birds

5.2.5. Coordination

The BOG is seeking to coordinate with other programs to leverage the funds for this survey and achieve more thorough studies relating to bioaccumulation in California lakes.

One significant collaboration will be with the US Geological Survey (USGS). The USGS will be collecting and analyzing the grebe tissues, as well as writing the report on that portion of the study. Furthermore, they are contributing approximately \$96,000 in In-Kind funds.

5.3. Tissue contamination criteria

Determination of effects-thresholds in wildlife species is complicated by variation in species sensitivity. However, extensive work on common loons in eastern North America suggest that

toxic effects become measurable and reproduction is impaired at blood concentrations of 3 ug/g wet weight or greater (Evers et al. 2007). Those values also relate to egg concentrations of approximately 1.8 ug/g wet weight (Evers et al. 2003).

A small fish criterion is presently in development by the State Board. However, it is not certain that this criterion will be available in time for the reporting of the results of the first year of this study. When the criterion does become available, results from this study will be assessed in comparison to this threshold.

Threshold levels for determining impairment of a body of water based on pollutants in fish tissue are listed in Table 3. Fish Contaminant Goals (FCGs), as described by Klasing and Brodberg (2008), are “estimates of contaminant levels in fish that pose no significant health risk to humans consuming sport fish at a standard consumption rate of one serving per week (or eight ounces [before cooking] per week, or 32 g/day), prior to cooking, over a lifetime and can provide a starting point for OEHHA to assist other agencies that wish to develop fish tissue-based criteria with a goal toward pollution mitigation or elimination. FCGs prevent consumers from being exposed to more than the daily reference dose for non-carcinogens or to a risk level greater than 1×10^{-6} for carcinogens (not more than one additional cancer case in a population of 1,000,000 people consuming fish at the given consumption rate over a lifetime). FCGs are based solely on public health considerations without regard to economic considerations, technical feasibility, or the counterbalancing benefits of fish consumption.” For organic pollutants, FCGs are lower than Advisory Tissue Levels (ATL)s.

ATLs, as described by Klasing and Brodberg (2008), “while still conferring no significant health risk to individuals consuming sport fish in the quantities shown over a lifetime, were developed with the recognition that there are unique health benefits associated with fish consumption and that the advisory process should be expanded beyond a simple risk paradigm in order to best promote the overall health of the fish consumer. ATLs provide numbers of recommended fish servings that correspond to the range of contaminant concentrations found in fish and are used to provide consumption advice to prevent consumers from being exposed to more than the average daily reference dose for non-carcinogens or to a risk level greater than 1×10^{-4} for carcinogens (not more than one additional cancer case in a population of 10,000 people consuming fish at the given consumption rate over a lifetime). ATLs are designed to encourage consumption of fish that can be eaten in quantities likely to provide significant health benefits, while discouraging consumption of fish that, because of contaminant concentrations, should not be eaten or cannot be eaten in amounts recommended for improving overall health (eight ounces total, prior to cooking, per week). ATLs are but one component of a complex process of data evaluation and interpretation used by OEHHA in the assessment and communication of fish consumption risks. The nature of the contaminant data or omega-3 fatty acid concentrations in a given species in a water body, as well as risk communication needs, may alter strict application of ATLs when developing site-specific advisories. For example, OEHHA may recommend that consumers eat fish containing low levels of omega-3 fatty acids less often than Table 3 would suggest based solely on contaminant concentrations. OEHHA uses ATLs as a framework, along with best professional judgment, to provide fish consumption guidance on an ad hoc basis that best combines the needs for health protection and ease of communication for each site.”

Table 3. Sport fish assessment thresholds

Thresholds for concern based on an assessment of human health risk from these pollutants by OEHHA (Klasing and Brodberg, 2008). All values given in ng/g (ppb). The lowest available threshold for each pollutant is in bold font. One serving is defined as 8 ounces (227 g) prior to cooking. The FCG and ATs for mercury are for the most sensitive population (i.e., women aged 18 to 45 years and children aged 1 to 17 years).

Pollutant	Fish Contaminant Goal	Advisory Tissue Level (3 servings/week)	Advisory Tissue Level (2 servings/week)	Advisory Tissue Level (No Consumption)
Chlordanes	5.6	190	280	560
DDTs	21	520	1000	2100
Dieldrin	0.46	15	23	46
Mercury	220	70	150	440
PCBs	3.6	21	42	120
Selenium	7400	2500	4900	15000

Element 6. Project Description

6.1. Work statement and produced products

This study will be completed in two years of sampling. Sampling will focus on the California lakes known to support grebe breeding with primary focus on those lakes previously studied under the 2 year BOG Lakes study. Chemistry and ancillary data will be collected from fish caught at these sites, and a report of the findings will be made publicly available in 2013.

6.2. Constituents to be analyzed and measurement techniques.

A detailed Sampling and Analysis Plan (SAP) is in Appendix II. Chemistry analytical methods are summarized in Section E. Constituents to be analyzed are summarized in Tables 4-6. All chemistry data will be reported on a wet weight basis. Analytical methods are listed in each table as appropriate.

Table 4. Constituents to be Analyzed – Grebe Attributes

Grebe Attributes
Body Mass (g)
Flattened wing chord (mm)
Tarsus length (mm)
Exposed culmen length (mm)
Moisture (%)
Collection Location (UTMs)

Table 5. Constituents to be Analyzed – Fish Attributes

Fish attributes are physical measurements or observations. These are not covered in any analytical method.

Fish Attributes
Total Length (mm)
Fork Length (mm)
Weight (g)
Sex (sport fish only)
Moisture (%)
Collection Location (UTMs)

Table 6. Constituents to be Analyzed – Metals and Metalloids

Analyte	Tissue Type	Analytical Method
Total Mercury	Grebe blood and eggs	EPA 7473 (USEPA 1998)
Total Mercury	Whole Body Small Fish and Sport muscle	EPA 7473 (USEPA 1998)

6.3. Project schedule and number of samples to be analyzed.

Key tasks in the project and their expected due dates are outlined in Table 7.

Table 7. Project Schedule Timeline

Item	Activity and/or Deliverable	Deliverable Due Date
1	Contracts	
	Subcontract Development	February 2012
2	Quality Assurance Project Plan & Monitoring Plan	
2.1	Draft Monitoring Plan	February 2012
2.2	Final Monitoring Plan	March 2012
2.3	Draft Quality Assurance Project Plan	April 2012
2.4	Final Quality Assurance Project Plan	May 2012
3	Sample Collection	Year 1 April-October 2012 Year 2 April-October 2013
4	Sample Selection and Chemical Analysis	
4.1	Selection of Tissue for Analysis	Year 1 September-October 2012 Year 2 September-October 2013
4.2	Creation of Sample Composites	Year 1 October-November 2012 Year 2 October-November 2013
4.3	Chemical Analysis	Year 1 November 2012-February 2013 Year 2 November 2013-February 2014
4.4	Small Fish Data Reported to SWAMP	Year 1 March 2013 Year 2 March 2014
4.4	Grebe Data Reported to CEDEN	Year 1 May 2013 Year 2 May 2014
5	Data Quality Assessment and Narrative	Year 1 May 2013 Year 2 May 2013
6	Interpretive Report	
6.1	Draft Report	Year 1 March 2014 Year 2 March 2015
6.2	Final Report	Year 1 May 2013 Year 2 May 2014

6.4. Geographical setting and sample sites

Sampling will occur in freshwater lakes throughout California that contain breeding grebe colonies. Site selection and timing will be determined based on breeding grebe locations and relative abundance of grebes. See the Proposal and Study Plan for a map depicted primary and alternate sampling lakes.

6.5. Constraints

All sampling must be completed by the end of the current year's sampling season in order to meet analysis and reporting deadlines set forth in Table 7.

Element 7. Quality Indicators and Acceptability Criteria for Measurement Data

Data quality indicators for the analysis of grebe and fish tissue mercury concentrations will include accuracy (bias), precision, recovery, completeness and sensitivity. Measurement Quality Indicators for analytical measurements in tissue are in Table 8.

Previously collected data will not be utilized in this study, therefore specific acceptance criteria are not applicable.

Table 8. Measurement quality indicators for laboratory measurements.

Parameter	Accuracy	Precision	Recovery	Completeness	Sensitivity
Trace metals (including mercury)	CRM 75% - 125%	Duplicate RPD <25%; n/a if concentration of either sample <RL Matrix Spike Duplicate RPD <25%	Matrix Spike 75% - 125%	90%	See Table 12

7.1. Accuracy

Evaluation of the accuracy of laboratory procedures is achieved through the preparation and analysis of reference materials with each analytical batch. Ideally, the reference materials selected are similar in matrix and concentration range to the samples being prepared and analyzed. The accuracy of the results is assessed through the calculation of a percent recovery.

$$\% \text{ recovery} = \frac{V_{\text{analyzed}}}{V_{\text{certified}}} \times 100$$

Where:

V_{analyzed} : the analyzed concentration of the reference material

$V_{\text{certified}}$: the certified concentration of the reference material

The acceptance criteria for reference materials are listed in Table 9.

Table 9. Measurement Quality Objectives – Inorganic Analytes in Tissues

SWAMP Measurement Quality Objectives* - General		
Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Calibration Standard	Per analytical method or manufacturer's specifications	Per analytical method or manufacturer's specifications
Continuing Calibration Verification	Per 10 analytical runs	80-120% recovery
Laboratory Blank	Per 20 samples or per batch, whichever is more frequent	<RL for target analyte
Reference Material	Per 20 samples or per batch, whichever is more frequent	75-125% recovery
Matrix Spike	Per 20 samples or per batch, whichever is more frequent	75-125% recovery
Matrix Spike Duplicate	Per 20 samples or per batch, whichever is more frequent	75-125% recovery, RPD ≤25%
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent	RPD <25%; n/a if concentration of either sample <MDL
Internal Standard	Accompanying every analytical run when method appropriate	75-125% recovery

*Unless method specifies more stringent requirements.

MDL = Method Detection Limit

RL = Reporting Limit

n/a = not applicable

7.2. Precision

In order to evaluate the precision of an analytical process, a field sample is selected and digested or extracted in duplicate. Following analysis, the results from the duplicate samples are evaluated by calculating the Relative Percent Difference (RPD).

$$RPD = \left| \frac{(V_{\text{sample}} - V_{\text{duplicate}})}{\text{mean}} \right| \times 100$$

Where:

V_{sample} : the concentration of the original sample digest

$V_{\text{duplicate}}$: the concentration of the duplicate sample digest
mean: the mean concentration of both sample digests

The acceptance criteria for laboratory duplicates are specified in Table 9.

A minimum of one duplicate per analytical batch will be analyzed. If the analytical precision is unacceptable, calculations and instruments will be checked. A repeat analysis may be required to confirm the results.

Duplicate precision is considered acceptable if the resulting RPD is $\leq 25\%$ for analyte concentrations that are greater than the Minimum Level (ML). The U.S. Environmental Protection Agency (EPA) defines the ML as the lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all standard operating procedure (SOP) or method-specified sample weights, volumes, and cleanup procedures have been employed.

7.2.1. Replicate Analysis

Replicate analyses are distinguished from duplicate analyses based simply on the number of involved analyses. Duplicate analyses refer to two sample digests, while replicate analyses refer to three or more. Analysis of replicate samples is not explicitly required; however it is important to establish a consistent method of evaluating these analyses. The method of evaluating replicate analysis is by calculation of the relative standard deviation (RSD). Expressed as a percentage, the RSD is calculated as follows:

$$\text{RSD} = \frac{\text{Stdev}(v_1, v_2, \dots, v_n)}{\text{mean}} \times 100$$

Where:

Stdev(v_1, v_2, \dots, v_n): the standard deviation of the values (concentrations) of the replicate analyses.

mean: the mean of the values (concentrations) of the replicate analyses.

7.3. Bias

Bias is the systematic or persistent distortion of a measurement process that skews data in one direction. Certified Reference Materials (CRM) and Matrix Spike (MS) samples are used to determine the analyte-specific bias associated with each analytical laboratory. CRMs are used to determine analytical bias, and MS are used to determine the bias associated with the tissue matrix.

A matrix spike (MS) is prepared by adding a known concentration of the target analyte to a field sample, which is then subjected to the entire analytical procedure. If the ambient concentration of the field sample is known, the amount of spike added is within a specified range of that concentration. Matrix spikes are analyzed in order to assess the magnitude of matrix interference and bias present. Because matrix spikes are analyzed in pairs, the second spike is called the matrix spike duplicate (MSD). The MSD provides information regarding the precision of the matrix effects. Both the MS and MSD are split from the same original field sample.

The success or failure of the matrix spikes is evaluated by calculating the percent recovery.

$$\% \text{ recovery} = \frac{(V_{\text{MS}} - V_{\text{ambient}})}{V_{\text{spike}}} \times 100$$

Where:

- V_{MS} : the concentration of the spiked sample
- $V_{ambient}$: the concentration of the original (unspiked) sample
- V_{spike} : the concentration of the spike added

In order to properly assess the degree of matrix interference and potential bias, the spiking level should be approximately 2-5 times the ambient concentration of the spiked sample but at least 3 times the reporting limit. If the MS or MSD is spiked too high or too low relative to the ambient concentration, the calculated recoveries are no longer an acceptable assessment of analytical bias. In order to establish spiking levels prior to analysis of samples, the laboratories should review any relevant historical data. In many instances, the laboratory will be spiking the samples blind and will not meet a spiking level of 2-5 times the ambient concentration. However, the results of affected samples will not be automatically rejected.

In addition to the recoveries, the RPD between the MS and MSD is calculated to evaluate how matrix affects precision.

$$RPD = \left| \frac{(V_{MS} - V_{MSD})}{\text{mean}} \right| \times 100$$

There are two different ways to calculate this RPD, depending on how the samples are spiked.

- 1) The samples are spiked with the same amount of analyte. In this case,
 - V_{MS} : the concentration for the matrix spike
 - V_{MSD} : the concentration of the matrix spike duplicate mean: the mean of the two concentrations (MS + MSD)
- 2) The samples are spiked with different amounts of analyte. In this case,
 - V_{MS} : the recovery associated with the matrix spike
 - V_{MSD} : the recovery associated with matrix spike duplicate mean: the mean of the two recoveries ($\text{recovery}_{MS} + \text{recovery}_{MSD}$)

The MQO for the RPD between the MS and MSD is the same regardless of the method of calculation; detailed in Table 9.

7.4. Contamination assessment – Method blanks

Laboratory method blanks (also called extraction blanks, procedural blanks, or preparation blanks) are used to assess laboratory contamination during all stages of sample preparation and analysis. At least one laboratory method blank will be run in every sample batch of 20 or fewer field samples. The method blanks will be processed through the entire analytical procedure in a manner identical to the samples. The QC criterion for method blank analysis states that the blanks must be less than the Reporting Limit (<RL) for target analytes. If blank values exceed the RL, the sources of the contamination are determined and corrected, and in the case of method blanks, the previous samples associated with the blank are re-analyzed. All blank analysis results will be reported. If it is not possible to eliminate the contamination source, all impacted analytes in the analytical batch will be flagged. In addition, a detailed description of the

contamination sources and the steps taken to eliminate/minimize the contaminants will be included in interim and final reports. Subtracting method blank results from sample results is not permitted, unless specified in the analytical method.

7.8. Representativeness

The representativeness of the data is mainly dependent on the sampling locations and the sampling procedures adequately representing the true condition of the sample site. Requirements for selecting sample sites are discussed in more detail in the SAP (Appendix II). Sample site selection, sampling of relevant media (water, sediment and biota), and use of only approved/documented analytical methods will determine that the measurement data does represent the conditions at the investigation site, to the extent possible.

7.9. Completeness

Completeness is defined as “a measure of the amount of data collected from a measurement process compared to the amount that was expected to be obtained under the conditions of measurement” (Stanley and Verner, 1985).

Field personnel will always strive to achieve or exceed the SWAMP completeness goals of 90% for fish samples when target species (SAP Table 1 Appendix II) are present. Due to the variability and uncertainty of species availability in each zone, this level of completeness may not be attainable. If grebes or fish cannot be collected from a particular location, another location may be chosen to replace it. Additional locations will be chosen by the PI with input from Regional Board staff. Additionally, colony size may limit grebe egg collection in some areas. In order to generate the most robust data necessary, any lakes where targeted egg collections are not possible the PIs will collect grebe feathers as the 2nd tissue for analysis.

In the event field documentation is incomplete, datasheets will be returned to the collection crew for amendment.

Laboratories will strive for analytical completeness of 90% (Table 8). In the event laboratory documentation is incomplete, datasheets will be returned to the dissector for amendment.

Occasionally digestates or extracts are rendered unusable for various reasons in the preparation process. If this occurs, the sample(s) affected will be re-processed.

Element 8. Special Training Requirements/Safety

8.1. Specialized training and safety requirements

Analysts are trained to conduct a wide variety of activities using standard protocols to ensure samples are analyzed in a consistent manner. Training of each analyst includes the use of analytical equipment and conducting analytical protocols, and other general laboratory processes including glassware cleaning, sampling preparation and processing, hazardous materials

handling, storage, disposal. All laboratory staff must demonstrate proficiency in all the aforementioned and required laboratory activities that are conducted, as certified by the Laboratory QAO.

8.2. Training, safety and certification documentation

Staff and safety training is documented at USGS and MPSL-DFG. Documentation consists of a record of the training date, instructor and signatures of completion. The Laboratory QAO will certify the proficiency of staff at chemical analyses. Certification and records are maintained and updated by the Laboratory QAO, or their designee, for all laboratory staff.

8.3. Training personnel

The USGS or MPSL-DFG Lab Director (LD) trains or appoints senior staff to train personnel. The Laboratory QAO ensures that training is given according to standard laboratory methods, maintains documentation and performs performance audits to ensure that personnel have been trained properly.

8.3.1. Laboratory Safety

New laboratory employees receive training in laboratory safety and chemical hygiene prior to performing any tasks in the laboratory. Employees are required to review the laboratory's safety program and chemical hygiene plan and acknowledge that they have read and understood the training. An experienced laboratory employee or the laboratory safety officer is assigned to the new employee to provide additional information and answer any questions related to safety that the new employee may have.

On-going safety training is provided by quarterly safety meetings conducted by the laboratory's safety officer or an annual laboratory safety class conducted by the USGS Safety Officers or MLML Chemical Safety Officer.

8.3.2. Technical Training

New employees and employees required to learn new test methods are instructed to thoroughly review the appropriate standard operating procedure(s) and are teamed up with a staff member who is experienced and qualified to teach those test methods and observe and evaluate performance. Employees learning new test methods work with experienced staff until they have demonstrated proficiency for the method both by observation and by obtaining acceptable results for QC samples. This demonstration of proficiency is documented and certified by the section leader, Laboratory QAO and the laboratory director prior to the person independently performing the test method. Training records are retained on file for each employee by their supervisor or QAO. On-going performance is monitored by reviewing QC sample results.

Element 9. Documentation and Records

The following documents, records, and electronic files will be produced:

- Quality Assurance Project Plan (submitted to contract manager in paper and electronic formats)
- Sampling and Analysis Plan (submitted to contract manager in paper and electronic formats)
- Archived Sample Sheets (internal documentation available on request)
- Chain-of-Custody Forms (exchanged for signatures with chemistry lab, and kept on file)
- Lab Sample Disposition Logs (internal documentation available on request)
- Calibration Logs for measurements of water quality standards (internal documentation available on request)
- Refrigerator and Freezer Logs (internal documentation available on request)
- Quarterly Progress Reports (oral format to contract manager)
- Data Tables (submitted to contract manager in electronic formats)
- Draft Manuscript (produced in electronic format)
- Final Manuscript (in electronic format)
- Data Appendix (submitted to contract manager in paper and electronic spreadsheet formats)

Copies of this QAPP will be distributed by the project manager to all parties directly involved in this project. Any future amended QAPPs will be distributed in the same fashion. All originals of the first and subsequent amended QAPPs will be held at MPSL-DFG. Copies of versions, other than the most current, will be discarded to avoid confusion.

The final report will consist of summary data tables and an appendix that contains all project data in electronic SWAMP compatible spreadsheet format. All laboratory logs and data sheets will be maintained at the generating laboratory by the Laboratory Manager for five years following project completion, and are available for review by the Contract Manager or designee during that time. Copies of reports will be maintained at SFEI for five years after project completion then discarded, except for the database, which will be maintained without discarding. Laboratories will provide electronic copies of tabulated analytical data (including associated QA/QC information outlined below) in the SWAMP database format or a format agreed upon by the Contract Manager. All electronic data are stored on computer hard drives and electronic back-up files are created every two weeks or more frequently.

Laboratories will generate records for sample receipt and storage, analyses and reporting.

Laboratories maintain paper copies of all analytical data, field data forms and field notebooks, raw and condensed data for analysis performed on-site, and field instrument calibration notebooks.

The PC will be responsible for sending out the most current electronic copies of the approved QAPP to all appropriate persons listed in Table 1.

Group B Elements. Data Generation and Acquisition

Element 10. Sample Process Design

The project design is described in the Sampling and Analysis Plan (SAP), Section B, pp. 6-8 (Appendix II). Twenty-four lakes and reservoirs identified as western grebe breeding areas will be sampled, where possible, for birds, small fish and sport fish. Effort will first be put into grebe blood and eggs collections. Once suitable samples have been taken, small fish and sport fish collections will commence within 2-3 weeks. It is important not to collect fish before grebe samples, since data on the birds is pivotal to the development of a model.

Potential small fish and sport fish sampling equipment and methods can be found in MPSL-102a (Appendix III). Once samples have been identified for composite creation, they will be processed according to the timeline in Table 7.

All measurements and analyses to be performed are critical to address the objectives laid out in Section III of the SAP (Appendix II), with the exception of grebe parameters, fish weight, sex, and moisture content. These parameters may be used to support other data gathered.

10.1. Variability

The grebe tissue and small fish samples will be analyzed individually as outlined in the SAP (Appendix II) and MPSL-DFG SOPs (Appendix III). Sport fish composites may be created for non-bass species collected because of variability within species.

10.2. Bias

Bias can be introduced by using fish of one particular species and/or total length for chemistry regressions and statistical analyses. The SAP (Appendix II) was reviewed by a Scientific Review Panel which approved of the inclusion of length ranges and multiple target species to reduce the associated bias.

Element 11. Sampling Methods

Grebe tissue samples will be collected in accordance with USGS standard operating procedures. One egg from up to 30 nests (but typically 5-10 eggs) from each breeding colony

will be collected (Egg Collection SOP, Appendix IV A). Blood will be collected via heparinized needles and syringes (Blood Collection SOP, Appendix IV B).

Fish will be collected in accordance with MPSL-102a, Section 7.4 (Appendix III B) except where noted here. Because habitats may vary greatly, there is no one method of collection that is appropriate. Field crews will evaluate each fishing site and species targeted to determine the correct method to be employed. Potential sampling methods include, but are not limited to: electroshocking, seining, gill netting, and hook and line. Field Crew will determine the appropriate collection method based on physical site parameters such as depth, width, flow, and accessibility. Field crew will indicate collection method on data sheets (Attachment 2).

Details on targeted fish species, number of individuals and size ranges can be found in the SAP (Appendix II, Tables 1-2).

The following adaptation to MPSL-102a, Section 7.4.5 (Appendix III) has been made: Collected fish may be partially dissected in the field. At the dock, the fish is placed on a measuring board covered with clean aluminum foil; fork and total length are recorded. Weight is recorded. Large fish such as carp will then be placed on the cutting board covered with a foil where the head, tail, and guts are removed using a clean cleaver (scrubbed with Micro™, rinsed with tap and deionized water). The fish cross section is tagged with a unique numbered ID, wrapped in aluminum foil, and placed in a clean labeled bag. When possible, parasites and body anomalies are noted. The cleaver and cutting board are re-cleaned with Micro™, rinsed with tap and deionized water between fish species, per site if multiple stations are sampled.

Special care is being taken to prevent the potential contamination of invasive species from one location to another. A 10% bleach solution is sprayed on all boat and personal gear components that come into contact with ambient water from each location. In addition, a visual inspection of the boat or equipment is conducted to ensure any algae or other organisms are not transferred between locations. Furthermore, boat bilges are verified to be dry before the boat is launched into a location.

Further details on sample collection and processing can be found in the SAP (Appendix II).

11.1. Corrective Action

In the event samples cannot be collected, the Sample Collection Coordinator will determine if corrective actions are appropriate. Table 10 describes action to take in the event of a collection failure.

Table 10. Field collection corrective actions

Collection Failure	Corrective Action
No Breeding Grebes Present	Inform PC and move on to another location – another location may be substituted; document the occurrence
Egg collection not possible	Collect feathers instead, or increase number of grebe blood samples at that lake
Only one species of small fish present	Collect from that species alone
No sport fish present	Inform PC and move on to another location

Element 12. Sample Handling and Custody

The field coordinator will be responsible for ensuring that each field sampling team adheres to proper custody and documentation procedures. A master sample logbook of field data sheets shall be maintained for all samples collected during each sampling event. A chain-of-custody (COC, Attachment 1) form must be completed after sample collection, archive storage, and prior to sample release.

Grebe blood samples will be stored in glass or plastic vials on wet or dry ice in the field, then stored in the laboratory at -20C. Grebe eggs will be stored in polyethylene bags on wet ice in the field and then transferred to a refrigerator, until dissection within 6 months. After dissection, egg contents will be stored frozen in glass or plastic jars at -20C. Samples delivered to USGS-FRESC or USGS-WERC will be logged upon arrival.

Fish samples will be wrapped in aluminum foil and frozen on dry ice for transportation to the storage freezer or laboratory, where they will be stored at -20°C until dissection and homogenization. Samples delivered to MPSL-DFG will be logged in according to MPSL-104 (Appendix III C).

Sport fish samples will be dissected according to MPSL-105 (Appendix III D) and data retained on the lab data sheets in Attachment 4. Small fish samples will be processed according to the USGS Sample Preparation SOP (Appendix IV C).

Lab homogenates will be frozen until analysis is performed. Frozen tissue samples have a 12 month hold time from the date of collection. If a hold-time violation has occurred, data will be flagged appropriately in the final results.

Element 13. Analytical Methods

Methods and equipment for laboratory analyses are listed in Table 11. EPA methods can be downloaded from www.epa.gov/epahome/index/nameindx.htm. EPA method numbers followed by “M” indicate modifications have been made. Modifications and non-EPA SOPs can be found

in Appendix III and IV. Method validation data for modifications and SOPs can be obtained by contacting the analytical laboratory (Table 1.)

Table 11. Methods for laboratory analyses

Parameter	Method	Instrument
Mercury in Grebe Tissues	EPA 7473 (USEPA 1998)	Milestone DMA 80
Mercury in Fish Tissues	EPA 7473 (USEPA 1998)	Milestone DMA 80

An AWS brand AMW-DISC digital pocket scale, or similar, is used to weigh fish in the field and is calibrated monthly in the lab with standard weights. Fish lengths are determined using a fish measuring board that does not require calibration. No other field measurements are being taken.

Mercury in fish tissues will be analyzed according to EPA 7473, “Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry” (USEPA, 1998) using a Direct Mercury Analyzer (DMA 80). Samples, blanks, and standards will be prepared using clean techniques. ASTM Type II water and analytical grade chemicals will be used for all standard preparations. A continuing calibration verification (CCV) will be performed after every 10 samples. Initial and continuing calibration verification values must be within $\pm 20\%$ of the true value, or the previous 10 samples must be reanalyzed. Three blanks, a certified reference material (DORM-3 or similar), as well as a method duplicate and a matrix spike pair will be run with each analytical batch of samples. Reporting Limits (RL) can be found in Table 12 and Measurement Quality Objectives (MQO) in Section 7, Table 9.

Table 12. Trace metal analytical parameters, reporting units, and reporting limits (RL) for tissue samples.

Parameter	Method	RL ($\mu\text{g/g}$ wet wt)
Mercury in Grebe Tissues	EPA 7473 (USEPA 1998)	0.004
Mercury in Fish Tissues	EPA 7473 (USEPA 1998)	0.009

13.2.1. Corrective Action

It is the responsibility of each analyst to take corrective action upon instrument failure. Corrective action will be conducted according to manufacturer or method specifications. Additional information on corrective actions can be found in Section 20.2.

13.2.2. Turn around time

All tissue analyses must be completed within the 1 year hold time. In addition, results need to be reported according to the timeline outlined in Table 7.

13.3. Sample Disposal

The laboratories are responsible for complying with all Federal, State and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions. Chemicals must be appropriately neutralized prior to disposal or must be handled as hazardous waste.

Element 14. Quality Control

MPSL-DFG and DFG-WPCL conduct quality control through several activities and methods. These methods of quality control are performed to identify possible contamination problem(s), matrix interference and the ability to duplicate/repeat results. When control limits are exceeded the Laboratory QAO will review with appropriate laboratory staff to ascertain the possible cause of the exceedance. A review of SOPs will be conducted and any deficiencies will be identified, documented, and corrected. A written report of the corrective action(s) will be provided to the PI and PM via email. The PM will contact the SWAMP QAO as needed.

Each aspect of laboratory quality control is listed in Table 9 for frequency as well as Measurement Quality Objectives (MQO) for each.

Element 15. Instrument/Equipment Testing, Inspection and Maintenance

Laboratory instruments are inspected and maintained in accordance with lab SOPs, which include those specified by the manufacturer and those specified by the method (Table 13). These SOPs have been reviewed by each respective Laboratory QAO and found to be in compliance with SWAMP criteria. Analysts are responsible for equipment testing, inspection, and maintenance. Appendices III and IV list the referenced SOPs. USGS SOPs are available upon request from the Laboratory Director by email: ceagles-smith@usgs.gov. Likewise, MPSL-DFG SOPs are available upon request from the Laboratory QAO by email: bonnema@mlml.calstate.edu.

Electronic laboratory equipment usually has recommended maintenance prescribed by the manufacturer. These instructions will be followed as a minimum requirement. Due to the cost of some laboratory equipment, back up capability may not be possible. But all commonly replaced parts will have spares available for rapid maintenance of failed equipment. Such parts include but are not limited to: batteries; tubes; light bulbs; tubing of all kinds; replacement specific ion electrodes; electrical conduits; glassware; pumps; etc.

The lead chemist, or designee, is responsible for the testing, inspection, and maintenance of equipment. Each instrument has its own logbook where the results of tests, inspections, maintenance and repairs are documented. When an instrument's test results fail to meet

accuracy and/or precision criteria after the lead chemist has performed maintenance, the manufacturer will be contacted.

Element 16. Instrument/Equipment Calibration and Frequency

Laboratory instruments (listed in Table 13) are calibrated, standardized and maintained according to procedures detailed in laboratory SOPs (Appendix I). Instrument manuals identify step-by-step calibration and maintenance procedures. Instruments and types of calibration required are listed in Table 13. If analytical instrumentation fails to meet performance requirements, the instrument(s) will be checked according to their respective SOP(s) and recalibrated. If the instrument(s) does again does not meet specifications, it will be repaired and retested until performance criteria are achieved. The maintenance will be entered in the instrument log. If sample analytical information is in question due to instrument performance, the PM will be contacted regarding the proper course of action including reanalyzing the sample(s).

At a minimum all calibration procedures will meet the requirements specified in the US EPA approved methods of analysis. The means and frequency of calibration recommended by the manufacturer of the equipment or devices as well as any instruction given in an analytical method will be followed. When such information is not specified by the method, instrument calibration will be performed at least once daily and continuing calibration will be performed on a 10% basis thereafter except for analysis by GC/MS. It is also required that records of calibration be kept by the person performing the calibration and be accessible for verification during either a laboratory or field audit.

Table 13. Equipment maintenance and calibration frequency.

Instrument	Inspection/Maintenance Frequency	Calibration Frequency
Milestone DMA-80 Direct Mercury Analyzer (USGS)	As needed	At least once every 2 weeks
Milestone DMA-80 Direct Mercury Analyzer (MPSL-DFG)	As needed	At least once every 2 weeks

16.1. Analytical Instrumentation

16.1.1. Instrument calibration

Upon initiation of an analytical run, after each major equipment disruption, and whenever on-going calibration checks do not meet recommended MQOs, the system will be calibrated with a full range of analytical standards. Immediately after this procedure, the initial calibration must be verified through the analysis of a standard obtained from a different source than the standards used to calibrate the instrumentation, prepared in an independent manner, and ideally having certified concentrations of target analytes of a CRM or certified solution. Frequently, calibration standards are included as part of an analytical run, interspersed with actual samples. However, this practice does not document the stability of the calibration and is incapable of detecting

degradation of individual components, particularly pesticides, in standard solutions used to calibrate the instrument. The calibration curve is acceptable if it has an R^2 of 0.990 or greater for all analytes present in the calibration mixtures. If not, the calibration standards, as well as all the samples in the batch are re-analyzed. All calibration standards will be traceable to a recognized organization for the preparation and certification of QC materials (e.g., National Institute of Standards and Technology, National Research Council Canada, US EPA, etc.).

Calibration curves will be established for each analyte and batch analysis from a calibration blank and a minimum of three analytical standards of increasing concentration, covering the range of expected sample concentrations. Only data which result from quantification within the demonstrated working calibration range may be reported (i.e., quantification based on extrapolation is not acceptable). Alternatively, if the instrumentation is linear over the concentration ranges to be measured in the samples, the use of a calibration blank and one single standard that is higher in concentration than the samples may be appropriate. Samples outside the calibration range will be diluted or concentrated, as appropriate, and reanalyzed.

16.1.2. Continuing calibration verification (CCV)

Calibration verification solutions traceable to a recognized organization are inserted as part of the sample stream. The sources of the calibration verification solutions are independent from the standards used for the calibration. Calibration verification solutions used for the CCV will contain all the analytes of interest. The frequency of these verifications is dependent on the type of instrumentation used and, therefore, requires considerable professional judgment. The required frequency for this project is listed in Table 9. All analyses are bracketed by an acceptable calibration verification; all samples not bracketed by an in control CCV should be reanalyzed. If the control limits for analysis of the calibration verification solution are not met, the initial calibration will have to be repeated. All samples analyzed before the calibration verification solution that failed the MQOs will be reanalyzed following the recalibration. Only the re-analysis results will be reported. If it is not possible or feasible to perform reanalysis of samples, all earlier data (i.e., since the last successful calibration control verification) are suspect. In this case, the laboratory QAO will contact the PM to determine proceedings, and will flag the data and note the issue in interim and final reports.

Element 17. Inspection/Acceptance of Supplies and Consumables

All supplies will be examined for damage as they are received. Laboratory ordering personnel will review all supplies as they arrive to ensure the shipment is complete and intact. All chemicals are logged in to the appropriate logbook and dated upon receipt. All supplies are stored appropriately and are discarded upon expiration date. Table 14 indicates items that are considered for accuracy, precision, and contamination. If these items are not found to be in compliance with the acceptance criteria, they will be returned to the manufacturer.

Table 14. Inspection/acceptance testing requirements for consumables and supplies.

Project-Related Supplies (source)	Inspection / Testing Specifications	Acceptance Criteria	Frequency	Responsible Individual
Certified pre-cleaned glass or plastic (I-Chem/Fisher Scientific or similar)	Carton custody seal is inspected	Carton custody seal intact	At receipt date of shipment	USGS or MSPL-DFG personnel
Nitrile Gloves (Fisher Scientific or similar)	Carton seal is visually inspected for damage or tampering	Carton is intact and gloves within are clean and intact	At receipt date of shipment	USGS or MSPL-DFG personnel
Polyethylene Gloves (Fisher Scientific or similar)	Carton seal is visually inspected for damage or tampering	Carton is intact and gloves within are clean and intact	At receipt date of shipment	USGS or MSPL-DFG personnel
Analytical Standards (Perkin-Elmer, VWR, Fisher Scientific or similar)	Solution bottles are inspected to verify factory seal	Manufacturer's seal intact	At receipt date of shipment	USGS or MSPL-DFG personnel

Element 18. Non-Direct Measures

Data will not be used from non-direct measures in this study.

Element 19. Data Management

Field data will be entered into the SWAMP Database version 2.5 upon return to the lab. Original field sheets (Attachment 1) will be retained in a log book, and copies of the COCs (Attachment 2) will be kept by each receiving laboratory. SWAMP Authorization forms will also accompany samples sent to each laboratory (Attachment 3).

All data generated by USGS will be maintained as described in USGS SOPs (Appendix IV) and the USGS QAP (Appendix I). The USGS QAO will be responsible for oversight of the collection of all organic chemical analysis data and entering QA-checked data into the SWAMP database.

Likewise, all MPSL-DFG data will be generated and maintained according to the Marine Pollution Studies Laboratory Quality Assurance Plan (Appendix I). The MPSL-DFG QAO will be responsible for oversight of the collection of all dissection and metals analysis data and entering QA-checked data into the SWAMP database.

All data collected will be entered into electronic spreadsheets that are SWAMP compatible. Each data element is checked at a minimum by the technician that entered the data and verified by the technician's signature on the data sheet. Tissue data will be provided to the PC in Microsoft Excel spreadsheets. Data will be reviewed to ensure they are consistent with the format of the database and other data records.

All raw and statistical analysis data are subject to a 100% check for accuracy by the PM and Laboratory QAOs. Data are analyzed and proofread for accuracy, and then QA checked against

the QAPP and SWAMP criteria before being entered into the SWAMP database. Original hard copies of the data are filed in a secure cabinet until requested by the PM and/or inclusion into the Final Report. Electronic copies are stored and backed up by each analyst and respective laboratory internal project manager.

Hardware and software will be updated as recommended by the manufacturer or as needed. Testing of each component is not required on a regular basis aside from day to day functionality. Each entity is responsible for the necessary updates or upgrades, whether provided regularly through an Information Technology department or otherwise.

Data management checklists are not required. Analytical completeness will be tracked through the SWAMP Database version 2.5.

Group C Elements: Assessment and Oversight

Element 20. Assessments and Response Actions

20.1. Audits

All reviews of QA data will be made by the QAO of each laboratory prior to submission of each batch to the USGS or SWAMP Tissue Database 2.5. Reviews of the sampling procedures will be made by the Field Collection Coordinator and the Project Coordinator in case problems occur. As SOPs are updated and refined, additional reviews will be made. Each data technician is responsible for flagging all data that does not meet established QA/QC criteria.

Project data review established for this project will be conducted once all data sets have been received, and includes the following:

- Initial review of analytical and field data for complete and accurate documentation, chain of custody procedures, compliance with analytical holding times, and required frequency of laboratory QA samples.
- Comparison of all spike and duplicate results with the MQOs in Table 9.
- Assigning data qualifier flags to the data as necessary to reflect limitations identified by the process.

If a review discovers any discrepancy, the QAO will discuss it with the personnel responsible for the activity. The discussion will include the accuracy of the information, potential cause(s) leading to the deviation, how the deviation might impact data quality and the corrective actions that might be considered. If the discrepancy is not resolved, the QAO will issue a stop work order until the problem is fixed.

Assessments by the QAO will be oral; if no discrepancies are noted and corrective action is not required, additional records are not required. If discrepancies are observed, the details of the discrepancy and any corrective action will be reported and appended to the report.

All assessments will be conducted as data is received by the laboratory QAO in accordance with the timeline in Table 7.

20.2. Deviations and corrective actions

Analyses are conducted according to procedures and conditions recommended by the US EPA and described in laboratory SOPs (Appendices III and IV), with the exception of those reported herein. Beyond those identified, deviations from these recommended conditions are reported to the Laboratory QAO. The PM will be notified within 24 hours of these deviations.

In the event of a SOP/QAPP deviation or corrective action, a deviation/corrective action form will be prepared, completed, signed and the PM notified. Best professional judgment will be used in interpretation of results obtained when deviations in the test conditions have occurred. All deviations and associated interpretations will be reported in interim and final reports. Protocol amendments will be submitted to the Laboratory QAO and PM. Upon approval, protocol amendments will be employed.

This study strives for 90% analytical data completeness. If this goal cannot be achieved, various corrective actions can be undertaken as described in Section D24.

Element 21. Reports to Management

The following products are to be delivered to PM:

- Each LD shall regularly brief the PC, LS and PM on the progress of all on-going chemical analyses in monthly emails or conference calls. When deemed necessary for decision making, other BOG participants will also be notified of progress.
- The LS will provide a draft final report and a final report to the PM in accordance with the dates listed in Table 7.

Group D Elements: Data Validation and Usability

Element 22. Data Review, Verification and Validation Requirements

All data reported for this project will be subject to a 100% check for errors in transcription, calculation and computer input by the laboratory internal project manager and/or laboratory QAO. Additionally, the Laboratory QAO will review sample logs and data forms to ensure that requirements for sample preservation, sample integrity, data quality assessments and equipment calibration have been met. At the discretion of the LD, data that do not meet these requirements will either not be reported, or will be reported with qualifiers which serve as an explanation of any necessary considerations.

Reconciliation and correction will be decided upon by the Laboratory QAO and LD. The Laboratory QAO will be responsible for informing data users of the problematic issues that were discussed, along with the associated reconciliations and corrections.

Data generated by project activities will be reviewed against the measurement quality objectives (MQOs) in Table 9. Furthermore, the final dataset as a whole will be scrutinized for usability to answer the three Management Questions.

Element 23. Verification and Validation Methods

Grebe tissue data will be reported electronically to the USGS database managers. The data will be validated according to USGS procedures.

Fish data will be reported electronically to the Project Coordinator, then to the SWAMP Database Management Team (DMT) for inclusion in the SWAMP Database version 2.5. The DMT will follow SWAMP SOP Chemistry Data Verification V1.1 (Appendix V A).

All data will be validated by according to BOG Data Validation (Appendix V B), outlined below. Please refer to the appended document for complete descriptions and validation steps, as well as examples of potential QC failures.

QA narratives will be produced to be incorporated in the BOG Wildlife Report. This narrative will summarize the data set from a QA standpoint. Validated data will be made available to users via the State Water Resources Control Board CEDEN website (<http://www.ceden.us/AdvancedQueryTool>).

23.1. Blank Contamination Check

Blank verification samples identify if the target analyte has contaminated field samples via lab contamination from any part of sample preparation and analysis. One method blank (laboratory derived) sample is run with each analytical batch (≤ 20 samples). The method blanks will be processed through the entire analytical procedure in a manner identical to the field samples. The ideal scenario is that method blank samples are non-detects. If a field sample is contaminated from laboratory procedures and the analytical quantification of that field sample is low, then a high proportion of the field sample value could be from laboratory contamination which results in that value being uncertain and not usable. Laboratory blank contamination could result in a false positive when field sample results are low. There is less concern of blank contamination affecting a field sample if field samples are some multiple higher than the method blank result (in this case 3 times the method blank concentration).

Please refer to BOG Data Validation Standard Operating Procedure (Appendix V B) for details on the steps taken to determine blank contamination.

23.2. Accuracy Check

Accuracy is the degree of agreement of a measurement with a known value and is utilized to assess the degree of closeness of field samples to their real value. Using the bull's-eye analogy, accuracy is the degree of closeness to the bull's-eye (which represents the true value). Over/under estimation of analytical quantification is important in this project. If the QA elements indicate overestimation of the field sample result than this could lead to false positives above particular human health consumption thresholds and potentially limit human consumption of particular sport fish species. If the QA elements indicate underestimated analytical quantification then low field sample values could falsely suggest that fish are below human health thresholds when they may actually be above the thresholds. Good accuracy in a data set increases the confidence and certainty that the field sample value is close to the true value. Accuracy is determined by such QC elements as: certified reference materials (CRM), laboratory control samples, blind spikes, matrix spikes, and performance samples.

Please refer to BOG Data Validation Standard Operating Procedure (Appendix V B) for details on the steps taken to determine accuracy.

23.3. Precision Check

Precision is the degree to which repeated measurements under unchanged conditions show the same result (usually reported as a relative standard deviation [RSD] or relative percent difference [RPD]). The repeatability measure indicates the variability observed within a laboratory, over a short time, using a single operator, item of equipment, etc. These QA elements also show the reproducibility of an analytical measurement. Good precision provides confidence that the analytical process is consistently measuring the target analyte in a particular matrix.

Please refer to BOG Data Validation Standard Operating Procedure (Appendix V B) for details on the steps taken to determine precision.

Element 24. Reconciliation with User Requirements

Data will be reported in the SWAMP Database version 2.5. Data that do not meet with the Measurement Quality Objectives in Table 9 will be flagged accordingly as discussed in Section D23. Rejected data will not be included in data analyses while data flagged as estimated will be evaluated for inclusion on a case-by-case basis in conjunction with the associated QA data and program objectives.

The project needs sufficient data, as represented by the completeness objective (Table 8), to address the management questions laid out in the Sampling Plan (Appendix II). A failure to achieve the number of data points cited could mean an inability to answer these questions.

To address MQ1, concentrations of mercury in avian tissues will be compared to effect thresholds from the literature and concentrations in small fish will be compared to the threshold for small fish to be established by the State Water Board.

To address MQ2, we will use mixed-effects general linear model to test whether lake-specific mean THg concentrations in avian tissues can be determined using lake-specific mean THg concentrations in small fish sampled during a similar timeframe. We will compare the strength of models that incorporate a suite of factors, including region, elevation, and lake size to determine if there are category specific factors that can be used to refine biomagnification factor estimates.

To address MQ3, successful elements of the sampling and analysis plan will be recommended as valuable components of TMDL-related monitoring.

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Appendix I: List of Associated QAPs

MPSL-DFG Laboratory QAP, Revision 5. February, 2006

Quality Assurance Plan for USGS Environmental Mercury Lab

Appendix II: Sampling and Analysis Plan

FINAL

Sampling Plan

Incorporating Wildlife Methylmercury Exposure and Risk Estimates Using Biomagnification Factors into California Lake Monitoring

Josh Ackerman, Collin Eagles-Smith, Alex Hartman, Tom Maurer,
and Mark Stephenson

Surface Water Ambient Monitoring Program

March 2012

Sampling Plan

Incorporating Wildlife Methylmercury Exposure and Risk Estimates Using Biomagnification Factors into California Lake Monitoring

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I. INTRODUCTION

This document presents a sampling plan for a two-year study that will relate methylmercury exposure in fish from California lakes to exposure and risk in fish-eating birds. Piscivorous birds likely face significant risks from methylmercury exposure in a large number of California lakes. The goal of this study is to assess those risks in a representative sample of lakes and to investigate development of a biomagnification factor to estimate methylmercury exposure in wildlife based on concentrations in lower trophic level prey fish and we will also correlate sport fish mercury concentrations with prey fish and birds.

This work will be performed as part of a two-year field study for the State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP). Oversight for this Project is being provided by the SWAMP Roundtable. The Roundtable is comprised of State and Regional Water Board staff and representatives from other agencies and organizations including USEPA, the California Department of Fish and Game (CDFG) the California Office of Environmental Health Hazard Assessment, and the University of California.

The Roundtable has formed a subcommittee, the Bioaccumulation Oversight Group (BOG) that focuses on the Bioaccumulation Monitoring Project. The BOG is comprised of State and Regional Water Board staff and representatives from other agencies and organizations including USEPA, the Department of Fish and Game, the Office of Environmental Health Hazard Assessment, the Southern California Coastal Waters Research Project, and the San Francisco Estuary Institute. The members of the BOG individually and collectively possess extensive experience with bioaccumulation monitoring.

The BOG has also convened a Bioaccumulation Peer Review Panel that is providing programmatic evaluation and review of specific deliverables emanating from the Project, including this Sampling Plan. The members of the Panel are internationally recognized authorities on bioaccumulation monitoring. The BOG was formed and began developing a

strategy for designing and implementing a statewide bioaccumulation monitoring program in September 2006. To date the efforts of the BOG have included a two-year screening survey of bioaccumulation in sport fish of California lakes and reservoirs (in 2007 and 2008), another two-year screening survey of the California coast (in 2009 and 2010), and a one-year screening survey of California rivers and streams (in 2011). This wildlife study (in 2012 and 2013) will begin the next phase of BOG studies to assess the impacts of bioaccumulation on beneficial uses in California water bodies.

II. GENERAL ASPECTS OF THE SWAMP BIOACCUMULATION MONITORING PROJECT

A. Addressing Multiple Beneficial Uses

Bioaccumulation in California water bodies has an adverse impact on both the fishing and aquatic life beneficial uses (Davis et al. 2007). The fishing beneficial use is affected by human exposure to bioaccumulative contaminants through consumption of sport fish. The aquatic life beneficial use is affected by exposure of wildlife to bioaccumulative contaminants, primarily piscivorous species exposed through consumption of small fish. Different indicators are used to monitor these different types of exposure. Monitoring of status and trends in human exposure is accomplished through sampling and analyzing sport fish. On the other hand, monitoring of status and trends in wildlife exposure can be accomplished through sampling and analysis of wildlife prey (small fish, other prey species) or tissues of the species of concern (e.g., bird eggs or other tissues of juvenile or adults of the species at risk).

Over the long-term, a SWAMP bioaccumulation monitoring program is envisioned that assesses progress in reducing impacts on both the fishing and aquatic life beneficial uses for all water bodies in California. In the near-term, however, funds are limited, and there is a need to demonstrate the value of a comprehensive statewide bioaccumulation monitoring program through successful execution of specific components of a comprehensive program. Consequently, the BOG initially focused on sampling that addressed the issue of bioaccumulation in sport fish and impacts on the fishing beneficial use. This approach was intended to provide the information that is the highest priority for the state government and the public. The present study represents a first step in evaluating the impacts of bioaccumulation on the aquatic life beneficial use.

B. Addressing Multiple Monitoring Objectives and Assessment Questions for Aquatic Life Beneficial Uses

The BOG has developed a set of monitoring objectives a statewide program evaluating the impacts of bioaccumulation on the aquatic life beneficial use. This framework is consistent with frameworks developed for other components of SWAMP, and is intended to guide the bioaccumulation monitoring program over the long-term. The four objectives can be summarized as 1) status; 2) trends; 3) sources and pathways; and 4) effectiveness of management actions.

Over the long-term, the primary emphasis of the statewide bioaccumulation monitoring program will be on evaluating status and trends. Bioaccumulation monitoring is a very effective and essential tool for evaluating status, and is most cost-effective tool for evaluating trends for many contaminants. Monitoring status and trends in bioaccumulation will provide some information useful for identifying sources and pathways and for evaluating the effectiveness of management actions at a broader geographic scale. However, other types of monitoring (i.e., water and sediment monitoring) and other programs (regional TMDL programs) are also needed for addressing sources and pathways and effectiveness of management actions.

In the near-term, the primary emphasis of the statewide bioaccumulation monitoring program is on evaluating Objective 1 (status). The reasons for this are:

1. systematic statewide assessment of status has never been performed and is urgently needed;
2. we are starting a new program and establishing a foundation for future assessments of trends;
3. past monitoring has established very few time series that are useful in trend analysis that this program could have built upon.

The study described in this sampling plan will generate information that supports assessment of status. This effort will be the first study of bioaccumulation in California wildlife with a statewide perspective. Information on methylmercury exposure and risk in wildlife will be obtained for a representative group of 24 lakes, providing 1) a direct measure of status in those lakes and 2) an assessment of the degree to which data on exposure in small fish and sport fish can be interpreted as an indication of exposure in wildlife.

C. Addressing Multiple Habitat Types

SWAMP has defined the following categories of water bodies:

- lakes and reservoirs;
- bays and estuaries;
- coastal waters;
- large rivers;
- wadeable streams; and
- wetlands.

Due to their vast number, high fishing pressure, and a relative lack of information on bioaccumulation (Davis et al. 2007), lakes and reservoirs were identified as the first priority for sport fish monitoring. Coastal waters, including bays and estuaries, were selected as the next priority, due to their importance for sport fishing and a relative lack of past monitoring. Rivers and streams were the last in the series of water body types to be covered with a statewide screening study. Wetlands were not covered due to the low fishing pressure in those habitats.

Following the sequence established for the fishing beneficial use, assessment of the impact of bioaccumulation on aquatic life beneficial uses is also beginning with a focus on lakes

and reservoirs. Methylmercury exposure and risk was identified as the greatest concern in this habitat type, and reproduction in piscivorous birds as the taxa and lifestage at greatest risk. The logistics of performing surveys of exposure and risk in wildlife, require much greater effort and time at each water body, and thus do not readily allow for statewide surveys of the same breadth as were performed for sport fish. However, a two-year study covering 24 lakes was considered to be feasible within the scope of available funding and staffing, and is expected to be sufficient to answer some critical general questions with regard to aquatic life beneficial uses. Including other contaminants or habitats is not feasible with existing funding at this time.

Bioaccumulation is likely having negative impacts on aquatic life beneficial uses in all of the habitat types identified by SWAMP, including wetlands, which are among the most important habitats for wildlife. Whether SWAMP will perform surveys in the other habitat types has not yet been determined. The results of this preliminary assessment of methylmercury impacts in lakes and reservoirs will be valuable in informing the decision on the priority of further assessments.

In summary, focusing on one habitat type (lakes and reservoirs), one objective (status), and one category of beneficial use (aquatic life) will allow us to provide reasonable coverage and provide an informative assessment of bioaccumulation in these habitats in a two-year study.

III. STUDY DESIGN

A. Management Questions Addressed by this Study

Management Question 1 (MQ1)

Does methylmercury pose significant risks to aquatic life in a representative sample of California lakes and reservoirs?

Management Question 2 (MQ2)

Can a biomagnification factor approach (for small fish) or correlation approach (for sport fish) be applied on a statewide basis to estimate risks to birds?

Management Question 3 (MQ3)

What are appropriate TMDL monitoring requirements to address methylmercury exposure in wildlife?

To answer these questions, over two consecutive field seasons in 2012 and 2013, we will sample birds and small fish simultaneously at 24 lakes throughout California during the breeding season when birds are particularly vulnerable to potential mercury-induced reproductive impairment. Specifically, the study will have four main components:

- 1) Sample grebes at 24 California lakes over 2 years to determine mercury levels in a species near the top of the food chain, and compare these data to known effects-thresholds for birds.

- 2) Simultaneously with grebe sampling, collect small fish (<100 mm) at these same 24 lakes over 2 years to determine if mercury concentrations are above current wildlife diet objectives.
- 3) Use these data in Objectives 1 and 2 to calculate a bird biomagnification factor, evaluate the biomagnification factor's usefulness for estimating wildlife exposure, and assess whether the biomagnification factor differs by lake type or geographic region.
- 4) Simultaneously with grebe and small fish sampling, collect sport fish at these same 24 lakes over 2 years to assess correlations of mercury concentrations in sport fish, small fish, and birds.

B. Methods

This project will be led by USGS for the wildlife component and Moss Landing Marine Labs for the small fish and sport fish components. Bird sampling will be conducted immediately before fish sampling, and then bird collection sampling locations will be communicated to fish sampling personnel for subsequent sampling by the fish team within two weeks of bird sampling. It is likely that southern California lakes will be sampled earlier in the summer, and northern California lakes will be sampled later in the summer as grebes nest earlier in southern California sites.

Grebes

We will use western and Clark's grebes as our index of mercury exposure to wildlife in California lakes. We will sample grebe blood (and eggs where possible) from 24 California lakes during April-October of 2012 and 2013. **Figure 1** shows the proposed primary and alternate lake sites which will be investigated further and the final 24 lakes will be chosen after scouting lakes in the field. We will sample up to 12 lakes each year and conduct the field research over a 2-year period in 2012 and 2013 so that we can travel to all 24 lakes and sample grebes and fish during a narrow time window.

Grebes will be captured using boats and a combination of dip nets, net guns, and gill nets. If necessary, we may use shotguns to lethally collect grebes at sites where capture proves too difficult and costly. Grebe eggs also will be sampled when possible, and we will collect 1 egg randomly from each nest (up to 30 nests per lake, but this will depend on colony size and typically only 10 eggs will be collected from most lake sites). For each grebe captured, we will measure wing, culmen, and tarsus lengths, and weight. Each grebe tissue sample will be marked with an individual tag ID. Whole blood will be transferred from the field on wet ice to the lab where it will be stored at -20°C until mercury analysis. For each grebe captured, we will record the latitude/longitude or UTM where it was captured.

After grebes are collected, grebe collection locations will immediately be transferred from USGS to MLML personnel for the capture of small fish and sport fish within 2 weeks of grebe collections.

Small Fish

Small fish (25-100 mm) will be sampled using traps, seines, and dip nets from areas near grebe collections. We will sample 10 individuals each from two different prey fish species from each lake. We will target the following prey fish at all 24 lakes: Mississippi silversides, young of year largemouth bass, young of year bluegill, threadfin shad, shiner, and young of year tui chubs. Efforts will be made to sample the same species across all lakes, and when not possible we will sample fish that overlap in trophic guild. We will keep extra species of fish in the correct size ranges, and then choose which fish to analyze for mercury after all fish are collected each year. Upon collection, each prey fish collected will be tagged with a unique ID. Fish collected will be linked to the latitude/longitude or UTM where it was collected. Several parameters will be measured in the field for each small fish, including total length (longest length from tip of tail fin to tip of nose/mouth), fork length (longest length from fork to tip of nose/mouth), standard length, and weight. The individual prey fish will be placed in uniquely labeled bags and frozen. Small fish will be analyzed as whole fish.

Sport Fish

Sport fish (10 individuals per lake) will also be sampled in these same locations as small fish and grebes were formerly sampled, targeting the same individual species among all 24 lakes to the extent possible. One species will be sampled per lake. Fish species are distributed unevenly across the State, with different assemblages in different regions (e.g., high Sierra Nevada, Sierra Nevada foothills, and Central Valley) and a variable distribution within each region (Moyle 2002). To cope with this, the sampling crew will have a prioritized menu of several potential target species (Table 1). If the primary targets are not available in sufficient numbers, secondary targets have been identified. Largemouth bass will be the primary target species where they are present. At higher elevation lakes, resident, self-sustaining trout species will be the primary targets. Other species will also be observed in the process of fish collection. This "bycatch" will not be collected, but the sampling crew will record estimates of the numbers of each species observed. This information may be useful if follow-up studies are needed at any of the sampled locations.

The sampling design includes analysis of mercury in individual sport fish samples. An analysis of covariance approach will be employed, in which the size:mercury relationship will be established for each location and an ANCOVA will be performed that will allow the evaluation of differences in slope among the locations and the comparison of mean concentrations and confidence intervals at a standard length, following the approach of Tremblay (1998). Experience applying this approach in the Central Valley indicates that 10 fish spanning a broad range in size are needed to provide robust regressions (Davis et al. 2003, Melwani et al. 2007).

Specific size ranges to be targeted for each species are listed in Table 2. The numbers and sizes indicated for these species will provide the size range needed to support ANCOVA. In addition, the size range for black bass takes the legal limit for these species (305 mm, or 12 inches) into account. The goal for black bass is to have a size distribution that encompasses the

standard length (350 mm) to be used in statistical comparisons. This length is near the center of the distribution of legal-sized fish encountered in past studies (Davis et al. 2003, Melwani et al. 2007).

Sport fish will be sampled using seines, gill nets, and electroshocking. Upon collection, each sport fish collected will be tagged with a unique ID. Fish collected will be linked to the latitude/longitude or UTM where it was collected. Several parameters will be measured in the field, including total length (longest length from tip of tail fin to tip of nose/mouth), fork length (longest length from fork to tip of nose/mouth), standard length, and weight. Whole fish will be wrapped in aluminum foil and frozen on dry ice for transportation to the laboratory, where they will be stored frozen at -20°C. Fish will be kept frozen wrapped in foil until the time of dissection. Consistent with past SWAMP sport fish monitoring, sport fish will have the skin dissected off, and only the fillet muscle tissue will be used for analysis.

Mercury Analysis

Methylmercury is the form of mercury that biomagnifies and poses risks to wildlife and humans. Methylmercury concentrations will be estimated through measurements of total mercury. Nearly all of the mercury present in fish and in bird blood and eggs is methylmercury, and analysis of tissue for total mercury provides a valid, cost-effective estimate of methylmercury concentration (Wiener et al. 2007).

We will determine mercury concentrations in avian tissues at the USGS Davis and Corvallis Environmental Mercury Labs, and in fish at the Moss Landing Marine Lab following EPA method 7473, "Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry" using a Direct Mercury Analyzer. Specifically, using an integrated sequence of sample drying and combustion, coupled with amalgamation and atomic absorption spectroscopy, we will evaluate mercury concentrations in avian and fish tissues in relation to established reference standards. Samples, blanks, and standards will be prepared using clean techniques. ASTM Type II water and analytical grade chemicals will be used for all standard preparations. A continuing calibration verification (CCV) will be performed after every 10 samples. Initial and continuing calibration verification values must be within $\pm 20\%$ of the true value, or the previous 10 samples must be reanalyzed. Three blanks, a standard reference material (such as IAEA-407 or NRCC DORM-3), as well as a method duplicate and a matrix spike pair will be run with each set of samples.

Quality Assurance

This effort will adhere to quality assurance requirements established for the SWAMP. A QAPP specific to this effort is in preparation (Bonnema 2012).

Archiving

Grebe Tissues

For the majority of grebe blood samples, we will likely not have any remaining sample mass after we have analyzed blood samples for mercury and performed QAQC. However, any remaining grebe tissue will be stored in short-term archives. Samples in the short-term archive are stored at -20 °C and are intended for use in the identification of short-term time trends (i.e. < 5-10 years), the investigation of yet unidentified chemical contaminants (but note archival jars may not be appropriate for all other contaminant types, and are stored with respect to potential future mercury analysis), and addressing quality assurance issues that may arise during the routine analyses of samples. The short-term archives will be located in a freezer at USGS at either Davis, Dixon, or Corvallis Field Stations. These facilities are not equipped with a backup generator; however, in the event of power failure the facility contingency plans are to keep the freezer closed, providing maintenance of low temperatures for several days.

Fish Tissues

Fish samples will be stored in short-term archives. Samples in the short-term archive are stored at -20 °C and are intended for use in the identification of short-term time trends (i.e. < 5-10 years), the investigation of yet unidentified chemical contaminants, and addressing quality assurance issues that may arise during the routine analyses of samples. The short-term archives will be located in an off-site freezer facility rented by Moss Landing Marine Laboratory. The facility is not equipped with a backup generator; however, in the event of power failure the facility contingency plan is to keep the freezer closed, providing maintenance of low temperatures for several days.

Timeline

Field work for this project will be conducted in the summers (April-October) of 2012 and 2013. Laboratory sample processing and mercury analysis will be conducted in winter and spring of 2012/2013 and 2013/2014. Data analysis and report writing will occur in spring and summer 2014. A draft year one report after the first field season will be delivered in April 2013, with description of any changes for year 2 field sampling. A final report on year one will be delivered in March 2014. A final report on the two year study will be delivered in March 2015.

Figure 1. Sampling locations for grebes and fish at 24 lakes during 2 field seasons in 2012 and 2013.

Xx list of likely sampling locations would be good
 Xx there is no symbol explaining Ramer Lake on the legend

Proposed lakes for sampling mercury concentrations in western grebes and fish for calculating a biomagnification factor. Red-scale color palette sites are those lakes where grebes are known to have recently bred. Blue-scale color palette sites are those lakes where grebes are known to have bred historically. Green-scale color palette sites are those lakes where grebes occur in the summer but it is not known whether they breed. Darker colored sites indicate a long-term BOG site for sport fish trend monitoring. Stars indicate sites that have been previously sampled by BOG for sport fish. Circles indicate the 24 primary lakes selected, whereas squares indicate alternate lakes that will be used if grebes cannot be sampled at a primary lake after scouting. Other lakes not depicted on this map may also be used for grebe and fish sampling if necessary and further information is found. The relative size of the symbol indicates mercury concentrations in sport fish from BOG sampling during 2007-2011.



Table 1. Target sport fish species and their characteristics.

Species	Foraging Type		Trophic Level	Distribution			Priority for Collection
	Water column	Bottom feeder		Low Elevation	Foot hills	High Elevation	
Largemouth bass	X		4	X	X		1
Smallmouth bass	X		4	x	X		2
Spotted bass	X		4	x	X		2
Sacramento pikeminnow	X		4	x	x		2
Rainbow trout	X		3	x	x	X	2
Brown trout	X		3/4		x	x	1
Eagle Lake trout	X		3			x	2

Trophic levels are the hierarchical strata of a food web characterized by organisms that are the same number of steps removed from the primary producers. The USEPA's 1997 Mercury Study Report to Congress used the following criteria to designate trophic levels based on an organism's feeding habits:

Trophic level 1: Phytoplankton.

Trophic level 2: Zooplankton and benthic invertebrates.

Trophic level 3: Organisms that consume zooplankton, benthic invertebrates, and TL2 organisms.

Trophic level 4: Organisms that consume trophic level 3 organisms.

X widely abundant X less widely abundant "1" primary target for collection "2" secondary target for collection

Table 2. Target sport fish species and size ranges.

	Numbers and Size Ranges (mm)
Black bass (largemouth, smallmouth, spotted)	2X(200-249), 2X(250-304), 5X(305-407), 2X(>407)
Sacramento pikeminnow	3X(200-300), 3X(300-400), 3X(400-500)
Rainbow trout	5X(300-400)
Brown trout	5X(300-400), and keep up to five fish > 400 if present
Eagle Lake trout	5X(300-400), and keep up to five fish > 400 if present

Appendix III: MPSL-DFG SOPs

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

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

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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

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- 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

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- 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

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- 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 # z140406redline
- 4.63 Zipper-closure Polyethylene Bags, 4milx6"x8": Packaging Store Part # z140608redline
- 4.64 Zipper-closure Polyethylene Bags, 4milx9"x12": Packaging Store Part # z1400912redline
- 4.65 Zipper-closure Polyethylene Bags, 4milx12"x15": Packaging Store Part # z1401215redline
- 4.66 Zipper-closure Polyethylene Bags, 4milx13"x18": Packaging Store Part # z1401318redline

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)

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- 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 (HNO₃), concentrated redistilled: Seastar Chemicals Part # BA-01
- 5.14 Nitric Acid (HNO₃), BAKER INSTRA-ANALYZED**, 69.0–70.0% (15N): VWR Part # JT9598-34
- 5.15 Nitric Acid (HNO₃), 7.5N (50%): prepared by adding 1 part Baker HNO₃ to 1 part MilliQ
- 5.16 Nitric Acid (HNO₃), 6%: prepared by adding 1 part Seastar HNO₃ to 16.67 parts MilliQ
- 5.17 Nitric Acid (HNO₃), 1%: prepared by adding 1 part Seastar HNO₃ 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).

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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% HNO₃ 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

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- 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 HNO₃, cap. Allow outside of bottle to dry.

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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 Micro/Tap solution.

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

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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 HNO₃ 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 HNO₃ 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 in 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.

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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.

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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.

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- 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% HNO₃ 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.

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- 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.

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- 7.2.3.2 Submerge in 50% Baker HNO₃ 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% HNO₃ 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

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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.

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7.4.2.3 Under a fume hood, rinse each jar and lid three times with Petroleum Ether by putting a small 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 HNO₃ coldbath overnight.

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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% HNO₃ by putting a small 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 HNO₃ 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 # MPSSL-105.
- 8.2 Trace Metal and Mercury Only digestion procedures can be found in EPA 3052, modified, and Method # MPSSL-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 # MPSSL-103 or by DMA and EPA 7473.
- 8.5 Methylmercury tissue samples are extracted and analyzed according to Method # MPSSL-109.
- 8.6 Methylmercury sediment samples are extracted and analyzed according to Method # MPSSL-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 B: MPSL-102a Sampling Marine and Freshwater Bivalves, Fish and Crabs for Trace Metal and Synthetic Organic Analysis

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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 # MPSSL-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 MPSSL 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 # z1401318redline

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 contaminants 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.

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- 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.

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- 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

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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.

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- 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.

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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 # MPSSL-105.
- 8.2 Trace Metal and Mercury Only digestion procedures can be found in EPA 3052, modified, and Method # MPSSL-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 # MPSSL-103 or by DMA and EPA 7473.
- 8.5 Methylmercury tissue samples are extracted and analyzed according to Method # MPSSL-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.
- 10.2 Goldberg, E.D., ed. 1980. The International Mussel Watch. National Academy of Sciences Publ., Washington, D.C.
- 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.

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- 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 C: MPSL-104 Sample Receipt and Check-In

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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

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

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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 # MPLS-106 and Method # MPLS-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 # MPLS-103 or by DMA and EPA 7473.

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

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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.

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- 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 # MPSTL-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

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- 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 (HNO₃), BAKER INSTRA-ANALYZED*, 69.0–70.0%: VWR Part # JT9598-34
- 5.10 Nitric Acid (HNO₃), 50%: prepared by adding 1 part Baker HNO₃ to 1 part MilliQ

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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.

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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.

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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.

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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.

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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% HNO₃ 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% HNO₃, 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.

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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 # MPSSL-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 # MPSSL-103 or by DMA and EPA 7473.
- 8.4 Methylmercury tissue samples are extracted and analyzed according to Method # MPSSL-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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- 11.3 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.
- 11.4 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

Appendix IV: USGS SOPs

USGS Standard Operating Procedures			
Page	Procedure/Equipment	SOP number	Revision Date
A	Western Grebe Egg Collection		March 2012
B	Collection of Avian Blood		March 2012
C	Sample Processing		December 2011

Appendix IV A: Western Grebe Egg Collection

Edited on 5/25/10 (CES)

Page 1

Standard Operating Procedure Western Grebe Egg Collections in CA Lakes 2012

Objectives

- (1) Collect up to 30 eggs per colony.
- (2) Collect only 1 egg randomly from each nest.
- (3) Target eggs from active nests but also collect salvage eggs when necessary.

Equipment

- Blue sharpies (please use blue to write on eggs)
- GPS unit (Decimal degrees in NAD83)
- Float cup & fresh water
- Soft-sided cooler for egg storage
- Whirlpaks & plastic Ziplocs for eggs
- Egg cartons

Data sheets

- Egg collection data sheet
- Floatation chart

Egg Collection Data Sheet – write in thin blue Sharpie provided

ID Code: Pre-printed ID codes will be provided prior to leaving for the field.

Date: Record collection date.

Site: Record the lake name

Coordinates Latitude/Longitude: Record nest location in UTM's with NAD83 projection

Number of Eggs in Nest: record the current number of eggs in the clutch before the egg was collected

Float Incubation Stage: record the age the collected egg is floating at, or the incubation age via candling in the field

Nest Status: only collect eggs from viable, currently active nests where the parent(s) are still actively incubating the clutch. If you must (see "Objectives" for only times to do this) collect an egg from an abandoned nest, or a nest with dead or infertile eggs, please note this.

Notes: record any notes about disturbance, failed-to-hatch eggs, abnormal nests, etc.

Egg Storage - Once You Have the Egg Collected

- (1) Please carefully write in **thick blue sharpie** on the egg shell:
 - a. Egg ID Code
 - b. Date
 - c. Site
 - d. Species

Edited on 5/25/10 (CES)

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- (2) Please write in thick blue sharpie on the whirl-pak or Ziplocs:
 - a. Egg ID Code
 - b. Date
 - c. Site
 - d. Species
- (3) Place the labeled egg into the labeled Ziploc/whirl-pak. **Do NOT** seal the bag; the eggs will mold.
- (4) Place the egg wrapped in the Ziploc/whirl-pak into a regular chicken egg carton.
- (5) Place the egg carton in a soft-sided cooler in the field (preferably on a small blue ice pack).
- (6) At the end of the day, place the egg carton in a large cooler with ice. Do not try to jam the egg carton closed! You may break eggs. It is better to leave it open then try to close it and break eggs.
- (7) Write in blue sharpie on the egg carton the general date, species and site where those eggs were collected.

Appendix IV B: Collection of Avian Blood

Edited on 3/30/12 (CES)

Page 1

Standard Operating Protocol for the Collection of Avian Blood

Supplies:

- Syringes (various volumes: 1ml, 3 ml, 5ml)
 - 3ml syringes are the most universal and generally recommended unless working with very small or large veins.
- Needles (various sizes: 27ga., 26ga., 25ga., 23ga., 22ga.)
 - 25 and 23 gauge needles are generally most effective. Use smaller if there is difficulty with 25 ga.
- Cryovials (Pre-labeled; 2.0 ml recommended, but 1.2 OK)
- Cryovial storage boxes
- Alcohol wipes
- Cotton absorbent pads
- Nitrile gloves
- Wet or dry ice
- Sharps/bio-waste container
- Data sheets
- Heat pads
- Folding table
- Sodium heparin

Blood Collection Site

- Several locations can be used for blood collection. Selection should depend upon species, bleeder experience and available supplies. In general, the brachial and jugular veins are preferred. However, the tarsal vein may also be used in some circumstances.

Bleeding Preparation

- Prior to bleeding, be sure to have pre-labeled cryovials and heparinized needles and syringes ready.
 - To heparinize needles/syringes, draw a very small amount of heparin through needle, into syringes and inject back into heparin bottle. Remove needle from heparin bottle and pump the plunger several times vigorously to eject most of the remaining heparin in the syringe.
 - This is important to avoid diluting the sample and skewing the overall weight, thus biasing the concentrations.

Edited on 3/30/12 (CES)

Page 2

Bird Preparation

- After capture hold birds in appropriate sized cage until processing.
- Remove an individual bird from cage, bring to table, and keep head covered with cotton bird bag or pillow case.
- One person should hold the bird on its back and display the ventral surface of the whole wing (for wing bleeding).
- Locate the brachial vein and wipe area with alcohol wipes.

Blood Collection

- Carefully insert needle (with bevel facing up) into selected vein and slowly pull back on plunger making sure there is continuous flow into the syringe.
- When target blood volume has been reached (MAX = 1% of body weight), slowly remove and immediately cap needle and place a cotton pad over the collection site. Place pressure over the collection site until bleeding has stopped.
- Remove needle from syringe, inject blood into cryovial, cap cryovial, and place in storage box on wet or dry ice.
- Discard needle and syringe into sharps/biohazard container.

Blood Storage (in the field)

- Keep blood on dry or wet ice (dry preferred),
 - Freeze upright so blood pools in bottom of cryovial.
 - Freeze as quickly as possible.

Blood Storage (in the lab)

- Upon return to laboratory, immediately place blood in freezer (-20 C).
- Freeze upright.
- Do not allow blood to freeze and thaw, keep frozen.

Appendix IV C: Sample Processing

Sample Processing Standard Operating Procedures



Forest and Rangeland Ecosystem Science Center

**CONTAMINANT ECOLOGY
RESEARCH PROGRAM**

Collin Eagles-Smith
Revised 12/27/2011



Quick View Processing SOP

1. Clean work space, tools, analytical equipment.
2. Thaw samples.
3. Prepare data sheets.
4. Generate unique tissue CERP ID Codes (if dissecting tissues from sample).
5. Generate tough tags for tissue samples.
6. Update google docs.
7. Weigh whole body sample (wet weight).
8. Dissect (this process differs by project and sample type and often includes additional steps not identified in this SOP. (For birds, refer to *S:\Projects\Eagles-SmithLab\SOPs\Birds\Egg dissection*).
9. Dry samples.
10. Weigh dry sample.
11. Grind samples.
12. Enter data.
 - a. Use *DataEntryTemplates_EasyAccesssTemplate* found in *S:\Projects\Eagles-SmithLab\Data*.
 - b. Save data in *S:\Projects\Eagles-SmithLab\Active Projects*.
13. Verify (proof) data.
14. Scan data sheets.
 - a. Save scans in *S:\Projects\Eagles-SmithLab\Scanned datasheets*
15. Store original data sheets in data repository filing cabinet.
16. Update google docs.
17. Once all data are verified, move folder into the “*Data*” folder (*S:\Projects\Eagles-SmithLab\Data*) indicating data are ready to be uploaded to database.

**USGS FRESC Contaminant Ecology Research Program
Standard Operating Procedure (SOP)
Fish Sample Processing
12/20/2011**

The sample processing component is the step in the workflow immediately after cataloging, in which samples are dissected (when applicable), dried, and homogenized prior to chemical analyses. This step often occurs over several days as samples are dried, then homogenized. Thus, special care must be taken to ensure that datasheets are carefully tracked.

In some cases, this step is merged with the cataloging step and both are completed simultaneously. Be sure to check with project leader(s) to verify the most appropriate approach for each sample set.

1. Laboratory conditions and equipment cleanliness

- 1.1. Tape clean lab bench paper or aluminum foil to the lab bench.
- 1.2. Prior to sample processing, the surfaces of all processing locations and tools shall be cleaned and thoroughly rinsed with DI water. All processing tools shall also be rinsed with DI water and wiped with a clean KimWipe between each sample.
- 1.3. Gently wipe the analytical balance (using care not to apply pressure to the weigh pan) and ensure that it is calibrated daily, prior to use.

2. Initial sample tracking and cataloging

- 2.1. From the freezer, remove only the number of samples that can be initially processed (cleaned, weighed, and inserted into drying oven) in the allotted time for the given day. These samples should not be thawed for ~1 hour before processing.
- 2.2. Prepare data sheets. Use the processing datasheet template Excel file in the "*Data sheet templates*" folder and add or remove columns as necessary. (See the *CERP_DATA_database* or previous project templates for reference).
- 2.3. Save this file as: "*ProjectID_Proc_MATRIX_##_mddy.xls*" (where: *ProjectID* is the unique ID for that project (refer to *tbl CERP_Project List* in the database: *S:\Projects\Eagles-SmithLab\Databases*), (where *MATRIX* is the type of sample being analyzed fish/bird eggs/invertebrates/etc...) and *##* is a unique number for that datasheet template). Put a copy of these datasheets in the *lab datasheet templates* subfolder within the *datasheet templates* folder on the share drive.
- 2.4. Open the Google Docs file entitled "*Lab project tracking*" and enter the page numbers you will be using under the Drying and grinding data sheet page numbers column.
- 2.5. Print datasheets and make sure each datasheet has the current date and page numbers in the header.

- 2.6. Prepare tough tags with appropriate *CERP ID Code* (refer to the *Master Sample ID codes_ToughTAGs* (*S:\Projects\Eagles-SmithLab\Sample ID Codes_ToughTags*) Excel sheet and the Access database to identify a list of unique codes. Note: all tissue samples have different codes (refer to the '*tbl TissueCode*' in the database (*S:\Projects\Eagles-SmithLab\Databases*)). See *ID Code SOP* for more detail.
 - 2.7. Store all data sheets in the respective binder until all samples have been processed.
3. **Sample cleaning and wet weight** – *This step may seem redundant with the weighing involved in the cataloging step. However, recording the exact wet weight of a sample just prior to drying is critical for data integrity. We often assess these values in comparison to the catalog weights to evaluate desiccation in the freezer due to sublimation.*
 - 3.1. Wearing powderless nitrile gloves remove sample from container and rinse the surface of each thawed sample with DI water and pat dry with clean KimWipe.
 - 3.2. Obtain one drying vessel (e.g. aluminum or plastic weigh boat, sample vial, etc.) for each sample and write the appropriate *CERP ID Code* on each weigh boat.
 - 3.3. With the balance empty, press the tare button to zero the balance. Place each weigh boat on the balance and record the weight in the “**Drying vessel weight**” cell on the hard copy data sheet.
 - 3.4. With a clean, dry KimWipe, pat the surface of the sample dry and place in weigh boat. Obtain a sample wet weight (be sure that the weight of the drying vessel is included in this – **i.e. DON'T TARE THE WEIGHT BOAT!**) and hand record in the “**Lab wet weight + drying vessel**” cell on the hard copy data sheet.
4. **Dissection** – *The dissection process varies by project. The following are general procedures outlining the most basic dissection steps. In most cases either 1) the whole organism will be processed and thus no dissection process is warranted, or 2) tissue samples will be extracted and processed for later analyses.*
 - 4.1. With the balance empty, press the tare button to zero the balance.
 - 4.2. Label each drying weigh boat with *CERP ID Code*.
 - 4.3. Place each weigh boat on the balance and record the weight in the “Drying vessel weight” cell on the data sheet.
 - 4.4. Dissect out appropriate tissue(s). Record *CERP ID* with respective tissue code in appropriate column(s).
 - 4.5. For samples in which a muscle tissue is extracted for processing instead of the whole organism, dissect the dorsal portion of muscle between the head and dorsal fin, along the side of the spine (see Figure 1). Remove at least **1 gram** of muscle (both sides of the fish can be pooled if necessary; when applicable make note on data sheet).
 - 4.6. Obtain a sample wet weight for each tissue (be sure that the weight of the drying vessel is included in this – **i.e. DON'T TARE THE WEIGHT BOAT!**) in the “**tissue wet weight + drying vessel**” cell on the data sheet. Be sure to indicate the tissue type on the data sheet or that the appropriate column exists for the tissue (e.g. IDMuscleAxial, IDKidney, etc.).

4.7. Place tissue in drying oven.

5. Sample drying

- 5.1. Turn on drying oven and set to 50 degrees C.
- 5.2. Load samples onto a plastic or aluminum tray, and place the tray in oven.
- 5.3. Log the sample on the drying oven log sheet (on the front of the oven).
- 5.4. Record the dry start date and drying temperature on the processing data sheet.
- 5.5. Allow sample to dry for 48 hours or until a constant mass is achieved (change in mass in 8 hours is <1%).
- 5.6. Open the Google Doc file "*Lab_project tracking*" and fill out the appropriate fields with your initials and % completed: (e.g. NB 50%)
 - 5.6.1. Samples dried (N) [N=number]

6. Sample dry weight

- 6.1. After samples have dried, remove them from the oven (while still warm) and place into large dessicator to cool (~ 20 minutes).
- 6.2. Log sample removal on drying oven log sheet.
- 6.3. Record total dry time in the appropriate cell on processing data sheet.
- 6.4. Remove 3-4 samples from dessicator (NOT the whole tray), place a dried sample **AND** its associated weigh boat onto a calibrated balance and record mass (in grams) in the "**Dry weight + drying vessel**" column on the data sheet.
- 6.5. Place sample in clean glass vial and label with a ToughTag that has *Project ID* and *CERP ID* code on it. Tissue may need to be broken into pieces to fit into vials.

7. Sample grinding

- 7.1. Clean grinding apparatus (Wiley Mill, Cryogrinder, IKA mill, or mortar and pestle). The specific apparatus will vary with sample type and many samples can be ground using a variety of methods (discuss with the project leader(s)). If the sample mass is very small, use mortar and pestle with wax paper, as it reduces sample loss during the grinding process.
- 7.2. Grind sample to a uniform consistency (fine powder) and record grind method and date on data sheet.
- 7.3. Carefully pour ground sample back into glass vial, using care not to lose material in the transfer, or contaminate other samples.
- 7.4. Clean grinding apparatus between samples. Mortar and pestle: clean with DI water and dry with KimWipe; Wiley Mill: clean with compressed air and brush; Cryogrinder and IKA mill: clean with either DI water and KimWipe or compressed air.

8. Post processing

- 8.1. Enter all data from hard copy datasheet into the appropriate electronic file and save as "*ProjectID_Proc_MATRIX_data_ddmmyy.xls*" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc...). Use the *DataEntryTemplate_Easy AccessUpload* excel file as a template for data entry. This file contains the correct column headings and additional information used in the database but not recorded in the laboratory during dissecting/processing samples. Note: column headings used on laboratory data sheets are identified in row 2 (under the headings used in access). Appropriate column headings may be found on different worksheets for database purposes (i.e., data collected during the "cataloging" process in the lab may actually be found in the "morphology/dissection" worksheet in the DateEntryTemplate excel file). Add and delete columns as necessary for each particular project. All data can be entered on one excel worksheet. For further description of column headings see the access database design view for the respective table
- 8.2. Proof (verify) all entered data.
- 8.3. Initial the data sheets following data entry and proofing data.
- 8.4. Scan hard copy datasheets and save the file as "*ProjectID_Proc_MATRIX_scandata_ddmmyy.xls*" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc...) Place PDF of datasheets into the Scanned datasheets folder on the share drive.
- 8.5. File the hard copy of the original datasheets in the current year's data folder in the data repository filing cabinet.
- 8.6. Data are ready for uploading to the database, so move excel files to data folder on the share drive (*S:\Projects\Eagles-SmithLab\Data*).
- 8.7. Open the Google Docs file entitled "*Lab_project tracking*" and update the processing fields with your initials and necessary information. The following fields **must** be completed on the Google Docs project tracking datasheet before moving on to processing:
 - 8.7.1. Samples dried (N) N= number of samples
 - 8.7.2. Samples ground (N) N=number of samples
 - 8.7.3. Drying and grinding data entered
 - 8.7.4. Drying and grinding data sheet file names and locations
 - 8.7.5. Drying and grinding data sheet page numbers
 - 8.7.6. Drying and grinding data sheet file names and locations
 - 8.7.7. Processing data proofed
 - 8.7.8. Drying data sheets scanned and filed

Appendix V: MPSL-MLML SOPs

MPSL-MLML Procedures			
Page	Procedure/Equipment	SOP Number	Revision Date
A	Verification of the Surface Water Ambient Monitoring Program Database		March 2011
B	BOG Data Validation SOP		April 2011

Appendix V A: SWAMP SOP Chemistry Data Verification v1.1

This document is an official SWAMP SOP and can be found at:

http://swamp.mpsl.mlml.calstate.edu/wp-content/uploads/2011/06/SWAMP_SOP_Chemistry_Data_Verification_03.23.11.pdf

Appendix V B: BOG Data Validation SOP

BOG Data Validation Standard Operating Procedure

Blank Contamination Check

Blank verification samples identify if the target analyte has contaminated field samples via lab contamination from any part of sample preparation and analysis. One method blank (laboratory derived) sample is run with each analytical batch (≤ 20 samples). The method blanks will be processed through the entire analytical procedure in a manner identical to the field samples. The ideal scenario is that method blank samples are non-detects. If a field sample is contaminated from laboratory procedures and the analytical quantification of that field sample is low, then a high proportion of the field sample value could be from laboratory contamination which results in that value being uncertain and not usable. Laboratory blank contamination could result in a false positive when field sample results are low. There is less concern of blank contamination affecting a field sample if field samples are some multiple higher than the method blank result (in this case 3 times the method blank concentration).

In order to determine if field samples have been contaminated, the following data validation method is applied:

1. If there is more than 1 method blank in a batch, use the method blank with the highest concentration.
2. Second, compare the highest method blank concentration to the method blank Method Detection Limit (MDL) (Note: SWAMP has a method blank MQO of $<$ Reporting Limit (RL) for all targeted analytes. If the method blank concentration is greater than the RL then corrective action needs to be taken by the lab prior to submitting data to the DMT. For the data validation exercise any quantitation of the method blank above the MDL is considered a detection and therefore the data validation exercise uses the MDL as the threshold for assessing blank contamination):
 - a. If the Method Blank concentration is less than ($<$) the Method Blank MDL then there is no detection of that analyte in the blank sample. This suggests that there was no laboratory contamination of field samples and no further action for that analyte, in that batch, is required.
 - b. If the Method Blank concentration is greater than ($>$) the Method Blank MDL then the method blank sample has been contaminated with the targeted analyte and there is possible contamination of associated field samples. For those cases where the method blank result is greater than the MDL, compare the field sample results to the highest Method Blank result for each batch. Be sure that the Method Blank results, MDLs, and field sample results are all in the same units and basis (wet weight or dry weight).
 - i. If the field result is less than ($<$) 3x highest Method Blank concentration then flag that field sample with a QACode of VRIP. This sample is considered a censored result (the blank contamination is likely too large a component of the field result to be differentiated). The compliance code is REJ.

- ii. If the field result is greater than ($>$) 3x highest Method Blank, then the sample should be flagged with QACode VIP if not already IP flagged. The compliance code is QUAL.

Accuracy check

Accuracy is the degree of agreement of a measurement with a known value and is utilized to assess the degree of closeness of field samples to their real value. Using the bull's-eye analogy (Figure 1), accuracy is the degree of closeness to the bull's-eye (which represents the true value). Over/under estimation of analytical quantification is important in this project. If the QA elements indicate overestimation of the field sample result than this could lead to false positives above particular human health consumption thresholds and potentially limit human consumption of particular sport fish species. If the QA elements indicate underestimated analytical quantification then low field sample values could falsely suggest that fish are below human health thresholds when they may actually be above the thresholds. Good accuracy in a data set increases the confidence and certainty that the field sample value is close to the true value. Accuracy is determined by such QC elements as: certified reference materials (CRM), laboratory control samples, blind spikes, matrix spikes, and performance samples.

Figure 1. Demonstration of target accuracy (black marks) to a known value (bull's-eye). The figure shows very good accuracy but poor precision.



Table 1. (Table 12a from BOG QAPP) shows BOG Measurement Quality Objectives for inorganic analytes in tissues

SWAMP Measurement Quality Objectives* - General		
Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Calibration Standard	Per analytical method or manufacturer's specifications	Per analytical method or manufacturer's specifications
Continuing Calibration Verification	Per 10 analytical runs	80-120% recovery
Laboratory Blank	Per 20 samples or per batch, whichever is more frequent	Blanks <ML for target analyte
Reference Material	Per 20 samples or per batch, whichever is more frequent	75-125% recovery
Matrix Spike	Per 20 samples or per batch, whichever is more frequent	75-125% recovery
Matrix Spike Duplicate	Per 20 samples or per batch, whichever is more frequent	75-125% recovery, RPD $\leq 25\%$
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent	RPD $\leq 25\%$; n/a if concentration of either sample <ML
Internal Standard	Accompanying every analytical run when method appropriate	75-125% recovery

*Unless method specifies more stringent requirements.

ML = minimum level (Puckett, 2002)

n/a = not applicable

Table 2. (Table 12b from BOG QAPP) shows BOG Measurement Quality Objectives for synthetic organic analytes in tissues

SWAMP Measurement Quality Objectives* - General		
Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Calibration Standard	Per analytical method or manufacturer's specifications	Per analytical method or manufacturer's specifications
Continuing Calibration Verification	Per 10 analytical runs	75-125% recovery
Laboratory Blank	Per 20 samples or per batch, whichever is more frequent	Blanks <ML for target analytes
Reference Material	Method validation: as many as required to assess accuracy and precision of method before routine analysis of samples; routine accuracy assessment: per 20 samples or per batch (preferably blind)	70-130% recovery if certified; otherwise, 50-150% recovery
Matrix Spike	Per 20 samples or per batch, whichever is more frequent	50-150% recovery or control limits based on 3x the standard deviation of laboratory's actual method recoveries
Matrix Spike Duplicate	Per 20 samples or per batch, whichever is more frequent	50-150% recovery, RPD $\leq 25\%$
Laboratory Duplicate	Per 20 samples or per batch, whichever is more frequent	RPD $\leq 25\%$; n/a if concentration of either sample <ML
Surrogate or Internal Standard	As specified in method	50-150% recovery

*Unless method specifies more stringent requirements.

MDL = method detection limit (to be determined according to the SWAMP QA Management Plan)

n/a = not applicable

For the accuracy data validation, SWAMP follows a multiple failure rule. The possible QC elements for the accuracy check are:

CRM, Reference Material, LCS, Matrix Spike/Matrix Spike Duplicate¹

Only samples in a quantitative range should be used for evaluation of accuracy, as non-quantitative results may be lucky passes or unlucky fails rather than true indications of the ability for the analysis to accurately determine concentrations

- For any of the accuracy QC samples, Expected Value must be at least 1xRL, otherwise it shouldn't be used.
- Additionally for MS/MSDs, the Matrix Spike Expected Value should be greater than or equal to 3x the Native Field Result.

Data Validation for Accuracy:

If there are no valid QC elements available based on the quantitative range screening from above, then apply QACode "VQCA" to all of the related results in that batch.

For the remaining QC samples in a quantitative range, the following apply where there is more than one usable measure.

1. Following SWAMP MQOs, one QC element is allowed to be outside the MQO for accuracy (occurs when the QC element is less than or greater than the MQO target range (see Tables 1 and 2 above) but less than 2 times the MQO range (see method for determining this "2x" range in item 3 below) in a batch and still be compliant. If one QC element in a batch is outside the MQO, then the individual QC sample is given a QACode of (EUM, GBC, or GB). The compliance code for the associated field samples is COM.
2. When more than one QC element is outside of the MQO, each QC element is given a QACode (EUM, GBC, GB). The compliance code for the associated field samples is QUAL. In these cases, a QACode of "VIU" is applied to the field samples.
3. **Rejection Point:** The QACode "VRIU" is applied to the field samples when the % Recovery is more than 2 times outside the MQO target range (see Tables 1 and 2) or when the lower rejection limit is <10%, in 2 or more QC elements (CRM, Reference Material, LCS, MS/MSD). In these cases, the compliance code is changed to REJ. The QACode is applied to all field samples in the affected batch including those that are not quantifiable (flagged with ND (not detected) in ResQualCode). Below is the method for determining the upper and lower rejection limits:
 - Lower Rejection Limit = $100 - (2 * (100 - \text{lower limit of the range}))$
 - Upper Rejection Limit = $100 + (2 * (\text{upper limit of the range} - 100))$

¹ Matrix Spike/Matrix Spike Duplicate, preferably, alone cannot be used to evaluate the precision and accuracy of individual samples. However, when exercising professional judgment, these QA elements should be used in conjunction with other available QC information.

As an example, the acceptable range for certified reference material for organics is percent recovery 70-130%. The lower rejection limit would be $100 - (2 * (100 - 70)) = 40$ and the upper rejection limit would be $100 + (2 * (130 - 100)) = 160$. Recoveries less than 40% and greater than 160% are more than 2 times outside the MQO target Range which would result in a compliance code of REJ and a QACode of VRIU.

If there is only one usable QC sample for accuracy evaluation, the individual QC sample is flagged as appropriate, and the following applies to the batch:

4. In the case where there is only one QC element reported in the batch and the % Recovery is more than 1 time outside the MQO target range (see Tables 1 and 2) but less than 2 times the target range then the compliance code would be QUAL and a QACode VIU is applied to the field samples in that batch.
5. **Rejection Point:** In the case where there is only one QC element reported in the batch and the %Recovery was more than 2 times outside the MQO target range (see Tables 1 and 2) or when the lower rejection limit is <10%, then the compliance code would be REJ and the QACode VRIU is applied to the field samples in that batch.

Table 3 summarizes the application of QACodes for the accuracy check scenarios above.

Table 3. Accuracy Data Validation Rules – where there are more than 2 quantitative (usable) measures, A & B are the two quantitative measures with the worst performance for any given analyte

Measure A Range	Measure B Range	QACode	Comment
>±2x range or when the lower rejection limit is <10%	>±2x range or when the lower rejection limit is <10%	VRIU	Both badly fail.
>±2x range or when the lower rejection limit is <10%	>±1x range - <±2x range	VIU	One badly, one marginally fail
>±2x range or when the lower rejection limit is <10%	Within range	None	One badly fail, remainder pass
>±2x range or when the lower rejection limit is <10%	Null	VRIU	One badly fail
>±1x range - <±2x range	>±1x range - <±2x range	VIU	Both marginally fail
>±1x range - <±2x range	Within range	None	One marginally fail, remainder pass
>±1x range - <±2x range	Null	VIU	One marginally fail
Within range	Within range	None	Both pass

Precision check

Precision is the degree to which repeated measurements under unchanged conditions show the same result (usually reported as a relative standard deviation [RSD] or relative percent difference [RPD]). The repeatability measure indicates the variability observed within a laboratory, over a short time, using a single operator, item of equipment, etc. These QA elements also show the reproducibility of an analytical measurement. Good precision provides confidence that the analytical process is consistently measuring the target analyte in a particular matrix.

The possible QC elements in the precision check are:

Lab duplicates, Matrix Spikes/Matrix Spike Duplicates, LCS/LCSD. See Tables 1 and 2 above for MQOs.

Similar to the case for evaluating accuracy, only results in a usable quantitative range should be used to calculate precision.

- Check for each sample (pair or set) analyzed in replicate that the average result is greater than (>) 1 times the RL. If the average result is greater than (>) 1 times the RL then include RPD or RSD in lab tests submission evaluation. Otherwise that set of sample replicates is not quantitative and thus not usable.

Data Validation for Precision:

If there are no valid precision QC elements available based on the quantitative range screening from above, then apply QACode "VQCP" to all of the related results in that batch.

For the remaining QC samples in a quantitative range, the following apply where there is more than one set of replicates.

1. When one or more QC elements for precision (e.g. lab duplicate or MS/MSD) is greater than 1 time to less than 2 times the target (for organics and metals RPD or RSD greater than 25% to less than 50%, Tables 1 and 2 above) then the field samples within that batch are flagged with a QACode of VIL. The compliance code is QUAL.
2. If one QC elements fails badly (> 50% RPD), then consider the RPD/RSD of the other QC elements (e.g. MS/MSD, LCS/LCSD) for that analyte. If other QC elements pass ($\leq 25\%$), or marginally fail ($25\% < \text{RPD} < 50\%$), and there are no other indications of ongoing QA problems, then assign the samples within that batch, for that analyte, with a QACode of VIL. The compliance code is QUAL.
3. **Rejection Point:** If more than one QC element fails badly (> 50% RPD), then assign a QACode of VRIL to the samples for that analyte in the batch and a compliance code of REJ.

If there is only one usable quantitative measure, the following apply:

4. If there is only one QC element reported in the batch and the RPD is greater than 1 time to less than 2 times the target (for organics and metals greater than 25% to less than 50%) then the field samples within that batch are flagged with a QACode of VIL. The compliance code is QUAL.
5. **Rejection Point** : If there is only one QC element reported in the batch and the RPD was more than 2 times outside the MQO target (> 50%) then the compliance code would be REJ and the QACode VRIL is applied to the associated field samples in that batch

Table 4 summarizes the application of QACodes for the precision check scenarios described above.

Table 4. Precision Data Validation Rules where there are more than two usable measures, use the two worst as A & B

Measure A	Measure B	QACode	Comment
>50%	>50%	VRIL	Both bad fail.
>50%	>25%	VIL	One bad, one marginal fail
>50%	<25%	VIL	One bad fail, rest pass.
>50%	Null	VRIL	One usable, bad fail
>25%	>25%	VIL	Both marginal fail
>25%	<25%	VIL	One marginal fail, one pass
>25%	Null	VIL	One usable, marginal fail
<25%	<25%	None	Both good

(for analytes where RPD or RSD limits are not 25%, substitute 1x those limits for 25% and 2x those limits instead of 50%)

Assumptions:

Measure A and B can be either different types of elements (duplicates, MS/MSD) or pairs of the same type of measure. Each measure is treated separately and not averaged when there are multiple pairs of the same measure (e.g. do not average RPD if there are 2 sets of replicates).

Glossary

Calibration Standard: Calibration standards are the measurement of an absolute value of a target analyte and in many cases, the standards are traceable back to standards at the National Institute for Standards and Technology. A **calibration curve** is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration. A calibration curve is one approach to the problem of instrument calibration.

Certified Reference Material: CRMs are similar in matrix and concentration range to the samples being prepared and analyzed. The accuracy of an analytical method can be assessed using CRMs only when certified values are provided for the target analytes.

Continuing Calibration Verification: Calibration verification solutions traceable to a recognized organization are inserted as part of the sample stream. The sources of the calibration verification solutions are independent from the standards used for the calibration. Calibration verification solutions used for the CCV will contain all the analytes of interest.

Expected Value: the concentration of the analyte in a reference standard, laboratory control sample or matrix spike sample, or the value expected to be obtained from analysis of the QC sample. This consists of the native sample result concentration plus the spike amount.

Internal (or Surrogate) Standard: To optimize gas chromatography mass spectrometry (GC-MS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analyses, internal standards (also referred to as “injection internal standards”) may be added to field and QC sample extracts prior to injection. Use of internal standards is particularly important for analysis of complex extracts subject to retention time shifts relative to the analysis of standards. The internal standards can also be used to detect and correct for problems in the GC injection port or other parts of the instrument.

Laboratory Control Sample: An LCS is a specimen of known composition prepared using contaminant-free reagent water or an inert solid spiked with the target analyte at the midpoint of the calibration curve or at the level of concern. The LCS must be analyzed using the same preparation, reagents, and analytical methods employed for regular samples.

Laboratory Duplicate: In order to evaluate the precision of an analytical process, a field sample is selected and digested or extracted in duplicate and analyzed according to the method.

Matrix Spike: A matrix spike (MS) is prepared by adding a known concentration of the target analyte to a field sample (spike amount), which is then subjected to the entire analytical procedure. If the ambient concentration of the field sample is known, the amount of spike added is within a specified range of that concentration. Matrix spikes are

analyzed in order to assess the magnitude of matrix interference. Because matrix spikes are analyzed in pairs, the second spike is called the matrix spike duplicate (MSD).

Method Blank: A laboratory blank prepared to represent the sample matrix as closely as possible and analyzed exactly like the calibration standards, samples, and quality control (QC) samples. Results of method blanks provide an estimate of the within-batch variability of the blank response.

Method Detection Limit or Method Limit: EPA defines the method detection limit as, "the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte." Any sample that is not quantifiable is considered to be not detected and below the MDL.

Measurement Quality Objectives: Numerical acceptance criteria for the quality attributes measured by project data quality indicators. During project planning, measurement quality objectives are established as quantitative measures of performance against selected data quality indicators, such as precision, bias, representativeness, completeness, comparability, and sensitivity.

Native Sample: the original sample to which a known spike amount is added. The native sample plus spike becomes a Matrix Spike.

Reference Material: The distinction between a reference material and a certified reference material does not involve how the two are prepared, rather with the way that the reference values were established. Certified values are determined through replicate analyses using two independent measurement techniques for verification. The certifying agency may also provide "non-certified or "reference" values for other target analytes. Such values are determined using a single measurement technique that may introduce bias.

Reporting Limit: A reporting limit is the minimum value below which chemistry data are documented as detected but not quantified.

References

Puckett, M. *Quality Assurance Management Plan for the State of California's Surface Water Ambient Monitoring Program*; California Department of Fish and Game, Monterey, CA, 2002.

Appendix VI: Signatures of Approval

Attachment 2: Field Data Sheets

SWAMP Tissue Sampling - Non-Trawl (Event Type = T1) SWB WildLk 2012				Entered in d-base (initial/date)		Pg of Pgs	
*StationCode: _____		*StationName: _____		*Purpose Failure Code: _____		Agency	
*FundingCode: 1 1 S W B G 0 1		*Date (mm/dd/yyyy): / /		WIND DIRECTION (from):		PHOTOS (RB & LB assigned when facing downstream; RENAME to StationCode_yyyy_mm_dd_uniquecode)	
*Sampling Crew:		Arrival Time:	BEAUFORT SCALE (see attachment):			1: (RB / LB / BB / US / DS / ##)	
		Departure Time:					
DOMINANT SUBSTRATE: Concrete, Cobble, Gravel, Sand, Mud, Other _____, unk			WATERCOLOR: Colorless, Green, Yellow, Brown			2: (RB / LB / BB / US / DS / ##)	
WATER CLARITY: Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis)			OTHER PRESENCE: Vascular, Nonvascular, Oily Sheen, Foam, Trash, O				
Comments:						3: (RB / LB / BB / US / DS / ##)	
Tissue Collection							
COLLECTION DEVICE: RV _____ Masta-Blasta, Big E, Sparky _____, Backpack Model _____, Net (length & mesh) _____							
Target:	Lat (dd.dxxxx)		Long (dd.dxxxx)		-		
GPS Model:			Datum: NAD83 WGS84 Other _____		*GPS / DGPS		Elevation (ft):
Location	*Depth (m):	Distance from Bank (m):		Accuracy (ft / m)	Latitude (dd.dxxxx)	Longitude (-ddd.dxxxx)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1		
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2		
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3		
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____	Geoshape: Line Poly Point		Coord. 4		
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.dxxxx)	Longitude (-ddd.dxxxx)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1		
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2		
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3		
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____	Geoshape: Line Poly Point		Coord. 4		
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.dxxxx)	Longitude (-ddd.dxxxx)	Depth (m)
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1		
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2		
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3		
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____	Geoshape: Line Poly Point		Coord. 4		
Failure Codes: Dry (no water), Instrument Failure, No Access, Non-sampleable, Pre-abandoned, Other							
Comments:							

SWAMP Tissue Sampling - Non-Trawl (Event Type = TI) SWB_WildLk_2012					Entered in d-base (initial/date)		Pg	of	Pgs
*StationCode: _____			*StationName: _____				*Purpose Failure Code:	Agency	
*FundingCode: 1 1 S W B G 0 1			*Date (mm/dd/yyyy): / /						
Tissue Collection									
Location	*Depth (m):	Distance from Bank (m):		Accuracy (ft / m)	Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)		
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____ Geoshape: Line Poly Point			Coord. 4				
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)		
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____ Geoshape: Line Poly Point			Coord. 4				
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)		
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____ Geoshape: Line Poly Point			Coord. 4				
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)		
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____ Geoshape: Line Poly Point			Coord. 4				
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)		
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____ Geoshape: Line Poly Point			Coord. 4				
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)		
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____ Geoshape: Line Poly Point			Coord. 4				
Location	*Depth (m):	Distance from Bank (m):			Latitude (dd.ddddd)	Longitude (-ddd.ddddd)	Depth (m)		
COLLECTION METHOD:	E-boat, Backpack shocker, Fyke net, gill net, seine, hook & line			Start Time	Coord. 1				
SAMPLE LOCATION:	Bank, Thalweg, Midchannel, Open Water, NA				Coord. 2				
HYDROMODIFICATION:	None, Bridge, Pipes, Concrete Channel, Grade Control, Culvert,			End Time	Coord. 3				
HYDROMODLOC(to sample):	US / DS / NA / WI	Other _____ Geoshape: Line Poly Point			Coord. 4				
Failure Codes: Dry (no water), Instrument Failure, No Access, Non-sampleable, Pre-abandoned, Other									
Comments:									

<u>Fish Species</u>	<u>Species Code</u>	<u>Fish Species</u>	<u>Species Code</u>
American shad	AMS	Sacramento sucker	SAS
black crappie	BCR	Sacramento blackfish	SBF
bluegill	BGL	sculpin ssp	SCP
black bullhead	BLB	shiner perch	SHP
blue catfish	BLC	smallmouth bass	SMB
brown trout	BNT	spotted bass	SPB
brown bullhead	BRB	Sacramento perch	SPH
brook trout	BRT	Sacramento pike minnow	SPM
carp, common	CAR	Sacramento splittail	SST
channel catfish	CHC	striped bass	STB
chinook salmon	CHS	steelhead	STH
coho salmon	COH	striped mullet	STM
California roach	CRH	sturgeon, white	WST
delta smelt	DTS	threadfin shad	TFS
flathead catfish	FHC	tilapia ssp	TIL
fathead minnows	FHM	topsmelt	TPS
goldfish	GLF	threespine stickleback	TSS
golden trout	GLT	tui chub	TUC
green sunfish	GRS	tule perch	TUP
hitch	HIT	warmouth	WAR
hardhead	HRH	white crappie	WCR
inland silverside	ISS	white catfish	WHC
killifish	KIL		
kokanee salmon	KOK		
lamprey	LAM		
longfin smelt	LFS		
lake trout	LKT		
largemouth bass	LMB		
mosquitofish	MQF		
pumpkinseed sunfish	PKS		
rainbow trout	RBT		
redestye bass	REB		
redestye sunfish	RES		
red shiner	RSR		

SWAMP Lab Data Sheet - FISH		ProjectID: SWB_WildLk_2012	PrepPres: Skin ON; Scales ON	LabID:	Pg: 1 of 2 Pgs
StationCode:	Tissue: Whole Body		Entered d-base (initial/date)		
StationName:	Homog. Method: USGS SOP Mortar/Pestle		Staff: Diss.	Homog.	
Species Name:	Date Diss. (mm/dd/yyyy): / /		Date Homog. (mm/dd/yyyy): / /		
CHEMISTRY JARS					
Individual ID: _____		Individual ID: _____		Individual ID: _____	
Analysis: Mercury		Analysis: Mercury		Analysis: Mercury	
Jar Weight Full (g): _____		Jar Weight Full (g): _____		Jar Weight Full (g): _____	
Jar Weight Empty (g): _____		Jar Weight Empty (g): _____		Jar Weight Empty (g): _____	
Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____	
Individual ID: _____		Individual ID: _____		Individual ID: _____	
Analysis: Mercury		Analysis: Mercury		Analysis: Mercury	
Jar Weight Full (g): _____		Jar Weight Full (g): _____		Jar Weight Full (g): _____	
Jar Weight Empty (g): _____		Jar Weight Empty (g): _____		Jar Weight Empty (g): _____	
Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____	
Individual ID: _____		Individual ID: _____		Individual ID: _____	
Analysis: Mercury		Analysis: Mercury		Analysis: Mercury	
Jar Weight Full (g): _____		Jar Weight Full (g): _____		Jar Weight Full (g): _____	
Jar Weight Empty (g): _____		Jar Weight Empty (g): _____		Jar Weight Empty (g): _____	
Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____	
Individual ID: _____		Individual ID: _____		Individual ID: _____	
Analysis: Mercury		Analysis: Mercury		Analysis: Mercury	
Jar Weight Full (g): _____		Jar Weight Full (g): _____		Jar Weight Full (g): _____	
Jar Weight Empty (g): _____		Jar Weight Empty (g): _____		Jar Weight Empty (g): _____	
Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____		Comp Tissue Wt (Jar Full - Empty; g): _____	
Individual ID: _____ Analysis: Mercury Jar Weight Full (g): _____ Jar Weight Empty (g): _____ Comp Tissue Wt (Jar Full - Empty; g): _____					
Comments: 					